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Effect on the Quality of Milk and Milk Products in Murrah Buffaloes (*Bubalus bubalis*) Fed Rumen Protected Fat and Protein

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ABSTRACT

Objectives of present study were to study the effect of feeding protected nutrients on quality of milk and milk products in buffaloes. Eighteen Murrah buffaloes (*Bubalus bubalis*) were divided into two groups (9 each) on the basis of Most Probable Production Ability (MPPA). Group 1 (control; MPPA 2204.17 kg) were fed wheat straw, green maize fodder and concentrate mixture as per requirements. However, group 2 (treatment; MPPA 2210.64 kg) were fed same ration as control group plus 2.5% rumen protected fat (on dry matter intake basis) and concentrate mixture containing formaldehyde treated mustard and ground nut oil cake (1.2 g formaldehyde/ 100 g crude protein). The average milk yield (90 days) was 19.07% higher ($p<0.01$) in group 2 than that of group 1 (13.11 vs 11.01 kg day^{-1}). Total unsaturated fatty acid content (% of total fatty acids) increased by 35.73% (41.78 vs 30.78%) and saturated fatty acids of milk decreased by 18.70% (52.91 vs 65.08%) in group 2 than that of group 1, respectively. There was no difference in flavor and overall acceptability between raw and pasteurized milk samples of group 1 and 2. Total sensory evaluation score of butter was 90.45 and 91.70 in group 1 and 2, respectively. The spreadability of butter was better in protected nutrient supplemented group. Total sensory evaluation score of ghee was 86.65 and 87.85 in group 1 and 2, respectively. Results of present study indicated that supplementation of protected nutrients to lactating buffaloes not only increased milk yield but quality of milk and milk products also.

Key words: Fatty acid profile, milk production, milk quality, protected fat, protected protein

INTRODUCTION

Supplementing ration of lactating animals with bypass fat enhanced the energy intake in early lactation which reduces deleterious effect of acute negative energy balance on lactation (Tyagi *et al*., 2010). Feeding of rumen protected fat and protein to lactating cows and buffaloes significantly increased milk yield and milk composition (Garg *et al*., 2003; McNamara *et al*., 2003; White *et al*., 2004; Salem and Bouraoui, 2008; Thakur and Shelke, 2010). Formaldehyde treatment has proved to be an efficient and cheaper method for protecting highly degradable protein sources in rumen (Chaturvedi and Walli, 2001). There is increasing evidence that polyunsaturated fatty acids play important roles in the normal development of infants (Hoffman *et al*., 1993) and in prevention of cardiovascular diseases in adults (Sheard, 1998; Nordoy *et al*., 2001). Various strategies have been pursued to increase the level of unsaturated fatty acids in the diet, mainly meat, milk and milk products. In this direction many efforts have been made in the past

successfully to increase unsaturated fatty acids and long chain fatty acids in milk fat by supplementing bypass fat (Kitessa *et al*., 2004; Titi and Obeidat, 2008; Theurer *et al*., 2009). Milk fat is responsible for many of the sensory, physical and manufacturing properties of dairy products. Consumers perceive milk and dairy products as important nutritional foods; full cream milk and butter have traditionally enjoyed a high market share, but their consumption is on the decline due to high content of saturated fats. Poor spreadability of butter, which again is due to the high content of saturated fatty acids is another reason why consumers are preferring other spreads (Gulati *et al*., 2000).

The influence of protected nutrients supplementation on quality of milk and milk products is not well documented as much of the published data emanates from studies having production and reproduction related objectives rather than those of quality of milk and milk products. Organoleptic properties are considered as basic traits in product characterization and qualification. Keeping these points in view, a feeding trial was conducted to study the quality of milk and milk products by feeding protected nutrients to Murrah buffaloes.

