Dept. of Animal Prod.,
Fac. of Agric., Mansoura Univ., Mansoura

MILK PRODUCTION AND NUTRIENT UTILIZATION RESPONSES TO REDUCED DIET-CRUDE PROTEIN LEVEL AND METHIONINE SUPPLEMENTATION FOR DAIRY COWS

(With 7 Tables)

By

A.A. GABR; M.K. EL-BANNA*; M.A. El-HARAIRY; A.E. El-SAYED** and M.B. ABOUL-ELA

* Animal Prod. Res. Institute, Dokki, Giza, Egypt.
** El-Mansoura for Agric. Develop. Dairy Farm.

(Received at 21/3/2006)

إستجابة الأبقار الحلابة من إنتاج اللبن ومكوناته والقيمة الغذائية لعليقه منخفضة البروتين مع إضافة الميثيونين المحمي

أحمد عبد الرازق جبر ، مصطفي قطب البنا ، مصطفي عبد الحليم الحرايري ، علي السيد الغندور السيد ، محمد بدر الدين أبو العلا

الغـــرض مـــن هذا البحث هو دراسة مدي تأثير إضافة الحامض الأميني الميثيونين المحمي للعلـــيقه المنخفضة في مستوي البروتين الخام بدون التأثير السلبي علي إنتاج اللبن ومكوناته وكذلـــك القـــيمة الغذائـــية للعليقه ومدي الإستفادة منها. أجريت هذه الدراسة علي ٢٨ بقره هولشـــتين فريـــزيان حلابـــة عالـــية الإدرار. قسمت الحيوانات إلي أربعة مجاميع متناسقة، المجموعة الأولي: غذيت علي عليقه منخفضة في البروتين الخام (١٦,٩% علي أساس المادة الجافـــة)، المجموعة الثانية: نفس عليقة المجموعة الأولي بالإضافة إلي الميثيونين المحمي، المجموعـــة الثالـــثة: غذيت علي مستوي عالي من البروتين (١٩,٣%)، المجموعة الرابعة: نفـــس علـــيقة المجموعـــة الثالثة بالإضافة إلي الميثيونين المحمي. أضيف الميثيونين بمقدار ١٢جـــم/بقـــره/يوم بحيث يعوض مقدار النقص المحسوب نظريا والناجم عن خفض مستوي البروتين الخام. إضافة الميثونين المحمي للعليقة المنخفضة في مستوي البروتين أعطي تأثيراً إيجابـــيا علي إنتاج اللبن ومحتواة من البروتين وكذلك أحدث تحسن في كفاءة تحويل العليقه والإســـتفاده مـــن بروتيـــنه وإنخفضت تكلفة العليقة لكل واحد كجم لبن وخاصة في مراحل الحليب الأولي ولم يختلف معنوياً في تأثيره عن بقية المعاملات في مراحل الحليب المتأخرة. زيادة مستوي بروتين العليقه عن ١٦,٩% تسبب في زيادة معنوية في تركيز نيتروجين يوريا الـــدم واللبـــن. وبذلـــك تكون التغذية علي أساس المحتوي من الاحماض الامينيه فضلا عن المحـــتوي من البروتين الخام نظام غذائي مقبول لتحسين الأداء الإنتاجي لماشية اللبن عالية الإدرار مـــع الأخذ في الإعتبار التأثير السيادى لأسعار المكون البروتيني في العليقه والبحث

عن أعلي معدل ربح عن طريق تكوين عليقه أقل تكلفه وكذلك تقليل تلوث البيئة بالنيتروجين المُنبعث في البول والروث.

SUMMARY

This study was carried out to determine whether the addition of rumen-protected Met to ration formulation allowed a reduction in dietary crude protein (CP) without jeopardizing total milk or milk protein yields. Twenty-eight multiparous Holstein-Friesian cows, in a commercial dairy herd, were randomly assigned to the trial. Rations differed in percentages of CP level (H, 19.3% or L, 16.9%), with or without supplementation by protected methionine. Milk yield was recorded individually at each milking and milk samples were taken during the experimental period. In addition, four digestion trials were conducted on three cows of each group to evaluate the digestibility coefficients of used diets using Acid Insoluble Ash technique. No effects of pMet supplementation or interactions between CP levels and methionine supplementation were detected on any of the parameters measured for digestibility coefficients of nutrient. In early lactation stage, milk production response to pMet supplementation was greater with the low level of CP diets compared with the high level (2.00 vs. 0.300 kg/d, respectively). The pMet supplementation by CP level interaction was significant for protein concentration, while it was not significant for fat and lactose concentration during early and mid lactation period. Every one percentage unit increase in dietary CP above 16.9%, MUN concentrations were, in average, increased 2.2, 2.0, and 1.6 mg/dl in early, mid, and late lactation, respectively. The feed and N utilization efficiencies were improved by feeding low CP diets supplemented with pMet in early lactation. Cows fed diets reduced in CP level had a reduction in cost of feed per kg milk in various stages of lactation. In early lactation stage, feeding diets supplemented with pMet reduced the cost of feed per kg milk by 0.04 L.E. It could be concluded that, a reduction in dietary protein to 16.9% and supplemented with protected AA, particularly the first limiting AA, is an acceptable new nutritional strategy for improving productive performance in dairy cattle and may be used as an approach for reformulating lactating cows rations based on AA profile rather than CP as simple or metabolizable protein. This strategy, with prevailing feed prices, could be compatible with maximum profit and a moderate amount of nitrogen excretion to the environment.

