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Effects of Rumen-Protected Methionine on Dairy Performance and Amino Acid Metabolism in Lactating Cows

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Abstract: Problem statement: Free Met as one of the most limiting AA in dairy cows would be mostly degraded in the rumen. This study was to determine the effect of different levels of Rumen-Protected Met (RPMet) on dairy performance and serum amino acid metabolism. **Approach:** Thirty-six Holstein cows in similar condition were randomly assigned to six experimental treatments with six replicates each. Levels of RPMet in six treatments were 0(control), 14, 28, 42, 56 and 70 g day^{-1} per cow, respectively. **Results:** Treatment had no effect on percentage of milk protein, lactose and SNF. However, milk yield of cows fed 42 g day^{-1} RPMet was significantly higher than that of the control group and milk fat percentage was significantly increased with 56 g day^{-1} RPMet supplementation. There was the trend to decrease the concentration of serum amino acids except Met and Arg with the supplementation of RPMet. Serum EAA contents of the group supplementation of 42 g day^{-1} RPMet were lowest although there were no significant differences among all treatments. Serum BCAA concentrations of cows fed 28 g RPMet were significantly lower than that of the control group. Supplementation of 42 g RPMet could significantly decrease the concentration of NEAA and TAA compared to the control group. **Conclusion/Recommendations:** Supplementation of rumen-protected methionine improved dairy performance and promoted amino acid utilization in lactating cows in the present experiment. The optimal level of RPMet in the diet was 42 g per cow day^{-1}.

Key words: Rumen-protected methionine, dairy performance, amino acid metabolism

INTRODUCTION

Protein available for absorption in the ruminant intestine is derived from ruminal microbes and dietary protein that escapes degradation during passage through the rumen (Dhiman and Satter, 1997). Protein is one of the major limiting nutrients in the diets of lactating dairy cows (Koenig and Rode, 2001). Feeding a diet containing more protein may lead to more waste and environment pollution because excess rumen degradable protein in the form of urea will excrete for the degradation of ruminal microbes. It is important how to increase greater milk yield, especially milk protein yield and the efficiency of protein utilization and to avoid protein deficiency in early lactation (Clark *at al*., 1992; Xu *at al*., 1998; Blum *at al*., 1999).

Methionine has been identified as one of the most limiting AA for the synthesis of milk and milk protein by dairy cows fed diets based on corn (Schwab *et al*., 1976; Leonardi *et al*., 2003). Other researches have shown that Met deficiencies have most often been suggested to affect milk fat synthesis because Met is a methyl donor in the transmethylation reactions of lipid biosynthesis (Robinson *at al*., 1998). Unfortunately, crystalline Met may be degraded by ruminal bacteria before it passes to the small intestine for absorption; therefore, passage of Met to the small intestine may not increase. Because free Met would be mostly degraded in the rumen, it needs to be supplemented in a rumen-protected form to be available in sufficient amounts for absorption in the duodenum and for metabolic purposes. In order to increase Met digestibility in small intestine, one approach by feeding protein sources with low ruminal degradabilities was proved to be nonideal, probably because the undegradable protein supplement may have to supply more dietary CP, which may result in a shortage of ruminally available N, leading to decreased microbial protein to the duodenum (Overton *at al*., 1996). Alternatively, one approach that has been used to supply additional Rumen-Protected Met (RPMet) to the cow has been to protect Met from ruminal degradation for subsequent absorption in the small intestine (Overton *at al*., 1998). Primarily, these efforts have focused on either various encapsulated

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forms of Met (Broderick *et al*., 1970; Papas *et al*., 1984; Schingoethe *at al*., 1988) or analogs of Met, such as Met hydroxy analog (Lundquist *et al*., 1983). Addition of methionine hydroxy analog to diets of dairy cows frequently has resulted in increased fat content of milk (Lundquist *et al*., 1983) and addition of encapsulated forms of Met to the diet has increased protein content of milk (Schingoethe *at al*., 1988; Casper *at al*., 1987) or has increased both protein content of milk and milk production. In addition, the prohibition of feed of animal sources increased the problem of ruminant feed formation because animal feed ingredients such as fish meal, meat and bone meal are the most common sources of rumen undegradable protein. It is necessary to research and use rumen-protected AA so as to relieve deficiency of protein feed sources and protect environment. The objective of this study was to evaluate the effect of rumen-protected met on milk yield and composition, plasma AA concentration of dairy cows.

