PRODUCTIVE RESPONSES OF EARLY LACTATING DAIRY COWS TO RUMINALLY PROTECTED METHIONINE SUPPLEMENTION.

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SUMMARY

Increased milk production requires high intakes of crude protein in the diet, and/or improved supply and profiles of amino acids (AA) delivered to the duodenum, in order to meet animal needs for milk and milk component synthesis. The objectives of this experiment were to estimate the impacts of a rumen protected methionine (RPM) supplementation on dry matter intake as well as milk production and composition and blood metabolites in high producing Holstein dairy cows at early lactation (62±4.1 days in milk). Forty early lactating multiparous Holstein dairy cows (570±13.5 kg) were randomly assigned into two experimental groups and fed a total mixed ration (TMR) without (CTRL) or with 240 g/pen/d of the RPM supplement (to deliver 12 g methionine/cow/d) for 10 weeks. The results of the current experiment showed that RPM supplementation did not influence dry matter intake (DMI) in early lactating cows (24.4 *vs* 24.1 kg/d), but improved ($P<0.05$) milk yield (37.9 versus 38.9 kg/d), as well as the yields of milk protein (1.250 versus 1.344 kg/d) and milk solid not-fat (SNF; 3.310 versus 3.560 kg/d). The inclusion of RPM in TMR of dairy cows enhanced the feed efficiency and achieved daily net profit of 6.62 EGP per cow. The concentrations of blood total protein and globulin were increased ($P<0.05$) with RPM supplementation, while the concentrations of serum albumin, glucose, urea nitrogen and creatinine were not affected by RPM supplementation. In conclusion, the inclusion of RPM in the diet of early lactating dairy cows has increased milk yield, increased milk protein percentages and yield, and improved the net return per cow.

Keywords: *milk composition; intake; blood metabolites; economic.*

INTRODUCTION

Methods to increase milk production through dietary changes have been extensively researched and it is generally accepted that milk protein proportion increases with increased dietary crude protein (CP) level. There is, however, no single source of rumen undegradable CP which provides an ideal balance of essential amino acids (AA) that matches the AA profile of milk thereby ensuring optimal milk production. It is difficult to formulate rations to provide all required AA concentrations using currently available feed sources and metabolic models. Methionine and lysine have been suggested to be the most limiting AA for milk production when corn-based diets are fed and diets containing soybean meal as the supplemental protein source (Schingoethe *et al.*, 1988 and NRC, 2001).

In today's high producing dairy cow rations, an ideal balance within specific nutrients is difficult to achieve especially for AA. Increased milk production requires high intakes of CP in the diet, and/or improved supply and profiles of AA delivered to the duodenum, in order to meet animal needs for milk and milk component synthesis (Misciattelli *et al.*, 2003; Socha *et al.*, 2005; Rulquin *et al.*, 2006). An imbalance of AA will compromise dairy cow performance and condition as far as health, fertility and milk yield and quality is concerned (Robinson *et al.*, 2000). It is well known that methionine is the first limiting AA for dairy cows (Schingoethe, 1996; and NRC, 2001), particularly in rations based on lucerne as the primary CP source and/or containing soybean or cottonseed meals as protein supplements. Supplementation with a highly bio-available source of methionine is essential in order to meet dairy cow needs (Ali *et al.*, 2009). Therefore, the objective of the current study was to determine the effect of feeding a ruminally protected methionine (RPM) supplement on dry matter intake (DMI), milk production, milk composition and blood metabolites of high producing dairy cows in their early stage lactation.

MATERIALS AND METHODS

Farm, animals and management:

This study was conducted on the commercial Holstein dairy farm, which belongs to the Alexandria Company of Agriculture (Talaat Moustafa), located at 75 km of the Cairo-Alexandria desert road, Egypt. The herd in which the study was performed consisted of 640 lactating Holstein cows with an average milk yield of 33.5 kg/head/day. Cows were housed in open barns with shades. Cows have access to fresh water *ad libitum*. The animals were healthy and free from any parasites and diseases. Cows were machine-milked three times daily (every 8 hours). Cows were randomly assigned to the experimental groups after being sorted by parity and days in milk in two pens. Each pen for each group held 20 cows, with cows averaging 62 ± 4.1 days in milk. This study was performed between November and February, of 2010-2011 and lasted for 10 weeks. All analysis (feed, milk and blood samples) were carried out at the Animal Nutrition Laboratory, Department of Animal Production, Faculty of Agriculture, Alexandria University.

