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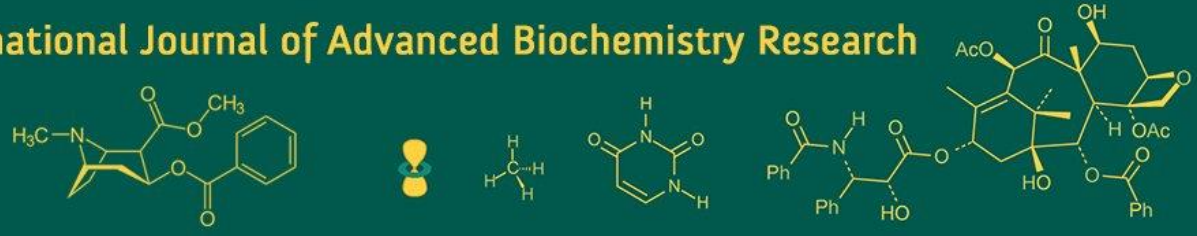

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# Effect of solid state fermented (SSF) biomass on *in-vitro* methanogenesis and dry matter digestibility in adult Surti buffaloes

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**Abstract**
Using the rumen fluid of adult Surti buffaloes, an *in-vitro* rumen fermentation study was carried out to assess the impact of adding Solid State Fermented Biomass (SSF) biomass to the total mixed ration (TMR) on methanogenesis, dry matter digestibility, and total gas generation. For *in-vitro* gas generation experiments, SSF biomass was supplemented with TMR (65% wheat straw and 35% concentrate) at 0, 1, 2, 3, 4, 5, 6, 7, and 8%. At a 3% level of SSF biomass addition in TMR, the *in-vitro* study's results showed considerably ($p$<0.05) higher IVDMD (58.43%) and decreased CH4 generation (3.58 ml CH4/100 mg DDM). The most appropriate level of SSF biomass supplementation for additional *in-vivo* research in adult Surti buffaloes was determined to be 3% based on the overall findings of *in-vitro* experiments.

**Keywords:** SSF biomass, *in-vitro*, digestibility, methane, gas production

## Introduction
Rumen microbial research aims to improve animal production, feed utilization, food safety, and health. By promoting optimal fermentation, reducing ruminal issues, and avoiding infections, these objectives can be achieved. Supplements should ideally be viewed as an adjunct to good feeding practices. A category of feed compounds called feed additives, which are only necessary at trace quantities, might affect an animal's behavior. Due to their negative effects on animal health, the residue they leave in animal products, and the potential for microorganisms to develop resistance to them, the use of anti-biowaste in feed has significantly decreased during the past ten years. The use of microorganisms in animal nutrition became more and more common as a result. Up until now, the primary focus has been on creating enzyme supplements that improve the digestion of fiber and reduce enteric methane emissions from large ruminants.

Solid-state fermentation (SSF) and submerged fermentation (SmF) are the two primary methods for enzyme extraction. Growing interest has been shown in SSF's bio-conversion of fibrous material since it requires less energy, produces less wastewater, and allows fermented products to be directly applied for feeding (Yang *et al.*, 2011) [17]. The process of fermenting solids without the presence of free water, or nearly without it, is known as "solid-state fermentation."

Solid-state fermentation has great potential for the production of enzymes by microbial flora. This process produces a raw, fermented product that may be used immediately as an enzyme source, which makes it especially interesting. Ideally, almost all known microbial enzymes can be produced using the SSF technique. Many studies have been conducted on the production of enzymes such as pectinases, cellulases, xylanases, amylases, proteases, and cellulases (Pandey *et al.*, 1999) [13]. Since they were introduced to animal feeds in recent years, exogenous fibrinolytic enzymes have greatly improved digestibility, reduced intestinal methane emission, and improved ruminant feed utilization efficiency both *in vitro* (Murad *et al.*, 2009) [12] and *in vivo* (Arriola *et al.*, 2011) [3]. The aim of the current study was to determine how SSF biomass supplementation affected *in-vitro* rumen fermentation.

## Materials and Methods

The Animal Nutrition Research Station at Kamdhenu University's College of Veterinary Science and Animal Husbandry in Anand, Gujarat, is where the current study was carried out. Molasses, groundnut cake, deoiled rice bran, wheat straw, mung gotar, and mineral combination are combined to make the Total Mixed Ration (TMR). This TMR was finely processed in a Wiley mill using a 1mm sieve after being oven dried at 70 °C. The TMR was examined for the fiber fraction (Van Soest *et al.*, 1991) [16] and proximal constituents (AOAC, 2005) [1].

SSF biomass was procured from Department of Microbiology, Gujarat Vidhyapeeth, Sadra, Gandhinagar, Gujarat, India. *Trichoderma spp*. and *Aspergillus oryzae* fungal cultures were used to analyze the solid state fermented (SSF) biomass of jowar hay. While TMR with SSF biomass supplementation at 1, 2, 3, 4, 5, 6, 7, and 8% were classified as $S_1$, $S_2$, $S_3$, $S_4$, $S_5$, $S_6$, $S_7$, and $S_8$, the experimental TMR without any SSF biomass supplementation was assigned as the control group and was marked as $S_0$.