Key words: *Crude protein, rumen-protected methionine, lactating cows.*

INTRODUCTION

Overfeeding of CP to obtain maximum milk yield is common in the dairy industry today, increasing feed cost, decreasing the efficiency of nutrient utilization (Tamminga, 1992), and contributing to higher levels of N in the waste (St-Pierre and Thraen, 1999). Dairy cattle secrete roughly 25 to 35% of the N consumed as milk N, and almost all the remaining N is excreted in urine and feces (Chase, 1994). Most urinary N is excreted as urea (Broderick, 2003) and may be lost as ammonia into the environment (Van Horn *et al.*, 1994). In Egypt, many of the applied strategies for meeting the nutritional requirements of high producing dairy cows may lead to feed diets with CP in excess. Dairy producers often feed CP over recommendations of NRC to early postpartum cows for higher milk peak. Close management of dietary protein is needed to maximize profits and to minimize risk of environmental damage caused by excessive nitrogen excretion in urine and feces. One possible way of reducing urinary N excretion is by reducing the amount of dietary CP fed to the cow. Decreasing dietary CP from 18.4 to 15.1% linearly decreased urinary N expressed as grams per day or as a percentage of N intake (Broderick, 2003). Unfortunately, dietary CP reduction also decreased milk production by 1.1 K/d (Broderick, 2003) and milk protein yield by nearly 100g/d (Kalscheur *et al.*, 1999).

Methionine has been reported to be the first –limiting AA for milk yield and milk protein production (Bach *et al.*, 2000). Under the assumption that dairy cattle require a certain amount of duodenal methionine to maximize production, the primary objective of this experiment was to verify the hypothesis that a lower protein diet with supplemented methionine would support milk and milk protein production as much as a higher CP diet.

In an experiment where treatments comprised two levels of CP high (19.3%) or low (16.9%) with or without protected methionine addition (by a dose designated to compensate the theoretically calculated deficiency level of methionine resulting from reducing dietary CP), the hypothesis would be supported by an interaction with supplemental methionine, improving performance and efficiency of the lower CP diet with less effect on the higher CP diet.

MATERIALS and METHODS

Experimental design and treatments

Twenty-eight multiparous lactating Holstein-Friesian cows were used in the study. The cows were housed in a free-stall barn belong to El-Mansoura for Agriculture Development Dairy Farm. The cows were 20 ±3 days post-calving and blocked into four similar groups, based on parity, average daily milk yield from the 7^{th} through the 20^{th} day of lactation. Cows were randomly assigned to one of four treatments. Group 1 (16.9B): received low CP level (16.9% on DM basis) diet, group 2 (16.9M): received low CP level (16.9% on DM basis) diet supplemented with pMet, group 3 (19.3B): received high CP level (19.3% on DM basis) diet and group 4 (19.3M): received high CP level (19.3% on DM basis) diet supplemented with pMet. The trial lasted for 220 days as experimental period.

Chemical compositions of feedstuffs (Table 1) of the experimental rations (barseem, corn silage, cottonseed meal; decorticated, soybean meal, wheat bran, and calcium soap of FA's) were analyzed according to A.O.A.C. (1984). Cows were fed Total Mixed Ration (TMR), ad libitum, (Table 2) that was based on a good quality of corn silage and soybean meal. The offered feed was calculated daily based on the weight of the refusals during the previous day (approximately 5% of the offered feed), which were measured each morning. Equal portions of the rations were fed at 0800, 1400, and 1700 h. The CP level was regulated by the concentrates, mostly SBM and cottonseed meal.

The NRC (2001) software was used to calculate the required amount of protected methionine (pMet) to be added and compensate partially the theoretically calculated deficiency level of methionine resulting from reducing dietary CP to the level 16.9% CP.

Protected Methionine (Smartamine M, 78% post-ruminally release of Met, Adisseo animal nutrition, France) supplementation to TMR at a rate of 12 g/cow/d was top-dressed and then mixed by hand into the TMR for each cow once daily at morning offered portion of TMR to ensure that the protective coats of Smartamine M was not destroyed by mechanical mixing.

All cows were milked three times daily. Milk yield was recorded individually at each milking for three days per week. Daily and weekly milk yield averages were calculated for each cow. The periods from wk 3 to 12 and from wk 13 to 21 were considered as the early and mid stage of lactation, respectively.

