Original study

Effect of protected methionine supplementation on milk production and reproduction in first calf heifers

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Abstract

Sixty Holstein heifers (30 days in milk) were used to examine the effects of protected methionine on milk production, milk composition and reproduction performance up to 100 days *post-partum*. Heifers were randomly divided into two dietary treatment groups (30 each) receiving either 14 or 16 % crude protein. Heifers of each treatment group were assigned into three subgroups with a daily supplementation of 0, 15 or 25 g/hd/d of rumen protected methionine. Diet fed was a total mixed ration consisting of fresh alfalfa, corn silage and a concentrate mixture. In addition, alfalfa hay was offered *ad libitum* during the whole experimental period.

Supplementation of the 14 % crude protein diet with 25 g/hd/d rumen protected methionine increased ($P<0.05$) milk yield, fat corrected milk, energy corrected milk, percentages of milk fat, total solids and casein as well as yields of milk fat and protein compared to those without or with 15 g/hd/d rumen protected methionine. Dry matter intake, feed to fat corrected milk ratio, body weight and body condition score were also improved ($P<0.05$) with the supplementation of rumen protected methionine at the same level compared to the other levels. Meanwhile, milk protein percentage and feed to milk ratio were unaffected. At 16 % crude protein, milk yield, fat corrected milk, energy corrected milk, fat %, yields of fat and protein as well as feed:milk ratio and feed :fat corrected milk ratio were all increased ($P<0.05$) with rumen protected methionine supplementation regardless of its level. However, milk content of protein and total solids were unaffected. Casein was only increased ($P<0.05$) when rumen protected methionine was supplemented to this diet at 15 g/hd/d. Dry matter intake, final body weight and body condition score were increased ($P<0.05$) when rumen protected methionine was supplemented at 25 g/hd/d. Reproductive performance measurements were unaffected

by rumen protected methionine supplementation to both diets. These results indicate that supplementation of rumen protected methionine would improve milk production performance at both dietary protein levels with no effect on reproductive performance.

Keywords: heifers, protected methionine, milk production, reproductive performance

Abbreviations: CP: crude protein, ECM: energy corrected milk, FCM: fat corrected milk, RPMet: rumen protected methionine

Introduction

Compared to milk protein or the amino acid profile of ruminal microbes, feeds are often deficient in lysine and methionine contents (NRC 2001). The protein which reaches the small intestine is adequate in cystine but deficient in methionine, making it the first limiting amino acid for high yielding dairy cows (Lara *et al.* 2006). Rumen microbial protein synthesis cannot supply sufficient quantities of amino acids to meet the requirements of cows producing large quantities of milk (Polan *et al.* 1991, Ali *et al.* 2009). Therefore, ruminally undegradable protein must be provided in the diet to maximize the amount of amino acids available for absorption in the intestine (Samuelson *et al.* 2001, Leonardi *et al.* 2003).

Many of the studies that evaluated production responses of dairy cattle to protein supplementation have not considered the lysine and methionine content across treatments but have used diets formulated for crude protein (CP) or ruminally undegradable protein content only (NRC 2001). In such studies, it was not clear whether production responses were the effect of ruminally undegradable protein content or amino acid supply. Further studies are still needed to consider amino acid supply in order to determine the causes of responses to changes in protein feeding. At the same time, Bobe (1997) and (Trinacty *et al.* 2009) revealed that high CP intake, in particular high intake of methionine, in early lactation is necessary for stimulating maximum milk production.

Many researchers reported inconsistent results concerning the effect of rumen protected methionine (RPMet) supplementation on milk yield. Numerous studies reported no increase in milk yield when RPMet was supplemented to dairy cows (Overton *et al.* 1998, Socha *et al.* 2005, Ali *et al.* 2009). Other studies did not show any response in milk protein percentage or yield with the addition of RPMet (Polan *et al.* 1991, Overton *et al.* 1998, Socha *et al.* 2005, Ali *et al.* 2009).

