

**Table 1 (Abstr. 486).** Effects of dietary supplementation with RPA on milk production by dairy cows (mean ± SEM; \*P = 0.016)

Treatment group	No. of cows	Milk yield (MY)		Change in MY (kg/cow/7d): d 7 – d 0	CV of MY change (%)
		d 0	d 7		
Non-handling control	14	27.7 ± 2.3	31.5 ± 3.4	3.77 ± 3.22 <sup>NS</sup>	321
Isonitrogenous control (Ala)	13	26.3 ± 3.5	30.9 ± 3.0	4.58 ± 3.36 <sup>NS</sup>	264
Unprotected Arg product	14	26.0 ± 3.3	31.3 ± 2.3	5.36 ± 3.59 <sup>NS</sup>	259
Rumen-protected Arg product	13	26.2 ± 2.6	33.6 ± 2.1	7.35 ± 2.70*	132

resulted in greatest milk yield, milk protein yield, and dairy efficiency when fed to early to mid-lactating dairy cows. However, the greatest amount of NALM supplementation (45 g/d) led to no beneficial effects on lactational performance. Overall results in the current study suggest that NALM supplementation can improve productive performance of dairy cows when added at 30 g/d.

**Key Words:** dairy efficiency, milk production, *N*-acetyl-L-methionine

**486 Dietary supplementation with a rumen-protected L-arginine product enhances milk production by dairy cows.** A. B. Keith, M. C. Satterfield, F. W. Bazer, and G. Wu\*, *Texas A&M University, College Station, TX.*

This proof-of-concept study was conducted on HAW Farms (Belen, NM) to test the hypothesis that L-arginine (Arg) can enhance milk production by lactating cows. A rumen-protected Arg product (RPA, which contained a by-pass matrix) and an unprotected Arg product (UPA) that had the same composition as RPA were manufactured by Biotechnology Services & Consulting Inc. (Coppell, TX). Before parturition, 54 healthy Holstein cows (parities 1 to 4), weighing 550–600 kg, were assigned randomly to receive either no dietary supplementation (non-handling control) or dietary supplementation of 500 g RPA, 500 g UPA or an isonitrogenous amount of L-alanine (Ala) per day beginning on d 1 to 4 after parturition (the initial day of supplementation = d 0 of the trial). Each cow was fed twice daily a typical silage- and alfalfa hay-based lactation diet containing 17% crude protein (25 kg DM/d) to meet the NRC-recommended requirements of nutrients, and had free access to drinking water. A supplement was administered to cows

twice daily (equally divided doses at 8 a.m. and 6 p.m.) by gavage immediately after consumption of their regular meals. On d 0 and 7 of the trial, milk yields of cows were determined using an auto-milking system. On either day, the composition of nutrients did not differ (*P* > 0.05) among the 4 groups of cows. Changes in milk yields between d 7 and 0 (analyzed by the paired *t*-test) were significant (*P* = 0.016) for the RPA group but not significant (*P* > 0.05; NS) for the other groups (Table 1). Interestingly, variation in milk yield change was much less for RPA-supplemented cows, compared with the other groups of cows. These results indicate that cows fed RPA had more consistent lactation performances and produced more milk.

**Key Words:** arginine, lactation, milk

**487 Efficiency of utilization of amino acid increased with energy supply at low and high metabolizable protein supply in dairy cows.** C. Omphalius<sup>1,2</sup>, H. Lapierre<sup>3</sup>, L. Bahloul<sup>2</sup>, and S. Lemosquet<sup>1</sup>, <sup>1</sup>PEGASE, INRA, Agrocampus-Ouest, Rennes, France, <sup>2</sup>Adisseo France S.A.S, Antony, France, <sup>3</sup>Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.

Implementing the concept of a variable efficiency of use of individual AA (AA<sub>ef</sub>) in feeding systems requires knowledge on the effect of net energy of lactation (E) in addition to the effect of metabolizable protein supply (P expressed as PDI, INRA). Therefore, AA<sub>ef</sub> were determined at 2 levels of E (LE: 24.8 vs. HE: 32.6 Mcal/d of NEL) and 2 levels of P (LP: 1,261 vs. HP: 2,227 g/d of PDI) using a 4 × 4 Latin square with 4 cows and 2-wk periods. Individual AA<sub>ef</sub> was calculated as the sum of milk protein yield (MPY), endogenous fecal (EFP) and scurf

