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RESEARCH ARTICLE

Post-partum supplementation of calcium salts of long-chain fatty acids and fibrolytic enzymes on reproductive performance and blood metabolites of lactating Surti buffaloes

J. K. Movaliya[1], B. Kumar[2], Dinesh Kumar[3](✉), A. P. Raval[4], V. R. Patel[4], N. S. Dangar[5]

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Abstract: Twenty-four lactating Surti buffaloes (BW=459.21±7.39 kg and parity of 2.58±0.15) after seven days of calving were randomly divided into four treatment groups. The control (CON) group was fed with basal diet (chaffed sorghum straw, green fodder hybrid napier and BIS Type-I compound cattle feed). The second group was fed the exogenous fibrolytic enzymes (EFE) supplement, while the third and fourth groups (Bypass Fat-1 and Bypass Fat-2)were fed EFE along with 1% and 2% of bypass fat of total DMI, respectively. Blood biochemical parameters were measured at the start (0 d), middle (75th d), and end (150th d) of the experiment. The treatments had no significant effect on the serum levels of glucose, total protein, T_3, T_4, urea, and lipid profile (including triglycerides, total cholesterol, HDL, LDL, VLDL and NEFA). However, animals that received these supplements reduced service period and fewer services per conception when compared to the control group. Thus, adding bypass fat and fibrolytic enzyme supplements to animals' ration could improve their reproductive performance without negatively impacting their blood biochemical indicators.

Keywords: Bypass fat, Buffaloes, Blood metabolites, Fibrolytic Enzymes

[1]College of Veterinary Science and A.H., Kamdhenu University, Navsari, Gujarat- 396450

[2]College of Agriculture, Navsari Agricultural University, Bharuch, Gujarat – 392012

[3]Department of Animal Nutrition, College of Veterinary Science and A.H., BAU, Kanke, Ranchi, Jharkhand – 834006

[4]Department of Animal Nutrition, College of Veterinary Science and A.H., Kamdhenu University, Navsari, Gujarat- 396450

[5]AGB, College of Veterinary Science and A.H., Kamdhenu University, Junagadh, Gujarat-362001

(✉)E-mail: kr.dinesh7@gmail.com
Telephone: 8909990530

Introduction

In tropical regions, buffaloes are often fed with cereal crop residues. However, these residues are not easily digestible due to the high content of crude fiber that is coated with lignin making them resistant to biological degradation (Mahesh and Mohini, 2013). As a result, the rumen microbes are unable to efficiently utilize the nutrients, leading to negative energy balance (NEB) and lower production and reproduction performance of the animals (Sirohi et al. 2010). To increase the productivity of lactating animals, energy supplementation is necessary. One way to do this is by incorporating fat into their diet which increases energy density. Small dairy farmers commonly supplement with locally available concentrate to improve energy density. Further NEB could be overcome by supplementation of calcium salts of long-chain fatty acids (Ca-LCFA) as bypass fat (BPF) to increase the energy density of the ration without adversely affecting the dry matter (DM) intake and nutrient digestibility (Naik et al. 2009). The National Dairy Development Board (https://www.nddb.coop/services/animalnutrition/bypass) recommends using bypass fat (BPF) in the diet of dairy animals 10 days before and 90 days after calving. The BPF can be added to the diet of dairy animals at a rate of 15-20 g/kg of milk production or 100-150 g per animal per day. It's important to note that fat does not hinder the digestion of fiber and is always a better option than feeding ghee or oil. Supplementation of calcium salts of long-chain fatty acids (Ca-LCFA) as BPF is partially resistant to biohydrogenation by the rumen microbes and also reduces the risk of metabolic acidosis (Naik et al. 2009). Calcium salts of long-chain fatty acids would be beneficial in dairy nutrition, but it couldn't completely overcome the challenge of fiber digestion inhibition, even with the addition of calcium. Beauchemin et al. (2003) have reported improvement in fiber utilization in animal diets by using exogenous supplementation of fibrolytic enzymes. Feed enzymes are active in the rumen in the presence of feed substrate, and the mechanism of effects includes direct hydrolysis changes in gut viscosity, complementary action with ruminal enzymes and change in the site of digestion. Most of the studies have been conducted either by using BPF or EFE individually, but the literature on its combined effects on lactating buffaloes is inadequate and scanty. Considering the importance of BPF supplementation with EFE in

milk production, an attempt was made to study the effect of EFE alone and in synergetic effect with BPF (1% and 2% of DMI) supplementation on productive performance through blood biochemical profile of lactating Surti buffaloes.

