RESEARCH ARTICLE

Influence of Direct-Fed Microbials on *In-Vitro* Dry Matter Digestibility, Rumen Fermentation Parameters and its Economics in Adult Sheep

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Abstract

The present study was aimed to find out the effect of direct fed microbials (DFM) on *in-vitro* dry matter digestibility (IVDMD), rumen fermentation parameters and its economics in 20 adult sheep. Based on the promising results from the laboratory-based *in-vitro* trials (49.55% IVDMD), the *in-vivo* study with a selected 2% level of DFM was further investigated. The sheep were arbitrarily split into two equal groups of ten each. The animals under T1 and T2 were fed compound concentrate mixture and roughage in conventional farm feeding as per ICAR (2013), additionally treatment group (T2) was supplemented with 2% DFM. The results for rumen fermentation pattern revealed that the average pH and TVFA values for T1 and T2 did not differ significantly. The experimental animals of DFM treatment group showed significantly ($p<0.05$) higher concentration of average total nitrogen (118.91 Vs. 100.71 mg/dL). The ammonia N, NPN concentration and TCA precipitable nitrogen concentration values were found to be non-significant ($p>0.05$) between two groups, while the ammonia N concentration was significantly ($p<0.05$) higher at 3 and 6 h post-feeding in both control and treatment group. The TCA precipitable nitrogen concentration values in rumen liquor neither differed significantly between groups nor at different time intervals post-feeding.

Key words: Ammonia nitrogen, DFM, IVDMD, pH, Sheep, Rumen Fermentation.

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Introduction

The sheep accounts for 13.83% of total livestock as per 20th livestock census (BAHS, 2022). Goats and sheep are valuable livestock animals in India. They are small ruminants which play a crucial role in Indian economy and they are a source of income and employment to millions of rural households. The nutrition plays significant role in maintaining productive and reproductive performance of livestock. Out of which dietary modifications could be helpful for future prospects in development of sheep farming. Feed additives, including enzymes, prebiotics, probiotics (direct-fed microbials, or DFM), and antimicrobial growth promotors, have been added to animal diets in order to achieve this set of objectives. Feed additives called direct-fed microbials (DFMs) are live, natural microorganisms that are able to improve the health and productivity of animals. Spore-forming bacteria are used in the development of DFMs because they are effective in distributing these strains to the targeted organs. In livestock production, lactic acid-producing bacteria such as *Enterococcus* species, *Streptococcus* species, *Lactobacillus* species and *Pediococcus* species are often utilized (Puniya *et al*., 2015).

In vitro digestion assays simulate the physiological conditions of digestion *in vivo* and are useful tools for studying and understanding changes, interactions, as well as the bioaccessibility of nutrients, drugs and non-nutritive compounds. The chemical and physical characteristics of animal feed play a significant role in influencing the process of fermentation within the rumen (Tedeschi *et al.,* 2009). The gas parameters, such as methane or other gases produced by browse species, can serve as indicators of the variations in their nutritional content. The IVGP technique is key for

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evaluating rumen digestibility, particularly the potential of browse species containing tannins, of ruminant diets (Salem *et al.,* 2007; Norman *et al.,* 2010). This helps optimize diet formulation for better digestion and animal performance (Getachew *et al.,* 2002; Salem *et al.,* 2007). This technique enables the calculation of the fraction degraded, which can be either fermented to produce volatile fatty acids (VFAs) or incorporated into the biomass of microbes and energy levels (Blummel *et al.,* 2003; Salem *et al.,* 2007).

DFMs such as *Saccharomyces cerevisiae* reduce lactate build up and provide organic acids and vitamins to cellulolytic bacteria (Wang *et al*., 2022). Direct-fed microbials, have shown great promise in manipulating rumen fermentation and improving productive animal performance. Antibiotic residues and antibiotic resistance are two consequences of using antibiotics as growth enhancers in animals. DFMs are being researched as a safer substitute for antibiotics to improve production and health in ruminants by reestablishing the balance of the gut flora. This study was aimed to evaluate the effect of direct fed microbials on rumen parameters and economics in adult sheep.

Materials and Methods

The study was conducted at Animal Nutrition Research Station, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand (Gujarat, India) for 70 days after approval of protocol from CPCSEA (approval No. 400/AN/23-CPCSEA), New Delhi on the recommendation of IAEC. The experiment was conducted on a total of twenty (n=20) adult sheep. The Department of Microbiology at Gujarat Vidhyapith, Sadra (Ahmedabad), prepared the direct-fed microbials (DFM) using vegetable waste with bacterial culture of *Lactobacillus lactis, Lactobacillus paracasei, Lactobacillus bifermentans, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bacillus coagulants, and Pediococcus acidilactici.* The animals were randomly allotted based on their body weight into following two treatments each comprised of ten adult sheep.