Diets and treatments:

Forty early lactating dairy cows were allocated into two groups according to parity and days in milk. The first group (n=20) was fed total mixed ration (TMR) eight times daily each 3 h and had free access to water without a supplement of RPM for 10 weeks. The second group (n=20) were fed TMR supplemented with 240 g RPM/pen/d (12 g methionine/cow/d). The Smartamine™ (rumen-protected methionine) product was used in this study (Kemin Europa N.V., Industriezone Wolfstee, Toekomstlaan 42, Belgium). Total mixed ration was formulated using Gavish computer operated cattle feeding system 2008 to cover or exceed NRC recommendations (NRC, 2001). The ingredients and proximate analysis of TMR are presented in Tables 1 and 2.

Table (1): Ingredients of the total mixed ration fed to the dairy cows (g/100g DM).

Item	%
Alfalfa hay	10.7
Green clover	7.3
Corn silage	26.2
Ground yellow corn	25.6
Soybean meal	24.6
Limestone	0.9
Minerals mixture	0.2
Vitamins[b]	0.1
Protected fat (Magnabac)	3.1
Sodium bicarbonate	1.0
Mono calcium phosphate	0.4

[a] *Supplied per kg of diet mixture: Ca, 190; P, 115; Mg, 63; Cl,167; K, 380; Na, 70; S, 53; Co, 0.15; Cu, 0.1; Fe, 0.9; Mn, 2.7; Se, 0.15; Zn, 2.6.*

[b]*Supplied per kg of diet mixture Vit.A, 940 (1000 IU/d); Vit.D, 165 (1000 IU/d); Vit.E, 374 (1000 IU/d).*

Table (2): Chemical composition of the total mixed ration (g/100g DM).

Item	%
Crude protein (CP)	19.08
Ether extract (EE)	4.4
Neutral detergent fiber (NDF)	34.0
Acid detergent fiber (ADF)	16.0
Hemicellulose	18.0
Cellulose	13.5
Acid detergent lignin (ADL)	4.5
Non-fiber carbohydrates (NFC)	36.6
Total digestible nutrients (TDN)	71.1
Metabolizable energy (MJ/kg DM)	3.13
Net energy of lactation (MJ/kg DM)	1.62

Total mixed ration was mixed before each feeding, after which it was loaded into trucks to be unloaded at the pens. The 'Feed Watch' system (Valley Ag Software, Tulare, CA, USA) recorded all feeding activities, keeping daily records of the total amount of TMR fed to each pen and the actual ingredient profile of each batch of TMR that was mixed. Feed refusals were weighed individually by pen every morning thereby allowing calculation of dry matter intake (DMI) per pen. The number of cows in each pen was retrieved from Dairy Comp 305 (Valley Ag Software, Tulare, CA, USA) weekly, allowing group intake expressed on a per cow basis to be calculated.

Samples collection and analysis:

During the entire experiment, the amounts of TMR offered and refused were recorded daily and represented samples of TMR were collected weekly and stored at -20 C° until chemical analysis for DM, organic matter (OM), CP, ether extract (EE), neutral detergent fibers (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970); Van Soest *et al.* (1991); AOAC, (2006). Cellulose and hemicellulose were calculated by differences among NDF, ADF and ADL. Total digestible nutrients (TDN), metabolizable and net energy for lactation were calculated (NRC, 2001).

Cows were milked 3 times daily at 4 am, 12 am and 8 pm. Milk yield for individual cows was measured daily using Delaval alpro program, rapid exit. Milk samples at the second milking time (12 am) were collected biweekly and analyzed immediately for fat, protein, lactose and solid not fat (SNF) content using infrared method by Milk Analyzer (Milko tester Instruments Inc, Bulgaria). Average fat and CP yields were calculated by multiplying milk yield by fat and CP content of milk on an individual cow basis.

