RESEARCH ARTICLE

Effect of Direct Fed Microbials (DFM) and Solid State Fermented (SSF) Biomass on Nutrients Digestibility and Rumen Fermentation in Surti Buffaloes

Gaurang P. Mathukiya[1*], Paresh R. Pandya[1], Sunil V. Rathod[1], Nitesh P. Sarvaiya[2], Kalpesh K. Sorathiya[1]

Abstract

Present study was carried out to evaluate the effect of supplementing Direct Fed Microbials (DFM) and Solid State Fermented (SSF) biomass for 70 days on body weight, nutrient intake, digestibility and rumen fermentation in adult Surti buffaloes (n=15). The animals were randomly allotted into three groups, with 5 animals in each group. The animals in the control group (T_1) were fed TMR (Roughage to concentrate ratio of 65:35) without supplement, while the animals in group T_2 and T_3 were fed TMR with 3% DFM and 3% SSF biomass on DM basis, respectively. The average initial and final body weight (kg) of animals in T_1 (411.8 and 428.8), T_2 (412.2 and 433.1), and T_3 groups (415.8 and 438.7, respectively) were more or less similar. None of the probiotics had significant effect on average DMI and CPI. The average daily DCPI was however significantly ($p<0.05$) higher in T_3 and T_2 than T_1. Average bi-weekly total digestible nutrient intake was significantly ($p<0.05$) higher in T_3 followed by T_2 and T_1 group. Digestibility of all nutrients was improved numerically by supplementation of DFM and SSF biomass in the diet of buffaloes, and was within the normal range. There was significantly ($p<0.05$) higher concentration of ammonia-N and TCA precipitable nitrogen in SRL of treatment groups of animals, while the values of SRL pH, TVFA, total-N, soluble-N and NPN were statistically similar between groups. The overall results suggested that supplementation of SSF biomass or DFM @ 3% in the ration of buffalo has no adverse effect on feed intake, digestibility and rumen fermentation. Further research is needed to explore their utilization in livestock ration.

Keywords: Buffalo, Direct fed microbials (DFM), Nutrients digestibility, Rumen fermentation, Solid state fermented (SSF) biomass.

Ind J Vet Sci and Biotech (2024): 10.48165/ijvsbt.20.1.21

Introduction

Improvements in feed utilization, animal production, health, and food safety are the goals of rumen microbial research. These goals can be accomplished by encouraging ideal fermentation, minimizing ruminal problems, and preventing infections. Feed additives have been used to increase animal performance, and feed efficiency, and prevent illness. When utilized properly, feed additives can assist dairy farmers to increase income while helping to enhance the nutrition of their cows. The use of anti-biowastes in the last ten years has shown their detrimental effects on animal health, the residue they left in animal products, and the possibility that microbes could become resistant to them. As a result, the idea of using microorganisms in animal nutrition gained popularity.

Yeast culture and probiotics when consumed support the growth of advantageous rumen microorganisms and keep the pH stable. Direct fed microbials (DFM) also enhances nutrient flow post-rumination, enhances nutrient digestion, productivity, and lowers methane emission and stress by enhancing immunological response (Yoon and Stern, 1995). When yeast *Saccharomyces cerevisiae* was added to the meal, nutritional digestibility and thereby FCR and body weight increased greatly including the activity of carboxy methyl cellulose in the rumen (Deendayal, 2008). Total VFA synthesis, the ratio of acetate to propionate, especially 4 h after feeding, and *in-vitro* dry matter digestibility were all increased in the rumen fluid of sheep (Rao *et al.,* 2001; Deendayal, 2008). When fed to cattle, yeast cultures have been proven to promote fibre digestion, activate cellulolytic bacteria in the rumen, and regulate rumen pH (Rossi *et al.,* 2006).

