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IN VITRO ASSESSMENT OF NUTRIENT DIGESTIBILITY AND MICROBIAL BIOMASS PRODUCTION OF TOTAL MIXED RATION SUPPLEMENTED WITH DIFFERENT LEVELS OF THERMOSTABLE YEAST IN CROSSBRED DAIRY COWS

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ABSTRACT

An experiment was conducted to study the effect of supplementation of thermostable yeast in total mixed ration on rumen fermentation parameters by *in vitro* gas production technique in crossbred cows. *Saccharomyces cerevisiae* was supplemented in the total mixed ration at various doses *viz*., 0 (control), 0.25×10^6, 0.5×10^6, 1×10^6, 2×10^6 and 4×10^6 CFU. The *in vitro* true dry matter degradability, i*n vitro* true organic matter degradability, total gas production, metabolisable energy, volatile fatty acid production and microbial biomass production increased proportionally to the dose of *S. cerevisiae*. Methane production was significantly lowered by supplementation. Results revealed that the *S. cerevisiae* supplementation can modify ruminal fermentation and were dose dependent.

Keywords: *In vitro*, Microbial biomass, *S. cerevisiae*, Total mixed ration

INTRODUCTION

Exploring natural growth-promoting substances like probiotics or their metabolites as feed additives to manipulate rumen fermentation and to enhance feed efficiency is the need of the hour due to the negative impact of inclusion of antibiotics and ionophores in animal feed. Probiotics have the ability to alter rumen fermentation and also improve animal performance. Probiotics such as *Lactobacillus sp., Propionibacterium sp., Saccharomyces sp., Bifidiobacterium sp. etc.* are generally used as feed additive. Among this *Saccharomyces*

sp. supplementation in ruminant's diet is widely used. *Saccharomyces cerevisiae* is a direct-fed microorganism (DFM) of fungal in origin and capable of fermenting carbohydrates. (Perdomo *et al*., 2020). Live *Saccharomyces cerevisiae* scavenges ruminal oxygen (Newbold *et al*., 1996), which reduces ruminal redox potential, favouring the activity of cellulolytic bacteria and lactate-utilising bacteria (Marden *et al*., 2008). It is also associated with increased fibre digestion in the rumen, and optimisation of the volatile fatty acid concentration, decreases rumen ammonia nitrogen and stimulate the growth of rumen microorganisms. The role of *S. cerevisiae* by in *vitro* and *in vivo* fermentation has been described by Elghandour *et al*. (2014) and Ahmed *et al.* (2015). Inclusion of yeast in ruminant diets leads to increased consumption of dry matter and nutrient utilisation (Wang *et al*., 2016). However, most of the commercial strains of yeast used is mesophilic in nature and the beneficial effect may vary due to the adverse conditions in the rumen (temperature and pH). Hence, this study was designed to evaluate the effect of thermostable *S. cerevisiae* as feed additive at various levels in paddy straw based total mixed ration (TMR) on nutrient digestibility and microbial biomass production in crossbred cows.

MATERIALS AND METHODS

The evaluation of TMRs were done by Hohenheim *in vitro* gas production technique (Menke and Steingass, 1988). Rumen liquor was collected from early lactating crossbred cows fed on standard TMR in accordance with ICAR (2013) using stomach tube against negative pressure created by a suction pump. It was strained through four layers of cheese cloth, transferred into pre-warmed CO_2-filled thermos flask. The temperature of the rumen fluid was maintained at 39 °C throughout the preparation of the incubation medium. Fermentation was conducted in 100 mL glass syringe. The syringes were prewarmed (39°C) for one hour, before the addition of 30 mL of buffered rumen fluid into each syringe under CO_2 flushing. The required volume of strained rumen liquor, water, micro and macro minerals solution, buffer and resazurin were mixed in flask and CO_2 was flushed continuously in the medium. Three blank syringes containing only 30 mL of buffered rumen fluid were incubated to estimate gas production due to endogenous substrates for the blank corrections. Two hundred milligram of TMR were fortified with different levels of yeast and were incubated in 30 ml of incubation medium (Makkar, 2004). The syringes were then placed in automatic shaker water bath incubator at 39 °C.