Blood samples were collected from Jugular vein of 10 cows only per group prior to morning feeding monthly. Serum were obtained by centrifugation the blood tubes for 20 min, 3000xg and stored at -20°C until blood metabolites analysis. Concentrations of serum total protein, albumen, glucose, urea and creatinine were determined colorimetrical using commercial kits (Stanbio Diagnostic company, Germany) according to Weicheselbaum (1964), Bartholomew and Delaney (1964); Folin and Wu (1920); Henry *et al.* (1974) and Jaffe (1886), respectively. The concentration of globulins in each serum sample was obtained by subtracting the value of albumin from the total blood serum protein concentration.

Economic efficiency expressed as the daily feed and supplement cost and price of milk. The price for TMR was estimated 2200 EGP per ton, while the price for RPM was at 120 EGP per kg. Milk (fat, 3.5%; SNF, 8.5%) was priced at 2.90 EGP per kg by using the average prices of years 2010-2011.

Statistical analysis:

Statistical analysis of the results was analyzed using the MIXED procedure (SAS, 2002) according to the following model $Yij = \mu + Ei + eijk$ where Yij is the observation of j cows of i treatment, μ the overall mean, Ei the fixed effect of i treatment and, eijk is the experimental error. The data related to the DMI were averaged daily for each pen and analyzed on a pen basis including treatment effect. Significant treatment effects were further separated by Duncan Test (Duncan, 1955).

RESULTS AND DISCUSSION

The chemical composition of the basal TMR is given in Table 2. There were no substantive differences in composition of the TMR fed to the two groups (Table 2). The National Research Council (NRC) recommends that lactating dairy cow rations should contain 30 to 40% non-fiber carbohydrates (NFC), 19 to 21% ADF and 25 to 28% NDF. However, NFC content was lower in TMR, while NDF and ADF are higher than that recommended by NRC (2001). Currently, many dairy nutritionists also evaluate the concentration of starch in the ration, with common recommendations ranging from a minimum of 21 to a maximum of 27 % (NRC, 2001).

Dry matter intake, milk yield, fat corrected milk, milk: feed ratio, milk composition and milk energy value are presented in Table 3. The results showed that DMI was not affected by RPM supplementation to the early lactating dairy cows. The effect of RPM supplementation on milk yield profile in early lactating dairy cows is shown in Figure 1. Milk yield (37.9 versus 39.9 kg/d) and FCM (35.3 vs 37.0 kg/d) were increased ($P<0.01$) by 5% with RPM supplementation. Milk protein percentage ($P<0.01$) and SNF percentage ($P<0.008$) were also increased with RPM addition to dairy cows diet in addition, inclusion of RPM improved ($P<0.05$) the milk: feed ratio from 1.55 to 1.66 kg milk/kg DM.

The effect of RPM on DMI of dairy cows has not been consistent. In the current study, DMI was similar (P>0.05) among treatments averaging 24.4 and 24.1 kg/d for control and RPM diets, respectively (Table 3). The supplementation of RPM in basal rations of pre-partum and post-partum dairy cows containing 18.5 and 16% CP showed no effects on DMI (Rulquin and Delaby, 1997 and Socha *et al.*, 2005), which is in agreement with our results. On the other hand, other studies reported significant increases in DMI when lactating dairy cows received RPM (Vanhatalo *et al.*, 1999 and Trinacty *et al.*, 2006). In addition, Piepenbrink *et al.* (1996) and Armentano *et al.* (1997) reported that addition of rumen protected amino acids improved the DMI of the ration containing 18% CP compared to ration containing 14% CP.

Figure (1): Effect of rumen protected methionine (RPM) inclusion on milk yield in early lactating dairy cows.

