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Review

Biological treatment of crop residues for ruminant feeding: A review

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Crop residues are often referred to as 'lignocellulosics' as they are rich in cellulose which is bound with a biopolymer lignin. Rumen microbiota (bacteria, protozoa and fungi), even with their hydrolytic enzymes, are not very competent enough to break these bonds efficiently. Biological treatment of such crop residues using white rot fungi (WRF) can break the ligno-cellulose complexes, liberating free cellulose and thus enhancing their feeding value for ruminants. Biologically treated roughages have higher digestibility for most of the nutrients (both cell walls and cell solubles) with an increase in crude protein content as compared to untreated material, besides ensuring more fermentable substrates in the rumen. Further, recent studies have indicated low methane emission from feedstuffs subjected to solid state fermentation (SSF) with ligninolytic fungi as a result of their improved digestion and nutrient assimilation. This review primarily deals with the nature and composition of crop residues, their inherent nutritive constraints as ruminant feed and to overcome the same by means of biological treatments. The progresses to date in *in vitro* and *in vivo* studies on biologically treated lignocellulosics have also been reviewed.

Key words: Crop residues, ligninolytic fungi, ruminant, digestibility, methane.

INTRODUCTION

Ruminant animals in many tropical countries subsist mainly on crop residue based diets. The increasing expansion of agro-industrial activity over the last few years has led to the accumulation of a large quantity of lignocellulosic residues all over the world. In India, the major agro-residues in terms of volumes generated (in million metric tons, MMT) were found to be rice straw (112), rice husk (22.4), wheat straw (109.9), sugarcane tops (97.8) and bagasse (101.3) (Saritha et al., 2012). Although a vast energy potential is locked in these lignocellulosic crop residues (Jung, 1989), these are not utilized to their fullest potential for ruminant feeding due to poor digestibility, low nitrogen and mineral contents which rendered them to be classified under non-maintenance type of feeds. Also, the covalent encrustation of plant cell wall with lignin prevents their biodegradation in the rumen. Therefore various physical and chemical treatments have been tried, which are known to improve feed quality either by increasing digestibility or by enhancing palatability. However, these treatments have their own limitations and few are environment unfriendly (Silverstein et al., 2007). Recently, the biological treatments of crop residues to improve the accessibility of cellulosic fractions, thus improving their digestibility and feeding value have been attracting the extensive interests among researchers (Zhang et al., 2007; Yu et al., 2009) although this process has a long history. The major obstruction in biological conversion of lignocelluloses is the physical protection of cellulose by lignin against cellulolytic enzymes. The potential of biological treatments has been explained by the ability of certain microbes (specifically basidiomycetes fungi) to disrupt plant cell wall by

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Table 1. Chemical composition of commonly used cereal straws (% DM) for livestock feeding.

Particular	Wheat straw	Rice straw	Barley straw	Oat straw
Lignin	8.5-15	6-10	11.0	14
NDF	75-82	65-80	80	70
ADF	51-64	49-59	59	47
SiO_2	1-5	13	2	2-5

Compiled from Singh and Oosting (1993), Prasad et al. (1993) and Van Soest (2006).

partial breakdown of the lignin-carbohydrate complex (Keller et al., 2003) thus improving their utilization in the rumen by increasing the availability of fermentable energy to ruminal microbes (Akin et al., 1993). This way, basidiomycetes seems to be the most promising microorganisms for biological pre-treatment and among them the selective lignin degrading WRF holds immense importance (Akhtar et al., 1997; Yu et al., 2009) in enhancing the utilization of poor quality roughages for animal feeding. WRF such as *Phanerochaete chrysosporium, Pleurotus* sp*., Lentinus edodes, Coriolus versicolor, Phlebia* sp. and *Ceriporiopsis subvermispora* have largely been studied for their ability to ferment different crop residues (wheat straw, olive mill solid waste, *madake* bamboo, tanniniferous lespedeza plants, oil palm fronds etc.) to produce improved animal feed (Zafar et al., 1989; Yadav and Tripathi, 1991; Moyson and Verachtert, 1991; Tripathi and Yadav, 1992; Akin et al*.,*1996; Basu et al., 2002; Shabtay et al., 2009; Okano et al., 2009; Hassim et al., 2012). *P. chrysosporium* fungi degrades lignin to the extent of 65 to 70% while others like *Ganoderma applanatum* and *Coriolus versicolor* degrade over 45% of lignin in lignocellulosic materials (Gupta et al., 1993). Thus, microbial conversion seems to be a practical and promising alternative for increasing the nutritional value of agro by-products by transforming them into animal feed and producing a value-added product (Villas-Bôas et al., 2002).

NATURE AND CHEMICAL COMPOSITION OF STRAWS AND OTHER AGRICULTURAL BYPRODUCTS

Straws are being used as a livestock feed ever since the advent of cereal cultivation as they are inevitably produced as cereal by-products.

Prasad et al. (1993) distinguished two major groups of straws as follows: a) Slender straws: rice, wheat, oats; b) Coarse straws: millet, sorghum.