The development of enzyme supplements that enhance fibre digestion and lower enteric methane emissions from large ruminants has been the main emphasis so far. The two main techniques for extracting enzymes are solid-state fermentation (SSF) and submerged fermentation (SmF). Due to its decreased energy need, less effluent generation, and direct application of fermented products for feeding, SSF's

[1]Animal Nutrition Research Station, College of Veterinary Science & A.H., Kamdhenu University, Anand-388110, Gujarat, India

[2]Reproductive Biology Research Unit, College of Veterinary Science & A.H., Kamdhenu University, Anand-388110, Gujarat, India

Corresponding Author: Gaurang P. Mathukiya, Animal Nutrition Research Station, College of Veterinary Science & A.H., Kamdhenu University, Anand-388110, Gujarat, India, e-mail: mathukiyagaurang@gmail.com

How to cite this article: Mathukiya, G. P., Pandya, P.R., Rathod, S. V., Sarvaiya, N. P., & Sorathiya, K. K. (2024). Effect of Direct Fed Microbials (DFM) and Solid State Fermented (SSF) Biomass on Nutrients Digestibility and Rumen Fermentation in Surti Buffaloes. Ind J Vet Sci and Biotech. 20(1), 102-107.

Source of support: Nil

Conflict of interest: None

Submitted 24/11/2023 **Accepted** 12/12/2023 **Published** 10/01/2024

bio-conversion of fibrous material has attracted increasing interest (Yang *et al.,* 2011). For the generation of enzymes by microbial flora, SSF has enormous promise. Ideally, the SSF system can be used to manufacture practically every known microbial enzyme (Pandey *et al.,* 1999). Exogenous fibrolytic enzymes have been shown recently to have positive effects on increasing the efficiency of feed utilization by ruminants both *in-vitro* (Murad *et al.,* 2009) and *in-vivo* (Arriola *et al.,* 2011). Furthermore, methane production was 9.0% lower when dairy cows were fed corn silage with extra enzymes (Beauchemin *et al.,* 2003). The present study was aimed to evaluate the effect of DFM and SSF biomas supplementation on nutrient digestibility and rumen fermentation in buffaloes.

Materials and Methods

An experiment of 70 days was carried out on 15 adult Surti buffaloes at Animal Nutrition Research Station, College of veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat (India) following approval of the Institutional Animal Ethics Committee. Buffaloes were selected on the basis of their body weight and dry physiological (non-lactating) status of animals. The experimental animals were randomly allotted into three treatment groups, with 5 animals in each group.

Feeding and Maintenance of Animals

All experimental animals were fed TMR to meet their nutrient needs, as per ICAR (2013). Animals in the T_2 and T_3 groups were fed TMR as per T_1 (control, Roughage to concentrate ratio of 65:35) but with additional supplement of 3% DFM and 3% SSF biomass on DM basis, respectively. Individual feeding of all the animals was carried out three times, *i.e.,* morning-evening (TMR; at 9 a.m. & 6 p.m.), and afternoon (green; at 3 p.m.). The animals were let loose for exercise for 2 h in the morning and 1 h in the afternoon under controlled conditions, during which they had free access to fresh, wholesome drinking water. Deworming of all the animals was carried out using broad spectrum anthelmintic before initiation of the experiment.

DFM and SSF biomass were procured from Department of Microbiology, Gujarat Vidhyapeeth, Sadra, Gandhinagar, Gujarat, India. The DFM of vegetable waste was carried out with cultures of *Lactobacillus lactis, Lactobacillus rhamnosus, Lactobacillus paracasel, Lactobacillus bifermentans, Lactobacillus acidophilus, Bacillus coagulans,* and *Pediococcus acidilactici* of bacteria. The SSF biomass of jowar hay was made with a culture of *Aspergillus oryzae* and *Trichoderma* spp. of fungi.

Digestibility Trial

During the experimental feeding period, the daily intake of feeds for each buffalo was carefully monitored. After 60 days of feeding, a digestibility trial was conducted for seven days to measure the digestibility of nutrients in the buffaloes fed three types of TMR. Throughout the trial period, detailed records were maintained, including the total amount of feed offered, the amount refused, and the amount of faeces voided by each buffalo. The oven dried samples of individual animals were combined over a week and ground to a fine consistency. They were then stored in sealed containers at room temperature for future analysis. The composite samples of the food provided, leftover food, and faeces were tested for their dry matter (DM), crude protein (CP), and organic matter (OM) content using the AOAC (2005) method. Additionally, the levels of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin, hemicellulose, and cellulose were determined following the approach outlined by Van Soest *et al*. (1991). Digestibility of each nutrient was then determined for each group using standard procedure.