Materials and methods

Location of the study

The experiment was conducted at the Livestock Research Station, Kamdhenu University, Navsari, Gujarat, India. The research site is located at 202 92°N and 722 89°E, with an altitude and average elevation of 9 m above sea level. The area experiences heavy rainfall, with an average annual rainfall of 122 cm. The experiment was conducted from September, 2019 to February, 2020 on an elite herd of Surti buffaloes.

Experimental animals and treatments

Multiparous lactating Surti buffaloes (24 No.) were divided into four groups based on their average body weight (459.21±7.39 kg), parity (2.58±0.15) and previous lactation yield (960.81±28.07 kg). The control group (CON) that was given a basal diet consisting of chaffed dry fodder (sorghum straw), green fodder (hybrid napier) and BIS Type-I compound cattle feed. The EFE group was given exogenous fibrolytic enzymes along with the basal diet while BF-1 and BF-2 groups had bypass fat added at 1% and 2% of DMI respectively along with exogenous fibrolytic enzymes, in addition to the basal diet. An EFE mixture weighing 08 g containing equal proportions of cellulase (with a minimum of 100000 IU/g) and xylanase (with a minimum of 50000 IU/kg) enzymes were used in a study conducted over a period of 150 days after a two week adaptation period. All animals were fed according to the ICAR (2013) feeding standards and were kept in well-ventilated byres with free access to fresh, clean drinking water. Feed intake was assessed on a biweekly basis, conducted over two consecutive days. During the afternoon session, the animals were permitted to roam freely in an open paddock area. The calculation of fortnightly dry matter and nutrient intake was derived from the quantity of feed offered and the leftover remaining. Additionally, daily milk production was recorded following hand milking procedures.

Sample collection and analysis

The compound cattle feed, sorghum straw and hybrid napier grass samples, both offered and leftover, were dried and then ground to pass through a 1-mm sieve using a MAC® Willey grinder. The pooled samples were analyzed for proximate composition (AOAC, 2005) and fiber fractions (Van Soest et al. 1991).

Blood sampling and analysis

Blood samples were collected from each animal during the experimental period. The collection was done by puncturing the jugular vein on the 0^{th}, 75^{th} and 150^{th} day at 08AM. The serum samples were separated from the blood by centrifugation at 2000 rpm for 15 min. and stored at "40°C for subsequent analysis of the biochemical profile. Diagnostic kits from Sigma Diagnostics and Randox Laboratories Company, India were used for analyzing various blood biochemical parameters, i.e., glucose (GOD–POD method), total protein, tri-iodothyronine (T_3), thyroxine (T_4), serum urea modified (UV/IFCC method), total cholesterol (CHOD–PAP method), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL) (Friedewald's equation), triglycerides (GPO-PAP), non-esterified fatty acids (NEFA).

Statistical analysis

The data were analyzed using a statistical model that estimates the least squares means of variables (Harvey, 1982) for the random effect of treatment and periods. The analysis of variance was performed using the PROC MIXED procedure of SAS with repeated measures (version 9.3; SAS Institute Inc., Cary, NC) and Tukey's HSD (honestly significant difference) multiple comparison tests. Differences were considered significant at $P<0.05$, and values of $P<0.10$ were interpreted as a trend towards significance.

Results and Discussion

Chemical composition of feeds and forage

The chemical composition of feeds has been shown in Table 1. The bypass fat had a total fat content of 82.10%. The concentrate mixture with or without the bypass fat was similar in chemical composition, except for the ether extract and total ash contents, which were non-significant ($P>0.05$) in the concentrate mixture fed to all the groups. The total ash content of the bypass fat (17.90%) was comparatively higher than other feeds and fodders, due to the high level of Ca contributed from the bypass fat. Although the diet was iso-nitrogenous, it was not iso-caloric because of the addition of bypass fat.

The total dry matter intake (DMI) showed no significant variation ($P>0.05$) among all groups, regardless of the treatment regimen, throughout the experimental period (Movaliya et al. 2021). The total DMI recorded was 11.43 kg/d for the control group (CON), 12.17 kg/d for the EFE group, 12.42 kg/d for the BF-1 group, and 11.27 kg/d for the BF-2 group. Notably, DMI was highest in the BF-1 group, followed by the EFE group, then the CON group, with the BF-2 group having the lowest intake.

