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Effects of rumen-protected methionine and lysine on milk yield and milk composition in Holstein dairy cows consuming a corn grain and canola meal-based diet

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Abstract: Methionine (Met) and lysine (Lys) are thought to be the two most important amino acids for lactation performance. Met and Lys dietary supplementation can thus be an effective approach to improve amino acid balance for lactation performance, particularly in early lactation. The purpose of this study was to determine how rumen-protected (RP) Met + Lys affect milk production, milk composition, and feed efficiency in primiparous Holstein dairy cows fed a corn grain and canola meal-based diet for 60 days (8.5 weeks) following calving. Two hundred primiparous Holstein dairy cows were randomly assigned to one of two dietary treatments based on their expected calving date: 1) a basal diet; or 2) a basal diet supplemented with RP Met + Lys. Milk production and feed consumption were tracked on a daily basis, and milk components were tested once a week. During the trial, drinking water was always available in front of the dairy cows. The RP Met + Lys considerably increased ($p < 0.05$) milk yield (+ 2.20 kg/d), fat corrected milk yield (+ 2.18 kg/d) and feed efficiency but had no effect on dry matter intake ($p > 0.05$). There was no effect of treatment on either starting or final body weight ($p > 0.05$). Cows receiving RP Met + Lys, on the other hand, showed a tendency ($p = 0.09$) of reduced body condition score (BCS) losses between weeks 1 and 8. The RP Met + Lys increased ($p < 0.05$) the proportion of milk fat, but had no effect on the other milk composition markers ($p > 0.05$). Except for milk crude protein, the yields of fat, true protein, lactose, and energy in milk were higher ($p < 0.05$) in cows fed RP Met + Lys vs. control cows. The results indicate that supplementing with RP Met + Lys after the first calving has a considerable impact on subsequent milk and milk fat production, as well as the yield of the majority of milk nutrients.

Keywords: Lysine, methionine, milk production, milk nutrients, feed consumption, primiparous cows

1. Introduction

The efficient use of the ration protein depends on the formulation of rations that can optimally meet the metabolizable amino acids (i.e. absorbed from the intestine) in the correct proportions to meet the cow's requirement. When a single essential amino acid is limited, it is not possible to synthesize proteins from other absorbed amino acids [1]. Spears et al. [2] and Nadeau et al. [3] mentioned that the conversion efficiency of dietary nitrogen into milk protein in dairy cows is rather low, around 25%, with the remaining consumed dietary nitrogen lost through feces and urine. Although efficiency can be enhanced by lowering dietary crude protein, this method typically results in lower milk and milk protein outputs [4,5]. Methionine (Met) and lysine (Lys) are the primary limiting essential amino acids used to maximize the use of metabolizable protein (MP) for milk production [1]. To enhance the usage of MP for milk production, the first two limiting amino acids, Lys and Met, recommended to be included in a 3:1 ratio [1,6,7]. During the prepartum and postpartum periods, continuous feeding of diets supplemented with rumen-protected lysine (RP Lys) and methionine (RP Met) can improve milk production and milk protein content in transition cows [8–12]. More recently, continual RP Met feeding prepartum and postpartum dramatically enhanced milk protein yield in postpartum cows [13]. Furthermore, during the transition phase (3 weeks prenatal to 3 weeks postpartum), diets enriched with Lys and Met improved daily milk output by 0.68 kg/day and milk protein by 80 g/day during the first 16 weeks of lactation [14,15]. Before calving, the administration of amino acid-rich diets increased milk output by 2.27 kg/day, milk protein by 112 g/day, and milk fat by 115 g/day [7,14]. This suggests that dietary amino acid patterns should be evaluated further during early lactation.

