EFFECT OF SUPPLEMENTING BYPASS FAT WITH AND WITHOUT RUMEN PROTECTED CHOLINE CHLORIDE ON MILK YIELD AND SERUM LIPID PROFILE IN JAFFARABADI BUFFALOES

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ABSTRACT

Jaffarabadi buffaloes (*n*=27) yielding 8-10 kg milk/head/day were divided into three groups of nine each, based on milk yield, fat per cent and stage of lactation. All animals were fed similar basal diet, comprising 12-15 kg green jowar and 4-6 kg groundnut straw. Concentrate mixture was given according to the level of milk production to meet the maintenance and milk production requirements. Buffaloes in Group 2 were supplemented daily with 150 g bypass fat per animal and in Group 3 along with 150 g bypass fat, 15 g rumen protected choline chloride was also fed. Observations on daily feed intake, daily milk yield, fat per cent etc. were recorded for three months. Average increase in milk yield and fat of Groups 2 and 3 were 1.26 kg ($p<0.05$) and 0.31% ($p<0.05$) and 1.55 kg ($p<0.01$) and 0.44% ($p<0.05$), as compared to Group 1. There was significant improvement in polyunsaturated fatty acids in the milk of Groups 2 and 3. Total unsaturated fatty acids also increased by 9.24 and 9.95% in Groups 2 and 3, respectively. Non-esterified fatty acids in the blood serum decreased by 7.69 and 18.46% ($p<0.05$) in Groups 2 and 3, respectively. There was significant ($p<0.05$) reduction in the cholesterol levels in the blood serum in the animals of Groups 2 and 3, as compared to Group 1. The study indicates supplementing bypass fat helps improving milk and fat yield, which can be further enhanced by fortification with rumen protected choline chloride.

Keywords: bypass fat, rumen protected choline chloride, milk yield, serum lipid profile, Jaffarabadi buffaloes

INTRODUCTION

The beginning of lactation is one of the most crucial periods in the lactation cycle of dairy animals. Despite having access to high energy diets *ad-libitum*, most dairy animals go through a period of negative energy balance, particularly during the first trimester of lactation. The process allows much higher production by changing energy flow so as to partition more energy to milk and less to body reserves for a longer period during lactation. Therefore, nutritional management during this period is crucial for the productivity of dairy animals. Dairy animals mobilize large amounts of fatty acids, also known as non-esterified fatty acids (NEFA), from adipose tissue to meet their energy requirement during early lactation, resulting in increased circulating concentrations of NEFA in the bloodstream.

Supplementation with calcium salts of long chain fatty acids is a good method for increasing energy density of the diet to improve productive

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performances. Choline, a component of phospholipid and methyl donor, plays an essential role in very low density lipoprotein synthesis and thereby contributes to fat export from the liver. Evidence suggests that the dietary supply of choline in early lactating dairy animals may be inadequate, even though choline can be synthesized by the animals (Pires and Grummer, 2008). As dietary choline get degraded in the rumen, it must be supplemented in a protected form (Elek *et al*., 2008). Jaffarabadi buffaloes yield 12-15 litres milk with milk fat in the range of 8 to 12 percent on diets that are usually low in energy density. In view of this, the present study was undertaken in Jaffarabadi buffaloes, to see the impact of supplementing bypass fat with and without rumen protected choline chloride (RPC) on milk yield and blood serum lipid profile.

MATERIALS AND METHODS

Site of study and breed

This study was conducted during August to November months at Galiawada village of Junagadh district in Gujarat State of India. It is sited in western part of India and geographically located between 69.40º to 71.05º east longitude and 20.44º to 21.40º north latitude, at an elevation of 107 metres above mean sea level. The winter temperature varies from 8 to 32ºC while maximum temperatures of 46ºC in summer have been recorded. The average rainfall is 787 mm during the monsoon season. Jaffarabadi *(Bubalus bubalis)* is the heaviest buffalo breed in India. This buffalo breed is native to the region around the Gir forest of Junagadh district. The average daily milk yield of the breed is 12-15 litres, having 8 to 12% fat in milk. Age at first calving, inter calving period and dry period vary from 50-55, 16-18 and 5-8 months, respectively.

