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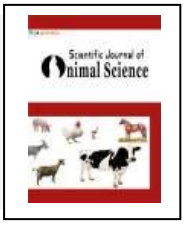

Original article

Influence of biologically treated wheat straw diet on *in vitro* rumen fermentation, methanogenesis and digestibility

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ABSTRACT

The present study was conducted to assess the nutritive worth of diets comprising of wheat straw (T1 diet) and basidiomycetes white-rot fungal (WRF) isolate RCK-SC treated wheat straw (T2 diet) for in vitro gas production, digestibility and rumen fermentation parameters. Diets (on dry basis) containing 70% straw, 10% berseem green forage, 17% groundnut cake, 2% mineral mixture and 1% salt were formulated. It was observed that in vitro total gas (GV 24 h), true DM and OM digestibility (48 h), in vitro NDF digestibility (48 h), microbial biomass production (MBP) and short chain fatty acid (SCFA) production were significantly ($P<0.05$) higher in T2 diet to the extent of 9.71, 17.39, 14.92, 17.45, 6.34, 9.34%, respectively when compared to T1. While, total N and NH3-N in rumen liquor of both the diet remained same. Volatile fatty acids like propionate concentration increased ($P<0.05$) with a decrease in acetate to propionate ratio (A:P). Methane production expressed as L/kg IVTDMD was significantly ($P<0.05$) lower (13.86%) in T2 diet. This study demonstrates that fungal treatment of wheat straw using basidiomycetes WRF isolate RCK-SC produces better quality straw and its incorporation benefits rumen fermentation in vitro.

1. Introduction

One of the reasons for suboptimal performance of livestock in the tropical countries can be the feeding of poor quality dry roughages like straws (Logeswari Monica et al., 2013), which constitute a major part of ruminant ration, including in India. Wheat straw, a major energy-rich cereal straw available in Northern India (up to 109.9 MMT, Saritha et al., 2012) is low in protein and minerals besides the presence of lignin-cell wall polysaccharide complex, which make it inaccessible by rumen microbiota leading to low digestibility (García-Cubero et al., 2012) that inturn limits its fullest nutritional utility in ruminants. Biological treatment by solid state fermentation (SSF) using basidiomycetes white-rot fungi (WRF) has been employed as a means of bioremediation of straws to improve nutritive value by selectively reducing lignin content (Villas-Bôas, 2002; Hassim et al., 2012), which is a non-hazardous and eco-friendly method. Different WRF like Ceriporiopsis sp., Cyathus stercoreus, Pleurotus sp., Lentinus edodes, Phlebia sp., Phanerochaete chrysosporium, Ganoderma sp. and Trametes versicolor have been used successfully to improve the nutritive value of various substrates viz. wheat straw, paddy straw, madake bamboo, bermuda grass, corn-stalks and oil palm fronds etc. under SSF (Okano et al., 2009; Akinfemi, 2010; Arora and Sharma, 2012; Hassim et al., 2012; Omer et al., 2012; Shrivastava et al., 2012).

Livestock are responsible for 18% of the global anthropogenic greenhouse gas emissions, accounting for about 37% of the total anthropogenic methane emissions (Patra and Yu, 2012). Methanogenesis represents a net loss of 2-12% of ingested gross energy, depending on the quality of feeds fermented in the rumen (Patra, 2012). Ruminants fed on crop residues are reported to emit a large quantity of methane (Zhu et al., 2008) due to longer ruminal residency period (Moss et al., 1994). A few in vitro studies also indicated that fungal treated substrates yield low methane in the rumen (Jalc et al., 1994; Akinfemi, 2010).

As the gas production technique (Menke and Steingass, 1988) is one of the best techniques currently available to study the rate of in vitro organic matter fermentation in rumen fluid (Krishnamoorthy et al., 2005), this technique is employed in the current study.

Objective of the present investigation was to evaluate the nutritional quality in terms of rumen fermentation and digestibility of diet comprising mainly of wheat straw treated with ligninolytic WRF RCK-SC by in vitro methods.

