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Protected Methionine Prolonged Provision Improves Summer Production and Reproduction of Lactating Dairy Cows

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Abstract: The objective was to establish prolonged effects of a rumen-protected Met (RPM) product (SmartamineM™) on milk production and reproduction of high-producing early-lactation cows under extended very high ambient temperatures. Twenty four fresh Holstein cows (27±9 days in milk, 617 kg b.wt., 2.8 body condition score) including 12 second lactation and 12 higher lactation cows were randomly assigned to either control or RPM-supplemented total mixed rations (TMR, 520:480 g kg^{-1} forage:concentrate), and were fed continuously for 5 months. Cows were offered TMR 3 times daily post-milking plus a top-dress alfalfa hay. The study was conducted from May through November of 2009 in central Iranian province of Isfahan. The RPM group had consistently greater 5-month-long average DM intake (21.9 vs. 19.1 kg day^{-1}), milk yield (42.4 vs. 37.4 kg day^{-1}), milk fat content (33 vs. 27.5 g kg^{-1}), fat yield (1.40 vs. 1.04 kg day^{-1}), milk protein content (29.6 vs. 27.5 g kg^{-1}) and protein yield (1.25 vs. 1.02 kg day^{-1}). Mature cows tended to produce mor e milk (42.2 vs. 37.6 kg day^{-1}) and milk fat (1.30 vs. 1.13 kg day^{-1}) than second lactation cows. The RPM significantly improved ovary function, estrus expression visibility and body condition score (3 vs. 2.6), while shortening days open (106 vs. 143) and calving interval (387 vs. 421 d). Findings provide compelling evidence for beneficial effects of prolonged RPM provision on feed intake, milk production, and reproduction of Holstein cows under concurrent metabolic pressures of early lactation and stressful high ambient temperatures.

Key words: Dairy cow, heat stress, production, protected methionine, reproduction

INTRODUCTION

Stressful summer conditions can depress nutrient intake, elevate metabolism, and thus depress cow production and reproduction (Jordan, 2003; St-Pierre *et al*., 2003). High Ambient Temperatures (AT) compromise oocyte development, visible estrus expression, and successful estrus detection (Edwards and Hansen, 1997; Payton *et al*., 2004). Heat stress stimulates synthesis of special immune proteins and increases maintenance amino acid (AA) requirements (Guerriero and Raynes, 1990). Methionine (Met) is obligatory for initiating and retaining polypeptide biosynthesis (Kozak, 1983). As a result, heat stress is hypothesized to increase Met requirements. Moreover, in addition to its lipotropic action, Met is required for hepatic biosynthesis of carnitine that instigates mitochondrial oxidation of fatty acids (Mayes, 2000). While acting as a gluconeogenic factor, Met helps to fuel glucose neogenesis from AA, propionate, and lactate through furnishing NADH and ATP from fat oxidation (Nikkhah *et al*., 2011). Furthermore, Met provides 1-C units continuously demanded for nucleic acid synthesis and gut cell proliferation (Allen, 1983; Stipanuk, 1986).

Dietary rumen-protected Met (RPM) can improve postrumen AA supply and milk biosynthesis (Overton *et al*., 1996; Socha *et al*., 2005). Recently, we demonstrated beneficial effects of RPM on the lactation curve persistency during a 14-week period (Ghorbani *et al*., 2007). Examining prolonged RPM intake for high-merit cows under stressful environments is of high commercial significance because, for instance, heat stress is a major cause of extended losses in dairy reproduction and farm economics (Jordan, 2003; St-Pierre *et al*., 2003). The objective was to establish prolonged effects RPM provision on production and reproduction indices of early lactation Holstein cows under very high ambient temperatures.