Total milk and fat-corrected milk (6% FCM) yield (kg/d) were significantly (PÂ0.05) higher in BF-1 (5.41 & 6.44), followed by

EFE (4.48 & 5.40), when compared to the CON (3.93 & 4.65) and BF-2 (3.89 & 4.47) groups (Movaliya et al. 2021).

Blood biochemical profile

The levels of serum glucose, total protein, tri-iodothyronine, thyroxine and urea were not influenced by supplemental bypass fat and fibrolytic enzymes. However, they differed (P<0.05) with the advancement of lactation trials (Table 2) except for serum urea. The serum glucose level was similar in all the groups (Ramteke et al. 2014a; Singh et al. 2014; Raval et al. 2017, 2019). Similar findings were observed by Zilio et al. (2019) with fibrolytic enzyme supplementation were serum glucose levels. This might be due to high metabolic rate of glucose utilization in body during early lactation and developed homeostatic mechanism of animal body that does not allow noticeable changes in glucose level. In contrary to present findings, bypass fat (Nirwan et al. 2019 and Vala et al. 2020) and fibrolytic enzymes (El-Bordeny et al. 2015) supplementation significantly increased (P<0.05) serum glucose level.

Average serum total protein (g/dL) was comparatively similar amongst treatment groups. The serum total protein showed non-significant difference due to supplementation of bypass fat (Wadhwa et al. 2012; Raval et al. 2017). El-Bordeny et al. (2015) and Beigh et al. (2017) also reported normal serum protein level after EFE supplementation. The probable reason might be improved digestibility of nutrients which made no major changes in protein level but reflected in improvement of milk production.

The concentration of tri-iodothyronine and thyroxine was similar in the groups. Overall, periodically T3 (mg/dl) significantly increased from 0.84 (0 d) to 0.92 (150 d) and T4 (mg/dl) decreased from 2.75 (0 d) to 2.42 (150 d). Wadhwa et al. (2012); Theodore et al. (2017) and Vala et al. (2020) indicated that bypass fat supply during transitional period in lactating animal has increased (P<0.01) serum levels of T3 while T4 decreased (P<0.05) consistently. The serum thyroid hormone levels are also shown to be influenced significantly by the stage of estrous cycle (Borady et al. 1985) and stage of lactation (Garg, 1998).

Serum urea nitrogen revealed no significant (P>0.05) difference amongst treatment groups as well as periods. Overall periodical effect shown non-significant (P>0.05) decreasing trend without treatment effect. In contrary, Wadhwa et al. (2012); Ramteke et al. (2014a) and Katiyar et al. (2019) observed that postpartum bypass fat supplementation reduced the blood urea nitrogen level in buffaloes and Morsy et al. (2016) also concluded that the blood urea nitrogen level remained unaffected with EFE supplementation in buffaloes.

Blood lipid profile parameters (Table 3) like total cholesterol including LDL, HDL, VLDL cholesterol and NEFA were also not influenced by dietary treatment but affected by time (P<0.05) with advancement of lactation. Serum total cholesterol level (mg/dL) was non-significant (P>0.05) with dietary treatment. However, total cholesterol was also increased gradually with advancement of sampling time due to continuous intake of lipids for long time in basal diet in the form of bypass fat supplementation. Wadhwa

Table 1: Chemical composition of feed and fodder (% DM basis)

Attributes	Concentrate mixture	Hybrid napier	Sorghum straw	Bypass fat
Dry matter	90.50	26.04	94.50	-
Organic matter	95.59	88.23	93.00	-
Crude protein	21.72	05.54	02.83	-
Ether extract	02.93	01.24	01.15	82.10
Crude fiber	07.36	33.77	38.19	-
Neutral detergent fiber	27.58	51.97	73.20	-
Acid detergent fiber	17.01	40.90	44.70	-
Nitrogen free extract	63.58	47.68	50.80	-
Hemi-cellulose	10.57	11.04	28.50	-
Cellulose	15.33	36.21	33.60	-
Acid detergent lignin	01.68	04.69	11.10	-
Total ash	04.41	11.77	07.03	17.90
Calcium	00.92	01.44	01.04	12.15
Phosphorus	00.93	00.22	00.21	03.56

et al. (2012) and Raval et al. (2017) found that rumen protected fat supplementation improved serum triglycerides and cholesterol levels in crossbred cows and Surti buffaloes, respectively.

