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The Comparison of the Amount of Methionine Supply by Different Rumen-Protected Methionine Sources

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ABSTRACT

Bioavailability of three rumen protected Methionine (Met) sources with different protection methods (Mepron® M85, Evonik Industries, Germany; Methioplus®, Soda Nutrition, Italy and Methilock®, Tehrandaneh Co. Iran) were evaluated in 2 experiments with 6 canulated non-lactating Holstein cows. In experiment 1, the ruminal *in situ* and mobile bag techniques were used for assessing ruminal degradability and intestinal digestibility of Met from the protected Met sources. The rate of disappearance of Met from Mepron® M85 was lower than Methioplus® (2.94 *vs.* 5.73 % h^{-1}). Mepron® M85 had more resistance to ruminal degradation than Methioplus® (82.78 *vs.* 68.51%), but the higher intestinal digestibility of Methioplus® resulted in similar amounts of available Met for two products. Because of high washing out loss from *in situ* bags, ruminal degradation was not estimated for Methilock®. In the second experiment, Met availability was assayed by the blood Met response after 5 days feeding each product in comparison to pretreatment levels utilizing a 3×3 Latin square design. Three Met sources increased blood Met concentration significantly after 5 days feeding (37.5, 52.23 and 44.39% for Methilock®, Mepron® and Methioplus® respectively). Results of the present study showed that the three RPM sources increased blood Met concentration. This study also suggests that the *in situ* method may not adequately characterize the availability of rumen protected amino acids, especially those of small particle size.

Keywords: Blood Response, Holstein cow, Intestinal Disappearance, Methionine, Ruminal degradability.

INTRODUCTION

As milk production of dairy cows continues to increase, meeting nutrient requirements, especially for Amino Acids (AA), becomes more difficult. Methionine (Met) has been identified as one of the most limiting AAs for synthesis of milk and milk protein by lactating dairy cows (Schwab *et al.,* 1976; NRC, 2001). Many studies have been focused on increasing amounts of limiting AA, especially Met, in the diet of high yielding dairy cows to increase milk and milk protein yield (Rulquin *et al.,* 2006) and the efficiency of protein utilization (Dinn *et al.,* 1998; Broderick *et al.,* 2008), reducing nitrogen excretion (Leonardi *et al.,* 2003) and reducing protein intake (Broderick *et al.,* 2008).

Three approaches have been used to supply additional AA to cows: (1) Optimizing ruminal fermentation makes extensive use of microbial protein synthesized within the rumen (Oba and Allen 2003; Brito *et al.,* 2007); (2) Inclusion of protein sources in the diet that are not readily degraded in the rumen and pass to

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the small intestine (Noftsger and St-Pierre, 2003), and (3) Supplementation of individual AA sources in the diet (Leonardi *et al.,* 2003; Broderick *et al.,* 2008).

Feeding protein sources with low ruminal degradability have been associated with varying results, perhaps because supplying 35% or more of the total dietary protein from rumen undegradable protein may result in a shortage of ruminally available N, leading to decreased passage of microbial protein to the duodenum (Clark *et al.,* 1992). Alternatively, individual AA may be added to the diet, but because unprotected AAs may be degraded by ruminal bacteria before it passes into the small intestine for absorption, different methods have been developed to protect them from ruminal degradation.

Feeding Ruminally Protected Met (RPM) to dairy cows has resulted in increased passage of Met to the small intestine and increased the amount of Met in the plasma (Overton *et al.,* 1996; Blum *et al.,* 1999; Berthiaume *et al.,* 2000). However, varying results have been observed when RPM was fed to dairy cows (Koenig and Rode, 2001; Sudeküm *et al.,* 2004). One possible reason for these results might be different bioavailability of the RPM sources used in these experiments. Bioavailability is determined by a combination of their AA content, resistance to ruminal degradation and intestinal digestibility (Berthiaume *et al.,* 2000). For example, Smartamine® M (a pH sensitive polymer coated Met product, Adisseo, Antony, France) has been reported to be more effective in increasing blood Met concentration than Mepron® M85 (a ethylcellulose and stearic acid coated Met product, Evonik Industries, Hanau, Germany) (Blum *et al.,* 1999; Südekum *et al.,* 2004), whereas Südekum *et al.* (2004) observed equal potential between Mepron and Methio-BY for changing blood Met levels.