MATERIALS AND METHODS

Experimental design and cow management: Twenty four fresh Holstein cows (27±9 days in milk, 617 kg^{-1} b.wt. 2.8 body condition score) including 12 second lactation

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and 12 higher lactation cows were used. The study was conducted from May through November of 2009 in a modern dairy farm (1500 dairy cattle) in central Iranian province of Isfahan. Cows were divided based on parity, milk yield of previous lactations and body condition score. Within divisions, cows were then randomly assigned to either control or RPM-supplemented total mixed rations (TMR, 520:480 g kg^{-1} forage: concentrate), and were fed continuously for 5 months. Of 12 control cows, 5 cows were in their second lactation and 7 cows were in their third or higher lactation. In the RPM group, 7 cows were in their second lactation and 5 cows were in their third or higher lactation. Cows were housed in free individual boxes, milked 3 times daily at 0000, 0800 and 1600 h in a milking parlor, and were offered TMR (Table 1) post-milking plus a top-dress alfalfa hay. The dairy farm had an approximate population of 1250 dairy cattle with average daily milk yield of 30.6 kg. The diet fed to the RPM group was supplemented with a RPM product (SmartamineM™) in the form of a white solid granular powder coated with a polymer (2-vinyl pyridine-Co Styren e sensitive to abomasum acidic pH) containing 750 g kg^{-1} DL-Methionine. The amount of the supplemental RPM provided daily for each cow was 15 g (Table 1). The two diets were similar in forage type and proportions. The RPM was thoroughly mixed with a small portion of concentrate and then added to the mixer. Forage and concentrate were then mixed before delivery to the cows.

Diets (Table 1) were formulated using NRC (2001). The total mixed rations were offered to allow for 50 g kg^{-1} daily orts. Alfalfa hay was chopped with a chopper machine (Agricultural Machinery Co., Tabriz, Iran) for an average theoretical chop length of 5 cm before mixing with the concentrate. The amount of TMR offered and orts for individual cows were measured daily for the entire experiment. Orts were collected just before the morning feed delivery and were analyzed for DM. Samples of TMR were taken weekly at 0700 h and were oven-dried at 100°C for 24 h, ground to pass through 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia) and stored at -20°C until analyzed for nutrients. Samples were analyzed for DM, ash (method 935.42; AOAC, 1990), CP (method 984.13; AOAC, 1990), and for ADF (method 973.18; AOAC, 1990), aNDF (Van Soest *et al*., 1991; using heat-stable α-amylase and sodium sulfite), and ether extract (EE; method 920.39; AOAC, 1990). Body condition score was recorded every month using a 5-score scoring procedure, with score of 1 being an emaciated cow and score of 5 describing an extremely obese cow (Edmonson *et al*., 1989). Animal were cared for according to the guidelines of the Iranian Council of Animal Care (1995). The study region has usual day ambient temperatures of > 38-40°C in summer (Table 2). The upper limit of AT to maintain body temperature in Holstein cows is 25-26°C (West, 2003).

Table 1: The DM-based ingredients (g kg^{-1}) and chemical composition of the control and rumen protected-supplemented (RPM) total mixed rations (TMR)

Item	RPM	TMR
Alfalfa hay	180	180
Corn silage	199	199
Beet pulp	141	141
Barley grain	137	137
Corn grain	135	135
Soybean meal	99	83
Cottonseed meal	49	49
Wheat bran	24	24
Corn gluten meal	-	16
Bypass fat	12	12
Minerals and vitamins supplement[1]	5	5
Sodium chloride	3.3	3.3
Sodium bicarbonate	1.3	1.3
Calcium carbonate	6.4	6.4
Magnesium oxide	1	1
Microsorb	3.4	3.4
Natural buffer (marine algae)	2.5	2.5
Direct fed microbials	0.8	0.8
Yeast	0.3	0.3
SmartamineM™ (RPM), g/d/cow	15	-
Chemical composition (DM-based)		
NE_L 4× (MJ kg^{-1})[2]	6.48	6.44
CP (g kg^{-1})	151	148
ADF (g kg^{-1})	190	190
aNDF (g kg^{-1})	346	349
Ca (g kg^{-1})	9	9
P (g kg^{-1})	4	4