MATERIALS AND METHODS

Cows and design: Thirty-six multiparous (2-4 parities) Holstein cows were randomly distributed among 36 pens with one cow per pen. The cows were then allocated to 6 dietary treatments that were arranged as a complete randomized block design. To be eligible for assignment, cows had to be in good health and to have produced an average of 19-22 kg day^{-1} of milk during 4-5 months postpartum.

The RPMet consisted of a DL-methionine (Sumitomo Chemical Co., Ltd., Japan) core coated with pH sensitive copolymer was fat-protected. The content of Met was about 70%. Our lab had proved that stability of RPMet in rumen and availability in postrumen was reliable. Levels of RPMet in six treatments were 0 (control), 14, 28, 42, 56 and 70 g per cow day^{-1}, respectively. The RPMet was premixed with concentrate supplement prior to being mixed with the total diet to achieve uniform distribution. The ingredient and nutrient compositions of the control diet were shown in Table 1. The experiment lasted 56 day and consisted of 14 day adaptation and 35 day trial period. During the adaptation period all cows received the control treatment (no RPMet). On day 15, cows were assigned randomly to one of six treatments. Cows were housed in individual tie stalls and had free access to water throughout the experiment.

Sampling, measurements and analyses: Cows were milked twice daily at 0530 and 1530 h and milk yield of individual cows was recorded at each milking. Milk was sampled weekly from two consecutive milkings, preserved with 2-bromo-2-nitropropane-1, 2-diol and composited according to milk yield.

Table1: Ingredients and nutrient compositions of the experiment basal diet (air-dry basis, (%))

Ingredients	Content	Calculated composition	Content
Corn silage	20.00	DM	70.660
Brewage distiller's grain	7.00	NEL (MJ Kg^{-1})	6.240
Alfalfa hay	3.00	Crude protein	15.200
Hay	10.00	Calcium	0.824
Corn	31.20	Phosphorus	0.529
Soybean meal	13.62	Lysine	0.486
Wheat bran	7.20	Methionine	0.168
DDGS	3.60	Methionine + Cystine	0.354
Calcium phosphate dibasic	0.90	Threonine	0.396
Limestone meal	1.08	ADF	39.910
Sodium bicarbonate	0.90	NDF	21.660
Salt	0.90		
Mineral and vitamin mix[a]	0.60		

[a]: Contained 5.0% Mg, 7.5% K, 1.0% S, 3% Zn, 3% Mn, 2% Fe, 0.5% Cu, 0.025% I, 0.015% Se, 0.004% Co, 22000 IU of Vitamin A g^{-1}, 660 IU of Vitamin D3 g^{-1} and 8 IU of Vitamin E g^{-1}

Composite milk samples were analyzed for contents of CP, fat, lactose and SNF. Milk CP was measured by orange G dye-binding and fat with Babcock method and milk SNF content was estimated by difference (gravimetric total solids minus percent milk fat).

Blood samples were obtained from the jugular vein with evacuated tubes containing coagulant (Haimen city, Jiangsu province, PR China) during the last day of the experiment at 3 h post-feeding. Sample tubes were centrifuged at 3000 × g for 10 min at a low speed table centrifuge (80-2 B, Shanghai, PR China). The serum was frozen at -20°C until analyzed. Three milliliters of serum were deproteinized with 90 mg of sulfosalicylic acid and analyzed for serum amino acids using ionexchange chromatography with an AA autoanalyzer (835-50, HITACHI, Japan).

Statistical analysis: Data were statistically analyzed by one-way ANOVA using GLM proceeding of SAS (2000) with individual pen as statistical unit. Differences among dietary treatment were compared using Duncan's multiple range tests. A significance level of $p<0.05$ was used.

RESULTS

Milk yield and milk compositions: Effects of different levels of RPMet on milk yield and milk compositions were shown on Table 2. The supplementation with 42 g day^{-1} of RPMet significantly increased milk yield ($p<0.05$) compared with control group and the group of supplementation with 14 g day^{-1} of RPMet and had no significant difference compared with other groups ($p>0.05$). The supplementation with RPMet did not influence milk protein, lactose and SNF percentage by Holstein cows.

