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Rumen-protected methionine and lysine: effects on milk production and plasma amino acids of dairy cows with reference to metabolisable protein status

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Two experiments were conducted to study the effects of rumen-protected Met (RPM) alone or with rumen-protected Lys (RPL) on milk yield and plasma amino acids of dairy cows. In experiment 1, 24 multiparous Holstein cows (154 DIM) were assigned to one of 3 groups where each cow received 0 g/d of RPM and RPL (C), 30 g/d of RPM (M), or 30 g/d of RPM plus 25 g of RPL (ML). The study lasted for 8 weeks where milk yield and composition were determined weekly. Daily milk yield averaged 28·0, 27·8, and 29·7 kg/cow for the C, M, and ML groups, respectively. Dietary treatments had no effects ($P \geq 0·54$) on milk contents of fat, lactose, solid non-fat or total solids. Milk protein content in the ML group was greater ($P < 0·05$) than the C and M groups. Plasma levels of all AA were not significantly ($P \geq 0·09$) affected by supplemental RPL and/or RPM. In experiment 2, 30 multiparous Holstein cows (100 DIM) were assigned to one of 3 groups where each cow received 0 g/d of RPM and RPL (C), 50 g/d of RPM (M), or 50 g/d of RPM plus 25 g/d of RPL (ML). The study lasted for 5 weeks. Cows in the M (30·5 kg) and ML (31·4 kg) groups produced ($P < 0·05$) more milk than those of the C group (29·1 kg). Under conditions of this study, RPM plus RPL improved milk yield and protein contents of dairy cows and was better than supplying RPM alone. Response in milk yield to RPM and RPL was affected by the MP status of cows which deserves further investigation.

Keywords: Dairy cows, lysine, methionine, milk, plasma amino acids.

Estimating protein requirements for dairy cows has been refined in the NRC publications from crude protein (1978) to digestible protein (1989) and later to metabolisable protein (MP; 2001). Recently, balancing dairy rations for individual amino acid (AA) is becoming more popular. Met and/or Lys were identified as the most limiting AA for milk protein synthesis in most dairy diets (NRC, 2001). The NRC (2001) concluded that milk protein was more responsive to improving duodenal Met and Lys supply than milk yield. However, improvements in milk yield and milk fat contents might be observed in high producing dairy cows during early lactation.

For optimal MP use for milk protein synthesis, the NRC (2001) recommended that Lys and Met percentages in MP should be 7·2 and 2·4%, respectively (i.e., 3 : 1 ratio). It is not only the percentages of Lys and Met in MP that are important, but also the ratio itself. For instance, increasing Lys% in MP decreased milk fat when Met supply was deficient or when the ratio was more than 3·0 (Swanepoel et al. 2010). However, the recommended percentages and ratio are typically hard to achieve in most dairy diets. The only efficient way to achieve the above recommendation is by using rumen-protected (RP) AA because dietary protein and crystalline AA are largely degraded by ruminal microbes.

Commercial RP Met (RPM) has been available for relatively long time and received intensive research. Relatively, RP Lys (RPL) is a new product with limited publications (Robinson, 2010; Wang et al. 2010). More commercial RPL products will be available in the future and more data is needed (Swanepoel et al. 2010; Elwakeel et al. 2012). Cow response (milk yield and composition) to supplemental Met and/or Lys has been variable and inconsistent (Robinson, 2010; Zanton et al. 2014). A systemic review of literature (Robinson, 2010) concluded that: Met alone improved milk protein and fat contents, Met plus Lys increased milk yield and milk protein contents, and Lys alone might have negative effects on DMI. Most of the published studies on

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RPM and RPL used corn grain-based concentrate and it is know that compared to corn, barley contains less Met and more Lys in proteins escaping the rumen (Robinson, 2010).

In light of this background, the objective of this study was to evaluate the effects of supplemental RPM alone or with RPL on lactation performance of dairy cows fed barley grain-based concentrate with reference to plasma AA levels and MP status.

