Bacteroidetes and Firmicutes, accounting for 49% and 39% of total sequences, respectively. The bacterial community compositions in both of liquid and solid fractions of effluent digesta were changed ($P < 0.01$) by dietary CC but not by dietary fat levels. Including CC in the diets decreased ($P < 0.05$) the relative abundance of *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Fibrobacter* spp., and *Butyrivibrio* spp. The most abundant genus, across treatments, *Prevotella*, was decreased ($P < 0.05$) by high dietary fat levels while *Megasphaera* was increased ($P < 0.01$) by CC in the liquid fraction. Correlatively, the concentration of acetate was decreased ($P < 0.01$) while propionate was increased ($P = 0.01$); saturated fatty acid (C16:0 and C18:0) were decreased ($P < 0.01$) and PUFA, especially C18:2 n-6 and C18:3 n-3 were increased ($P < 0.01$) by dietary CC. Based on the correlation analysis between genus and metabolites, this study revealed that CC could be energetically beneficial to dairy cows and useful at suppressing ruminal bacteria associated with biohydrogenation; however, attention should be given to avoid negative effects of CC on suppressing cellulolytic bacteria.

Key Words: PUFA, microbiome, biohydrogenation

T210 First-lactation performance of Holstein cows fed milk replacer or pasteurized or raw non-saleable milk as preweaning heifers. M. Garcia*, S. R. Montgomery, L. E. Hulbert, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

A growing body of research has shown that strategic feeding and nutritional management of preweaning heifers can have life-long effects on their performance. Our objective was to assess the performance of first-lactation heifers that were fed either accelerated milk replacer (MR; 3.98% CP, 2.56% fat) or non-saleable milk (3.59 ± 0.28% true protein; 4.12 ± 0.37% fat) that was either pasteurized (PM) or raw (RM). Heifers were randomly assigned to feeding treatments after birth and were fed 3 times daily either 1.4 L (calves <36.3 kg, until the target weight was achieved) or 1.9 L (calves ≥36.3 kg). After weaning, all heifers were managed uniformly. Monthly test day data were used to generate DHIA estimates of 305-d mature equivalent milk (MEM), fat (MEF), and protein (MEP) yields; predicted transmitted ability (PTA) estimates for these traits were also collected. Data were analyzed using mixed, regression, and logistic procedures of SAS and significance declared at $P \leq 0.05$. The model included the main effect of milk treatments, amount fed daily at enrollment, and their interaction. The corresponding PTA was used as covariate for milk yield variables. The sample size (n = 152) provided sufficient power (80%) to detect statistical differences of 950 kg MEM between treatments before accounting for the covariate. Treatments did not influence ($P > 0.10$) the number of inseminations at first calving, age at first calving, days open, or retention in the herd by 36 mo of age (left/enrolled: 18/50, 18/50, 16/52 for MR, PM, and RM, respectively). Heifers fed 5.7 L/d produced more MEM ($P = 0.04$; + 695 kg), tended ($P = 0.07$) to produce more MEF (+26 kg), and produced more MEP ($P = 0.05$; +18 kg) compared with heifers fed 4.2 L/d, which may be related to either prenatal factors causing low BW or to the level of nutrition early in life. An interaction of treatment and feeding rate was observed for MEM and MEF ($P = 0.07$); within the RM-treatment, low-BW heifers produced less MEM and MEF compared with high-BW heifers (−1,705 kg MEM and −68 kg MEF, both $P < 0.01$). Although no overall treatment effects on first-lactation performance were observed, feeding RM may impair first lactation performance of low-BW heifers.

