RESPONSE OF LACTATING BUFFALOES FOR RUMINALLY PROTECTED FAT AND PROTECTED AMINO ACIDS SUPPLEMENTATION

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SUMMARY

Four multiparous early lactating buffaloes (3-5 years old, 532 kg average body weight and 52 days in milk) were used in single 4x4 Latin Square experiment. All animals were subjected to four different experimental diets; 1) control diet, consisted of concentrate feed mixture, berseem clover and rice straw without supplementation, 2) control diet supplemented with 400 g protected fat source (calcium soap, Magnapac®); 3) control diet supplemented with ruminal protected amino acids (15g Methionine + 40g Lysine); and 4) control diet supplemented with (400g Magnapac® + 15g Methionine + 40g Lysine). Results obtained indicated that apparent digestibility of nutrients, amount of total digestible nutrients, and digestible crude protein were not significantly differed among treatments. However, milk composition also not significantly changed, actual milk yield not significantly increased, but the amount of 7% fat corrected milk (FCM) increased significantly ($p<0.05$) in response to protected fat and protected amino acid supplementations. Blood serum total lipid content and alanine-aminotransferase (ALT) activity were significantly increased ($p<0.05$) as a result of protected fat with protected amino acids supplemented diets. Creatinine as an endogenous protein metabolite was significantly increased ($p<0.05$) as protected amino acids was added to the diet of buffalos. In respect of economical perspective, supplementing lactating buffalo's diet with protected fat and/or protected amino acids is infeasible.

Keywords: *buffalo, protected fat, protected amino acids, digestibility, milk, blood metabolites*

INTRODUCTION

During early lactation, cows are often in negative energy and nitrogen balance because of the maximal DMI does not occur until after peak milk production (Oldham, 1984). Energy (fibre and fat) and amino acids are the main nutritional factors which are limit the amount of milk production. The relationship between nitrogen (N) and

energy requirements for dairy cows is complex because there are two requirements to be met: one for the host animal and another for ruminal microbes (Oldham, 1984).

Adding fat or high fat containing feedstuffs to increase the energy density of diets and energy intake for early lactating dairy cows is becoming common practice (Bull, 1987 and Oldham, 1981). Use of dietary fat may continue to increase as the genetic potential for milk production increases. However, feeding large amounts of ruminally unprotected fat may have detrimental effects on fiber digestibility (Palmquist, 1987). Oilseeds and animal or animal-vegetable blends are acceptable fat supplements; however, partial substitution with ruminally inert fats might be warranted if the previously mentioned fat supplements are adversely affecting ruminal fermentation, milk fat percentage, or DM intake (Jerred *et al.*, 1990; Chilliard, 1993). Added dietary fat particularly if those containing high percentage of unsaturated fatty acids, often depress milk protein content by 0.1 to 0.2 percentage units (Wilks, *et al.* 1991), although total protein yields are similar (Klusmeyer, *et al.*, 1991). In general, added dietary fat depresses total milk N, casein N, and total protein N (DePeters and Cant, 1992). The development of Ca salts of fatty acids (Ca-FA), which are considered mostly inert in the rumen, offers a method of increasing the energy density of diets without affecting fiber digestion.

Lysine (Lys.) and methionine (Met.) have been identified as potentially limiting amino acids for milk protein synthesis, particularly when corn-based diets are fed (Schwab, *et al.*, 1976). Ruminally protected amino acids (RPAA) have increased milk protein percentage in cows fed diets based on corn silage (Donkii, *et al.*, 1989 and Rogers, *et al.*, 1987), and grass silage (Girdler, *et al.*, 1988). Dietary supplementation of ruminally protected Lys and Met prevents the reduction in milk casein percentage that often occurs in the case of fat supplementation (Canale, *et al.*, 1990 and Chow, *et al.*, 1990). It suggest that the AA profile of ruminally undegradable protein is important to prevent the decrease in milk protein content of cows fed supplemental fat. Lysine is the first limiting AA for milk and milk protein yield in cows fed diets based on corn (King, *et al.*, 1991, and Schwab, *et al.*, 1992).

