ajava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 2 (4): 184-195, 2007
ISSN 1683-9919

Rumen-protected Methionine Improves Early-Lactation Performance of Dairy Cattle Under High Ambient Temperatures

[1]G. R. Ghorbani, [1]D. Kianzad, [1]M. Alikhani and [2]A. Nikkhah
[1]Department of Animal Science,
Isfahan University of Technology, Isfahan, 84156, Iran
[2]Department of Animal Science, University of Manitoba,
Winnipeg, Manitoba, R3T 2N2, Canada

Abstract: High Ambient Temperatures (AT) would be an additional pressure on early-lactation cows already undergoing physiological and metabolic adaptations of early-lactation. Methionine is the initiator amino acid in any polypeptide biosynthesis. The high AT can stimulate the synthesis of specific proteins by the immune system to maintain cell integrity. The high AT was hypothesized to increase maintenance methionine requirements in dairy cattle already facing large methionine demands of early-lactation. The primary objective was to determine the effects of a Rumen-Protected Met (RPM) product (SmartamineM^{TM}) on productivity and estrous expression visibility in early-lactation dairy cattle under high AT. Ten second-calf and fifteen third- and fourth-calf Holstein cows were grouped based on parity, previous milk production records and body condition score. Cows within each group were randomly assigned to either a control (n = 13) or a RPM treatment (n = 12) at 2 week prepartum and monitored through 14 week in milk. The cows were housed in loose stalls receiving 12 g daily RPM from 2 week prepartum to 2 week postpartum and 17 g RPM from 3 week through 14 week postpartum. The control cows received the same diets but without RPM. Body condition was scored at -14, 25, 60 and 110 day of calving. The visibility of estrous expression was scored on the basis of standing and mounting activities with the scorers blind to the treatments. The average maximum daily AT was 42°C in August. Across the experimental weeks, RPM increased ($p<0.01$) milk contents of protein, lactose and SNF in all cows. The RPM increased ($p = 0.04$) milk protein yield and tended ($p = 0.09$) to increase milk yields of energy and fat in second-calf cows but not in older cows. In addition, feeding RPM improved ($p = 0.05$) visibility of estrous expression across parities. Analysing the polynomial coefficients of individual lactation curves suggested that RPM-fed cows had a more persistent milk yield than control cows ($p = 0.05$). Results demonstrated that RPM can benefit early-lactation dairy cattle under high ambient temperatures.

Key words: Early-lactation, hot weather, dairy cattle, rumen, methionine

INTRODUCTION

High Ambient Temperatures (AT) can depress AA and energy intakes (Jordan, 2003) and jeopardize estrous expression visibility (Payton *et al.*, 2004). The high AT stimulate synthesis of specialized proteins by the immune system (Guerriero and Raynes, 1990). Methionine (Met) is mandatory for any polypeptide biosynthesis to initiate and retain (Kozak, 1983). The high AT is thus hypothesized to increase Met demands in early-lactation cows.

Corresponding Author: A. (Akbar) Nikkhah, Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada Tel: ++1-204-474-9087 Fax: ++1-204-474-7628

Methionine offers methyl group for the biosynthesis of apolipoprotein-B100 and Very Low Density Lipoproteins (VLDL) (Bauchart *et al*., 1998). In so doing, Met promotes triglyceridemia in early-lactation cows (Huber *et al*., 1984), suggesting an increased VLDL export by the liver and small intestine (Auboiron *et al*., 1994). Blended with Lys, Met can reduce liver triglycerides by stimulating apolipoprotein B100 synthesis (Bauchart *et al*., 1998; Durand *et al*., 1992). While still unverified directly, Met has, thus, long been speculated to ease the hepatic VLDL export and attenuate the metabolic stress of tissue mobilization in early-lactation cows (Overton and Waldron, 2004). Methionine may, moreover, facilitate mitochondrial oxidation of Long-Chain Fatty Acids (LCFA) by contributing to carnitine synthesis (Mayes, 2000). Thus, Met could fuel gluconeogenesis from AA, propionate and lactate by furnishing adequate NADH and ATP from LCFA oxidation (Armentano *et al*., 1991). Alongside is a role Met plays in providing 1-carbon units for nucleic acid synthesis and gut cell proliferation (Allen, 1983; Stipanuk, 1986). Such considerable Met and other essential AA demands of early lactation (Lapierre *et al*., 2006) will be superimposed on the elevated maintenance Met requirements under high AT to sustain the immunity. Feeding RPM was hypothesized to ease the metabolic stress of early-lactation cows under high AT, thus improving productivity. The primary objective was to determine the effect of feeding RPM from 2 week prepartum through 14 week in milk on lactation performance and estrous expression visibility of dairy cattle under high AT.

MATERIALS AND METHODS

Experimental Design and Cow Management

Twenty-five multiparous Holstein cows were used in an early-lactation study from 2 week prepartum (BCS = 3.7±0.05; mean±SE) through 14 week postpartum. The cows had an average production record of 10.057±1.489 (mean±SD) kg milk in their previous lactations. The maximum record was about 14,000 kg and the minimum record was 6,200 kg milk yield in a 305 day cycle. Cows were blocked based on parity, previous milk yield records and body condition score. Cows within each block were assigned randomly to either a control diet (Con, n = 13) or a diet supplemented with Rumen-Protected Met (RPM, n = 12) at 2 week prepartum. There were five second-calf cows and seven third- and fourth-calf cows in the RPM group. The Con group had five second-calf cows and eight third- and fourth-calf cows. Cows in their second lactation were in parity group 1 and cows in their third and fourth lactation were in parity group 2. Seven cows in each of control and RPM groups had an average previous record of >10,000 kg 305 day milk yield. Cows were housed in loose stalls at Sharifabad Dairy Facilities (Qazvin, Iran). The average milk yield in the farm was 35 kg day^{-1} at the time of the current study. The complex was located in Qazvin plain in the northwest of Tehran. The region has cold winters but long and hot summers. As a result, cows are usually exposed to high AT (>35-40°C) for most of June, July, August and September (Table 1). This experiment was conducted between June and September 2005.