Although straw is an important feedstuff, and indeed the staple feed in large parts of the developing world for ruminants, it is not preferred by animals. A characteristic of straw is that it mainly consists of highly lignified cell wall material, which often constitutes upto 80% of the dry matter. This consists of major proportions of cellulose, hemicellulose and lignin which occur in the ratio 4:3:3, respectively (Theander and Aman, 1984), while the rest comprises of nitrogenous compounds and ash. Cereal straws normally contain at least 70% carbohydrates and are therefore, a potential source of energy for livestock, which can be utilized through microbial fermentation in the rumen. Nutritive value of straw is controlled by the chemical attributes of straw that limit the digestion of cellulose and hemicellulose. These attributes include lignification, silicification, crystallinity of cellulose, etc. which are known to be the inherent nutritive constraints in straw. Even though these residues contain enough cellulose to make them an excellent source of energy for ruminants, these are poor quality feeds in their natural state because of their low protein content (2.5 to 6%) (Khilberg, 1972) associated with high lignin (7 to 14%). The major part of the protein is most likely associated with the cell-walls which are known to have low digestibility. Thus, it is unlikely that any straw without supplementation can sustain the nitrogen balance in animals. There appears to be a curvilinear effect of supplementation on intake of straw (Prasad et al., 1993). Generally, straws like wheat straw contains (on dry matter basis) 85 to 94% organic matter (OM), 2.5- to 5% crude protein (CP), 40 to 44% crude fibre (CF), 45 to 46% nitrogen free extractives (NFE), 0.16 to 0.22% calcium and 0.05 to 0.14% phosphorus. The chemical composition and energy contents of agricultural by-products are presented in Tables 1, 2 and 3, respectively.

CHARACTERISTICS OF STRUCTURAL POLYSACCHARIDES IN STRAWS

Plant cell wall contains three types of structural polysaccharides, namely cellulose, hemicelluloses and pectic polysaccharides. In straws, the polysachharide composition is rather simple, with cellulose and xylans as the predominant components (Theander and Aman, 1984) along with smaller amount of polysaccharides containing mannose, galactose and probably pectic components. Cellulose in plants is composed of both crystalline and amorphous structure. The degree of crystallinity is believed to affect the rate of its decomposition by cellulolytic organisms; the greater the degree of crystallinity, the slower is the rate of microbial cellulose degradation (Fan et al., 1981). Although the cellulose of wheat straw can be utilized by ruminant animals, digestibility often is limited by its low protein content and a high degree of

Table 2. Cell wall composition of some agricultural byproducts (g kg^{-1} DM).

Roughage	Cell wall	Hemicellulose	Cellulose	Lignin
Barley straw	810	270	440	70
Oat straw	730	160	410	110
Paddy straw	790	260	330	70
Wheat straw	800	360	390	100
Sorghum stover	740	300	310	110
Chickpea straw	620	200	300	100
Lucerne straw	690	190	380	110
Sugarcane bagasse	820	290	400	130
Sugarcane trash	800	260	360	100
Paddy hulls	860	140	390	110
Cottonseed hulls	910	150	590	130

Adapted from Jackson (1977).

Table 3. Energy content of straws (MJ kg^{-1} DM).

Straw	GE	DE	ME	NE_m	NE_g
Wheat straw	18.0	7.5	6.2	2.7	0.5
Rice straw	16.7	7.5	6.2	2.7	0.4
Barley straw	16.3	7.5	6.1	2.5	0.3
Bermuda straw	18.4	11.3	9.1	5.5	3.1

Adapted and modified from NRC (1982). GE, Gross energy; DE, digestible energy; ME, metabolizable energy; NEm, net energy for maintenance and NEg, net energy for gain, respectively.

lignification. Hemicelluloses are alkali soluble cell wall polysaccharides that are closely associated with cellulose (McDonald et al., 2009). Other organic molecules like cutin and suberin have been reported to be closely associated with carbohydrates in the cell wall of plants (Kolattukudy et al., 1981).

Physical incrustation of plant fibers by means of lignocarboydrate complex renders them inaccessible to chemical degradation and for enzymes that would normally digest them. Strong chemical bonds which exist between lignin and many plant polysaccharides and cell wall proteins render these compounds unavailable during digestion as the digestibility is generally inversely correlated to the amount of lignin in the substrate (Han, 1975). The soluble phase of silica is also associated with lowered digestibility of straw (Van Soest and Jones, 1968). Further, Van Soest (1994) stated that lignin is the single most responsible factors for reducing digestibility of forages and the reported digestibility values for the straws of wheat, barley, rice and oats ranges 40-55, 48-50, 40-55 and 45-50%, respectively (Van Soest, 2006). Therefore, removal of lignin has become a prerequisite for the efficient utilization of carbohydrates from lignocelluloses besides improving their palatability.

LIGNINOLYTIC MICROORGANISMS

Ligninolytic microorganisms are mainly wood inhabiting fungi. They are able to colonize different plant residues (Zadražil, 1976, 1979) and increase the digestibility of the substrate (Kirk and Moore, 1972). The ideal microorganism for upgrading lignocellulosics into animal feed should combine high ligninolytic capability with low degradation of cellulose and hemicelluloses. Following three major aerobic ligninolytic fungi are known to play a major role in lignin degradation of straw (FAO, 2011).

