RESEARCH ARTICLE

Effect of Bypass Fat and Fibrolytic Enzymes on Milk Yield and Milk Composition of Surti Buffaloes

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Abstract

The investigation was planned to study the effect of bypass fat @ 1 and 2 % of DMI with exogenous fibrolytic enzymes (EFE) supplementation for 150 days on nutrients utilization and milk production in lactating buffaloes. Recently calved twenty-four lactating buffaloes were assigned to four different treatment groups (n=6 animals in each) that included control, EFE (8 g mixture containing an equal proportion of cellulase minimum 100000 IU/g and xylanase minimum 50000 IU/kg) alone, and EFE with bypass fat @ 1 % and 2 % of DMI, respectively. The digestibility trial of seven days was conducted at the end of the experiment. Any treatment did not influence feed, dry matter and nutrients intake in buffalo. EFE supplementation alone or with 1% bypass fat had significantly ($p < 0.05$) improved digestibility of organic matter, crude protein, ether extract, detergent fiber, and cellulose. The milk production of buffaloes was significantly ($p < 0.05$) higher in groups supplemented with EFE and EFE with 1% bypass fat. Milk compositions did not alter significantly with these supplementations. The study concluded that bypass fat supplementation @ 1% of DMI with 8 g exogenous fibrolytic enzymes mixture mixture (containing equal proportion of cellulase minimum 100000 IU/g and xylanase minimum 50000 IU/kg) improved digestibility of nutrients, which was reflected by higher milk production in Surti buffaloes.

Keywords: Buffaloes, Bypass fat, Digestibility, Exogenous fibrolytic enzymes, Milk production, Milk compositions.

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Introduction

The major reasons for lower productivity of dairy animals in India are both intrinsic (low genetic potential) and extrinsic (poor nutrition/feed management). The cereal crop residues fed to them are low in nutrients, high in crude fibre and lignin that restrict intake and digestibility by the rumen microbes, and cause negative energy balance (NEB), adversely affecting milk production, and often result in low daily milk yields, short lactation periods with long calving intervals. Supplementation with the locally available concentrate is a common practice among smallholder farmers to improve the energy density of ruminants' diets. NEB leads to mobilization of body reserve fat to satisfy energy requirements resulting in loss of body weight and affect reproduction. 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 nutrients digestibility (Naik *et al.,* 2009). Calcium salts of 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 The roughages contain cellulose and hemicellulose, which are not completely digested by the ruminants. Several studies (Beauchemin *et al.,* 2003) have reported improvement in fiber utilization in animal diets by using exogenous fibrolytic enzymes (EFE). Feed enzymes are stable in the rumen in the presence of feed substrate, and the mechanism of effects includes direct hydrolysis, improvement in palatability, changes in gut viscosity, complimentary 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 scarce. Considering the importance of BPF supplementation with EFE in buffalo milk production, this experiment was carried out to investigate the effect of EFE supplement alone and in combination with BPF (1% and 2% of DMI) on nutrient intake, digestibility, milk production and milk composition in lactating Surti buffaloes.

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Materials and Methods

Animal, Diet, and Experimental Design

The experiment was executed from 1st September 2019 to 28th February 2020 on elite Surti buffaloes at Livestock Research Station, Navsari Agricultural University, India. Recently calved twenty-four (n=24) lactating Surti buffaloes were selected based on their average body weight (459.21 ± 7.39 kg), milk yield (960.81 ± 28.07 L) of the previous lactation, and parity (2.58 ±0.158). They were divided randomly into four equal groups (6 animals in each group) as Control (T1) and Treatments (T2, T3 & T4). Buffaloes of control group were fed basal diet, *i.e.,* chaffed dry fodder (Jowar straw, *ad libitum*), green fodder (Hybrid Napier grass, 10 kg per animal/d) and compound cattle feed BIS Type-I according to their nutrient requirements as per ICAR (2013) feeding standards. Whereas, the animals of T2 group were fed basal diet along with 8 g exogenous fibrolytic enzyme (EFE) mixture that contained equal proportion of cellulase minimum 100000 IU/g and xylanase minimum 50000 IU/kg, while T3 and T4 groups were fed basal diet along with EFE and bypass fat @ 1% and @ 2% of total DM intake, respectively, for a period of 150 days. Animals were provided fresh and clean water *ad libitum* three times daily. The animals were also let loose in an open paddock area in the afternoon session.