Because soybean meal (SBM) is a protein supplement extensively used in dairy cow diets, the majority of the studies for amino acid supplementation was focused on diets based on soybean meal. Canola meal (CM), on

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the other hand, has become a viable alternative protein supplement [16,17] due to its excellent protein content and better availability [18]. Furthermore, when SBM or other protein supplements are replaced with CM in the diets of lactating dairy cows, there is an increase in milk yield [16,17] as well as a decrease in milk urea nitrogen concentration and urinary urea nitrogen (percent of total urinary N) excretion [16,19,20]. There is a knowledge gap in the production responses of canola meal fed dairy cows to enhanced RP Met + Lys nutrition. As far as we know, no studies of this trait in primiparous Holstein dairy cows during early lactation have been done. As a result, the purpose of this study was to determine the effect of supplementing canola-based diets with RP Met + Lys on lactation performance in early postpartum cows.

2. Material and methods

2.1. Animal care

The Institutional Animal Care and Use Committee (UÜHADYEK) (approval date: 17.04.2018; no: 2018-06/02) approved all procedures involving animals. No health problems were detected in the experiment and the cows remained free of disorders during the overall experimental period.

2.2. Animals, diets, and experimental design

The research was carried out with Holstein dairy cows raised on a commercial dairy farm, which operates with a capacity of 2500 cattle in the Marmara region of Karacabey district of Bursa. The animals were housed in modern shelter conditions with a free circulation system and a fan system with rubber bedding, which was frequently cleaned using manure scraper. Illumination was done by daylight during the day and artificial light sources at night with 24 h continued illumination. The water requirement of the animals was provided unlimitedly from automatic drinkers. The trial was carried out for 8.5 weeks from 15 August to 15 October.

A total of 200 Holstein cows in their first lactation (approximately 23–24 months of age) were employed in this study. The animals were divided into two groups at calving, depending on the date of calving, and two treatments were applied at random within each block starting from calving. The control group animals were fed a basal diet without Met + Lys, whereas the RP Met + Lys groups were fed a basal diet with additional Met (10 g/d; Novimet®, Belgium) and Lys (100 g/d; NoviLys®, Greece). Tables 1 and 2 show the ingredient composition of the grain feeds and TMR used in the study, respectively, while Table 3 provides the nutrient composition of the total mixed ration of ingredients. Cows were penned by treatment (two pens per treatment), group fed for ad-libitum intake three times a day. Cows were fed ad-libitum a total mixed ration (TMR) formulated to meet or exceed the predicted requirements [1] of energy, protein, minerals and vitamins primarily consisting of corn silage, grain silage and concentrate mix in a 47:53 forage:concentrate ratio (F:C; Table 1). The application rates of RP Lys and RP Met were calculated assuming that the optimal dLys and dMet at approximately 7.2% and 2.4% of MP [1], resulting in a dLys-to-dMet ratio close to 3.0 (Table 2). To calculate dry matter intake (DMI), feed refuels were recorded daily prior to morning feeding. Daily adjustments were made to

Table 1. Ingredient composition of grain mix.

Ingredient	Fresh grain mix, % (1–21 DIM)	Post fresh grain mix, % (22–60 DIM)
Corn, fine ground (0.06 mm)	35.30	36.51
Barley, ground	8.20	7.94
Wheat middling	4.10	6.35
Corn gluten feed, dehydrated	4.10	6.35
Corn gluten meal, 60% CP	4.10	6.35
Canola meal, solvent	24.59	19.05
Sunflower meal	8.20	6.35
Fractionated palm fatty acids (Bergafat F100)	2.87	4.44
Vitamin mineral premix Molasses, sugar beet	5.33 3.28	2.86 3.81

DIM, days in milk; CP, crude protein

Vitamin mineral premix composition, %: marble powder, 36.90; sodium bicarbonate, 25.00; salt, 14.64; potassium carbonate, 10.71; magnesium oxide, 4.14; wheat bran, 3.12; toxin binder, 1.43; yeast, 1.43; zinc + copper + manganese, 1.43; organic selenium, 0.43; manganese sulfate, 0.34; vitamin E, 0.21; biotin, 0.14; copper sulfate, 0.04; vitamin A, 0.02; selenium, 0.01; vitamin D, 0.01.