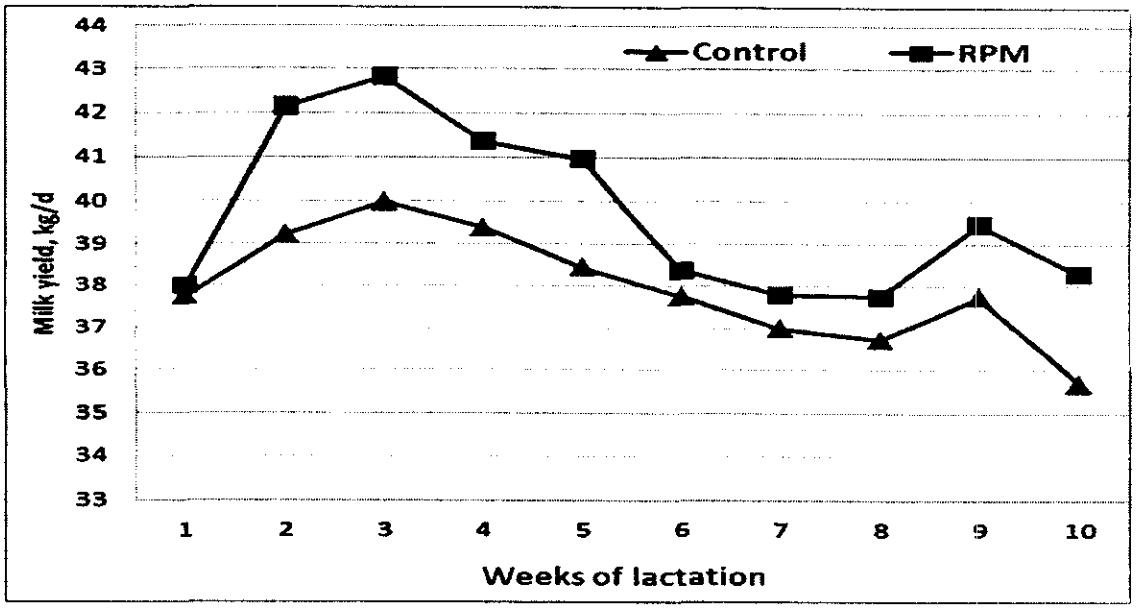

Table (3): Effect of rumen protected methionine (RPM) supplementation on dry matter intake (DMI), milk yield and composition at early lactation of dairy cows

Item	Control	RPM	MSE	*P-value*
DMI, kg/d	24.4	24.1	2.45	0.421
Milk yield, kg/d	37.9	39.9	6.66	0.011
FCM, kg/d	35.3	37.0	6.12	0.011
Milk: Feed ratio	1.55	1.66	0.007	0.050
Milk composition				
Fat, %	3.55	3.51	0.907	0.825
Fat, kg/d	1.349	1.404	0.135	0.825
Protein, %	3.29	3.36	0.018	0.011
Protein, kg/d	1.250	1.344	0.003	0.011
SNF,%	8.71	8.90	0.137	0.008
SNF, kg/d	3.310	3.560	0.021	0.008

FCM: Fat corrected milk; SNF: solid not fat, MSE: Mean square of error

The increase of milk yield with RPM supplementation was in agreement with Robinson *et al.*, (1995) and Watanabe *et al.* (2006). Rumen-protected methionine added to diets containing soybean meal as the supplemental protein source has increased milk production (Watanabe *et al.*, 2006). Generally, the requirements of high producing animals for dietary essential AA are increased from those supplied by the microbial and escaped protein pool. Thus the dietary supplementation of rumen protected protein and AA is recommended to support the physiological and productive needs of livestock for AA. The enhancement of milk yield at the current study may be due to supply the ruminal microorganisms an optimal level of methionine to improve ruminal fermentation and post-ruminal supply of metabolizable AA. The response to feeding protected AA in the literature is variable depending on the protein source in the basal diet (Piepenbrink *et al.* (1996) and Armentano *et al.* (1997).

Supplementing the diets of dairy cows or buffaloes with RPM and ruminally protected lysine has been shown to increase milk yield (Robinson *et al.*, 1995; Xu *et al.*, 1998; and Kholif *et al.*, 2006), milk lactose (Robinson *et al.*, 1995), and more frequently milk protein (Robinson *et al.*, 1999; Wu *et al.*, 1997; Xu *et al.*, 1998). Improvement in milk yield from RPM and ruminally protected lysine supplementation, when observed, is generally limited to cows in early lactation (Xu *et al.*, 1998). Milk protein synthesis may be limited by the supply of precursors reaching the mammary gland, in particular the essential AA (Clark, 1975). If an essential AA is the key limiting substrate for milk protein synthesis and the AA transport systems are operating well below saturation in the mammary gland (Baumrucker, 1985), then increased delivery of a limiting AA should increase milk protein synthesis.