The objectives of present investigation were to study the effects of supplementing diets with Ca-FA and/or ruminal protected amino acids on milk yield, composition, nutrients digestibility, and some blood metabolites of lactating buffaloes in early lactation.

MATERIALS AND METHODS

This investigation was carried out at the Agricultural Experimental Farm in Faculty of Agriculture, Cairo University, Giza, in collaboration with Dairy Science Department, National Research Centre, Dokki, Giza, during spring seasons in the years 2005-2006.

Animals and treatments:

Four multiparous early lactating buffaloes (3-5 years old, 532 kg average weight and 52 days in milk)

were used in single 4x4 Latin Square. The experiment consisted of four periods (21 days each) and extended for 84 days entire period through restricted feeding system. Dietary treatments were (1) control, consisted of concentrate feed mixture (CFM) (consisted of 15% whole cotton seed meal, 20% wheat bran, 53% yellow corn, 10% soybean meal, 1% limestone and 1% sodium chloride), berseem and rice straw; (2) control + 400 g calcium soap of palm oil (Magnapac®, Norel S.A., Spain); (3) control + 15 g protected methionine (Met) + 40g protected lysine (Lys), produced by ADWIA company, Egypt and treated with formaldehyde (40%) according to the method suggested by Fergueson (1975); and (4) control + 400 g calcium soap of palm oil + 15 g methionine + 40 g lysine. The ingredients and chemical composition of the experimental diets are presented in Table (1). Nutrients requirements were calculated according to NRC (2001). The CFM allowance was offered twice daily in equal portions during milking times (6.00 a.m. and 6.00 p.m.), berseem was offered at 10.00 a.m. after animals have freely accessed to drinking water, while rice straw was offered overnight. The daily supplemental calcium soaps of palm oil (Ca-FA) and rumen protected amino acids (RPAA) were daily mixed with CFM just before morning feeding to ensure that each animal had consumed its own supplement.

Sample collection and analysis:

Feces grab samples were withdrawn by the end of each experimental period at 10.00 a.m. and 4:00 p.m. for three successive days from each animal. Subsample (10%) of total collected feces of each individual was taken and sprayed with 10% formaldehyde, 10% sulfuric acid, and then dried at 70°C for 24 hours. Concentrate feed mixture, berseem, rice straw, calcium soaps, protected amino acids and feces grab samples were analyzed for dry matter (DM), ash, crude protein (CP), crude fiber (CF), and ether extract (EE) according to A.O.A.C. (1995) while, nitrogen free extract (NFE) was calculated. Acidic ether extract of Magnapac was determined according to (Drackley, *et al.* 1985). Acid insoluble ash (silica) as internal marker was applied for digestibilities determination according to (Gallup *et al.*, 1945 and Forbes and Garrigus 1948).

The animals were hand-milked twice daily and yield was recorded during the entire experimental period. During the last four days of each period, 100 ml milk samples were collected from each animal at evening and morning milking, respectively. Composite daily milk sample (relative to the quantity of milk produced were used for total solids (TS), fat, total protein (TP), lactose, solids not fat (SNF) and urea determinations using infrared spectroscopy (Milkcoscan, B. Foss Electric, France), pH and ash were measured according to Ling (1963).

Blood samples were taken at the final day of milk sampling at four hours after morning feeding from jugular vein from all animals. Collected blood samples were centrifuged at 4000 r.p.m. for 20 min. and the supernatant was stored in glass vials at -18°C till

Table (1): Chemical analysis of Dietary ingredients (%DM).

Item	Diet ingredients*					
	CFM	B	RS	Met	Lys	Magnapac
Dry matter	90.39	20.00	94.28	99.82	99.15	96.55
Organic matter	92.4	86.42	83.94	99.83	99.96	83.46
Ash	7.60	13.58	16.06	0.17	0.04	15.96
Crude protein	16.4	18.22	3.85	70.32**	62.57***	-
Crude fiber	6.9	23.04	33.94	-	-	-
Ether extract	4.17	3.59	2.10	1.58	2.17	83.46
Nitrogen-free-extract	64.93	41.57	41.47	26.93	35.22	-

* concentrate feed mixture (CFM), berseem (B), rice straw (RS), protected methionine (Me), protected lysine (Ly) and magnapac (Calcium soaps of palm oil).
** Total nitrogen is multiplied by (9.4).
*** Total nitrogen is multiplied by (5.26).