Rumen Parameters

About 150 mL of rumen liquor was collected from each experimental animal at 0, 3, and 6 h post-feeding, through a stomach tube against negative pressure created by a suction pump. The collected rumen liquor was strained through four-layered muslin cloth and referred to as Strained Rumen Liquor (SRL). The SRL was brought to the laboratory in a pre-warmed (39±1°C) thermos flask. The pH of SRL was determined immediately after collection, using a portable digital pH meter. Then one mL of saturated $HgCl_2$ solution was added to each collected sample to kill the microbes and stop the metabolic activity. The samples of SRL were analyzed for ammonia-N and total-N by Kjeldahl's method. After centrifugation of SRL, Soluble-N in the supernatant was estimated by Kjeldahl's method and the same procedure was applied for non-protein-nitrogen determination, except the addition of Trichloroacetic acid before centrifugation of SRL. The concentration of TVFA was determined in SRL by the steam distillation method, using the Markham micro-distillation apparatus.

The cost of feeding of experimental animals was calculated from records of daily feed consumption and procurement price of feeds and fodder used in the experiment.

Statistical Analysis

The data generated during the experiment were analyzed by two-way analysis of variance (ANOVA) using WASP 2.0 method as prescribed by Snedecor and Cochran (1994).

Results and Discussion

The proximate composition and fibre fractions (NDF and ADF) of three TMR fed to animals were almost same. However, green fodder on DM basis contained relatively less DM, CP, ash, lignin and calcium, with higher OM, CF, EE, NDF, ADF, hemicelluloses and cellulose as compared to TMR.

Body Weight

There was no significant (p>0.05) effect of DFM and SSF biomass supplementation on body weight of buffaloes (Table 1). Similar observations were also recorded by the earlier researchers (Beauchemin *et al.*, 2003; Nocek and Kautz, 2006; Dangi, 2022; Kumar and Sirohi, 2013; Sherasia *et al.,* 2018).

Table 1: Average (± SE) bi-weekly body weight (kg) of experimental animals

Bi-wkly Periods	Dietary treatments		
	T_1	T_2	T_3
P0	411.8±30.91	412.2±29.04	415.8±30.72
P1	417.0±30.24	422.0±27.29	431.0±32.82
P2	426.8±28.70	433.8±26.82	438.6±32.11
P3	432.0±27.65	438.0±26.55	442.2±32.69
P4	438.0±26.66	441.8±27.23	447.4±33.82
P5	446.8±25.36	451.0±27.59	457.0±33.23
Overall	**428.8±5.34**	**433.1±5.72**	**438.7±5.79**

Note: The periodic and overall mean values of three dietary treatments did not vary significantly.

Nutrients Intake

There was no significant (P>0.05) effect of DFM and SSF biomass feeding on average DMI and CPI (Table 2) of buffaloes. Similar findings were also reported by Anjum *et al*. (2018) with DFM in buffaloes, while Shekhar *et al.* (2010) observed similar results with SSF biomasss in the diet. Intakes of DCP and TDN were significantly (p<0.05) higher in both DFM and SSF biomass supplemented groups (Table 3). Sadrsaniya *et al*. (2015) and Patel (2019) supplemented DFM and SSF biomass, respectively, in diet of buffaloes and found significant (p<0.05) effect on DCP and TDN intake.