[1]Contained 10 g kg^{-1} Ca, 65 g kg^{-1} P, 80 g kg^{-1} Mg, 20.8 g kg^{-1} Fe, 8.5 g kg^{-1} Mn, 14.3 g kg^{-1} Zn, 3 g kg^{-1} CO, 210 ppm I, 0.1 ppm Se and 100000 IU kg^{-1} vitamin A, 250000 IU kg^{-1} vitamin D3 and 15000 IU kg^{-1} vitamin E. [2]Calculated using NRC (2001)

Table 2: Air temperature (T) and relative humidity (RH) (data provided by the climatology centre, Isfahan, Iran)

	Mean		Maximum		Minimum	
Month (2009)	T°C	RH%	T°C	RH%	T°C	RH%
June	35.4	36.6	39.5	57.5	28.5	15.7
July	38.3	34.3	41.7	53.8	32.5	14.8
August	39.1	40.1	43.4	60.5	34.2	19. 7
September	30.1	47.5	32.2	62.3	29.1	32.6

Table 3: The prolonged effects of supplemental rumen-protected methionine (RPM) on milk production across experimental months

	Treatment (Trt)			Fixed effect, P		
Item	RPM	Control	SEM	Trt	Parity	Trt×Time
DM intake (kg day^{-1})	21.9	19.1	0.7	0.01	0.11	0.35
Actual milk yield (kg day^{-1})	42.4	37.4	1.8	0.06	0.05	0.26
Milk energy density[1] (Mcal L^{-1})	2.76	2.51	0.08	0.002	0.99	0.46
Milk energy output (Mcal day^{-1})	116.6	93.6	5.4	0.005	0.10	0.23
Milk fat (g kg^{-1})	33.0	27.5	1.3	0.007	0.85	0.48
Milk fat yield (kg day^{-1})	1.40	1.04	0.07	0.002	0.11	0.31
Milk protein (g kg^{-1})	29.6	27.5	0.6	0.02	0.44	0.70
Milk protein yield (kg day^{-1})	1.25	1.02	0.05	0.006	0.19	0.11

[1]Milk energy density = 4.18 [(0.0929×milk fat%)+(0.0547×milk protein%)+0.192 (NRC (2001).

Milking and milk processing and analyze: Milking was performed in a milking parlour with no water or concentrate provision. Milk yield and composition were recorded monthly for all cows. The amount of milk produced for each cow at each milking was measured by reading the predetermined standard values graduated on individual jars. Milk was sampled at each milking into small vials containing potassium chromate ($K_2Cr_2O_7$) as preservative. Upon sampling, milk was stored at 4°C until analysis of fat, protein, SNF and lactose by Milk-O-Scan (134 BN Foss Electric, Denmark). The milk samples from individual cows were analyzed for milk fat and protein.

Reproduction data collection: Post-calving reproduction data including the time of the first heat-estrus expression, the time of the first Artificial Insemination (AI), the number of AI required for successful conception, ovary function score, the time of pregnancy declared, pregnancy status in the course of the study, days open, and calving interval were also recorded. Ovary function was scored based on a 4-point scoring system with scores of 1, 2, 3 and 4 representing active, moderately active, minimally active, and inactive (e.g., cystic) ovaries, respectively. Estrus expression visibility was scored using a 3-point scoring scale as follows: 1 = evident standing and mounting, 2 = scantily-evident standing and evident mounting, and 3 = no evident standing and mounting (Ghorbani *et al*., 2007).

Statistical analysis: The repeated milk production measurements were analyzed as repeated measures linear mixed models with the best fitted covariance structure (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, 2003). The final models included fixed effects of treatment, parity, time, treatment ×time, parity×time and treatment×parity×time. The effect of cow within 'parity×treatment' plus residuals were considered random. The distinct pregnancy data were analyzed using the non-parametric chi-square procedure (SAS, 2003). For other reproduction data, mixed models with fixed effects of treatment, parity and their interaction, plus the random effect of cow within the interaction of treatment by parity were utilized. Where significant, treatment means were separated using PDIFF option of (SAS, 2003). Significance levels were declared at p = 0.05 and tendencies were discussed at p = 0.10.