Brown-rot fungi

Brown-rot fungi preferentially attack cellulose and hemicellulose, leaving lignin intact, thus, decaying residue turning brown. Brown rot fungi are mainly humifiers causing only limited changes in lignin. They do not cleave lignin's aromatic ring efficiently, or if they open the rings, they are unable to make significant decomposition resulting in lignin fragments. These results in lower *in vitro* digestibility compared to untreated substrate (Zadražil et al., 1999). The examples are *Agrocybe aegerita* and *Flammulina velutipes.*

White-rot fungi

White-rot fungi are capable of degrading lignin without affecting much of cellulose and hemicelluloses (Zadražil and Brunnert, 1982) thus causing decayed residue to turn white. WRF attack unaltered lignin polymers causing cleavage of interlignol bonds and aromatic ring cleavage,

which ultimately results in an increase in *in vitr*o digestibility (Zadražil et al., 1999). They mainly degrade polysaccharides by hydrolytic enzymes like cellulases and xylanases, and lignin by oxidative ligninolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. The examples are *Abortiporus biennis, Agaricus bisporus, Dichomitus squalens, Pleurotus eryngii, Pleurotus sajor-caju, Pleurotus ostreatus, Pleurotus flabellatus, Pleurotus floridanus, Phanerochaete chrysosporium, Ganoderma* sp. rckk02, *Crinipellis* sp., *Pycnoporous sangeus, Coriolus versicolor, Lenzites striata, Poria plascenta,* etc.

Soft-rot fungi

Soft-rot fungi leaves the attacked lignocellulosic material watery-soft and breaks down cellulose and hemicelluloses (Chahal and Moo-Young, 1981). Example include *Chaetomium cellulolyticum*.

BIOCONVERSION OF LIGNOCELLULOSIC RESIDUES AS RUMINANT FEEDS BY SOLID STATE FERMENTATION (SSF)

Zadražil et al. (1999) defined SSF as a process, in which solid substrates are decomposed by known pure or mixed cultures of microorganisms (mainly fungi, which can grow on and through the substrate) under controlled conditions, with the aim of producing a high quality standardized products (different from composting). SSF is a polyfactorial event, in which the fungus, its enzymes, physical structure of substrate, physiological factors of fermentation and, culture and nutritional conditions play an important role in controlling lignin degradation and digestibility of fermented substrate (Zadražil, 1986). Kamra and Zadražil (1988) suggested that an improvement in lignocellulose digestibility must be the aim of the bioconversion process when the product is destined for ruminant nutrition that is proposed process of biological upgrading of lignocellulosics into animal feed should be characterized by marked lignin decomposition and liberation of nutrients from the lignocellulose-matrix with contemporary accumulation of digestible substances (Zadražil et al., 1999) along with enriching the final product with microbial protein (Villas-Bôas et al., 2002). In order to promote the delignification of a lignocellulosic substrate, it is also essential to maximize the rate as well as the specificity of lignin molecule degradation, avoiding polysaccharide consumption (Kerem and Hadar, 1995). However, the knowledge of regulating mechanisms that pro-mote selective delignification is limited (Reid and Deschamps, 1990; Kerem and Hadar, 1995; Ardon et al., 1998).

Villas-Bôas et al. (2002) stated that the bioconversion of lignocellulose into protein-rich animal feed results in a relatively lower digestibility of the fermented product than the unfermented lignocelluloses, as the basic principles of improvement in digestibility and protein enrichment are known to be antagonistic to each other. Furthermore, lignin content alone does not explain digestibility or intake sufficiently, and the degree of lignin-hemicellulose bonding might also be responsible for poor nutritive quality of biologically treated straws (Singh and Schiere, 1993). Some fungi grow on the straw but reduce the digestibility of fermented product (Ulmer et al., 1981). Zadražil (1980) also reported decreased digestibility in some cases after fungal treatment with *Agrocybe aegerita* which was unable to break the lignocellulose complex. Such organisms, which are generally brown rot and sweet rot fungi, utilize cellulose, hemicellulose and other soluble carbohydrate fractions for their growth leaving behind residues of low digestibility (Kundu et al., 2005). Likewise, Jalc et al. (1994) also showed that bioconversion of wheat straw with *Polyporus ciliates* improves the digestibility whereas with *Lentinus tigrinus,* the digestibility was reduced.

CHEMICAL COMPOSITION AND *IN VITRO* DIGESTIBILITY OF FUNGAL TREATED CROP RESIDUES

Digestibility of the straw is dependent on the deploymerisation of its structural carbohydrates. Enzymatic degradation of these macromolecules in the straw will result in degradation and increase in digestibility and availability of carbohydrates (Giovannozzi-Sermanii et al*.,* 1989; Fazaeli et al., 2004).

Although several species of higher fungi possess ligninolytic activity, *Pleurotus* sp. is the most studied fungi since they improved the digestibility (Kundu et al., 2005) and nutritional quality of straws (Streeter et al., 1982; Kakkar et al., 1990). These straws contain more of free sugars, more protein with less cellulose and lignin and an increased content of ash compared to the beginning material (Rajarathnam and Bano, 1989). According to the study of Zadražil (1997), the *in vitro* dry matter digestibility (IVD) of spent wheat straw increased to the extent of 4.4 to 8.9% after culture and harvesting of *Pleurotus ostreatus* mushrooms. Calzada et al. (1987) found that for SSF of wheat straw by *P. ostreatus* for a 30 day period, the lignin content decreased significantly and IVD increased from 14.3 to 29.5%. Ramirez-Bribiesca et al. (2010) reported that *P. ostreatus* treatment for 15 days on corn straw increased crude protein (39.5%) and soluble protein (165%), soluble carbohydrates (621%), ash (188.32%) and decreased neutral detergent fibre (14.5%). Langar et al. (1980) cultivated edible mushrooms such as *Agaricus bisporus* (26-30 days) and *Volvariella diplasia* (28-30 days) on wheat straw and showed an increase (% increase in fermented straw) in CP (14.3, 8.6), cell-solubles (52.2, 24.1) and lignin (13.2, 10.8) contents in the post-fungal harvested straw compared to original straw, whereas CF (20.5, 28.5), cellulose (12.4, 31.5) and hemicellulose (0.06, 16.5) contents decreased for *A. bisporus and V. diplasia,* respectively. In the case of *Sporotrichum pulverulentum,* Nikhat et al. (1983) observed

