International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM February 2025, 16(2): 01-06 Article AR5794

Research Article Natural Resource Management

DOI: HTTPS://DOI.ORG/10.23910/1.2025.5794

Effect of Direct Fed Microbials on Body Weight, Nutreint Intake and Nutrient Digestibility in Sheep

Rahul K. Dangi[1], Makbul A. Shekh[1], Rakesh J. Modi[2], Ashish C. Patel[3], Priyam H. Agravat[2] and Sunil V. Rathod[1]

[1]Dept. of Animal Nutrition Research Station, [2]Dept. of Livestock Production Management, [3]Dept. of Animal Genetics and Breeding, Kamdhenu University, Anand, Gujarat (388 001), India

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Corresponding drpriamagravett@gmail.com

0009-0005-4877-4050

ABSTRACT

The present study was conducted during mid May to July, 2023 (70 days) at Animal Nutrition Research Station, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat (388001), India to investigate the effect of direct fed microbials on nutrient intake and digestibility in adult Sheep. Twenty adult sheep, which were apparently healthy, of nearly the same body weight were used. The experiment was conducted in two phases: *in-vitro* study to decide the level of DFM and *in-vivo* study. The results of *in vitro* study showed, significantly ($p<0.05$) higher IVDMD (49.55%) at 2% level of DFM. Thus, an additional *in-vivo* investigation was carried out at the 2% DFM level on DM basis. The sheep were allotted into two equal groups, both groups T_1 and T_2 were fed compound concentrate mixture and roughage in conventional farm feeding, additionally treatment group (T_2) was supplemented with 2% DFM. During experimental period the animals were weighed at biweekly interval. The weight gain was not differed significantly in both the groups. The average daily DMI of experimental animals was significantly lower in T_2 as compared to T_1 group, respectively. The study revealed no adverse effect of DFM on dry matter and other nutrient intakes. The digestion trial was conducted on all the twenty experimental sheep once during the experimental period. The digestibility coefficient (%) of crude protein was significantly higher in treatment group T_2 as compared to control T_1 group. Digestibility of DM, OM, EE, CF, ADF, NDF and hemicellulose was not affected by 2% of DFM supplementation in feed over the control diet.

KEYWORDS: DFM, body weight, nutrient intake, digestibility

Citation* (VANCOUVER)*: Dangi et al., Effect of Direct Fed Microbials on Body Weight, Nutreint Intake and Nutrient Digestibility in Sheep. *International Journal of Bio-resource and Stress Management,* 2025; 16(2), 01-06. HTTPS://DOI.ORG/10.23910/1.2025.5794.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 19th September 2024 RECEIVED in revised form on 14th January 2025 ACCEPTED in final form on 05th February 2025 PUBLISHED on 11th February 2025

1. INTRODUCTION

The study of rumen microbes aims to improve feed utilization, enhance animal production and health, and ensure the safety of animal food products. These objectives can be realized by elevating nutrient intake and digestibility, promoting beneficial fermentation processes, minimizing ruminal disorders, and eliminating pathogens. A variety of feed additives have been utilized to enhance animal performance, increase feed efficiency, and prevent disease (Ban et al., 2021). Research has focused on antibiotics and probiotics (growth promoters) to manipulate the microbial ecosystem and fermentation characteristics within the rumens and intestinal tracts of livestock (Kassa et al., 2022).

The use of growth-promoting antibiotics in animal feeds is prohibited in Europe due to potential risks, including the spread of antibiotic resistance genes (Prieto et al., 2014) and the contamination of milk and meat with antibiotic residues. Consequently, many livestock producers have turned to alternative strategies to improve animal performance and health. In recent years, direct-fed microbials (DFM) have gained attention as a viable replacement or a means to reduce antibiotic usage. The term "probiotics" describes "a live microbial feed supplement that can improve the intestinal microbial balance of host animals" (Anee et al., 2021). This includes viable microbial cultures, extracts, and enzyme preparations. Direct Fed Microbials (DFM) are a more specific type of microbial feed additive (Kassa et al., 2022).