Milk fat percentage has been reported to be increased when RPMet was supplemented (Samuelson *et al.* 2001, Trinacty *et al.* 2009). Lara *et al.* (2006) showed no effects on fat content of the milk when dairy cows received RPMet supplementation.

A limited number of studies discussed the effects of methionine supplementation on reproductive performance. Ardalan *et al.* (2010) reported that the reproductive performance of cows fed methionine hydroxy analog was improved. Dietary supplementation with RPMet in the first 90 days of lactation did not affect the time to first observed oestrus, days to first service, services per conception, calving interval or days of retained placenta and metritis (Rogers *et al.* 1989, Polan 1991, Ardalan *et al.*, 2010). However, the same methionine source increased the days to first conception, services per conception, incidences of cysts and did not affect reproductive cycling of dairy cows (Bobe 1997, Ardalan *et al.* 2010).

Most of the above mentioned studies were obtained with adult dairy cows and none were with first calf heifers. Heifers have higher CP requirements to sustain growth requirements (NRC 2001). Therefore, the objective of this study was to investigate the effects of RPMet supplementation on early lactation performance and reproductive performance of first calf dairy heifers from day 30 to day 100 *post-partum*.

Material and methods

Dietary treatments and animal care

Sixty Holstein Friesian first calf heifers were randomly assigned into two dietary treatment groups (14 and 16 % CP) with 30 cows in each group. Each treatment group was randomly divided into three subgroups of equal numbers of animals. The subgroups were supplemented with 0, 15 or 25 g/hd/d of RPMet (MET55; DL-Methionine 55 %, hydrogenated vegetable oil 45 %; JEFO Nutrition Inc., St-Hyacinthe, Québec, Canada). Diets were balanced according to the NRC (2001) requirements.

The experiment started on day 30±3 *post-partum* and continued up to 100 days *post-partum*. The average body weight of heifers at the beginning of experiments was 453±4 kg and after 100 days it was 466±4 kg. Body condition score was measured on a five-point scale (1=emaciated to 5=obese) (Edmonson *et al.* 1989).

Treatment diets were offered as a total mixed ration. Diets were based on corn silage, alfalfa hay, 44 % soybean, wheat bran, barley grain and corn grain. Ingredient per cent composition of the experimental diets is listed in Table (1) and chemical analysis of these rations is listed in Table (2). Cows were fed at the level of 19 kg dry matter/hd/d of the total mixed ration. In addition, alfalfa hay was offered *ad libitum* during the whole experimental period and average individual consumption was estimated. Daily intake of the hay was recorded on group basis. Feed was offered individually after milking three times daily with free access to water. The methionine supplement for each group was mixed with the total mixed ration at mixing time and diets were prepared every day. Samples for analysis were taken after each mixing and kept frozen until time of analysis. All cows were handled and housed in free stall shadow barns.

Table 1
Ingredient composition of the experimental diets

Ingredients	14 % CP	16 % CP
Barley	28	25
Soybean meal, 44 %	8	13.6
Corn grain	13	13.6
Wheat bran	13	13.6
Fresh alfalfa	10	8
Corn silage	24.73	22.93
Wheat straw	3	3
Salt	0.1	0.1
Sodium carbonate	0.05	0.05
Limestone	0.1	0.1
Mineral and vitamin mix*	0.02	0.02

*Ruminant Premix (Nutristar International, Cergy-Pontoise, France) 1 kg contains: Vitamin A 5 000 000 IU, Vitamin D3 1 000 000 IU, Vitamin E 10 000 µg, Choline Chlorides 4 000 µg, Manganese 25 000 µg, Copper 2 000 µg, Zinc 10 000 µg, Selenium 0.200 µg, Cobalt 0.250 µg, Iron 25 000 µg, Iodine 0.5000 µg, Phosphorus 15 000 µg, Antioxidant 11 200 µg

Table 2
Chemical composition of the experimental diets

Chemical composition	14 % CP	16 % CP	Alfalfa hay	Corn silage
Dry matter, %	68	70	89	37
Crude protein, %	14	16	13.8	8
Neutral detergent fibre, %	36.6	37	43	49
Acid detergent fibre, %	19.6	20	32	31
Ash, %	6	4.3	7	4.5
Net energy of lactation, Mcal/kg	1.6	1.7	1.22	1.5

Cows were milked three times daily at 8-h intervals using a complete computerized milking system (Herringbone parlour 2x12; Delaval, Tumba, Sweden). Milk production of each cow was recorded after each milking time.