Analyses were completed in sextuplicate (two times) with readings of gas production recorded after incubation for 0 and 24 hours. The fermented fluid was collected for the estimation of volatile fatty acids, *in vitro* true dry matter and organic matter degradability. The metabolisable energy and microbial protein was calculated from the data as per Blummel and Lebzien (2001).

Total Gas Production

Gas produced (ml/ 200 mg substrate) by fermentation of substrate feed during 24 hour was measured after correcting corresponding blank values.

In vitro True DM and OM Digestibility

Goering and Van Soest (1970) method were followed for the determination of true dry matter digestibility (TDMD) and true organic matter digestibility (TOMD)

$$\text{TDMD \%} = \frac{(\text{DM taken for incubation} - \text{NDF residue})}{(\text{DM taken for incubation})} \times 100$$

$$\text{TOMD \%} = \frac{(\text{OM taken for incubation} - \text{OM residue})}{(\text{OM taken for incubation})} \times 100$$

Microbial Biomass Production (MBP)

Microbial biomass production (MBP) of the TMR tested was calculated from TDOM using equation

MBP (mg) = TDOM (mg) – (Corrected gas production for 24 hrs×2.20)

Where 2.20 is the stoichiometric factor for roughages (Blummel *et al*., 1997) and for mixed diets (Blummel and Lebzien, 2001)

TDOM- Truly digested organic matter

Metabolizable Energy (ME)

ME of target TMR was calculated by formula given by Menke and Stiengass (1988)

ME (KJ/kg DM) = 1.24+ 0.146 × gas (ml / 200mg DM) + 0.007× CP + 0.0224× EE

Where, CP - Crude protein, EE - Ether extract

Methane Estimation

The percentage of methane production was estimated by collecting and injecting the gas produced on *in vitro* study into the Methane Gas Analyser (0-100%; Precision Equipment Private Limited) at Central Instruments Laboratory, CVAS, Mannuthy. **(**Purushothaman *et al.,* 2019; Sadan *et al*., 2019 and Neenu, 2021).

Estimation of Volatile Fatty Acids

Volatile fatty acid composition of the inoculum was found out using 7890A

NUCON 5700 gas chromatograph, as per standard procedure described by Filipek and Dvorak (2009). On completion of the incubation, the buffered rumen liquor was filtered through four layers of muslin cloth and approximately 0.8 mL of the sample was preserved with 200 µL of 25 per cent metaphosphoric acid and allowed to stand for half an hour, then centrifuged at 7000 rpm for 20 min at 4 °C. The samples preserved in this way were immediately analysed or stored at -20 °C temperature for the further analysis.

Observations made on the various parameters *viz*. true dry matter digestibility (TDMD) and true organic matter digestibility (TOMD), microbial biomass production, metabolisable energy and methane production were subjected to cluster analysis and based on this, the best level of yeast was identified.

Table 1. Ingredient composition of total mixed ration used in *IVGPT*

Ingredient	Quantity (parts per quintal)
Maize	23
Rice polish	10
Corn gluten fibre	10
De-oiled rice bran	10
Alfalfa pellet	8
Coconut oil cake	8
Paddy straw	29
Mineral mixture	1
Salt	1
Total	100

Feed samples were analysed for proximate principles (AOAC, 2016). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the method described by Van Soest *et al.* (1991). Data gathered on the various parameters were analysed statistically as per Snedecor and Cochran (1994) by analysis of variance (ANOVA) technique, using the software, statistical product and service solutions (SPSS) version 24.0.

RESULTS AND DISCUSSION

Proximate Composition

The chemical composition and fibre fractions of the evaluated samples are presented in Table 1. The CP, EE, CF, total ash, NFE, acid insoluble ash, calcium, phosphorus, NDF and ADF contents of the basal substrate were found to be 13.28, 3.74, 12.55, 11.45, 58.99, 5.11, 0.90, 0.56, 35.25 and 24.74 per cent on dry matter basis.