The RPM product (SmartamineM™) was a white solid granular powder coated with a polymer (poly 2-vinylpyridine-co-styrene) sensitive to acidic abomasal pH and contained 75% DL-methionine.

Table 1: Air temperature (T) and Relative Humidity (RH) during the experiment (data provided by the climatology centre, Qazvin, Iran)

	Mean		Maximum		Minimum	
Month (2005)	T°C	RH (%)	T°C	RH (%)	T°C	RH (%)
June	32.60	44.0	37.0	68.5	26.0	19.5
July	36.57	43.3	40.0	66.7	33.0	19.9
August	34.60	47.1	42.0	72.3	30.0	22.0
September (10 days)	27.40	57.5	28.0	72.5	27.0	42.5

The treatment cows received a transition diet (Table 2) supplemented with 12 g RPM from 2 week prepartum until 2 week postpartum. Starting at 3 week postpartum and continuing until 14 week postpartum, the treatment group was offered a lactation diet (Table 2) with 17 g of RPM. The Met contents of the basal and RPM-supplemented lactation diets were respectively 1.9 and 2.3% of total dietary CP (Table 4). The Con cows received the same diets but without RPM. The dry matter-based forage to concentrate ratio was 47.8:52.2 for the transition diet and 38.3:61.7 for the lactation diet. The RPM was mixed with a small portion of the concentrate and then added to the rest of the concentrate. Next, the forage and concentrate portions were mixed before delivery to the cows. Diets (Table 3) were formulated using the diet formulation program of NRC (2001). The RPM-supplemented and Con Total

Table 2: Dry matter-based ingredients and chemical composition of the transition (2 week prepartum to 2 week postpartum) and lactation (2 week through 14 week in lactation) Totally Mixed Rations (TMR)

Items	Transition TMR	Lactation TMR
Ingredients		
Alfalfa hay	27.70	18.90
Corn silage	20.10	19.40
Beet pulp	4.60	3.40
Barley grain	18.30	22.40
Corn grain	6.10	7.00
Soybean meal	9.60	11.70
Cottonseed meal	6.70	8.20
Whole linted cottonseed	4.20	5.30
Ca-long chain fatty acid	0.85	1.10
Minerals and vitamin supplement[1]	0.45	0.58
Sodium chloride	0.25	0.58
Sodium bicarbonate	0.70	0.87
Calcium carbonate	0.40	0.58
Niacin supplement	0.05	0.03
Chemical composition (DM-based)		
NE_L 4× (Mcal/kg)	1.51	1.58
CP (%)	15.52	16.04
CP-RDP[2] (%)	9.71	10.20
CP-RUP[2] (%)	5.81	5.84
Met (%) (% of dietary CP)	0.29 (1.9)	0.30 (1.9)
ADF (%)	22.20	20.00
NDF (%)	37.00	33.90
NFC[3] (%)	36.03	38.60
Ash (%)	7.08	7.90
Ca (%)	0.79	0.77
P (%)	0.36	0.39
Total fat (%)	3.43	3.61

[1] Contained 19.6% Ca, 9.6% P, 7.1% Na, 1.9% Mg, 0.3% Fe, 0.03% Cu, 0.2% Mn, 0.3% Zn, 100 ppm Co, 100 ppm I, 0.1 ppm Se and 50×10^5 IU of vitamin A,10×10^5 IU of vitamin D and 0.1 g of vitamin E kg^{-1}, [2] Calculated from NRC (2001). [3] Non fiber carbohydrates = 100-(NDF% + CP% + total fat% + ash%)

Table 3: Chemical composition of the dietary feed ingredients (DM basis)

Ingredients	DM ($g\ kg^{-1}$)	CP ($g\ kg^{-1}$)	NE_L 4×(Mcal kg^{-1})	NDF ($g\ kg^{-1}$)	ADF ($g\ kg^{-1}$)	EE ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	Met (gkg^{-1})
Alfalfa hay	900	150	1.11	515	350	25	14.0	2.0	2.5
Corn silage	300	88	1.38	510	280	38	2.8	2.6	2.5
Beet pulp	850	100	1.35	458	231	11	9.1	0.9	1.2
Barley grain	870	117	1.76	205	72	22	0.6	3.6	1.8
Corn grain	880	87	1.90	100	40	40	0.5	2.8	1.8
Whole cottonseed	891	195	1.83	503	403	193	1.7	6.0	4.0
Cottonseed meal	930	260	1.61	380	240	45	2.0	11.5	6.3
Soybean meal	891	409	2.13	140	100	16	3.6	6.5	6.6
Calcium carbonate	970	-	-	-	-	-	390.0	-	-
Vitamin and mineral supplement[1]	980	-	-	-	-	-	196.0	96.0	-

[1] Contained 196 g kg^{-1} Ca, 96 g kg^{-1} P, 71 g kg^{-1} Na, 19 g kg^{-1} Mg, 3 g kg^{-1} Fe, 0.3 g kg^{-1} Cu, 2 g kg^{-1} Mn, 3 g kg^{-1} Zn, 100 ppm Co,100 ppm I, 0.1 ppm Se and 50×10^5 IU of vitamin A, 10×10^5 IU of vitamin D and 0.1 g of vitamin E kg^{-1}. The values for NE_L were adopted from NRC (2001) and Met were based on the recent data by Taghizadeh *et al.* (2005)

