

## Ruminant Nutrition: Protein metabolism

**615 Water partitioning in lactating Holstein cows fed two levels of dietary forage and crude protein contents.** J. A. D. R. N. Appuhamy\*<sup>1</sup>, M. Niu<sup>1</sup>, A. Leytem<sup>2</sup>, R. Dungan<sup>2</sup>, and E. Kebreab<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of California, Davis, CA, <sup>2</sup>USDA-ARS, Northwest Irrigation Research Lab, Kimberly, ID.

Knowledge on relationships between diet composition and water kinetics of dairy cows will assist in understanding water utilization and determining water footprint. The objective of the study was to investigate the effects of dietary forage and CP contents on water partitioning in lactating dairy cows. Twelve Holstein cows were randomly assigned to a 2 × 2 factorial arrangement of 2 forage levels [38 (LF) vs. 53% (HF)] and 2 CP levels [15.2 (LP) vs. 18.5% (HP)] in a 4 × 4 Latin square design with 4 periods. Cows were kept in metabolic cages indoors, where drinking water intake (dWI), DMI, urine weight, feces weight, and milk yield were measured over 3 d. The dietary treatment effects were analyzed in mixed-effect models including random cow effect. Average DMI, dWI, water via feed (fWI), milk yield, and ambient temperature (T) were 20.0 ± 2.2, 100.6 ± 14.3, 2.5 ± 0.2 and 31.4 ± 5.8 kg/cow/d, and 25.9 ± 1.4°C, respectively. Drinking water intake was positively related to DMI ( $P < 0.01$ ). Independent of DMI, dWI increased respectively by 5.0 ± 2.1 and 5.9 ± 2.2 kg/cow/d ( $P < 0.05$ ) for the increases in dietary fiber and CP contents. Irrespective to DMI and the diet composition, dWI increased by 2.2 ± 0.8 kg/cow/d ( $P = 0.02$ ) for unit increase in T. Fecal water output (fWO) increased as DMI increased ( $P < 0.01$ ). Regardless of DMI, fWO increased (FL = 27.2 vs. FH = 30.4 kg/cow/d) as dietary fiber content increased ( $P < 0.01$ ). Urinary water output (uWO) was positively related to dWI ( $P < 0.01$ ). When adjusted for dWI, HP and HF diets were associated with greater uWO (by 3.4 ± 1.1 and 2.0 ± 1.1 kg/cow/d, respectively) than LP and LF diets ( $P < 0.08$ ). Each dietary nutrient modification independently and equally increased total manure water output (fWO+uWO) by 4.7 ± 1.2 kg/cow/d ( $P < 0.01$ ). Water in milk (mWO) and BW were not affected by the dietary treatments. Overall, 26.8 ± 5.4, 28.1 ± 5.7, and 23.5 ± 6.8% of total water intake (fWI+dWI) were partitioned to mWO, fWO, and uWO, respectively. The majority of the rest (21.6 ± 10.7%) may be respiratory-cutaneous water losses. Forage content, dietary composition and temperature should be included for a better estimate of water utilization and partitioning in dairy cows.

**Key Words:** diet composition, lactating cow, water balance

**616 Milk yield and composition responses to changes in supply of net energy and metabolizable protein: A meta-analysis.** Jean-Baptiste Daniel\*<sup>1,2</sup>, Nicolas C. Friggens<sup>1</sup>, Patrick Chapoutot<sup>1</sup>, Harmen Van Laar<sup>2</sup>, and Daniel Sauvant<sup>1</sup>, <sup>1</sup>INRA-AgroParisTech UMR791, Paris, Îles-de-France, France, <sup>2</sup>Nutreco R&D, Boxmeer, North Brabant, the Netherlands.

This study aimed to quantify, through a meta-analysis of literature data, average milk yield, milk composition and energy balance responses to changes in supply of: net energy (NE<sub>L</sub>), metabolizable protein (MP), and their interaction, for dairy cows. Experiments with designs focused on the effects of protein and/or energy levels on milk performance were selected. The dietary NE<sub>L</sub> and MP supplies were estimated for all treatments using the updated digestive model from INRA (Sauvant and Nozière, 2013). From all studies included in the database, 308 experiments (917 treatments) with experimentally induced variations in either NE<sub>L</sub> or MP supplies were kept for this analysis. These treatments