Table 2: Effects of Rumen-Protected Methionine (RPMet) on milk yield and composition in lactating cows

	RPMet (g day^{-1})						
Item	0	14	28	42	56	70	SEM[Y]
Milk yield (kg day^{-1})	18.95[b]	19.04[a]	19.74[ab]	21.55[a]	19.87[ab]	19.42[ab]	0.299
Protein (%)	2.95	3.08	3.09	3.21	3.22	3.25	0.141
Fat (%)	3.05[b]	3.59[ab]	3.08[b]	3.52[ab]	3.78[a]	3.60[ab]	0.120
Lactose (%)	4.57	4.69	4.51	4.54	4.59	4.63	0.091
SNF (%)	8.17	8.43	8.19	8.41	8.47	8.54	0.126

[a and b]: Means within a row with different superscript differ (p<0.05); [Y]SEM: Standard Error of the Mean

Table 3: Effects of Rumen-Protected Methionine (RPMet) on concentrations of serum AA in lactating cows (mg/100 mL)

	RPMet (g day^{-1})						
Amino acid[Z]	0	14	28	42	56	70	SEM[Y]
EAA	15.71	15.32	13.89	13.41	14.24	14.04	0.891
Cys	0.61	0.64	0.59	0.61	0.60	0.59	0.028
Val	3.73	3.72	2.92	3.09	3.20	3.09	0.339
Met	0.47	0.49	0.48	0.49	0.49	0.51	0.042
Ile	1.74	1.63	1.50	1.55	1.48	1.61	0.145
Leu	3.15[a]	2.93[ab]	2.35[b]	2.51[ab]	2.54[ab]	2.50[ab]	0.272
Phe	1.05[a]	0.98[a]	0.99[a]	0.67[b]	0.99[a]	0.85[ab]	0.105
Lys	2.07[a]	1.56[ab]	1.53[b]	1.58[ab]	1.64[ab]	1.49[b]	0.186
Arg	1.91	2.49	2.57	1.99	2.39	2.54	0.454
His	0.99	0.89	0.93	0.78	0.88	0.83	0.164
BCAA	8.62[a]	8.28[ab]	6.77[b]	7.15[ab]	7.22[ab]	7.19[ab]	0.654
NEAA	12.01[a]	11.98[a]	11.57[ab]	9.95[b]	10.21[b]	10.60[ab]	0.771
Asp	6.38	6.02	5.33	5.44	5.32	5.57	0.381
Ser	1.21	1.27	1.17	1.17	1.06	1.04	0.092
Glu	3.21[ab]	3.50[b]	3.32[ab]	2.89[ac]	2.66[c]	2.81[ac]	0.195
Gly	2.77[ab]	3.19[a]	2.76[ab]	2.46[b]	2.63[b]	2.51[b]	0.192
Ala	2.56[a]	2.31[ab]	2.35[ab]	2.37[ab]	1.95[b]	2.01[ab]	0.195
Tyr	1.03	1.07	1.00	0.88	0.97	1.01	0.082
Pro	1.24	1.13	1.10	0.83	1.00	0.88	0.180
TAA	34.32[a]	33.52[ab]	30.98[ab]	28.98[b]	29.96[ab]	30.41[ab]	1.652

[a-c]: Means within a row with different superscript differ (p<0.05); [Z]BCAA: Bracched-Chain AA (Ile, Leu and Val); EAA: Essential AA (Cys, Val, Met, Ile, Leu, Phe, Lys, Arg, His); NEAA: Nonessential AA (Asp, Ser, Glu, Gly, Ala, Tyr, Pro); TAA: EAA+NEAA; [Y]SEM: Standard Error of the Mean

However, milk fat percentage was increased (p<0.05) by 23.93 and 22.73% for the Holstein cows receiving 56 g day^{-1} RPMet compared with the control treatment and the treatment of supplementation with 28 g day^{-1} RPMet.