**Table 1 (Abstr. 487).**

In mol/12h of N	Diet				SEM	P-value		
	LELP	HELP	LEHP	HEHP		E	P	E × P
AA <sub>S-EU</sub>	7.4	7.1	13.6	13.8	0.5	0.76	<0.01	0.18
MPY	3.7	4.2	4.1	5.0	0.2	<0.01	<0.01	0.15
EFP	1.1	1.4	1.0	1.5	0.1	<0.01	0.68	0.02
Uptake								
EAA	2.3	2.6	3.0	3.3	0.1	0.02	<0.01	0.92
NEAA	1.3	1.5	1.2	1.4	0.2	0.10	0.40	0.81
AA <sub>eff</sub>								
EAA	0.74	0.92	0.44	0.54	0.01	<0.01	<0.01	<0.01
NEAA	0.68	0.84	0.40	0.50	0.01	<0.01	<0.01	<0.01
MU: milk output								
EAA	1.30	1.26	1.51	1.38	0.04	0.06	0.01	0.28
Group 1 <sup>1</sup>	1.08	1.02	1.01	1.03	0.03	0.44	0.31	0.15
Group 2 <sup>2</sup>	1.23	1.28	1.50	1.34	0.08	0.45	0.06	0.18

<sup>1</sup>His, Met, Phe, Tyr, and Trp.

<sup>2</sup>Lys, Ile, Leu, and Val.

proteins (INRA, 2018) multiplied by their respective AA profile (Lapierre et al., 2016; CNC: 205) and divided by the AA supply minus the AA endogenous urinary losses ( $AA_{S-EU}$ ). Mammary net uptake (MU) of AA (6 samples in 12 h) was determined on d 13 of each period. Data were analyzed by MIXED procedure (SAS) with cow as random effect. Increasing E and P supply increased MPY, essential AA (EAA) MU and only increasing E supply tended to increase non-EAA (NEAA) MU. Increasing E supply was partially achieved by increased DMI (14.5 vs. 19.1 kg/d) increasing EFP. Therefore, the  $AA_{ef}$  increased with increasing E supply but at a higher extent in LP diets. The gain in  $AA_{ef}$  with increasing E was mainly explained through a change in the partition of AA toward MPY and EFP at whole body level and of MU AA toward MPY as suggested by the lower EAA MU: milk output ratio. The decreased  $AA_{ef}$  with increased P supply was related to a decreased NEAA and EAA MU relative to the  $AA_{S-EU}$  and to an increased MU: milk output ratio of group 2 AA (Lys, Ile, Leu and Val), both suggesting a higher AA catabolism (Table 1).

**Key Words:** amino acid, efficiency, mammary gland

**488 Leucine and lysine alter inflammatory response of immune cells from growing cattle.** M. Garcia\*, K. A. Pearl, E. C. Titgemeyer, and B. J. Bradford, *Kansas State University, Manhattan, KS.*

Bovine neutrophils alter their inflammatory response upon amino acid supplementation, yet research evaluating effects of specific amino acids is scarce. We examined effects of lysine (Lys) and leucine (Leu) on inflammatory responses of neutrophils (PMN) and mononuclear cells (PBMC) from growing Holstein steers ( $173 \pm 3.7$  kg BW). Seven steers were used in a  $6 \times 6$  Latin square design with a factorial arrangement of 2 levels of Lys (0 and 6 g/d) and 3 levels of Leu (0, 15, and 30 g/d). Steers were fed a basal diet and infused with all required nutrients except Lys. Treatments were delivered by abomasal infusion. Blood was collected on d 7 of each 7-d period. A hematology analyzer was used to measure blood cell composition, and immune cells were isolated using density gradients. To harvest media for cytokine measures, isolated cells were treated with lipopolysaccharide (LPS, 0 or 1  $\mu$ g/mL) for 2 (PMN) or 24 h (PBMC); PBMC were treated with concanavalin-A (10  $\mu$ g/mL) to enhance proliferation. PMN were exposed to dihydrorhodamine and labeled *E. coli* for 50 min to assess oxidative burst and phagocytosis. Period and steer were considered random effects, and data were transformed (square root or log10) to attain normality. Lys reduced lymphocyte numbers ( $6.40$  vs.  $6.61 \pm 0.52 \times 10^3/\mu$ L,  $P = 0.04$ ). Leu (30 g/d) almost doubled eosinophil numbers but only for non-Lys fed steers ( $0.36, 0.31$ , and  $0.63 \pm 0.12 \times 10^3/\mu$ L,  $P = 0.02$ ). PMN phagocytic and oxidative burst responses were not impacted by treatment. In the presence of LPS, TNF- $\alpha$  produced by PMN ( $1.7, 8.5, 17.1 \pm 6.9$  pg/mL,  $P = 0.08$ ) and IFN- $\gamma$  produced by PBMC ( $384, 431, 523 \pm 87$  pg/mL,  $P = 0.01$ ) increased or tended to increase linearly as Leu increased. In the absence of LPS, TNF- $\alpha$  produced by PBMC tended to increase quadratically as Leu increased ( $424, 630, 431 \pm 99$  pg/mL,  $P = 0.08$ ). Regardless of LPS, Lys tended to increase production of IFN- $\gamma$  by PBMC ( $353$  vs.  $400 \pm 83$  pg/mL,  $P = 0.10$ ). These findings point to a role for dietary Lys and Leu in regulating immune responses.