Key Words: heifer, pasteurized milk, programming

T211 Enriching bovine milk fat with α-linolenic acid, an n-3 fatty acid, through feeding of a rumen-protected flax-based supplement. H. Peterson*[1], R. Day[2], J. E. Williams[1], W. J. Price[3], B. Shafii[3], and M. A. McGuire[1], [1]*University of Idaho, Moscow, ID,* [2]*N3 Feed LLC, Tualatin, OR,* [3]*Statistical Programs, College of Agriculture and Life Sciences, University of Idaho, Moscow, ID.*

The objective of this study was to increase the α-linolenic acid (ALA) concentration in milk fat by feeding a rumen protected flax-based (RPF) supplement. Eight lactating Holstein cows at 194 ± 16.3 DIM were randomly assigned to 4 treatment sequences in replicated 4 × 4 Latin squares with 16-d periods. The 4 treatments were 0, 0.9, 1.8, and 2.7 kg of RPF (26.6% protein and 31.9% lipid, of which 49.9% was ALA) added to a base ration (3% lipid DM basis) daily. Milk samples were collected on d 15 and 16 of each period. Milk components were assessed by near-infrared analysis and fatty acids by gas chromatography. Data were analyzed using a generalized linear mixed model with cow nested within period as the random effect and treatment, period, and treatment by period interactions as the fixed effects. A β distribution was assumed for the proportion fatty acid data and a normal distribution was assumed for dry matter intake, milk yield, and milk components. RPF did not affect dry matter intake, milk yield, or most milk components. Milk fat concentration tended to increase ($P = 0.02$) from 3.75% to 3.91% and milk urea nitrogen concentration tended to decrease ($P = 0.08$) from 14.2 to 11.9 mg/dL as the amount of RPF supplement added to the diet increased. Many fatty acids in milk were altered by the RPF dose showing a generally decreasing trend in short- and medium-chain saturated fatty acids and increasing trends in long-chain fatty acids. Compared with the diet with no added RPF (ALA concentration 0.53 g/100 g total fatty acids), RPF added at 0.9, 1.8, and 2.7 kg/d increased ($P < 0.0001$) the concentration of ALA in milk to 1.43, 2.14, and 2.77 g/100 g total fatty acids, respectively. The n-6-to-n-3 fatty acid ratio was lowered (6.6 to 1.3) as the amount of RPF supplement added to the diet increased. This novel rumen protected ALA source enriched milk ALA greater than any previous method.

Key Words: lipid supplement, milk fatty acid, α-linolenic acid

T212 Determination of the bioavailability of lysine in the latest generation of a rumen-protected lysine product exposed to TMR using the in vivo plasma lysine response method. K. Hultquist[1], C. S. Ballard*[1], M. Miura[2], T. Fujieda[2], and I. Shinzato[3], [1]*William H. Miner Agricultural Research Institute, Chazy, NY,* [2]*Ajinomoto Co. Inc., Kawasaki-ku, Kawasaki-shi, Japan,* [3]*Ajinomoto Heartland, Inc., Chicago, IL.*

The objective of this study was to estimate bioavailability of the third generation of a rumen-protected Lys (RPL) product, AjiPro-L (A3G; Ajinomoto Heartland Inc.) using the currently marketed AjiPro-L (A2G; Ajinomoto Heartland Inc.) after products were exposed to TMR. Fourteen multiparous lactating Holstein cows (114 ± 8 d in milk) housed in tie stalls were used in a replicated 7 × 7 Latin square design with 7-d periods. A common basal diet adequate in Lys was prepared 1 × /d and fed proportionately at 0500 h, 1300 h, and 2100 h. Treatments included Lys supplemented at 0, 75, 112.5 and 150 g/d from A2G and A3G and were mixed with a small amount of total mixed ration (TMR) once daily to mimic inclusion and exposure to a diet fed 1 × /d. Treatments were fed 3 × /d 1 h before feeding on d 2 through 7 of each period in amounts proportional to feed offered. Four blood samples were obtained from cows on d 6 and 7 of each period from the tail vein at 2-h intervals starting at 0600 h. Plasma, pooled by day, was analyzed for AA concentrations. Data were reduced to period mean for each cow and analyzed using the MIXED procedure of SAS. The REG procedure was used to generate linear regression models for each RPL product using the values of Lys (μmol/L) and Lys as a percent of total