Trial design and treatments

A farm-level feeding trial was undertaken to evaluate the effect of supplementing bypass fat, with or without RPC, on milk yield, composition, and blood serum lipid profile in early lactating Jaffarabadi buffaloes. Buffaloes (*n*=27), yielding 8-10 kg milk/head/day were divided into three similar Groups (1, 2 and 3) of nine each, based on level of milk production (8.78 kg), fat (8.17%) and stage of lactation (2-3 weeks post partum). Buffaloes in all the three groups were fed a similar basal diet, comprising 12-15 kg green jowar fodder and 4-6 kg groundnut straw. Concentrate mixture was given according to the level of milk production to meet the maintenance and milk production requirements (Kearl, 1982). No supplement was offered to the buffaloes in Group 1. However, the buffaloes in Groups 2 were supplemented with bypass fat 150 g per animal per day and for those in Groups 3, along with 150 g bypass fat, 15 g RPC was also fed to each of the animals. Feeds and feed supplements were offered to all the animals in three different groups for three months, and observations on daily feed intake, daily milk yield, fat per cent etc. were recorded.

Sample collection and analytical methods

The chemical composition of feeds and fodder offered during the trial period was carried out as per AOAC (2005). Feeds and fodder were also analyzed for neutral detergent fibre (NDF), neutral detergent insoluble nitrogen (NDIN), acid detergent fibre (ADF), acid detergent insoluble nitrogen (ADIN), cellulose, hemi-cellulose and acid detergent lignin (ADL) as per Goering and Van Soest (1970). The degree of rumen protection in bypass fat and RPC supplements were measured as per procedures of Gulati *et al*. (1993) and Sharma and Erdman (1989). The milk yield of individual buffaloes was recorded in the morning and evening. Milk samples from each buffalo, pooled from each milking were collected weekly for analysis of fat and protein contents by using Milkoscan. Blood samples were collected by jugular venipuncture of each buffalo. The blood was allowed to clot and centrifuged at 1500 rpm for 10 minutes. The serum was then harvested for estimation of serum NEFA, triglyceride and cholesterol using commercially available test kits (Brishketu and Thakur, 2007).

Preparation of fatty acid methyl esters and estimation by using GC

Milk samples were also drawn for preparation of fatty acid methyl esters (FAME), during the trial period. Milk fat from a 2 ml sample was saponified with ethanol and 5N sodium hydroxide, followed by acidification with 5N hydrochloric acid. The collected supernatant was then methylated using methanol. Methyl esters were extracted by adding 3 ml petroleum ether to the solution and removing a portion of the top phase into gas chromatograph (GC) vials. Individual fatty acids were determined by using a Perkin-Elmer gas chromatograph with a flame ionization detector and fitted with a BPX70 capillary column (50 m x 0.32 mm ID). Helium gas was used as a carrier, and the detector temperature was set at 210^{o}C (Ashes *et al*., 1992).

RESULTS AND DISCUSSION

Quality analysis

Analysis of feeds and fodder offered to animals in various groups is given in Table 1. The total fat content in bypass fat supplement was 84.24%, and the degree of rumen protection was 78.76%. The predominant fatty acid in the bypass fat supplement was palmitic acid (43.37%), whereas, levels of oleic, linoleic and linolenic acids were 38.22, 9.64 and 0.22%, respectively. The degree of rumen protection of the choline chloride supplement was 68.29%. Since animals in all the three groups were fed a similar ration, there was no significant difference in daily dry matter intake (DMI) amongst the groups (12.89, 12.36 and 12.72 kg). Other workers too have reported that daily dry matter intake of the ration is not affected by supplementing with rumen bypass fat (Tyagi *et al*., 2009).

Milk production and composition

On supplementing with bypass fat alone or with RPC in lactating Jaffarabadi buffaloes, the average increases in milk yield (kg) were 1.26 ($p<0.05$) and 1.55 ($p<0.01$) in Groups 2 and 3, respectively, as compared to the control. The average fat percent increased by 0.31 ($p<0.05$) and 0.44 ($p<0.05$) in Groups 2 and 3, respectively (Figure 1). As choline is used for phospholipid synthesis, supplementation facilitates lipid absorption and transport, thereby favoring milk fat synthesis. However, the milk protein content remained unaffected amongst the groups. Significant effects of supplementing with bypass fat on milk production and daily fat yield in lactating animals have been reported earlier (Barley and Baghel, 2009; Sirohi *et al*., 2010). Elek *et al*. (2008) and Lima *et al*. (2007) observed significant improvement in milk yield on supplementing with RPC in dairy cows. It is also reported that RPC may improve the milk yield of dairy animals by elevating the export of triglycerides from the liver and by sparing methionine as a methyl donor. Collectively, the study indicated that further improvements in milk

Table 1. Chemical composition (% on DM basis) of feeds and fodder.