an increased digestibility of wheat straw from 16 to 34% at the expense of the lignin and cellulose and Zadražil and Brunnert (1980) obtained even a higher digestibility upto 40 to 50%.

Zadražil (1985) tested around 200 white rot fungal cultures on wheat straw and found an increase in IVD from 15 to 32% in most of the cases, depending upon temperature and period of fermentation. Kamra and Zadražil (1987) suggested that the IVD of lignocellulosic substrates is one of the most important criteria for the selection of fungal cultures; however, if OM loss and OM intake by animals are not taken into account, the results of IVD alone could be misleading as many edible fungi consume majority of soluble sugars and hemicelluloses which are easily digestible by ruminants (Kewalramani et al., 1988). In most of the studies, dry matter (DM) losses varied widely from 6 to 40% depending on the organism used, duration of fermentation, type of substrate and environmental conditions (Agosin and Odier, 1985). Gupta (1988) developed 'Karnal process' at National Dairy Research Institute (Karnal) which was essentially a biological treatment of lignocellulosics in a SSF using WRF *Coprinus fimetarius* under non-sterile conditions, consisting of two stages. The total period for fungal treatment of cereal straws through two stage Karnal process was 35 days (first stage: 30 days for urea treatment + second stage: 5 days for SSF). Although CP content increased to 13 to 14%, the DM loss was more than 25% and was also found to be uneconomical (Gupta et al., 1993).

Jung et al. (1992) also noted large losses of DM (11.7 to 42.3%) for five white-rot basidiomycetes (*Phanerochaete chrysosporium, Scytinostroma galactinum, Phlebia tremellosa, Phellinus pini* and *Pholiota mutabilis*) grown on oat straw and alfalfa stems. Further, they observed that cell wall polysaccharides were removed from both substrates by fungal activity and only *P. chrysosporium* increased IVD of oat straw (44.1%), but all other species decreased IVD of alfalfa stems, presumably because the fungi removed the most readily fermentable polysaccharides. In spite of increased IVD and efficient lignin degradation, *P. chrysosporium* was declared unfit for practical animal feed production owing to huge OM loss (Jung et al., 1992). Zadražil and Puniya (1995) also noted a raise in *in vitro* OM digestibility (3 to 16%) of bagasse by culturing with *P. eryngii.* Karunanandaa and Varga (1996a) reported that fungal (*Cyathus stercoreus*) treated rice straw diet (75:25 straw: concentrate) increased the digestion of cellulose (27%) and glucose (38%) during continuous culture but that of hemicellulose (37%), arabinose and xyloses were decreased in addition to reduced CP availability for microbial digestion. Karunanandaa and Varga (1996b) also showed an increased IVD of rice leaf colonized by *C. stercoreus* and *Pleurotus sajor-caju* with an increased digestion of cellulose. Akin et al. (1996) reported that cell walls in alfalfa stems are more resistant to biological delignification than those in grasses, which can be biodegraded using *C. stercoreus* fungi.

Adamovic et al. (1998) cultivated *P. ostreatus* on wheat straw and after seeding, neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were decreased to the tune of 58 and 73.44%, respectively without pronounced lignin decomposition (Table 4). Díaz-Godínez and Sánchez (2002) found that maize straw after harvesting edible mushroom (*P. ostreatus*) on it, contained less NDF whereas ash, water soluble protein and water soluble carbohydrates increased favourably resulting in better *in situ* digestibility. Silva et al. (2002) reported reduced fibre and CP levels with a concomitant raise in ash content of *Pleurotus pulmonaris* grown substrates. Nutritional profile of fermented finger millet (*Eleucine coracana*) straw using brown-rot fungi *Ceratocystis culmi, Tyromyces palustris* and *Aspergillus terries* showed a favorable decrease in lignin content from the initial value of 6.68 to 3.68% after SSF (Sridhar and Senani, 2007). They concluded that *C. culmi* was the most effective with regard to lignin break down followed by *T. palustris* while *A. terries* failed to cause any significant change. In another study of SSF of finger millet straw with different strains of white-rot fungi viz. *Pleurotus sajor-caju, P. ostreatus, Voriella volvoraceae, Phanerochaete chrysosporium* and *Trametes hirsuta,* a linear increase in IVD was recorded with all the five fungi (Sridhar et al., 2007). Lignin degradation of mustard (*Brassica compestris*) straw was highest with *Phanerochaete chrysosporium* treated straw than *Ganoderma applanatum* while, IVD and CP enrichment was more with *Coriolus versicolor* cultured straw (Tripathi et al., 2008). A synergistic response with different fungal strains (*Pycnoporus sanguineus* and *Oideodendron echinula*) on lignin biodegradation and enrichment of wheat straw with microbial protein was achieved by Wadhwa et al. (2008).