On day 30±3 each heifer was rectally palpated to ensure uterus and ovaries were normal. Hormonal protocol was applied to all cows to synchronize oestrus starting on day 45±3 *post-partum* with a 25 mg intramuscular injection of prostaglandin (PGF$_{2\alpha}$; Lutalyse, Pfizer, Belgium). Heifers that did not show oestrus after the first PGF$_{2\alpha}$ injection received another one on day 57±3. One heifer of each subgroup was excluded from the synchronization protocol due to reproductive disorders.

Oestrus was determined by visually monitoring the heifers throughout the day. Heifers that returned oestrus after the first artificial insemination were re-inseminated, while those found non-pregnant on day 50 post artificial insemination were injected with PGF$_{2\alpha}$ and inseminated after observed oestrus. Heifers were bred from a single ejaculate of a single Holstein sire (Lyster, Semex, Canada). Insemination was conducted approximately 12 h after heifers were observed in standing heat. A single inseminator was responsible for breeding all heifers in the study. Pregnancies were determined by rectal palpation on day 50 post artificial insemination. Observation was continued beyond the end of the experimental period to monitor the pregnancy results up to the third insemination. Reproductive data were recorded for days to first, second and third insemination and pregnancy rates.

Sample collection and analytical procedures

During the experimental period, milk was sampled weekly from each cow in the experiment and analysed for percentage of milk fat, protein and total solids (AOAC 1995). Fat was determined using the Gerber method, while protein content of both milk and feed was determined by Kjeldahl method (N×6.38). Casein was determined according to AOAC (1995).

Feed samples were dried at 105 °C overnight. Ash was determined by burning sample in a muffle furnace at 650 °C. Neutral detergent fibre and acid detergent fibre were determined following the procedure of Van Soest *et al.* (1991).

Energy corrected milk was determined following the equation of AbuGhazaleh *et al.* (2002):

$$ECM = (0.3246 \times milk\ yield) + (12.86 \times fat\ yield) + (7.04 \times protein\ yield) \tag{1}$$

Fat corrected milk (4 %) was determined using the equation provided by the NRC (2001) as follows:

$$4\%\ FCM = (0.4 \times milk\ yield) + (15 \times fat\ yield) \tag{2}$$

Statistical analysis

The effects of supplementing RPMet to the diet on total dry matter intake, milk yield and milk composition were analysed as a repeated measure analysis using the MIXED procedure of SAS v7 (SAS Institute Inc., Cary, NC, USA). Individual animal (heifer) was considered as the experimental unit for data analysis. Data of production response collected in the pre-experimental period were used as a covariate for statistical model that included CP level (14 % and 16 %), methionine level (0, 15 and 25 g/hd/d) and period effect. For reproductive performance, the statistical model was protein and methionine levels and their interactions. Also, Chi square test was applied for pregnancy test data. All data are presented as least squares means and separated using the protected least significant difference tested at $P<0.05$ (Steel & Torrie 1986).

Results and discussion

Milk yield and composition

Milk production during the first 30-d period was used as a covariate during analysis. Heifers fed the 14 % CP diet had maximum ($P<0.05$) milk production and fat corrected milk (4 % FCM) at level of 25 g/hd/d RPMet supplementation (Table 3). When fed the 16 % CP diet, both parameters increased ($P<0.05$) linearly as RPMet in the diet increased. Milk production was analysed according to different periods of the experiment: 5-8 week (time of expected peak lactation) and 9-14 week (remaining period of the study). During both stages of the experiment, maximum ($P<0.05$) daily milk production in heifers fed the 14 % CP diet was found with 25 g/hd/d RPMet while in those fed the 16 % CP diet, average daily milk production increased ($P<0.05$) linearly with RPMet level in the diet.