Sample Collection and Analysis

Feed intake was measured fortnightly on two consecutive days. Feed and fodder samples were collected at fortnightly intervals and were analyzed for proximate composition according to AOAC (2005) and Van Soest (1991). Fortnightly dry matter and nutrient intake was calculated on the basis of feed offered and leftover. The digestibility trial for seven days was conducted at the end of the experiment.

Daily milk production was recorded after hand milking. Fortnightly pooled milk samples were taken of each animal and analyzed on pre-calibrated ultrasonic milk analyzer Lactoscan MCCWS 3080 (Milkotronic Ltd, Bulgaria) for various milk constituents like fat, SNF, protein, lactose, salts, density, pH, and conductivity. The milk yields in terms of 6% FCM, SCM, ECM and TGEM were calculated using standard formulae.

Fatty acids of feed and milk were analyzed as per the direct-transesterification method of O'Fallon *et al*. (2007). Fatty acid methyl ester (FAME) was prepared directly from milk without prior organic solvent extraction and was analyzed on gas chromatography-mass spectrometer (GCMSQP 2010 Plus) equipped with an auto-sampler injector. The FAME was separated by 100% biscyanopropyl polysiloxane capillary column (100 m x 0.25 mm ID, 0.20 µm film), Rt-2560 column with a mass spectrometer. The effluent from the column was mixed with helium, and air gets ionization. Helium was used as carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were 250°C and 240°C, respectively. Samples (2 µL) were injected by split injection (split ratio 50:1). The mass spectrometer was operated under the following conditions: source temperature 230 °C; interface temperature 240 °C with electrospray ionization (EI) and a scan range of 50-1000 m/z. Identification of FAME was performed from the retention times by using standards of 37 individual FAME (Supelco, Sigma-Aldrich Co. LLC) to determine response factors. The peak areas in the chromatograph were calculated and normalized using response factors.

Statistical Analysis

The data were statistically analyzed by a model designed to estimate least squares means of variables for the random effect of treatment and periods. Least squares analysis of variance (Harvey, 1982) by using the PROC MIXED procedure of SAS with repeated measures (version 9.3; SAS Institute Inc., Cary, NC) using Tukey's HSD (honestly significant difference) multiple comparison test was used. Differences were considered significant at $p < 0.05$, with values of $p < 0.01$ being interpreted as a trend towards significance.

Results and Discussion

The data pertaining to feed intake, dry matter intake (DMI), nutrient intake, and digestibility in different treatment groups are presented in Table 1. The results revealed that values of feed and DM intake from concentrate, dry fodder, roughages, and total DM intake were statistically not significant ($p>0.05$) among all groups throughout the experimental period. The calculated total DMI and DMI percent of body weight were also statistically non-significant ($p>0.05$), but it was higher in T3 group followed by T2 and T1 group, while it was lowest in T4 group. However, the period effect was found to be highly significant ($p < 0.01$) among all these traits during the study, with significant increase in feed and DM intake from different feedstuff by day 15 postpartum and then it continued to increase further till day 130 of experiment, while the DCI and TDN intake dropped significantly by day 30 postpartum and then TDNI continued at same lower level around 9.5 to 10.5 kg (Fig. 1), but the interaction between treatment and period was observed to be non-significant on all these traits, except TDNI, suggesting that the parameters studied for period effect were independent of treatment used (Table 1). These results corroborated with the reports of Sirohi *et al.* (2010); Raval *et al.* (2017) and Lunagariya *et al.* (2019).

Table 1 further presents that dry matter digestibility was numerically higher in T3, but the difference was non-significant ($p>0.05$), and crude fiber digestibility also was statistically similar in all groups, while OM digestibility was significantly ($p < 0.01$) higher in T2 and T3 groups. Crude protein and ether extract digestibility were significantly ($p < 0.01$) higher in T3 than the T2, T4 and T1 groups. Digestibility of NDF and ADF were significantly ($p < 0.01$) higher in T2 group followed by T3 group as compared to the non-supplemented control group (T1). The same pattern

