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IMPROVEMENT IN NUTRITIVE VALUE OF PADDY STRAW THROUGH BIOTECHNOLOGICAL APPROACH USING SOIL FUNGI

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ABSTRACT

Three soil fungi viz., *Aspergillus niger*, *Humicola fuscoatra* and *Penicillium notatum* were used for biological treatment of paddy straw for a period of 10, 20 and 30 days. The pH of the urea treated straw has been changed by fungi from basic to acidic (7.8 to 6.5), which is favourable for rumen microflora. Substantial increase in cellulolytic (C_x and C_1), lignolytic (lignin peroxidase-LiP and Laccase - Lac), amylolytic, proteolytic urease and phosphatase enzymes was noticed during the solid state fermentation. Combined treatment using chemical and biological methods enhanced the nutrient availability of paddy straw. Biomolecules like carbohydrates (reducing sugars) increased seven times, proteins (amino acids) 18 times and inorganic phosphorus and total organic matter four times that of untreated straw. Solid state fermentation treatment using soil fungi in presence of 1% urea for a period of 10 days is considered to be appropriate to improve the nutritive value of paddy straw. Among the three soil fungi used, *Humicola fuscoatra* and *Penicillium notatum* exhibited better performance.

INTRODUCTION

In India and other developing countries, about 70 percent of the population depends upon agriculture and its allied sectors. Among these dairying is the important source of employment and income for the rural households. Agriculture byproducts like cereal straws are being used extensively for feeding milk producing animals such as cattle and buffaloes. Paddy straw has long been used as maintenance feed for ruminants like cattle and buffaloes. Approximately 200 million tons of paddy straw is produced annually in India and most part of it is used for feeding cattle and buffaloes. Despite its high production, the nutritive value of paddy straw is very low (Staniforth 1982). The proportion of total digestible nutrients (TDN) is about 45 percent while digestible crude protein (DCP) is almost zero. The presence of some anti-nutritional factors like lignin and silica decrease the quality of these lignocellulosic feeds. Yet it could be an important energy feed for animals as it contains at least 70% carbohydrates (Hartely et al. 1974) provided, some measures are taken to improve its utilization. Therefore, it is necessary to improve the nutritive value of paddy straw through suitable means in order to improve the per animal milk yield.

A number of physical, chemical and biological methods have been developed for the improvement of straw quality for bioavailability of nutrients. Physical treatments like pressure cooking, though alters the cell wall, does not increase the digestibility (Chaudhry 1998). The chemical methods involve the use of alkali solutions, though widely used, are toxic to animals and eliminate the rumen microflora. Hence, the use of microorganisms or their enzymes for bioconversion of straw has become a promising method and received much attention (Crawford & Crawford 1984). Thus, efforts have been made to find out suitable ways of microbial and enzymatic modifications of cereal straws to improve their biodegradability and to upgrade their feeding value for ruminants (Jackson 1977, Klopferstein 1981, Sundstol & Owen 1984, Garmo 1986). Among the microorganisms, soil

fungi received much attention in the production of cellulolytic and lignolytic enzymes in delignification of paddy straw for improving digestibility (Kirk et al. 1980, Hadar et al. 1993, Hatakka 1994, Vijaya & Singaracharya 2003).

At present various dairy development organizations are promoting chemical method using urea in order to improve the nutritive value of paddy straw (NDRI 1988, Chaudary 1998). Due to poor palatability of the treated straw, the intake of the straw by the animal is low. This might be due to alkalinity of straw in the presence of urea. However, urea can not be eliminated from the straw because of it supplies non-protein nitrogen to the straw which in turn is converted into proteins by rumen microflora. Therefore, the development of appropriate method of bioconversion of urea treated straw using microorganisms or their enzymes is necessary in the present scenario of feed scarcity (Crawford & Crawford 1984). After critically analysing the influence of effective chemical treatments and ecofriendly biological treatments, an effort has been made in this study to develop an innovative method by combining the chemical and microbiological treatments to improve the nutrient availability of paddy straw, along with the conversion of non protein nitrogen (NPN) like urea into microbial protein in the straw before feeding to the animal.

The upgrading of paddy straw by using fungi has been suggested by many workers (Kirk et al. 1980, Crawford & Crawford 1984, Vijaya & Singaracharya 2003 a). Similarly, the application of chemical oxidising agents have been used to improve the quality of straw (Chandra & Jackson 1971, Flachowsky & Sundstol 1988, Chaudhry & Miller 1994). However, attempts on the combined effect of chemical and biological treatment methods on the improved biodigestibility are scanty (Vijaya & Singaracharya 2003 b).