Table 4: Estimated dry matter and methionine (Met) intake from dietary ingredients and whole diet

Items	Alfalfa hay	Corn silage	Beet pulp	Barley grain	Corn grain	Whole cottonseed	Cottonseed meal	Soybean meal	RPM ($g\ day^{-1}$)
As-fed intake ($kg\ day^{-1}$)	5.500	17.00	1.00	6.700	2.10	1.58	2.470	3.520	0.017
DMI ($kg\ day^{-1}$)	4.950	5.10	0.85	5.830	1.85	1.40	2.290	3.130	0.017
Met intake ($g\ day^{-1}$)	12.375	12.75	1.02	10.494	3.33	5.60	14.427	20.658	12.75

Met intake, $g\ day^{-1}$ (% of basal diet CP) = 80.65 (1.9%), Met intake of rumen protected Met (RPM) added diet, $g\ day^{-1}$ (% of diet CP) = 93.41 (2.3%)

Mixed Rations (TMR) were offered three times daily at 0800, 1500 and 2100 h, allowing for 3-5% orts at each feed delivery. The samples of feed ingredients were analysed for crude protein (AOAC, 1990), NDF (Van Soest *et al*., 1991; using Na-sulfite) and ADF (AOAC, 1990).

Milking and Milk Composition Analysis

Cows were milked three times daily in a milking parlour at 0400, 1200 and 2000 h. Milk yield was recorded once weekly for all cows. The amount of milk produced for each cow at each milking was read using the standard, graduated jars. Milk was sampled at each milking into small vials containing potassium chromate as preservative. Upon sampling, milk was stored at 4°C until analysis. Daily milk samples were composited based on milk yield at each milking for Individual cows. The composited daily milk samples from individual cows were analyzed for fat, protein, SNF and lactose by Milk-O-Scan (134 BN Foss Electric, Hillerød, Denmark). Milk fat and protein were measured once every two weeks throughout the study. Milk lactose and SNF were measured at weeks 2, 4 and 12 of lactation. The yield of Energy Corrected Milk (ECM) was calculated by the following formula:

$$[(0.327\times \text{kg milk}) + (12.95\times \text{kg fat}) + (7.2\times \text{kg protein});\ \text{DeFrain } et\ al.,\ 2006].$$

Scoring Estrous Expression Visibility

The visibility of estrous expression was scored based on visual observations. The scorers were blind to the treatments. The estrous visibility scores were: 1 = evident standing and mounting, 2 = poorly evident standing and evident mounting and 3 = no evident standing and mounting. For cows scored 1, conception occurred mostly after the first AI and some after the second AI. For cows scored 2, conception occurred either after 2nd or 3rd AI. For cows scored 3, no conception occurred throughout the experiment.

Statistical Analysis

The weekly milk yield data were regressed against time for individual cows. The resulting second-order polynomial regression coefficients were subjected to variance analysis to reflect the curvature of milk yield during the study (Allen *et al*., 1983; Morris, 1999). The repeated milk yield measurements were analyzed as a linear Mixed Model with the best fitted covariance structure (Littell *et al*., 1998; Wang and Goonewardene, 2004). The least square means were estimated by Restricted Maximum Likelihood method and denominator degrees of freedom were calculated by Satterwaith method (SAS Institute, 2003). The final model included fixed effects of treatment, parity, time (week of lactation), treatment × time and parity × time. Cows in their second lactation were defined as parity group 1 and cows in their third and fourth lactation as parity group 2. The effect of cow within parity × treatment was considered random. The covariance structure used to analyze the repeated measures of milk component was Spatial Power (Wang and Goonewardene, 2004). For milk yield estimates, first-order heterogeneous autoregressive structure was adopted, as it produced the least Akaike's Information Criterion (Littell *et al*., 1998; Wang and Goonewardene, 2004). Where significant, treatment means were separated using PDIFF option of SAS (SAS Institute, 2003). Significance levels were declared at $p<0.05$ and trends were discussed at $p<0.10$. The standard errors presented were for the differences of least square means. The chi-square test was used to compare pregnancy rates between RPM-fed and Con cows. Due to the incidence of displaced abomasums in one of third-calf control cows, data for this cow were excluded from the analysis.

RESULTS

Measurements Across Lactation

The RPM did not significantly affect actual milk yield across lactation weeks (Table 5). The ECM milk fat yield tended to be greater ($p = 0.09$) in RPM-fed second-calf cows than in Con second-calf cows. Across weeks, the older cows showed no significant milk yield response to RPM. The RPM increased ($p = 0.04$) milk protein yield in second-calf cows but not in older cows. Across parities, the RPM-fed cows had higher ($p<0.01$) milk percent of protein, lactose and SNF than Con cows (Table 5). The RPM did not affect milk yields of SNF and lactose across lactation weeks. The overall changes in BCS from 2 week prior to calving through 25, 60 and 110 days in milk were not affected by RPM ($p = 0.24$). The quadratic coefficients of lactation curve were closer ($p = 0.05$) to zero in RPM-fed cows than in Con cows (Table 7). This means that RPM-fed cows had a more persistent ($p = 0.05$) early-lactation milk yield than did control cows (Table 7 and Fig. 2B).

Measurements Repeated in Time

Milk fat content was higher at weeks 4, 6 ($p<0.01$) and 8 ($p<0.05$) of lactation in RPM fed cows than in Con cows (Fig. 1A). Milk fat yield was greater at week 4 ($p<0.05$) and week 6 ($p<0.01$) and tended to be greater at week 8 ($p = 0.08$) in RPM-fed cows than in Con cows (data not shown). Milk protein percent was higher ($p<0.05$) through weeks 6, 8, 10, 12 and 14 of lactation in cows receiving RPM compared to Con cows (Fig. 1B). Milk protein yield was greater in RPM-fed cows ($p<0.05$) only at weeks 2 and 14 of lactation, when compared to con cows (data not shown).