Using a stomach tube, the rumen liquor from two adult Surti buffaloes was extracted for *in-vitro* rumen fermentation research. Individual buffaloes were given unrestricted access to water and TMR that was prepared to fulfill their nutritional needs. Prior to incubation, the collected rumen liquor—known as strained rumen liquor, or SRL—was strained through four layers of muslin cloth and combined with prepared artificial saliva (McDougall's) in the appropriate amounts. In a shaker twin water bath, 200 mg of substrates containing different amounts of SSF biomass were incubated for 48 hours in a quadruplet at 39±1 °C with artificial saliva combined with 40 ml of SRL (Menke *et al.*, 1979) [11]. Total gas production (TGP) was calculated after subtracting gas output from blank after 48 hours of incubation. Gas generated in 100 ml glass syringes following a 24-hour incubation period was utilized to measure *in-vitro* methane generation. Each syringe's gas sample was directly injected into a Gas Chromatograph (GC), and the concentration of CH4 was measured in comparison to the standard methane gas (22.54%). A GC apparatus furnished with a flame ionization detector (FID) and an SS column (4 feet long, 3.2 mm inner diameter) packed with Porapack N (80 to 100 mesh) was used to analyze all samples. Nitrogen was utilized as a carrier gas, with a flow rate of 30 milliliters per minute, and the column temperature was kept at 50 degrees Celsius. Using standards (22.54%) purchased from CHEMIX Specialty Gases & Equipment, Bangalore, the calibration was finished. Each syringe's contents were filtered and dried in a Gooch crucible that had been previously weighed when the incubation period was over. The IVDM was stated as a percentage and was computed by deducting residues left over after incubation from the volume of substrate incubated.

## Statistical analysis

According to Snedecor and Cochran's (1994) [15] recommendations, the experiment's data were subjected to a two-way analysis of variance (ANOVA) using the WASP 2.0 method.

## Results and Discussion

Table 1 displays the prepared TMR's proximal composition and fiber fraction data. Table 2 shows how IVDMD, Total Gas Production (TGP), and methane (ml/100 g of digestible DM) are affected by solid state fermented (SSF) biomass.

**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 level of SSF biomass

| Substrates | IVDMD | TGP | Methane |
|---|---|---|---|
| $S_0$ | $53.12^{ab}$±0.37 | 67.67±2.96 | 4.01±0.44 |
| $S_1$ | $48.97^{a}$±0.35 | 35.33±15.07 | 4.01±0.77 |
| $S_2$ | $51.90^{ab}$±2.78 | 59.00±11.27 | 3.85±0.83 |
| $S_3$ | $58.43^{b}$±4.53 | 52.33±11.79 | 3.58±0.22 |
| $S_4$ | $50.80^{ab}$±1.53 | 42.00±14.00 | 4.02±0.21 |
| $S_5$ | $52.83^{ab}$±0.88 | 23.37±14.66 | 4.27±0.22 |
| $S_6$ | $48.03^{a}$±1.27 | 58.00±4.04 | 4.35±0.36 |
| $S_7$ | $56.83^{ab}$±2.90 | 50.33±14.15 | 4.23±0.22 |
| $S_8$ | $56.73^{ab}$±5.70 | 64.00±3.06 | 4.32±0.49 |
| SEM | 3.03 | 11.24 | 0.48 |
| CD(0.05) | 8.99 | NA | NA |
| CV% | 9.88 | 38.76 | 20.14 |

* a, b, c superscripts in a column differ significantly ($p$<0.05)

The results showed that the $S_0$, $S_1$, $S_2$, $S_3$, $S_4$, $S_5$, $S_6$, $S_7$, and $S_8$ groups had *in-vitro* dry matter digestibility percentages of 53.12, 48.97, 51.90, 58.43, 50.80, 52.83, 48.03, 56.83, and 56.73, respectively. The equivalent *in-vitro* total gas production (ml) values for the $S_0$, $S_1$, $S_2$, $S_3$, $S_4$, $S_5$, $S_6$, $S_7$, and $S_8$ groups were 67.67, 35.33, 59.00, 52.33, 42.00, 23.37, 58.00, 50.33, and 64.00. For the $S_0$, $S_1$, $S_2$, $S_3$, $S_4$, $S_5$, $S_6$, $S_7$, and $S_8$ groups, the corresponding in-vitro methane production values were 4.01, 4.01, 3.85, 3.58, 4.02, 4.27, 4.35, 4.23, and 4.32.

Analysis of the data showed that the IVDMD was substantially ($p$<0.05) higher in the group supplemented with 3% SSF biomass (58.43%) than in the control group (53.12%). Higher fermentation rates were seen in the current study as a result of the addition of SSF biomass, which may have increased digestibility. Furthermore, adding enzymes promoted the development of cellulolytic bacteria, sped up the breakdown of fiber, and made it easier for microbial protein to leave the rumen (Azzaz *et al.*, 2013) [4]. Likewise, other authors have reported a significant ($p$<0.05) improvement in IVDMD (Bhasker *et al.*, 2012; Arati, 2013; Reddy *et al.*, 2016; Chaudhari, 2018) [6, 2, 14, 7].

The total amount of gas produced throughout the 48-hour *in-vitro* incubation investigation was recorded, and Table 2 shows the results. There was no discernible difference between the total gas production values. Since methane contributes significantly to greenhouse gas emissions, reducing its impacts is also essential for a healthier

environment. Probiotics, supplements, and other nutrients have been shown to have an impact on the production of methane (CH4) Lamba *et al.* (2014) [8] and Mamuad *et al.* (2014) [9] in feed. The effect of SSF biomass supplementation on methane production levels was found to be quantitatively ($p$>0.05) lower in the group supplemented with 3% SSF biomass than in the control group.

## Conclusion

At the 3% level, SSF biomass was found to exhibit a noticeably increased IVDMD. SSF biomass fed at a 3% level in the diet is the most appropriate for additional *in-vivo* research because *in-vitro* methane production quantitatively decreased at this level.

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