Serum HDL, LDL and VLDL cholesterol in treatment groups remained statistically (P>0.05) similar but overall periodically increased (P<0.05) from beginning to end of experiment. Greater concentration of lipid metabolites in animals supplemented with bypass fat can be explained by increased intestinal secretions of lipoprotein (especially HDL cholesterol). Higher level of cholesterol in portal circulation is dependent on how it is transported within lipoproteins (HDL or LDL). Shelke et al. (2012b) also reported increased level of HDL cholesterol using bypass fat supplementation. Likewise, Ranjan et al. (2012) found that supplementation of bypass fat to lactating buffalo's increased HDL cholesterol in fat supplemented group. Increased HDL cholesterol in bypass fat supplemented group might be contributed by long-chain fatty acids incorporation.

Average serum triglyceride was similar in all the groups, however there periodic increase (16.26 at 0 d to 20.20 mg/dL at 150 d). Raval et al. (2017) also showed that supplementation of rumen protected fat caused energy enrichment of diets for buffaloes at early stage of lactation, which improved serum triglycerides and

Table 2: Impact of adding fibrolytic enzymes and bypass fat on Surti buffalo blood biochemical parameters

Day	CON	EFE	BF-1	BF-2	Mean	SEM	P value		
							D	T	D x T
Glucose (mg/dl)									
0	26.60	31.50	29.00	29.94	29.26[b]				
75	45.43	50.10	45.31	48.30	47.28[a]				
150	44.61	42.66	38.97	44.37	42.65[a]				
Mean	38.88	41.42	37.76	40.87	39.73	2.84	0.781	0.0001	0.93
Total protein (mg/dl)									
0	6.91	7.00	6.76	6.54	6.80[b]				
75	7.05	7.13	7.42	7.32	7.23[a]				
150	7.19	7.50	7.29	7.07	7.26[a]				
Mean	7.05	7.21	7.16	6.98	7.10	0.16	0.714	0.007	0.618
T_3 (mg/dl)									
0	0.87	0.95	0.82	0.75	0.84[b]				
75	0.85	0.87	0.82	0.82	0.84[b]				
150	0.88	0.92	0.92	0.96	0.92[a]				
Mean	0.87	0.91	0.85	0.84	0.87	0.03	0.376	0.0012	0.051
T_4 (mg/dl)									
0	2.68	2.80	2.87	2.67	2.75[a]				
75	2.64	2.90	2.57	2.32	2.61[ab]				
150	2.21	2.66	2.62	2.19	2.42[b]				
Mean	2.51	2.78	2.69	2.39	2.59	0.11	0.03	0.003	0.351
Serum urea nitrogen (mg/dl)									
0	41.13	42.96	40.89	40.89	41.47				
75	37.70	38.83	39.76	40.93	39.30				
150	36.21	39.65	39.39	37.99	38.31				
Mean	38.34	40.48	40.01	39.94	39.69	1.73	0.833	0.289	0.983

[a,b]Means bearing different superscripts in a row differ significantly (P<0.05)
CON- control group that was given a basal diet
EFE- basal diet + exogenous fibrolytic enzymes
BF-1- basal diet + bypass fat added at 1% + exogenous fibrolytic enzymes
BF-2- basal diet + bypass fat added at 2% + exogenous fibrolytic enzymes

cholesterol concentration, respectively. In contrary to present findings, Kumar and Thakur (2007) reported significantly increased triglycerides in buffalo calves with bypass fat supplementation. Most of research showing positive energy balance might be responsible for normal serum triglycerides level.

Mean NEFA (mg/dL) concentration was non-significant (P>0.05) within the treatment but periodically it was declined significantly (P<0.05) from 0.69 (0 d) to 0.55 (150 d) indicating the utilization of NEFA for energy purposes, in addition to fatty acid synthesis. Shelke et al. (2012b) and Mohamed et al. (2013) reported positive energy balance with bypass fat and EFE supplementation and no effect on NEFA level in serum. In contrary to present findings, significantly increased level of serum NEFA level with bypass fat and EFE supplementation has been reported (Kumar and Thakur 2007, Ranaweera et al. 2019).