Methilock® (Tehrandaneh Company, Tehran, Iran) is a commercial RPM supplement that contains 50% DL-Met and is protected by molecular attachment to phenolic compounds. The objective of the present study was to assess the ruminal degradability, post ruminal disappearance and ability to raise peripheral blood Met levels of 3 sources of RPM. These sources were Mepron (85% DL-Met), Methioplus (55% DL-Met: Soda Feed Ingredients, Monaco) and Methilock.

MATERIALS AND METHODS

Ruminal Degradability

Ruminal Disappearance of the three RPM products was assessed by *in situ* technique as described by Berthiaume *et al.* (2000). In this experiment, 6 cannulated nonlactating Holstein cows (3-4 years old with average body weight= 638.7±34 Kg) were used. Animals were fed a total mixed ration with forage to concentrate ratio of 60:40 which was balanced based on NRC (2001) recommendations for 10% greater than maintenance requirements (Vahdani *et al.,* 2014). The ingredients and chemical composition of the diet was presented in Table 1. Cows were housed in tie stalls and were fed twice daily at 0800 and 1400. For each RPM product, 16 nylon bags (3×5 cm; pore size, 51 µm) were filled with 1.5 g RPM, inserted into large mesh bags and suspended in the rumen. Two randomly chosen small bags were removed from the rumen of each animal after 1, 2, 4, 8, 10, 12 and 16 hours of incubation. The 0 time samples were placed into a washing machine for 20 minutes to calculate true DM and Met disappearance which were considered as washing loss. After removal, bags were washed by running cold tap water until no color was visible in the rinse water (Berthiaume *et al.,* 2000). Bag residuals were dried in a forced-air oven at 40°C for 72 hours. After drying, bag contents were weighed and duplicates of bags within animals were pooled by time and analyzed for DM and Met contents. The means reported in Table 2 are the means of duplicate samples in 6 cows at 8 time points.

Table 1. Ingredient and nutrient composition of the total mixed diet on DM.

Composition	
Ingredients (%)	
Alfalfa hay	11.0
Corn silage	10.5
Wheat straw	26.1
Barley grain	23.7
Corn grain	3.6
Wheat bran	10.0
Soybean meal	4.6
Canola meal	8.9
Calcium carbonate	0.69
Dicalcium phosphate	0.13
salt	0.21
Vitamin and mineral premix [a]	0.57
Nutrients (%)	
DM	68.5
CP	13.8
EE	2.7
NDF	42.6
ADF	25.6
Ca	0.7
P	0.5

[a] Contained 195.0 g kg^{-1} calcium; 21.0 g kg^{-1} magnesium; 1000.0 mg kg^{-1} cobalt; 300.0 mg kg^{-1} copper; 120.0 mg kg^{-1} iodine; 3000.0 mg kg^{-1} iron; 2200.0 mg kg^{-1} manganese; 3000.0 mg kg^{-1} zinc; 1.1 mg kg^{-1} selenium; 600.0 KIU kg^{-1} vitamin A; 200.0 KIU kg^{-1} vitamin D; 200.0 mg kg^{-1} vitamin E, 2500.0 mg kg^{-1} antioxidant .

Post Ruminal Disappearance

For comparing post ruminal disappearence and availability of Met of the 3 RPM products, a 3-step technique was used as described by Berthiaume *et al.* (2000). This experiment was performed using 2 ruminally and duodenally cannulated cows and repeated 2 times for each RPM product. At each time, 13 small nylon bags, similar to those used in experiment 1, were placed into a large mesh bag and suspended in the rumen for 4.5 hours (Berthiaume *et al.,* 2000). After removal from the rumen, four bags were washed by hand under cold tap water until no color was visible. The remaining 9 bags were immediately transferred into a pepsin-HCL solution (pH= 2) for 2.5 hours at 39°C to mimic abomasal digestion. After incubation in pepsin, another 4 bags were washed as described earlier. Thereafter, the 5 remaining bags were inserted into the small intestine through the duodenal cannula at a rate of 1 bag in each 30 minutes (De Boer *et al.,* 1987). All 5 remaining bags were recovered from the feces within 24 hours, thoroughly washed as described above and analyzed for DM and Met contents.