Parameter	Cotton seed oil cake	Maize bhardo	Green jowar fodder	Groundnut straw
Moisture	6.46	8.90	82.14	6.62
Crude protein	24.40	8.08	9.59	6.14
Ether extract	6.60	2.88	1.23	0.78
Acid detergent fibre	36.86	2.72	45.48	26.55
Neutral detergent fibre	44.89	17.56	49.51	33.14
Acid detergent lignin	10.64	0.30	14.66	5.23
Cellulose	25.88	2.21	27.61	16.87
Hemi-cellulose	8.03	14.84	4.03	6.59
Silica	0.34	0.21	3.21	4.45
Acid detergent insoluble nitrogen	1.56	0.87	1.92	1.46
Neutral detergent insoluble nitrogen	2.88	1.65	3.55	2.37

Table 2. Milk fatty acid profile (% of total fatty acids).

Fatty acids	Group I	Group II	Group III
Caprylic acid ($C_{8:0}$)	2.36 ± 0.02	2.30 ± 0.03	2.27 ± 0.02
Capric acid ($C_{10:0}$)	3.45 ± 0.03	2.62 ± 0.03	2.69 ± 0.02
Lauric acid ($C_{12:0}$)	2.23 ± 0.01	1.45 ± 0.02	1.29 ± 0.01
Myristic acid ($C_{14:0}$)	12.84 ± 1.20	11.88 ± 1.22	11.95 ± 1.00
Myristoleic acid ($C_{14:1}$)	1.23 ± 0.02	1.25 ± 0.01	1.28 ± 0.02
Palmitic acid ($C_{16:0}$)	30.11 ± 2.22	31.37 ± 3.14	30.40 ± 2.10
Palmitoleic acid ($C_{16:1}$)	1.38 ± 0.03	1.10 ±0.05	0.98 ± 0.03
Stearic acid ($C_{18:0}$)	11.12 ± 0.82	11.08 ± 0.90	10.98 ± 0.85
Oleic acid ($C_{18:1}$)	27.32 ± 1.65	29.68 ± 1.92	29.97 ± 2.12
Linoleic acid ($C_{18:2}$)	1.81 ± 0.04	2.74 ± 0.05	2.73 ± 0.04
Linolenic acid ($C_{18:3}$)	0.71 ± 0.05	0.68 ± 0.05	0.72 ± 0.03
Arachidic acid ($C_{20:0}$)	0.40 ± 0.01	0.39 ± 0.00	0.39 ± 0.00
Total saturated fatty acids	62.51 ± 2.45	61.09 ± 2.44	59.97 ± 3.15
Total unsaturated fatty acids	32.45 ± 3.10	35.45 ± 2.42	35.68 ± 2.31
LCFA ($C_{16:0 \text{ to } 20:0}$)	72.85 ± 2.50	77.04 ± 2.53	76.17 ± 3.14
MUFA ($C_{14:1,\ 16:1,\ 18:1}$)	29.93 ± 2.11	32.03 ± 2.10	32.23 ± 2.55
PUFA ($C_{18:2,\ 18:3}$)	2.52 ± 0.08	3.42* ± 0.06	3.45* ± 0.05

*($p<0.05$)

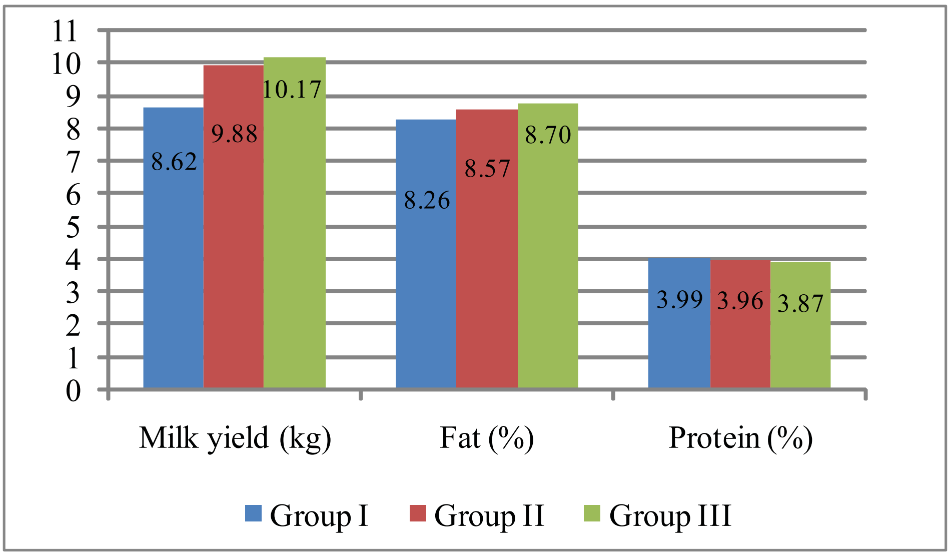

Figure 1. Effect of treatments on milk production and composition.