Table 3
Average milk production and composition of heifers fed deferent levels of protected methionine in their diets

Diets	14 % CP			16 % CP				
Methionine g/d	0	15	25	0	15	25	SE	*P*
Milk yield								
Total (5-14 week)	21.12[b]	21.30[b]	22.62[a]	22.84[c]	24.75[b]	25.97[a]	0.154	0.001
5-8 week	21.59[b]	22.21[b]	23.29[a]	23.17[c]	25.61[b]	27.04[a]	0.040	0.001
9-14 week	20.65[b]	20.39[b]	21.95[a]	22.51[c]	23.89[b]	24.90[a]	0.092	0.001
4 % FCM[2]	19.24[b]	19.62[b]	21.27[a]	21.07[c]	23.10[b]	24.19[a]	0.162	0.001
Fat, %	3.42[c]	3.49[b]	3.61[a]	3.48[b]	3.54[a]	3.55[a]	0.016	0.001
Fat, kg/d	0.72[c]	0.74[b]	0.81[a]	0.79[c]	0.88[b]	0.92[a]	0.007	0.001
Protein, %	3.10	3.13	3.10	3.04	3.03	3.08	0.023	ns
Protein, kg/d	0.65[b]	0.66[b]	0.70[a]	0.69[c]	0.75[b]	0.80[a]	0.007	0.001
Casein, %[1]	2.45[b]	2.50[b]	2.57[a]	2.36[b]	2.40[a]	2.35[b]	0.011	0.001
Total Solids, %	11.45[b]	11.61[ab]	11.79[a]	11.61	11.71	11.77	0.013	0.481
ECM[3]	20.70[c]	21.07[b]	22.68[a]	22.43[c]	24.63[b]	25.89[a]	0.173	0.001

[a,b,c]Means with different superscripts within the same row are different ($P<0.05$), [1]Casein as % of milk composition, [2]4 % FCM=(0.4×kg milk)+(15×fat kg), (NRC, 1989), [3]ECM=(0.3246×milk yield)+(12.86×fat yield)+(7.04×protein yield), (AbuGhazaleh *et al.*, 2002)

Interaction of treatment × week on milk production is illustrated in Figures 1 and 2. Although no differences were detected among heifers fed the 14 % CP diet (Figure 1), those supplemented with 25 g/hd/d RPMet were able to maintain higher and stable production during the first 4 weeks before starting to decline. Other heifers on the same diet exhibited declined milk production starting at week 2 or 3 of the experiment. In Figure 2, heifers fed 16 % CP diet and 25 g/hd/d RPMet showed higher ($P<0.05$) milk production during the first 4 weeks of the experiment. However, persistency of milk production was maintained for the first 4 weeks in heifers that received 25 and 15 g/hd/d RPMet but only for the first 3 weeks in the control heifers.
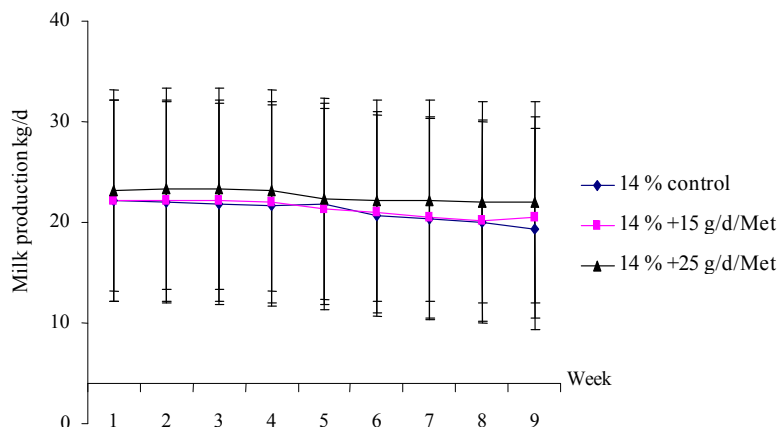