Table (2): Dietary Ingredients composition and chemical analysis (%DM).

Item	Control	Ca-FA	AA	Ca- FA+AA
Ingredient, %				
CFM	57.64	56.88	55.70	52.71
Berseem	34.60	35.03	35.21	٣5.16
Rice straw	7.76	5.23	8.70	9.05
Ca-FA	-	2.86	-	2.69
Methionine	-	-	0.11	0.11
Lysine	-	-	0.28	0.28
R:C ratio	42 : 58	41: 59	44:56	45 :55
Chemical analysis, %				
Dry matter	90.85	90.93	91.13	91.12
Organic matter	89.67	89.63	89.79	89.33
Crude protein	16.06	15.90	16.21	15.67
Crude fiber	14.54	13.73	14.89	14.78
Ether extract	3.81	6.14	3.79	5.92
N-free extract	55.05	53.56	54.68	52.73
Ash	10.31	10.36	10.34	10.56
Feeding value, %				
DCP	10.62	11.15	11.05	10.74
TDN	62.50	65.88	63.08	65.13
DE, M Cal/Kg*	2.76	2.90	2.78	2.87
ME, M Cal/Kg**	2.34	2.48	2.36	2.45
NE_l, M Cal/Kg***	1.39	1.49	1.43	1.48

The calorific values of the experimental diets were estimated as follows: * DE (M Cal/Kg DM) = 0.04409 (TDN %, ** ME (M Cal/Kg DM) = 1.01 (DE, M Cal/Kg DM) – 0.45, *** NE_l (M Cal/Kg DM)= 0.0245 (TDN %) – 0.12

analysis. Serum total protein was determined according to Gornal *et al.*, (1949), albumin (Doumas *et al.*, 1971), urea (Fawcett and Soctt 1960), transaminases (AST and ALT) activities (Reitman and Frankel 1957), glucose (Trinder 1969), creatinine (Larsen, 1972), total lipids (Zollner and Kirsch 1962), and total cholesterol (Allain *et al.*, 1974). Globulin, and albumin to globulin ratio (A/G) were calculated.

Statistical analysis:

The data of milk yield, milk composition, dry matter intake (DMI), nutrients digestibility, and blood serum metabolites were analyzed according to Statistical Analysis System (SAS, 1998) using Latin Square design where the model was:

$$Y_{ijk} = U + T_i + P_j + A_k + E_{ijk}$$

where, Y: stands for every observation of the K^{th} animal in the J^{th} period given I^{th} treatment, T: treatment effect, P: periods effect, A: animal effect and E: the experimental error. Duncan's multiple range test (Duncan, 1955) was ran out for means separation.

RESULTS AND DISCUSSION

Ingredients analysis and diets composition:

The ingredients analyses are presented in Table (1). Based on dry matter percentage, the organic matter of the amino acids supplements were the highest comparing to the other ingredients. As calcium soap is mainly protected through binding with calcium cation, the ash content is relatively high *vs.* the other ingredients.

The ingredients composition and chemical analyses of the experimental diets are shown in Table (2). The roughage concentrate ratio was comparable for both the control diet and Ca-FA supplemented diet, while slightly higher for the rest of the other experimental diets. All the nutrients analyses are fairly similar to all the experimental diets, whereas ether extract was remarkably higher in the diets supplemented with Ca-FA alone or mixed with the amino acids (AA) supplemented diet. Nevertheless, protected amino acids were supplemented; all diets were fairly iso-nitrogenous. Digestible energy (DE), metabolizable energy (ME), and net energy for lactation (NE_l) were estimated according to NRC (2001). For all the experimental diets, the ether extract percentages were within the limit (6-7% of dietary dry matter) as recommended by (Schauff and Clark 1992).