MATERIALS AND METHODS

Study area: The study was conducted in the experimental cattle shed of National Dairy Research Institute, Karnal, India located at 29° 42" 20 sec N and 76° 58" 52.5 sec E at an altitude of 227 meters amsl. Minimum and maximum ambient temperature range from near freezing point in winter to 45°C in summer with annual rainfall of 700 mm. The experiment was conducted in December 30, 2008 to May 30, 2009 with daily minimum and maximum temperature averaging 5.6 and 40°C.

Animals and dietary treatments: Eighteen Murrah buffaloes (*Bubalus bubalis*) were selected from the herd maintained at NDRI, Karnal and divided into two groups (9 each) on the basis of Most Probable Production Ability (MPPA) and lactation number (2nd to 4th lactation). Buffaloes in group 1 (control group; MPPA 2204.17 kg) were fed chaffed wheat straw (particle size-1.5 to 2.0 cm), chopped green maize fodder (particle size-2.0 to 2.5 cm) and concentrate mixture as per requirements (Kearl, 1982). However, animals in group 2 (treatment group; MPPA 2210.64 kg) were fed the same ration as control group plus 2.5% rumen protected fat (on DMI basis) and concentrate mixture containing formaldehyde treated Mustard (MC) and Ground nut oil (GNC) cake (1.2 g HCHO/100 g CP) in place of untreated cakes as a rumen protected protein source.

Composition (%) of the concentrate mixture was: maize 33, groundnut cake 21, mustard cake 12, wheat bran 20, deoiled rice bran 11, mineral mixture 2 and common salt 1. Green maize forage was fed separately whereas wheat straw and concentrate mixture was mixed before feeding and fed as per weekly calculated requirements (Kearl, 1982) of each buffalo. The concentrate mixture was offered 2 times a day in equal parts at the milking time i.e., 05.00 and 18.00 h. Bypass fat was fed through concentrate mixture at one time i.e., 05.00 h. Fresh green maize forage were fed at 10.00 and 19.00 h in addition to wheat straw which was offered at 05.00 h. Left over, if any, was weighed next morning. Dry Matter (DM) content of forage and left over was determined to calculate the daily DM intake. Buffaloes were milked manually and milk yield of individual buffalo was recorded. Fresh and clean water was provided free choice to each buffalo three times a day. Rumen protected fat and protein was supplemented 60 days pre partum to 90 days postpartum.

Formaldehyde treatment of cakes: Rumen protected fat (Ca salts of fatty acids) was purchased from market. Individual GNC and MC were crushed in feed mill and treated with formaline

(40% formaldehyde) at the level of 1.2 g formaldehyde/100 g CP of cake, in a horizontal mixer of feed mill (Poshak Feed Mill, G. T. Road, Karnal). After treatment, the cakes was mixed thoroughly and then finally stored in tightly sealed plastic bags for at least four to five days, as per the procedure standardized for the protection of cake to make it bypass protein (Chaturvedi and Walli, 2001). The RDP (rumen degradable protein) and UDP (undegradable protein) values of cakes were estimated by *in sacco* nylon bag technique (Mehrez and Orskov, 1977). These formaldehyde treated cakes were used for preparation of compounded concentrate mixture for feeding of treatment group buffaloes.

Analytical techniques: The degree of protection of rumen protected fat was judged through estimating the degree of saponification of the Ca Soaps (Garg and Mehta, 1998). The dried samples of concentrate mixture, wheat straw and green maize forage were ground to pass through 1 mm sieve, pooled samples were analyzed for proximate principals (AOAC, 2005) and cell wall constituents (Goering and van Soest, 1970). The fatty acid analysis of feed samples (green maize forage, wheat straw, concentrate mixture of control and treatment group), rumen protected fat and pooled milk samples of both groups was done using saponification method of Tyagi *et al.* (2010). The analysis was carried out on gas liquid chromatography fitted with flame ionization detector and 50 m Length of capillary column. Initial temperature of the column was 140°C. The RAMP rate was 2°C min^{-1}. Identification of peaks was made through retention time of the reference standards purchased from Supelco, Bellefonte PA, USA.