Materials and methods

The Institutional Animal Care and Use Committee at JUST approved all procedures used in this study.

Experiment 1

Twenty four multiparous Holstein cows averaging (±SD) 154 ± 59 DIM and 31·6 ± 4·3 kg/d of milk at the beginning of the study were selected. Cows were housed and handled in tie-stall shadow barns. Lactation number of cows ranged from 1–4 and averaged 2·35 ± 0·95 (±SD). Selected cows were grouped based on DIM, lactation number, and milk amount and assigned to one of 3 groups (8 cows/treatment) where each cow received 0 g/d of RPM or RPL (C), 30 g/d of RPM that supplied 7·65 g of metabolisable Met (M), or 30 g/d of RPM plus 25 g/d of RPL that supplied 7·65 g of metabolisable Met plus 5·13 g of metabolisable Lys (ML). Dietary treatments were top-dressed on offered concentrate for each cow during the morning feeding to ensure complete consumption.

Level of RPM in the M group was selected to initially increase Met% in MP from 1·79 to 2·04 and to decrease Lys : Met ratio from 3·64 to 3·18. Levels of RPM and RPL in the ML group were selected to initially increase Met and Lys% in MP to 2·03 and 6·70 (respectively) and to reach a 3·30 ratio. However, using post-experiment evaluation, Lys : Met ration was actually decreased from 3·68 in the C group to 2·88 and 3·09 in the M and ML groups, respectively. The study lasted for 8 weeks.

Diet (Table 1) was formulated to meet nutrient requirements of cows (NRC, 2001) and was offered as separate forage and concentrate. Concentrate was offered individually to each cow twice a day at the time of milking (at 0700 and 1900) and each cow completely consumed her allocated concentrate portion (10·9 kg DM/cow/d). Silage and wheat straw were group-offered three times a day (at 0800, 1400, and 2000) and the estimated DMI was 1·8 and 3·9 kg/cow/d, respectively. Cows were milked twice a day and individual milk weights were recorded weekly on the day of obtaining milk samples. Milk samples from individual cows were collected and analysed for protein, fat, lactose, SNF, and total solids (Milkoscope Julie C8 automatic, Milscope, Scope Electric, Regensburg, Germany).

Five cows from each group were selected to obtain blood samples. The same cows were used to obtain blood samples at all blood-collecting times (week 1, 5, and 7). Immediately after obtaining morning milk samples, blood samples were collected from the coccygeal vein into vacuum tubes (containing sodium heparin) and were immediately chilled on ice, centrifuged for 20 min at 1000 ***g*** to obtain plasma, and stored (−20 °C) after deproteinised with sulfosalicylic acid for later analysis of amino acids (SYKAM S433 amino acid analyser, SYKAM GmbH, Munich, Germany) with post-column derivatisation.

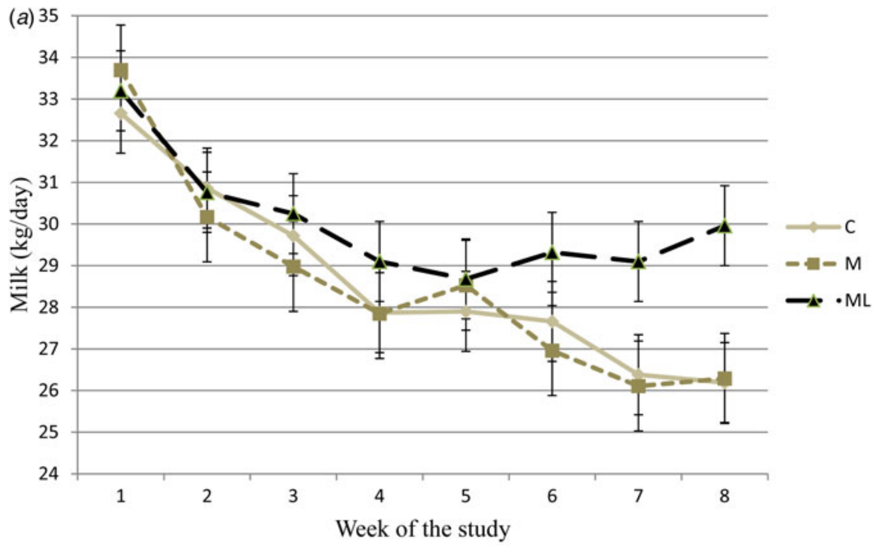
Table 1. Ingredient and chemical composition of the basal diets used in both experiments

