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Biodegradation of urea-NH_3 treated wheat straw using anaerobic rumen fungi

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ABSTRACT

In present study, the *in vitro* dry matter digestibility and cell-wall content degradation of urea-NH_3 treated wheat straws were reported by incubating with rumen liquor and anaerobic fungi i.e. *Orpinomyces* sp. C-14 or *Piromyces sp.* WNG-12. The maximum digestibility of dry matter (55.8%) in urea-NH_3 treated wheat straws was shown by *Piromyces* sp. WNG-12, when compared to *Orpinomyces* sp. C-14 (54.3%) and control (45.5%) after 72 h. The cell-wall contents in terms of percent neutral detergent fibre, acid detergent fibre and acid detergent lignin were found to decrease significantly when wheat straw was treated with *Piromyces* sp. WNG-12, followed by *Orpinomyces* sp. C-14 and controls, for 48 or 72 h.

KEY WORDS: anaerobic fungi, wheat straw, biodegradation, urea-NH_3 treatment

INTRODUCTION

Cereal straws, that are available in large quantities for ruminants feeding, are poor in nutritional quality because of low protein and high lignin contents in these feeds. Yet, these straws are the potential source of cell-wall polysaccharides such as cellulose and hemicellulose. However, lignin prevents a close contact between the polysaccharides and rumen microorganisms. Therefore, upgrading of straw

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quality through chemical and/or biological treatments is an important strategy for improving the livestock productivity. In these directions, urea-NH_3 treatment received a major attention as an appropriate technology for chemical treatment of straws (Rai and Gupta, 1990; Flachowsky et al., 1996; Dayal et al., 2002). During treatment of straws with urea, NH_3 is released to form NH_4OH that improves the nutritive value of cereal straws by degrading lignin (Sehgal and Patnayak, 1993; Prakash et al., 2004). Similarly, anaerobic rumen fungi are also known for their ability to attack, penetrate and utilize all categories of recalcitrant plant biomass (Orpin, 1977). The ruminal fungi have been shown to degrade the cereal straws to a significant extent under *in vitro*, i.e. 34.4% (Thareja et al., 2006) and *in vivo* conditions, i.e. 59.95 (Dey et al., 2004) and 41.8% (Lee et al., 2000). However, scientific evidences are not known on the biodegradation of urea-NH_3 treated wheat straw using ruminal anaerobic fungi so as to get the double benefits of chemical and biological treatments. Hence, in this study, an effort was made to undertake the degradation of urea-NH_3 treated wheat straw using rumen fungi to study the further enhancement in the digestible energy of straws, in order to prepare a good quality animal feed.

MATERIAL AND METHODS

Substrate

Wheat straw particles (100 g), grounded to mesh size of 0.5 mm, were taken into polypropylene bags (25 × 17 cm) and mixed with 4% urea (40% moisture). The bags were sealed and stored for 30 days at ambient temperature (28-32°C) and used as urea-NH_3 treated wheat straw substrate for further biological treatments and degradation analyses (Singh et al., 1989).

Rumen fungal isolates

Rumen fungi (*Orpinomyces* sp. C-14 and *Piromyces* sp. WNG-12) were procured from Fungal Biotechnology Laboratory, Dairy Microbiology Division, NDRI, Karnal, and tested morphologically on the basis of number of flagella per zoospore, thallus morphology (monocentric or polycentric), and rhizoid type (filamentous or a vegetative cell) to check the purity of the cultures before being used as inoculums (Thareja et al., 2006; Tripathi et al., 2007). The fungal cultures were grown and maintained as per the modified Hungate's roll-tube technique (Joblin, 1981). The roll-tubes were kept in CO_2 incubator at 39±1°C and the colonies were sub-cultured routinely in Joblin's broth supplemented with anti-bacterial antibiotics (penicillin and streptomycin).