Digestibility of Nutrients

The average values of digestibility of nutrients are presented in Table 4. Digestibility of DM, OM, CP, EE, CF, NFE, NDF, ADF, cellulose and hemicellulose were non-significantly higher in SSF supplemented (T_3) group followed by DFM

Table 2: Average (± SE) bi-weekly DMI (kg/d) and CPI (g/d)

Period	Dietary treatments					
	Dry matter intake (DMI)			Crude protein intake (CPI)		
	T_1	T_2	T_3	T_1	T_2	T_3
P1	8.97±0.11	8.45±0.14	8.68±0.16	1178.47±14.05	1126.25±17.81	1160.16±21.21
P2	9.58±0.01	9.51±0.04	9.48±0.05	1255.39±1.21	1263.71±4.87	1264.46±6.26
P3	9.47±0.04	9.51±0.04	9.38±0.08	1241.33±5.28	1263.10±5.50	1251.67±9.73
P4	9.40±0.08	9.53±0.04	9.51±0.06	1221.15±10.19	1255.47±4.98	1259.09±7.49
P5	9.63±0.00	9.63±0.00	9.63±0.00	1237.70±0.00	1259.30±0.00	1265.60±0.00
Overall	**9.41±0.12**	**9.33±0.22**	**9.34±0.17**	**1226.81±13.26**	**1233.57±26.87**	**1240.20±20.16**

Note: The periodic and overall mean values of three treatments did not vary significantly.

Table 3: Average (± SE) bi-weekly DCPI (g/d) and TDNI (kg/d)

Period	Dietary treatments					
	Digestible CP intake (DCPI)			TDN intake (TDNI)		
	T_1	T_2	T_3	T_1	T_2	T_3
P1	641.49±13.11	682.30±10.25	752.78±18.37	4.34±0.07	4.28±0.07	4.59±0.12
P2	684.15±12.00	767.31±5.38	815.40±11.03	4.65±0.06	4.84±0.04	4.98±0.07
P3	675.76±11.72	766.98±5.70	808.21±12.63	4.59±0.06	4.84±0.04	4.93±0.08
P4	664.16±12.23	762.25±5.28	811.52±10.88	4.55±0.07	4.84±0.04	4.98±0.07
P5	674.55±11.83	764.92±5.07	514.72±8.95	4.66±0.07	4.88±0.04	5.03±0.06
Overall	**668.02^{a}±7.35**	**748.75^{b}±16.64**	**800.53^{c}±12.00**	**4.56^{a}±0.06**	**4.74^{ab}±0.11**	**4.90^{b}±0.08**

Means bearing superscripts a, b, c in a row differ significantly *(P<0.05).

Table 4: Effect of dietary treatments on digestibility of nutrients

Nutrient digestibility (%)	Dietary treatments				
	T_1	T_2	T_3	Sig.	CV (%)
DM digestibility	50.77±3.51	54.06±2.06	58.98±2.47	NS	11.25
OM digestibility	55.16±3.18	58.24±1.95	60.95±2.93	NS	10.53
CP digestibility	54.50±3.97	60.74±1.67	64.38±2.94	NS	11.24
EE digestibility	60.94±4.78	63.90±3.35	68.53±1.92	NS	12.31
CF digestibility	48.48±3.65	52.14±0.99	55.58±3.70	NS	13.13
NFE digestibility	58.16±3.16	58.77±6.25	60.19±5.36	NS	14.91
NDF digestibility	46.35±4.18	47.80±5.54	50.12±3.98	NS	16.85
ADF digestibility	40.71±5.43	40.37±2.72	42.24±4.52	NS	23.78
Cellulose digestibility	50.88±3.56	52.41±1.45	54.49±3.70	NS	13.11
Hemicellulose digestibility	56.87±2.18	60.44±3.78	63.54±3.67	NS	12.20

NS= Non-significant.

supplemented (T_2) group and control (T_1) group. Similarly, Kumar and Sirohi (2013) and ElKatcha *et al.* (2016) also did not observe significant effect of DFM supplementation on nutrients digestibility in buffaloes and lambs, respectively, and Muwalla *et al.* (2007) with SSF biomass in the diet of lambs.

