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Effect of Feeding Solid-State Fermentation Biomass on Nutrients intake, Digestibility and Microbial Protein Synthesis in Lactating Buffaloes

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ABSTRACT

Increasing livestock population with limited availability of feed resources requires a paradigm shift in implementing newer arena of nutritional research which would enable efficient utilization of available low-quality feed resources. The objective of the present study was to evaluate the effect of feeding of solid state fermentation (SSF) biomass on nutrients intake and digestibility of lactating buffaloes. Two treatments *i.e.* basal diet (T_1) and o basal diet supplemented with SSF at 4% of DM (T_2). Twenty lactating buffaloes (2^{nd} -3^{rd} week post-calving) were selected and randomly assigned to one of the either treatment for a duration of 139 days. Towards the end of feeding trial provision was made for individual fecal collection from buffaloes to measure nutrients digestibility. The SSF supplementation had not resulted in any significant improvement in dry matter (DM) and crude protein (CP) intake, but intake of digestible crude protein (DCP) and total digestible nutrients (TDN) was significantly ($P<0.05$) improved in SSF supplemental group. Digestibility of fibre fractions had also shown significant ($P<0.01$) improvement due to SSF supplementation. Numerically increased microbial protein synthesis was also observed in SSF supplemental group but mean value showed non-significant effect. Supplementing SSF biomass as crude fermented product with fibrolytic enzymes has resulted in improvement in digestibility of nutrients without affecting DMI of lactating buffaloes which signifies that SSF biomass helps in effective utilization of feeds and potentiation of energy availability for productive performance.

Key Words: Buffalo, Digestibility, Microbial protein, Nutrient intake, SSF

INTRODUCTION

Agricultural by-products and cereals crops residues usually form the major part of ration of lactating ruminants which are low in nutrients and less digestible too due to presence of lignocellulose bonds, high levels of silica and anti-nutritional factors. This not only reduces the production potential but also affects the profitability of farmers. In such conditions use of feed additives appears to be an attractive solution. Ruminants are equipped with huge array of microorganisms which can utilize such feeds but suitable manipulation of ruminant ecosystem can further improve feed digestibility and economic returns. Use of enzymes, probiotics, prebiotics and nutraceuticals (Patel *et al.,* 2018) are found to be effective in such conditions.

A biotechnological tool *i.e.* SSF appears to be an attractive and suitable technique for enrichment of microbial protein, efficient digestion and utilization of lignocellulosic agricultural fibrous feeds and fodder residues. SSF is a cultivation technique where microbes and fungi (Doelle *et al.,* 1992) are grown on a moist solid substrate under controlled conditions which are devoid of free water which eventually can produce a variety of enzymes (Pandey *et al.,* 1999) and can be fed directly as crude fermentable mass to animals. Positive effects of supplementation of fibrolytic enzymes like cellulase, xylanase, pectinase, laccase *etc.* was also documented by several authors (Beauchemin *et al.,* 2003; Miachieo and Thakur, 2007; Arif *et al.,* 2019). We had hypothesized that supplementation of SSF biomass would improve intake and digestibility of nutrients. Accordingly, the current study was aimed to examine the effect of SSF biomass supplementation digestibility and intake of nutrients of lactating buffaloes.

MATERIALS AND METHODS

An experiment of 139 days was carried out (excluding adaptation of 30 d) on 20 lactating buffaloes

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of private dairy farms of livestock farmers of Sojitra village of Anand District, Anand, Gujarat (India). Animal care and procedures were conducted under the established standard of the Institutional Animal Ethics Committee (IAEC). Lactating buffaloes (2^{nd} to 3^{rd} week post calving) were selected on basis of their parity (2^{nd} to 4^{th} parity) and milk yield and were randomly divided into two groups of 10 buffaloes each as either control group or SSF supplemental group. All the lactating buffaloes were fed upon as per farmer's schedule. Ration of experimental buffaloes that included commercial concentrate mixture of Amul dairy like Amul buffalo special concentrate mixture (*Dan*), Amulnutri power and Amul crushed maize (*bhardo*) and cotton seed cake and maize cake along with *ad libitum* cereal straw (paddy/maize/wheat) and green fodder (*jowar* forage) (10 kg/d) as a basal ration in control group of animals. Buffaloes of treatment (T_2) group were supplemented with SSF biomass at 4% of DM intake by mixing it with concentrates at the time of milking during evening.