In vitro degradation experiments

In vitro dry matter digestibility (IVDMD) trials were conducted in 100 ml wide mouthed conical flasks capped with rubber cork containing two inlets fitted with glass tubes and incubated at 39±1°C for 48 to 72 h (AOAC, 1995). The four different treatments designated as T_1 to T_4 in triplicates were made as follows:

T_1: untreated wheat straw + 40 ml McDoughall's buffer + 10 ml strained rumen liquor (SRL) + 5 ml cell-free anaerobic broth;

T_2: urea-NH_3 treated wheat straw + 40 ml McDoughall's buffer + 10 ml SRL + 5 ml cell-free anaerobic broth;

T_3: urea-NH_3 treated wheat straw + 40 ml McDoughall's buffer + 10 ml SRL + 5 ml of *Orpinomyces* C-14 culture (≈10^6 cfu/ ml);

T_4: urea-NH_3 treated wheat straw + 40 ml McDoughall's buffer + 10 ml SRL + 5 ml of *Piromyces* WNG-12 culture broth (≈10^6 cfu/ ml).

For this, fresh rumen liquor was collected before feeding from the rumen of permanently fistulated crossbred male calf (Sahiwal × Holstein-Friesian; age ≈ 3 yr; average weight ≈ 250 kg) fed on a standard diet containing wheat straw, green fodder maize and concentrate mixture (%: groundnut cake 40, maize 20, barley 20, wheat bran 17, mineral mixture 2, salt, 1) to meet their nutritional requirements (NRC, 2001). Rumen liquor was strained through four layers of muslin cloth and clarified by centrifugation at 16,000 *g* for 20 min before being added to the medium. All the flasks were flushed thoroughly with CO_2 and then placed in incubator at 39±1°C for 48 and 72 h. After incubation, samples from each trial were centrifuged at 2800 *g* for 10 min. The pellets were dried at 100°C for 24 h and analysed for percent IVDMD (Tilley and Terry, 1963), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents (VanSoest et al., 1991). Data were recorded as mean ± S.D. of three independent replicates and were statistically analysed using ANOVA after suitable transformation, and standard deviation was calculated by the method of Snedecor and Cochran (1980).

RESULTS

Figure 1 depicts that among the four different chemical and biological treatments given to wheat straw, the IVDMD of treated straw was maximum (55.8%) after 72 h in treatment T_4 i.e. *Piromyces* sp. WNG-12, followed by other treatments in the order of T_3 (54.3%), T_2 (45.5%) and T_1 (38.8%) irrespective of the period of incubation. Similar to IVDMD, there was also maximum reduction of NDF contents in treatment T_4 when compared with T_1 after 72 h. The NDF contents in all the treatments were found to decrease with increased incubation periods from

48 to 72 h. The percent decrease in ADF contents were more in treatments T_2, T_3 and T_4, in the increasing order when compared to treatment T_1. Following the pattern of NDF and ADF, the ADL contents in treatment T_2 differed significantly with other treatments, i.e. T_1, T_3 and T_4. The percent decrease in ADL was highest after 72 h in treatment T_4 when compared to any other treatment including control (Figure 1).