In Vitro Gas Production

Ruminal fermentability characteristics evaluated by *IVGPT* are presented in Table 3. Net gas production for 24 hours in different treatments ranged from 28.62 to 38.11 mL. *S. cerevisiae* supplementation to basal diet improved the total gas production irrespective of the level of supplementation. Significantly highest

Table 2. Chemical composition of TMR used in IVGPT (% DM basis)

Parameter	Nutrient composition (%)
Dry matter	92.63
Crude protein	13.28
Ether extract	3.74
Crude fibre	12.55
Total ash	11.45
Nitrogen free extract	58.99
Acid insoluble ash	5.11
Calcium	0.90
Phosphorus	0.56
Neutral detergent fibre	35.25
Acid detergent fibre	24.74

total gas production was documented at 4×10^6 CFU of *S. cerevisiae* supplementation. The increased total gas production on yeast supplementation to TMRs could be attributed to increased nutrient degradation. Malik and Singh (2009) and Elghandour *et al.* (2014) also reported increased *in vitro* gas production in groups supplemented with *S. Cerevisiae* than control group.

Digestibility and Metabolisable Energy

Metabolisable energy varied from 5.62 to 7.02 MJ/kg DM. The corresponding TDMD and TOMD values ranged from 67.29 to 78.66 and 70.48 to 83.32, per cent respectively. Supplementation of yeast to basal diet significantly ($P < 0.01$) improved ME, *in vitro* TDMD and TOMD (Table 3). Higher levels of supplementation showed significantly higher *in vitro* TDMD and TOMD and ME when yeast was supplemented at 4×10^6 CFU *S. cerevisiae* level. Increase in nutrient digestibility in the present study may be due to the stimulation of rumen microbial growth. Higher *in vitro* rumen degradability of feed was also reported by Rodriguez *et al.* (2015) by supplementing TMR with *S. cerevisiae* as a feed additive than control. *S. cerevisiae* was reported to effectively scavenge O_2 from the rumen, enhance anaerobiosis and improved fermentation thereby making a favourable condition for the action of various microorganisms (Newbold *et al*., 1996).

Methane production

Methane production in different treatments varied from 16.94 to 19.26 per cent. The results showed significantly lowered methane production in *S. cerevisiae* supplemented treatment groups than control group. *S. cerevisiae* might have stimulated the acetogens through competition and co-metabolisation of H_2 with methanogens (Hristov *et al*., 2013) and thereby lowering methane production. Newbold and Rode (2006) and Seo *et al*. (2010) also reported a decrease in methane production by supplementing live *S. cerevisiae*. On the contrary Elghandour *et al.* (2017) reported increased methane production with supplementation of *S. cerevisiae.*

Microbial Biomass Production

The MBP (mg/200mg DM) calculated from the gas production data are listed in Table 3. The MBP production ranged from 63.32 to 68.24 mg respectively. The statistical analysis of the data on MBP revealed a significant difference ($P < 0.01$) between the treatments. Microbial biomass was highest for T4 where the *S. cerevisiae* was supplemented at 1×10^6 CFU. This could probably be due to the ability of *S. cerevisiae* to remove oxygen from the rumen (Kumar *et al.,* 2013) and making the rumen environment more suitable for microbial growth. *Saccharomyces cerevisiae* fermentation metabolites (i.e., B vitamins, amino acids, organic acids) and may also have contributed to the enhanced total anaerobic and cellulolytic bacteria counts.

Increased *in vitro* total bacterial count on *S. cerevisiae* supplementation was also observed by Lila *et al*. (2006) and Malik and Singh (2009).

Volatile fatty acids

Data related to total volatile fatty acids (TVFA (mmol/L), acetic acid, propionic acid, butyric acid and acetate: propionate ratio are presented in Table 4. Acetic acid concentration ranged from 45.20 to 46.77, propionic acid from 19.04 to 22.06 and TVFA ranged from 71.09 to 73.51 mMol/L. Total volatile fatty acids (TVFA) and propionate production increased ($P < 0.01$) with increase in dose of *S. cerevisiae*, whereas, acetate production ($P <0.05$) and the ratio of acetate: propionate decreased ($P < 0.01$) proportionately with increase in dose of *S. cerevisiae*. However, butyrate

Table 3. *In vitro* gas, CH4, ME, TDMD, TOMD and MBP production of TMR supplemented with yeast in cross bred cows