Concentrations of serum amino acids: Table 3 summarized the effects of RPMet on concentrations of serum amino acids. Dietary treatment had no effect (p>0.05) on contents of EAA in cows. As to EAA, concentration of serum Leu in cows fed 28 g RPMet per day was significant lower compared to the control group, whereas this difference was not observed compared to other treatments. Cows fed 42 g RPMet per day had nonsignificant different serum Phe concentration with cows fed 70 g RPMet per day, but had significant lower concentration than those of other treatments. Supplementation of 28, 70 g RPMet to diets increased (p<0.05) serum contentration of compared to the control group. There were not significant differences mong other EAA. Interestingly, concentration of serum Arg increased with the supplement of RPMet. Cows fed 28 g RPMet had lower (p<0.05) concentration of serum BCAA than cows fed control diet, but had similar concentration (p<0.05) to other groups. Concentration of serum NEAA of cows fed 42 g and 56 g RPMet was significant higher (p<0.05) than those fed control diet and 14 g RPMet. With regard to NEAA, cows fed 56 g RPMet had lowest concentration of serum Glu. Moreover, supplementation of RPMet to diet had no significant effect on concentration of Gly compared to the control group. Concentration of serum Ala of cows consuming the diet added 56g RPMet was lower (p<0.05) than that of cows consuming the control diet, but was similar (p>0.05) to other groups. Concentration of serum TAA of cows added 42 g RPMet was significantly low (p<0.05) compared with control group. In conclusion, RPMet had the trend to decrease serum amino acids except for Met and Arg.

DISCUSSION

The results in the study had shown that milk yield of treatment groups supplemental RPMet was higher than that of control group. Moreover, the group of supplemental 42 g day^{-1} RPMet per cow had significant difference in milk yield compared with the control group ($p<0.05$). Berthiaume *et al*. (2001; 2006) found there was no effect of RPMet on milk yield. In the experiment of Casper and Schingoethe (Casper and Schingoethe, 1988) with cows fed barley and corn silage-based total mixed diet, supplementation of ruminally protected Met did not increase milk production but increased milk protein percentages. They concluded that Met increased in mammary synthesis, but it was not first factor limiting milk production. The effect on milk yield of RPMet was also consistent with the results of Robinson *et al*. (1995) and Varvikko *et al*. (1999). However, milk yield decreased with the addition of 56 and 70 g day^{-1} of RPMet. This suggested that as the diet went from a deficit (control) to adequate (42 g day^{-1} of RPMet) and excessive (56 and 70 g day^{-1} of RPMet) Met levels, amino acid imbalance led to negative effects. Percentage of milk protein may be more sensitive index than milk yield to estimate the effect of RPMet on cows (Samuelson *at al*., 2001). Rumen-protected Met tended to increase protein percentage in milk, which agreed with the data from other experiments (Wu *et al*., 1997; Misciattelli *at al*., 2003). The non significant effect of PRMet on protein percentage in milk observed in the experiment may be due to low bioavailability of methionine from PRMet for protein synthesis (Blum *et al*., 1999). Increased percentage of milk fat was obtained in this experiment when RPMet was fed to cows. These findings were in agreement with those of previous studies documenting increased milk fat synthesis following feeding of RPMet (Robinson *at al*., 1998; Overton *et al*., 1996) or abomasal infusion of methionine (Oldham, 1984). Clark and Oldham had suggested that supplementary dietary Met was associated with an increase in milk fat production (Clark, 1975; Oldham, 1984)[l]. Rogers *et al*. (1987) reported that RPMet and RPLys increased the percentage of milk fat and protein. The specific reason for the increased percentage of milk fat in our experiment was unknown; however, several possibilities had been suggested in the literature. McCarthy *et al*. (1968) reported that Met might be important for synthesis of serum lipoprotein and as a methyl donor for synthesis of phospholipids, suggesting that a post-absorptive effect of Met on lipid metabolism is possible. Sharma and Erdman (1988) speculated that choline synthesized from Met was likely to have been at least partially responsible. In the study, percentages of lactose and SNF in milk were not significantly affected when RPMet was fed, which was agreeable with Overton *et al*. (1996). Previous studies reported that encapsulated Met products fed to lactating dairy cows had no effect on milk yield or composition, increased milk protein content (Schingoethe *at al*., 1988; Casper *at al*., 1987), increased both milk yield and milk protein content. Differences in results from these experiments might have been caused by differences in the status of Met or other AA of the cows, the amount of Met supplied in the protected product and the efficacy of the protection scheme in delivering Met to the small intestine.

In the study concentrations o EAA, BCAA, NEAA and TAA of groups added RPMet were lower than those of control group, which indicated that addition of RPMet improved the balance of plasma amino acids and increased the utilization of all amino acids. In theory, when the supply of the most limiting amino acids is increased postruminally, other essential amino acids in plasma should decrease because of an increased synthesis of milk protein (Nimrick *et al*., 1970; Bull and Vandersall, 1973).