Dry matter intake and nutrients digestibility:

Effect of calcium soap of palm oil and rumen protected amino acids (Met and Lys) supplementations on DMI and nutrients digestibility of lactating buffaloes are shown in Table (3). DM intakes did not differ among treatments, these result is in agreement with (Bertrand *et al.*, 1998; and Garcia-Bojalil, *et al.*, 1998). Similarly, not significant differences were observed among diets in apparent digestibilities of DM, OM, CP, EE, CF, and NFE which agreed with the findings of (Anderson, *et al.*, 1984; El-Bedawy, *et al.*, 1994; Palmquist, 1995; Aboul-

Fotouh, *et al.*, 1999 and Deluca and Jenkins, 2000) .All the nutrients digestibilities of buffaloes fed on diet supplemented with protected fat were slightly higher with the exception of fiber digestibility, which recorded the lowest value among all treatments as described also in some previous studies (Ferlay *et al.* 1992; Smith, *et al.*, 1993; Bernard, *et al.*, 1999 and Deluca ad Jenkins, 2000). But these data are coincide with the findings of Karalazos, *et al.* (1992) with sheep. Devendra and Lewis (1974) proposed the following factors to explain the reduction always occurred in ruminal fibre digestion in response to dietary fat supplementation; 1) modification of the ruminal microbial population; 2) inhibition of ruminal microbial activity; 3) reduced cation avilability.

Milk yield and composition:

Effect of protected fat and rumen protected amino acids (Met and Lys) supplementation on milk yield and its composition of lactating buffaloes are shown in Table (4). Milk yield response to supplemental fat can be influenced by several factors, including basal diet, stage of lactation, energy balance, fat composition, and amount of supplemental fat (National Research Council, 2001). Supplemental fat has increased milk yield as was found in many studies; however, responses have been variable. Milk yield was not significantly (P>0.05) increased in lactating buffaloes in response to dietary protected fat and or amino acids supplementation as compared to those fed on the control diet. In contrary, 7% fat corrected milk was significantly increased in buffaloes fed on diets supplemented with both protected fat and protected amino acids compared to the control diet as reported by (Klusmeyer *et al.*, 1991). These results indicate that the associated effect and potential of both protected fat and protected amino acids is greater than their separate individual effect on milk yield. Milk yield response to supplemental fat is curvilinear; the response diminishes as supplemented fat in the diet increases (Palmquist, 1983 and Jenkins 1994). Fat, total solids, solids-non-fat, total protein, lactose, urea, and ash percentages were not significantly higher (P >0.05) in treatments received Ca-FA or protected amino acids than that control ration. These findings are in agreement with that reported by (Palmquist, 1991; Schauff and Clark, 1992; Polidori *et al.*, 1997; Moallem *et al.*, 1997, and Garcia-Bojalil *et al.*, 1998). In the present study, protein percentage was not affected by protected fat supplementation, however it was significantly depressed in other investigations (Canale *et al.*, 1990; Elliott *et al.*, 1996; Chouinard *et al.*, 1997, and Bertrand *et al.*, 1998). Milk pH values were not significantly differed among treatments.

Blood serum metabolites:

Data in Table (5) showed no significant differences (P>0.05) in the amount/activity of the blood serum metabolites (total protein, albumin, globulin, A/G ratio, urea, AST, glucose and cholesterol) among the different treatments. Serum glucose concentrations were not significantly higher in control than the other treatments (P>0.05), that was accompanying with not significantly higher lactose levels. The treatment

Table (3): Dry matter intake and nutrients digestibility as affected by protected fat and / or protected amino acids supplementation in lactating buffalo's diet.

Item	Treatments				±SE
	Control	Ca-FA	AA	Ca-FA+AA	
Dry matter intake (kg/d)	13.60[a]	13.50	14.20	14.36	0.62
Nutrient digestibility (%)					
DM	62.50	65.00	62.75	65.00	2.80
OM	66.15	68.12	65.90	68.45	2.56
CP	66.05	70.35	68.21	68.55	2.45
CF	58.00	53.00	56.33	57.60	3.59
EE	66.85	70.40	65.97	65.30	2.79
NFE	68.35	70.65	69.42	70.45	8.02

Each value of means obtained from 4 animals.
Dissimilar superscripts (a,b) at the same row means significant (P<0.05)

Table (4): Milk yield and composition as affected by protected fat and / or protected amino acids supplementation in lactating buffalo's diet.