covered a wide range of diet characteristics and therefore a large part of the plausible NE<sub>L</sub> and MP supplies that can be expected in practical situations. The average predicted MP supply was (mean ± SD) 2080 ± 467 g/d and average NE<sub>L</sub> supply was 143 ± 25 MJ/d. The majority of those studies used multiparous Holstein-Friesian cows between 50 and 200 d in milk. Between and within-experiment variations were split to estimated milk responses. Linear and quadratic effects of NE<sub>L</sub> and MP supplies and their interaction on milk responses were systematically tested, and removed from the model when non-significant ( $P > 0.05$ ). Increasing either NE<sub>L</sub> or MP supplies resulted in positive responses for milk, lactose, protein and fat yields. Interactions between NE<sub>L</sub> and MP supplies were significant ( $P < 0.01$ ) for milk yield, milk protein yield and milk lactose yield. Greater responses were found with increasing NE<sub>L</sub> at higher MP supply, and vice versa. Energy balance was related, positively with NE<sub>L</sub> supply, and negatively with MP supply. The negative interaction found between NE<sub>L</sub> and MP supply diminished the effect of MP on energy balance at high NE<sub>L</sub> supply. The current meta-analysis provides accurate estimation of milk yield (RMSE = 1.04 kg) and milk composition responses to change in supply of NE<sub>L</sub> and MP. This study also found significant interactions between NE<sub>L</sub> and MP supply for milk, protein and lactose yields.

**Key Words:** dairy cow, milk composition, meta-analysis

**617 Updating protein requirements and responses of metabolizable protein efficiency in dairy cows and goats.** Daniel Sauvant\*<sup>1</sup>, Gonzalo Cantalapiedra-Hijar<sup>2</sup>, and Pierre Nozière<sup>2</sup>, <sup>1</sup>AgroParisTech-INRA, Paris, France, <sup>2</sup>INRA-VetagroSup, Theix, France.

One of the major target of renewing the INRA feed evaluation system for ruminants was to update their metabolizable protein (MP) requirements as well as their responses to changes in protein supply. These updates have been made from meta-analyses of large databases of INRA and of literature. For the requirements, the nitrogen losses observed in practical situations were quantified. The 3 main routes of losses, which result in non-productive protein requirements, are, in decreasing order of importance (1) metabolic fecal protein (MFP) which was calculated from non-digestible organic matter intake, (2) urinary endogenous nitrogen losses (NUE) which was the NU losses corresponding to digestible N intake = 0 and corrected for purine-N derivative from rumen microorganisms, (3) loss of protein from phaneres (PPH). After, the values of “non-productive” MP requirements were applied to 873 treatments of 296 experiments performed on lactating cows or goats with the aim to study the milk response to MP supply. MP supply was calculated using the renewed INRA model (Sauvant and Nozière, 2013). The efficiency of available MP utilization (EffMP%) was determined with an iterative calculation, assuming the same common EffMP value not only for milk protein synthesis but also for MFP, for PPH as well as for the accretion or mobilization of body proteins which was related to the calculated energy balance. This led to the most accurate prediction of EffMP in response to the dietary concentration of MP (g/kg DM). EffMP presented the same decreasing function to the MP concentration for cows and goats. Moreover, for a given MP content, inter-experiments variations were significantly explained, positively by actual or potential milk protein yield (MPY, g/d) and negatively by the level of dry matter intake (DMI, kg/d). Therefore, for dairy cows, the following regression was calculated: EffMP = 68.3 - 0.54 (MP-100) + 0.029 (MPY-1000) - 1.04 (DMI - 21) [n = 873, R<sup>2</sup> = 0.83, RMSE = 2.9]. In conclusion, the major

novelties of these updates were the fecal and urinary nitrogen losses and the common value of EffMP for all the functions of proteosynthesis.

**Key Words:** protein requirement, efficiency, dairy

**618 The effect of concentration allocation strategy on the performance, health and fertility of high-genetic-merit dairy cows offered a grass silage-based diet.** Mark W. Little\*<sup>1,2</sup>, Niamh O'Connell<sup>2</sup>, and Conrad P. Ferris<sup>1</sup>, <sup>1</sup>Agri-Food and Biosciences Institute, Hillsborough, UK, <sup>2</sup>School of Biological Sciences, Queens University Belfast, Belfast, UK.