The absorption of methionine can be determined by measuring amino acid content as the levels of serum free methionine are positively related to the levels of methonine in small intestine (Diao, 2007). In the study, serum Met content of all treatment groups with addition of PRMet was slight higher than that of control group, which realized that RPMet was absorbed in small intestine through rumen. The report was in agreement with the result of Overton *et al*. (1998) and Blum *et al*. (1999). Overton *et al*. (1998) reported that the concentration of Met in plasma increased when PRMet was fed to cows. Blum *et al*. (1999) showed that concentration of plasma sulfur amino acids (Met and Cys) were evaluated of cows fed PRMet coated with a pH-sensitive polymer coating, however, only concentration of plasma Met increased of cows fed PRMet which was fat-protected. Rogers *et al*. (1987) indicated that a significant ($p<0.01$) linear increase in plasma Met and Lys was observed but other plasma essential and nonessential amino acids when cows were fed increasing amounts of RPMet and RPLys.

Overton *et al*. (1998) reported concentration of Met in plasma increased, in contrast, concentrations of Gly, Ile, Leu, Thr, Tyr and Val in plasma tended to decrease and the concentration of Phe in plasma decreased when RPMet was fed to cows. In this experiment content of serum Val, Ile, Leu, Phe, Lys, His, Asp, Ala and Pro of treatment groups added with RPMet was lower than that of control group, indicating RPMet affects the

metabolism of other amino acids and promotes absorption and utilization of amino acids so as to decrease the concentration of serum amino acids. The concentrations of AA in blood plasma reflect the supply of these AA from intestinal absorption and endogenous synthesis as well as the demand for protein synthesis and degradative metabolism (Harper, 1968). Feedig ruminally protected Met resulted in decrease of total amino acids concentration in lactating dairy cows equipped with duodenal cannulae.

Varvikko *et al*. (1999) observed despite large increases in plasma Met, TAA concentrations were not changed as Met infusion increased, which suggests a compensating decline in plasma concentrations of some other individual AA, such as BCAA. Blum *et al*. (1999) indicated that concentration of plasma Val, Ile and BCAA (Val + Ler + Ile) decreased when cows fed RPMet coated with a pH-sensitive polymer coating. BCAA were decomposed into acetic acid, propionic acid and citric acid through blood into mammary gland and was used for the carbon frame to synthesize NEAA. Unlike other essential AA, the liver has less capacity to degrade BCAA and concentrations of BCAA in blood plasma have therefore been used as an indicator of protein absorption from the intestine Bergen *et al*., (1973). The reduced plasma BCAA agrees with the study by Guinard and Rulquin (1995). Yang *et al*. (2004) reported that Met embedded with fat and absorptive MHA could decrease concentration of serum Val, Ile, Leu and significantly decrease the content of serum TAA, EAA, which suggested ability of tissue to synthesize protein using amino acids was improved. Jiang *et al*. (2004) indicated reduced concentration of NEAA, TAA with graded DL-Met infusions by duodenal cannulas suggested increased availability of amino acids.

However, some studies showed that RPMet did not decrease plasma concentration of other amino acids, perhaps because the level of Met in diet exceeded the requirement of animals.

The concentrations of free amino acids in blood plasma reflect a dynamic equilibrium between intestinal absorption, endogenous synthesis of amino acids and protein synthesis and catabolism (Bergen, 1979). Amino acid concentrations in plasma provide a sensitive criterion for qualitative determination of postruminal delivery of rumen-protected amino acids. Accurate quantification of the amount delivered is difficult for limiting amino acids because their concentrations in plasma remain constant or increase slowly until tissue requirements are met. Inflection points of blood response curves defining the point of change in the rate of accumulation have been used as indices for preliminary estimates of amino acid requirements in monogastric animals and less in ruminants. These estimates serve only as guidelines and need to be confirmed with measurements of metabolic and production parameters before they are used in final conclusions.

CONCLUSION

In conclusion, milk yield was significantly increased with the addition of 42 g RPMet per cow day^{-1} and had the trend to promote the synthesis of milk protein and milk fat. In addition, RPMet could decrease the concentration of serum amino acids and supplementation of 42 g RPMet significantly decreased the content of serum NEAA and TAA compared to the control group. This study demonstrated that addition of 42 g RPMet in the diet could improve dairy performance and utilization of amino acids. However, further evaluation is needed to determine the usefulness of RPMet in improving milk production of high producing dairy cows during an entire lactation.

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