Table 5: Effects of supplemental Rumen-Protected Methionine (RPM) on productive indices of lactating Holsteins across the lactation weeks (W). Con = Control cows fed diets without RPM

	Treatment (Trt)			p-value		
Items	RPM	Con	SEM	Trt	Parity	Trt×week
Actual milk yield (kg day^{-1})	38.60	36.50	3.30	0.88	0.33	0.03
ECM^1 (kg day^{-1})	36.70	34.30	2.10	0.26	0.11	<0.01
Milk fat (%)	3.18	3.08	0.15	0.51	0.55	0.01
Fat yield^2 (kg day^{-1})	1.23	1.13	0.08	0.28	0.15	<0.01
Milk protein (%)	2.93	2.76	0.03	0.007	0.39	0.03
Protein yield^3 (kg day^{-1})	1.13	1.03	0.06	0.12	0.11	0.01
SCC (×1000 mL^{-1})	217.60	293.30	75.80	0.67	0.06	0.22
BCS difference	-0.048	-0.092	0.03	0.24	0.42	<0.01

[1] In second-calf cows, ECM was 30.9 kg day^{-1} in Con group vs. 36.7 kg day^{-1} in RPM group ($p = 0.09$), [2] In second-calf cows, milk fat yield was 1.0 kg day^{-1} in Con group vs. 1.24 kg day^{-1} in RPM group ($p = 0.09$), [3] In second-calf cows, milk protein yield was 0.92 kg day^{-1} in Con group vs. 1.14 kg day^{-1} in RPM group ($p = 0.04$)

Table 6: Effects of supplemental Rumen-Protected Methionine (RPM) on milk SNF and lactose at weeks (W) 2, 4 and 12 in lactation. Con = control cows fed diets without RPM

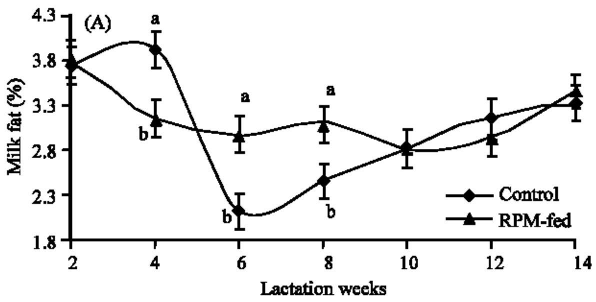
	Week-2		Week-4		Week-12			p-value^1		
Items	RPM	Con	RPM	Con	RPM	Con	SEM	RPM	W	RPM×W
Lactose (%)	4.40^a	4.12^b	4.52	4.54	4.73^a	4.56^b	0.05	0.004	0.66	0.03
Lactose yield (kg day^{-1})	1.65^a	1.44^b	1.84	1.81	1.76	1.71	0.09	0.32	0.92	0.32
SNF (%)	8.44^a	7.99^b	8.15	8.28	8.74^a	8.29^b	0.09	0.002	0.70	0.002
SNF yield (kg day^{-1})	3.16^a	2.75^b	3.31	3.31	3.25	3.12	0.07	0.31	0.83	0.20

[1] Within each week, means with different superscripts differ i.e., lactose content: week 2, $p<0.01$; week 12, $p<0.05$; lactose yield, $p = 0.09$; SNF content, $p<0.001$; SNF yield, $p = 0.07$

Table 7: Regression coefficients of early-lactation curve and the visibility of estrous expression in control and Rumen-Protected Methionine (RPM) fed cows

	Treatments			p-value
Items	RPM	Con	SEM	RPM vs.Con
Actual milk yield[1]				
Quadratic coefficient	-0.0037	-0.0062	0.0012	0.05
Linear coefficient	0.4033	0.6274	0.1400	0.13
Intercept	31.5000	29.0000	2.5000	0.63
Heat expression visibility[2]	1.4800	2.1200	0.3000	0.05

[1] Polynomial equations of actual milk yield were developed for individual cows and then milk yields at 10 days intervals (10 to 100 days in milk) were acquired. The repeated measures acquired plus linear and quadratic coefficients of the regression equations were subjected to variance analysis. The quadratic and linear coefficients describe the curvature of milk yield through the study; The closer the coefficients to zero, the more persistent milk yield through early-lactation, [2] Estrous visibility scores given by blind scorers: 1 = evident standing and mounting, 2 = poorly evident standing and evident mounting and 3 = no evident standing and mounting



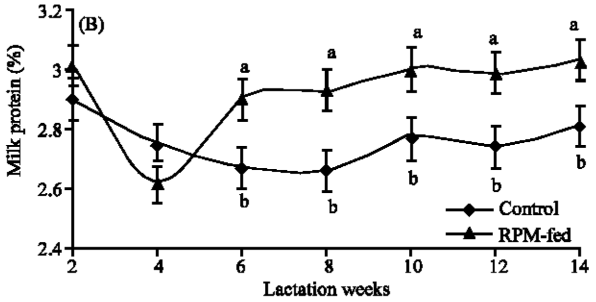


Fig. 1: Milk contents of fat (A) and protein (B) in Holstein cows fed control (Con) or rumen-protected methionine supplemented (RPM) diets through 14 week in lactation. (A) weeks 4 and 6, $p<0.01$; week 8, $p<0.05$ and (B) week 8, $p<0.01$; weeks 6, 10, 12 and 14, $p<0.05$

Dietary addition of RPM increased milk percent of lactose at weeks 2 ($p<0.01$) and 12 ($p<0.05$) but not at week 4 of lactation (Table 6). Milk lactose yield tended to increase ($p = 0.09$) only at week 2 but not at weeks 4 and 12, when the lactation diet was supplemented with RPM (Table 6). Milk percent of SNF was increased ($p<0.001$) at weeks 2 and 12 and milk SNF yield tended to increase ($p = 0.07$) at week 2 (Table 6) by feeding RPM. Weekly yield of ECM tended to be greater ($p = 0.07$) at weeks 2 and 6 and was greater ($p = 0.04$) at week 14 of lactation in RPM-fed cows than in Con cows (Fig. 2A).