SSF biomass used in the present study was prepared on *jowar* hay which was used as substrate for inoculation of microorganisms in fermentation process. Fermentation process was carried out at Department of Microbiology, Gujarat Vidhyapeeth, Sadra, Gandhinagar, Gujarat (India). *Jowar* hay was inoculated with various group of fungi *i.e. Aspergillus spp., Trichoderma spp., Fusarium spp., Pleurotus spp.* and *Phanerochaete spp.* Enzymes were produced due to fermentation activity during SSF biomass preparation, and activity of various enzymes *i.e.* carboxy methyl cellulase (CMCase) (170 U/g), filter paperase (FPase) (60 U/g), xylanase (468 U/g), laccase (270 U/g), manganese peroxidase (MnP) (488 U/g) and lignin peroxidase (LiP) (298 U/g) was also assessed using enzymatic assay technique.

Daily intake of feeds of each buffalo was recorded by weighing feeds offered to buffaloes and analyzing nutrients present in feeds at regular intervals. After 100 days of experimental feeding, a digestibility trial of seven days collection period was conducted to measure digestibility of nutrients. A proper record of total amount of feed offered, refusal and faeces voided by each buffalo was maintained during the trial period. Representative samples of offered feeds, refusal (if any) and $1/300^{th}$ part of faeces voided were taken in tray and kept in a hot air oven at 100±2°C for 24 h to estimate the DM content of samples.

Oven dried samples of individual animals were further pooled for seven days of collection and wre then grinded to pass through 2.0 mm sieve and stored in air tight bottles at room temperature for further analysis. Composite samples of feeds offered, refusal feeds and faeces were analyzed for DM, CP, OM as per AOAC (1995). Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin, hemicellulose and cellulose were analyzed as per Van Soest *et al.* (1991). Urine samples (50 ml) was collected from individual buffalo which was preserved with sufficient quantity of 1.87 mol/l H_2SO_4 to maintain pH<3.0 which was further analyzed for purine derivatives (allantoin, uric acid and creatinine) using spectrophotometric method (Hawk *et al.,* 1976). Purines absorbed and microbial protein was calculated from the daily urinary purine derivatives excreted IAEA (1997).

Statistical Analysis

The experimental data generated were subjected to analysis of variance using the General Linear Model (GLM) procedure of the Statistical Software Package (SPSS for windows, V21.0; Inc., Chicago, IL, USA) and analyzed as per the methods of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The proximate composition and fibre fractions of feeds offered to lactating buffaloes are presented in Table 1 along with composition of SSF biomass. Supplementation of SSF biomass in the diet of lactating buffaloes had not resulted in any significant increase in daily intake of DM and CP (Table 2). Other authors had also reported that supplementation of several enzymes had not resulted in any effect on DM and CP intake in cows (Herrick *et al.,* 2012; El-Bordeny *et al.,* 2015; Daniel *et al.,* 2016). Supplementation of enzymes like cellulase and xylanase (Shekhar *et al.,* 2010) and

Table 1. Proximate composition and Fiber fractions (% on DM basis) of offered feed and SSF biomass