Table 2. The total mixed ration composition (kg/day, as fed)

	Fresh (1–21 DIM)	Post fresh (22–60 DIM)
Wheat silage	7.00	5.00
Corn silage	10.00	15.00
Alfalfa hay, 15% CP	3.75	5.00
Fresh grain mix	12.20	-
Post fresh grain mix	-	15.75
Cottonseed whole	1.00	1.50
Water	5.00	2.00
Total	38.95	44.25
Nutrient composition (% DM basis)		
DM	52.50	58.80
NEL, Mcal/kg	1.60	1.71
CP	18.18	18.00
ADF	19.60	18.70
NDF	32.71	32.00
NFC	35.51	36.76
RDP	66.38	64.24
RUP	33.62	35.80
Fat	2.00	5.40
TDN	72.49	73.71
Methionine, % of MP	0.22	2.03
Lysine, % of MP	0.72	6.00
Met: Lys in MP	3.3:1	2.96:1
Ca	0.79	0.78
P	0.46	0.44
Se	0.68	0.68

DIM, days in milk; CP, crude protein; DM, dry matter; NEL, net energy lactation; ADF, acid detergent fiber; NDF, neutral detergent fiber; RDP, rumen degradable protein; RUP, rumen undegradable protein; NFC, nonfiber carbohydrate, TDN, total digestible nutrients; Met; methionine; MP, metabolizable protein; Lys, lysine, Ca; calcium, P; phosphorus, Se; selenium.

the feed offered in order to achieve 5% to 10% orts. Cows were milked thrice a day in a double twelve parallel milking parlor and milk yield was recorded daily. Milk samples were taken from the morning milking weekly and bulk samples from each cow were analyzed for fat, protein, lactose and somatic cell count (SCC) with MilkoScan FT120 (Foss, Denmark). The body weight of cows was recorded on the 3rd and 4th day after calving, after the afternoon milking, and the final body weight was recorded on the final day of the experiment. All cows were individually scored for body condition after calving on day 3 and at the end of the experimental period on d 60 by 2 trained professionals (1–5 scale) [1].

2.3. Chemical analysis

Feed samples were collected on a daily basis when meals were weighed and pooled to generate a sample for analysis. The dry matter (DM) concentration was determined by drying at 100 °C for 24 h, and the crude protein (CP) was determined by micro-Kjeldahl analysis [21]. The ash content was determined by burning at 550 °C for 8 h [21]. The fat was evaluated using the Randall method, which included submersion in petroleum ether [21]. Neutral detergent fiber (NDF) was evaluated with sodium sulfite and amylase, and acid detergent fiber (ADF) was analyzed with an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). The nonfiber carbohydrate (NFC) fraction was calculated as NFC = 100-(CP + FAT + ASH + NDF). The NRC [1] was used to predict the values of net energy lactation (NE_L), rumen degradable protein (RDP), RUP, MP, Met, and Lys content for the diets.

2.4. Calculations

Feed efficiency was evaluated by dividing the kg of milk produced by the kg of DM consumed. Milk energy (MJ/kg) was calculated according to [22] as gross energy value (kcal/kg of milk); E = (40.72 (% F) + 22.65 (% TP) + 102.77) * 2.204 * 4.185

Symbols in equations represent the following: E: gross energy value (kcal/kg of milk); % F: % of fat; % TP: % of true protein; 4.185 converts Mcal to MJ and 2.204 converts Mcal/lb to Mcal/kg.

Milk energy output (MJ/d) was calculated: Milk energy (MJ/kg) × milk yield (kg/d). The crude protein of milk was found by multiplying the true protein of milk by a coefficient of 0.934 [22,23]. According to Tyrrell and Reid 4% fat corrected milk yield (FCM) was calculated [22].