Bioavailability

Six Holstein non lactating ruminally cannulated cows (BW= 638.7±34 Kg) were assigned to a change-over design with 3 periods and 3 treatments. Treatments were rumen protected Met sources (Mepron® M85, Methioplus® and Methilock®) that differed in protection technology while periods were 14 d. Cows were fed the basal diet (Table 1) during the first 9 days of each period which was the control period. During the second week, from day 10 to 14, each

Table 2. Mean ruminal disappearance of Met from two rumen protected Met sources.

		Ruminal incubation time (h)							
RPM source		0	1	2	4	8	10	12	16
Methioplus	Mean (%)	18.16	15.81	22.60	32.52	46.12	50.43	60.78	68.44
	SE	3.5	4.3	2.8	3.9	4.1	5.02	6.17	5.89
Mepron	Mean (%)	6.38	4.33	11.50	15.82	27.64	29.24	32.00	44.50
	SE	2.98	1.23	1.09	3.44	3.02	4.12	4.46	5.06
	P-value	**	**	**	**	**	**	**	**

* $P \leq 0.05$, ** $P \leq 0.01$.

cow received the control diet plus the equivalent of 50 g of *D, L*-methionine from one of three RPM sources as a top-dress with the morning meal. Blood samples were taken from coaccygeal vein on day 9 (one day before feeding RPM sources) and on day 14 (after 5 days feeding RPM sources) at 2, 6 and 10 hours after the morning meal by heparinzed tubes. Blood was immediately centrifuged at 3,000×g at 4°C for 15 minutes. Blood plasma samples were pooled on days 9 and 14 within day for each cow and then analyzed for Met content (Blum *et al.,* 1999, Sudeküm *et al.,* 2004).

Analytical Procedures

Feed ingredients and mixed diet were analyzed for Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), and ash using AOAC methods (AOAC 1990). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed as described by Van Soest *et al.* (1991). The NDF and ADF contents of the basal ration were expressed without residual ash. For Met analysis, samples of the *in situ* experiment were pretreated with performic acid and then with hydrobromic acid for removing performic before being digested with 6 *N* HCL (method 994-12; AOAC, 1997). For determination of Met concentrations in plasma, the blood plasma samples of days 9 and 14 were pooled within day and cow and deproteinized using sulfosalicylic acid. Amino acids were quantified by a High Performance Liquid Chromatography system (HPLC) that set up only for AA separation (Acme 9000, YOUNG LIN, Korea) in a commercial labolatory (Masood Lab., Tehran, Iran).

Statistical Analysis

Because Methilck was washed out of the bags, calculation of degradability was not possible. Differences for mean *in situ* degradation and digestibility values between Methioplus and Mepron were assessed using GLM procedure of SAS (1998). Comparisons of mean plasma Met values for the 3 RPM products were evaluated in a changeover design with model effects for period, treatment (RPM sources) fixed effects and animal as random effect by the following model:

$$y_{ijkl} = \mu + T_i + P_j + A_k + e_{ijk}$$

y_{ijkl} : Observed Met concentration
μ : Overall mean
T_i : Treatment effect
P_j : Period effect
A_k : Animal effect
e_{ijk} : Random error

Comparison mean plasma Met levels were performed using the Mixed procedure of SAS (1998). Significant differences were declared at $P< 0.05$ for both analyses.