Ingredients	Proximate composition (% on DM basis)									Fiber fractions (% on DM basis)						
	CP	EE	CF	NFE	OM	Ash	Silica	Ca	P	NDF	NDS	ADF	Hemi-cellu-lose	Cellu-lose	Lignin	Silica
SSF	9.55	2.91	31.89	42.63	86.98	13.00	3.77	0.73	0.18	63.30	39.65	37.19	26.10	29.21	7.65	0.88
Green *jowar*	12.00	1.96	28.54	37.52	80.02	19.98	7.53	1.28	0.37	70.25	40.23	38.39	31.86	34.36	4.89	0.98
Bajra straw	10.74	1.73	27.76	48.75	88.97	11.03	4.32	1.57	0.53	68.72	41.12	37.74	30.98	34.58	4.14	0.65
Wheat straw	3.15	1.92	36.29	47.42	88.78	11.22	6.62	1.01	0.28	78.42	30.23	46.47	31.95	41.31	5.81	0.97
Amul Concentrate Mixture (*Dan*)	18.55	8.37	11.15	50.41	88.48	11.52	3.13	1.37	1.69	35.13	78.52	16.28	18.85	12.83	3.79	0.22
Amul Nutri Power	23.05	4.30	12.85	53.29	93.48	6.52	1.46	1.07	0.98	32.73	81.54	18.00	14.73	13.95	3.71	0.67
Crushed maize (*bhardo*)	8.30	2.65	1.57	85.52	98.05	2.11	0.23	0.56	0.26	14.73	87.15	3.57	11.16	3.45	0.23	0.23
Cotton seed cake	23.14	10.14	20.39	41.01	94.69	5.31	0.33	0.81	0.96	47.54	62.10	27.60	19.94	20.70	7.22	0.11
Maize cake	14.49	11.09	4.79	68.34	98.73	5.21	0.32	0.77	0.51	42.57	65.39	8.92	33.65	9.02	0.64	0.21

commercial fibrolytic enzymes (Morsy *et al.,* 2016) did not cause any significant change of DM and CP intake in buffaloes.

Intake of DCP and TDN were significantly ($P<0.05$) higher in SSF supplemented groups as compared to control. Similar, to present the findings Gaafar *et al.* (2010) reported that supplementation of cellulase and xylanase as enzyme in the diet of lactating buffaloes caused significant increase in DCP and TDN intake. Similar response was also observed by Silva *et al.* (2016) on supplementing xylanase. However, several authors had also reported non-significant effect of enzymes supplementation on DCP and TDN intake (Miachieo and Thakur,2007; Bassiouni *et al.,* 2010; Shekhar *et al.,* 2010). The difference in responses of supplementation between the studies might be due to different methods of preparation, application, concentration, activities and nature of enzymes. An enzymatic effect also depends on the type of feeds and their processing. Proteins are also subjected to proteolysis by several enzymes which might be responsible for inconsistent results on protein intake Ravindran (2013).

Data pertaining to digestibility of nutrients are presented in Table 3. Digestibility of DM, OM, CP, CF, EE and NFE was higher ($P<0.05$) in SSF biomass supplemented group as compared to control group. Similar to the findings of present investigation several authors have also reported a significant increase in digestibility of proximate principles in buffaloes due to supplementation of various enzyme preparations containing cellulase and xylanase (Gaafar *et al.,* 2010; Azzaz *et al.,* 2013; Morsy *et al.,* 2016). Similar, results were also observed on supplementing the enzymes to buffaloes at increasing levels in ration (Abd ElT awab *et al.,* 2016; Azam *et al.,* 2017; Arif *et al.,* 2019).

Digestibility (%) of various fibre fractions were found to be significantly increased ($P<0.05$) in treatment group as compared to control group. Similarly, several earlier researchers had reported that supplementation of fibrolytic enzymes improved digestibility of fibre fractions of feed. Supplementation of commercial fibrolytic enzymes like Asperozyme,

Table 2. Cumulative DM, CP, DCP and TDN intake (kg/day) of buffaloes

Buffalo No.	Treatments							
	DMI		CPI		DCPI		TDNI	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
1	16.76	16.76	2.52	2.52	1.44	1.56	9.66	10.40
2	14.68	16.54	2.13	2.50	1.23	1.66	7.97	10.26
3	14.59	16.77	2.11	2.51	1.12	1.54	7.07	9.63
4	15.05	16.62	2.16	2.49	1.42	1.52	7.89	9.54
5	15.19	16.63	1.93	2.49	1.32	1.50	8.72	10.07
6	14.86	15.53	2.13	2.21	1.11	1.46	8.03	9.87
7	14.82	15.04	2.12	1.92	1.20	1.23	8.16	8.83
8	16.71	15.15	2.51	1.93	1.44	1.20	9.41	8.57
9	15.24	15.12	1.93	1.93	1.16	1.25	8.14	8.74
10	16.62	16.72	2.50	2.51	1.23	1.51	8.16	9.77
Average	15.45±0.28	16.09±0.24	2.20±0.07	2.30±0.08	1.27^b±0.04	1.44^a±0.05	8.32^b±0.24	9.57^a±0.20
SEM[1]	0.26	0.07	0.04	0.22				
CD[2] (0.05)[3]	NS	NS	0.13	0.66				
CV[4] %	5.25	11.10	10.56	7.88				

