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Effect of Solid-state Fermentation Biomass Supplementation to Mixed Substrate on Digestibility and Methane Mitigation *in vitro*

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ABSTRACT

An experiment was conducted to assess the effect of supplementation of various levels of solid-state fermentation (SSF) biomass on *in vitro* digestibility, total gas production and methane production. Eight different types of substrates were prepared *viz.* SSF0 (control), and the basal substrates supplemented with SSF biomass at 1%, 2%, 3%, 4%, 5%, 6% and 7% replacing mixed cereal straw was designated as SSF1, SSF2, SSF3, SSF4, SSF5, SSF6 and SSF7, respectively. *In vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) were 10.62 and 11.70% higher (P<0.01) in SSF4 treatment than that of SSF0 treatment. *In vitro* total gas production was increased numerically due to SSF biomass supplementation as compared to control with maximum increment at 5% level, but statistically the change was not significant. Methane production after 24 h of incubation was lowest (P<0.01) in SSF4 treatment, it reduced by 38.4% as compared to control. Results revealed an improved digestibility, gas production and reduced methane production at 4% level of SSF supplementation, and was found to be most suitable level for further *in vivo* studies in dairy animal.

Key words: SSF, *in vitro*, Digestibility, Methane, Gas production

INTRODUCTION

Animal husbandry plays an important role in rural economy of India as almost about 73% of rural population of India possesses some form of livestock. Moreover, the importance of livestock in India's economy can be gauged from the fact that 90 million farming families rear over 90 million milch animals. According to livestock census 2012, there is an increment of 4.51% livestock population in India as compared to previous one while for the state of Gujarat increment in livestock population was even more up to 15.36%. As population grows it leads to increased demand for feeds and fodder leading to widening of the gap between demand and supply. The deficit in the requirement and availability of dry fodder, green fodder and concentrates during 2015 was to the extent of 21 MT, 26 MT and 34 MT which will increase further to 23 MT, 40 MT and 38 MT, respectively, by the year 2025 (NIANP, 2016). This scenario demands a paradigm shift for implementing newer arena of nutritional research on the way of developing proper feeding strategies to utilize available feed resources for its optimum utilization. Ruminant animals have complex ecosystem of various microorganisms which eventually utilize low quality agricultural by-products (rich in cellulose, hemicellulose and lignin) and convert them into quality food resources.

Utilization of such agricultural by-products can be improved by appropriate use of feed additives like prebiotics, probiotic, enzymes *etc.* and also through appropriate application of biotechnological tools. Solid state fermentation (SSF) technique is the one of the most suitable and economical technique for enrichment of microbial protein, efficient digestion and utilization of lignocellulosic agricultural fibrous feeds and fodder residues for enhancing their feed values. SSF is a cultivation technique where microbes and fungi (Doelle *et al.,* 1992) are grown on a moist solid substrate under controlled conditions which are devoid of actual free water which eventually can produce a variety of enzymes (Pandey *et al.,* 1999) and can be fed directly as crude fermentable mass to animals. Beneficial effects of supplementing such fibrolytic enzymes produced by microorganisms on overall productive performance of lactating ruminants was also reported

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by several authors (Miachieo and Thakur, 2007; Gallardo *et al.,* 2010; Lopuszanska-Rusek and Bilik, 2011; Ahmad Para *et al.,* 2019; Arif *et al.,* 2019). It was hypothesized that SSF would improve the fermentation kinetics and digestibility of nutrients. Hence, this study was planned to assess the effect of supplementation of various levels of SSF biomass on *in vitro* digestibility, total gas production and methane production.