2.5. Statistical analysis

For analysis, daily measurements (daily milk yield and feed intake) were transformed to weekly averages. Any week with fewer than four daily values per animal was excluded from the data set and regarded as a missing data point. SAS (SAS Institute Inc., Cary, NC) PROC MIXED technique was used to analyze production data, with week as a repeated measure and an autoregressive covariance structure. Single measurements (milk components, body weight, BCS) were analyzed using the PROC MIXED technique (SAS Institute Inc., Cary, NC) with day as a repeated measure and an autoregressive covariance structure. Somatic cell counts were logarithmically transformed for the statistical analysis. The data provided at least squares means standard error means (SEM), and differences between means were considered significant at $p < 0.05$, whereas trends were examined at $p < 0.10$, unless otherwise noted.

Table 3. Composition of nutrients in total mixed ration ingredients.

Nutrients, % of DM	Wheat silage	Corn silage	Alfalfa hay	Cottonseed whole	Fresh grain mix	Post fresh grain mix
DM	30.00	30.00	90.00	89.00	90.60	90.10
NEL, Mcal/kg	1.16	1.53	1.23	1.88	1.83	2.05
CP	12.50	8.50	15.00	23.00	22.50	22.00
ADF	36.00	23.00	37.00	32.50	9.20	8.50
NDF	58.00	44.00	50.00	44.60	18.60	18.50
RDP	78.00	70.00	72.00	60.00	64.17	61.46
RUP	22.00	30.00	28.00	40.00	35.80	38.50
NFC	19.50	39.50	24.10	7.60	43.30	46.60
Fat	2.50	3.00	2.00	20.00	6.00	6.20
TDN	57.97	70.69	56.01	81.15	80.05	85.42
Met	0.15	0.13	0.22	0.37	0.45	0.47
Lys	0.37	0.21	0.72	0.96	0.89	0.84
Ca	0.27	0.23	1.25	0.21	0.95	0.92
P	0.27	0.22	0.22	0.64	0.61	0.59
Se	0.00	0.04	0.29	0.00	1.17	1.13

DM, dry matter; NEL, net energy lactation; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; RDP, rumen degradable protein; RUP, rumen undegradable protein; NFC, nonfiber carbohydrate, TDN, total digestible nutrients; Met; methionine; Lys, lysine, Ca; calcium, P; phosphorus, Se; selenium.

3. Results and discussion

The effects of first-limiting amino acids on the lactation performance of primiparous Holstein dairy cows were investigated using two grain mixes based on ground corn and canola meal (Table 1). All diets were similar in protein, protein fractions, NDF, and net energy lactation (NE_L) (Table 2). Wheat silage, corn silage, alfalfa hay, whole cotton seed, and grain mixes were among the TMR ingredients (Table 3).

During the trial, there was no treatment effect on either the starting or ultimate body weight ($p > 0.05$, Table 4). Cows given RP Met + Lys, on the other hand, demonstrated a trend ($p = 0.09$, Table 4) of mitigating BCS losses between weeks 1 and 8. Rumen-protected Met + Lys supplementation may compensate cows for losing more BCS early in lactation [24]. Overall, cows fed RP Met + Lys produced + 2.2 kg/day more milk than control cows ($p < 0.05$, Table 4, Figure 1). Cows fed the RP Met + Lys diet had higher ($p < 0.05$) fat corrected milk (FCM) yield (30.78 kg/d) than control cows (28.60 kg/d, Table 4). The peak milk yield in the control group was 35.2 kg/day, while the peak milk yield in the RP Met + Lys group was 38.7 kg/day ($p < 0.05$, Figure 1). The days when the cows in the control and RP Met + Lys groups reached peak milk yield were determined to be the 46th and 48th days of lactation, respectively ($p < 0.05$, Figure 1). Cows administered RP Met + Lys consumed more dry matter during weeks 1–3, but DMI did not differ across treatments during weeks 4–8 ($p > 0.05$, Figure 2). During weeks 1–3, the treatments had no influence on feed efficiency ($p > 0.05$, Figure 3); nevertheless, it was shown to be higher in the supplemented group during weeks 4–8 ($p < 0.05$, Figure 3). Overall, feed efficiency improved as milk yield increased without a substantial change in consumption in the supplementary group (Milk yield/DMI, 1.42 vs. 1.38, $p < 0.05$, Table 4, Figure 3). The effects of RP Met + Lys supplementation on DMI and feed efficiency were found to be dependent on stage of lactation (Treatment × week interaction; $p < 0.05$; Figures 2 and 3, respectively). Some of the observed increase in milk production in this study could be linked to the mild increase in DMI in the RP Met + Lys supplemented cows. Cows have negative energy and protein balances after parturition because nutrient demands for milk production exceed food intake [25]. This results in a lot of nutrients (fatty acids and amino acids) being mobilized to enhance milk production during early lactation. Nutritional control during the transition phase is critical since excessive mobilization can create a variety of health problems following parturition, limiting milk production. Amino acid supplementation to reduce excessive amino acid mobilization after parturition can be an approach to mitigate this challenge. Osorio et al. [26] discovered that cows fed two different forms of methionine produced +3.1 kg/day and +4.4 kg/day more milk than