Sensory evaluation of milk and milk products: The pooled milk samples of control and treatment groups were collected during supplementation period of rumen protected fat and protein. From these samples the raw and pasteurized milk samples of both groups were analyzed for its quality by panel of expert committee (8 members) from Dairy Technology Division, NDRI, Karnal. Milk samples were scored for appearance, texture, flavor and overall acceptability using 9-point hedonic scale.

The pooled milk samples of both groups were processed for preparation of butter and ghee in Experimental Dairy, Dairy Technology Division, NDRI and Karnal. Butter was prepared as per method described by Robinson (1994). Ghee was prepared by commercial method as described by Kumar and Singhal (1992).

Statistical analysis: Statistical analysis of the data was carried out by Students 't' test as per Snedecor and Cochran (1986) with SPSS package programme.

RESULTS

Chemical composition and fatty acid profile of feeds and forage: The chemical composition of feed ingredients is presented in Table 1. Total fat content in the supplemented bypass fat was 76.23% and the protection level from rumen hydrolysis (saponifiable portion) was 57.35%. The predominant fatty acids (% of total fatty acids) in green fodder, wheat straw and concentrate feed of control and treatment group are presented in Table 2. However, predominant fatty acids content of bypass fat was palmitic (13.09), stearic (11.83), elaidic (8.32), oleic (26.45) and linoleic acids (32.67). Formaldehyde treatment of MC increased the UDP content from 4.97 to 28.55%. Similarly, in case of GNC, formaldehyde treatment increased UDP content from 15.86 to 32.66%.

Table 1: Chemical composition of feed ingredients offered (% dry matter basis)

Parameter	Concentrate (control)	Concentrate (treatment)	Green maize	Wheat straw
Dry matter	89.14	89.23	12.98	90.07
Organic matter	91.17	91.25	88.94	91.56
Crude protein	20.24	20.65	8.74	3.12
Crude fiber	8.07	9.13	25.06	39.90
Ether extract	3.89	3.91	2.01	1.32
NFE	58.97	57.56	53.13	47.22
NDF	37.11	38.24	52.47	77.78
ADF	13.48	14.40	32.80	50.43

NFE = Nitrogen free extract, NDF = Neutral detergent fiber, ADF = Acid detergent fiber. Total fat content in the supplemented bypass fat was 76.23% and the protection level from rumen hydrolysis (saponifiable portion) was 57.35%

Table 2: Fatty acid profile (% of total fatty acids) of feed ingredients and milk samples

	Feed ingredients					Milk samples	
Fatty acids	Green fodder	Wheat straw	Concentrate (control)	Concentrate (treatment)	Bypass fat	Group 1	Group 2
Caprylic acid (C8:0)	0.59	ND	0.56	0.30	0.23	0.88	0.86
Capric acid (C10:0)	0.71	0.51	0.78	0.43	0.30	3.06	2.45
Lauric acid (C12:0)	3.31	8.67	1.89	2.05	0.65	3.55	2.15
Myristic acid (C14:0)	2.40	2.03	1.56	1.13	0.35	10.62	8.23
Myristoleic acid (C14:1)	0.67	ND	ND	0.00	0.54	1.20	2.13
Palmitic acid (C16:0)	21.77	21.56	14.37	15.83	13.09	27.45	24.84
Palmitoleic acid (C16:1)	1.02	0.32	1.48	1.23	0.53	1.56	1.85
Margaric acid (C17:0)	ND	3.12	ND	0.25	ND	0.62	0.42
Stearic acid (C18:0)	3.02	39.50	32.98	30.08	11.83	18.45	13.34
Elaidic acid (C18:1t9)	2.06	ND	2.65	3.11	8.32	2.41	2.55
Oleic acid (C18:1c9)	4.68	14.89	21.06	23.03	26.45	23.65	29.85
Linoleic acid (C18:2)	17.12	ND	2.73	1.98	32.67	1.23	4.45
Linolenic acid (C18:3)	38.87	ND	15.41	16.26	2.01	0.73	0.95
Arachidic acid (C20:0)	0.79	4.34	0.33	0.78	0.46	0.45	0.62
Total	97.01	94.94	95.80	96.46	97.43	95.86	94.69
Total unsaturated						30.78	41.78
Total saturated						65.08	52.91
Total LCFA						76.55	78.87