Few studies have examined the effect of concentrate allocation strategy on the performance high yielding cows (approximately 40 kg/day). This 140-d study (commencing at calving) examined the effect of concentration allocation strategy: group fed (GF) or individual cow fed (ICF) on feed intake, milk production and composition, tissue changes, energy balance, health and fertility of Holstein Friesian dairy cows (n = 72). With GF, cows were offered a total mixed ration comprising grass silage and concentrates (50:50 DM ratio) plus 0.35 kg chopped straw/cow/day throughout the study. With ICF, cows were offered a basal ration consisting of grass silage, concentrates (6 kg/cow/day) and chopped straw (0.35 kg/cow/day), with this diet designed to meet the cows energy requirements for maintenance plus 24 kg milk/cow/day. With this treatment additional concentrates were offered 'feed-to-yield' via an out-of-parlor feeding system (0.45 kg concentrate/kg milk) based on each individual cow's milk yield during the previous week. Production data were analyzed using a Residual Maximum Likelihood (REML) mixed model analysis; fertility and health data were analyzed using generalized linear model regression analysis with the logit link function, using GenStat Version 16.2. Total concentrate intakes were 1616 kg/cow and 1635 kg/cow for GC and ICF, respectively. Neither silage DM intake ( $P = 0.059$ ) nor total DM intake (22.4(GF), 22.2(ICF) kgDM/day:  $P = 0.243$ ) was affected by allocation strategy. Similarly, concentrate allocation strategy had no effect on milk yield (39.3 (GF), 38.0 (ICF) kg DM/day:  $P = 0.113$ ), milk fat content ( $P = 0.819$ ), milk protein content ( $P = 0.308$ ), somatic cell score ( $P = 0.125$ ), BCS loss to nadir ( $P = 0.521$ ) or mean daily energy balance ( $P = 0.356$ ). Concentrate allocation strategy has no effect on the numbers of cows with mastitis ( $P = 0.617$ ), lameness ( $P = 0.144$ ) or pneumonia ( $P = 0.451$ ). Cows on the ICF treatment tended to have more abomasal disorders ( $P = 0.096$ ), and an improved pregnancy rate to first and second service ( $P = 0.014$ ). Offering concentrates according to milk yield had no effect on cow performance or health, while improving fertility performance.

**Key Words:** concentrate allocation, performance, fertility

**619 How the efficiency of utilization of histidine varies with supply in dairy cows.** Helene Lapiere\* and Daniel R. Ouellet, *Agri-culture and Agri-Food Canada, Sherbrooke, QC, Canada.*

To determine the effect of a limited supply of His, 5 multiparous Holstein cows fitted with catheters were used in a 4 × 4 Latin square plus one cow, with 14-d periods. Cows were fed a diet balanced to supply 103% of NE<sub>L</sub> and 72% of MP requirements (1610 g/d; NRC, 2001). Treatments were abomasal infusion of His at 0, 7.6, 15.2 or 22.8 g/d plus a mixture of AA (595 g/d, CN profile; from Ajinomoto). At each period, 6 sets of blood samples were collected from arterial, portal, hepatic and mammary sources. Splanchnic plasma flow was calculated by downstream dilution of deacetylated para-amino-hippurate and mammary plasma flow, using the Fick principle. Results are given in Table 1. Mammary plasma flow was not affected ( $P = 0.75$ ) by His deletion,

averaging 690, 644, 682, and 690 ± 34 L/h, for incremental doses of His. Portal absorption accounted for 108% of the infused His at the highest dose suggesting very little catabolism of His across the gut. The mammary uptake:output ratio did not differ from unity across infusion rates. However, the splanchnic release was not sufficient to account for MG uptake at the 0 dose, in line with decreased muscle carnosine (a labile pool of His dipeptide) concentration at low His supply (JDS 97 E-Suppl.1:757). The increased hepatic removal observed as His supply increases was the sole responsible of the decreased His efficiency and was directly related to liver inflow of His.

**Table 1 (Abstr. 619).**

His flow (mmol/h)	His infused (g/d)				SEM	P-value	
	0	7.6	15.2	22.8		Linear	Quadratic
Digestive flow <sup>1</sup>	8.9	8.9	9.1	9.1	0.12	0.08	0.92
Total supply (Tsupply)	8.9	10.9	13.2	15.2	0.12	<0.001	0.92
Portal absorption	7.2	8.7	11.2	14.2	0.88	<0.001	0.39
Hepatic removal	-1.2	-1.4	-3.8	-5.0	0.83	0.005	0.53
Splanchnic release	5.9	7.5	7.5	9.8	1.48	0.11	0.82
Mammary (MG) uptake (U)	-6.7	-7.7	-7.5	-9.0	0.49	0.01	0.64
Milk output (O) <sup>2</sup>	6.3	7.5	7.8	8.3	0.24	<0.001	0.21
Liver fractional removal	0.04	0.02	0.04	0.04	0.016	0.72	0.55
MG fractional removal	0.65	0.40	0.22	0.23	0.033	<0.001	0.004
U:O	1.06	1.02	0.97	1.08	0.054	0.92	0.21
Splanchnic/MG	0.92	0.98	0.99	1.09	0.174	0.50	0.91
Maintenance net rqt <sup>3</sup>	1.9	1.9	2.0	2.0	0.02	0.08	0.91
Efficiency <sup>4</sup>	0.95	0.90	0.76	0.70	0.022	<0.001	0.93

<sup>1</sup>NRC (2001).