MATERIALS AND METHODS

The present study was conducted at Animal Nutrition Research Department, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat. Ingredients for experimental substrates were procured from farmers where further *in vivo* trial will be carried out. Mixed cereal straw (paddy straw, *bajra* straw and wheat straw), green fodder (jowar CoFS-29) and Amul buffalo special concentrate mixture (*Dan*), Amulnutri power and Amul crushed maize (*bhardo*) and cotton seed cake and maize cake were procured, which were then oven dried at 70°C and finely grounded in Wiley mill using 1 mm sieve. Substrates were prepared for *in vitro* by mixing the ingredients in ration as per the feeding schedule followed by farmers i.e. by mixing green fodder, mixed cereal straw, and concentrate at 4:5:4 (kg on dry matter basis). The ingredients and substrates were analyzed for proximate constituents (AOAC, 1995) and fibre fractions (Van Soest *et al.,* 1991).

SSF biomass was procured from Department of Microbiology, Gujarat Vidhyapeeth, Sadra, Gandhinagar, Gujarat, India. SSF biomass was prepared using inoculations of various fungi species *i.e. Aspergillus spp., Trichoderma spp., Fusarium spp., Pleurotus spp.* and *Phanerochaete spp.* on *jowar* hay which was used as solid substrate for SSF biomass preparation. Activity of various microbial enzymes *viz.* carboxy methyl cellulase (CMCase), filter paperase (FPase), xylanase, laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP) were at the tune of 170, 60, 468, 270, 488, 298 Units/g of wet weight of SSF biomass, respectively. The experimental substrate without any supplementation of SSF biomass was designated as SSF0 (control), while mixed cereal straw of the SSF0 was replaced with 1, 2, 3, 4, 5, 6 and 7% of SSF in groups SSF1, SSF2, SSF3, SSF4, SSF5, SSF6 and SSF7, respectively.

Rumen liquor for *in vitro* studies was collected from two lactating buffaloes using stomach tube. Buffaloes were fed individually with TMR prepared to meet their nutrients requirements (ICAR, 2013) with free access of water. Collected rumen liquor was strained through four-layers of muslin cloth which was termed as strained rumen liquor (SRL) and was mixed in prepared artificial saliva (McDougall's) in proper proportions prior to incubation. Substrates (200 mg) with various levels of SSF biomass was 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 24 h of incubation period was used. A gas sample was directly injected into Gas Chromatograph (GC) from each syringe and CH_4 concentration was determined against standard methane gas (10.4 ppmv and 101.9 ppmv). All samples were analysed using GC instrument, fitted with a SS column (4 ft. long, 3.2 mm inside diameter) packed with Porapack N (80 to 100 mesh), 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 (10.4 ppmv and 101.9 ppmv) procured from Scott-Marrin Inc., USA. Peak areas were integrated using a Perkin Elmer integrator. 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 remained after incubation from the amount of substrate incubated and was expressed in percentage.

Statistical analysis

Data obtained were subjected one way analysis of variance as per the methods of Snedecor and Cochran

(1994).Treatment means were separated by using Duncan's multiple range tests. All analysis was performed using a statistical package (SPSS 9.00 software).

RESULTS AND DISCUSSION

The data on proximate composition and fibre fractions of prepared substrate are presented in Table 1. The data on IVDMD and IVOMD are presented 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 significant ($P<0.05$) improvement in digestibility of DM and OM in 4% SSF group as compared to control. In the present study higher fermentation rates was observed due to addition of SSF which might have improved digestibility. Furthermore, addition of enzymes stimulated the population of cellulolytic bacteria, increased fibre digestion and flow of microbial protein from the rumen (Azzaz *et al.,* 2013). This improvement in digestibility could be also due to synergetic effects of enzyme and rumen microbes (Morgavi *et al.,* 2000), better attachment of rumen microbes and feed particles (Yang *et al.,* 1999). Similarly, significant improvement in IVDMD (Faramarzi-Garmroodi *et al.,* 2013; Rajamma *et al.,*2015; Lopez *et al., 2*016; Vallejo *et al.,* 2016; Lunagariya *et al.,* 2017) and IVOMD (Elghandour *et al.* 2013; Dey *et al.,* 2014; Lunagariya *et al.,* 2017). was also reported by earlier authors.