2. Materials and methods

2.1. SSF of wheat straw

Wheat straw was dried at 60 0C and sieved to attain a uniform particle size (1.5-2.0 cm) before SSF (Shrivastava et al., 2012). The unfermented and fermented straws were ground in the laboratory mill (Remi Motors, Delhi, India) and sieved (30 mesh) for analytical purposes.

The fungus used in the study was isolated from the leaf litter in Delhi ridge forest area and temporarily named as basidiomycetes WRF RCK-SC, maintained on malt extract agar (MEA) in the University of Delhi South Campus, New Delhi. The cultures were stored at 4°C and subcultured every fortnight. Inoculation of wheat straw with fungal inoculums was carried out as per Shrivastava et al. (2012) with a final substrate to moisture ratio of 1:3 and incubated at 30 ºC and 60% relative humidity for 10 days. The uninoculated trays without fungal inocula served as control. The inoculated wheat straw trays in triplicates were harvested at regular intervals and weight loss of wheat straw was determined by deducting the weight of oven dried fermented straw (at 60 ºC to a constant weight) from the weight of dried control straw (Shrivastava et al., 2011).

2.2. Experimental diets

The two experimental diets were prepared as below considering maximum inclusion of wheat straw.

T1 diet: 70% wheat straw (WS) + 10% berseem fodder + 17% groundnut cake + 2% mineral mixture + 1% salt.

T2 diet: 70% fungal treated wheat straw (FT-WS) + 10% berseem fodder + 17% groundnut cake + 2% mineral mixture + 1% salt.

2.3. Proximate, cell wall analysis, in vitro gas production, methane and digestibility

Proximate and cell wall analysis were performed according to AOAC (2005) and Van Soest et al. (1991), respectively. Hemicellulose was the difference between neutral detergent fibre (NDF) and acid detergent fibre

(ADF). Acid detergent lignin (ADL) was assayed by solubilizing ADF with 72% (w/w) sulfuric acid (Van Soest et al., 1991). Cellulose was calculated by subtracting ADL from ADF. Total carbohydrates (T-CHO) were calculated by subtracting crude protein, ether extracts and ash contents from 100 (Sniffen et al., 1992). All the chemical analyses were in triplicates.

For in vitro gas production study (Menke and Steingass, 1988), rumen fluid was collected from two cannulated Karan-Fries bulls (5-6 years, body weight 450 ± 7.3 kg) fed on maintenance ration. Around 200 ± 10 mg of air equilibrated substrates of both T1 and T2 diets were taken with 30 ml of buffered rumen inoculum (10 ml rumen fluid, 5ml bicarbonate buffer, 5 ml macro-mineral solution, 0.0025 ml micro-mineral solution and 10 ml distilled water) under continuous flushing with CO2 into 100 ml calibrated syringes (Fortuna optima, Germany) along with blanks. After 24 h of incubation, net gas produced (GP24 h) was recorded from visual assessment of the calibrated scale on the syringe and was corrected for blanks. In vitro microbial biomass production (MBP) was calculated (Blümmel et al., 1997) considering 2.20 as the stoichiometric factor, and in vitro short chain fatty acids (SCFA) were calculated by the equation developed by Getachew et al. (2002), which are as follows.

MBP (mg) = TDOM (mg) – (GP24 h×2.20)
where TDOM is truly digestible organic matter
In vitro SCFA (mM/0.2g DM) = (0.0239×GP24 h) – 0.0601

For in vitro true dry matter digestibility (IVTDMD), separate incubations (48 h) of the 2 diets were carried out (Menke and Steingass, 1988) and, IVTDMD and in vitro NDF digestibility (IVNDFD) was calculated after recovering syringe contents with neutral detergent solution (Blümmel and Becker, 1997). Upon ashing the residue at 550 0C, in vitro true organic matter digestibility (IVTOMD) was estimated.

For methane estimation, 1 ml gas was sampled from the head space of syringe in an air tight syringe (Hamilton) and injected into gas chromatograph (Nucon 5700, India) equipped with stainless steel column packed with Porapak-N and flame ionization detector (FID). Methane volume (ml) was calculated as methane (%) × total gas produced (ml) in 24 h as suggested by Bhatta et al. (2013) and expressed per unit of IVTDMD and IVTOMD.