Sampling

The TMR was routinely sampled once biweekly for each diet, composited monthly, and frozen for later analysis of 3-mo pooled samples. Milk samples were collected at each milking from all tested cows at wk 3, 8, 12, and 18 of lactation. Samples of different milkings of the day were composited and preserved using potassium dichromate until analyzed for TS, SNF, lactose, protein, and fat by infrared analysis with a Fossmatic, Milk Scan System (Foss Electric, Hillerød, Denmark) and urea N (MUN) using a colorimetric procedure (Crocker, 1967).

Digestion trials

At wk 18 of lactation period, four digestion trials were conducted on three cows of each group to evaluate the nutrient digestibility coefficient of used diets. The previous period of the study was considered as a preliminary period, followed by eight days as a collection period. Feed intake was individually recorded; Feces samples were collected directly from the rectum twice daily. The collected amount of feces was thoroughly mixed, and a sample was frozen for chemical analysis. Acid Insoluble Ash (AIA) technique was used as an internal marker to measure the digestibility coefficients of used diets and was determined according to Van Keulen and Young (1977). Proximate chemical analysis of TMR and feces samples were carried out for dry matter (DM), crude protein (CP), crude fiber (CF) and ash according to the standard methods outlined by A.O.A.C. (1984). Acid ether extract (AEE) in calcium soap of FA's, TMR, and feces were determined by modified method, as was described by (Abo-Donia *et al.* 2003).

Calculation and Statistical analysis:

The *N* utilization efficiency was calculated as follows:

The N utilization efficiency = Milk N (kg) /feed N (kg) x 100

Milk N (kg) = (average milk yield, kg x (milk protein %/100))/ 6.38

Feed N (kg) = (average DMI x (CP % in the ration/100))/ 6.25

The crude protein in milk has a different multiplier (N x 6.38) than in feed (N x 6.25) because milk protein contains a different proportion of N (15.7%), while in feed about 16%.

The cost of feed per 1 kg milk was calculated based on the following:

Cost of pMet (L.E./day) = 0.13 (L.E./1g) x 12 (g/cow/ day) = 1.56 L.E./ cow/ day

Cost of pMet/1kg DM = Cost of pMet (L.E./day)/ average DMI (kg) for each group

Cost of 1kg dry matter = 1.55 L.E. for high dietary CP level and 1.35 L.E. for low dietary crude protein level (calculated based on farm data).

The data obtained were statistically analyzed using SAS computer program (SAS, 1996).

The following mode was used for analyzing data of daily average milk yield, contents and yields of milk total solids (TS), solids not fat (SNF), lactose, protein, and fat, milk urea nitrogen (MUN) concentration, and digestibility coefficients of nutriént through the studied period:

$$Y_{ijk} = \mu + P_i + S_j + PS_{ij} + e_{ijk}$$

Where: μ = overall mean,
P_i = effect of the i^{th} CP level and i= 1(low CP level) or 2 (high CP level),
S_j= effect of j^{th} pMet supplementation where j= 1(non-supplemented) or 2 (supplemented)
PS_{ij} = the interaction between CP level and pMet supplementation,
e_{ijk} = common error term

The differences among means were tested using Duncan's (Duncan, 1955) Multiple Range Test.

RESULTS

The apparent digestibility coefficients of nutrients, except for EE, (Table 3) was reduced (P <0.01) for cows fed diet with low CP compared with those fed diet with high CP. No effects of pMet supplementation or interactions between CP levels and methionine supplementation were detected on any of the parameters measured for digestibility coefficient of nutrients.

Cows fed diet (16.9% CP) supplemented with pMet (16.9M) produced daily milk yield numerically higher than those fed high level of crude protein without (19.3B) or with (19.3M) pMet during early, Mid, and late lactation (33.7 vs. 33.1 and 33.4, 38.7 vs. 38.3 and 38.6, 23.5 vs.23.2 and 22.9 kg/d, respectively, Tables 4, 5, and 6).

As indicated in Tables 4, 5, and 6 pMet supplementation significantly (P<0.01) increased daily milk yield in early lactation period, but in mid or late stage of lactation it did not; as similar as CP level did. It is worthy noting also that the production response to pMet supplementation was greater with the low level of CP diets compared with the high level (2.00 vs. 0.300 Kg/ d, respectively). Milk protein percentages (Tables 4, 5, and 6) were similar in groups 16.9M and 19.3B through the different stages (early, mid, and late) of lactation (2.7, 2.6, and 2.7, respectively). Supplementing the basal diets of lactating cows with pMet increased (P<0.01) percentage of protein in milk. Moreover, responses were consistent across dietary CP level (CP x pMet interaction, P<0.05). Supplementing 19.3B or 16.9B with pMet increased milk protein content 0.2 percentage unit. As shown in Tables 4, 5, and 6, pMet supplementation of the basal diets had no effect on milk fat content during the early and mid stage of lactation. In the late stage of lactation, adding pMet to 16.9B or 19.3B increased (P<0.01) milk fat content. No differences across treatments were detected in percentage of lactose throughout the full lactation. The percentages of TS and SNF in milk were significantly increased (P<0.01) by pMet supplementation in mid and late lactation.