Table 1: Effect of supplementing bypass fat and fibrolytic enzymes on feed and nutrient intakes in lactating Surti buffaloes

						p-Value		
Attributes	*T1*	*T2*	*T3*	*T4*	*SEM*	*T*	*P*	*T x P*
Feed Intake (kg/d)								
Concentrate	2.46	3.05	3.38	2.43	0.28	0.052	<0.0001	0.551
Dry fodder	6.99	7.20	7.02	6.60	0.35	0.671	<0.0001	0.101
DM Intake (kg/d)								
Concentrate	2.22	2.76	3.06	2.20	0.25	0.056	<0.0001	0.551
Dry fodder	6.60	6.80	6.63	6.23	0.33	0.672	<0.0001	0.101
Roughages	9.21	9.40	9.23	8.88	0.33	0.672	<0.0001	0.101
Total DMI	11.43	12.17	12.42	11.27	0.52	0.324	<0.0001	0.518
DMI % of BW	2.67	2.75	2.89	2.58	0.12	0.360	<0.0001	0.482
Nutrient intake								
DCPI (gm)*	569^{b}	661^{ab}	721^{a}	566^{b}	0.04	0.018	<0.0001	0.050
TDNI (kg)	6.35	7.18	7.43	6.44	0.32	0.054	<0.0001	0.049
Digestibility (%)								
Dry matter (DM)	64.14	66.48	68.26	65.73	1.62	0.367		
Organic matter (OM)*	62.23^{b}	64.90^{a}	63.70^{ab}	61.61^{b}	0.56	0.002		
Crude protein (CP)**	56.06^{b}	58.47^{ab}	61.24^{a}	59.19^{ab}	0.79	0.007		
Ether extract (EE)**	67.16^{c}	82.15^{ab}	89.02^{a}	78.15^{b}	2.68	<0.0001		
Crude fiber (CF)	48.39	48.49	49.35	49.59	0.45	0.178		
Nitrogen free extract**	61.51^{b}	66.20^{a}	65.93^{a}	63.04^{b}	0.60	<0.0001		
NDF**	57.32^{c}	62.74^{a}	59.13^{b}	55.80^{c}	0.43	<0.0001		
ADF**	52.00^{ab}	52.23^{a}	51.62^{a}	47.81^{b}	0.53	0.0003		
Cellulose**	56.24^{b}	56.77^{ab}	58.27^{a}	54.01^{c}	0.50	<0.0001		
Hemicellulose*	62.86^{b}	64.80^{a}	64.82^{a}	62.67^{b}	0.44	0.002		

**Highly significant ($p < 0.01$), *Significant ($p < 0.05$), Means with different superscript in a row differ significantly ($p < 0.05$).
T - Treatment; P - Period; T x P - Treatment and period interaction; SEM- Standard error of mean

was seen in cellulose and hemicellulose digestibility, which was also significantly ($p < 0.01$) higher in the T3 and T2 groups. Present findings were in agreement with Thakur and Shelke (2010) and Sihag *et al.* (2020), who reported improved digestibility with bypass fat supplementation in cattle and buffaloes. Similar results were also reported by Siddeswara *et al.* (2018) and Morsy *et al.* (2016), on the addition of EFE in diets of cattle and buffaloes. While, in contrast, to present findings, Sirohi *et al.* (2010), Savsani *et al.* (2016), Shankhpal *et al.* (2016) and Raval *et al.* (2017) observed non-significant ($p>0.05$) difference in apparent digestibility of DM, OM, CP, EE, CF and NFE with the addition of bypass fat in the ration of cattle and buffaloes. Silva *et al.* (2016) and Zilio *et al.* (2019) also reported a non-significant difference in apparent digestibility after supplementation of fibrolytic enzymes in cows. This may be attributed to EFE supplementation as it improves metabolic process especially of protein and organic matter in the rumen and flow of microbial protein from the rumen. Improvement in T2 and T3 group digestibility in the present study with an increased total hydrolytic capacity of the rumen might be due to synergistic effect to the exogenous enzymes with the hydrolases of the ruminal microorganisms (Morgavi *et al.,* 2001). The increase in the digestibility of the

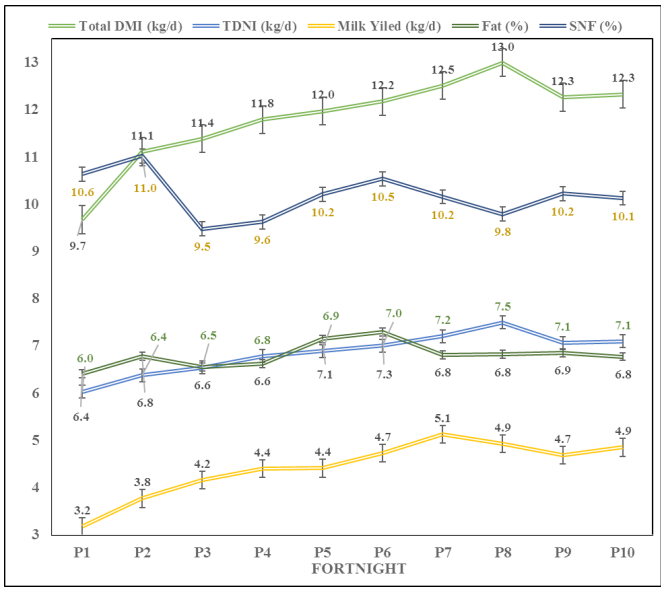

Fig. 1: Effect of fortnightly postpartum periods/intervals on DM and TDN intake, milk yield and milk composition of experimental Surti buffaloes