RESULTS

Ruminal Disappearance and Intestinal Digestibility

Because of high wash out loss of Methilock from *in situ* bags, the data of its ruminal disappearance was not reported. Ruminal disappearance of two RPM sources are presented in Table 2. As expected, ruminal disappearance of Met from Mepron and Methioplus increased with residence time. Disappearance of Met from Methioplus was greater than Mepron likely because of difference in protection type (5.73 vs. 2.94 % h^{-1}). Estimation of disappearance of Met from Mepron and Methioplus in different parts of gastrointestinal tract of cows, as presented in Table 3, were higher than Mepron ($P< 0.05$) whereas, resistance of Mepron to ruminal degradation was higher than Methioplus ($P< 0.05$).

Bioavailability

There were no significant differences among treatments for dry matter intake of

Table 3. Mean disappearance of Met from two rumen protected Met in different parts of the gastrointestinal tract determined *in situ*.

Item	Methioplus	Mepron	SEM	*P-value*
Rumen disappearance [a] (%)	31.49	17.22	0.9	**
Post rumen disappearance [b] (%)	63.57	50.54	1.20	**
Ruminal resistance [c] (%)	68.51	82.78	0.9	**
Postruminal Digestibility [d] (%)	91.02	61.07	2.10	**
Available Met [e] (%)	43.61	41.83	1.27	ns

[a] Met disappearance after 4.5 hours in rumen, [b] Met disappearance in HCL solution and Intestine, [c]100-Rumen, [d] Post rumen/(100-rumen), [e] Post rumen*(100-rumen), ns: Not significant; * $P \leq 0.05$, ** $P \leq 0.01$.

cows fed experimental diets. Values of basal plasma Met 3 days before starting of feeding RPM sources were similar among treatments (Table 4). All RPM sources increased blood Met concentration in a similar manner after 5 days of feeding and there were no significant differences among them for changing blood Met level.

DISCUSSION

Met in the Mepron is protected by ethylcellulose and stearic acid but protection in Methioplus is achieved by microencapsulation in a lipidic layer and the different ruminal disappearance of these two RPM sources is due to different protection types.

The ruminal fractional rate of Met disappearance from Mepron in the present study was similar to the results of Berthiaume *et al.,* (2000) (2.94 and 2.66% h^{-1}, respectively), but higher than the results of Overton *et al.,* (1996) and Koenig and Rode (2001) (1.82 and 2.25% h^{-1}, respectively). The methodological differences such as physiological status of animals, bag sizes and ratio of sample size to bag surface (Vanzant *et al.,* 1998; Berthiaume *et al.,* 2000) might explain differences reported between the present study and other studies. The *in situ* technique is one of the approaches that is most commonly used for assessing ruminal resistance and availability of RPM products. However, the *in situ* technique may underestimate ruminal degradation as well as bioavailability of certain ruminally protected Met products (Berthiaume *et al.,* 2000; Koenig and Rode, 2001).

The tradeoff between ruminal resistance and intestinal availability, which was observed in the present study, was reported earlier by Koenig and Rode (2001). There was no significant difference between available Met of Mepron and Methioplus. Estimation of Met disappearance from Mepron in different parts of the gastrointestinal tract in the present study is similar to results of Berthiaume *et al.* (2000), but, it is not in agreement with others (Overton *et al.,* 1996; Koenig and Rode, 2001). Higher postruminal diapearance of Mepron in this study relative to results of Koenig and Rode (2001) is

Table 4. Plasma methionine concentrations before and after the start of feeding ruminally Protected methionine sources.

	Methilock	Mepron 85	Methioplus	SEM	*P-value*
Pre-feeding level (µmol l^{-1})	23.67	25.33	22.50	0.93	ns
Post-feeding level (µmol l^{-1})	32.67	38.50	33.30	2.13	ns
Blood Met change (µmol l^{-1})	9.00	13.17	10.50	1.72	ns

ns: Not significant; * $P \leq 0.05$, ** $P \leq 0.01$.

likely due to different physiological status of animals between their experiment and the present study (lactating vs. dry cows). They reported that higher dry matter intake by cows and faster rate of passage through the lower tract in their experiment may have reduced intestinal digestibility of RPM from Mepron. The amount of available Met in the present study is lower than the results of Berthiaume *et al.* (2000) that is due to different calculations used for estimation of available Met between two experiments.