Arora and Sharma (2009) also conducted the SSF of wheat straw obtained from different regions of India with four different WRF viz. *Phlebia brevispora, P. fascicularia, P. floridensis* and *P. radiata,* and observed that *P. brevispora* was found to be the best organism which degraded more than 30% lignin in all the straw samples irrespective of the region, along with good laccase production, which might be a reason for its better ligninolytic ability. As a result of higher ligninolysis, the organism was also able to enhance the digestibility (from 17.2 to 28.7 %) upto a significant level and a strong positive correlation between the two were observed. In other experiment, 50% increase in IVD was observed with *P. floridensis* (Sharma and Arora, 2010). Increased *in vitro* digestibility of *Madake* bamboo (*Phyllostachys bambusoides*) by SSF with white rot *Ceriporiopsis subvermispora* for 10 weeks was observed by Okano et al. (2009). Vadiveloo et al. (2009) reported that SSF of rice husk for 25 days with *Pleurotus sajor-caju* increased IVD and CP content without any organic matter loss.

Akinfemi (2010) reported that CP content of peanut husk improved with *P. ostreatus* (9.29%) and with *P. pulmonaris*

Table 4. Changes in the composition of wheat straw incubated with *Pleurotus ostreatus* mycelium (g kg^{-1} DM).

Day of incubation	DM	CP	EE	Ash	NDF	ADF	Hemicellulose	Cellulose	Lignin
0	195	38	19	63	824	561	263	453	109
15	183	40	15	63	770	560	210	472	88
30	193	43	16	64	714	523	192	436	87
45	215	44	16	70	689	513	176	433	80
60	223	46	17	74	657	473	184	398	76
90	245	45	15	84	561	421	141	346	75
120	250	41	12	98	485	412	74	341	71

Adapted from Adamovic et al. (1998).

(16.11%) compared to untreated husk (7.39%) when fermented for 21 days and depleted CF levels. Increased CP content of fermented substrates was associated with increased fungal biomass (Chen et al., 1995). Similar higher nutritive values were obtained for fungal treated maize cobs (Akinfemi et al., 2009a) and maize straw (Akinfemi et al., 2009b). *Cyathus stercoreus* was observed as the best among four WRF tested (others being *Phonerochaete chrysosporium*, *Auricularia polytricha* and *Sporotrichum pulverulentum*) with respect to maximum ligninolytic and minimum cellulolytic and hemicellulolytic activity leading to minimum nutrient losses (Bakshi et al., 2011). Shrivastava et al. (2011) also reported significant decrease in cell wall constituents like ADF, NDF, hemi-celluloses, lignin and cellulose to the extent of 35.00. 38.88, 45.00, 37.48 and 37.86%, respectively in *P. ostreatus* fermented straw, while 30.04, 33.85, 39.90, 31.29 and 34%, respectively in *T. versicolor* fermented straw. However, maximum efficiency of fermentation in terms of low carbohydrate consumption per unit of lignin degradation was observed for *P. ostreatus* on the 10^{th} day (17.12%) as compared to *T. versicolor* on the 30th day (16.91%). The myco-straw thus produced was found to contain high crude protein (CP; 4.77% *T. versicolor*, 5.08% *P. ostreatus*) as compared to untreated straw (3.37%). Shrivastava et al. (2012) reported an improved organic matter digestibility (OMD) of wheat straw (32.22%) with *Ganoderma* sp. rckk02 and as a result of fungal growth, a significant decrease in ADF (24.77%), NDF (31.03%), hemicelllose (42.51%), lignin (34.95%) and cellulose (34.33%) contents were observed on 15^{th} day. These recent studies clearly indicate the nutritional improvement of poor quality straws by fungal fermentation under SSF. Recently, Hassim et al. (2012) observed an increase in *in vitro* apparently degradable carbohydrates of oil palm fronds inoculated with WRF *Ceriporiopsis subvermispora* (3 weeks) and *Lentinula edodes* (9 weeks) up to 13 and 10%, respectively.

FUNGAL TREATED STRAW IN THE RATION OF RUMINANTS

Effect on nutrient utilization and growth performance

Majority of the animal trials on utilization of fungal treated crop residues reported a positive response in terms of nutrient utilization, nitrogen (N) balance as well as gain in body weight (Walli et al., 1988; Fazaeli et al., 2002; Kabirifard et al., 2007; Mahesh 2012; Omer et al., 2012; Shrivastava et al., 2012) although it is not consistent with all types of WRF. Spent straw remaining after edible mushroom harvesting (called spent mushroom substrate), generally contains an increased CP, cell-wall solubles (Langar et al., 1980), total and acid insoluble ash and reduced cell wall components which might be more useful than the original straw for feeding ruminants. Meanwhile, inclusion (up to 25%) of spent wheat straw obtained from cultivation of *Agaricus bisporus* mushroom in the diet of buffaloes resulted in a similar nutrient digestibility but a lower DM intake (Langar et al., 1982). Ward and Perry (1982) found an improved digestibility of DM and NFE of corn cobs treated with *Trichoderma viride* in lambs. Bakshi et al. (1985) also reported that the spent straw had lower digestibility of nutrients and was thus, of poor quality due to high losses of nutrients during *Pleurotus* growth and much increase in the ash content.