During the initial phase of *in-vitro* analysis (Menke *et al.* 1979), the focus was on determining the optimal inclusion level of Direct Fed Microbials (DFM) in the farm feed. Each animal was provided with a daily feed consisting of 300 g concentrate, 2.5 kg of green feed, and 1.5 kg of gotar for 10 days prior to the *in-vitro* studies. DFM was supplemented at levels ranging from 1 to 7% in the feed, and the desired DFM level for the subsequent *in-vivo* study in phase-II was determined based on *in-vitro* dry matter digestibility (IVDMD). Rumen liquor was collected from the experimental animals via a stomach tube 15 min prior to the *in vitro* investigations, 2 h after the morning feeding without water, using the negative pressure created by a suction pump. It was sieved through a four-layered muslin cloth. The SRL was then treated with carbon dioxide gas and stored at a temperature of 39±1°C in a thermos flask for further analysis.

For *in vitro* research, fresh artificial saliva (McDougall buffer) was prepared. After this, rumen liquor was collected from 3 different sheep for additional studies. A total mixed ration (TMR) was prepared for the *in-vitro* study based on the sheep's feeding regimen on the farm. The feed sample was ground using a 1.0 mm screen, and approximately 500 mg samples were placed into 100 mL glass syringes in three replicates for digestibility testing. Blank syringes were also prepared in triplicate. A specific quantity of water, fresh micro and macro minerals solution, buffer, and resazurin was mixed in a flask and preserved in an incubator at 39°C. The required volume of SRL was added with continuously flooded CO_2 in the medium. The necessary volume of rumen inoculum was transferred into the syringe using a silicone tube and placed in a shaker water bath at 39°C. The *in-vitro* digestibility was determined after 24 h of incubation. For the *in-vitro* digestibility assessment, the contents of each syringe were filtered through pre-weighed Gooch crucibles, dried, and then weighed. The Gooch crucibles containing undigested residues were then oven-dried at 70°C for 24 h, cooled in desiccators, and weighed to determine the *in-vitro* DMD.

In Phase II, animals were fed on a farm feed and the quantity of the same were attuned at a biweekly interval according to change in body weight (ICAR, 2013). In supplement, the sheep under group II (Treatment 2) were given 2% DFM based on their DMI. Animals were fed twice daily, in the morning and evening. The dry matter content and CP% of feed were estimated weekly. The samples of feeds offered and left-over feeds in both groups were collected and stored for detailed chemical analysis. The daily feed intake of each experimental animal was diligently recorded. The animals were weighed bi-weekly before feeding and watering using electronic weighing balance.

About 150 mL of rumen liquor was collected at 0, 3, and 6 h after feeding *via* a stomach tube against the suction pump-created negative pressure from five animals of each group. The samples were subjected to pH, TVFAs and nitrogen fractions analysis. The SRL was brought to the laboratory in a pre-warmed (39±1°C) thermos flask. The pH was determined immediately using digital pH meter. The samples of SRL were evaluated for ammonia-N (Pearson and Smith, 1943) and total-N by Kjeldahl's method. The concentration of TVFA was determined from SRL by the steam distillation method, using the Markham micro-distillation apparatus. Records of the daily feed intake and the purchase price of feed and fodder ingredients used in the experiment were used to compute the cost of feeding the experimental animals. The data were analyzed by completely randomized design (Snedecor and Cochran, 1994).

Results and Discussion

In-Vitro Dry Matter Digestibility (IVDMD)

The effects of direct fed microbials (0 to 7%) on *in-vitro* dry matter digestibility (%) and average total gas production (mL) are shown in Table 1. The *in-vitro* results showed, significantly

higher (p<0.05) IVDMD at 2% level of DFM supplementation (49.55%), at par with 1% level (48.15±0.85), than 0 or higher levels of DFM, though the average total gas production did not differ significantly between different DFM treatments.

Table. 1: *In-vitro* dry matter digestibility (IVDMD) percent

Group	DFM (%)	Average total gas production (ml)	Average IVDMD (%)
DFM-0	0	101.50 ± 01.85	39.60^{c} ± 2.12
DFM-1	1	081.75 ± 01.80	48.15^{ab} ± 0.85
DFM-2	2	091.25 ± 03.52	49.55^{a} ± 0.21
DFM-3	3	087.25 ± 14.26	41.25^{c} ± 0.62
DFM-4	4	086.25 ± 09.10	42.35^{c} ± 1.33
DFM-5	5	095.33 ± 06.28	43.85^{bc} ± 3.46
DFM-6	6	106.00 ± 01.35	40.30^{c} ± 2.20
DFM-7	7	081.67 ± 10.63	40.20^{c} ± 0.91
CV%		16.58	8.19
CD @ 5%		NS	5.16
CD @ 1%		NS	6.99

Values bearing different superscripts (a, b, c) within a column differ significantly (p<0.05).