<sup>2</sup>In protein synthesized within the MG, 28.9 mg His/g true protein.

<sup>3</sup>Maintenance requirement = scurf + endogenous urinary + metabolic fecal (Florida Symposium, 2014:166) excluding efficiency of utilization.

<sup>4</sup>(Milk output + maintenance)/Total supply.

**Key Words:** histidine, metabolism, dairy cow

**620 Effects of rumen-protected methionine, lysine, and histidine on lactation performance of dairy cows.** F. Giallongo\*<sup>1</sup>, J. Oh<sup>1</sup>, M. Harper<sup>1</sup>, J. Lopes<sup>1</sup>, A. N. Hristov<sup>1</sup>, H. Lapiere<sup>2</sup>, R. A. Patton<sup>3</sup>, I. Shinzato<sup>4</sup>, J. Tekippe<sup>4</sup>, and C. Parys<sup>5</sup>, <sup>1</sup>Department of Animal Science, The Pennsylvania State University, University Park, PA, <sup>2</sup>Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, <sup>3</sup>Nittany Dairy Nutrition Inc., Mifflinburg, PA, <sup>4</sup>Ajinomoto Co. Inc., Tokyo, Japan, <sup>5</sup>Evonik Industries AG, Hanau, Germany.

The objective of this study was to evaluate the effects of rumen-protected (RP) Met, Lys and His supplementation to a metabolizable protein (MP)-deficient diet on performance of dairy cows. The experiment was a 9-wk randomized complete block design with 36 Holstein cows (DIM, 132 ± 30 d; BW, 611 ± 81 kg). After a 2-wk covariate period, cows were blocked by DIM, milk yield, and parity, and randomly assigned to 1 of the following 6 treatments: control [AMP; +245 g/d of MP over NRC (2001) requirements]; MP-deficient diet (DMP; -118 g/d of MP); DMP supplemented with RPMet (30 g/d of Mepron; Evonik Industries AG; DMPM); DMP supplemented with RPLys (130 g/d of AjiPro-L; Ajinomoto Co., Inc.; DMPL); DMP supplemented with RPHis (120 g/d of an

experimental product; DMPH); and DMP supplemented with RPMet, RPLys and RPHis (DMPMLH). The AMP and DMP diets consisted of (DM basis): 42% corn and 21% alfalfa silages and 37% concentrates and contained 16.5 and 14.5% CP, respectively. DMI tended to be decreased ( $P = 0.07$ ) by DMP compared with AMP (28.0 vs. 29.4 kg/d). Milk and energy-corrected milk yields were decreased ( $P < 0.03$ ) by DMP (40.5 and 36.1 kg/d) vs. AMP (44.1 and 42.1 kg/d). Milk protein content was increased ( $P \leq 0.03$ ) by DMPH and DMPL (3.17 and 3.20%) compared with DMP and AMP (3.01%), and tended ( $P = 0.06$ ) or was numerically higher ( $P \leq 0.15$ ) for DMPMLH and DMPM (3.15 and 3.12%) vs. DMP and AMP. Milk fat content was decreased by DMP vs. AMP (3.33 and 3.90%;  $P = 0.04$ ) and was increased by DMPH and DMPMLH (3.93 and 4.01%;  $P \leq 0.03$ ) compared with DMP. Yields of milk protein and milk fat were decreased ( $P = 0.01$ ) by DMP vs. AMP (by 10 and 20%, respectively). Cows fed AMP had higher MUN (11.7 mg/dL;  $P < 0.01$ ) compared with cows fed the DMP diets (on average 8.09 mg/dL). Overall, feeding an MP-deficient diet decreased DMI and yields of milk, protein, and fat. Addition of RPAA to the DMP diet generally increased milk protein content but did not affect protein yield. Supplementation of RPHis alone or in combinations with RPMet and RPLys also increased milk fat content.