EE indicates that added fat is less digestible than the basal diet fat, and fat supplementation dilutes the endogenous

Table 2: Effect of supplementing bypass fat and fibrolytic enzymes on milk production and its compositions in lactating Surti buffaloes

						p value		
Attributes	*T1*	*T2*	*T3*	*T4*	*SEM*	*T*	*P*	*T × P*
Milk Production (kg/d)								
Daily milk yield*	3.93^{b}	4.48^{ab}	5.41^{a}	3.89^{b}	0.33	0.0047	<0.0001	0.009
6 % FCM*	4.65^{b}	5.40^{ab}	6.44^{a}	4.47^{b}	0.41	0.0034	<0.0001	0.963
SCM**	5.58^{b}	6.56^{ab}	7.71^{a}	5.40^{b}	0.48	0.0027	<0.0001	0.983
ECM**	5.82^{b}	6.79^{ab}	8.08^{a}	5.66^{b}	0.51	0.003	<0.0001	0.945
TGEM* (Mcal/d)	4.18^{bc}	5.43^{ab}	5.78^{a}	4.05^{c}	0.48	0.012	<0.0001	0.543
Milk composition (%)								
Fat	6.8	7.12	6.84	6.48	0.29	0.497	<0.0001	0.84
SNF	10.04	10.40	10.06	10.24	0.17	0.360	<0.0001	0.16
Protein*	3.56^{b}	3.76^{ab}	3.66^{ab}	3.77^{a}	0.06	0.029	<0.0001	<0.0001
Total solid	16.84	17.52	16.90	16.72	0.30	0.238	<0.0001	0.43
Lactose	5.48	5.69	5.52	5.71	0.09	0.209	<0.0001	0.09
MUN (mg/mL)	9.88	10.27	9.97	10.32	0.16	0.1506	<0.0001	<0.0001
Physical properties of milk (%)								
Density	29.26	31.48	30.63	31.25	0.66	0.3059	<0.0001	0.123
Conductivity (mS)	3.74	3.59	3.71	3.99	0.08	0.057	<0.0001	0.092
pH	6.58	6.57	6.59	6.55	0.03	0.894	<0.0001	0.058
Salt %	0.84	0.82	0.81	0.81	0.01	0.331	<0.0001	0.189

**Highly significant ($p < 0.01$), *Significant ($p < 0.05$), Means with different superscript in a row differ significantly ($p < 0.05$).
T- Treatment; P - Period; T x P77 - Treatment and period interaction; SEM- Standard error of mean

Table 3: Fatty acids (% of total FA) composition of milk

	Milk					
Attributes	*T1*	*T2*	*T3*	*T4*	*SEM*	*p value*
C4:0	2.536	2.419	2.614	2.506	1.190	1.000
C6:0	2.458	1.642	2.434	1.796	0.71	0.784
C8:0	1.741	0.869	1.076	1.031	0.345	0.522
C10:0	2.497	1.644	1.941	2.121	0.68	0.430
C12:0	2.914	1.953	2.265	2.504	0.671	0.350
C13:0	0.165	0.068	0.075	0.088	0.038	0.293
C14:0	16.967	12.406	13.576	13.167	1.584	0.230
C14:1	0.748	0.401	0.268	0.381	0.156	0.196
C15:0	1.233	0.817	1.197	1.255	0.195	0.369
C16:0	41.415	48.868	47.642	47.139	4.04	0.402
C16:1	1.069	0.996	0.951	1.329	0.128	0.201
C18:0	9.342	11.645	11.609	10.234	1.131	0.426
C18:1n9t	0.142	0.07	0.111	0.123	0.037	0.576
C18:1n9c	13.649	13.665	12.36	13.761	1.182	0.811
C18:2n6c	0.52	0.474	0.475	0.568	0.085	0.844
C20:0	0.204	0.242	0.241	0.239	0.027	0.722
C22:0	0.342	0.140	0.118	0.136	0.077	0.176
SFA	83.71	83.37	85.43	82.84	1.02	0.345
UFA	16.286	16.626	14.564	17.157	1.026	0.345
MUFA	14.796	15.857	13.935	16.271	1.164	0.501
PUFA	0.881	0.769	0.629	0.885	0.204	0.791
ω-6	0.635	0.541	0.505	0.64	0.107	0.754
ω-3	0.369	0.187	0.108	0.199	0.094	0.289
SCFA (4 -10)	9.233	6.574	8.065	7.454	2.664	0.911
MCFA (12 -16)	64.368	65.508	65.945	65.901	2.249	0.955
LCFA (>16)	26.274	27.834	25.893	26.537	1.833	0.887