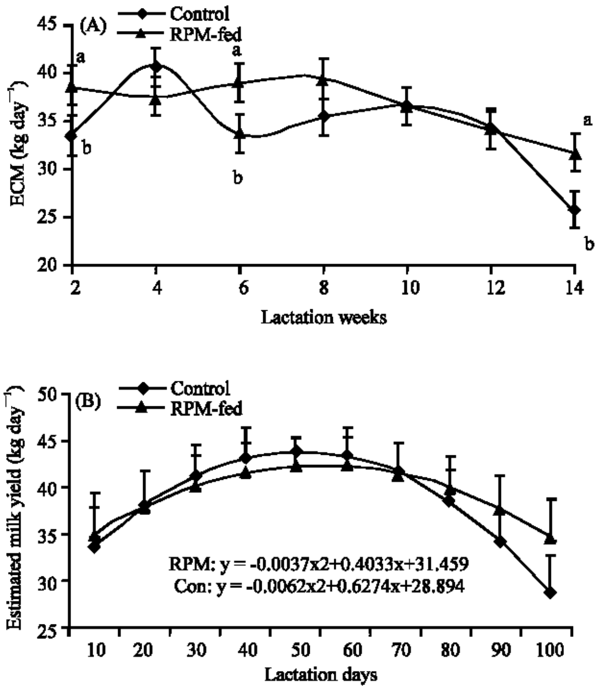


Fig. 2: Energy-Corrected Milk yield (ECM) (A) and lactation curve (B) of Holstein cows fed control (Con) or rumen-protected methionine supplemented (RPM) diets through 14 week in lactation. (A) weeks 2 and 6, $p = 0.07$; week 14, $p<0.05$. (B) Polynomial regression equations were fitted using actual milk yield and then milk yield estimates at 10 day intervals were aqcuired for individual cows. The absolute quadratic term of the equations was greater in Con group than in RPM group ($p = 0.05$) i.e., the early lactation curve of Con cows was less persistent than that of RPM cows

Estrous Expression Visibility

Across parities, estrous signs were more visible ($p = 0.05$) in RPM-fed cows than in Con cows (Table 6). Of 12 cows in each group, pregnancy test proved positive for 8 RPM-fed cows but only for 3 Con cows, by the end of the experiment ($p = 0.04$).

DISCUSSION

Overall and Weekly Productive Indices

The overall trend for Rumen-Protected Met (RPM) to increase Energy-Corrected Milk yield (ECM) and milk fat yield in second-calf cows agrees with the reports by Overton *et al.* (1996). Almost comparable to our trial, Overton *et al.* (1996) monitored the cows from 7-10 day pre-calving until 18 week post-calving. In contrast, Overton *et al.* (1996) observed no RPM effects on milk percent of fat, protein and SNF. The discrepancies could be related to the lower dietary NDF (25 vs. 34%) and higher CP (19 vs. 16%) in their study compared to that in the present study. Dietary CP and fiber have previously been reported to affect the impact of RPM on milk protein (Bateman *et al.*, 1999). Low dietary NDF may reduce rumen pH, thereby reducing the efficiency of microbial protein synthesis

(MPS) (Van Soest, 1994). In addition, high intake of CP and RDP may increase rumen ammonia and MPS (Reynal and Broderick, 2005) and consequently mask the potential impact of RPM on milk protein. This is because the greater MPS can reduce demands for essential AA and, hence, dilute the benefits of RPM. In addition, debatably (Firkins and Reynolds, 2005), the higher rumen ammonia may increase ATP cost for urea biosynthesis and excretion in the liver and kidney. However, Leonardi *et al.* (2003) found that RPM can improve milk protein content to the same extent regardless of whether it was added to a diet with 14% or a diet with 18% CP. Their finding would suggest that excess CP elevates N excretion (NRC, 2001) but may not essentially neutralize the RPM mechanism of action (Leonardi *et al.*, 2003). Thus, the positive impact of RPM on cow productivity (e.g., in the current study) appears to be mediated not exclusively via protein metabolism.

The high AT reduce DMI (Jordan, 2003; St-Pierre *et al.*, 2003; West, 2003). Reduced DMI may subsequently reduce the possibility of N oversupply to rumen microbes. This is apparent particularly when diets are moderate in CP, as was the case in the current study. The high AT can also increase water intake, which can hasten ruminal fluid outflow rate (Van Soest, 1994). The higher rumen outflow rate could inhibit excessive N turnover and so improve MPS efficiency (Bach *et al.*, 2005). Reduced ruminal N input along with increased rumen outflow rate and post rumen Met supply (by feeding RPM) may, hence, contribute to the increased milk percent and yield of protein. These results concur with that of Socha *et al.* (2005) who also observed rises in milk percents of protein and fat. Likewise, Rulquin and Delaby (1997) reported a positive response in milk protein percent to RPM with a corn silage and soybean meal based diet during negative energy balance. The higher milk percents of lactose and protein at weeks 2 and 12 in RPM-fed cows than in Con cows confirmed the higher milk SNF percent in RPM-fed cows.