MATERIALS AND METHODS

Solid state fermentation technique is selected for the microbiological treatment of paddy straw. Three pits were dug, each with measurements of 15 × 15 cm and allowed to dry for two days. The conditions of the pit provide the required temperature and humidity necessary for the fungal growth. Paddy straw was chopped (2 cm long), presoaked in water overnight and spread in the pit in three loose layers each 5 cm thick. Fungal species such as *Aspergillus niger, Humicola fuscoatra and Penicillium notatum* were selected for treating the straw. The selected fungi were inoculated into 50 mL of Saboraud's dextrose broth and allowed for maximum sporulation for a period of 5-6 days at 37°C. About 100 mL of this fungal spore suspension was sprayed on each of these straw layers. Five hundred mL of 1 percent urea solution was sprinkled on these layers. The pit was covered with banana leaves, news papers or with plastic sheets to facilitate the impregnation of ammonia gas, released from urea, into the straw. After every 3-4 days of incubation the layers were moistened by spraying normal tap water. The same procedure was followed for the other pits also. The pit filled with straw only and the pit with straw and urea were used as controls. After 10, 20 and 30 days of incubation, 20 g of straw from middle layer of the each pit was carefully taken and ground in 50 mL acetate buffer having pH 5.3. It was then centrifuged and filtered through Whatman No. 1 filter paper and the extract was used to estimate biomolecules and the activity of microbial enzymes released during solid state fermentation.

Estimation of enzymes and biomolecules: During the process of incubation, soil fungi produce many lignolytic and cellulolytic enzymes to convert the less digestible lignin and cellulose into easily digestible carbohydrates and other nutrients. The relative enzyme activity of cellulolytic enzyme (C_x) was assayed by the method as suggested by Akin et al. (1995). The other cellulolytic

enzyme, C_1 activity was estimated according to the procedure of Plummer (1993) and expressed as mg/mL of reducing sugars liberated in 6 hours. The two lignolytic enzymes, LiP and Lac from the culture extract were estimated by the method suggested by Tien & Kirk (1984). The activity of amylase in the culture filtrate is assayed and expressed as mg/mL of reducing sugars, as per the method suggested by Ross & Weilly (1973). The protease, urease, phosphatase and organic matter contents of the extract were analysed by procedures suggested by Nannipieri et al. (1980). The total proteins, total reducing sugars and total inorganic phosphorus of the sample were estimated by Lowery's method, anthrone method and Fisky-Subbaraow method respectively (Plummer 1993).

RESULTS AND DISCUSSION

Enzymatic activity: During the combined chemical and biological treatment of paddy straw, the activity of cellulolytic enzymes (C_x, $C_{1)}$, lignolytic enzymes (LiP, Lac), amylase, protease, phosphates and urease were estimated and are reported in Table 1. An efficient microbiological treatment method shall exhibit maximum activity of lignolytic as well as cellulolytic enzymes in order to convert the less digestible lignin and cellulose into more digestible carbohydrates. It could be noted that all the inoculants used exhibited higher activity of these enzymes during the solid state fermentation process. The reduction in the pH from alkaline to acidic due to microbial fermentation is favourable for the growth of these inoculants and also for rumen microbial flora (Flachowsky & Sundstol 1988).

The activity of the cellulolytic enzymes, c_x and c_1, is highest after 10 days of incubation in the case of all the three inoculants used. The two cellulolytic enzymes showed a substantial improvement in the activity with the treatment of *P. notatum* with production abilities of 125 REA (C_x) and 1680 mg/mL (C_1). There is decline in the activity of these enzymes with the increase in the period of incubation for all the inoculants except *H. fuscoatra*. The organism *H. fuscoatra* showed maximum enzyme activity after 20 days of fermentation, which is greater than the enzyme activity shown by other organisms. The activity of lignolytic enzymes, Lip and Lac, exhibited a different pattern than that of cellulolytic enzymes. The lignolytic enzyme activities are low at 10 days of incubation and increased along with the increase in incubation period. Therefore, average incubation period of 20 days may be necessary in order to have the benefit of the activity of both cellulolytic as well as lignolytic enzymes during the fermentation of urea treated straw. *H. fuscoatra* proved to be better for the production of lignolytic enzymes (Lip-40 U/mL and Lac-80 U/mL) during the treatment of straw.