**Key Words:** methionine, lysine, histidine

### 621 Evaluation of a rumen-protected methionine product for lactating dairy cows at 2 concentrations of dietary crude protein.

Shane M. Fredin<sup>\*1</sup>, Heather M. Dann<sup>1</sup>, Kurt W. Cotanch<sup>1</sup>, Catherine S. Ballard<sup>1</sup>, Richard Paratte<sup>2</sup>, Kai Yuan<sup>3</sup>, and Rick J. Grant<sup>1</sup>, <sup>1</sup>William H. Miner Agricultural Research Institute, Chazy, NY, <sup>2</sup>Vetagro S.p.A., Reggio Emilia, Italy, <sup>3</sup>Vetagro Inc., Chicago, IL.

This study evaluated the effect of a supplemental rumen-protected Met product (Timet; VETAGRO S.p.A.; Reggio Emilia, Italy) on lactation performance and rumen measures of dairy cows fed 2 concentrations of dietary CP. Sixteen multiparous Holstein cows (8 ruminally-cannulated) were used in a replicated 4 × 4 Latin square design with 28-d periods. Cows were fed 1 of 4 diets: (1) a control diet containing (DM basis) 40.8% corn silage, 9.5% hay crop silage, and 49.7% grain mix (CON); (2) the CON diet plus Timet (TMT); (3) the CON diet plus Smartamine (Adisseo USA Inc.; Alpharetta, GA; SMT); and (4) a reduced-CP diet plus Timet (TMT-RCP). The TMT, SMT, and TMT-RCP were balanced to contain the same supply of metabolizable Met and Lys, and contained (DM basis) 16.2, 16.2, 15.9, and 13.7% CP, respectively. All diets contained 30.9% NDF and 26.8% starch. Dry matter intake and milk yield were measured on d 22–28. Milk samples were collected d 26–27 to determine composition. Rumen NH<sub>3</sub>-N concentrations were measured on d 26–27 at 4-h intervals. Data were analyzed by ANOVA using the MIXED procedure of SAS. Dry matter intake was unaffected by diet, averaging 26.8 kg/d ( $P > 0.10$ ). Milk yield was unaffected by diet and was 45.6, 46.8, 44.4, and 44.9 kg/d for the CON, TMT, SMT, and TMT-RCP diets, respectively ( $P > 0.10$ ). Milk protein percentage was increased for cows fed SMT (3.33%) compared with the other 3 diets (3.24%;  $P = 0.01$ ), but protein yield was unaffected by diet and was 1.44, 1.49, 1.45, and 1.46 kg/d for the CON, TMT, SMT, and TMT-RCP diets, respectively ( $P > 0.10$ ). Milk lactose percentage was decreased for cows fed SMT (4.55%) compared with the other 3 diets (4.61%;  $P = 0.01$ ). Consistent with reduced CP, MUN (mg/dL) was decreased for cows fed TMT-RCP (11.3) compared with the other 3 diets (13.5;  $P \leq 0.01$ ), and ruminal NH<sub>3</sub>-N (mg/dL) was decreased for cows

fed TMT-RCP (3.4) compared with the other 3 diets (6.9;  $P = 0.001$ ). In conclusion, milk and protein yields were unaffected by sources of rumen-protected Met products. Supplementation of Timet maintained the lactation performance of cows fed reduced CP diets.

**Key Words:** amino acid, reduced CP, rumen measure

### 622 Milk protein yield and plasma concentration of methionine increase in dairy cows fed field peas supplemented with rumen-protected amino acids.

Andre B. D. Pereira<sup>\*</sup>, Andre F. Brito, Kayla M. Aragona, Simone F. Reis, and Kelsey A. Juntwait, *University of New Hampshire, Durham, NH.*