Numerous studies have definitively established methods to enhance the productivity of ruminant animals by strategically manipulating the rumen environment. This enhancement leads to improved feed digestibility and nutrient utilization, ensuring that adequate nutrients are available to support elevated levels of milk production (Sallam et al., 2014). A particularly promising approach that has emerged is the use of direct-fed microbial (DFM) preparations, which has been extensively researched and shown to be effective. Recent studies in small ruminant nutrition have focused on improving feed conversion rates. Antimicrobial feed additives, widely used but now restricted due to concerns about antibiotic resistance, have sparked interest in alternatives like direct-fed microbials (DFMs), which consist of beneficial microorganism cultures (Elam et al., 2003). Sheep and goat are highly prolific livestock in India, playing a crucial role in the economy and providing income for rural households. With their ability to convert crops and household residues into meat, fiber, skins, and milk, they are important for development. Additionally, ensuring proper nutrition is essential for maintaining their productivity and reproductive performance. In ruminants, direct-fed microbials are used to reduce stress and antibiotics use in calves, while increasing milk yield in dairy cows and improving weight gain and feed conversion in beef cattle. (Krehbiel et al., 2003). Adding direct-fed microbials to the ration of growing lambs reduced harmful microorganisms in the intestines and improved fattening performance and feed conversion rate (Lema et al., 2001). Feed additives like enzymes, prebiotics, probiotics (DFM), and antimicrobial growth promoters have been used to improve the health and productivity of sheep in farming. DFMs are live microorganisms added to animal diets for these benefits. Spore-forming bacteria are used in developing direct-fed microbials (DFMs) because they effectively distribute strains to targeted organs. In livestock production, lactic acid-producing bacteria such as *Enterococcus species, Streptococcus species, Lactobacillus species and Pediococcus species* are commonly utilized (Puniya et al., 2015 ; Elghandour et al., 2015 and Kholif et al., 2024). Direct-fed microbials show promise in improving animal performance and rumen fermentation (Azzaz et al., 2016). The use of antibiotics in animals can lead to antibiotic residues and resistance, disrupting the microbial balance in ruminants. Research is exploring direct-fed microbials as a safer alternative to antibiotics to enhance production and health in small ruminants by restoring gut flora balance (Parvez et al., 2006). Therefore, the present study was conducted to compare the responses of sheep to supplementation of commercial DFM and determine their effect on feed intake, nutrient digestibility and utilization.

2. MATERIALS AND METHODS

The was conducted at Animal Nutrition Research Station, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat (388001), India for 70 days after approval of protocol from CPCSEA (approval No. 400/AN/23-CPCSEA), New Delhi on the recommendation of Institutional Animal Ethics Committee (IAEC). The experiment was conducted on a total of thirty (n=20) adult sheep. The Department of Microbiology at Gujarat Vidhya pith, Sadra, prepared the direct-fed microbial (DFM) using vegetable waste with a culture of *Lactobacillus lactis, Lactobacillus paracasei, Lactobacillus bifermentans, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bacillus coagulants and Pediococcus acidilactici* of bacteria. The animals were randomly allotted based on their body weight into following two treatments each comprised of ten adult sheep. The duration of experiment was 70 days. 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 (Anonymous, 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 animals will be released for exercise under controlled circumstances for two hours in the afternoon, during which time they will have to free access to fresh, clean drinking water. The deworming of all the animals was done using broad-spectrum anthelmintic drug throughout experimental period.

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 using electronic weighing balance. A digestion trial was conducted on all the experimental animals to determine the digestibility of various nutrients. Throughout the whole trial period, an accurate record of the feed that each animal eaten, refused, and of faeces voided was kept. The dried samples collected were pooled, ground and preserved for chemical analysis as per Anonymous (2005) and for fiber fractions as per Van Soest et al., 1991. The wet faeces were preserved in acid and they were used for further estimation of faecal nitrogen & ultimately CP. The experimental data were analyzed by completely randomized design (Snedecor and Cochran, 1994).