Rumen Parameters

Rumen pH in SRL of T_1, T_2 and T_3 groups decreased gradually from 0 h to 6 h, and it was non-significantly higher in T_3 than T_1 at all intervals. The average concentration of TVFA was non-significantly (p>0.05) higher in T_2 group followed by T_3 and T_1 groups, and also at 4 h than 6 h and 0 h (Table 5). Similarly, Raeth-Knight *et al.* (2007) and Chaudhary *et al.* (2008) observed non-significant effect of DFM supplementation on pH and TVFA concentrations in SRL of crossbred cattle. Similar results were also observed for SSF biomass supplemented diet of crossbred cattle (Sherasia *et al.,* 2018; Chaudhari, 2020).

There was significantly (p<0.05) higher concentration of ammonia-N in T_2 and T_3 groups than T_1 group and it was higher at 3 h than 0 h and 6 h in both T1 and T2 groups. The average concentration of TCA precipitable nitrogen was significantly (p<0.05) higher in T_3 and T_2 groups than T_1 group, with T_2 statistically at par with T_1 and T_3 groups. The levels were also high at 3 h than 6 h and 0 h in all the groups (Table 5). Similar results were reported with DFM supplementation in diet of bullocks by Pandey and Agarwal (2001).

The average concentration of total-N was non-significantly (p>0.05) higher in T_3 group followed by T_2 and T_1 groups, and it increased significantly at 3 h in all three groups with grater magnitude in T_2 and T_3 groups (Table 5). Similarly, Hossain *et al.* (2012) also observed non-significant effect of DFM

Table 5: Average ruminal pH, TVFA, total-N, NH_3-N, soluble-N, NPN and TCA precipitable nitrogen concentration in different treatment groups

Treatment	Hours of Sampling			
	0 h	3 h	6 h	Overall
Ruminal pH				
T1	7.48±0.21	7.25±0.19	7.09±0.13	**7.27±0.11**
T2	7.44±0.19	7.25±0.17	7.25±0.17	**7.31±0.01**
T3	7.55±0.06	7.27±0.09	7.30±0.07	**7.37±0.05**
Total Volatile Fatty Acids in mM/dL SRL				
T1	7.02±0.22	12.96±0.55	11.28±0.38	**10.42±0.32**
T2	8.96±0.75	14.66±1.54	12.02±0.85	**11.88±1.03**
T3	8.18±0.44	12.84±0.45	12.06±0.59	**11.03±0.40**
Ammonia – N (mg/dL SRL)				
T1	15.96±0.71	13.16±0.71	12.88±1.62	**14.00^a±0.42**
T2	18.76±3.50	23.52±2.14	18.76±1.86	**20.35^b±1.90**
T3	16.24±1.14	25.76±1.14	14.28±1.12	**18.76^b±0.85**
Total nitrogen (mg/dL SRL)				
T1	61.60±3.19	71.46±1.24	63.84±3.12	**65.63±2.45**
T2	62.16±1.86	85.12±7.33	64.40±1.98	**70.56±3.02**
T3	66.08±7.44	84.40±4.10	67.76±6.47	**74.67±5.88**
Soluble – N (mg/dL SRL)				
T1	39.20±3.07	38.98±1.12	37.52±2.10	**38.56±1.98**
T2	34.72±2.27	50.96±7.53	33.60±2.80	**39.76±2.80**
T3	33.60±6.74	42.00±6.80	36.40±5.67	**37.33±5.78**
Non-protein nitrogen (mg/dL SRL)				
T1	45.36±1.05	56.56±2.41	49.84±1.63	**50.59±1.52**
T2	49.28±0.69	58.80±1.25	52.08±0.69	**53.39±0.62**
T3	45.36±2.71	56.00±2.50	49.28±1.90	**50.21±2.12**
TCA precipitable nitrogen (mg/dL SRL)				
T1	22.40±0.89	32.48±0.69	26.32±1.43	**27.07^a±2.93**
T2	27.44±0.56	34.16±1.05	30.80±0.89	**30.80^{ab}±0.78**
T3	32.48±1.43	48.16±3.69	31.36±3.47	**37.33^b±0.83**

Mean values with different superscripts (a, b) within column differ significantly between treatments (p<0.05) for a parameter.

supplementation on total-N concentration in SRL of Kankrej cows, and Sherasia *et al.* (2018) and Chaudhari (2020) with SSF biomass supplemented in diet of crossbred cattle.