RESULTS

The RPM increased DM intake ($p<0.01$) and milk percentages of fat ($p<0.01$) and protein ($p<0.02$) and tended to increase actual milk volume ($p<0.06$). As a result, milk yields of fat and protein as well as milk energy density and milk energy output were increased ($p<0.01$) (Table 3). Cows on RPM had greater overall Body Condition Score (BCS) than control cows ($p<0.01$) (Table 4). The RPM group expressed their first postpartum estrus earlier than the control group ($p<0.01$). Consequently, the RPM cows had their first AI also earlier ($p<0.01$). Pregnancy tended to be declared sooner for RPM vs. control cows ($p<0.06$). As a result, days open were lower ($p<0.04$) and calving interval trended to be shorter ($p<0.06$) for RPM. Ovary function and estrus expression visibility both tended to be improved (p = 0.09) when heat stressed cows received prolonged RPM during early and mid lactation.

Table 4: The effects of supplemental rumen-protected methionine (RPM) on reproductive parameters in lactating Holstein cows

	Treatment (Trt)			P		
Item	RPM	Con	SEM	Trt	Par	Trt×Par
First estrus detection, d postpartum	30.0	52.7	3.9	<0.01	0.93	0.33
First Artificial Insemination (AI) (day)	50.5	78.0	7.0	0.01	0.79	0.38
Declared pregnancy, d postpartum	137.0	173.0	13.3	0.06	0.91	0.98
Number of AI required	2.8	3.1	0.3	0.69	0.69	0.44
Days open, (day)	106.2	142.7	11.7	0.04	0.74	0.88
Calving interval (day)	386.8	421.3	12.9	0.06	0.61	0.87
Ovary function[1]	1.4	2.1	0.26	0.09	0.88	0.82
Heat expression visibility[2]	1.96	2.61	0.27	0.09	0.88	0.27
Body condition score	3.07	2.62	0.1	<0.01	0.43	0.33

[1]Scores of 1, 2, 3 and 4 representing active, moderately active, minimally active and inactive (e.g., cystic) ovaries, respectively. [2]Heat visibility scores: 1 Evident standing and mounting, 2: Poorly evident standing and evident mounting and 3: No evident standing and mounting

DISCUSSION

The findings of the current experiment establish the hypothesis that prolonged provision of supplemental RPM to high-producing cows improves feed intake and productivity when metabolic pressures of early lactation are superimposed on extended heat stress. A tendency for improved energy-corrected milk yield and milk fat yield was recently observed in second-calf cows on RPM in a shorter-period study (Ghorbani *et al*., 2007). However, Overton *et al*. (1996) observed no RPM effects on milk percent of fat and protein. The discrepancies could be related to dietary and non-dietary environmental differences between studies that would affect cow responsiveness to RPM. High intake of CP and RDP in the study of Overton *et al*. (1996) may have increased microbial protein and rumen ammonia production (Reynal and Broderick, 2005), thereby masking any potential significant impact of RPM on milk protein synthesis. Greater microbial protein would reduce demands for essential AA and, hence, dilute RPM effects on milk production. In addition, debatably, greater rumen ammonia may increase energy costs of urea biosynthesis and excretion by the liver and kidney (Firkins and Reynolds, 2005). However, Leonardi *et al*. (2003) found that RPM can improve milk protein percent to the same extent regardless of whether it was added to a diet with 140 g kg^{-1} or a diet with 180 g kg^{-1} CP. Their finding would suggest that extra CP elevates N excretion, but may not essentially neutralize the RPM mechanism of action at least on milk protein content (Leonardi *et al*., 2003). Collectively, the positive impact of RPM on cow productivity may be mediated not exclusively via protein metabolism. This gains support from the increased milk fat production by the RPM in the present experiment.