Table 4. Effects of ruminally protected methionine and lysine (RP Met+Lys) on feed intake, milk production and milk composition in Holstein cows.

Parameters	Control	RP Met+Lys	SEM	P-value
n = 200 cows				
Initial BW,kg	570	550	20.9	0.64
Final BW, kg	540	530	13.7	0.96
BCS week 1	3.50	3.25	0.04	0.79
BCS week 8.5	2.50	2.50	0.08	0.49
Change in BCS	–1.00	–0.75	0.02	0.09
DMI, kg/d	21.83	22.29	0.14	0.40
Yield				
Milk yield, kg/d	31.00	33.20	0.23	<0.01
4% FCM yield, kg/d	28.60	30.78	1.81	<0.01
Feed efficiency, kg/kg	1.34	1.42	0.15	<0.01
Fat yield, kg/d	1.17	1.33	0.09	<0.01
True protein yield, kg/d	0.97	1.14	0.07	<0.01
Crude protein yield, kg/d	1.04	1.12	0.081	0.42
Lactose yield, kg/d	1.49	1.66	0.08	<0.01
Energy, MJ/d	91.51	104.63	1.26	<0.01
Composition				
Total solids, %	8.61	8.63	0.04	0.16
Fat, %	3.77	3.87	0.02	<0.01
True protein, %	3.14	3.15	0.02	0.53
Crude protein, %	3.36	3.37	0.07	0.81
Lactose, %	4.85	4.88	0.03	0.57
Energy, MJ/kg	3.01	3.05	0.015	0.21
SCC, (x10^3/mL)	400	360	50	0.39

BW, body weight; BCS, body condition score; change in BCS, difference between the final and initial body condition scores (week 8–week 1); DMI, dry matter intake; FCM, fat corrected milk yield; feed efficiency, milk yield/DMI; SCC, somatic cell count.

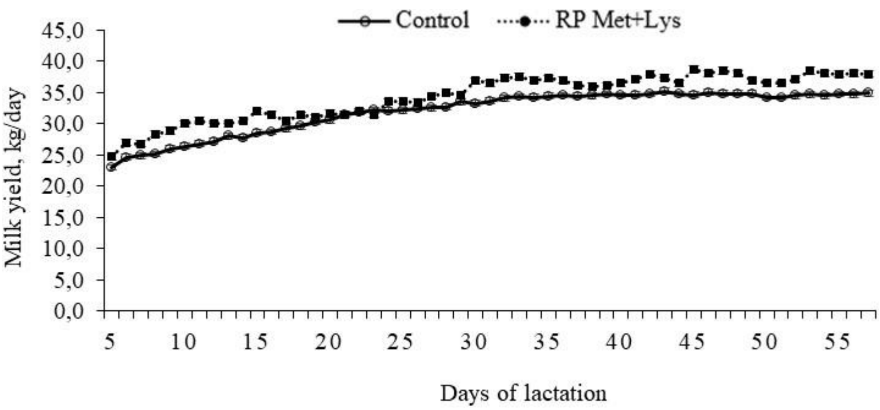

Figure 1. Effects of rumen-protected methionine and lysine (RP Met+Lys) (10/100) on milk yield in Holstein cows during early lactation (n = 200). The error bars represent the standard error of the mean.