The present results (Table 4, 5, and 6) indicated that for every one percentage unit increase in dietary CP above 16.9%, MUN concentrations were, in average, increased 2.2, 2.0, and 1.6 mg/dl in early, mid, and late lactation, respectively.

Table 7 shows that the feed efficiency (average kilograms of milk per average kilograms of dry matter intake) was improved by feeding diets supplemented with pMet in early lactation. Furthermore, the highest value of feed efficiency (1.48) was recorded in group 2 (16.3M). N utilization efficiency was improved by feeding diets supplemented with pMet (in various stages of lactation). However, it is worthy noting that the rate of improvement in N utilization efficiency was a diminished response by advance in days in milk, given its highest value in early lactation and the lowest value was recorded in late stage of lactation. The cost of feed per kg milk was reduced by 0.04 L.E. in cows fed diets supplemented with pMet at early stage of lactation.

Table 1: Chemical composition of feedstuffs

Feedstuffs	Chemical analysis						
	DM	OM	CP	CF	AEE	NFE	Ash
	--------------% of DM --------------						
Barseem	21.72	90.52	10.68	11.46	0.78	67.59	9.48
Corn silage	34.33	89.95	9.35	33.56	3.06	43.98	10.05
Corn grain, dry	88.00	98.82	8.75	2.61	4.32	83.14	1.18
Cottonseed meal, decort.	90.00	91.00	47.41	10.11	5.56	27.92	9.00
Soybean meal, 44% CP	87.00	92.53	50.57	8.39	1.72	31.84	7.47
Wheat bran	88.00	93.18	13.65	12.50	4.55	62.49	6.82
Calcium soaps of FA's*	97.00	82.51	--	--	82.51*	--	17.49
Di-calsium phosphate	97.00	--	--	--	--	--	--
Limestone, ground	94.10	--	--	--	--	--	--
Salt	94.02	--	--	--	--	--	--
Sodium bicarbonate	95.01	--	--	--	--	--	--
Vitamin & mineral premix	97.63	--	--	--	--	--	--

*EE in Calcium soap of FA's was determined as Acid Ether Extract (AEE).
Chemical compositions of Di-calcium phosphate, Limestone, Salt, Sodium bicarbonate, and Vitamin & mineral premix were cited from NRC, 2001.

Table 2: Ingredient and nutrient composition of diets that vary in CP content.

Ingredient, % of DM	High CP level	Low CP level
Barseem, fresh	12.87	12.92
Corn silage	28.72	30.02
Corn grain, ground, dry	26.84	31.03
Cottonseed meal (decort.)	12.92	12.43
Soybean meal, 44% CP	12.92	7.61
Wheat bran	1.6	1.84
Calcium soaps of FA's (Magnapac)	1.43	1.44
Di-calcium phosphate	0.85	0.85
Limestone, ground	0.80	0.81
Salt	0.45	0.45
Sodium bicarbonate	0.50	0.50
Vitamin & mineral premix	0.1	0.1
Nutrient :		
Dry Matter (DM, kg/d)	20.0	19.95
Dry Matter (DM, %)	48.36	47.83
Crude Protein (CP, % of DM)	19.29	16.90
RUP, % of DM*	12.74	11.03
RDP, % of DM*	6.55	5.87
MP from RUP*, g/d	1233	1018
Microflora Protein (MP total, g/d)*	2456	2243
Non-digested Fiber (NDF, % of DM)*	26.8	26.9
NE_L, Mcal/kg*	1.73	1.72
EAA, g/kg DM)*	65	61

*Calculated by using the NRC, 2001 software program.
RUP: Rumen Undegradable Protein, RDP: Rumen Degradable Protein
NE_L: Net Energy for Lactation, EAA: Essential Amino Acids

Table 3: Least square means and standard errors for nutrient digestibility coefficient of the experimental ration.

Item	Nutrient digestibility coefficient, (%)					
	DM	OM	CP	AEE	CF	NFE
CP level:						
Low level	63.7±0.93[a]	66.9±0.84[a]	64.5±0.95[a]	79.8±2.19	45.9±1.53[a]	72.6±0.67[a]
High level	68.7±1.38[b]	73.3±0.84[b]	72.1±1.45[b]	84.4±1.29	56.9±1.58[b]	76.8±1.17[b]
pMet suppl.:						
Non-suppl.	66.6±1.60	71.1±1.54	68.9±2.56	84.2±1.53	51.7±2.83	75.5±1.62
Supplemented	65.8±1.62	69.1±1.45	67.7±1.40	80.0±2.25	51.1±2.99	73.8±0.82
CP level x pMet supplementation:						
16.9B	64.5±0.47	67.2±0.51	64.1±1.55	81.2±0.70	45.6±1.09	72.7±1.40
16.9M	62.8±1.86	66.6±1.77	65.0±1.38	78.5±4.67	46.2±3.23	72.5±0.50
19.3B	68.6±2.90	75.0±1.47	73.8±2.57	87.1±1.59	57.9±1.06	78.4±1.71
19.3M	68.7±1.05	71.7±0.95	70.3±0.88	81.6±1.10	55.0±3.24	75.2±1.15

a, b: Means followed by different superscript in the same column are significantly different.
16.9B = Basal diet (16.9%CP), 16.9M = Basal diet (16.9%CP) + pMet,
19.3B= Basal (19.3%CP), 19.3M= Basal (19.3%CP)+ pMet.