Previous studies showed that diets with more than 15% field peas (FP; DM basis) resulted in reduced milk and milk protein yield in dairy cows. We hypothesize that decreased yields were caused by limited supplies of MP-Lys and MP-Met due to extensive degradation of FP RDP in the rumen. The objective of this study was to evaluate diets with 25% of FP supplemented with rumen-protected (RP) Lys (AjiPro-L) and Met (Smartamine-M) as a substitute for corn meal (CM) and soybean meal (SBM). Twelve multiparous and 4 primiparous lactating Holstein cows were blocked by DIM, milk yield and parity, and randomly assigned to 1 of 4 diets in a replicated 4 × 4 Latin square design. Diets were 35.5% corn silage, 15.5% grass-legume haylage, 5.9% roasted soybean, and: (1) 36% CM and 1.3% urea (3.59:1 MPLys:MPMet ratio; negative control: NC), (2) 29.7% CM, 9.8% SBM, and RPLys-Met (3.07:1 MPLys:MPMet ratio; positive control: PC), (3) 25% FP, 12.3% CM, and 2.4% SBM (3.88:1 MPLys:MPMet ratio; PEAS), and (4) 25% FP, 12.2% CM, 2.4% SBM, and RPLys-Met (3.13:1 MPLys:MPMet ratio; PEASAA). Data were analyzed using the MIXED procedure of SAS and Tukey adjustment for means separation ( $\alpha = 0.05$ ). All dietary treatments had 15.1% CP and 1.58 Mcal/kg. As expected, cows fed NC had decreased DMI, milk protein yield and total concentration of ruminal VFA and increased excretion of MUN. Cows fed NC and PEAS had decreased plasma concentration of Met. Feeding cows PC, and PEASAA mitigated these negative responses. Results showed that plasma Lys was higher for diets fed FP, suggesting that Met is the first limiting AA in FP-based diets.

**Table 1 (Abstr. 622).** Feeding field peas to dairy cows

Item	Diet				SEM	P-value
	NC	PC	PEAS	PEASAA		
DMI, kg/d	23.30 <sup>b</sup>	24.96 <sup>a</sup>	24.63 <sup>a</sup>	25.00 <sup>a</sup>	0.39	<0.01
Milk, kg/d	41.79	42.93	42.69	42.71	1.04	0.53
Milk fat, kg/d	1.40	1.41	1.45	1.49	0.04	0.09
Milk protein, kg/d	1.15 <sup>b</sup>	1.25 <sup>a</sup>	1.21 <sup>ab</sup>	1.24 <sup>a</sup>	0.03	<0.01
MUN, mg/dL	9.85 <sup>c</sup>	7.93 <sup>a</sup>	9.09 <sup>b</sup>	8.77 <sup>b</sup>	0.27	<0.01
Ruminal total VFA, mM	103.08 <sup>c</sup>	113.23 <sup>b</sup>	111.92 <sup>b</sup>	120.92 <sup>a</sup>	1.84	<0.01
Plasma Lys, mM	67.20 <sup>b</sup>	69.94 <sup>b</sup>	77.21 <sup>a</sup>	76.97 <sup>a</sup>	3.96	0.03
Plasma Met, mM	19.92 <sup>c</sup>	27.71 <sup>b</sup>	19.63 <sup>c</sup>	31.89 <sup>a</sup>	1.06	<0.01

**Key Words:** field peas, dairy cow, rumen-protected amino acid

**623 Increased pre-wilting increases metabolizable protein concentration in grass-clover silage.** Marianne Johansen\* and Martin R. Weisbjerg, *Department of Animal Science, AU Foulum, Aarhus University, Tjele, Denmark.*

In organic dairy farming supply with metabolizable protein (MP) is often limited. Therefore, the potential for increased MP concentration by increased pre-wilting of grass-clover before ensiling was investigated. Spring growth and first regrowth grown by 2 Danish organic farmers were cut and pre-wilted to a planned dry matter (DM) content of 35% and 70%, respectively, giving in total 8 silages with DM contents ranging from 28.3 to 72.5%. Four rumen, duodenal and ileal fistulated Holstein dairy cows (551 ± 33 kg body weight, 216 ± 23 d in milk, mean ± SD) were fed ad libitum with the silages in a crossover design, with 5 periods of 3 weeks. No concentrate was offered, but minerals and vitamins were offered daily. Three markers (Cr<sub>2</sub>O<sub>3</sub>, TiO<sub>2</sub> and YbCl<sub>3</sub>·6H<sub>2</sub>O) were dosed in the rumen twice a day for measurement of flow at duodenum and ileum. In the last week of each period daily feed intake was registered and 12 subsamples from duodenum and ileum, respectively, were collected over 94 h to cover the diurnal variation, pooled, and subsequently analyzed. Microbes were isolated from the rumen and analyzed for amino acids (AA) and purines to estimate the flow of microbial protein at the duodenum. Statistical analyses were done in R using a linear random regression model with DM content as fixed effect, cow and growth x farmer as random intercepts and with a growth x farmer random slope. Results given below are per 10% units increase in DM content of grass-clover silage. The absorption of AA in the small intestine was increased ( $P = 0.03$ ) with 5.59 g/kg DM intake. The increased absorption was caused by a higher small intestinal digestibility of AA (1.27 g/100g AA,  $P = 0.03$ ) and a higher flow of AA in the duodenum (5.63 g/kg DM intake,  $P = 0.08$ ). The higher flow of AA in the duodenum derived from both a higher flow of microbial AA (2.12 g/kg DM intake,  $P = 0.06$ ), and a lower true rumen degradation of AA from the silage (-4.04 g/100g AA,  $P = 0.04$ ). In conclusion, the MP concentration in grass-clover silage can be improved by pre-wilting to a higher DM content before ensiling.

