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*Research Article*

# Effect of Direct Fed Microbial (DFM) on *in-vitro* Methanogenesis and Dry Matter Digestibility

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**ABSTRACT**

*The in-vitro rumen fermentation study was conducted to evaluate the effect of supplementation of direct-fed microbial (DFM) in the total mixed ration (TMR) on total gas production, methanogenesis and dry matter digestibility by using rumen liquor of adult Surti buffaloes. DFM was supplemented at 0, 1, 2, 3, 4, 5, 6, 7, and 8% with TMR (65% Straw and 35% concentrate) for in-vitro gas production trials. The result of the in-vitro study revealed significantly (P<0.05) higher IVDMD (57.03%) and lower $CH_4$ production (3.20 ml $CH_4$/100 mg DDM) at a 3% level of DFM supplementation in TMR. Based on the overall results of in-vitro studies 3% level of DFM supplementation was found to be most suitable for further in-vivo studies in adult Surti buffaloes.*

***Keywords:*** *DFM, in-vitro, Digestibility, Methane, Gas production.*

## 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. It is best to consider supplements as an addition to sound feeding procedures. An animal's behaviour can be influenced by a class of feed chemicals known as feed additives, which are only required at trace levels. The use of antibiotics in feed has substantially reduced in the last ten years due to their detrimental effects on animal health, on account of the residue they leave 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.

Direct-fed microbials (DFM) have many advantages when they are consumed. They support the growth of advantageous rumen microorganisms and keep the pH stable.

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DFM enhances nutrient flow post-ruminantly, enhances nutrient digestion, and lowers stress by enhancing immunological response (Yoon & Stern, 1995). When yeast was added to the meal, nutritional digestibility greatly improved, and the activity of carboxy methyl cellulose in the rumen increased. The animals were fed *Saccharomyces cerevisiae* to increase their production and ability to digest food. Both the feed conversion ratio and body weight dramatically increased. Total VFA synthesis, the ratio of acetate to propionate, especially four hours after feeding, and *in-vitro* dry matter digestibility all increased in sheep (Deendayal, 2008; & Rao et al., 2001). Yeast cultures have been proven to promote fibre digestion, activate the rumen's cellulolytic bacteria, and regulate the cattle's rumen pH (Rossi *et al.,* 2006). There are many advantages of using DFM in ruminants, such as positively affecting ruminant productivity, immunity, defence against pathogens and infections, health protection, and methane emission reduction.

## MATERIALS AND METHODS

The present study was conducted at Animal Nutrition Research Station, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat. Wheat straw, Mung Gotar, Groundnut cake, Deoiled rice bran, Molasses and mineral mixture were used for the preparation of Total Mixed Ration (TMR). This TMR was oven-dried at 70° C and finely grounded in a Wiley mill using a 1mm sieve. The TMR was analyzed for proximate constituents (AOAC, 2005) and fibre fraction (Van Soest et al., 1991).

DFM was procured from the Department of Microbiology, Gujarat Vidhyapeeth, Sadra, Gandhinagar, Gujarat, India. The direct-fed microbial (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* species of bacteria. The experimental TMR without supplementation of DFM was control group and designated as D0, while TMR with DFM supplementation @ 1, 2, 3, 4, 5, 6, 7 and 8% were designated as D1, D2, D3, D4, D5, D5, D6, D7 and D8.

Using a stomach tube, Rumen liquor for *in-vitro* rumen fermentation studies was collected from two adult Surti buffaloes. Buffaloes were fed individually with TMR prepared to meet their nutrient requirements (ICAR 2013) with free access to water. Collected rumen liquor was strained through four- layers of muslin cloth, termed strained rumen liquor (SRL) and mixed in prepared artificial saliva (McDougall's) in proper proportions before incubation. Substrates (200 mg) with various levels of DFM were incubated with artificial saliva mixed with SRL (40 ml) for 48 h in quadruplet at 39 ± 1°C in a shaker twin water bath (Menke et al., 1979). After 48 h of incubation, total gas production (TGP) was recorded after subtracting gas production from blank. To determine *in-vitro* methane production, gas produced in 100 ml glass syringes after a 24-hour incubation period was used. A gas sample was directly injected into a Gas Chromatograph (GC) from each syringe, and $CH_4$ concentration was determined against standard methane gas (22.54%). All samples were analysed using a GC instrument, fitted with an SS column (4 ft. long, 3.2 mm inside diameter) packed with Porapaek N (80 to 100 mesh) and equipped with a flame ionization detector (FID). Column temperature was maintained at 50°C, and nitrogen was used as a carrier gas, with flow rate of 30 ml/min. Calibration was completed using standards (22.54%) procured from CHEMIX Specialty Gases & Equipment., Bangalore. After completion of incubation, the content of each syringe was filtered and dried in a pre-weighed Gooch crucible. The IVDM was calculated by subtracting residues that remained after incubation from the amount of substrate incubated and was expressed in percentage.

**Statistical analysis**

The data generated during the experiment were analyzed by two-way analysis of variance

(ANOVA) using the WASP 2.0 method as prescribed by Snedecor and Cochran (1994).

## RESULTS AND DISCUSSION

Table 1 presents the data on the proximate composition and fibre fraction of prepared TMR. Table 2 presents the effects of direct-fed microbes on IVDMD (%), Total Gas Production (TGP, ml), and methane (ml/100 g of digestible DM).