	Experiment	
Item	1	2
Ingredients, % of DM		
Barely grain	39·1	34·9
Soybean meal	13·3	10·5
Wheat bran	13·3	10·5
Corn silage	9·0	12·2
Alfalfa silage	1·6	1·1
Alfalfa hay	–	23·1
Wheat straw	23·3	7·3
Vitamin & mineral premix	0·4	0·4
Nutrients, % of DM		
CP	16·2	16·9
RDP	11·6	11·9
RUP	4·6	5·0
NDF	37·6	35·2
Forage NDF	21·8	23·1
ADF	20·9	21·4
NFC	40·5	41·5
EE	2·4	2·5
NE_L, Mcal/kg	1·63	1·55

Experiment 2

This experiment was conducted to test for repeatability of obtained results in experiment 1. Thirty multiparous Holstein cows averaging (±SD) 100 ± 40 DIM and 29·5 ± 3·9 kg/d of milk at the beginning of the study were selected. Cows were housed and handled in tie-stall shadow barns. Lactation number of cows ranged from 2–5 and averaged 3·40 ± 1·00 (±SD). Selected cows were grouped based on DIM, lactation number, and milk amount and were assigned into one of 3 groups (10 cows/treatment) where each cow received daily 0 g/d of RPM and RPL (C), 50 g/d of RPM that supplied 12·75 g of metabolisable Met (M), or 50 g/d of RPM plus 25 g/d of RPL that supplied 12·75 g of metabolisable Met plus 5·13 g of metabolisable Lys (ML). Dietary treatments were top-dressed on offered concentrate for each cow during the morning feeding to ensure complete consumption.

Level of RPM in the M group was selected to initially increase Met% in MP from 1·88 to 2·46 and to decrease Lys : Met ratio from 3·59 to 2·72. Levels of RPM and RPL in the ML group were selected to initially increase Met and Lys% in MP to 2·45 and 6·99 (respectively) and to reach a 2·85 ratio. However, using post-experiment evaluation, Lys : Met ration was actually decreased from 3·59 in the C group to 2·76 and 2·90 in the M and ML groups, respectively. The study lasted for 5 weeks.