Few RPM-studies (Robinson *et al.*, 1995) have reported concomitant rises in milk yield of fat, protein, lactose and SNF; and none has reported a simultaneous increase in milk percent of fat, protein and lactose through lactation; as found in the current study. Such positive responses in percent and yield of milk components to RPM could rationally be attributed to the ecological situation of the present study. The exposure of cows to long, hot summer days was determining in designing the current trial. The high AT challenges the immune system and stimulates the synthesis of specialized polypeptides (Guerriero and Raynes, 1990). Methionine is the initiator AA during translation process of any polypeptide biosynthesis (Kozak, 1983). The high AT would, hence, increase Met requirements of early-lactation cows already needing much Met to sustain normal hepatic metabolism and milk secretion. At greater Met demands, cow response to RPM is expected to be more pronounced (Socha *et al.*, 2005). Thus, the parallel improvements in milk yield and its composition of fat, protein and lactose in the present study support the hypothesis that RPM can greatly benefit high-producing cows exposed to high AT.

The increased milk lactose yield at week 2 agrees with the reports of Robinson *et al.* (1995). The increased milk lactose percent at week 2 and 12, however, contrasts with the results of Robinson *et al.* (1995) and Blum *et al.* (1999) who reported a decline in milk lactose percent. The statistical decline in milk lactose reported by Robinson *et al.* (1995) seems too low (4.87 vs. 4.83%) to be of biological implication. Additionally, unlike feeding only RPM in our study, Robinson *et al.* (1995) fed a blend of RPM and Lys through 40 week in milk. Also in contrast to about 17 g in the present study, Blum *et al.* (1999) fed 50 g of RPM in a cross-over experiment. The different experimental conditions among studies should thus explain the different milk lactose results. In view of the positive ECM response to RPM, the rise in milk lactose percent may indicate that RPM had a direct stimulatory effect on lactose synthesis in the Mammary Gland (MG).

Typically, the increased milk fat by RPM is not as highly expected as is the boosted milk protein. To explain, both forage and non-forage fibers could adequately supply milk fat precursors to MG unless the rumen fermentation is truly compromised (NRC, 2001). However, high-quality N sources, even if containing sufficient RUP, yet may not necessarily enhance milk protein synthesis (Santos *et al*., 1998; Noftsger and St-Pierre, 2003). Three main challenges may illuminate the more complex nature of manipulating milk protein compared to milk fat. First, optimizing the capture of ammonia-N, peptides and AA by rumen microbes depends on a multitude of fluctuating elements, notably rumen pH and outflow rate (Bach *et al*., 2005). Second, supplying highly digestible RUP with a proper balance in the post rumen AA profile, although possible in research (Noftsger and St-Pierre, 2003), may not be feasible under many commercial circumstances e.g., when lacking safe access to animal-protein sources or when using cereal grains (corn vs. barley) and forages (corn silage vs. grass and barley silages) with entirely different ruminal fermentation patterns. The dissimilar post feeding fermentation patterns of dietary ingredients can markedly change the efficiency and yield of MPS. Consequently, both quantitative and qualitative demands for RUP will change. Third, the gut, liver and associated tissues may oxidize a significant portion of AA from arterial and luminal sources (Berthiaume *et al*., 2006; Blouin *et al*., 2002). These challenges are expected to be more manifest in soybean meal and cottonseed meal based diets (Schingoethe, 1996) including the diets of the present study. As a result, large gaps may exist between the AA profile programmed to reach the duodenum, the AA profile truly arriving at the duodenum, the AA profile appearing in the portal vein and eventually the AA profile available to the periphery (Lapierre *et al*., 2006). Given the aforementioned challenges in meeting AA requirements of high-producing cows, a parallel improvement in milk content/yield of fat, protein and lactose suggests a multi-aspect role for Met. Such a role could involve augmenting: a) the epithelial proliferation to facilitate nutrient absorption across the gut (Stipanuk, 1986), b) Met supply to initiate and retain protein synthesis (Kozak, 1983) in MG, c) fat movement from adipose tissue to MG to complement milk fat secretion (Auboiron *et al*., 1994; Durand *et al*., 1992; Huber *et al*., 1984) d) gluconeogenesis in the liver and kidney to support lactose synthesis in MG and e) high-energy phosphate bonds (i.e., ATP) supply to fuel biochemical reactions concerning a, b, c and d (Mayes, 2000). The latter would be mediated by an enhanced carnitine-dependent LCFA oxidation (Mayes, 2000). Canale *et al.* (1990) found a synergistic rise in milk fat by adding RPM to a fat-added diet when compared to either of fat-added or RPM-supplemented diets alone. The greater response to RPM with higher dietary fat may suggest that Met contributes to fat metabolism. The results of Canale *et al.* (1990) support the improved milk fat percent and yield in the present study with diets containing supplemental fat and cottonseed. Considering the large milk volume, the RPM should have induced an inhibitory impact on dilution of milk components during peak lactation (Leonardi *et al*., 2003; Socha *et al*., 2005).

The RPM increased milk lactose percent at weeks 2 and 12 but not at week 4 in milk. Importantly, ECM was significantly greater at week 4 (39.04 kg) than at week-2 (36.14 kg) and week 12 (34.17 kg). The greater ECM requires greater glucose and AA to sustain. Glucose is essential to provide 1) NADPH for *de novo* milk fat synthesis (Bauman *et al*., 1970) and 2) ATP for protein synthesis from AA (Mayes, 2000). Hence, large non-lactose demands for glucose by MG at week-4, when ECM was greater than at weeks 2 and 12, may have minimized the potential RPM effect on milk lactose at week 4. The RPM tended to increase ECM at weeks 2 and 6 followed by a significant rise at week 14. Increased ECM suggested that RPM can positively affect milk energy output when the early-lactation curve peaked and dropped. Evidently, RPM moderated the curvature of early-lactation arch.