Table 4: Effects of crude protein level and methionine supplementation on milk yield and its composition in early lactation.

Item	Dietary treatments[1]				Effect[3] (*P*- value)		
	16.9%CP		19.3%CP				
	16.9B	16.9M	19.3B	19.3M	CP	Met	CP x Met
Milk yield, Kg/d	31.7	33.7	33.1	33.4	0.017	0.002	0.064
Milk composition, %							
Protein	2.5	2.7	2.7	2.9	0.0001	0.0001	0.034
Fat	3.6	3.6	3.7	3.7	0.0151	0.587	0.734
Lactose	4.6	4.7	4.7	4.7	0.221	0.540	0.790
Total Solids (TS)	12.1	11.9	12.1	12.3	0.166	0.092	0.633
Solids Not Fat (SNF)	8.5	8.3	8.4	8.6	0.357	0.048	0.642
Milk component, g/d							
Protein	792.5	909.9	893.7	968.6	0.0001	0.004	0.120
Fat	1141.2	1213.2	1224.7	1235.8	0.059	0.072	0.899
Lactose	1438.2	1583.9	1555.7	1569.8	0.089	0.059	0.343
Total Solids (TS)	3835.7	4010.3	4005.1	4108.2	0.286	0.539	0.635
Solids Not Fat (SNF)	2694.5	2797.1	2780.4	2872.4	0.515	0.934	0.559
MUN[2], mg/dl	17.4	16.7	22.6	21.8	0.0001	0.0001	0.899

[1]Treatments: 16.9B = Basal diet (16.9%CP); 16.9M = Basal diet (16.9%CP) + pMet; 19.3B= Basal (19.3%CP); 19.3M= Basal (19.3%CP) + pMet.
[2]MUN= Milk Urea Nitrogen.
[3]Effect: CP = main effect of CP; Met = main effect of methionine; CP x methionine interaction.

Table 5: Effects of crude protein level and methionine supplementation on milk yield and its composition in mid lactation.

Item	Dietary treatments[1]				Effect[3] (*P*- value)		
	16.9%CP		19.3%CP				
	16.9B	16.9M	19.3B	19.3M	CP	Met	CP x Met
Milk yield, Kg/ d	38.2	38.7	38.3	38.6	0.861	0.402	0.888
Milk composition, %							
Protein	2.5	2.6	2.6	2.8	0.0001	0.0001	0.0001
Fat	3.6	3.6	3.7	3.7	0.005	0.357	0.390
Lactose	4.5	4.5	4.5	4.5	0.115	0.572	0.505
Total Solids (TS)	11.5	11.8	11.8	12.0	0.0001	0.0001	0.045
Solids Not Fat (SNF)	7.9	8.2	8.1	8.3	0.0001	0.0001	0.004
Milk component, g/ d							
Protein	955.0	1006.2	955.8	1080.8	0.0001	0.020	0.120
Fat	1375.2	1393.2	1417.1	1428.2	0.077	0.485	0.899
Lactose	1719.0	1741.2	1723.5	1737.0	0.711	0.250	0.389
Total Solids (TS)	4393.0	4566.6	4519.4	4632.0	0.075	0.860	0.537
Solids Not Fat (SNF)	3017.8	3173.4	3102.3	3203.8	0.091	0.960	0.423
MUN[2], mg/ dl	20.1	19.6	24.9	24.5	0.0001	0.010	0.899

[1]Treatments: 16.9B = Basal diet (16.9% CP); 16.9M = Basal diet (16.9% CP) + pMet; 19.3B= Basal (19.3% CP); 19.3M= Basal (19.3% CP) + pMet.
[2]MUN= Milk Urea Nitrogen.
[3]Effect: CP = main effect of CP; Met = main effect of methionine; CP x methionine interaction.

Table 6: Effects of crude protein level and methionine supplementation on milk yield and its composition in late lactation.

Item	Dietary treatments[1]				Effect[3] (*P*- value)		
	16.9%CP		19.3%CP				
	16.9B	16.9M	19.3B	19.3M	CP	Met	CP x Met
Milk yield, Kg/ d	23.4	23.5	23.2	22.9	0.580	0.699	0.226
Milk composition, %							
Protein	2.5	2.7	2.7	2.9	0.0001	0.0001	0.738
Fat	3.6	3.7	2.7	3.7	0.0001	0.0001	0.0001
Lactose	4.5	4.6	4.6	4.6	0.861	0.614	0.871
Total Solids (TS)	11.9	12.1	12.2	12.4	0.0001	0.0001	0.850
Solids Not Fat (SNF)	8.3	8.4	9.5	8.7	0.0001	0.0001	0.866
Milk component, g/ d							
Protein	585.0	634.5	626.4	664.1	0.0001	0.0001	0.673
Fat	842.4	869.5	626.4	847.3	0.0001	0.0001	0.0001
Lactose	1053.0	1081.0	1067.2	1053.1	0.458	0.669	0.805
Total Solids (TS)	2784.6	2843.5	2830.4	2839.6	0.021	0.371	0.752
Solids Not Fat (SNF)	1942.2	1974.0	2204.0	1992.3	0.010	0.198	0.786
MUN[2], mg/ dl	18.8	18.0	22.6	22.0	0.0001	0.0001	0.311