The high AT reduces DM intake, microbial protein synthesis and milk protein secretion (St-Pierre *et al*., 2003). Increased DM intake of approximately 2.8 kg day^{-1} in the current study demonstrates that RPM was effective in overcoming the adverse effects of heat stress on DM intake, thereby contributing to increased milk, protein, and energy outputs. Socha *et al*. (2005) also observed increases in milk contents of protein and fat in a normal environment. Rulquin and Delaby (1977) reported an increased milk protein percent to RPM with corn silage and soybean meal based diet during negative energy balance under no heat stress. The exposure of cows to extended high AT was central to designing the current study. The prolonged heat stress challenges the immune system and stimulates special polypeptides synthesis (Guerriero and Raynes, 1990). Met is the initiator AA during translation process of polypeptides biosynthesis (Kozak, 1983). The high AT would further stress the early-lactation cows already undergoing tissue mobilization and hepatic modulations in nutrient metabolism (Drackley *et al*., 2001). As a result, Met requirements will rise to the levels at which more pronounced cow responses to RPM would be expected (Socha *et al*., 2005). Hence, the parallel improvements in milk yields of protein and fat in the present study establish that prolonged RPM intake can greatly alleviate simultaneous heat and metabolic pressure.

Parallel improvements in milk fat and protein contents and yields suggest multiple mechanisms of action for Met. Such mechanisms would include increasing epithelial proliferation to facilitate nutrient absorption (Stipanuk, 1986), enhancing Met supply to initiate and retain protein biosynthesis in the mammary gland (Kozak, 1983), augmenting fatty acids transport from adipose tissue to the mammary gland to sustain milk fat secretion (Auboiron *et al*., 1994; Durand *et al*., 1992), increasing gluconeogenesis to maintain mammary lactose synthesis and supplying high-energy phosphate bonds to fuel reactions concerning the aforementioned processes (Mayes, 2000; Lapierre *et al*., 2006). Canale *et al*. (1990) found a synergistic rise in milk fat by adding RPM to a fat-added diet compared to either of fat-added or RPM-supplemented diets alone. This alongside the current data suggest that RPM can induce to inhibit dilution of milk components in early lactation and improve their yields by increasing precursors availability under stretched stressful conditions. The greater energy-corrected milk yield requires increased glucose and AA supplies. Glucose is essential to provide NADPH for de novo milk fat synthesis and ATP for protein synthesis from AA (Mayes, 2000; Van Soest, 1994). The increased energy-corrected milk yield demonstrated that RPM could improve the lactation curve persistency under protracted exposure to unfavorable ambient temperatures, which substantiates the results of Ghorbani *et al.* (2007). Results also revealed that RPM benefited cow productivity constantly in second and higher lactation cows, conveying global commercial implications.

Increased milk production over the few recent decades has depressed reproduction. This has become more obvious in stressful environments when estrus detection and timely insemination face real challenges, thus elongating days open and calving interval. The current findings suggest that feeding strategies such as prolonged RPM provision can significantly attenuate such adverse effects and improve estrus expression visibility and ovary function. Since high-producing cows experience extended periods of negative nutrient balance and are more susceptible to heat stress, RPM would be expected to produce more manifest benefits on reproductive indices (Ravagnolo and Misztal, 2002). As such, the improved production and reproduction were consistent.

CONCLUSIONS

The early lactation negative nutrient balance concurrent with extended periods of heat stress introduce multiple challenges to profitable global dairy cattle management. Prolonged dietary provision of a rumen protected methionine (RPM) product to high-producing lactating cows for 5 months under very high ambient temperatures resulted in significant increases in DM intake and milk energy, fat, and protein outputs while improving reproduction. Shortened days open and calving interval by prolonged RPM provision have long-term economic and environmental implications.

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