**Highly significant ($p < 0.01$), *Significant ($p < 0.05$), Means with different superscript in a row differ significantly ($p < 0.05$).
SEM- Standard error of mean

lipid secretions, resulting in a more accurate estimate of the true lipid digestibility.

The average milk yield (kg/d) during the experimental period in different groups is presented in Table 2. The results revealed that not only average daily milk yield, but fat corrected milk (6% FCM) yield, solid corrected milk (SCM), energy corrected milk (ECM), and total gross energy of milk (TGEM) were also significantly ($p < 0.05$) higher in T3 followed by T2 as compared to T1 and T4 groups. Periods are also shown significant ($p < 0.01$) effect on average daily milk yield (Fig. 1), and other milk corrected traits, but the interaction of particular treatment over the period effect was non-significant, except milk yield, which increased significantly ($p < 0.001$) and followed normal lactation curve reaching to peak over the treatments.

These findings were in accordance with many of the earlier reports (Ramteke *et al.,* 2014; Rohila *et al.,* 2016; Raval *et al.,* 2017; Saxena *et al*., 2019) that bypass fat supplementation significantly improves the daily milk yield in cows and buffaloes. Lunagariya *et al.,* (2019) reported significant increase ($p < 0.05$) in milk yield with supplementation of fibrolytic enzymes in cows and buffaloes. On the contrary, no significant effect of supplementation of fibrolytic enzyme and bypass fat on milk yield was found by Ranjan et al. (2012) Singh and Singh (2018) and Zilio *et al*. (2019).

Supplementation of bypass fat and fibrolytic enzymes may improve the energy balance through the availability of energy and improved digestion in lactating animals, maintain the production level, and alleviate problems of negative energy balance. The improved milk yield may be a direct result of enhanced digestible nutrient intake and digestion coefficients. Increased available nutrients and metabolizable energy are the possible reasons for the increased milk yield with supplementation in this experiment.

Effects of supplementing bypass fat and fibrolytic enzymes on milk quality parameters like milk fat, SNF, total solid, milk urea nitrogen, and lactose presented in Table 2 revealed that all these parameters were statistically non-significant between treatments, except milk protein which was significantly higher in T4 than T1 group. Further, the period effect was highly significant on all these traits, with peak levels of Fat and SNF around day 45-60 postpartum (Fig. 1), and the period x treatment interaction was also significant ($p < 0.01$) on milk protein, lactose and urea nitrogen content (Table 2). Milk density, pH, conductivity, and salt percent were also not influenced significantly by treatments and treatment over period interaction. However, the period effect was significant ($p < 0.01$) on all these physical parameters. The milk density is responsible for the solid content of milk, and it varies with temperature. The milk salts have an important impact on the formation and stability of casein micelles, acid-base buffering, and various collative properties, as well as having a powerful influence on protein stability during processing (Lucey and Horne, 2009).

Fatty acid profile of milk revealed that bypass fat and EFE supplementation had non-significant effect, but numerically decreased the C10:0 and C12:0 in T2 and T3 as compared to T1 and T4 group (Table 3). In contrast to the present study, Elliot *et al.* (1996) found decreased contents of short and medium-chain fatty acids with increased long-chain fatty acids when supplemental rumen-bypass fat was fed. These results were consistent with earlier research utilizing supplemental dietary fat. Differences in fatty acid composition of the milk reflected differences in the fatty acid composition of the rations.

Conclusions

The present study indicated that supplementation of bypass fat@1 % of DMI with EFE (8 g mixture containing equal proportion of cellulase minimum 100000 IU/g and xylanase minimum 50000 IU/kg) does not affect the feed intake but improves nutrient digestibility, milk yield, milk composition without significant changes in fatty acids composition of milk of lactating buffalo.

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