P442 Influence of supplementing high fiber diets with abomasally infused methionine and lysine, or isoleucine, or all three amino acids. P.H. Robinson*, W. Chalupa[1], C.J. Sniffen[2], W.E. Julien[3], H. Sato, K. Watanabe[4], T. Fujieda[4] and H. Suzuki[4], *Atlantic Dairy and Forage Institute, Fredericton Junction, NB, Canada; [1]Univ. of Penn., Kennett Square, PA; [2]W.H. Miner Agric. Res. Inst., Chazy, NY; [3]Julien and Assoc., 7207 Surey Hill S., Omaha, NE; [4]Ajinomoto Co., Inc., Tokyo, Japan.*

Numerous studies have investigated responses of dairy cows to supplemental rumen protected (RP) lysine and methionine. While improvements in productivity have been reported, results are inconsistent. A possible reason may be that one or more other amino acids (AA) may be more deficit than lysine or methionine, at the intestinal absorptive site. Isoleucine has been suggested to be such an AA under some conditions. Four multiparous Holstein cows were fed a basal ration designed to be co-limiting in intestinally absorbable supplies of methionine, lysine and isoleucine. Cows were supplemented with none of these amino acids (Control), lysine and methionine in a RP form (LM), isoleucine by abomasal infusion (I) or lysine, methionine and isoleucine (LMI) in a 4 x 4 Latin square arrangement of treatments with 28 d periods. Performance of cows on all treatments was lower than expected due to low intake of dry matter (DM) which was probably due to the high fibrosity of the basal diet. This resulted in very high daily chewing times on all diets which was associated with high rumen pH values. Intake of DM and its components were not influenced by any treatment. Milk protein % tended (P=0.07) to be higher when cows were supplemented with RP lysine and RP methionine. However production of milk and its components were not affected. Cows tended (P=0.09) to have a higher milk lactose %, and tended (P=0.08) to produce more milk and (P=0.06) to produce more milk lactose when abomasally infused with isoleucine alone. However when cows were supplemented with all three AA, milk yield and composition did not differ from the control diet. Evaluation of results suggested that unsupplemented cows were actually co-limited in intestinally absorbable supplies of lysine, methionine and leucine and that intestinally available leucine may have limited performance on all diets. Results demonstrate that isoleucine has the ability to stimulate milk lactose synthesis although this may be compromised by deficiency of another AA.

P443 Ruminally protected lysine or lysine and methionine for lactating dairy cows fed to meet requirements for microbial and postruminal protein. P.H. Robinson*, W. Chalupa[1], C.J. Sniffen[2], W.E. Julien[3], H. Sato, K. Watanabe, T. Fujieda and H. Suzuki[4], *Atlantic Dairy and Forage Institute, Fredericton Junction, NB, Canada; [1]Univ. of Penn., Kennett Square, PA; [2]W.H. Miner Agric. Res. Inst., Chazy, NY; [3]Julien and Assoc., 7207 Surey Hill S., Omaha, NE; [4]Ajinomoto Co., Inc., Tokyo, Japan.*

This study was designed to separate the effects of ruminally protected (RP) lysine (lys) from those of RP methionine (met) on performance of lactating dairy cows fed a diet 1st limiting in intestinally available (IA) lys and 2nd limiting in IA met. Multiparous Holstein cows (30) were examined in a 20 wk study starting wk 5 post-partum. Diets contained timothy silage, corn silage, barley, corn, corn gluten meal and soybean meal. Treatments were: no supplemented amino acids; 21 g/d IA lys; and 22 g/d IA lys and 6 g/d IA met. Post-experiment calculations suggest that, in contrast to the objective, the unsupplemented diet was co-limiting in IA isoleucine and histidine (0.98 of requirements), followed by IA protein (1.02), lysine (1.04), valine (1.07), leucine (1.08) and met (1.18). Thus the virtually identical performance of the unsupplemented and lys groups demonstrated that these cows did not respond to enhanced IA lys where lys was not calculated to be 1st limiting. In contrast, cows supplemented with both lys and met numerically increased production of milk protein and fat to an extent consistent with earlier reported studies, even though calculations did not indicate that performance was limited by IA lys or met. This result, which, due to a lack of statistical significance, will be disputed by some, suggests that met, apparently unlike lys, may have a role in enhancing production of milk components beyond its role as a limiting amino acid.

	Control	Lys	Lys/Met	SEM	C vs L	C vs LM	L vs LM
DM intake (kg/d)	23.26	23.04	23.31	0.43	0.74	0.94	0.69
CP intake (kg/d)	3.23	3.21	3.27	0.07	0.83	0.73	0.57
Milk (kg/d)	33.85	33.53	33.92	0.74	0.79	0.95	0.75
Fat (kg/d)	1.27	1.26	1.31	0.04	0.88	0.53	0.45
Protein (kg/d)	1.07	1.07	1.11	0.03	0.90	0.51	0.47