AA (µmol/L basis) to determine the degree of elevation of plasma Lys in response to treatment. Relative to A2G, estimated bioavailability of A3G was determined using the slope-ratio assay technique. Plasma Lys was greatest ($P \leq 0.05$) for 150 g/d A2G (119.8 ± 3.2 µmol/L) and 150 g/d A3G (123.8 ± 3.5 µmol/L) when compared with 0 g/d Lys (110.7 ± 3.3 µmol/L). The slope for A3G treatment was numerically greater (0.009; $r^2 = 0.92$) when compared with the slope for A2G treatment (0.006; $r^2 = 0.93$) when expressing the concentration of plasma Lys relative to total AA. Calculated bioavailability of A3G was 146.7% of the bioavailability of A2G. To our knowledge, this is the first study to measure relative bioavailability of RPL products in a TMR to simulate on-farm exposure when TMR is fed 1 × /d.

Key Words: lysine, bioavailability, rumen-protected

T213 Characteristics of a rumen-protected lysine product. 1: Bioavailability of the third-generation AjiPro-L. M. Miura*[1], A. Haruno[1], H. Sato[1], S. Shimizu[1], M. Nakamura[1], Y. Miyazawa[1], T. Fujieda[1], and I. Shinzato[2], [1]*Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co. Inc., Kawasaki, Kanagawa, Japan,* [2]*Ajinomoto Heartland Inc., Chicago, IL.*

A new generation of a rumen protected lysine product, the third-generation AjiPro-L (A3G, Ajinomoto Co. Inc.), was recently developed. Because the particle size of A3G is smaller than the previous generation product (the second-generation AjiPro-L, A2G), it was expected that A3G has higher intestinal digestibility than A2G due to an increased surface area while the rumen protection of A3G might be compromised. This study was conducted to evaluate the bioavailability of A3G by examining the rumen escape and the fecal excretion of the product. Four dry cows (BW 650–720 kg), fistulated both ruminally and duodenally and fed a corn silage based diet (DMI 13–14 kg/d), were used. Ruminal escape of A2G and A3G was determined by the duodenal digesta collection method with using highly protected L-arginine as a control maker (HP-Arg; Robinson et al., 2011). A2G or A3G was ruminally administrated along with HP-Arg and duodenal digesta was collected every 2 or 3 h for 48–54 h. The digesta was heated with hot water to dissolve fat-containing A2G or A3G particles and homogenized to extract free Lys and Arg. Changes of Lys and Arg concentrations in duodenal digesta were plotted to calculate the area under the curve (AUC). The proportion of AUC of Lys to Arg was defined as the rate of ruminal escape of A3G, based on in situ rumen protection of HP-Arg (>90%). Fecal excretion of A2G or A3G was measured by the 72 h-fecal collection method (Miura et al., 2015). Feces collected individually after ruminal ingestion of the products were homogenized with hot water to extract Lys from A2G or A3G particles in feces. Amounts of Lys excreted in feces were calculated by analyzing free Lys concentration in the extract. Statistical differences were tested by a one-way ANOVA. Rumen escape of A3G (68 ± 7% of initial, mean ± SE) was not different from A2G (73 ± 6%, $P = 0.60$), but fecal excretion of A3G (18 ± 3%) was significantly lower than that of A2G (32 ± 4%, $P < 0.05$). These results indicated that A3G can deliver more bioavailable Lys to the absorption site of dairy cows than A2G, thanks to improved intestinal digestibility due to an increased surface area of A3G.