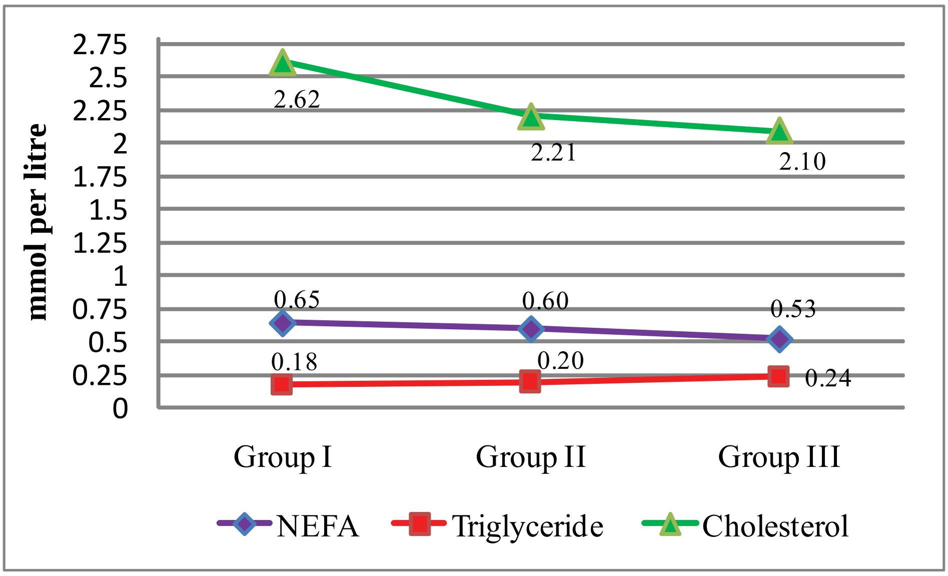

Figure 2. Effect of supplements on blood serum lipids.

production in response to RPC supplementation may be attributed to a methyl donor sparing effect. Thus, enhanced intestinal supply of choline might have further improved milk production in these Jaffarabadi buffaloes.

Milk fatty acid profile

Calcium salts of fatty acids provide partial resistance to lipolysis and bio-hydrogenation in the rumen by ruminal microbes and modify fatty acid profile of milk fat. Significant improvement was observed in poly unsaturated fatty acid (PUFA) content in Groups 2 (35.71%) and 3 (36.90%). Total unsaturated fatty acids increased by about 9.24 and 9.95% in Groups 2 and 3, respectively. Long chain fatty acids (LCFA; $C_{16:0}$ to $C_{20:0}$) and mono unsaturated fatty acids (MUFA; $C_{14:1}$, $C_{16:1}$ and $C_{18:1}$) contents were higher in experimental groups as compared to the control (Table 2). There are several reports indicating that supplementation with bypass fat in the ration of cows and buffaloes increased the proportion of unsaturated and long chain fatty acids of milk fat (Garg *et al*., 2008; Mahecha *et al*., 2008; Sajith *et al*., 2008).

NEFA - An indicator of energy balance and fat mobilization

The presence of NEFA in the blood is a direct indicator of energy balance and massive fat mobilization, suggesting more energy demands than supplied in the diet. Changes in blood serum lipid profile in buffaloes, subjected to the three feeding regimes are presented in Figure 2. Mean serum NEFA level (mmol/l) was reduced to 0.60 and 0.53 in Groups 2 and 3, respectively, as compared to Group 1 (0.65). Significant reduction in serum NEFA level has been reported on feeding RPC (Zahra *et al*., 2006). At the beginning of the lactation cycle, the blood NEFA originating from mobilization of adipose tissue is elevated, mainly due to a negative energy balance. Increased concentrations indicate lipolysis, which occurs in response to increased energy demand. Levels of blood serum triglycerides (mmol/l) increased on feeding bypass fat with and without RPC and cholesterol levels (mmol/l) reduced significantly ($p<0.05$). Brishketu Kumar and Thakur (2007) also reported increased levels of triglycerides on feeding bypass fat supplement to buffalo calves. Cholesterol is a component of the serum lipoproteins and its concentration in serum gives an indication of overall lipoprotein concentrations. Zahra *et al*. (2006) observed reduction in the level of cholesterol on supplementation of RPC to dairy cows. The results obtained in this study are also in agreement with the previous findings of Janovick Guretzky *et al*., 2006 and Pinotti *et al*., 2004.

CONCLUSION

The study revealed that supplementing with bypass fat in the ration of Jaffarabadi buffaloes helped in improving milk yield and fat per cent, which can be further enhanced by supplementing the ration with rumen protected choline chloride.

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