ND = Not detected

Table 3: Nutrients intake and milk production in buffaloes supplemented with rumen protected fat and protein*

Parameter	Group 1	Group 2
Nutrients intake (kg day^{-1})		
Dry matter	14.42±0.10	14.70±0.01
Crude protein	1.75±0.03^{a}	2.03±0.04^{b}
Total digestible nutrients	8.72±0.07^{c}	10.12±0.15^{d}
Milk production kg day^{-1} (90 days)		
Milk yield	11.01±0.39^{c}	13.11±0.43^{d}
6% FCM yield	12.93±0.48^{c}	16.43±0.56^{d}

*Values are given as Mean±SE. $^{a, b}$Values within one row with no common superscript are significantly different at p<0.05. $^{c, d}$Values within one row with no common superscript are significantly different at p<0.01

Feed intake and milk production: The overall mean DM intake (Table 3) was 14.42 kg and 14.70 kg d^{-1} in group 1 and 2, respectively. The overall average mean Crude Protein (CP) intake

was 1.75 and 2.03 kg d^{-1} in the group 1 and group 2, respectively, which was higher ($p<0.05$) in group 2 than that of group 1. Overall average Total Digestible Nutrients (TDN) intake was 8.72 kg and 10.12 kg d^{-1} in group 1 and 2, respectively. The average TDN intake was higher ($p<0.01$) by 16.05% in group 2 over that of group 1. Overall average milk production (Table 3) was 11.01 kg d^{-1} in group 1 and 13.11 kg d^{-1} in group 2 which was 19.07% higher ($p<0.01$) in group 2 over that of group 1. Overall average daily 6% FCM yield was 12.93 kg in group 1 and 16.43 kg in group 2. Group 2 had 27.07% higher ($p<0.01$) FCM yield over that of group 1.

Fatty acid profile of milk samples: There was an increase in the contents of myristoleic acid (C14:1), palmitoleic acid (C16:1), elaidic acid (C18:1t9), oleic acid (C18:1c9), linoleic acid (C18:2), linolenic acid (C18:3) and arachidic acid (C20:0) in the milk fat of group 2 buffaloes as compared to that of group 1 buffaloes (Table 2). Total unsaturated fatty acids content (% of total fatty acids) was 30.78 and 41.78 in group 1 and 2, respectively, showing an increase of 35.73% in group 2 over that of group 1. The saturated fatty acids contents (% of total fatty acid) were 65.08 and 52.91% in group 1 and 2, respectively, showing a decrease of 18.70% in group 2 over that of group 1. The total long chain fatty acid content was also higher in group 2 as compared to that of group 1.

Sensory evaluation of milk and milk products: Sensory evaluation scores of raw and pasteurized buffalo milk samples of control and treatment group are shown in Table 4. Raw milk of group 2 had higher score than that of group 1 (8.05 vs 8.20) in overall acceptability but the difference was not significant ($p = 0.80$). The other parameters viz. texture, flavor, color and appearance were also on higher side in group 2 than that of group 1 though, and the difference was not significant. Similarly, pasteurized milk of group 2 had higher score (8.00 vs 8.20) in overall acceptability but the difference was not significant. Similarly, texture, flavor, color and appearance were on higher side in group 2 than that of group 1 but the difference was not significant ($p>0.05$).