Walli et al. (1988) observed that the N intake, its digestion and retention in cross-bred calves fed fungal treated wheat straw supplemented with groundnut cake was higher than urea treated straw fed group. Kakkar et al. (1990) investigated the effect of replacement of wheat straw with *Pleurotus* treated straw in the diets of buffaloes where the animals received larger quantities of treated straw upto 2 to 4 kg per day and their daily gain was lower between which indicated that spent straw was as good as untreated straw for feeding buffaloes. Bakshi and Langar (1991) showed that *Agaricus bisporus* spent wheat straw (SWS) mixed with untreated wheat straw and 200 g cereal can meet the daily digestible crude protein (DCP) and total digestible nutrients (TDN) requirements of an adult ruminant as SWS contained 5.56% DCP and 29.3% TDN. Mushroom (*P. ostreatus*) harvested spent compost in the diet of Simmenthal heifers significantly reduced weight gain at more than 17% inclusion level in the total mixed ration (Adamovic et al., 1998). In contrast, increased voluntary daily intake as well as gain in body weight of Pelibuey sheep fed with spent maize straw of *P. ostreatus* was observed by Díaz-Godínez and Sánchez (2002). Intake and digestibility of DM and OM was increased by more than 10% in cattle

consuming fungal treated wheat straw diet (Fazaeli et al., 2002) and palm leaves treated with *Pleurotus florida* for sheep (Kabirifard et al*.,* 2007). Fazaeli and Shafeyi (2003) suggested that *Agaricus bisporus* harvested spent wheat straw could be included up to 15% of the diet for finishing lambs beyond which intake as well as nutrient balance would be reduced due to high mineral content. Fermentation of wheat straw by *Coprinus fimetarius* is effective in improving N status of the straw but it was found to be unfit for feeding goats as the fermented material was less palatable and reduced the intake and also adversely affected the growth rate (Dahiya et al*.,* 2004).

While assessing the effect of incorporation of wheat straw-rice straw (WS-RS in 50:50 ratio) or *Pleurotus florida* harvested spent WS-spent RS (50:50) in kids, Kaur et al. (2010) observed a higher DM intake (0.80 vs. 0.65 kg d^{-1}), digestibility of majority of nutrients (except cellulose which was depressed), N-retention (5.36 vs. 4.87 g), apparent biological value (63.08 vs. 53.43%) and daily live weight gain were comparable in both the groups. Ramirez-Bribiesca et al. (2010) evaluated the influence of *P. ostreatus* spent corn straw on the performance of feedlot Pelibuey lambs and found that average daily gain (ADG) increased to 17.5% in treatment group which received 9% of pro-farming straw from *P. ostreatus.* A significantly increased DM intake and growth rates were noted by Akinfemi and Ladipo (2011) in West African dwarf lambs fed with biologically treated maize cobs replacing wheat offal in guinea grass *(Panicum maximum)* based diets. Abdel-Azim et al. (2011) treated rice straw and corn stalks with *Trichoderma viride,* which improved their feeding value resulting in higher intake, N balance and growth rate in cross-bred lambs. Kim et al. (2011) fermented the oyster mushroom (*P. ostreatus*) spent substrate with selective lactic acid producing bacteria and supplemented at 10% level in calf starter diet which improved average daily gain and feed efficiency of post weaning calves. Recently, Shrivastava et al. (2012) reported that feeding wheat straw fermented with WRF *Ganoderma* sp. rckk02 improved DM intake, DCP, TDN and N retention in goats suggesting that the fungi holds potential in improving the nutritive value of straw. Omer et al. (2012) had shown that biologically treated corn stalks (using *Trichoderma ressi*) can completely replace clover hay in the ration of growing sheep which was evident by a favourable increase in DM intake, and an improvement in the digestibility of all nutrients with higher ADG. These reports clearly indicate that majority of the fungal treated spent substrates are less palatable, which can easily be improved by either ensiling or mixing with more palatable feeds (Kamra and Zadražil, 1988).

Effect of fungal treated wheat straw based diets on lactation performances in cows

Adequate levels of high quality forage NDF in the lactating cow diets are necessary to maintain optimum rumen functions and to maximize milk yield (Robinson and McQueen, 1992). In this regard, Fazaeli et al. (2002) studied the effect of fungal (*Pleurotus ostreatus* coded P-41) treated wheat straw in the diet of lactating Holstein cows at 0, 10, 20 and 30% levels. The daily intake of DM, OM, CP and TDN was not affected by substitution of alfalfa hay with fungal treated wheat straw. Inclusion of treated straw at these levels did not affect the digestibility of nutrients, except for the ADF that was significantly reduced (35.4% vs. 38.7) at 30% level of inclusion. Body weight gain was more at 20% inclusion level, but the daily milk yield and its composition were not affected by treated straw. Further, Fazaeli et al. (2004) reported that inclusion of fungal treated straw upto 30% of the total mixed ration in late lactating Holstein cows improved the nutrients digestibility and also noted an increase in fat corrected milk yield by 13% and daily average body weight gain by 2.7 times.