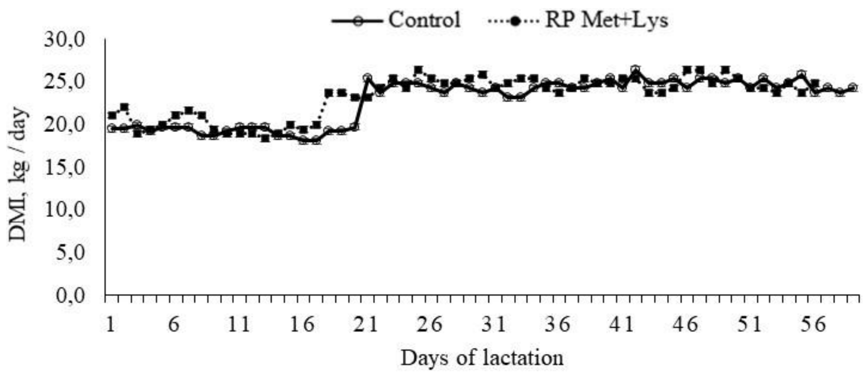

Figure 2. Effects of rumen-protected methionine and lysine (RP Met+Lys) (10/100) on dry matter intake (DMI) in Holstein cows during early lactation (n = 200). The error bars represent the standard error of the mean.

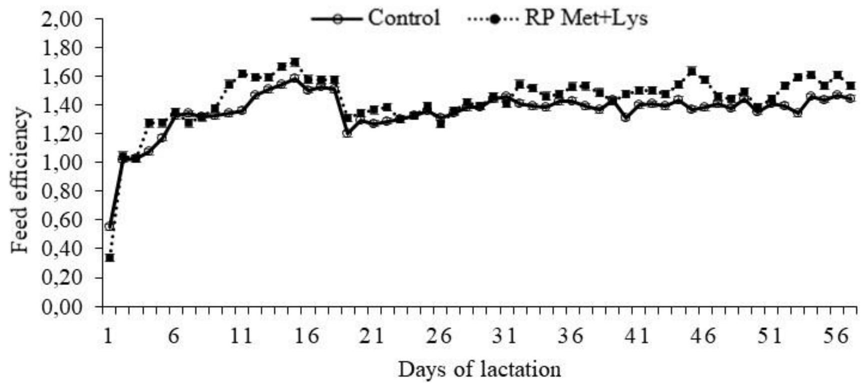

Figure 3. Effects of rumen-protected methionine and lysine (RP Met+Lys) (10/100) on feed efficiency in Holstein cows during early lactation (n = 200). The error bars represent the standard error of the mean.