The activity of amylase and protease was also estimated (Table 1). The activity of amylase was measured as the amount of reducing sugars liberated during the process while the activity of protease was estimated as the amount of amino acids liberated. The activity of these enzymes shall be low in order to supply maximum amount of nutrients to the animals through paddy straw. The results of the study indicate that the activity of these enzymes is higher and increased with the increase in the incubation period. Among the cultures used, *P. notatum* showed maximum amylase activity (2174 mg/mL of reducing sugars) and it increased with prolonged incubation. Similarly, maximum protease activity is noted at late incubations by *A. niger* (262.5 mg/mL). With regard to other enzymes estimated in the process, phosphatase activity is more in the straw treated with *P. notatum* (69.8 mg/mL) in its 10 days of incubation. Maximum production of urease was recorded in *P. notatum* (23.7 mg/mL) in 30 days of incubation. Higher phosphatase activity is desired because it enhances the bioavailability of phosphorus to the animals through treated straw. Among the three inoculants

Table 1: Extracellular enzymes in paddy straw treated with soil fungi in the presence of 1% urea.

Fungi	Days of Incubation	pH	C_x	C_1 (mg/mL)	LiP (U/mL)	Lac (U/mL)	Amylase (mg/mL)	Protease (mg/mL)	Protease (mg/mL)	Urease (mg/mL)
Control - A	-	7.0	0	0.4	0	0	40	8.5	3.7	0.5
Control - B	-	7.5	10	9.6	0	0	60	9.5	3.7	3.3
A. niger	10	7.0	66.7	800	10	30	333	37.5	41.8	3.3
	20	6.5	55.6	666	20	50	666	131.3	55.8	9.9
	30	6.0	50.0	400	30	80	1520	262.5	55.8	13.3
H. fuscoatra	10	7.0	66.7	1400	10	20	300	31.3	27.9	8.3
	20	6.5	76.9	1600	20	40	600	68.2	41.9	13.3
	30	6.5	100	533	40	80	1000	106.3	27.9	13.3
P. notatum	10	7.5	125	1680	00	10	400	31.3	69.8	6.6
	20	7.0	111	1400	10	30	1400	112.5	55.8	16.7
	30	7.0	100	466	30	50	2174	181.5	55.8	23.7

Control A - Straw; Control B - Straw + 1% urea solution; C_x - REA (Relative Enzyme Activity)
C_1 – Amount of reducing sugars liberated in 6 hrs; LiP – 0.01 increase in O.D. equal to one unit of enzyme
Lac – 0.01 increase in O.D. equal to one unit of enzyme; Amylase – Amount of reducing sugars liberated
Protease - Amount of total aminoacid liberated; Phosphatase - Amount of *p*-nitrophenol liberated in 1 min
Urease – Amount of NH_4 liberated in 15 minutes.

tried, *H. fuscoatra* is useful for treating the straw because it showed lower level of amylase and protease activities compared to other organisms. Since this organism has higher lignolytic and cellulolytic activities and lower level of amylase and protease activities, it appears to be suitable organism which could be used for the improvement of nutritive value of paddy straw through biological treatment.

Availability of nutrients: The enzymatic activity of lignocellulases and cellulases is expected to convert less digestible and indigestible lignin and cellulose portion of the paddy straw into easily digestible carbohydrates so that more nutrients will be available to the cattle through feeding of biologically treated paddy straw. The present study indicates that the availability of carbohydrates in the untreated straw (control - A) is 46.8 mg per mL of the culture extract while the availability in the urea treated straw is 55 mg/mL indicating that there is marginal increase of 16 percent in the availability of carbohydrates (Table 2). However, in the case of combined treatment with urea and soil fungi, the availability of carbohydrates increased from 46.8 mg/mL to 344-386 mg/mL after 10 days of fermentation. Thus, it indicates that joint treatment by chemical and biological methods would increase the nutrient availability of straw by about 7 times, i.e. by about 600 percent (Reid 1989). Among the three fungal inoculants used, *H. fuscoatra* has shown marginally higher performance than the other two inoculants. On the other hand, the availability of carbohydrates declined after 10 days of incubation in all the cases of three inoculants indicating that 10 days of treatment is optimum in this case. The decline in the availability of carbohydrates in the straw after prolonged incubation could be due to the utilization of nutrients by these organisms as well as by other organisms.