The average concentrations of soluble-N and NPN were statistically similar in all three groups, and also increased at 3 h post-feeding particularly in T_2 and T_3 groups over 0 h and 6 h values (Table 5). Similar results were also observed with DFM supplemented diet of cattle (Dangi, 2022; Asediya, 2022). Similarly, Chaudhari (2020) revealed non-significant effect of SSF biomass supplementation on NPN concentration in SRL of cattle.

The total feed cost (Rs./h/70 days) and daily feed cost (Rs./h/d) did not vary between three groups, although it was higher in T3 (10880.68 and 155.64, respectively) group followed by T2 (10579.39 and 153.83, respectively) and T1 (10660.35 and 152.29, respectively).

Conclusions

The overall results of the present study suggested that SSF biomass and DFM can be supplemented up to 3% in the ration of buffalo for beneficial effects on nutrient intake, digestibility and rumen fermentation.

Acknowledgement

Authors are grateful to the Animal Nutrition Research Station, Veterinary College, Anand, and the authorities of Kamdhenu University for providing necessary facilities and financial support for undertaking this study.

References

Anjum, M.I., Javaid, S., Ansar, M.S., & Ghaffar, A. (2018). Effects of yeast (*Saccharomyces cerevisiae*) supplementation on intake, digestibility, rumen fermentation and milk yield in Nili-Ravi buffaloes. *Iranian Journal of Veterinary Research*, *19*(2), 96-100.

AOAC (2005) Official method of Analysis. 18th edn., *Association of Official Analytical Chemists,* Washington DC, Method 935.14 and 992.24.

Arriola, K.G., Kim, S.C., Staples, C.R., & Adesogan, A.T. (2011). Effect of fibrolytic enzyme application to low and high concentrate diets on the performance of lactating dairy cattle. *Journal of Dairy Science*, *94,* 832-841.

Asediya, V.S. (2022). Growth performance of post-weaned Kankrej calves on direct fed microbials based ration. *M.V.Sc. Thesis.* Kamdhenu University, Anand, Gujarat, India.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., & Yang, W.Z. (2003). Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. *Journal of Animal Science, 81,* E37-E47.

Chaudhari, F.N. (2020). Effect of solid state fermentation (SSF) biomass on growth performance of crossbred heifers. *M.V.Sc. Thesis.* Anand Agricultural University, Gujarat, India. Krishikosh. https://krishikosh.egranth.ac.in/handle/1/5810184025

Chaudhary, L.C., Sahoo, A., Agrawal, N., Kamra, D.N., & Pathak, N.N. (2008). Effect of direct fed microbials on nutrient utilization, rumen fermentation, immune and growth response in crossbred cattle calves. *Indian Journal of Animal Science*, *78*(5), 515-521.

Dangi, R. (2022). Effect of direct fed microbials on digestibility and rumen fermentation in cattle. *M. V. Sc. Thesis.* Kamdhenu University, Anand, Gujarat, India.

Deendayal, (2008). Efficiency of utilization of leguminous straw based complete feed blocks alone and in combination with probiotics *(Saccharomyces cerevisiae)* in ration of sheep. *M.V.Sc. Thesis*. Rajasthan Agricultural University, Bikaner. http:// krishikosh. egranth.ac.in/handle/1/5810137196

El-Katcha, M.I., Soltan, M.A., & Essi, M.S. (2016). Effect of *Pediococcus* spp. supplementation on growth performance, nutrient digestibility and some blood serum biochemical changes of fattening lambs. *Alexandria Journal for Veterinary Sciences, 49*(1), 44-54.

Hossain, S.A., Parnerkar, S., Haque, N., Gupta, R.S., Kumar, D., & Tyagi, A.K. (2012). Influence of dietary supplementation of live yeast (*Saccharomyces cerevisiae*) on nutrient utilization, ruminal and biochemical profiles of Kankrej calves. *International Journal of Applied Animal Science*, *1*(1), 30-38.