P444 Effect of ruminally protected lysine and methionine on microbial yield, amino acid profile in the intestine, milk production and composition. S. Xu*, J. H. Harrison[1], W. Chalupa[2], C. Sniffen[3], H. Sato[4], T. Fujieda[4], K. Watanabe[4], T. Ueda[4], H. Suzuki[4], W. Julien[5], *[1]WSU Dairy Forage Facility, Puyallup, [2]U. of Pennsylvania, Kennett Square, [3]Miner Institute, NY, [4]Ajinomoto Inc., Tokyo, [5]Julien & Associates, NE.*

Six lactating Holstein cows fitted with ruminal and duodenal cannulas were used to determine the effect of ruminally protected Lys (RPLys) and Met (RPMet) on microbial yield, amino acid flow to duodenum, and milk production and composition. The experimental design was a 3x3 Latin square. Cows averaged 98 DIM at the beginning of the experiment. Two basal diets were formulated to provide either 86 and 90% required lysine and methionine (Neg) or 112 and 103% (Pos), using corn distillers grains or blood meal, fish meal, and meat bone meal as by-pass amino acid sources, respectively. The RPLys and RPMet were added as a top-dress to the Neg diet to provide 8 g/d methionine and 27 g/d lysine. Yb was used as a particular flow marker and the concentration of purines in the duodenal digesta was determined for estimation of microbial N. Milk protein, microbial synthesis in the rumen, and total EAA flow to duodenum were significantly improved by supplementation of RPLys+Met.

Item	Neg	Pos	RPLys +Met[1)]	SEM	P <
Milk yield, kg/d	35.6[b]	39.4[a]	37.1[b]	.64	.05
Protein, %	3.14[b]	3.15[b]	3.25[a]	.03	.05
N intake, g/d	609	649	646	23.7	NS
Duodenal N flow, g/d	480	539	515	20.16	NS
Microbial N, g/d	286	310	316	12.9	NS
Microbial N,g/kg OMTDR[2)]	26.9[b]	34.8[a]	32.8[a]	1.84	.05
Total EAA flow to Duod, g/d	1184[c]	1456[a]	1293[b]	42.83	.05
Lys flow, g/d	164[b]	204[a]	232[a]	13.71	.05
Met flow, g/d	48	56	62	3.83	NS
Lys/Met ratio	3.4	3.6	3.7	.09	NS

[1)] Ruminally protected Lys and Met were provided by Ajinomoto Co., Inc., Tokyo, Japan and non-available Lys and Met were corrected.
[2)] Organic matter truly digested in the rumen.

P445 Estimation of intestinal digestibility and absorbability of animal-marine protein blend using *in vivo*, *in vitro*, and *in situ* techniques. S. Xu*[1], J.H. Harrison[1], B. Riley[1], and M. Halstead[1], P.T. Chandler[2], *[1]WSU Dairy Forage Facility, Puyallup, [2]Chandler & Associate, Dresden, TN.*

Four Holstein cows fitted with ruminal, duodenal, and ileal cannulas were used to determine the intestinal protein and AA digestibility and absorbability of animal-marine protein blend (AMPB[1)]) in two experiments. The degradability of protein and AA of AMPB in the rumen and intestine were evaluated by *in situ* and mobile nylon bag techniques. The experimental design was a 2 × 2 Latin square and cows in the first experiment were in their dry period and cows in the second experiment averaged 80 DIM. One basal grain mix was formulated using soybean meal, barley, and corn. Cows were fed equal portions of a TMR consisted 18% of grass silage, 18% of corn silage, 13% of alfalfa hay, and 51% grain mix at 6 hour intervals for 5 days. Animal-marine protein blend as 4% of dietary DM was also dosed into rumen through a fistula at the time of feeding. Ytterbium labeled silage was used as a particulate flow marker. The protein degradability of ruminally undegraded AMPB was also determined by the three-step *in vitro* procedure (J. Anim. Sci. 73:1459). The results indicated that absorbability of AA in the small intestine was numerically greater when cows were offered supplemental AMPB. Protein digestibility of ruminally undegraded AMPB by the three--step *in vitro* procedure across experiments was highly correlated with its estimation using the mobile nylon bag technique ($r = 0.98$, $P < 0.05$).

	Exp. 1				Exp. 2			
Item	Control	AMPB	S.E.	P<	Control	AMPB	S.E.	P<
Total AA intake, kg/d	1.81	2.22	0.1	0.1	2.11	2.90	0.3	0.2
Duodenal AA flow, kg/d	2.07	2.34	0.2	0.4	1.95	2.97	0.1	0.03
Ileal AA flow, kg/d	0.71	0.70	0.1	0.9	0.69	0.83	0.1	0.4
Absorbed from small intestine, % of AA entering	65.78	69.95	3.2	0.5	64.55	72.00	3.7	0.3

Protein degradability of AMPB	Mobile nylon bag	Three-step	Mobile nylon bag	Three-step
16 hr. in the rumen, %	20.00 ± 1.67	—	28.70 ± 3.51	—
Duodenum to anus, %	62.48 ± 2.04	—	49.95 ± 6.05	—
Total digestive tract, %	82.67 ± 0.37	—	78.65 ± 2.54	—
Small intestine, %	75.89 ± 2.58	76.66 ± 1.60	63.57 ± 3.29	66.94 ± 4.73

[1]AMPB = Animal-marine protein blend: PRO-LAK®, H. J. Baker & Bro., Inc., Atlanta, GA.