[1]Treatments: 16.9B = Basal diet (16.9%CP); 16.9M = Basal diet (16.9%CP) + pMet; 19.3B= Basal (19.3%CP); 19.3M= Basal (19.3%CP) + pMet.
[2]MUN= Milk Urea Nitrogen.
[3]Effect: CP = main effect of CP; Met = main effect of methionine; CP x methionine interaction.

Table 7: Feed and economical efficiencies for various groups of cows**.

Item		Group				pMet		CP level	
		1	2	3	4	0	1	H	L
Early	DMI (kg/ d)	22.7	22.8	22.5	22.9	22.6	22.9	22.8	22.7
	Milk (kg)/ kg DMI	1.40	1.48	1.47	1.46	1.36	1.47	1.46	1.44
	N utilization efficiency	20.24	23.13	20.16	21.47	18.4	22.5	19.6	21.3
	Cost of feed/ kg milk	0.96	0.96	1.05	1.11	1.07	1.03	1.09	0.96
Mid	DMI (kg/ d)	23.5	23.9	24.0	23.8	23.8	23.9	23.7	23.9
	Milk (kg)/ kg DMI	1.63	1.62	1.60	1.62	1.61	1.62	1.62	1.61
	N utilization efficiency	23.56	24.40	21.06	23.05	23.9	24.4	25.0	23.3
	Cost of feed/ kg milk	0.83	0.87	0.97	0.99	0.90	0.94	0.98	0.86
Late	DMI (kg/ d)	18.2	18.1	17.9	18.4	18.3	18.1	18.2	18.2
	Milk (kg)/ kg DMI	1.29	1.30	1.30	1.24	1.27	1.28	1.29	1.27
	N utilization efficiency	18.63	20.32	17.76	18.32	19.1	20.3	20.0	19.4
	Cost of feed/ kg milk	1.05	1.10	1.19	1.31	1.14	1.18	1.22	1.09

** Group 1: received low CP level (16.9% on DM basis) diet non-supplemented with pMet,
Group 2: received as that of group 1 diet, but supplemented with pMet,
Group 3: received high CP level (19.3% on DM basis) diet non-supplemented with pMet,
Group 4: received as that of group 1 diet, but supplemented with pMet,
O= nonsuplemented, 1= supplemented, H=high CP level and L=low CP level.

DISCUSSION

Apparent Digestibility of Nutrients

This study showed that, except for EE, digestibility coefficients of nutrients was significantly improved by increasing dietary CP diet. Ha and Kenelly (1984) have suggested that higher apparent digestibility of DM and CP at higher dietary CP concentrations result from a combination of improved microbial fermentation and greater digestion in the gastrointestinal tract. Supplementation of pMet had no effect on digestibility coefficient of nutrients. These results are consistent with those reported by Vazquez-Anon *et al.* (2001), who reported no improvement was observed in nutrient digestibility when liquid methionine was added to the diet. In agreement with these results, Blum *et al.* (1999) reported that supplementation with different types of pMet did not affect nutritive value.

Lactation Responses

Supplementation of pMet increased daily milk yield mainly in early lactation period, as similar as high CP level did. This may be attributed to the challenge in diet formulation in achieving the desired concentration of methionine by relying solely on feed protein supplements during early lactation period; the period in which dry matter intake is relatively low and the level of milk production progressively increases. In agreement with the results of this study, others have noted that Met was limiting for milk yield, especially in early lactation cows (Samuelson *et al.,* 2001; Noftsger and St. Pierre, 2003). However, the present finding disagrees with previous reports (Leonardi *et al.,* 2003) that have reported a lack of significant effect of pMet supplementation on milk production, especially when corn silage in the ration was the main forage. Possible explanations for these inconsistent responses are the variable doses used in the studies, the strategies that by which pMet was used, the quantity of CP in the diet, the nature of the supplemental protein source, particularly its AA contents, and the level of milk production.

The production response to pMet supplementation was greater with the low level of CP diets. This finding agrees with that reported by NRC (2001). Who concluded from a number of reviews that production response to pMet supplementation were greater when CP level approximates the normal levels (14 to 18 percent) than when it was lower or higher.