ICAR (2013). *Nutrient requirements of cattle and buffalo*. Indian Council of Agricultural Research, New Delhi, India.

Kumar, B., & Sirohi, S. K. (2013). Effect of isolates of fibre degrading bacteria on body weight changes, milk production and its composition, nutrient intake and nutrient utilization in lactating Murrah buffaloes. *African Journal of Biotechnology*, *12*(21), 3302-3308.

Murad, H.H., Hanfy, M.A., Kholif, A.M., Abdel Gawad, M.H., & Murad, H.A. (2009). Effect of cellulases supplementation to some low quality roughages on digestion and milk production by lactating goats. *Journal of Biological Chemistry and Environmental Sciences, 4,* 791-809.

Muwalla, M.M., Haddad, S.G., & Hijazeen, M.A. (2007). Effect of fibrolytic enzyme inclusion in high concentrate fattening diets on nutrient digestibility and growth performance of Awassi lambs. *Livestock Science*, *111*(3), 255-258.

Nocek, J.E., & Kautz, W.P. (2006). Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and post-partum dairy cattle. *Journal of Dairy Science*, *89*(1), 260-266.

Pandey, A., Selvakumar, P., Soccol, R.C., & Nigam, P. (1999). Solid state fermentation for the production of industrial enzyme. *Current Science*, *77(1),* 149-162.

Pandey, P., & Agrawal, I.S. (2001). Influence of dietary supplementation of antibiotic and probiotic on rumen fermentation in crossbred bullocks. *Indian Journal of Animal Nutrition*, *18*(1), 19-22.

Patel, K.P. (2019). Effect of feeding solid state fermentation biomass on performance of lactating buffaloes. *M.V.Sc. Thesis*. Anand Agricultural University, Anand, Gujarat, India. https://krishikosh.egranth.ac.in/handle/1/5810169724

Raeth-Knight, M.L., Linn, J.G., & Jung, H.G. (2007). Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of Holstein dairy cows. *Journal of Dairy Science*, *90*(4), 1802-1809.

Rao, T.N., Rao, Z.P., & Rama Prasad, J. (2001). Supplementation of probiotics on growth performance in sheep. *Indian Journal Animal Nutrition*, *20,* 224-226.

Rossi, Sgoifo, C.A., Dell-Orto, V., Bassini, A.L., Chevaux, E., & Savoini, G. (2006). Effects of live yeast in beef cattle studied. *Feedstuffs*, *78,* p. 12-13.

Sadrsaniya, D.A., Raval, A.P., Bhagwat, S.R., & Nageshwar, A. (2015). Effects of probiotics supplementation on growth and nutrient utilization in female Mehsana buffalo calves. *Indian Veterinary Journal, 92*(9), 20-22.

Shekhar, C., Thakur, S.S., & Shelke, S.K. (2010). Effect of exogenous fibrolytic enzymes supplementation on milk production and nutrient utilization in Murrah buffaloes. *Tropical Animal Health and Production*, *42*(7), 1465-1470.

Sherasia, P.L., Pandya, P.R., Parnerkar, S., Prajapati, M.V., & Murty, S. (2018). Influence of supplementing solid state fermented biomass on digestibility, microbial nitrogen supply and enteric methane emissions in cattle. *Indian Journal of Animal Nutrition*, *35*(2), 138-143.

Snedecor, G.W., & Cochran, W.G. (1994), *Statistical methods*. 8th Edn., The Iowa State University press, Ames, Iowa, USA.

Van Soest, P.J., Robertson, J.B., & Lewis, B.A. (1991). Methods of dietary fiber. neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74,* 3583-3597.

Yang, H.E., Son, Y.S., & Beauchemin, K.A. (2011). Effects of exogenous enzymes on ruminal fermentation and degradability of alfafa hay and rice straw. *Journal of Animal Science, 24*(1), 56-64.

Yoon, I. K., & Stern, M.D. (1995). Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants - A review. *Asian Australasian Journal of Animal Sciences*, *8*(6), 533-555.