[ab]Means with different superscripts in a row for a parameter differ significantly; [1]Standard Error of Mean, [2]Critical Difference, [3]Level of significance, [4]Co-efficient of variation

Tomoko (TOM), Vet-zyme (VET) *etc.* having cellulase, xylanse, xylose, pectinase, protease and amylase activity with different types of feeds and their ratios between roughage and concentrates has shown a significant increase in digestibility's of fibre fractions in buffaloes (Shekhar *et al.,* 2010; Azzaz *et al.,*2013; Rajamma *et al.,* 2014; Morsy *et al.,* 2016; Abd ElTawab *et al,.* 2016; Azam *et al.,* 2017; Arif *et al.,* 2019). Increase in digestibility of nutrients in the present study was observed due to combined effect of several

Table 3. Apparent digestibility (%) of nutrients

Nutrient	T_1	T_2	SEM[1]	CD[2](0.05)[3]	CV[4] %
Dry Matter	58.18^b	64.73^a	0.99	2.96	5.14
Organic Matter	62.14^b	67.60^a	0.95	2.83	4.65
Crude Protein	57.81^b	62.90^a	1.39	4.13	7.28
Crude Fiber	48.31^b	57.28^a	1.11	3.29	6.65
Ether Extract	65.07^b	72.51^a	1.22	3.63	5.62
Nitrogen Free Extract	58.31^b	63.30^a	1.21	3.60	6.31
Neutral Detergent Fiber	48.43^b	55.36^a	1.20	3.58	7.35
Acid Detergent Fiber	39.09^b	45.84^a	1.34	3.98	9.99
Hemi-cellulose	60.06^b	65.05^a	1.13	3.37	5.74
Cellulose	52.27^b	59.24^a	1.56	4.65	8.88
Intestinal Flow of Microbial Nitrogen (g N/d)	168.71	180.10	10.79	NS	19.57

[ab]Means with different superscripts in a row for a parameter differ significantly; [1]Standard Error of Mean, [2]Critical Difference, [3]Level of significance, [4]Co-efficient of variation

enzymes like xylanase, carboxy methyl cellulase (CMCase), and lignin degrading enzymes like laccase, lignin peroxidase and manganese peroxidase which due to their hydrolytic and oxidizing actions breaks the bonds between fibre and renders them easy to digest.

Microbial protein was calculated on the basis of spectrophotometric analysis of purine derivatives (uric acid, allantion and creatinine) from urine samples of individual buffaloes. Amount of urinary microbial protein (g/d) on an average was found to be 168.71 g/d in control group of buffaloes while for treatment group of buffaloes it was 180.10 g/d. Statistically the amount of microbial protein excreted from buffaloes of two groups was found to be not differing significantly but still there was a numerical increase in microbial protein synthesis in SSF biomass supplemental group by 6.75% as compared to control. Synthesis of protein by ruminal microbes usually depends on type of feeds and its quality and fibre degradation by ruminal microbes. Increased overall digestibility of feeds (Table 3) might be responsible for increased activity and growth of microbes that ultimately increased intestinal flow of microbial nitrogen (Table 3). In agreement with the present study that several authors have reported that yeast supplementation resulted a non-significant change in microbial protein synthesis despite of numerical improvement (Erasmus *et al.,* 1992; Putnam *et al.,* 1997; Hriostav *et al.,* 2010; Noziere *et al.,* 2014; Robinson and Erasmus, 2016).

CONCLUSIONS

It was observed that supplementation of SSF biomass in the diets of lactating buffaloes had significantly increased the intake DCP and TDN. Further, improvement in digestibility of proximate nutrients by 8.50% to 11.50 % and that of fibre fractions by 8% to 14.5% is attributable to enzymatic degradation of nutrients and fibre in feed under the influence of enzymes and microbes present in SSF biomass. This signifies that more energy was available for enhancement of productive performance of buffaloes under SSF biomass supplementation. Rumen microbial protein synthesis was also found to be increased numerically due to increased microbial activity in SSF supplemental group.

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