These findings aligned with Carro *et al.* (1992), who reported significantly (p<0.001) higher IVDMD of a high concentrate diet (30% roughage : 70% concentrate) in the yeast culture-supplemented group (87.0%) as compared to the control (83.5%) group, using rumen simulation technique (Rusitec) and SRL collected from cattle. Sherasia *et al.* (2017) also reported significantly (p<0.01) higher IVDMD (78.17%) of TMR of cattle with 5% level of SSF biomass containing *Aspergillus oryzae* and *Trichoderma reesei* spp. of fungi. According to Patel *et al.* (2020), IVDMD was significantly (p<0.01) higher at the 4% level of SSF biomass supplementation. However, Lynch and Martin (2002) observed significantly (p<0.05) lower digestibility of alfalfa hay on supplementation of *Saccharomyces cerevisiae* at 0.35 g/L (56.9%) and 0.73 g/L (56.9%) as compared to control (59.4%). Another *in-vitro* study on yeast culture *(Saccharomyces cerevisiae)* was carried out by Biricik and Turkmen (2001) supplementing 4 g of yeast culture in diet containing 70% alfalfa hay and 30% concentrate, which resulted in significantly (p<0.001) greater IVDMD as compared to control (66.36 Vs. 64.54%) group.

Rumen Fermentation Parameters

Based on *in-vitro* trial results, a 2% level of DFM was used for this *in-vivo* study in the T2 group. The average values of various ruminal parameters studied in SRL are presented in Table 2. The average pH values were non-significant between groups, until 6 h post-feeding, which shows that DFM supplementation had maintained the ruminal pH value in sheep. The TVFAs concentration was non-significantly higher in the experimental animals in the DFM supplemented group T2 than in the control group, which might be due to enhanced microbial activity and microbial population in the rumen. The higher concentration of TVFAs was noted at 3 h post-feeding, which consequently declined gradually up to 6 h of post-feeding in both the groups. Statistically the pH and TVFAs concentration differed significantly (p<0.05) on interaction between the periods. Similarly, Elseed and Abusamra (2007) and Soren *et al.* (2013) observed non-significant effect of DFM supplementation on pH and TVFA concentrations in SRL of lambs. While, Sheikh *et al.* (2019) reported that supplementation of *Saccharomyces cerevisiae* (2×10^{10} cfu/g) plus *Lactobacillus acidophilus* (6×10^{9} cfu/g) with paddy straw based complete feed significantly (p<0.01) increased the pH value in probiotic mix supplemented group in male Corriedale lambs. As compared to the control group T1, the DFM-supplemented group T2 had significantly (p<0.05) greater value of average total nitrogen (118.91 Vs. 100.71 mg/dL). Similar results were reported with DFM supplementation in diet of lambs by Sheikh *et al.* (2019) and Pradhan *et al.* (2021). In contrast, Soren *et al.* (2013) reported that concentration of total nitrogen in the SRL was similar in control and treatment groups, irrespective of probiotic supplementation of *Saccharomyces cerevisiae* (SC) or combination of *S. cerevisiae* and *Lactobacillus sporogenes* (SCLS) at 1.5% of concentrate mixture.

The concentration of NH3-N in both the groups varied within a narrow range and did not reflect any treatment effect. The increased concentration of ammonia nitrogen may be due to increase in the population of protein synthesizing bacteria in rumen by supplementation of DFM. Similar findings with non-significant effect on NH3-N by yeast supplementation at 1.5% of concentrate mixture in lambs was observed by Soren *et al.* (2013) and Sallam *et al.* (2014). Compatibly, Zhong *et al.* (2014) found that ruminal concentrations of ammonia-N was unaffected between inoculation treatments, but varied between sampling times with the significantly lower values at day 56 compared with those at day 0 (p<0.05). Direkvandi *et al.* (2020) reported numerically higher average ammonia-N (mg/dL) concentration in treatment groups (26.0, 25.4 and 24.8) compared to control group (22.1).