Table 1
Chemical composition of the experimental diets (% DM basis).

Parameter	T1 diet	T2 diet
Organic matter	91.54	88.51
Crude protein	12.42	13.73
Ether extract	2.36	2.10
Total ash	7.86	10.89
NDF (ash free)	60.33	54.96
ADF (ash free)	41.28	36.94
Hemicellulose	19.05	18.02
Cellulose	32.39	30.32
Acid detergent lignin	9.03	6.76
T-CHO	76.76	72.67

2.4. Rumen fluid analysis

After noting the gas volume at 24 h, contents of each syringe were centrifuged (Heraeus, Germany) at 12,000 × g for 20 min at 4 0C to get clarified rumen liquor, which was used immediately for ammonia nitrogen (NH3-N) estimation and preserved at –20 0C for determining individual volatile fatty acids (IVFA) as per Erwin et al. (1961) using gas chromatograph (Nucon 5700, India) equipped with flame ionization detector (FID) and stainless steel column (length 4I; o.d ¼II; i.d 3 mm) packed with Chromosorb–101. Total nitrogen (N) and NH3–N were estimated by Kjeldahl method by following only distillation and titration steps.

2.5. Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) as per Snedecor and Cochran (1994) by Sigmastat software, considering the treatment as a factor with the following model.

$Yij=\mu + Ti + eij$

Where μ= population mean; Ti= effect of treatment; eij= random error.
The final data were tabulated and presented as mean ± SE.

3. Results

The chemical composition of the experimental diets is presented in Table 1. Results of in vitro gas production test (IVGPT) revealed a significant (P<0.05) rise in GP24 h in T2 diet. Further, there was an increase (P<0.05) in IVTDMD, IVTOMD and IVNDFD in T2 diet (Table 2). MBP and SCFA were improved significantly (P<0.05) in T2 diet (Table 2). Methane production was lower (P<0.05) in T2 diet compared to T1. Rumen liquor analysis with respect to total N and NH3–N showed no difference among the two diets (Table 3). Among the volatile fatty acids, the two diets did not differ significantly (P>0.05) for butyrate, whereas they differed (P<0.05) in acetate and propionate concentration, and acetate to propionate (A:P) ratio.

Table 2
Effect of fungal treated wheat straw based diet on *in vitro* $GP_{24\,h}$, digestibility (%) and associated parameters.

Parameter	T1 diet	T2 diet
$GP_{24\,h}$ (ml/g DM)	$145.83^{a} \pm 4.41$	$160.00^{b} \pm 6.29$
IVTDMD (%)	$47.37^{a} \pm 0.66$	$55.61^{b} \pm 1.17$
IVTOMD (%)	$52.0^{a} \pm 0.80$	$59.76^{b} \pm 0.76$
IVNDFD (%)	$35.30^{a} \pm 0.39$	$41.46^{b} \pm 1.02$
CH_4 (L/kg IVTDMD)	$78.86^{a} \pm 2.19$	$67.93^{b} \pm 4.89$
CH_4 (L/kg IVTOMD)	$78.26^{a} \pm 1.99$	$72.17^{b} \pm 4.41$
MBP (mg/g DM)	$199.31^{a} \pm 1.74$	$211.95^{b} \pm 2.47$
SCFA (mM/g DM)	$3.21^{a} \pm 0.09$	$3.51^{b} \pm 0.14$

[a,b]Means bearing different superscripts within a same row differ significantly (P<0.05).
IVTDMD, IVTOMD, IVNDFD, MBP and SCFA are in vitro true dry matter digestibility, in vitro true organic matter digestibility, in vitro NDF digestibility, microbial biomass production and short chain fatty acid production, respectively.

Table 3
Effect of fungal treated wheat straw diet on *in vitro* rumen fermentation parameters.