**Key Words:** amino acid, immunity, steer

**489 Alterations in amino acid transporters and the mTOR pathway in adipose tissue of Holstein cows during the periparturient period in response to methionine supply.** Y. Liang\*, F. Batistel<sup>1</sup>, C. Parys<sup>2</sup>, and J. J. Loo<sup>1</sup>, <sup>1</sup>*Department of Animal Sciences*

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Recent studies in non-ruminants have revealed that mammalian target of rapamycin (mTOR) signaling pathway plays a critical role in the regulation of adipose tissue function. The objective of the current study was to investigate whether increasing the methionine supply would alter mRNA expression of amino acid transporters and mTOR pathway components in adipose tissue during the periparturient period. Sixty multiparous Holstein cows were used in a block design and assigned to a control diet or the control plus rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH, Germany). Mepron was fed from -28 to 30 d relative to parturition at 0.09% and 0.10% of the diet dry matter during the prepartum and postpartum period, respectively. Blood sampled from the coccygeal vessel at -14, 7, and 30 d relative to calving date was used for amino acid analysis. Adipose tissue obtained from each group on d -10, 10 and 30 relative to calving date was used for RT-PCR analysis. The statistical model included the random effect of block and fixed effect of treatment, time and its interaction. A treatment  $\times$  time was observed for the plasma concentrations of Asn, Cys, and Gly due to higher responses in MET-supplemented cows ( $P < 0.05$ ). Compared with control, MET-fed cows had greater concentration of Met, Lys, Thr, Leu, Val, and Phe in plasma ( $P < 0.05$ ). Among the 7 amino acid transporters measured, MET-supply increased the mRNA expression of Gln (*SLC38A1*), Glu (*SLC1A1*), L-type amino acid (Met, Leu, Val, Phe; *SLC3A2*), small zwitterionic  $\alpha$ -amino acid (*SLC36A1*), and neutral amino acid (*SLC1A5*) transporters in adipose tissue ( $P < 0.05$ ). Expression of *AKT1*, *RPS6KB1*, and *EIF4EBP1* were also upregulated in MET-supplemented cows in adipose tissue ( $P < 0.05$ ), suggesting enhanced insulin signaling and activation of the mTOR pathway. Overall, the data indicate that enhanced methionine supply during the periparturient period enhances circulating amino acid concentrations and the availability and uptake of amino acids by adipose tissue.

**Key Words:** amino acid, dairy cow, mTOR

**490 Methionine supply during the periparturient period alters glutathione metabolism in adipose tissue of Holstein cows.** Y. Liang\*, F. Batistel<sup>1</sup>, C. Parys<sup>2</sup>, and J. Loo<sup>1</sup>, <sup>1</sup>*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, Urbana, IL, <sup>2</sup>Evonik Nutrition & Care GmbH, Hanau/Wolfgang, Germany.*

The periparturient period is characterized by increased oxidative stress status. Glutathione is one of the major intracellular antioxidants and is important for alleviating oxidative stress. Nuclear factor (erythroid-derived 2)-like factor 2 (NFE2L2) is a transcription factor that induces antioxidant-responsive genes. The hypothesis was that enhanced methionine supply has a positive effect on molecular pathways associated with glutathione synthesis and activation of NFE2L2 in adipose tissue. Sixty multiparous Holstein cows were used in a block design and assigned to a control diet or the control plus ethylcellulose rumen-protected methionine (MET; Mepron, Evonik Nutrition & Care GmbH, Germany). Mepron was fed from -28 to 30 d relative to parturition at 0.09% and 0.10% of diet dry matter during the prepartum and postpartum period, respectively. Adipose tissue obtained on d -10, 10, and 30 relative to calving date was used for RT-PCR analysis. Blood was sampled at -14, 7, and 30 d relative to calving date. The statistical model included the random effect of block and fixed effect of treatment, time and its interaction. Compared with control, MET cows had lower plasma concentrations of reactive oxygen metabolites ( $P = 0.08$ ) and higher