Due to DFM supplementation, the ruminal NPN and TCA precipitable nitrogen concentrations changed within a limited range, indicating more or less consistent rumen fermentation. The differences for hours of feeding and the differences for interaction of hours of feeding and treatment period were non-significant on both these parameters. Over the course of the study, the DFM supplemented group exhibited a numerically higher concentration of NPN and TCA precipitable nitrogen than the control group (Table 2). Pradhan *et al.* (2021) also reported similar results in lambs by supplementing yeast (*Saccharomyces Cerevisiae*). In contrast, Sheikh *et al.* (2019) reported significantly (p<0.01) increased TCA-precipitable nitrogen concentration in probiotic mix supplemented group (62.36±2.18 mg/dL) compared to control group (51.46±1.81 mg/dL) in male Corriedale lambs.

Table. 2: Average ruminal pH, TVFA, total-N, NH3-N, soluble-N, NPN and TCA precipitable nitrogen concentration in different treatment groups

Ruminal parameters	Treatment	Hours of sampling			
		0 h	3 h	6 h	Average
Ruminal pH	T_1	7.44	6.53	6.71	6.89±0.28
	T_2	7.67	6.56	6.50	6.91±0.38
TVFA (mmol/dL)	T_1	11.48	14.19	13.73	13.13±0.84
	T_2	11.76	18.24	15.52	15.17±1.88
Average total N (mg/dL)	T_1	91	107.52	103.6	100.71^{a}±4.98
	T_2	97.44	137.2	122.08	118.91^{b}±11.59
Average ammonia N (mg/dL)	T_1	8.4	13.16	15.12	12.23±2.00
	T_2	8.4	14.28	17.36	13.35±2.63
Average NPN (mg/dL)	T_1	29.12	36.96	35.84	33.97±2.45
	T_2	35.28	40.32	43.12	39.57±2.29
Av. TCA precipitable N (mg/dL)	T_1	61.88	70.56	67.76	66.73±2.56
	T_2	62.16	96.88	78.96	79.33±10.02

Values with different superscripts (a, b) within column differ significantly ($p<0.05$) for a parameter.

Economics of Feeding

The mean daily feed cost (₹/head/day) was slightly lower in DFM supplemented (17.25±0.31) group as compared to control (17.54±0.21) group. Similarly, total feed cost (₹/h/70 days) was 1228.07±15.00 and 1207.77±22.00 in T1 and T2 group, respectively. The total feed cost and daily feed cost for feeding sheep did not differ significantly in both the treatment groups. The average cumulative consumption of green, CCM and moong gotar (kg/h/70 days) in T1 and T2 groups were 83.57±2.56 & 75.08±4.24; 25.65±0.46 & 25.35±0.32 and 29.73±0.20 & 29.51±0.31, respectively. The daily feed cost reduced by 1.65% in T2 group compared to T1 group.

Conclusions

The findings of this study revealed that the *in vitro* DFM supplementation at the rate of 2% with farm feeding gave the highest IVDMD compared to higher or lower levels, without adversely affecting the total gas production. In *in vivo* study, DFM supplementation at 2 % did not adversely affect the body weight of sheep, but significantly increased total nitrogen concentration, while insignificantly increased average TVFAs, NPN, ammonia nitrogen and TCA precipitable nitrogen concentration in SRL, suggesting better rumen microbial activity. The daily feed cost (₹/head/day) and total feed cost (₹/head/70 days) were insignificantly ($p>0.05$) lower in DFM supplemented group.

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References

BAHS (2022). *Basic Animal Husbandry Statistics*. Department of Animal Husbandry, Dairying and Fisheries, Government of India, New Delhi.

Biricik, H., & Turkmen, I.I. (2001). The effect of Saccharomyces cerevisiae on *in-vitro* rumen digestibilities of dry matter, organic matter and neutral detergent fibre of different forage: concentrate ratios in diets. *Journal of the Faculty of Veterinary Medicine*, *20,* 29.

Blummel, M., Karsli, A., & Russell, J. R. (2003). Influence of diet on growth yields of rumen microorganisms *in vitro* and *in vivo*: Influence on growth yield of variable carbon fluxes to fermentation products. *British Journal of Nutrition*, *90*, 625-634.

Carro, M.D., Lebzien, P., & Rohr, K. (1992). Influence of yeast culture on the *in vitro* fermentation (Rusitec) of diets containing variable portions of concentrates. *Animal Feed Science and Technology*, *37*(3-4), 209-220.

Direkvandi, E., Mohammadabadi, T., & Salem, A.Z. (2020). Oral administration of lactate producing bacteria alone or combined with *Saccharomyces cerevisiae* and *Megasphaera elsdenii* on performance of fattening lambs. *Journal of Applied Animal Research*, *48*(1), 235-243.