3. RESULTS AND DISCUSSION

3.1. Body weight

The animals in control (T_1) and DFM treatment (T_2) had average initial body weights of 29.40 and 29.34 kg, respectively (Table 1). Over the course of the trial, they gained a total of 2.90 and 3.05 kg, resulting in final average body weights of 32.30 and 32.39 kg. The results indicated that while the animals in both groups did not lose weight during the trial, the average total body weight increase in the T_2 group (3.05 kg) during the course of the study which was insignificantly higher than that of the T_1 group (2.90 kg). DFM treatment though did not affect the final body weight, the average weight observed was insignificantly ($p>0.05$) higher in T_1 compared to T_2 DFM treatment (Table 1). In agreement with our study, Hagg et al. (2010) and ElKatcha et al. (2016) did not found any significant effect of DFM supplementation (*Megasphaera elsdeni* and *Pediococcus* spp., respectively) on body weight of animals. Similarly, Bata et al. (2022) did not find any significant effect of ammoniated rice straw treatment using direct-fed microbial and *Hibiscus tiliaceus* leaf meal supplementation with TMR on body weight of animals. Similar findings were reported by Wang et al. (2022) by supplementation of yeast (*Saccharomyces cerevisiae*) culture as a DFM with peanuts straw, corn, soyabean meal, wheat bran and tofu residue which offered during the experiment in fattening sheep.

3.2. Nutrient intake

DFM supplementation significantly decreases the dry matter intake and crude protein intake (Table 1). These findings were in accordance with Sallam et al. (2014), Soren et al. (2013), Zhong et al. (2014), Direkvandi et al. (2020) and Wang et al. (2022), they also reported no adverse effects of DFM supplementation on nutrient intake. In contrast to the current study's results, Hassan et al. (2020) reported that the increased total DM intake (p=0.02) for lambs fed the probiotics product diets versus lambs fed the control diet. Likewise, Elseed and Abusamra (2007), Bata et al. (2022) and Pradhan et al. (2021) observed increased DMI with adding of DFM sources in the diet of sheep.

The use of DFM @ 2% with farm feed in sheep had no adverse effect on DCP and TDN intake, though apparently

Table 1: Effect of DFM on body weight and nutrients intake

Variable	T_1	T_2	SEm±	p value
Initial BW (kg)	29.40±0.48	29.34±0.42	0.45	NS
Final BW (kg)	32.30±0.61	32.39±0.50	0.56	NS
Avg. wt. (kg)	31.72±0.47	31.65±0.48	4.33	NS
DMI (g h^{-1} d^{-1})	1129.90^a±33.76	1087.45^b±35.27	10.834	S
DMI (g 100 kg^{-1} BW d^{-1})	3525.03^a±112.87	3388.99^b±115.80	37.735	S
DMI (g kg^{-1} BW0.75 d^{-1})	83.84^a±2.63	80.64^b±2.71	0.821	S
CPI (g d^{-1})	120.30^a±2.46	116.22^b±2.55	0.921	S
CPI (g 100 kg^{-1} BW d^{-1})	374.81^a±7.46	362.15^b±7.59	2.991	S
DCPI (g d^{-1})	75.97±1.55	78.94±1.72	1.058	NS
DCPI (g 100 kg^{-1} BW d^{-1})	236.74±4.74	245.84±5.15	3.297	NS
TDNI (g d^{-1})	590.69±17.68	606.77±18.42	5.953	NS
TDNI (g 100 kg^{-1} BW d^{-1})	1841.77±58.39	1891.29±61.14	19.933	NS