Figure 1
Effect of different levels of RPMet on average weekly milk yield of heifers fed a 14 % CP diet. Data points represent weekly least squares means ± SEM for 14 % CP dietary treatments
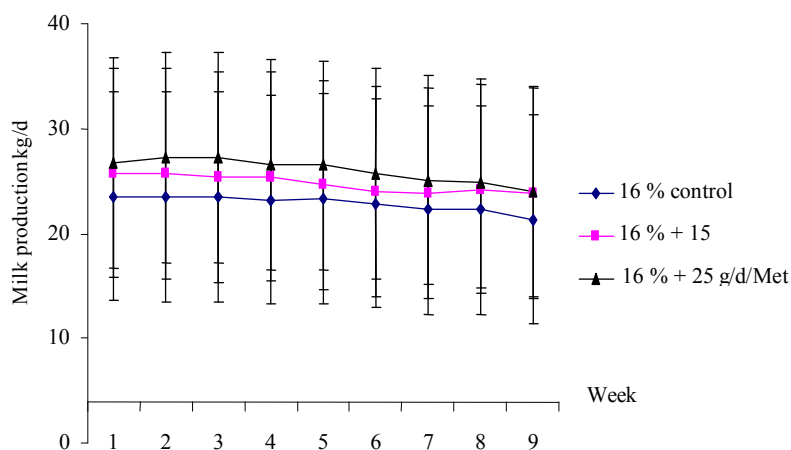

Figure 2
Effect of different levels of RPMet on average weekly milk yield of heifers fed a 16 % CP diet. Data points represent weekly least squares means ± SEM for 16 % CP dietary treatments

Production response of lactating dairy cows to supplemental RPMet is often controversial. The found increase in milk yield following RPMet supplementation to both diets agreed with Lara *et al.* (2006) and Kudrna *et al.* (2009). Moreover, Dinn *et al.* (1998) showed that feeding diets with 15 % CP supplemented with RPMet supported milk production as much as feeding 18 % CP without addition of amino acid. The positive response in milk production to RPMet would possibly indicate that methionine was mostly a limiting factor to production. Similar results indicated that methionine was a limiting amino acid for high milk production in dairy cows fed corn and soybean meal during early lactation (Samuelson *et al.* 2001, Lara *et al.* 2006, Ali *et al.* 2009, Benefield *et al.* 2009). The increased milk production of the 16 % CP group confirms the dose-dependent response to supplemental methionine (Lara *et al.* 2006, Benefield *et al.* 2009).

In contrast, milk yield of dairy cows supplemented with RPMet was either decreased (Benefield *et al.* 2009), not changed or only minor effects were reported (Noftsger *et al.* 2005, Kudrna *et al.* 2009, Trinacty *et al.* 2009). Girard *et al.* (2005) reported that in a non-exhaustive review between 1993 and 2003, only 1 of 22 studies looking for the effect of RPMet to dairy cows showed increased milk production following supplementation. Differences in milk yield among experiments might be attributed to the degree to which methionine was limiting in the diets fed, an excess of amino acids, an imbalance in amino acids, increased intake of compounds used to protect the amino acids from ruminal degradation or other factors (Lara *et al.* 2006).

In the present case, the studied herd consisted of first calf heifers that have higher protein requirements than adult cows (NRC 2001). This might explain the positive response to RPMet supplementation when heifers were fed either diet (14 % or 16 % CP) supplemented with 25 g/d RPMet, or with 15 g/d only when fed the 16 % CP diet. Feeding diets with 14 % CP to growing heifers might be not adequate and limits the response to the 25 g/d RPMet level that compensated partially the limited protein content of the diet. However, the 16 % CP diet seemed to be somehow nearly adequate and might had enabled heifers to present increased milk production with increased level of supplementation. However, Leonardi *et al.* (2003) found that RPMet can improve milk protein content to the same extent regardless of whether a diet with 14 % or 18 % CP was fed.