First post-partum heat (d) was observed earliest in BF-1% and last in CON but effect of treatment on it did not reach up to significant (P>0.05) level. Service period (d) and number of

Table 3: Consequence of supplementing bypass fat and fibrolytic enzymes on serum lipid profile of Surti buffaloes

Day	CON	EFE	BF-1	BF-2	Mean	SEM	P value		
							D	T	D x T
Cholesterol (mg/dl)									
0	83.13	86.15	84.90	90.59	86.19[b]				
75	115.37	126.11	115.33	108.84	122.84[a]				
150	118.04	132.43	120.89	119.99	116.41[a]				
Mean	105.51	114.90	107.04	106.48	108.48	8.85	0.868	<0.0001	0.755
LDL Cholesterol(mg/dl)									
0	29.10	34.07	31.51	32.67	31.84[c]				
75	43.17	51.26	51.07	48.87	48.59[b]				
150	52.69	59.76	51.16	51.37	53.75[a]				
Mean	41.65	48.36	44.58	44.30	44.72	4.62	0.784	<0.0001	0.613
HDL Cholesterol (mg/dl)									
0	56.11	64.8	55.17	63.92	60.00[c]				
75	73.17	73.98	71.98	77.31	74.11[b]				
150	81.87	93.00	78.04	81.35	83.57[a]				
Mean	70.38	77.26	68.40	74.19	72.56	0.270	0.695	<0.0001	0.748
VLDL Cholesterol (mg/dl)									
0	3.08	3.30	3.76	2.87	3.25[b]				
75	3.03	2.84	3.88	3.33	3.27[b]				
150	3.64	3.76	4.50	4.26	4.04[a]				
Mean	3.25	3.30	4.04	3.49	3.52	0.270	0.152	0.0004	0.655
Triglycerides (mg/dl)									
0	15.40	16.51	18.78	14.34	16.26[b]				
75	15.13	14.19	19.39	16.67	16.34[b]				
150	18.21	18.81	22.48	21.31	20.20[a]				
Mean	16.25	16.50	20.22	17.44	17.60	1.33	0.152	0.0004	0.655
Non-esterified fatty acids (mg/dl)									
0	0.67	0.70	0.61	0.77	0.69[a]				
75	0.66	0.68	0.58	0.74	0.66[a]				
150	0.55	0.54	0.54	0.58	0.55[b]				
Mean	0.70	0.64	0.63	0.58	0.64	0.05	0.461	0.0001	0.068

[a,b,c]Means bearing different superscripts in a row differ significantly (P<0.05)

Table 4: Result of supplementing bypass fat and fibrolytic enzymes on reproductive performance

Attributes	CON	EFE	BF-1%	BF-2%	SEM	P value
Post-partum heat (d)	94.83	87.17	65.33	77.67	13.38	0.580
Numbers of services per conception	03.10[a]	02.80[b]	02.60[b]	02.10[c]	00.50	0.038
Service period (d)	112.65[a]	108.6[ab]	106.78[ab]	95.92[b]	23.72	0.027

services per conception were significantly (P<0.05) decrease in both bypass fat supplemented group as compared to control. Present findings revealed that positive energy balance with bypass fat supplementation may be responsible for better performance. Higher cholesterol level may have favourable effect on the synthesis of reproductive hormones and in turn may affect the reproductive performance of animals in the bypass fat supplemented group. Present findings are in line with the findings of Patel et al. (2020) and Sihag et al. (2020) that bypass fat supplementation, enhanced early onset of first postpartum estrus and reduced number of services required per conception with improved conception rate and significantly improve the postpartum fertility. The role of fatty acids in the reproductive performance of dairy animals, as noted by Patel et al. (2020), includes several key factors. Enhanced energy balance results in an earlier return to ovarian cycling after postpartum periods. An increase in linoleic acid may elevate PGF2α levels, stimulating ovarian cycling and promoting follicular recruitment. Additionally, an increase in progesterone secretion either from improved energy balance or altered lipoprotein composition due to dietary fat can further enhance fertility.

Conclusions

The supplementation of bypass fat and exogenous fibrolytic enzymes did not have any adverse effect on the total dry matter intake (DMI), blood biochemical and lipid profile, however, total milk and fat-corrected milk yield were significantly higher in BF-1 group of Surti buffaloes. Nevertheless, both types of supplementation improved their reproductive efficiency.

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Competing interests

The authors declare that they have no competing interests.

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