Parameter	T1 diet	T2 diet
NH_3- N (mg/dl)	$14.82^{a} \pm 0.22$	$15.61^{a} \pm 0.15$
Total N (mg/dl)	$56.37^{a} \pm 0.31$	$58.31^{a} \pm 0.63$
Fractions of VFA (%)		
Acetic acid	$64.50^{a} \pm 0.60$	$60.53^{b} \pm 0.52$
Propionic acid	$30.41^{a} \pm 0.70$	$33.25^{b} \pm 0.25$
Butyric acid	8.25 ± 0.63	9.18 ± 0.08
A:P ratio	$2.12^{a} \pm 0.05$	$1.82^{b} \pm 0.02$

[a, b] Means bearing different superscripts within a same row differ significantly (P<0.05).

4. Discussion

As better quality of straw results in an increase in productivity of ruminants (Walli, 2009), the present experiment was conducted with the objective of testing the nutritional superiority of WRF treated wheat straw diet over the control. The tested fungus was selectively ligninolytic and reduced the lignin content of the treated straw up to 40.33% (Mahesh et al., 2013), thus availing more carbohydrates for optimal ruminal degradation

(Akinfemi, 2010) which lead to the higher digestibility of T2 diet. Similarly, Shrivastava et al. (2012) documented higher in vitro gas volume coupled with increased organic matter digestibility in wheat straw fermented with Ganoderma sp. rckk02. Higher gas production and digestibility in T2 diets were in consistent with several previous reports on different biologically treated substrates (Akinfemi, 2010; Shrivastava et al., 2011; Shrivastava et al., 2012; Omer et al., 2012). Recently, Hassim et al. (2012) observed an increased in vitro apparently rumen degradable carbohydrates of oil palm fronds inoculated with Ceriporiopsis subvermispora (3 weeks) and Lentinula edodes (9 weeks) up to 13 and 10%, respectively, proving the potential of WRF treatment in enhancing digestibility. MBP depends upon a balanced ruminal supply of ammonia, energy and carbon skeletons for amino acid synthesis (Wanapat et al., 2009) and diets with low structural carbohydrate contents are known to increase the efficiency of MBP (Tas and Susenbeth, 2007) as in T2 diet. Higher MBP implies a maximum substrate fixation into microbial cells leading to higher microbial protein flow to the intestine as absorbable amino acids. Furthermore, higher gas volume is positively correlated to in vitro MBP (Krishnamoorthy et al., 1991) which substantiates the present findings. A higher SCFA synthesis reflects an active fermentation of carbohydrates, and a high gas production is expected to yield higher value of SCFA (Blümmel and Ørskov, 1993) as observed in our study for T2 diet. Akinfemi (2010) also recorded a significantly higher SCFA (µM) in Pleurotus ostreatus (0.928) and P. pulmonaris (0.896) treated peanut husks as compared to untreated husk (0.370). The present study shows a positive relationship between GP24 h, IVTOMD and in vitro SCFA synthesis (Menke and Steingass, 1988). Further, two diets differed with respect to in vitro methane production. Reduced methane from fungal treated straw diets could be ascribed to the indirect effect of improved fibre digestion (Sallam et al., 2007) with concurrent availability of fermentable nutrients (Mohini and Singh, 2001).

Ruminal NH3-N concentration is an index of the extent of protein degradation, and the observed values were above the minimum level required for microbial growth (5–8 mg/dl) in the rumen (Satter and Slyter, 1974), which is in agreement with Tripathi et al. (2008). Pattern of volatile fatty acids in the rumen generally follow the trend of feed digestion in the rumen (Patra and Saxena, 2009), which differed among the two diets. This is because, fungal treated straw contained microbial protein in addition to those found within the straw, whose fermentation in the rumen probably yielded more propionate, and thus decreasing the ratio of A:P making ideal fermentation pattern as propionate is the principal glucose precursor in ruminants (over 75% of total blood glucose; Brockman, 1993). Similar VFA patterns were also noted by Karunanandaa and Varga (1996) in rice leaves treated with different white-rots.

5. Conclusion

This study demonstrated that fungal treatment of wheat straw using basidiomycetes WRF RCK-SC for 10 days produces better quality straw compared to untreated one as evidenced through IVGPT and associated parameters. Further, enhanced digestibility observed in treated straw by SSF proves its potential in ruminant nutrition, which needs further investigation through feeding trials involving different categories of ruminants.

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