The availability of total protein in the chemical and biological treatment of straw has shown similar pattern that of carbohydrates. While the urea treatment of straw alone could increase the total protein content of the paddy straw by 6 times (from 17 mg/mL to 125 mg/mL), the joint chemical and biological treatment could increase the protein content as high as 18 times that of untreated straw (Table 2). This could be attributed to the conversion of non-protein nitrogen (urea) into proteins by soil fungi. The fact is that the soil fungi utilize urea and grow enormously in the straw thereby adding

Table 2: Biomolecules in paddy straw under the treatment of three soil fungi in presence of 1% urea.

Fungi	Days of Incubation	Total carbohydrates (mg/mL)	Total proteins (mg/mL)	Total Inorganic Phosphorus (mg/mL)	Total Organic matter (%)
Control - A	-	46.8	17	10.4	0.27
Control - B	-	55.0	125	10.4	0.26
A. niger	10	338	368	40.0	0.35
	20	276	373	48.0	0.51
	30	228	270	11.0	0.60
H. fuscoatra	10	386	330	35.5	0.46
	20	307	368	44.4	0.50
	30	147	165	31.1	0.58
P. notatum	10	344	305	44.4	0.32
	20	307	330	48.0	0.40
	30	276	255	22.2	0.58

to the protein content of the straw. *Aspergillus niger* and *H. fuscoatra* have shown better performance than that of *P. notatum* in terms total protein content. However, the increase in the protein content is marginally higher after 20 days of incubation compared to that of 10 days of incubation. In the case of availability of inorganic phosphorus, urea treatment alone did not improve its availability whereas biological treatment improved the availability of phosphorus by about four times that of untreated straw. The maximum bioavailability of phosphorus in the fungal treated straw is caused by *P. notatum* (48 mg/mL). Biological treatment of paddy straw has also resulted in the enhancement of total organic matter of the straw. The rise in the organic matter of straw increased along with the elongation of incubation period and this could be attributed to the growth of soil fungi in the straw.

The solid state fermentation and its relevance to economic production of exoenzymes and different nutritional biomolecules by various microorganisms revealed the importance of this method for improvement of fodder quality in ruminants (Lonsane 1990). Ramesh & Lonsane (1991a,b) and Lekha & Lonsane (1993) have successfully exploited *Aspergillus niger* for effective biotreatment of paddy straw while producing sufficient amounts of amylase and phosphatases during solid state, liquid state and submerged fermentations. The growth characteristics of microorganisms in solid state fermentation for upgrading the protein values of lignocelluloses and cellulase production was extensively investigated by Chahal (1993). Mandles (1975) also noticed the potentiality of *T. resei* as a microbial source of cellulase in the improvement of nutritional value of paddy straw. Gayal & Khandeparker (1998) studied the cellulase from *P. funiculosum* for the saccharification of cellulose materials in the improvement of fodder quality. Krishna (1985) and Kahlon & Das (1987) tried for nutritional improvement of rice straw by solid state fermentation by variety of lignin degrading fungi. Arora et al. (1998) used *Trametes versicolor*, *Coprinus cinereus* and *Pleurotus sajor-caju* for potentiality of their secretions of LiP and Lac to upgrade the rice straw as feed for ruminants. The role of amylase, protease, urease and phosphatase in the bioavailability of various nutrients through fermented paddy straw was established (Dale 1987, Harvey et al. 1987, Rajarathnam et al. 1992, Kaur et al. 1998). Reid (1989) noticed the substantial improvement of reducing sugars and amino acids during the solid state fermentation of paddy straw for biological delignification. Khanh et al. (2005) and Chinnusamy et al. (2006) also emphasized on crop ecology/organic farming and also

analysed the role of microbial consortia under controlled conditions. The present study also reveals the importance of soil fungi as potential organisms in increasing the nutritive value of paddy straw.

CONCLUSIONS

The results indicate that treatment of paddy straw by chemical method using urea and microbiological method using soil fungi has undoubtedly improved the digestible carbohydrates content by seven times and protein content by 18 times. The optimum period of incubation appears to be 10-20 days. Among the three soil fungi used in the biological treatment of straw, *H. fuscoatra* and *P. notatum* appears to be more promising in this regard. However, the mycotoxins secreted by the fungi under study and their influence on health of the animal is to be critically analysed and experimented. These results shall be appropriate and useful after thorough examination of these toxins on the animal health. Further, feeding trials are to be carried out to establish the acceptability of the treated paddy straw by cattle and buffaloes.

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