Recently, Socha *et al.* (2005) underlined the greater RPM effect on milk protein at times of greater Met need. Their insight gains support by the enhanced milk protein percent from weeks 6 through 14, the increased milk fat percent at weeks 6 and 8, plus the trend for increased ECM at weeks 2 and 6. The clear reason for decreased milk fat content and yield at week 4 in RPM-fed cows is unknown. Increased milk SNF yield at week 2 was consistent with the increased milk yield of protein and lactose, the two main non-fat solids in milk.

Estrous Expression Visibility

The visibility of heat signs was improved by RPM regardless of parity. The high AT introduces an additional challenge to early-lactation cows already undergoing negative AA and energy balances. As such, early lactation cows are usually more susceptible to high AT and may experience longer periods of negative AA and energy balances compared with cows in later stages of lactation. Due to a more negative nutrient balance, high-yielding cows in early lactation may be at increased risks of reproductive failure than are cows in later stages of lactation (Ravagnolo and Misztal, 2002). Thus, RPM was expected to enable early-lactation cows to maintain productivity but not at the expense of a compromised estrous expression visibility. The pregnancy rate was improved by feeding RPM. Although significant, it should be noted that pregnancy rate is a variable trait depending upon several factors. This necessitates careful interpretation of the results. This finding denotes the importance of future research on the impact of Met on reproductive function under high AT.

CONCLUSIONS

Exposure to high ambient temperatures is an additional pressure on high-producing cows already facing physiological and metabolic pressures of transition and early-lactation. Feeding rumen protected methionine to transition and early-lactation cows under high ambient temperatures benefited milk fat, protein and lactose and above all, improved lactation persistency. Hence, feeding rumen protected methionine to early-lactation cows can reduce nutrient deficiencies under high ambient temperatures. Consequently, the environment can also benefit by less nutrient excretion. Maximizing the two benefits would be an ultimate goal for today’s human populations demanding safe animal-food resources in a viable environment.

ACKNOWLEDGMENTS

This project was financed by Isfahan University of Technology. The authors sincerely thank the management board and staff at Sharifabad dairy complex for providing field facilities and Dr. G. Crow of the Department of Animal Science, University of Manitoba for statistical discussion.

REFERENCES

Allen, J., 1983. Review: Biochemistry of the polyamines. Cell Biochem. Funct., 1: 131-40.

Allen, O.B., J.H. Burton and J.D. Holt, 1983. Analysis of repeated measurements from animal experiments using polynomial regression. J. Anim. Sci., 57: 765-770.

AOAC, 1990. Official Methods of Analysis. 15th Edn., Assoc. Offic. Anal. Chem., Arlington, V.A.

Armentano, L.E., R.R. Grummer, S.J. Bertics, T.C. Skaar and S.S. Donkin, 1991. Effects of energy balance on hepatic capacity for oleate and propionate metabolism and triglyceride secretion. J. Dairy Sci., 74: 132-139.

Auboiron, S., D. Durand, D. Bauchart, J.C. Robert and M.J. Chapman, 1994. Lipoprotein metabolism in the preruminant calf: Effect of a high fat diet supplemented with L-methionine. J. Dairy Sci., 77: 1870-1881.

Bach, A., S. Calsamiglia and M.D. Stern, 2005. Nitrogen metabolism in the rumen. J. Dairy Sci., 88: E9-21E.

Bateman II, H.G., J.N. Spain, M.S. Kerley, R.L. Belyea and R.T. Marshall, 1999. Evaluation of ruminally protected methionine and lysine or blood meal and fish meal as protein sources for lactating Holsteins. J. Dairy Sci., 82: 2115-2120.

Bauchart, D., D. Durand, D. Gruffat and Y. Chilliard, 1998. Mechanism of liver steatosis in early lactation cows-effects of hepatoprotector agents. In: Proceedings of the Cornell Nutrition Conference, pp: 27-37.

Bauman, D.E., R.E. Brown and C.L. Davis, 1970. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow and cow. Arch. Biochem. Biophys., 140: 237-244.

Berthiaume, H.G., M.C. Thivierge, R.A. Patton, P. Dubreuil, M. Stevenson, B.W. McBride and H. Lapierre, 2006. Effect of ruminally protected methionine on splanchnic metabolism of amino acids in lactating dairy cows. J. Dairy Sci., 89: 1621-1634.

Blouin, J.P., J.F. Bernier, C.K. Reynolds, G.E. Lobley, P. Dubreuil and H. Lapierre, 2002. Effect of supply of metabolizable protein on splanchnic fluxes of nutrients and hormones in lactating dairy cows. J. Dairy Sci., 85: 2618-2630.

Blum, J.W., R.M. Bruckmaier and F. Jans, 1999. Rumen-protected methionine fed to dairy cows: Bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. J. Dairy Sci., 82: 1991-1998.

Canale, C. J., L.D. Muller, H.A. McCahon, T.J. Whitsel, G.A. Varga and M.J. Lormore, 1990. Dietary fat and ruminally protected amino acids for high producing dairy cows. J. Dairy Sci., 73: 135-141.

DeFrain, J.M., A.R. Hippen, K.F. Kalscheur and D.J. Schingoethe, 2006. Feeding lactose to increase ruminal butyrate and the metabolic status of transition dairy cows. J. Dairy Sci., 89: 267-276.