Milk fat content of heifers fed a 14 % CP diet was increased (*P*<0.05) with level of RPMet supplementation (Table 3), while it was higher (*P*<0.05) in heifers fed the 16 % CP diet regardless of the level RPMet. Fat yield followed a similar trend in both diets (14 and 16 % CP) and increased with RPMet level in the diet. No differences were observed in average milk protein percentage of heifers fed either diet (14 or 16 % CP) regardless of methionine level. Meanwhile, protein yield in milk of the 14 % CP heifers was higher (*P*<0.05) at 25 g/hd/d RPMet level. Opposite to that, protein yield increased (*P*<0.05) with RPMet level within heifers fed the 16 % CP diet.

Total solids were higher (*P*<0.05) in the milk of heifers that received 25 g/hd/d RPMet in the 14 % CP diet, whereas no differences were observed among heifers fed the 16 % CP diet. Energy corrected milk was increased (*P*<0.05) with RPMet level in both diets. Milk casein of heifers fed the 14 % CP diet was higher (*P*<0.05) with 25 g/hd/d but when fed the 16 % CP diet, casein was higher (*P*<0.05) for those that received 15 g/hd/d.

The general expectation is that the major effect of the inclusion of RPMet in the ration of dairy cows is that milk protein content and yield will often increase (Girard *et al.* 2005).

Increased content and/ or yield of protein were reported earlier (Noftsger *et al.* 2005, Lara *et al.* 2006, Socha *et al.* 2008, Benefield *et al.* 2009, Kudrna *et al.* 2009, Trinacty *et al.* 2009) but in these experiments more cows were involved and longer periods of supplementation were used. However, Leonardi *et al.* (2003) reported that the addition of RPMet to diets of dairy cows does not always result in an increase of milk protein yield. No response in protein percentage or yield was observed with the addition of RPMet (Overton *et al.* 1998, Madsen *et al.* 2005, Socha *et al.* 2005, Kudrna *et al.* 2009). Inconsistent results are obviously due to many other factors interacting in this complex process, among them being the stage of lactation as well as the source, form and digestibility of protein that may play a very significant role (Ali *et al.* 2009, Kudrna *et al.* 2009).

Increased milk fat synthesis following feeding RPMet was reported previously (Samuelson *et al.* 2001, Benefield *et al.* 2009). On the other hand, no or low milk fat content was evident in many studies investigating the effects of RPMet supplementation (Samuelson *et al.* 2001, Girard *et al.* 2005; Lara *et al.* 2006, Trinacty *et al.* 2009).

The specific reason for the increased fat is unknown. However, several possibilities have been suggested. NRC (2001) reported that methionine supplementation would increase post-ruminal energy compounds (acetate) strongly associated with fat synthesis. Methionine may also increase *de novo* synthesis of short and medium chain fatty acids in the mammary gland or may act as a methyl donor for the synthesis of choline, which is essential for the synthesis of phospholipids that are required for the synthesis of chylomicrons and very low density lipoproteins (Girard *et al.* 2005, Benefield *et al.* 2009). Other studies proposed that methionine might serve as a substrate for triglyceride transport to the mammary gland (Madsen *et al.* 2005, Benefield *et al.* 2009). It is most likely that the increase in milk total solids, when cows were supplemented with RPMet, resulted from a combined increase in fat and protein concentration. Results of casein are in line with those from other experiments of Armentano *et al.* (1997), Girard *et al.* (2005) and Trinacty *et al.* (2009) who reported that the primary response to added methionine was increased secretion of casein and that the increased milk protein synthesis of cows fed rumen protected amino acids in some studies was mostly caused by increased synthesis of casein (Armentano *et al.* 1997).