the control group during the first 30 days of lactation. According to the researchers, the amino acid supplement increases growth hormone concentration after delivery and improves the immune system in dairy cows during the transition period. In another study [27], 60 Holstein dairy cows with an average milk yield of 32 kg/day were fed RP Lys or Met (0.15% dry matter) or a combination of the two for 8 weeks. At the end of the experiment, milk yield in the Lys group was +1.5 kg/day, while milk yield in the Met group was + 2.0 kg/day [27]. This implies that supplementary RP Met + Lys can enhance milk production when MP supply is adequate [27,28]. Other studies [11,29,30] have also shown that using RP amino acids during the transition phase increases milk production in dairy cows. The amino acid supply from microbial protein alone cannot cover the requirement for milk production in high-producing dairy cows [31], and these cows should be supplemented with protein sources high in rumen undegradable protein (RUP). According to NRC [1], for maximum milk yield, Lys and Met proportions in MP should be at 7.2% and 2.4%, respectively (ideal Lys: Met ratio of 3:1). Rumen protected Met + Lys supplementation may raise arterial concentrations of Lys, Met, and total amino acids, hence improving amino acid delivery to the mammary gland for milk production [27]. Awawdeh [28] reported that feeding RP Met + Lys to dairy cows increased fat-corrected milk production to +4.5 kg/day. Other studies [8,24] discovered that supplementing the diet with RP Met enhanced fat-corrected milk production by +1.2– 4.6 kg/day which was similar to our findings. In contrast to our findings, in some studies, addition of RP Met did not influence fat-corrected milk production [30,32,33]. Robinson [34], Třináctý et al. [10] and Wang et al. [27] stated that Lys plus Met supplementation gave higher improvements in lactation performance of cows than either one alone.

Except for milk fat, there was no effect of RP Met + Lys supplementation on total solids, true protein, crude protein, lactose, energy, or SCC content in milk ($p > 0.05$, Table 4). Milk fat content was found to be higher in cows fed with the RP Met + Lys diet than in control cows (3.87

% vs 3.77 %, $p < 0.05$, Table 4). Socha et al. [8] found that feeding RP Met + Lys to cows shortly after calving in diets containing 18.5 % CP enhanced milk fat content. The findings in this investigation were likewise consistent with those found in cows fed RP Met with or without Lys [5,27]. According to Ordway et al. [35] and Osorio et al. [26], peripartum methionine supplementation increased milk fat yield. Methionine has been found in vitro to stimulate microbial growth, cellulose digestion, and microbial fatty acid production, all of which appear to be particular polar lipids [36,37]. Adding various types of RP Lys or Met to dairy cow diets has been investigated in a number of studies; milk fat content [8,9] and milk protein content [8–12] have all been reported responsively. In our study, lack response in milk protein when RP Met + Lys was used may indicate that the protein sources were similarly utilized [27,37]. Why an additional supply of amino acids did not alter milk protein content is not clear and needs further investigation. On the other hand, variations in animal lactation stage, amount of supplementary Lys or Met, proportions of other amino acids in MP, and experimental design could all contribute to these variances.

In the current study, milk fat, protein, lactose and energy yield increased as milk production increased in RP Met + Lys-fed cows ($p < 0.05$, Table 4, Figures 4–6). Increased protein availability would improve milk yield as well as protein yield [10–13,38] and lactose synthesis [12,24,39]. In contrast to earlier results, the increased yield of milk protein in the current experiment was mostly due to an increase in milk production rather than an increase in milk protein content [8,11]. Previous findings also observed that feeding RP Lys and RP Met to dairy cows during the periparturient phase increased milk nutrients yield [26,30,38].

4. Conclusion

The results of this experiment, which was carried out under farm-like but controlled conditions, revealed a clear and significant increase in milk production (+2.20 kg/d) and fat corrected milk production (+2.18 kg/d) when RP Met + Lys was included in the canola meal based diet. Rumen-protected Met + Lys may improve feed efficiency by increasing milk

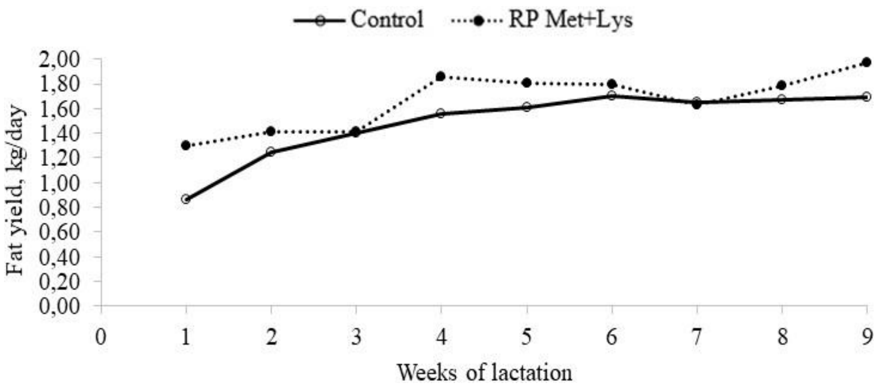

Figure 4. Effects of rumen-protected methionine and lysine (RP Met+Lys) (10/100) on milk fat yield (kg/day) in Holstein cows during early lactation (n = 200).