Milk protein percentage has increased in most studies (Leonardi *et al.*, 2003; Berthiaume *et al.*, 2006, Patton, 2010) when RPM was supplemented, but was also unaffected in several other studies (Younge *et al.*, 2001 and Davidson *et al.*, 2008). The increase in milk protein percentage as a response to feeding RPM has resulted in both significant increases (Lara *et al.*, 2006 and Patton, 2010) and decreases (Benefield *et al.*, 2009) in milk protein yields. Schwab *et al.* (2001) have suggested that changes in milk protein percentage may be a more sensitive method of determining effectiveness of RPM supplementation. Although milk protein percentage may be important, increases in grams of milk protein secreted may be a more sensitive measure of methionine utilization than milk protein percentage because of possible declines in milk production due to feeding RPM. Also, it has been demonstrated that the RPM allows a significant methionine supply to cows (Graulet *et al.*, 2005) and an increase in their milk protein yield (Noftsger *et al.*, 2005 and St-Pierre and Sylvester, 2005). Polan *et al.* (1991) reported that feeding of these RPM to dairy cows with basal rations containing corn silage, ground corn with soybean meal and corn gluten meal, fat corrected milk and milk protein yield were greater during early, mid and total lactation periods. Post-ruminal infusion of methionine has been shown to improve yield of milk protein, especially casein (Pisulewski *et al.*, 1996).

Additionally, some studies have reported increased milk fat percentage and yield (Samuelson *et al.*, 2001 and Krober *et al.*, 2000), and other studies have found a significant decline in milk fat yield (Socha *et al.*, 2005 and Patton, 2010), while Liu *et al.*, 2000 and Berthiaume *et al.*, 2006) reported that milk fat percentage and yield was not affected with RPM supplementation in dairy cows as observed at the current study or in buffaloes (Ebeid *et al.*, 2007). The main effects of RPM on milk production and composition were an increase in fat yield, especially when RPM was given repeatedly at doses of 25 to 35 g/d to multiparous dairy cows in early lactation receiving an adequate protein supply in the diet, whereas protein yield was not modified and milk production was rarely increased by RPM supplementation (St-Pierre and Sylvester, 2005). Moreover, postruminal infusion of methionine has been shown to improve milk fat percentage (Rulquin *et al.*, 1993),

Serum concentrations of total protein, albumin, globulin, glucose, urea and creatinine are given in Table 4. Our results showed that total protein ($P<0.02$) and globulin ($P<0.05$) were increased when RPM was included in the diet, while blood urea N, creatinine, albumin and glucose were not affected by RPM supplementation. Increased AA supply to small intestine is expected to change AA concentrations in blood plasma and, possibly, improve availability of AA for milk protein synthesis. The serum urea concentration in early lactating dairy cows received RPM was not changed ($P>0.05$) compared with control group suggesting efficient AA uptake by cows tissues. Mackle *et al.*, 1999 indicated that although plasma glucose concentrations did not change, the 2-fold higher insulin concentration would have stimulated glucose use in the body such that entry rate of glucose must have increased to maintain euglycemia in lactating cows. An increase in glucose entry rate with no effect on plasma glucose concentration has been observed in lactating cows given postruminal casein (Konig *et al.*, 1984). The absence of a large glucagon response in peripheral circulation whereas AA catabolism apparently increased in this experiment may be related to the ruminant liver remaining highly gluconeogenic irrespective of diet.

Economic evaluation of RPM supplementation to early lactating dairy cows is presented in Table 5. The results revealed that average daily feed cost (53.68 vs. 53.02 EGP) had no difference when RPM was supplemented to dairy cows but output of milk increasedfrom 109.91 to 117.31 EGP for untreated and RPM-treated cows, respectively. Moreover, the net profit achieved for RPM inclusion in diet of early lactating dairy cows was higher 6.62 EGP than untreated cows and economic efficiency increased from 2.05 to 2.15 by the treatment.