**Key Words:** pre-wilting, metabolizable protein, grass-clover silage

**624 Precision-feeding dairy heifers different levels of dietary fiber and high rumen undegradable protein.** Louisa Bowen\*, Ashley Bowyer<sup>1</sup>, Sonya Weeks<sup>1</sup>, Gustavo Lascano<sup>1</sup>, and N. A. Gomez<sup>2</sup>, <sup>1</sup>Clemson University, Clemson, SC, <sup>2</sup>The California Polytechnic State University, San Luis Obispo, CA.

The objective of this experiment was to determine the effects of feeding a high rumen undegradable protein (RUP) diet when dietary fiber level is manipulated within differing forage to concentrate ratio (F:C) on nutrient utilization of precision-fed dairy heifers. Six rumen cannulated Holstein heifers (555.4 ± 31.4 kg BW) were randomly assigned to 2 levels of concentrate, HC [55%] and LC [40%] and to a fiber level sequence [100% oat hay and silage OA, 0% wheat straw WS (low fiber); 83% OA, 17% WS (medium fiber); and 67% OA, 33% WS (high fiber)] administered according to a split-plot 3 × 3 Latin square design (21-d periods). Similar levels of N intake (1.70 g N/kg BW<sup>0.75</sup>) and RUP (55% of CP) were provided. All dependent variables were analyzed as a 3 × 3 Latin square using the PROC MIXED procedure of SAS. No differences were observed for dry matter (DM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) apparent digestibility

(AD) between HC and LC-fed heifers. Heifers receiving HC diets had greater starch AD ( $P < 0.01$ ) than LC heifers. Increasing the fiber level through WS addition resulted in a linear reduction of organic matter AD ( $P = 0.05$ ). There was a linear interaction for DM AD ( $P = 0.05$ ) with a concurrent linear interaction in NDF AD. Nitrogen intake, AD, and retention were not different among treatments, however, urine N excretion increased linearly ( $P = 0.03$ ) with added fiber. Uric acid excretion differed among F:C treatments ( $P < 0.01$ ) but total purine derivatives (PD), microbial CP, and protozoa did not differ between F:C treatments. Microbial CP flow and PD quadratically increased ( $P < 0.05$ ) with WS inclusion. Mean rumen fluid pH quadratically increased ( $P = 0.04$ ) with WS addition, although no F:C effect was detected. Ruminal ammonia concentration did not differ among treatments. Apparent digestibility, purine excretion, and ruminal pH were affected differently as dietary fiber was added through the addition of WS in the diet, suggesting that high RUP can have differential effects on nutrient utilization as dietary fiber is manipulated in precision-fed dairy heifers.

**Key Words:** fiber level, protein degradability, precision-feeding

**625 Dietary fiber and crude protein contents can be modified to minimize enteric methane emissions and nitrogen excretions from dairy cows simultaneously.** M. Niu\*, J. A. D. R.N. Appuhamy<sup>1</sup>, A. Leytem<sup>2</sup>, R. Dungan<sup>2</sup>, and E. Kebreab<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of California, Davis, Davis, CA, <sup>2</sup>USDA-ARS, Northwest Irrigation and Soils Research Lab, Kimberly, ID.