Supplementing the diet of low CP level with pMet increased milk protein percentage to the same level produced from cows fed diet with high CP level. These results are similar to those reported by both of Misciattelli *et al.*, (2003), Socha *et al.*, (2005). According to these authors, pMet supplementation increased milk protein content during early- and mid-lactation when the main source of protein was soybean meal. During early and mid lactation, cows have a greater demand for Met to meet the demands of high milk yield and, consequently, the dietary Met content is expected to be deficient in the low level CP diet, but in late lactation, these demands decline as milk yield decreases. So, added Met might partially compensated the shortage of dietary Met resulting from decreasing CP level in the diet during early and mid lactation, whereas it had no revenue when was supplemented to the low CP diet in late lactation that might be originally sufficient in Met content. Literature summaries confirm that responses to postruminal lysine (Lys.) and methionine (Met.) supplementation are greater when basal levels of Lys and Met in Rumen undegradable protein (RUP) are low rather than high, when cows are in early rather than mid or late lactation, and in high- producing cows rather than low producing cows (Rulquin and Verite, 1993; NRC, 2001). pMet supplementation had no effect on milk fat and lactose content during the early and mid stage of lactation. These results are in consistent with those reported in most researches (Pacheco-Rios *et al.*, 1999; Leonardi *et al.*, 2003). The increases in TS content observed during mid and late lactation are mainly attributed to the increases in milk fat and protein concentrations during those periods, while that observed in SNF percentage may be due to the increase in milk protein content.

Cows fed high CP diets had greater concentrations of milk urea nitrogen (MUN) and plasma urea nitrogen (PUN; data not shown), probably due to the higher N intake on the diet. Similar results were reported by Leonardi *et al.* (2003), who found an increase of 3.8 mg/ dl of milk urea nitrogen when dietary protein increased by 2.7-percentage unites. Hutjens and Barmore (1995) suggested that MUN in the range of 12 to 17 mg/dl would indicate optimal balance of RDP and ruminally fermentable energy. Therefore, elevated MUN above the upper level of this range may indicate excess protein has been fed to the dairy cow for her given level of production (Jonker *et al.*, 1998). Others have also noted that MUN had a positive relationship with levels of dietary CP (Frank and Swenson, 2002) and dietary CP content was the best single predictor of MUN (Nousiainen *et al.*, 2004). The primary field application of these results is that elevated MUN, above target levels, would indicate that excess protein is being consumed. Reformulating the diet with a lower protein concentration could reduce MUN concentration.

Feed efficiency:

Feed efficiency is a measure of how well cows convert the nutrients they eat into products. In the current study, the feed efficiency was determined as milk (Kg) / dry matter intake (Kg), milk nitrogen/ intake nitrogen, and cost of feed per kg milk. Supplementing the diet of low CP level with pMet improved the feed efficiency. The N utilization efficiency gives an index of feed protein utilization, and should likely go in the opposite direction that MUN values go. N utilization efficiency was improved by feeding diets supplemented with pMet. Which may indicate that feeding dietary CP level close to NRC recommendations and supported with adjusted amount of pMet is an acceptable nutritional strategy for improving feed efficiency and N utilization efficiency in dairy cattle. The cost of feed per kg milk was reduced in cows fed diets supplemented with pMet at early stage of lactation, but in mid and late stage of lactation it increased by the same value, which may indicate that the supplementation of pMet is profitable in early lactation, but not profitable in mid or late stage of lactation. Although, increasing of dietary CP level increased milk production in the current study, cows fed diets reduced in CP level had a reduction in cost of feed per kg milk in various stages of lactation. This may indicate that the point of maximum profitability is likely to be at a dietary protein concentration below that needed for maximum milk production.

Collecting all the results together, Cows receiving 16.9% CP plus pMet (16.9M), consumed the same amount of DM, produced numerically more average daily milk, same milk protein content, reduced in cost of feed per kg milk in various stages of lactation and converted dietary N to milk N with a higher gross efficiency when compared to cows receiving 19.3% CP. These results suggested that 16.9M were similar, if not superior, in nutritive value to 19.3B.

In conclusion, the results obtained in the present study indicate that close management of dietary protein is needed to maximize profits. Furthermore, feeding dietary CP lowered to the level that achieves the optimal concentration of MUN with supplementation of pMet to compensate for low dietary CP level, as that in group 2, (16.9M) improved feed and N utilization efficiencies and reduced the cost of feed per kg milk. It could be an acceptable new nutritional strategy for improving productive performance and may be used as an approach for reformulating lactating cows rations based on AA profile rather than CP as simple or metabolizable protein.

REFERENCES

Abo-Donia, F.M.A.; Ibrahim, S.A.; El-Shabrawy, H.M. and Salama, A.M.A. (2003): Milk production and composition of Friesian crossbred cows fed rations containing different sources of energy. The 9th Scientific Conference on Animal Nutrition October 2003-Horgada. (507).

Association of Official Analytical Chemists (A.O.A.C), (1984): Official Methods of Analysis. 12th ed. AOAC, Arlington, VA.

Bach, A.; Huntington, G.B.; Calsamiglia, S. and Stern, M.D. (2000): Nitrogen metabolism of early lactation cows fed diet with two different levels of protein and different amino acid profiles. J. Dairy Sci. 83: 2585-2595.