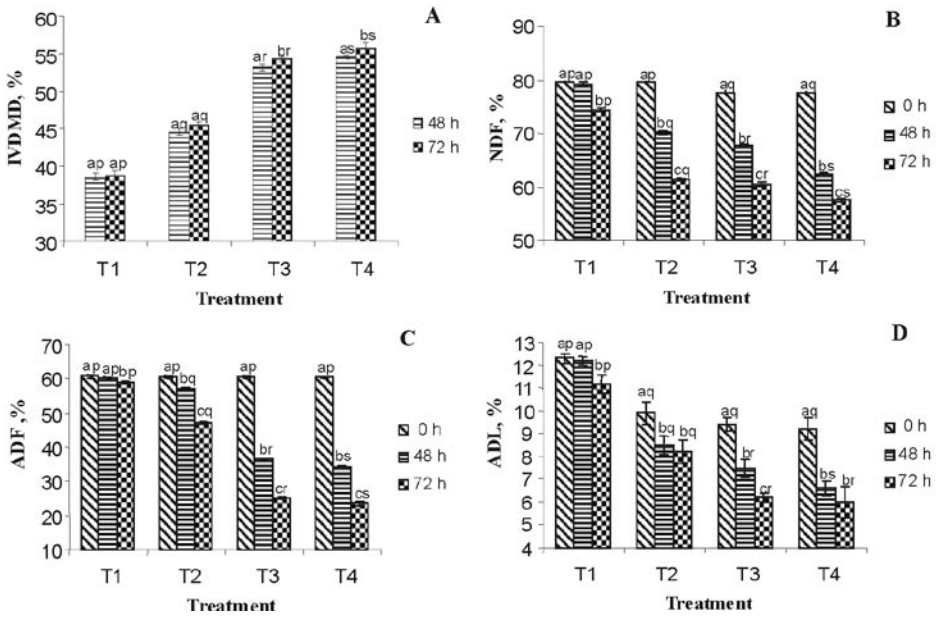

Figure 1. Effect of rumen fungi on IVDMD and cell-wall contents of urea-NH_3 and untreated wheat straw (T_1: untreated wheat straw + 40 ml McDoughall's buffer + 10 ml strained rumen liquor (SRL) + 5 ml cell-free anaerobic broth; T_2: urea-NH_3 treated wheat straw + 40 ml McDoughall's buffer + 10 ml SRL + 5 ml cell-free anaerobic broth; T_3: urea-NH_3 treated wheat straw + 40 ml McDoughall's buffer + 10 ml SRL + 5 ml of *Orpinomyces* C-14 culture; T_4: urea-NH_3 treated wheat straw + 40 ml McDoughall's buffer + 10 ml SRL + 5 ml of *Piromyces* WNG-12 culture broth). Error bars indicate the standard deviation of the mean (n = 3). Mean bars with different letters (a–c) in same treatment at different incubation periods differ significantly ($P<0.05$). Mean bars with different letters (p–s) at same incubation period in different treatments differ significantly ($P<0.05$)

DISCUSSION

The maximum dry matter digestibility shown by treatment T_4 might be due to the greater hydrolytic activity of *Piromyces* sp. WNG-12, as reported earlier by Thareja et al. (2006) and Tripathi et al. (2007). Anaerobic fungi can break

the ester linkages that connect lignin to hemicellulose by producing feruloyl and p-coumaroyl esterases (Borneman et al., 1990), ultimately increasing the digestibility of lignified plant fibres. Dey et al. (2004) also reported similar results for increase in digestibility of wheat straw with the oral administration of *Orpinomyces* sp. C-14 in growing male cross-bred calves. The increase in digestibility in treatment T_2, when compared with T_1, might be due to the loosened lignocellulolytic bonds after urea-NH_3 treatment of wheat straw. The similar results of increase in digestibility of straws after urea-NH_3 treatment were obtained by other workers (Singh et al., 1989; Kumar and Singh, 1990). Maximum reduction of NDF and ADF contents in treatment T_4 when compared with T_1 might also be due to the maximum hydrolytic activity of *Piromyces* sp. WNG-12. The difference between treatment T_1 and T_2 indicates that the urea-NH_3 treatment improved the digestibility of wheat straw, as also reported by Rai and Gupta (1990). However, higher degradation in T_3 and T_4 indicated that hydrolytic enzymes produced by respective fungal culture further acted upon, loosened bonds and digested the cell-wall constituents of wheat straw. The results of NDF disappearance after anaerobic fungal incubation are well in agreement with that of Singh et al. (1989), Dey et al. (2004), Manikumar et al. (2004) and Thareja et al. (2006). Ruminal anaerobic fungi are also efficient degraders of lignin and it further assists in digestion of plant cell-wall by ruminal bacteria (Ushida et al., 1997). The ADL content in treatment T_2 differed significantly with T_1, certainly because of the pre-treatment of wheat straw with urea for 30 days that led to solublization of ADL. The treatments T_3 and T_4 also differed significantly with each other indicating the varying degree of lignolytic activities shown by the two different fungal isolates. The results of ADL reduction are also well in agreement with the work of other researchers (Dey et al., 2004; Manikumar et al., 2004), who reported a decrease in ADL contents of wheat straw after fungal treatments.

CONCLUSIONS

Based on overall results obtained, it could be concluded that *Piromyces* sp. WNG-12 is more effective in enhancing the *in vitro* digestibility of urea-NH_3 treated wheat straw when compared with *Orpinomyces* sp. C-14 and control. Hence, this strain could be exploited in future for administration in ruminants after making some feeding trials to authenticate the reproducibility of results under *in vivo* conditions.

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