Item	Treatments				±SE
	Control	Ca-FA	AA	Ca-FA+AA	
Milk yield (Kg/h/d.)					
Milk yield	10.08	10.72	10.82	11.32	0.49
7% Fat-corrected milk*	8.33[b]	8.79[ab]	9.11[ab]	9.57[a]	0.31
Milk composition (%)					
Fat	5.38	5.35	5.51	5.53	0.32
Total solids	14.31	14.58	14.61	14.79	0.36
Solids-not-fat	8.89	9.26	9.10	9.25	0.21
Total protein	3.21	3.38	3.24	3.36	0.11
Urea	21.05	24.66	23.73	23.11	1.91
Lactose	4.69	4.82	4.82	4.82	0.12
Ash	0.85	0.88	0.86	0.98	0.05
pH value	6.75	6.78	6.75	6.74	0.02

*Fat- corrected milk was calculated as (7%FCM) = 0.265 x milk yield (Kg) + 10.5 x fat yield (Kg) (Raafat *et al.* 1963).
Dissimilar superscripts (a,b) at the same row means significant (P<0.05)

Table(5): Blood serum metabolites as affected by protected fat and / or protected amino acids supplementation in lactating buffalo's diet.

Item	Treatments				±SE
	Control	Ca-FA	A.A	Ca-FA+A.A	
Total protein (g/dl)	7.36	7.65	7.33	7.7	0.39
Albumin (g/dl)	3.16	3.39	3.08	3.16	0.11
Globulin (g/dl)	4.19	4.26	4.24	4.53	0.36
A/G ratio	0.77	0.79	0.73	0.70	0.06
Urea (mg/dl)	36.57	35.60	37.58	34.69	2.14
Creatinine (mg/dl)	1.80^{ab}	1.46^{b}	2.37^{a}	2.04^{ab}	0.21
AST (units/L)	52.74	52.79	53.32	53.68	065
ALT (units/ L)	27.27^{ab}	27.97^{ab}	26.93^{b}	28.13^{a}	0.32
Glucose (mg/dl)	86.50	79.13	76.74	69.75	8.05
Total lipids (mg/dl)	345.05^{b}	403.44^{ab}	426.64^{ab}	525.45^{a}	40.94
Cholesterol (mg/dl)	120.28	128.94	124.45	144.14	57.74

Each value of means obtained from 4 animals.
Dissimilar superscripts (a,b) at the same row means significant ($P<0.05$)

Table (6): Economic efficiency of milk production as affected by protected fat and / or protected amino acids supplementation in lactating buffalo's diet.

Item	Control	Ca-FA	A.A	Ca- FA+A.A
Feeding cost				
CFM	9.53	9.33	9.62	9.20
Berseem	2.35	2.36	2.50	2.52
Rice straw	0.09	0.06	1.05	1.09
Ca-FA	-	1.68	-	1.68
A.A	-	-	1.25	1.25
Total cost, L.E./day	11.98	13.43	14.42	15.75
Price of milk, L. E.	30.24	32.16	32.46	33.96
Cost of produced Kg milk, LE	1.19	1.25	1.33	1.39
Return over feeding cost, L.E/day	18.26	18.73	18.04	18.21
Relative economic efficiency %	100	102.6	98.80	99.7

The price of feedstuffs and supplementations: CFM/ Ton = 1100 LE; Berseem/ Ton = 100 LE; Rice straw = 80 LE; Ca-FA/ Ton = 4200 LE; Methionine/ Kg = 22 LE; Lysine/ Kg = 23 LE and raw milk (farm gate price/ Kg) = 3.00 LE.