Total amount of gas produced under the anaerobic microbial fermentation during *in vitro* incubation study of 48 h was observed and presented in Table 2. The values for the gas production were found to be numerically increased due to SSF supplementation but did not differ significantly. Numerical increase in gas production with increasing levels of SSF biomass in this study indicates better nutrients availability for rumen microbes which further increases their fermentation activity resulting in better feed digestion Morgavi *et al.* (2000); Azzaz *et al.* (2013).Methane contributes for major greenhouse gas emissions thus its mitigation measures are also required for better environment. Effect of SSF biomass supplementation on levels of methane production was estimated with gas chromatography. There is a significant ($P<0.05$) decrease in methane production (38.4%) in SSF4 group as compared to control.

Table 1. Proximate composition of substrate, jowar hay and SSF

Parameters (%)	Substrate	*Jowar* Hay	SSF
Crude protein	11.27	6.74	9.55
Ether extract	3.93	2.78	2.91
Crude fibre	28.84	38.40	31.89
Nitrogen free extract	45.92	42.24	42.63
Organic matter	89.96	90.16	86.98
Ash	10.04	9.81	13.00
Silica	4.10	3.25	3.77
Ca	1.11	0.66	0.73
P	0.89	0.18	0.18
Neutral detergent fibre	64.17	72.94	63.30
Neutral detergent solubles	44.35	34.22	39.65
Acid detergent fibre	34.84	40.04	37.19
Hemi-cellulose	29.33	32.89	26.10
Cellulose	22.07	35.43	29.21
Lignin	12.29	5.03	7.65

Table 2. Average *in vitro* dry matter digestibility (IVDMD, %) and organic matter digestibility (IVOMD, %), total gas production (TGP, ml) and methane (ml/ 100 g digestible DM) of substrates containing different levels of SSF biomass

Substrates	IVDMD	IVOMD	TGP	Methane
SSF0	60.64^{c}±0.54	60.47^{c}±0.66	68.00±1.77	1.38^{ab}±0.08
SSF1	61.48^{c}±1.81	60.89^{c}±1.82	66.25±4.98	0.91^{c}±0.20
SSF2	61.77^{c}±0.15^{c}	61.20^{c}±0.16	71.50±1.75	0.97^{bc}±0.16
SSF3	62.65^{c}±1.18	62.37^{c}±1.27	73.75±3.14	1.08±0.14^{bc}
SSF4	67.07^{a}±1.62	67.55^{a}±1.63	71.50±2.72	0.85^{bc}±0.07^{c}
SSF5	65.92^{ab}±0.90	65.78^{ab}±1.07	78.00±2.04	1.63^{a}±0.005
SSF6	62.98^{bc}±1.05	63.13^{bc}±0.77	77.50±1.93	1.36^{ab}±0.16
SSF7	63.00^{bc}±0.47	62.70^{bc}±0.62	72.75±4.67	1.62^{a}±0.19
[1]SEM	1.10	1.33	3.12	0.14
[2, 3] CD (0.05)	3.22	3.30	NS	0.34
[4]CV %	3.50	3.59	8.64	20.34

^{abc}Means with different superscripts in a column for a parameter differ significantly; [1]SEM, standard error of mean; [2]CD, critical difference; [3]level of significance, [4]CV, co-efficient of variation; SSF0 was made by blending jowar hay mixed cereal straw, and concentrate at 4:5:4; while mixed cereal straw of the SSF0 was replaced with 1, 2, 3, 4, 5, 6 and 7% of SSF in groups SSF1, SSF2, SSF3, SSF4, SSF5, SSF6 and SSF7, respectively.

CONCLUSION

It was observed that SSF biomass at 4% levels has shown most significant improvement in IVDMD and IVOMD, and significant reduction in methane emission *in vitro*. Therefore, SSF biomass at 4% level of supplementation was found to be most suitable for further *in vivo* studies in dairy animals.

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