In vitro gas production profiles and associated parameters of fungal treated substrates

Gases produced during rumen fermentation are waste products and of no nutritive value to the ruminants, but gas production tests are routinely used in feed research as gas volumes are related to both the extent and rate of substrate degradation (Blümmel et al., 1997). Thus *in vitro* gas production (IVGP) technique (Menke and Steingass, 1988) is used widely in animal nutrition for feed evaluation and to study the kinetics of microbial fermentation processes in the digestive tract. Krishnamoorthy et al. (1995) also suggested that IVGP technique should be considered for estimating metabolizable energy (ME) in tropical feedstuffs, as other methods are time consuming and costly. Gas production and associated *in vitro* parameters are presented in Table 5.

Gupta et al. (1992) described that the gas produced in the straw comprises of two phases; one being the soluble phase (rapid gas production) contributed by soluble portion of the straw and the other contributed by the insoluble fibrous portion of cell-wall. Further, Cone et al. (1997) described third phase of gas production, which is contributed by microbial turnover. As cell wall components (NDF and ADF) are known to have a negative correlation with gas production (Sallam et al., 2007), and thus readily available soluble carbohydrate fractions found in fungal treated substrates are expected to produce more gas (Chumpawadee et al., 2007) and short chain fatty acids (SCFA), with an increased ME contents (Akinfemi, 2010; Shrivastava et al., 2011, 2012; Mahesh, 2012). Accordingly, Okano et al. (2005) reported a higher gas production in wheat straw treated with *Pleurotus* sp. Higher cumulative gas production was also observed by Suzuki et al. (1995), Valizadeh et al. (2008) and Akinfemi (2010) for white rotted (shiitake and nameko mushrooms) woody

Table 5. *In vitro* evaluation of fungal treated substrates using gas production test.

Reference	Substrate(s)	Organism(s)	GV-24 h[#] (ml g^{-1} DM)	OMD (%)	ME (MJ kg^{-1})
Okano et al. (2005)	Wheat straw	*Pleurotus* sp.	135-140	-	-
Okano et al. (2009)	*Madake* bamboo	*Ceriporiopsis subvermispora*	151*	65.90**	-
Akinfemi et al. (2009a)	Maize cobs	*P. pulmonaris*	116.65	42.09	6.04
		P. sajor-caju	113.35	41.57	5.94
Akinfemi et al. (2009b)	Maize straw	*P. pulmonaris*	131.5	49.11	6.41
		P. sajor-caju	138.5	51.12	6.75
Akinfemi (2010)	Pea nut husk	*P. ostreatus*	206.50	60.90	8.40
		P. pulmonaris	200	63.20	8.61
Kaur et al. (2010)	Wheat straw: Rice straw (1:1)	*P. florida*	74.82	44.54	-
Shrivastava et al. (2011)	Wheat straw	*P. ostreatus*	91.25	33.39	4.92
		Trametes versicolor	81.25	31.74	4.66
Shrivastava et al. (2012)	Wheat straw	*Ganoderma* sp. rckk02	88.75	33.40	4.87
Mahesh (2012)	Wheat straw	*Crinipellis* sp.	155	40.58	5.45
		RCK-3 isolate	160	44.63	5.67

[#]Net *in vitro* gas volume produced by the feed substrate after 24 h of incubation; *expressed as GV-48 h (ml g^{-1} OM); **after 48 h of incubation.

Table 6. Rumen fermentation parameters (pH, TVFA, NH_3-N) as affected by fungal treated substrates.

Reference	Substrate(s)	Organism(s)	pH	TVFA (mmol 100ml^{-1})	NH_3-N (mg dl^{-1})
Karunanandaa and Varga (1996a)	Rice straw	*Cyathus stercoreus*	6.0*	14.18	0.77*
	Rice leaf	*Cyuthus stercoreus*	-	30.3	18.1
	Rice stem	*Cyuthus stercoreus*	-	6.7*	15.3
Karunanandaa and Varga (1996b)	Rice leaf	*Phanerochaete chrysosporium*	-	7.8*	16.8
	Rice stem	*Phanerochaete chrysosporium*	-	4.4*	16.4
	Rice leaf	*Pleurotus sajorcaju*	-	27.1	16.1
	Rice stem	*Pleurotus sajorcaju*	-	29.5	13.3
Omer et al. (2012)	Corn stalks	*Trichoderma ressi*	6.98	13.46	18.81
Tripathi et al. (2008)	Mustard straw	*Coriolus versicolor*	7.19	7.98	20.34
Salman et al. (2008)	Sugar beet pulp	*Trichoderma viride*	6.47	12.81	23.57
Akinfemi and Ladipo (2011)	Maize cob	*Pleurotus tuber-reguim*	9.08	12.84	26.40
Mahesh (2012)	Wheat straw	*Crinipellis* sp.	-	2.25	7.52
	Wheat straw	RCK-SC isolate	-	2.33	6.65

*Values decreased significantly ($P<0.05$) as compared to the control.

materials, *Pleurotus ostreatus* treated wheat straw and peanut husk fermented with *Pleurotus ostreatus* and *P. pulmonaris,* respectively. Okano et al. (2009) also correlated a decrease in OM, cell wall components and lignin with an increase in *in vitro* digestibility of OM, NDF and IVGP after 10 weeks of SSF of *Madake bamboo*. However, Kaur et al. (2010) found lower net gas production in spent wheat straw and rice straw (*Pleurotus florida)* at 1:1 ratio with a higher partitioning factor, a measure of efficiency of microbial protein synthesis (Blümmel et al., 1997).