Key Words: rumen-protected lysine, dairy cow, bioavailability

T214 Characteristics of a rumen protected lysine product. 2: Handling properties of the third-generation AjiPro-L in feeding practices. M. Miura*[1], A. Haruno[1], M. Tanida[1], Y. Miyazawa[1], T. Fujieda[1], and I. Shinzato[2], [1]*Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co. Inc., Kawasaki, Kanagawa, Japan,* [2]*Ajinomoto Heartland Inc., Chicago, IL.*

Our results of in vivo animal tests showed that the third-gneration AjiPro-L (A3G, Ajinomoto Co. Inc.) has higher bioavailability than the previous generation product (the second-generation AjiPro-L, A2G) mainly due to an increased intestinal digestibility which is attributed to the smaller particle size of A3G compared with A2G. Because the smaller particle size may affect the handling properties such as mixing homogeneity in feeds, durability at TMR mechanical mixing, and stability in TMR contact, however, the following studies were conducted to evaluate those properties of A3G. To examine the mixing homogeneity in feeds, A3G was added into a grain mix at 4.0 wt. % by using an auger mixer. The mix was transported by truck for 100 miles and unloaded into a feed bin. Four sub-samples of the mix (1 kg) were collected from a discharger of the bin. A3G particles in the mix were separated, weighed, and calculated for the inclusion rate. Statistical differences ($P < 0.05$) were tested by a one way-ANOVA. Mean inclusion rates of A3G in the mix before and after the transportation and subsequent discharge from the bin were 4.3 ± 0.2% and 3.8 ± 0.1%, respectively (mean ± SD, $P < 0.05$). Assuming the general CV of such feed mixing test is about 5%, those 2 rates fell within an acceptable range of the theoretical inclusion rate ± 2 SD. Next, in situ ruminal protection of A3G after TMR mechanical mixing was evaluated. A3G (1 g) were weighed and placed into a Dacron Bag. The bags were mixed with TMR by the Data Ranger (American Calan, Inc.) and were collected. Then the bags were placed in the rumen for 24 h. Comparing with intact A3G (Control), in situ protection of A3G was not affected by TMR mechanical mixing ($P = 0.09$). Lastly, a loss of Lys from A3G after contact with TMR was evaluated by measurements of Lys released from A3G into TMR after extraction with water at 0, 6, 12, 18, and 24 h. When A3G was mixed with TMR, about 5% of Lys in A3G was released into TMR within the first 6 h but no further Lys was released during the following 18 h. These results demonstrate that handling properties of A3G are not compromised regardless of reduction in the particle size.

Key Words: rumen-protected lysine, handling, stability

T215 Impact of tannins and grazing schedule on ruminal inoculum activity of dairy cows: Evaluation using the in vitro gas-production technique. C. A. Pozo*[1], J. L. Repetto[2], G. V. Kozloski[1], M. Cuffia[3], A. Ramírez[2], and C. Cajarville[2], [1]*Departamento de Zootecnia, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil,* [2]*Facultad de Veterinaria, Universidad de la República, San José, Uruguay,* [3]*Facultad de Agronomía, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina.*

The aim of this study was to evaluate the effects of dietary Acacia mearnsii tannin extract (TE) and managing the grazing schedule, on in vitro fermentative activity of the rumen inoculum (RI) collected from dairy cows. The trial was conducted with 9 Holstein cows averaging 197 ± 12 d in milk, in a triplicate 3 × 3 Latin square design, through three 22-d experimental periods. The treatments consisted of morning grazing and afternoon TMR (AM), morning grazing and afternoon TMR added with 15 g/kg of TE (AMt), or morning TMR and afternoon grazing (PM). Cows were fed twice a day at 0700 and 1600 h, having access to TMR or pasture during 5 h. After 20 d of adaptation, RI of each cow was collected twice a day, 4 h after the morning and afternoon meal. The pH was measured and the fermentative activity of the RI was estimated through a 96-h in vitro gas-production assay. The substrates used were 2 whole-crop oat silages incubated in triplicate. Data were fitted to a simple exponential model with lag. Statistical analysis was carried out using the PROC MIXED of SAS, where treatments were compared