possibly lowers serum glucose concentration, because of synthesizing greater quantities of lactose and other milk constituents than in control animals. Blood lipid metabolites reflect the positive influence of protected fat supplementation to lactating buffaloes diet on animal health and consequently also on human health because total lipids showed significant increase ($P<0.05$) as response to feeding protected fat and protected amino acids supplemented diets. Serum cholesterol concentration was not significantly increased in buffaloes fed on protected fat supplemented diets. The serum total lipids and cholesterol results are confirmed by (Hawkins, *et al.*, 1985; Barrza *et al.*, 1991; Deluca and Jenkins, 2000; Abdel Gawad, 2003, and El-Bedawy, *et al.*, 2004). Nestel, *et al.*, (1978) proposed that, an increase in dietary fat stimulates intestinal cholesterol synthesis to meet the increased demand for absorption and transport of fat. Creatinine was significantly increased with buffaloes fed on protected amino acids diet. Alanine-aminotransferase (ALT) activity showed slight significant difference with protected fat and amino acids supplementation compared to the other treatments.

Economic efficiency:

The economic efficiency of supplementing lactating buffaloes diet with protected fat and /or protected amino acids has been calculated (Table 6). Based on feeding cost and milk selling return, cost per one kg produced milk, return over feeding cost and the relative economic efficiency were calculated. The economic efficiency calculations indicted that there were no differences among the different experimental diets in term of return over feeding (LE) or relative efficiency to the basal diet (control diet). The slight increase in milk production in the diets supplemented with protected fats or protected amino acid was diminishing as a result of increasing feeding cost.

CONCLUSION

Adding the protected fat and/or protected amino acids to the diet of buffaloes in early lactation had a slight increase effect on milk yield. In addition, digestibility of nutrients, nutritive value, and milk composition did not change significantly. Blood lipid metabolites tended to increase in response to protected fat supplementation. In term of money (LE), the economic efficiency analysis indicated that the gain of milk yield is almost equal to the extra feeding cost. Addition of protected fat and/or protected amino acids seems to be more efficient with high yielding animals, which in a negative energy balance occurred and dietary energy demand increases.

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استجابة الجاموس الحلاب لإضافات الدهون المحمية والأحماض الأمينية المحمية من الهدم فى الكرش

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تم استخدام ٤ إناث جاموس حلاب عمرها من (٣-٥ سنوات) ومتوسط الوزن ٥٣٢ كجم بعد ٥٢ يوم من الولادة فى تجربة باستخدام تصميم المربع اللاتيني ٤x ٤. وزعت الحيوانات على ٤ مجموعات لتتلقى العلائق التالية: ١) العليقة المقارنة (عبارة عن مخلوط علف مركز و برسيم أخضر و قش أرز بدون إضافات)، ٢) العليقة المقارنة مع ٤٠٠جم صابون كالسيومى (مجنابك®)، ٣) العليقة المقارنة مع الأحماض الأمينية المحمية (١٥جم ميثيونين + ٤٠جم ليسين)، ٤) العليقة المقارنة مع ٤٠٠جم مجنابك® + ١٥جم ميثيونين و ٤٠جم ليسين. و أوضحت النتائج المتحصل عليها ما يلي:

١- لم تتأثر معاملات الهضم الظاهرية معنويا وكذلك المواد الكلية المهضومة و البروتين المهضوم بالمعاملات المختلفة.

٢- أرتفع إنتاج اللبن ارتفاع غير معنوي بالمعاملات بينما لم تتأثر مكونات اللبن المختلفة بالمعاملات، أما اللبن معدل نسبة الدهن ٧% فقد أرتفع معنويا (على مستوى ٥%) بإضافة الدهن المحمى مع الأحماض الأمينية المحمية إلى العلائق.

٣- ارتفع محتوى سيرم الدم من الليبيدات الكلية و الإنزيم الناقل لمجموعة الأمين (ALT) معنويا (على مستوى ٥ %) مع المعاملة الرابعة، بينماارتفع تركيز الكرياتينين فى سيرم الدم معنويا (على مستوى ٥%) عند اضافة الأحماض الأمينية المحمية.

٤- من الناحية الاقتصادية لم يلاحظ اى تأثير عند إضافة الدهن المحمية مع أو بدون إضافة الأحماض الأمينية المحمية إلى علائق الجاموس الحلاب.