Effect on rumen fermentation parameters

Fermentation pattern observed with fungal treated substrates upon microbial digestion favourably altered ruminal parameters because of bio-delignification by WRF which enables faster accessibility by rumen microbes. Conse-

quently, higher levels of total volatile fatty acids (TVFA), acetate to propionate ratio (A:P) and variable ammonia nitrogen (NH_3-N) are produced (Table 6). Karunanandaa and Varga (1996a) found that fungal (*Cyathus stercoreus*) treated rice straw diet produced an increased TVFA with an increased molar proportion of propionate and butyrate. But pH and NH_3-N production was reduced. Higher TVFA concentration was also observed by Suzuki et al. (1995) with fungal (Shiitake and nameko mushrooms) rotted woody materials. In another study, Karunanandaa and Varga (1996b) compared rumen fermentation pattern of different morphological fractions of rice straw colonized by different WRF (for 30 days) and found that TVFA production increased by 75% and 25% for leaf and stems colonized by *Cyathus stercoreus* (Cs) and *Pleurotus sajor-caju* (Ps), respectively compared to *Phanerochaete chrysosporium*. Further, A:P ratio and NH_3-N were higher in both fractions colonized by Cs and Ps. Abo-Donia et al*.* (2005) and Omer et al*.* (2012) reported that ruminal pH and the NH_3-N concentration in the rumen liquor increased significantly in biologically treated peanut hulls and sugarcane bagasse, and *Trichoderma ressi* treated corn stalks, respectively besides a higher TVFA concentration (Omer et al*.,* 2012). Higher NH_3-N levels were also recorded by Salman et al*.* (2008), Akinfemi and Ladipo (2011) and Mahesh (2012) with fungal treated sugar beet pulp, maize cobs and wheat straw, respectively. Tripathi et al. (2008) found that bio-processed mustard straw with *C. versicolor* (21 days) increased rumen pH and TVFA after 6 h of feeding in sheep. Further, cultured straw increased small holotricks but reduced large holotricks population in rumen liquor, while no effect on ruminal microbial enzyme activities was observed. These studies imply that most of the microbially converted feeds are safer and the potential biohazards associated with them are very low (Villas-Bôas et al., 2002) for ruminants.

Methane production from fungal treated substrates

Enteric methane (CH_4) production arises principally from microbial fermentation of hydrolyzed dietary carbohydrates such as cellulose, hemicellulose, pectin and starch. The amount of CH_4 produced during ruminal fermentation is dependent upon the nature of the substrate being fermented. Diet composition alters the digestion efficiency of animals thereby CH_4 production. In general, methanogenic potential of ruminal microflora is greatest for the fermentation of structural carbohydrates compared to that of non structural carbohydrates (Torrent et al*.,* 1994; Johnson and Johnson, 1995; Boadi et al., 2004). This is the reason why ruminants emit more CH_4 on fibrous (straw and stover) diets. Jalc et al. (1994) reported a reduction in *in vitro* total and individual gas (methane and CO_2) production from wheat straw treated with *Polyporus ciliates* for four weeks. Akinfemi (2010) reported that CH_4 (ml 200 mg^{-1}) production *in vitro* from peanut husk fermented for 21 days with WRF *Pleurotus ostreatus* (7) and *P. pulmonaris* (5) was significantly lower compared to untreated husk (8). Mahesh (2012) observed a linear reduction in CH_4 (%) from fungal treated wheat straws which contained lesser fibre fractions (NDF and ADF) than untreated straw. This could probably due to indirect effect via fibre digestion leading to lesser residency of feed particles in the rumen (Moss et al., 1994; Sallam et al., 2007). The role of quality forages in reducing enteric CH_4 production in ruminants has been evident from several studies (Varga et al*.*, 1985; Das and Singh, 1999; Singh and Mohini, 1999a, 1999b, Benchaar et al., 2001; Mohini et al., 2007). It can be concluded that enteric CH_4 emissions are highest when the animal is presented with poor quality forages. Thus, by fungal treatment (via SSF), an improvement in the forage quality with respect to cell wall digestion and overall enhancement in carbohydrates digestibility as well as increased DM intake will be expected to reduce the CH_4 emissions relative to nutrients digestibility, in ruminants (Mahesh, 2012).

CONCLUSION AND POSSIBLE FUTURE AREAS OF THE TECHNOLOGY

Biological treatments can be employed for improving the feeding value of low quality fibrous crop residues. The inevitable organic matter losses during biological treatments imply that an increased OM digestibility is needed to compensate for the losses. Hence, SSF for a period of 6-8 days has been recommended as the maximum time of fermentation in order to reduce DM loss (Owen et al., 2012). Although improvement in the nutritional worth of biologically treated crop residues is achieved, many are not economical and the process has not yet optimized under field conditions. Hence, the possible future of the technology should focus on the following areas.

Isolation and identification of selective and highly lignin-lytic fungus in the nature and cultivating it for the commercial production of ligninase enzyme (FAO, 2011). Further, biotechnological means of genetic manipulation of lignin-lytic fungus such that only lignin is degraded without any greater change in cell wall carbohydrates needs to be developed. Once the proven fungus is identified, its potential to upgrade (enhancing digestibility) various agro by-products (husks, straw, stovers, bagasse and other fibrous lignocellulosics) that are traditionally used as livestock feeds should be considered. In addition, focus should be given to develop a simple and economic technology for effective implementation especially at small and mixed farming systems in developing countries which may partially solve the ever increasing problems of feed crisis to livestock.

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