Sensory evaluation scores of butter and ghee prepared from buffalo milk samples of control and treatment group are shown in Table 5. Total sensory evaluation score of butter was 90.45 and 91.70 in group 1 and 2, respectively. Flavor and color was scored higher in group 2 than that of group 1 but the difference was not significant ($p>0.05$). The spreadability of butter was better in group 2 than that of group 1. Total sensory evaluation score of ghee was 86.65 and 87.85 in group 1 and 2, respectively. The other parameters were also on higher side in group 2 than that of group 1 except body and texture but the difference was not significant ($p>0.05$).

Table 4: Sensory evaluation of raw and pasteurized milk*

	Raw milk		Pasteurized milk	
Sensory attributes	Group 1	Group 2	Group 1	Group 2
Color and appearance	8.05±0.42	8.20±0.40	8.20±0.37	8.10±0.40
Texture	8.25±0.33[a]	8.45±0.25[a]	8.40±0.24[a]	8.50±0.22[a]
Flavor	8.25±0.31	8.45±0.25	8.00±0.47	7.95±0.35
Overall acceptability	8.05[b]±0.42	8.20[b]±0.40	8.00[b]±0.44	8.20[b]±0.40

*Values are given as Mean±SE. [a, b]Values within one row with similar superscript are not significantly different ($p>0.05$)

Table 5: Sensory evaluation of butter and ghee*

Sensory attributes (Max. Score)	Group 1	Group 2
Butter		
Flavor (45)	39.80±2.26[a]	40.40±1.94[a]
Body and texture (25)	23.40±0.29	23.40±0.24
Color (15)	13.50±0.50[b]	13.90±0.33[b]
Salt (10)	8.75±0.11	9.00±0.24
Package (5)	5.00	5.00
Total score (100)	90.45	91.70
Ghee		
Flavor (60)	52.60±1.98[a]	53.60±1.36[a]
Body and texture (25)	21.40±0.50	21.00±0.70
Color (10)	7.85±0.38[b]	8.30±0.30[b]
Ghee residue (5)	4.80±0.12	4.95±0.05
Total score (100)	86.65	87.85

*Values are given as Mean±SE. [a, b]Values within one row with similar superscript are not significantly different ($p>0.05$)

DISCUSSION

In this study, efforts have been made to improve the quality of milk and milk products by feeding protected nutrients to buffaloes. This study has provided valuable information on the fatty acid profile of milk and quality of milk products which may prove useful for dairy farmers and ruminant nutritionists in developing appropriate feeding strategies for ruminants in tropical regions with desirable milk composition for public health.

Chemical composition and fatty acid profile of feeds and forage: The chemical composition of green maize forage and wheat straw was within the normal range (Ranjhan, 1998). The bypass fat mainly consisted of unsaturated fatty acids i.e. oleic and linoleic acid whereas degree of rumen protection of fat supplement was 57.35%. Formaldehyde treatment of MC and GNC cake increased the UDP content. Thus, the dietary supplementation of these protected fat and protein to animals increase supply of unsaturated fatty acids and amino acids to the host postruminally.

Feed intake and milk production: The results of the present study indicated that there was no adverse effect of protected fat and protein supplementation on DM intake of lactating buffaloes. Group 2 had higher TDN intake than group 1 due to supplementation of rumen protected fat to group 2 buffaloes. The present results are similar to that of Gulati *et al.* (2003), Garg *et al.* (2003), Kudrna and Marounek (2008) and Thakur and Shelke (2010). Higher milk production observed in group 2 may be attributed to bypass fat and protein supplementation which increased the energy density of the ration resulting in reducing the deleterious effect of negative energy balance. These results are in agreement with those of Ghorbani *et al.* (2007), Ghoreishi *et al.* (2007) Salem and Bouraoui (2008), Foda *et al.* (2009) and Tyagi *et al.* (2010). The average milk and 6% FCM yield was 19.07 and 27.07% higher ($p<0.01$) in group 2 than that of group 1, respectively. The supplementation of protected nutrients lower stress during early lactation which support higher yield till late lactation (Sampelayo *et al.*, 2004), this may be the reason for significant increase in milk yield of present study. On the other hand, some workers reported no effect of rumen inert fat supplementation on milk yield in lactating cows (Juchem *et al.*, 2008; Lounglawan *et al.*, 2008).