Elseed, F.A.M.A., & Abusamra, R.M.A. (2007). Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on NDF digestibility and rumen fermentation of forage sorghum hay in Nubian goat's kids. *Research Journal of Agriculture & Biological Sciences*, *3*(3), 133-137.

Getachew, G., Makkar, H.P.S, & Becker, K. (2002). Tropical browses: Contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. *Journal of Agricultural Science*, *139*, 341-352.

ICAR (2013). *Nutrient Requirements of Sheep, Goat and Rabbit*, Indian Council of Agricultural Research, Krishi Bhawan, New Delhi, India.

Lynch, H.A., & Martin, S.A. (2002). Effects of Saccharomyces cerevisiae culture and Saccharomyces cerevisiae live cells on

in vitro mixed ruminal microorganism fermentation. *Journal of Dairy Science*, *85*(10), 2603-2608.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., & Schneider, W. (1979). The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *The Journal of Agricultural Science*, *93*(1), 217-222.

Norman, H.C., Revell, D. K, Mayberry, D. E., Rintoul, A. J. Wilmot, M. G., & Masters, D.G. (2010). Comparison of *in vivo* organic matter digestion of native Australian shrubs by sheep to *in vitro* and *in sacco* predictions. *Small Ruminant Research*, *91*, 69-80.

Patel, K.P., Katole, S.B., Pandya, P.R., Sorathia, K.K., & Patel, S. (2020). Effect of solid-state fermentation biomass supplementation to mixed substrate on digestibility and methane mitigation *in vitro*. *Indian Journal of Animal Nutrition*, *37*(2), 127-131.

Pearson, R.M., & Smith, J.A.B. (1943). The utilization of urea in the bovine rumen. 1. Methods of analysis of the rumen ingesta and preliminary experiments *in vivo*. *Biochemical Journal*, *37*(1), 142.

Pradhan, S.K., Deshpande, S.B., Singh, R.R., & Dabas, V.S. (2021). Effect of active dry yeast supplementation on nutrient digestibility, rumen metabolites and performances of Surti goat kids. *Indian Journal of Animal Nutrition*, *38*(1), 23-30.

Puniya, A.K., Salem, A.Z., Kumar, S., Dagar, S.S., Griffith, G.W., Puniya, M., & Kumar, R. (2015). Role of live microbial feed supplements with reference to 33 anaerobic fungi in ruminant productivity: A review. *Journal of Integrative Agriculture*, *14*(3), 550-560.

Salem, A.Z.M., Robinson, P.H., El- Adawy, M.M., & Hassan, A.A. (2007). *In vitro* fermentation and microbial protein synthesis of some browse tree leaves with or without addition of polyethylene glycol. *Animal Feed Science and Technology*, *138*, 318-330.

Sallam, S.M.A., Allam, A.M., & Najadi, S.A. (2014). Comparison of two products of direct-fed microbial supplementation on the nutrient utilization and ruminal fermentation in sheep. *Journal of Agricultural Science*, *6(*3), 159.

Sheikh, G.G., Masood, D., Ganai, A.M., Muzamil, S., Afzal, Y., & Ahmad, H.A. (2019). Effect of probiotics mix supplementation on haemato-biochemical parameters and bacterial faecal shedding in Corriedale lambs fed paddy straw based complete feed. *Indian Journal of Animal Research*, *53*(8), 1049-1053.

Sherasia, P.L., Pandya, P.R., Parnerkar, S., Murty, S., & Devalia, B.R. (2017). *In vitro* digestibility and methane production as affected by incorporation of different levels of solid-state fermented biomass in total mixed rations. *Indian Journal of Animal Nutrition*, *34*(4), 420-424.

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

Soren, N.M., Tripathi, M.K., Bhatt, R.S., & Karim, S.A. (2013). Effect of yeast supplementation on the growth performance of Malpura lambs. *Tropical Animal Health and Production*, *45,* 547-554.

Tedeschi, L.O., Kononoff, P.J., Karges, K., & Gibson, M.L. (2009). Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co) products. *Journal of Dairy Science*, *92*, 401-413.

Wang, J., Zhao, G., Zhuang, Y., Chai, J., & Zhang, N. (2022). Yeast (*Saccharomyces cerevisiae*) culture promotes the performance of fattening sheep by enhancing nutrients digestibility and rumen development. *Fermentation*, *8*(12), 719.

Zhong, R.Z., Sun, H.X., Li, G.D., Liu, H.W., & Zhou, D.W. (2014). Effects of inoculation with rumen fluid on nutrient digestibility, growth performance and rumen fermentation of early weaned lambs. *Livestock Science*, *162,* 154-158.