Energy corrected milk is a function of milk fat and CP content. Therefore, the increase in ECM would be due mostly to the increase in both contents observed in both diets when supplemented with methionine, as well as to the increase in milk yield obtained with methionine supplementations. To our best knowledge, published literature available regarding the effect of RPMet on ECM is very scarce. Only Socha *et al.* (2005, 2008) showed no effect of RPMet on ECM values. Moreover, Ardalan *et al.* (2010) suggest that the increased ECM results from a positive impact of RPMet on milk energy output when early-lactation curve peaks and drops.

In both diets, daily feed intake (kg DM/d) was higher ($P<0.05$) at 25 g/hd/d RPMet compared to the other two groups (Table 4). Increased dry matter intake following RPMet supplementation was reported by Trinacty *et al.* (2009) while it tended to increase linearly following RPMet supplementation as reported by Socha *et al.* (2008). However, others obtained reduced (Dinn *et al.* 1998, Benefield *et al.* 2009, Kudrna *et al.* 2009) or unaltered dry matter intake (Overton *et al.* 1998, Girard *et al.* 2005, Madsen *et al.* 2005, Noftsger *et al.* 2005, Lara *et al.* 2006).

Table 4
Feed intake and body weigt of heifers fed different levels of protected methionine in their diets

| Diets | 14% CP | | | 16% CP | | | | |
Methionine g/d	0	15	25	0	15	25	SE	P
Number of animals	10	10	10	10	10	10		
Feed intake, kg DM/d	21.45[b]	21.52[b]	22.46[a]	22.62[b]	22.63[b]	23.28[a]	0.07	0.001
Feed:Milk[1]	1.02	1.01	0.99	0.99[b]	0.91[a]	0.90[a]	0.01	0.001
FCM efficiency[2]	1.11[a]	1.10[a]	1.06[b]	1.07[b]	0.98[a]	0.96[a]	0.01	0.001
Initial body weight, kg	449.33	452.33	459.00	447.66	449.77	464.55		
Final body weight, kg	460[b]	464[b]	473[a]	459[b]	462[b]	478[a]	3.40	0.001
Initial body condition score[3]	2.65	2.66	2.71	2.62	2.73	2.77		
Final body condition score	2.70[b]	2.72[b]	2.77[a]	2.71[b]	2.78[b]	2.84[a]	0.02	0.001

[a, b, c]Means with different superscripts within the same row are different ($P<0.05$),   [1]Feed:Milk: daily dry matter intake: daily milk production, kg/kg,   [2]Fat corrected milk efficency = kg feed/kg 4% fat corrected milk,   [3]Body condition score measured on a five-point scale with 1=emaciated and 5=obese (Edmonson et al., 1989)

Feed:milk ratio (feed efficiency) expressed as kg dry matter of daily intake per kg daily milk production showed no differences among heifers fed the 14% CP diet (Table 4). Those fed the 16% CP diet showed an improved ($P<0.05$) ratio when supplemented with RPMet regardless its level. When expressed based on 4% FCM, heifers fed the 14% CP diet had the best ($P<0.05$) ratio at 25 g/hd/d RPMet supplementation. Meanwhile, when fed the 16% CP diet, the best ratio ($P<0.05$) was achieved regardless of the RPMet level.

Final body weight and body condition score were higher ($P<0.05$) for heifers that received 25 g/hd/d RPMet in both diets (Table 4). The increase in final body weight might be due to the increased dry matter intake observed in this experiment. Overton et al. (1996) indicated that dairy cows fed RPMet tended to have greater body weights. Noftsger & St-Pierre (2003) and Lara et al. (2006) reported no change in body weight of dairy cows after supplementation with RPMet. Higher body weight and body condition score of cows that received 25 g/hd/d RPMet in both diets might indicate that supplementation with RPMet aided to improve these measures. This might suggest that increased levels of RPMet might favour more energy partition into body tissues (Samuelson et al. 2001). However, it should be noted that dietary effects on body weight may not be accurately reflected in experiments with short term experimental periods.