Blum, J.W.; Bruckmaier, R.M. and Jans, F. (1999): Rumen-protected methionine fed to dairy cows: bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. J. Dairy Sci. 82: 1991-1998

Broderick, G.A. (2003): Effects of varying protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370-1381.

Chase, L.E. (1994): Environmental considerations in developing dairy rations. Pages 56-62 in Proc. Cornell Nutr. Conf. Feed Manuf., Rochester, NY. Cornell Univ., Ithaca, NY.

Crocker, C.L. (1967): Rapid determination of urea nitrogen in serum or plasma without deproteination. Am. J. Med. Technol. 33: 361-365.

Duncan, D.B. (1955): Multiple range and multiple F test. Biometrix 11: 1-42.

Frank, B. and Swenson, C. (2002): Relationship between content of crude protein in rations for dairy cows and milk yield, concentration of urea in milk and ammonia emissions. J. Dairy Sci. 85: 1829–1838.

Ha, J.K. and Kennelly, J.J. (1984): Effect of protein on nutrient digestion and milk production by Holstein cows. J. Dairy Sci. 67: 2302-2307.

Hutjens, M.F. and Barmore, J.A. (1995): Milk urea test gives us another tool. Hoard's Dairyman. May 25, p. 401.

Jonker, J.S.; Kohn, R.A. and Erdman, R.A. (1998): Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cattle. J. Dairy Sci. 81: 2681–2692.

Kalscheur, K.F.; Vandersall, J.H.; Erdman, R.A.; Kohn, R.A. and Russek-Cohen, E. (1999): Effects of dietary crude protein concentration and degradability on milk production responses of early, mid, and late lactation dairy cows. J. Dairy Sci. 82: 545-554.

Leonardi, C.; Stevenson, M. and Armentano, L.E. (2003): Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. J. Dairy Sci. 86: 4033-4042

Misciattelli, L.; Kristensen, V.F.; Vestergaard, M.; Weisbjerg, M.R.; Sejrsen, K. and Hvelplund, T. (2003): Milk production, nutrient utilization, and endocrine responses to increased postruminal lysine and methionine supply in dairy cows. J. Dairy Sci. 86: 275-286.

National Research Council (NRC, 2001): Nutrient requirements of dairy cattle. (7th rev. ed.). National Academy Press, Washington, DC, USA.

Noftsger, E. and St-Pierre, N.R. (2003): Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J. Dairy Sci. 86: 958-969.

Nousiainen, J.; Shingfield, K.J. and Huhtanen, P. (2004): Evaluation of milk urea nitrogen as a diagnostic of protein feeding. J. Dairy Sci. 87: 386-398.

Pacheco-Rios, D.; McNabb, W.C.; Hill, J.P.; Barry, T.N. and Mackenzie, D.D.S. (1999): The effects of methionine supplementation upon milk composition and production of forage-fed dairy cows. Can. J. Anim. Sci.: 235-240.

Rulquin, H. and Verite, R. (1993): Amino acid nutrition of dairy cows: Production effects and animal requirements. Page 55 in Recent Advances in Animal Production, Proc. 27th Univ. Nottingham Feed Manufacturer's Conf., P. C. Gainsworthy, ed. Nottingham University Press, Nottingham, UK.

Samuelson, D.J.; Denise, S.K.; Roffler, R.; Ax, R.L.; Armstrong, D.V. and Romagnolo, D.F.J. (2001): Response of Holstein and Brown Swiss cows fed alfalfa hay-based diets to supplemental methionine at two stages of lactation. J. Dairy Sci. 84: 917-928.

SAS User's Guide: Statistics. (1996): Version 5 Edition. SAS Inst., Inc., Cary, NC.

Socha, M.T.; Putnam, D.E.; Garthwaite, B.D.; Whitehouse, N.L.; Kierstead, N.A.; Schwab, C.G.; Ducharme, G.A. and Robert, J.C. (2005): Improving intestinal amino acid supply of pre-and postpartum dairy cows with rumen-protected methionine and lysine. J. Dairy Sci. 88: 113-1126.

St-Pierre, N. and Thraen, C.S. (1999): Animal grouping strategies, sources of variation, and economic factors affecting nutrient balance on dairy farms. J. Anim. Sci., 77 (Suppl. 2): 72-83.

Tamminga, S. (1992): Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75: 345-357.

Van Horn, H.H.; Wilkie, A.C.; Powers, W.J. and Nordstedt, R.A. (1994): Components of dairy manure management systems. J. Dairy Sci. 77: 2008-2030.

Van Keulen, J. and Young, B.A. (1977): Evaluation of acid-insoluble ash as natural marker in ruminant digestibility. J. Anim. Sci. 44: 262-266.

Vazquez-Anon, M.; Cassidy, T.; McCullough, P. and Varga, G.A. (2001): Effects of aliment on nutrient digestibility, bacterial protein synthesis, and ruminal disappearance during continuous culture. J. Dairy Sci. 84: 159-166.