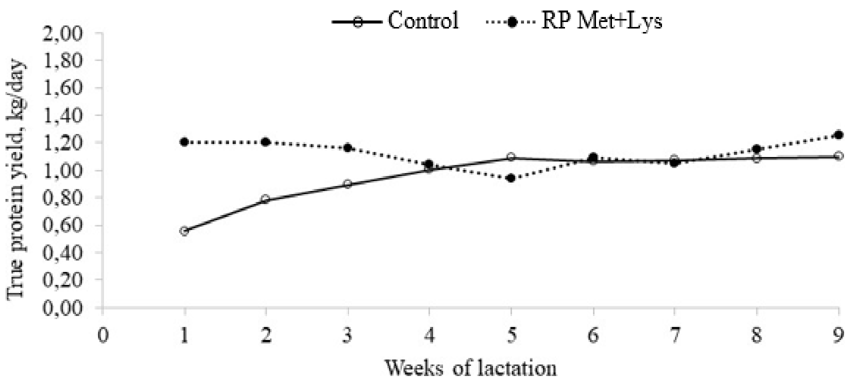

Figure 5. Effects of rumen-protected methionine and lysine (RP Met+Lys) (10/100) on milk true protein yield (kg/day) in Holstein cows during early lactation (n = 200).

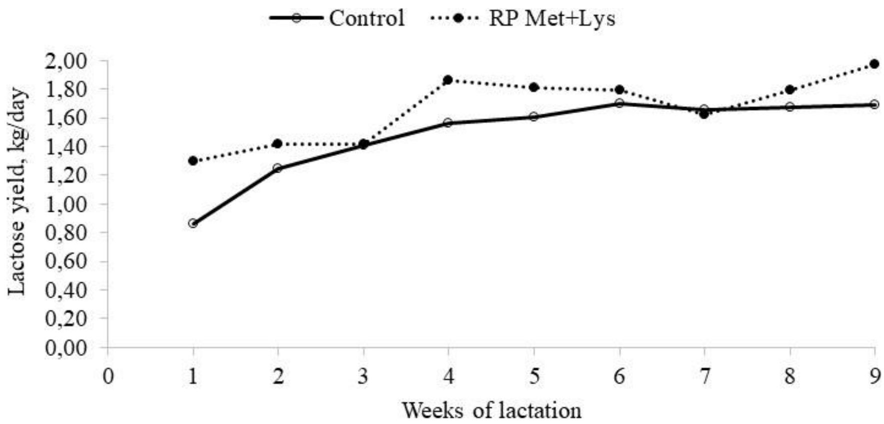

Figure 6. Effects of rumen-protected methionine and lysine (RP Met+Lys) (10/100) on milk lactose yield (kg/day) in Holstein cows during early lactation (n = 200).

production while having no effect on DM consumption. These findings show that RP Met + Lys fed cows were able to manage body reserves more efficiently during lactation, as they lost less BW in early lactation. Rumen-protected Met + Lys supplementation enhanced milk component yields especially milk protein yield. This suggests that the increase in volume of milk is important to increase milk protein yield. More research is needed to evaluate amino acid supplementation with different combinations and amounts in different dietary regimes. This is especially significant for animals fed total mixed diets, since the feed constituents change at a faster rate.

Conflict of interest

Regarding the material presented in the manuscript, we assure that there is no conflict of interest with any financial entity.

This study is a part of the author's MSc thesis.

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