Reproduction measurements

Results on reproductive performance indicate no differences in pregnancy rates from first artificial insemination of heifers fed either diet (14 or 16% CP) regardless of the RPMet level (Table 5). Likewise, no effect was observed on pregnancy rates from first to second insemination on the 14% CP diet. However, when heifers were fed the 16% CP diet, there was a tendency ($P=0.08$) in improved pregnancy rates. No differences were observed in the mean number of services among cows fed the 14% CP diet. Days from calving to first, second or third insemination, as well as calving interval were also not different regardless of diet or RPMet levels (Table 6).

Table 5
Pregnancy rates for heifers receiving different levels of rumen protected methionine in postpartum period

Diets	14 % CP			16 % CP				
Methionine g/d	0	15	25	0	15	25	SE	P
Number of animals	9	9	9	9	9	9		
Pregnancy rate %								
From first AI	44 (4)±0.17	44 (4)	44 (4)	33 (3)	55 (5)	55 (5)	0.17	ns
First and second AI	55 (5)±0.19	44 (4)	66 (6)	44 (4)	66 (6)	66 (6)	0.20	ns
Over whole[2]	66 (6)±0.16	55 (5)	66 (6)	66 (6)	66 (6)	77 (7)	0.16	ns
Number of service[1]	1.5	1.4	1.33	1.8	1.2	1.4	0.3	ns

[1]Number of service for all cows,   [2]First, second and third AI

Table 6
Average days from calving to first, second and third AI for heifers receiving different levels of rumen protected methionine in postpartum period

Diets	14 % CP			16 % CP				
Methionine g/d	0	15	25	0	15	25	SE	P
Days to first AI[1]	64 (9)	62 (9)	64 (9)	62 (9)	65 (9)	64 (9)	3	ns
Days to second AI	87 (5)	87 (5)	85 (5)	82 (5)	84 (4)	91 (4)	4	ns
Days to third A I	106 (4)	105 (5)	102 (3)	104 (5)	102 (3)	108 (3)	4	ns
Calving interval[2]	351 (6)	352 (5)	353 (6)	358 (6)	352 (6)	354 (7)	7	ns

[1]average days from calving to first artificial insemination,   [2]calving interval for all heifers,   number of animals in brackets

Present results agreed with Rogers *et al.* (1989) who reported that supplementation with RPMet did not effect reproductive performance of dairy cows. In a previous study, Fuquay *et al.* (1975) obtained a tendency to increased conception rate at first service when dairy cows were supplemented with 25 g methionine hydroxy analog/day during early lactation, while Polan *et al.* (1991) and Bobe (1997) observed that supplementation with RPMet did not alter the number of services per conception, days to first service, services per conception and calving interval in dairy cows. On the other hand, Ardalan *et al.* (2010) reported that supplementation of RPMet can improve reproductive performance of dairy cows.

   Present results indicate that reproductive performance was not affected when RPMet was supplemented to both diets. Dairy cows usually may experience periods of negative energy balance and therefore may be at greater risk of reproductive/conception failure. Therefore, providing RPMet was expected to enable the higher-producing cows to maintain productivity but at reduced expenses of reproductive malfunction. It is possible that the limited number of cows in this study may have diminished the ability to detect a significant difference in reproductive parameters with non-normally distributed data like conception and pregnancy rates (Santos *et al.* 2001). Other possible factors are associated with the chance grouping of animals, semen quality or many other factors involved in reproductive efficiency (Fuquay *et al.* 1975).

   It should be emphasized here that due to the short duration of the experimental period as well as the very limited number of heifers utilized throughout the experiment, present results might not be realistic or provide a good judgement.

In conclusion, addition of RPMet at a level of 15 g/hd/d to the ration of dairy heifers produced a very limited effect on the studied parameters regardless of the CP level. However, addition of 25 g/hd/d RPMet to dairy heifers regardless of CP level increased milk yield, FCM, ECM and improved milk composition except milk protein content which was unaffected. Reproductive performance was not affected by RPMet supplementation at both dietary protein levels. However, results on reproductive performance are not certain.

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