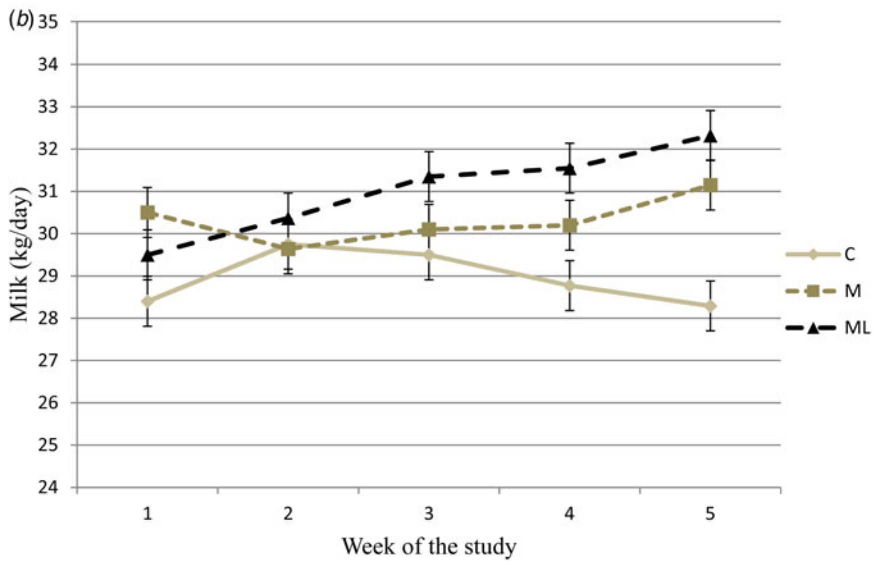


Fig. 1. Effects of supplemental RPM alone or with RPL on weekly milk yield. A: Experiment. 1, where C = no RPM or RPL, M = 30 g/d/cow of RPM, ML = 30 g/d/cow of RPM plus 25 g/d/cow of RPL. B: Experiment. 2, where C = no RPM or PL, M = 50 g/d/cow of RPM, ML = 50 g/d/cow of RPM plus 25 g/d/cow of RPL.

Diet (Table 1) was formulated to meet nutrient requirements of cows (NRC, 2001) and was offered as separate forage and concentrate. Concentrate was offered individually to each cow twice a day at the time of milking (at 0700 and 1900) and each cow completely consumed her allocated concentrate portion (13·0 kg DM/cow/d). Corn silage, alfalfa hay, and wheat straw were group-offered three times a day (at 0800, 1400, and 2000) and the estimated DMI intake was 2·8, 5·3, and 1·7 kg/cow/d, respectively. Cows were milked twice a day and individual milk weights were recorded weekly.

Statistical analysis

Data were analysed as repeated measures by MIXED procedure using the SAS software (SAS institute, 2000). Initial milk yield (week 1) was used as a covariate. The main effects included effects of treatment, time, and time x treatment interaction. The appropriate covariance structure of the data was selected for each variable. Means were separated using the PDIFF option in the LSMEANS statement. *P*-values of <0·05 were considered as significant effects and 0·05–0·15 values were considered as trends.

Results

Experiment 1

There was no treatment by week interaction in milk yield (Fig. 1) and all data presented in Table 2. Daily milk yield averaged 28·0, 27·8, and 29·7 kg/cow for the C, M, and ML groups, respectively. Dietary treatments had no effects

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Table 2. Effects of supplemental RPM alone or with RPL on milk yield and composition

	Treatment†				
Item	C	M	ML	SEM	*P*-value
Milk, kg/d	28·0	27·8	29·7	0·72	0·13
Protein, %	3·09[b]	3·09[b]	3·14[a]	0·013	0·02
Fat, %	3·38	3·61	3·50	0·157	0·61
Lactose, %	4·63	4·58	4·67	0·029	0·19
Solid non-fat, %	8·40	8·34	8·49	0·050	0·13
Total solids, %	11·77	11·92	11·94	0·185	0·77
Protein, g/d	866	864	928	20·5	0·06
Fat, g/d	928	1006	1028	43·3	0·13
Lactose, g/d	1293	1280	1377	30·3	0·07
Solid non-fat, g/d	2347[b]	2328[b]	2505[a]	56·6	<0·01
Total solids, g/d	3290	3313	3518	88·2	0·14

†C = no RPM or RPL, M = 30 g/d/cow of RPM, ML = 30 g/d/cow of RPM plus 25 g/d/cow of RPL.
[a,b]Means within same row with different superscripts differ at ($P < 0·05$).

($P > 0·05$) on milk contents of fat, lactose, solid non-fat or total solids. However, milk protein content of the ML group was greater ($P < 0·05$) than the C and M groups. Cows in the ML group produced more ($P < 0·01$) milk solid non-fat than the C and M groups.

Plasma levels of all AA (data not shown), were not significantly ($P \geq 0·09$) affected by supplemental RPL and/or RPM.

Experiment 2

There was no treatment by week interaction in milk yield (Fig. 1). Daily milk yield averaged 29·1, 30·5, and 31·4 kg/cow for the C, M, and ML groups, respectively. Cows supplemented with RPM alone (M group) or with RPL (ML group) produced ($P < 0·05$) more milk than those of the C group.