Durand, D., Y. Chilliard and D. Bauchart, 1992. Effects of lysine and methionine on *in vivo* hepatic secretion of VLDL in the high yielding dairy cow. J. Dairy Sci.,75(Suppl. 1): 279.

Firkins, J.L. and C.K. Reynolds, 2005. Whole Animal Nitrogen Balance in Cattle. In: Nitrogen and Phosphorus Nutrition of Cattle and Environment. Hristov, A.N. and E. Pfeffer (Eds.), CAB International, Wallingford, UK., pp: 167-185.

Guerriero, V. Jr. and D.A. Raynes, 1990. Synthesis of heat stress proteins in lymphocytes from livestock. J. Anim. Sci., 68: 2779-2783.

Huber, J.T., R.S. Emery, W.G. Bergen, J.S. Liesman, L. Kung, K.J. King, R.W. Gardner and M. Checketts, 1984. Influences of methionine hydroxyl analog on milk fat production, blood serum lipids and plasma amino acids. J. Dairy Sci., 67: 2525-2531.

Jordan, E.R., 2003. Effects of heat stress on reproduction. J. Dairy Sci., 86: E104-114E.

Kozak, M., 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes and organelles. Microbiol. Rev., 47: 1-45.

Lapierre, H., D. Pacheco, R. Berthiaume, D.R. Ouellet, C.G. Schwab, P. Dubreuil, G. Holtrop and G.E. Lobley, 2006. What is the true supply of amino acids for a dairy cow? J. Dairy Sci., 89: E1-14E.

Leonardi, C., M. Stevenson and L.E. Armentano, 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. J. Dairy Sci., 86: 4033-4042.

Littell, R.C., P.R. Henry and C.B. Ammerman, 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci., 76: 1216-1231.

Mayes, P.A., 2000. Oxidation of Fatty Acids: Ketogenesis. In: Harper's Biochemistry. 25th Murray, R.K., D.K. Granner, P.A. Mayes and V.W. Rodwell (Eds.), Appleton Lang, Connectiut, pp: 238-249.

Morris, T.R., 1999. Experimental Design and Analysis in Animal Sciences. Chapter 12: Repeated Measures. CAB Int., Wallingford, UK, pp: 113-118.

National Research Council, 2001. National Research Council. Nutrient Requirements of Dairy Cattle. 7th Rev. Edn., National Acad. Sci. Washington, DC.

Noftsger, S. and N.R. St-Pierre, 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J. Dairy Sci., 86: 958-969.

Overton, T.R., D.W. Lacount, T.M. Cicela and J.H. Clark, 1996. Evaluation of a ruminally protected methionine product for lactating dairy cows. J. Dairy Sci., 79: 631-638.

Overton, T.R. and M.R. Waldron, 2004. Nutritional management of transition dairy cows: Strategies to optimize metabolic health. J. Dairy Sci., 87: E105-119E.

Payton, R.R., R. Romar, P. Coy, A.M. Saxton, J.L. Lawrence and J.L. Edwards, 2004. Susceptibility of bovine germinal vesical-stage oocytes from antral follicles to direct effects of heat stress *in vitro*. Biol. Rep., 71: 1303-1308.

Ravagnolo, O. and I. Misztal, 2002. Effect of heat stress on nonreturn rate in Holsteins: Fixed-model analyses. J. Dairy Sci., 85: 3101-3106.

Reynal, S.M. and G.A. Broderick, 2005. Effect of dietary level of rumen-degraded protein on production and nitrogen metabolism in lactating dairy cows. J. Dairy Sci., 88: 4045-4064.

Robinson, P.H., A.H. Fredeen, W. Chalupa, W.E. Julien, H. Sato, T. Fujieda and H. Suzuki, 1995. Ruminally protected lysine and methionine for lactating dairy cows fed a diet designed to meet requirements for microbial and postruminal protein. J. Dairy Sci., 78: 582-594.

Rulquin, H. and L. Delaby, 1997. Effects of the energy balance of dairy cows on lactational responses to rumen-protected methionine. J. Dairy Sci., 80: 2513-2522.

Santos, F.A.P., J.E.P. Santos, C.B. Theurer and J.T. Huber, 1998. Effects of rumen-undegradable protein on dairy cow performance: A 12-year literature review. J. Dairy Sci., 81: 3182-3213.

SAS, 2003. SAS User's guide v. 9.1: Statistics. SAS Institute, Inc., Cary, NC.

Schingoethe, D.J., 1996. Balancing the amino acid needs of the dairy cow. Anim. Feed Sci. Technol., 60: 153-160.

Socha, M.T., D.E. Putnam, B.D. Garthwaite, N.L. Whitehouse, N.A. Kierstead, C.G. Schwab, G.A. Ducharme and J.C. Robert, 2005. Improving intestinal amino acid supply of pre and postpartum dairy cows with rumen-protected methionine and lysine. J. Dairy Sci., 88: 1113-1126.

Stipanuk, M.H., 1986. Metabolism of sulfur-containing amino acids. Ann. Rev. Nutr., 6: 179-209.

St-Pierre, N.R., B. Cobanov and G. Schnitkey, 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci., 86: E52-77E.

Taghizadeh, A., M. Danesh Mesgaran, R.Valizadeh, F. Eftekhar Shahroodi and K. Stanford, 2005. Digestion of feed amino acids in the rumen and intestine of steers measured using a mobile nylon bag technique. J. Dairy Sci., 88: 1807-1814.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. 2nd Ed. Cornell University Press. Ithaca, New York.

Wang, Z. and L.A. Goonewardene, 2004. The use of mixed models in the analysis of animal experiments with repeated measures data. Can. J. Anim. Sci., 84: 1-11.

West, J.W., 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci., 86: 2131-2144.