Fatty acid profile of milk samples: Dietary bypass fat alters the fatty acid profile of the milk towards the fatty acid content of the supplemental fat. The pattern of incorporation of dietary bypass fat into milk was similar to that reported earlier by Fahey *et al.* (2002) with calcium salts of fatty acids and calcium salt of methionine hydroxyl analogue and Sampelayo *et al.* (2004) with PUFA rich protected fat feeding. Similar pattern of fatty acid profile of milk were observed by Gulati *et al.* (2003) with protected nutrients and Kudrna and Marounek (2008) with dietary supplementation of progressively more unsaturated fatty acids of protected palm fat, whole cotton seed, and extruded linseed oil supplementation to dairy cows.

The increase in unsaturated fatty acid concentrations may also have been in part due to additional supply of theses fatty acids from the supplement originating from its protected fat (Table 2). Changes in the concentrations of palmitic acid (16:0) in different experiments were incoherent. They remained similar in unsupplemented and supplemented groups (Donovan *et al.*, 2000; Baer *et al.*, 2001) and were reduced in supplemented groups (Gulati *et al.*, 2003; Fatahnia *et al.*, 2007; Thakur and Shelke, 2010). However, in some experiments they were observed greater in supplemented groups (Franklin *et al.*, 1999). The rationale behind the lack of consistency in different studies is difficult to explain, but may relate to the difference in the basal rations used in the different experiments, as about 50% of palmitic acid in milk is of dietary origin (Moore and Christie, 1979). In addition, differences in the stage of lactation and degree of mobilization of body fat reserves may contribute to this variation in experimental results.

Fatty acids in milk with less than fourteen C atoms originate from endogenous synthesis in the mammary gland (Moore and Christie, 1979); this synthesis relies on the supply of acetate and butyrate from the rumen. In the present study, the unsaturated fatty acids increased and saturated fatty acids decreased in group 2 buffaloes may be due to higher supply of unsaturated fatty acids towards mammary gland. In all studies there was significant shift in the indicators of potential health benefit from milk due to protected nutrient supplementation of lactating animals; that is, a decrease in total saturated fatty acids and increase in total unsaturated fatty acids.

Sensory evaluation of milk and milk products: There was no difference in the sensory data between milk samples of control and treatment group buffaloes. Feeding of rumen protected fat and protein had no effect on flavor of raw and pasteurized milk samples. All raw and pasteurized milk samples were judged to be acceptable and free from flavor. Sensory characteristics of milk samples was similar to that reported earlier by Cadden *et al.* (1984) with protected lipid supplement, Kitessa *et al.* (2004) with rumen protected tuna oil and Jones *et al.* (2005) with fish and sunflower oil supplementation to cows.

There was no difference between butter samples prepared from milk of buffaloes fed control rations and milk from buffaloes fed protected nutrients. However, when butter samples were compared, the panel was able to distinguish between butter from buffaloes fed protected nutrients and butter from control buffaloes, primarily because of the slight to moderate difference in butter spreadability. Feeding protected fat and protein increase unsaturated fatty acids in milk fat (Table 2), this may be the reason for better spreadability of butter in supplemented group buffaloes. Similar reports were observed by Cadden *et al.* (1984) and Kitessa *et al.* (2004). Freshly prepared ghee samples were evaluated by panelists and they did not observe any difference between two groups. However, ghee from protected nutrient supplement group had higher ghee residue than unsupplemented group.

CONCLUSION

The results of present study indicated that feeding of Ca salts of fatty acids (protected fat) and formaldehyde treated cakes (protected protein) to lactating buffaloes increased the milk yield as well as quality of milk and milk products.

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