Treatments	Total gas (mL)	CH_4 (%)	ME (MJ/kg DM)	TDMD (%)	TOMD (%)	MBP (mg)
T1	28.62± 0.38 ^{a}	19.26 ± 0.24^{c}	5.62 ± 0.06^{a}	67.29 ± 0.18^{a}	70.48 ± 0.24^{a}	63.32 ± 1.13^{a}
T2	33.47± 0.39 ^{b}	17.58 ± 0.16^{b}	6.34 ± 0.06^{b}	72.92 ± 0.27^{b}	76.73 ± 0.19^{b}	64.14 ± 1.13^{a}
T3	33.91± 0.24 ^{b}	17.43 ± 0.03^{b}	6.40 ± 0.04^{b}	74.11 ± 0.7^{c}	78.37 ± 0.29^{c}	65.8 ± 0.24^{ab}
T4	36.29± 0.26^{c}	17.27 ± 0.04^{ab}	6.75 ± 0.04^{c}	77.85 ± 0.15^{d}	83.01 ± 0.28^{d}	68.24 ± 0.47^{c}
T5	36.85± 0.30 ^{c}	17.29 ± 0.06^{ab}	6.83 ± 0.04^{c}	77.78 ± 0.23^{d}	82.95 ± 0.09^{d}	66.87 ± 0.6^{bc}
T6	38.11± 0.36^{d}	16.94 ± 0.12^{a}	7.02 ± 0.05^{d}	78.66 ± 0.21^{d}	83.32 ± 0.13^{d}	64.95 ± 0.83^{ab}
F-value	107.34	39.40	107.34	158.40	554.24	5.066
P-value	0.001**	0.001**	0.001**	0.001**	0.001**	0.002**

[1]Mean values are based on six replicates with S.E.

**Mean± S.E. of different treatment having different alphabets as superscripts within a column differ significantly at $p<0.01$

production did not differ (P > 0.05) between the treatment groups. The VFA represent the main supply of energy for ruminants, and therefore an increase in their production would be favourable for the host animal.

Similarly, Diaz *et al*. (2017) reported that *S. cerevisiae* supplementation increased the total volatile fatty acid production and decreased acetate proportion and acetate: propionate ratio compared to control.

CONCLUSION

The results of the present experiment revealed that there was significant difference in *in vitro* rumen fermentation parameters with addition of *S. cerevisiae*. Supplementation of *S. cerevisiae* for manipulating rumen fermentation was effective and showed positive results on total gas production, *in vitro* true dry matter degradability, volatile fatty acid production and microbial biomass production. *S. cerevisiae* supplementation also decreased the methane production *in vitro* in paddy straw-based TMRs. It can be inferred that *S. cerevisiae* supplementation can be used as an environmental-friendly approach for the enhancement of nutrient utilisation in animals and its productivity.

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Table 4. Volatile fatty acid concentration[1] of TMR supplemented with yeast in cross bred cows assessed *in vitro*

Treatments	Acetic acid (mMol/L)	Propionic acid (mMol/L)	Butyric acid (mMol/L)	Total volatile fatty acids (mMol/L)	Acetate: propionate ratio
T1	46.77±0.29^{c}	19.04±0.52^{a}	5.28±0.40	71.09±0.73^{a}	2.47±0.19^{b}
T2	46.46±0.46^{bc}	19.10±0.52^{a}	5.78±0.63	71.35±0.49^{a}	2.44±0.22^{b}
T3	45.82±0.26^{abc}	20.89±0.50^{b}	5.60±0.51	72.31±1.46$^{a\,b}$	2.20±0.13^{a}
T4	45.66±0.23^{ab}	21.65±0.50^{b}	5.82±0.76	73.13±0.77^{b}	2.11±0.14^{a}
T5	45.60±0.22^{ab}	21.87±0.31^{b}	5.85±0.50	73.32±1.12^{b}	2.09±0.06^{a}
T6	45.20±0.53^{a}	22.06±0.18^{b}	6.25±0.75	73.51±1.95^{b}	2.05±0.06^{a}
F- value	2.803	9.662	1.678	4.556	9.803
P- value	0.034*	0.000**	0.170^{ns}	0.003**	0.000**

[1]Mean values are based on six replicates with S.E.

**Mean± S.E. of different treatment having different alphabets as superscripts within a column differ significantly at p<0.01

ns- non significant

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