Simulation of enteric methane (CH<sub>4</sub>) emissions and nitrogen (N) excretion from cows fed grass silage-based diet indicated that there may be a trade-off between reduced N excretions and increased CH<sub>4</sub> emissions when certain dietary manipulations were considered. The study aimed to examine effects of changing dietary forage and CP contents on CH<sub>4</sub> emissions and N excretions from lactating dairy cows. Twelve post-peak lactating Holstein cows were randomly assigned to a 2 × 2 factorial arrangement of 2 forage (alfalfa hay) levels [37 (LF) vs. 53% (HF)] and 2 CP levels [15.2 (LP) vs. 18.5% (HP)] in a 4 × 4 Latin square design with 4 periods. Cows were fed and milked twice daily. During the first 14 d, cows were housed in a free-stall barn, where enteric CH<sub>4</sub> emissions were measured using the GreenFeed system from d 8 to 14 in each period. Cows were then moved to metabolic cages, where total feces and urine output (kg/cow/d) were measured using total collection approach for 3 d. Treatment effects were analyzed by a mixed model with the random effect of cow. Dietary forage content was negatively associated with DMI ( $P = 0.09$ ) and milk yield (MY,  $P = 0.02$ ) and positively related ( $P < 0.05$ ) to CH<sub>4</sub> emission per unit of DMI (HF = 20.4 (0.83) vs. LF = 17.8 (0.84) g/kg) and MY (HF = 13.7 (0.80) vs. LF = 11.6 (0.80) g/kg). Dietary CP content did not affect CH<sub>4</sub> emissions but was positively related to milk urea nitrogen content, urine, urine N (UN), and total manure N outputs (MN) per kg of DMI or MY ( $P < 0.01$ ). Dietary forage content was positively associated with only UN (HF = 170 (8.5) vs. LF = 152 (8.5) g/cow/d,  $P < 0.01$ ). Fecal N output was not affected by dietary CP levels. Dietary forage content was positively associated with only UN (HF = 170 (8.5) vs. LF = 152 (8.5) g/cow/d,  $P < 0.01$ ). The enteric CH<sub>4</sub> emissions and MN per unit of milk yield were positively correlated ( $r = 0.67$ ,  $P < 0.01$ ). There were no interactions between dietary CP and forage contents on CH<sub>4</sub> emissions or N excretion. Overall, increasing dietary forage and CP contents independently

increased CH<sub>4</sub> emissions (g/kg of MY) and total manure N excretions (g/kg of MY), respectively.

**Key Words:** dairy cow, methane emissions, nitrogen excretions

**626 Effects of substituting true protein with non-protein nitrogen in Holstein dairy heifers precision-fed different forage to concentrate ratios.** Prabha G. Ranasinghe\*<sup>1</sup>, Noe A. Gomez<sup>2</sup>, Krysztina Rowland<sup>1</sup>, Ashley Caprio<sup>1</sup>, and Gustavo J. Lascano<sup>1</sup>, <sup>1</sup>*Clemson University, Clemson, SC*, <sup>2</sup>*California Polytechnic State University, San Luis Obispo, CA*.

The objective of this experiment was to determine the effects of feeding different combinations of true protein (TP) and non-protein nitrogen (NPN) when forage to concentrate (F:C) is manipulated on nutrient utilization in precision-fed dairy heifers. Eight ruminally fistulated Holstein heifers (386 ± 9.1 kg BW) were randomly assigned to 2 concentrate levels: HC (25% forage) and LC (75% forage) and to a combination of TP and NPN sequence [U:C; 0% from urea U, 100% casein C (0% U, 100% C; 33% U, 67% C; 67% U, 33% C; 100% U, 0% C)] within concentrate level administered according to a split-plot, 4 × 4 Latin square design (21 d periods). Data were analyzed using the PROC Mixed

procedure from SAS. Intake of N was similar and use U and C to provide 1.80 g N/kg BW 0.75. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) apparent digestibility (AD) were positively affected in HC diets ( $P \leq 0.05$ ). ADF AD linearly increased ( $P < 0.01$ ) in HC, and the opposite occurred in the LC group. Fecal N, urine N, and total excreted N were reduced ( $P \leq 0.05$ ) in the HC treatment (TRT) which resulted in an increased retained N. No U:C effects were observed in AD of DM, OM, and NDF, but NAD, N (% of intake), N (% of digested) decreased linearly as U level increased ( $P < 0.05$ ). A quadratic interaction ( $P < 0.01$ ) was observed; decreasing total excreted N and retained N (g/d) in the HC and the opposite in LC. Total purine derivatives (PD) were positively affected by the HC group ( $P = 0.04$ ) but did not affect microbial protein flow. There was a positive quadratic ( $P < 0.01$ ) response in total PD as U increased in both F:C TRT. Protozoa numbers were higher in the HC diets ( $3.3 \times 10^5$  vs.  $2.8 \times 10^3$ ;  $P < 0.01$ ) and increase quadratically ( $P < 0.01$ ) with U inclusion. No effects for ruminal NH<sub>3</sub>N between F:C TRT was observed, but there was a linear increase ( $P < 0.01$ ) as U level increased. Ruminal pH was lower for HC diets (6.3 vs. 6.6) and there was a quadratic interaction as U increased. Nutrient utilization was significantly affected by manipulation of F:C and U:C.

**Key Words:** heifer, true protein, urea