Discussion

Several studies have demonstrated that enhancing Met and Lys percentages in MP by using RPM and RPL improved lactation performance of dairy cows (Piepenbrink et al. 2004; Noftsger et al. 2005; Socha et al. 2005). However, improvements were variable and inconsistent (Robinson, 2010). In our study and consistent with others (Armentano et al. 1997; Socha et al. 2005; Třináctý et al. 2009), supplemental RPM alone did not improve milk yield or protein contents (Experiment. 1).

In the current study, RPM plus RPL improved milk protein contents (Experiment. 1) and milk yield (Experiment. 2). This is in agreement with previous studies (Robinson et al. 1995; Nichols et al. 1998; Wang et al. 2010). The increase in milk yield in response to supplemental RPL and/or RPM was most probably achieved by improvement in the efficiency of MP use for milk protein synthesis (NRC, 2001). Milk fat content was numerically greater for cows supplemented with RPL and/or RPM in the current experiment. This is in accordance with previous reports (Robinson et al. 1995; Wang et al. 2010).

It has been reported that improvements in milk yield in response to supplemental Met plus Lys were more than supplying each AA lone (Trináctý et al. 2009; Wang et al. 2010). In his review, Robinson (2010) concluded that Lys plus Met supplementation provided more benefits in lactation performance of cows than supplying each one alone. We observed that supplying RPM plus RPL numerically improved milk yield more than RPM alone. Inconsistent with our results, RPM plus RPL failed to improve milk yield of dairy cows (Trináctý et al. 2009; Mullins et al. 2013; Lee et al. 2015). Several factors can contribute to this inconsistency including different diets (barley *vs* corn based), basal Met and Lys percentages of MP and ratio, level and type of supplemental AA, level of milk production, etc.

In the light of Robinson et al. (1998) emphasis, post- rather than pre-experiment dietary evaluation should be considered to assess the response of cows to supplemental RPM and RPL. Evaluating our diets (NRC, 2001) using the actual milk yield and DMI revealed that the basal diet was marginally deficient (−275 g) in MP supply in experiment 1, but adequate (+303 g) in experiment 2. This suggests that when MP supply was deficient, supplemental RPL and/or RPM to improve Lys : Met ratio failed to improve milk yield (Experiment. 1). On the other hand, supplying both RPM plus RPL improved milk protein contents in the same experiment. This implies that Lys was more limiting than Met or that other AA (i.e., His) was co-limiting. It was suggested that His might be more limiting than Met or Lys when cows were fed grass-based diets with barley as the main energy source (Kim et al. 2000, 2001). This is supported by lower His contents of barley compared to corn (NRC, 2001). However, because RPM plus RPL improved milk protein, it is unlikely that His was co-limiting in the current experiment. Indeed, Lys was probably the first and Met the second limiting AA.

However, when MP supply was adequate, improving Lys : Met ratio by supplemental RPM alone or with RPL

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improved milk yield of cows (Experiment. 2) by improving the efficiency of MP use for milk protein synthesis. Supplemental RPM alone might have improved milk yield beyond its role as being a limiting AA as suggested by (Robinson et al. 1998). The fact that RPM plus RPL improved milk yield of cows not limited by MP supply might show benefits of supplying Met and Lys above the NRC (2001) requirements. Alternatively, MP supply might have been overestimated.

In the current study, plasma levels of Met and Lys did not simply respond to increases in supply from RPL and/or RPM. It is known that plasma levels of a given AA increases in response to supplementation only when supply exceeds requirements (Nichols et al. 1998). In experiment 1, MP supply was deficient and it is not expected to see increases in plasma levels of Met or Lys.

Conclusions

Under conditions of the current study, supplying RPM plus RPL to improve Lys : Met ratio improved milk yield and milk protein contents of dairy cows fed barely-based concentrate regardless of MP status. RPM improved milk yield only when MP supply was adequate. Lys was more limiting than Met, thus, supplying both RPM plus RPL was superior to RPM alone in improving lactation performance of cows. The relationship between MP status and response of dairy cows to RPL and/or RPM deserves further investigation.

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