**Table 1: Chemical composition and fibre fraction of Total mixed ration**

| Parameters (%, on DM basis) | TMR |
|---|---|
| **Crude protein** | 11.08 |
| **Ether extract** | 2.33 |
| **Crude fibre** | 27.42 |
| **Nitrogen free extract** | 45.30 |
| **Total Ash** | 13.87 |
| **Organic matter** | 78.84 |
| **Neutral detergent fibre** | 53.13 |
| **Acid detergent fibre** | 35.32 |
| **Cellulose** | 28.12 |
| **Hemicellulose** | 17.81 |
| **Lignin** | 5.62 |
| **Calcium** | 1.50 |

**Table 2: Average *in-vitro* dry matter digestibility (IVDMD, %), total gas production (TGP, ml) and methane (ml/100 g digestible DM) of substrates containing different levels of DFM**

| Substrates | IVDMD (%) | TGP (ml) | Methane |
|---|---|---|---|
| **D0** | 53.12 ± 3.69 | 67.67 ± 2.96 | 4.18 ± 0.30 |
| **D1** | 46.13 ± 6.83 | 51.33 ± 4.91 | 4.09 ± 0.54 |
| **D2** | 38.13 ± 7.41 | 57.67 ± 12.84 | 3.98 ± 0.70 |
| **D3** | 57.03 ± 0.98 | 70.33 ± 0.88 | 3.20 ± 0.31 |
| **D4** | 51.17 ± 0.87 | 51.00 ± 7.55 | 3.76 ± 0.04 |
| **D5** | 49.73 ± 2.24 | 63.33 ± 5.78 | 3.33 ± 0.09 |
| **D6** | 53.09 ± 0.30 | 67.67 ± 7.86 | 3.46 ± 0.18 |
| **D7** | 54.17 ± 1.97 | 66.67 ± 3.71 | 3.36 ± 0.11 |
| **D8** | 56.43 ± 6.80 | 71.33 ± 4.91 | 3.65 ± 0.63 |
| **SEM** | 4.37 | 6.57 | 0.40 |
| **CD(0.05)** | NA | NA | NA |
| **CV%** | 14.86 | 18.06 | 18.65 |

The data on proximate composition and fibre fractions of the prepared substrate are presented in Table 1. The data on IVDMD are shown in Table 2, while values of TGP and methane (ml) produced per 100 g of digestible DM are presented in Table 2. Perusals of the data revealed a non-significant (P>0.05) improvement in the digestibility of DM in the 3% DFM group compared to the control. In the present study, higher fermentation rates were observed due to the addition of DFM, which might have improved digestibility.

The findings indicated that the *in-vitro* dry matter digestibility per cent was 53.12, 46.13, 38.13, 57.03, 51.17, 49.73, 53.09, 54.17, and 56.43 for the D0, D1, D2, D3, D4, D5, D6, D7, and D8 groups, respectively. For the D0, D1, D2, D3, D4, D5, D6, D7, and D8 groups, the corresponding *in-vitro* total gas production (ml) values were as follows: 67.67,

51.33, 57.67, 70.33, 51.00; 63.33, 67.67, 66.67; and 71.33. The *in-vitro* methane production values for the D0, D1, D2, D3, D4, D5, D6, D7, and D8 groups were 4.18, 4.09, 3.98, 3.20, 3.76, 3.33, 3.46, 3.36, and 3.65, respectively.

The present data revealed 7.36 % higher IVDMD at a 3 % level of DFM than the control group. Similarly, statistically higher IVDMD was also observed by Sullivan & Martin (1999).

The total amount of gas produced during the 48-hour *in-vitro* incubation study is presented in Table 2. Methane contributes to major greenhouse gas emissions, and thus, mitigation measures are also required for a better environment. The numerical increase in total gas production indicates better *in-vitro* rumen fermentation.

There was a 21% numerically ($P>0.05$) decrease in methane production at a 3% level of DFM supplementation compared to the control.

The rumen microbiome is crucial in fermentation as it significantly influences the overall production of the gas, methane ($CH_4$), and the concentration of volatile fatty acids (VFA). The addition of probiotics, additives, or various nutrients in the feed has an established effect on methane ($CH_4$) generation. (Mamuad et al., 2014, & lamba et al., 2014). We found no significant reduction in $CH_4$ yield with or without DFM. The CH4 results indicated that there was no effect of the additives; our finding corroborates with Dhakal et al. 2023, while our results are not in agreement with other research (Pan et al., 2022; Ellis et al., 2016; Ridwan et al., 2018, & Qiao et al., 2010). Doyle et al. (2019) found that lactic acid bacteria can decrease CH4 emissions by directly affecting methanogenesis through the use of bacteriocins or by reducing the availability of substrates for methanogenesis given by rumen microbes. In addition, yeast cultures can potentially decrease ruminal methane (CH4) generation by many mechanisms, such as reducing the number of protozoa, boosting the production of butyrate or propionate, and encouraging acetogenesis (Newbold et al., 1998). In an in-vitro investigation, Chaucheyras et al. (1995) provided evidence that extracts from *Saccharomyces cerevisiae* enhanced the utilisation of hydrogen by acetogens and reduced the emission of $CH_4$.

## CONCLUSION

It can be concluded that the in-vitro total gas production technique (IVTGP) showed 7% higher IVDMD and 21% lower methane production at the 3% level of DFM supplementation. Hence, DFM supplemented at this level in the ruminant's diet is suitable for further *in-vivo* studies.

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**Conflict of Interest:**

There is no such evidence of conflict of interest.

**Author Contribution**

All authors have participated in critically revising the entire manuscript and approving the final manuscript.

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