Table (4): Effect of rumen protected methionine (RPM) supplementation on blood metabolites in dairy cows at early lactation

	Control	RPM	MSE	*P-value*
Total protein, g/dL	7.38	8.14	1.48	0.017
Albumin, g/dL	3.93	4.05	0.135	0.225
Globulin, g/dL	3.44	4.09	1.57	0.047
Urea, mg/dL	23.03	23.38	4.09	0.751
Glucose, mg/dL	57.38	61.15	14.38	0.318
Creatinine, mg/dL	2.55	2.42	2.42	0.947

MSE: mean square of error

Table (5): Econmic evaluation of the rumen protected methionine (RPM) supplementation to the early lactating dairy cows

	Control	RPM
Feed cost, EGP/cow	53.68	53.02
Supplement cost, EGP/cow	-	1.44
Milk Price*, EGP/kg	2.90	2.94
Output of milk, EGP/cow/d	109.91	117.31
Return, EGP/cow/d	56.23	62.85
Net profit over the control, EGP/cow	-	6.62
Economic efficiency	2.05	2.15

* *The difference in milk price between two groups was due to higher 0.2% in SNF, which represent 4 piasters.*

CONCLUSION

The results of the current study concluded that feeding a ruminally protected methionine applied to the total mixed ration of dairy cows in early lactation has the potential to increase milk production, SNF and economic efficiency.

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الإستجابات الإنتاجية فى أبقار اللبن الحلابة في بداية موسم الحليب لإضافة الميثيونين المحمى من التحلل فى الكرش.

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زيادة إنتاج اللبن يحتاج كمية كبيرة من البروتين المأكول الخام فى العليقة، و/أو تحسين الإمداد ونوعية الإحماض الأمينية الواصلة للإثنى عشر لكى تغطي إحتياجات الحيوان لإنتاج اللبن وتخليق مكونات اللبن. لذلك كان الهدف من هذه التجربة هو تقدير تأثيرات إضافة الميثيونين المحمى بالكرش (سمارتمين) على المادة الجافة المأكولة و إنتاج وتركيب اللبن وقياسات الدم فى أبقار اللبن الهولشتين عالية الإنتاج فى بداية موسم الحليب (62يوم من الحليب).

تم إستخدام 40 بقرة حلابة متوسط وزنها حوالي 570 كجم وتم تقسيمها عشوائيا لمجموعتين و تم تغذيتها على عليقة متكاملة الخلط (TMR) مع أو بدون إضافة 240 جم مثيونين محمى بالكرش/ حظيرة/ يوم بحيث يكون معدل الإضافة 12 جم مثيونين محمي/ بقرة/ يوم لمدة 10 أسابيع. و كانت النتائج كما يلى:

إضافة الميثيونين المحمى بالكرش لم تؤثر معنويا على كمية المادة الجافة المأكولة (26.8 كجم/ يوم) لكن حسنت معنويا إنتاج اللبن (37,9 مقابل 38,9 كجم/ يوم)، و أيضا دهن البن (1,349 مقابل 1,404 كجم/ يوم)، و البروتين (1,250 مقابل 1,344 كجم/ يوم) و المواد الصلبة غير الدهنية (3,310 مقابل 3,560 كجم/ يوم).

إحتواء العليقة متكاملة الخلط TMR على الميثيونين المحمى بالكرش لأبقار اللبن أدي إلي زيادة الكفاءة الغذائية و حقق صافى ربح يومى لكل بقرة حوالى 6,62 جنية مصرى.

أوضحت قياسات الدم حدوث زيادة معنوية فى الدم، البروتين الكلى والجلوبيولين عند إضافة الميثيونين المحمى بالكرش للأبقار الحلابة بينما الألبيومين والجلوكوز واليوريا و الكرياتينين لم تتأثر معنويا بالإضافة. نستخلص من نتائج هذه الدراسة أن إضافة الميثيونين المحمى بالكرش لأبقار االلبنفى المرحلة المبكرة من موسم الحليب له تأثيرات إيجابية و يحسن إنتاج وجودة اللبن فى الأبقار الحلابة إقتصاديا.