Mean±SE values with different superscripts (a, b) within row differ significantly ($p<0.05$)

the values were higher in T_1 than in T_2 treatment (Table 1). In agreement with our present findings, Chaudhary (2020) reported that supplementation of SSF biomass @ 3% of DM, significantly increased the DCPI (g d^{-1}) as compared to the control group in crossbred heifers (402.42 *vs.* 361.49). Similarly, Sadrsaniya et al. (2015) reported significantly (p<0.001) higher DCPI (kg d^{-1}) in probiotics supplemented group compared to the non-supplemented group in buffalo calves. Similarly, Tripathi and Karim (2010) reported that supplementation of mixed yeast culture (*Kluyveromyce smarximanus* NRRL3234, *Saccharomyces cerevisiae* NCDC42, *Saccharomyces uvarum* ATCC9080 all in a 1:1:1, ratio) insignificantly (p>0.05) increased TDNI in lambs. While Hassan et al. (2020) and Sadrsaniya et al. (2015) observed increased TDN intake with adding of DFM in the diet of sheep.

3.3. Digestibility of nutrients

The data pertaining to digestibility of nutrients were presented in Table 2. The study revealed that there was no adverse effect of DFM on digestibility of nutrients like DM, OM, CP, EE, NFE, ADF, and hemicellulose, though the values gradually increased with 2% DFM supplement over the control diet. In fact, the digestibility of CP was significantly improved in DFM supplemented group over control group. Further, the digestibility of fibre fractions *i.e.*, CF, NDF, ADF and hemicelluloses were found to be increased numerically but not significantly with inclusion of DFM in the diet of sheep over control. The non-significance results of nutrient digestibility (DMD and OMD) were in accordance with Soren et al. (2013), ElKatcha et al. (2016) and Sallam et al. (2014). In contrast, Madkour et al. (2018) found that direct fed microbial supplementation to improve utilization of the low-quality roughages in lambs significantly improves (p<0.001) the DM digestibility in DFM supplemented group (72.87, 72.45 and 72.90) as compared to control (68.37).

The average CP digestibility percent of experimental animals under T_1 and T_2 groups were 63.09±1.47 and 67.97±1.63, respectively. Our findings supported the previous observations of Madkour et al. (2018), Hassan et al. (2020), and Pradhan et al. (2021) for an increased (p<0.05) in CP digestibility while Soren et al. (2013), Sallam et al. (2014) and ElKatcha et al. (2016) found non-significant effect of DFM supplementation on CP digestibility. The improvement in CP digestibility observed might be due to limited CP intake in DFM supplemented group which might results in high microbial fermentation and ultimately higher CP digestibility. The non-significance results of CF, EE and nitrogen free extract digestibility were in accordance with ElKatcha et al. (2016), while in contast to Madkour et al. (2018) in lambs.

Table 2: Average digestibility percent of nutrients

Attributes (%)	T_1	T_2	SEm±	Sig. (p<0.05)	CV %
DMD	55.01±2.59	59.39±2.35	2.47	NS	13.68
OMD	61.94±2.44	66.02±2.04	2.25	NS	11.11
CPD	63.09^{a}±1.47	67.97^{b}±1.77	1.63	S	7.86
EED	57.12±2.63	61.94±2.29	2.47	NS	13.11
CFD	42.14±2.96	47.15±3.59	3.29	NS	23.31
NFED	69.98±2.68	73.99±1.65	2.23	NS	9.77
NDFD	49.14±2.60	53.93±2.67	2.64	NS	16.18
ADFD	34.19±3.40	40.64±3.53	3.47	NS	29.33
HCD	66.85±1.86	69.74±1.80	1.83	NS	8.49

NS: Non significant, S: Significant, Mean±SE values with different superscripts (a, b) within row differ significantly (p<0.05)

4. CONCLUSION

The DFM supplementation at the rate of 2% with farm feeding did not adversely affect the body weight, nutrient intake and digestibility, except crude protein, which was significantly improved. Though the 2 percent level of DFM in diet of sheep enhanced the flow of microbial protein from rumen and observed higher CP digestibility in sheep.

5. ACKNOWLEDGEMENT

We extend my sincere thanks to the Animal Nutrition Research Station, College of Veterinary Science & AH, Kamdhenu University, Anand for providing research facilities and financial supports for this study. First author would like to extend sincere and heartfelt obligation towards all who have helped him in this endeavor.

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