The amount of changes in blood Met level by Mepron in the present study (13.17 µmol l^{-1}) was similar to earlier studies with similar blood sampling approach and similar amount of RPM feeding (13.6 and 14.3 µmol l^{-1} respectively for Blum *et al.,* 1999; Sudeküm *et al.,* 2004). Despite the different results on the degree of ruminal degradation of RPM sources found *in vitro* and *in vivo* incubations, this similarity to results of earlier studies for blood Met response to oral administration of ruminally protected Met shows that the blood sampling assay can be a useful method for assessing protection and availability of ruminally protected Met sources (Blum *et al.,* 1999 and Sudeküm *et al.,* 2004). There is no previously published information for Methilock and Methioplus about their effects on blood Met concentration.

Methilock is an RPM product that contains 50 percent RPM and protected from ruminal degradation by binding methionine to some phenolic compounds. Although, we could not estimate ruminal resistance and intestinal digestibility of this product via i*n situ* technique, measuring bioavailability by a blood sampling method (Blum *et al.,* 1999 and Sudeküm *et al.,* 2004) showed that it can increase blood Met significantly (9.5 µmol l^{-1}) which was in line with the blood Met increase observed with other products.

CONCLUSIONS

The ultimate goal of supplying ruminally protected methionine, or other AA, is to improve performance of dairy cattle. Selection of the ruminally protected AA products by dairy producers should be based on the effectiveness of the product at escaping the rumen intact and releasing absorbable AA in the small intestine. Based on the result of the present study, there were no major differences between investigated RPM sources in increasing blood Met concentration, and all of them increased blood Met level, significantly.

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مقایسه میزان تامین متیونین از طریق منابع مختلف متیونین محافظت شده شکمبه ای

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چکیده

زیست فراهمی سه منبع متیونین محافظت شده شکمبه ای با روشهای مختلف محافظت (میران M85 ساخت شرکت اوونیک، آلمان، متیوپلاس ساخت شرکت سودا، ایتالیا و متیلاک ساخت شرکت تهران دانه، ایران) در ۲ آزمایش با استفاده از ۶ گاو شیری غیر شیرده کانولادار شکمبه ای مورد ارزیابی قرار گرفت. در آزمایش اول، روشهای کیسه گذاری شکمبه ای و کیسه گذاری متحرک جهت ارزیابی تجزیه پذیری شکمبه ای و قابلیت هضم روده ای متیونین منابع محافظت شده متیونین بکار گرفته شدند. نرخ ناپدید شدن شکمبه ای برای میران کندتر از متیوپلاس بود (۲/۹۴ در مقابل ۵/۷۶ درصد در ساعت). میران مقاومت شکمبه ای بیشتری نسبت به متیوپلاس داشت (۸۲/۷۸ در مقابل ۶۸/۵۱ درصد) ولی قابلیت هضم روده ای بالاتر متیوپلاس به میزان متیونین در دسترس مشابهی در دو محصول منجر شد. تجزیه پذیر شکمبه ای برای متیلاک به علت میزان بالای شسته شدن و خارج شدن آن از کیسه های تجزیه پذیری برآورد نگردید. در آزمایش دوم، زیست فراهمی متیونین با استفاده از پاسخ متیونین خون پس از ۵ روز تغذیه هر کدام از محصولات نسبت به سطوح قبل از اعمال تیمارهای آزمایشی در قالب یک طرح مربع لاتین ۳×۳ ارزیابی شد. هر سه منبع متیونین غلظت متیونین خون را پس از ۵ روز تغذیه به طور معنی داری افزایش دادند (۳۷/۵، ۵۲/۲۳ و ۴۴/۳۹ میکرومول در لیتر بترتیب برای متیلاک، میران و متیوپلاس). نتایج مطالعه حاضر نشان داد که سه منبع متیونین محافظت شده شکمبه ای موجب افزایش غلظت متیونین خون می شوند. همچنین، این مطالعه پیشنهاد می کند که روش کیسه گذاری ممکن است به طور کامل نتواند فراهمی اسیدهای آمینه محافظت شده بخصوص منابع با ذرات ریز را مشخص نماید.