BIOLOGICAL TREATMENT OF CROP RESIDUES AS AN OPTION FOR FEED IMPROVEMENT IN TROPICS

Nguyen Van Quang (collection)

National Institute of Animal Science

Corresponding author: Nguyen Van Quang, Tel: 0989637328. Email: quangvcn@gmail.com

ABSTRACT

In tropical countries, roughages feeds are the major diets of ruminant animals which are poor in quality. Hence, improving the nutritive value of feeds of such kind is vital for best utilization for ultimate goal of increased animal production. Animal feeds and feeding practices can be changed by biological catalysts such as fungi with the objective to improve nutritive value and to reduce environmental waste. Biological treatment of such crop residues using white rot fungi can break the ligno- cellulose structure, liberating free cellulose and thus enhancing their feeding value. Biologically treated roughages have higher digestibility for most of the nutrients with an increase in crude protein content as compared to untreated material, besides ensuring more fermentable substrates in the rumen. In addition, treatment of low-quality animal feeds with white rot fungi species increases the protein and ash contents with a reduction of its fibrous fraction. Moreover, biological treatment roughages feed increases the feed intake, digestibility and eventually livestock production and reproduction. However, the application of biological treatment of roughages is limited by lack of biological agents such as the typical fungi or its products (enzymes) and knowledge of utilization of such agents. The other setback is reduction of weight of the final substrate after the treatment in which case a dry matter loss of substrate can be as high as 40% in prolonged incubation with the fungus. Moreover, there is lack of know how to use such technology in most of tropical Africa including Ethiopia. This review was then organized to create awareness on utilization of biological treatment as remedy for poor quality of roughages, optimizing mechanisms must be sought.

Keywords: biological treatment, Ethiopia, white rot fungi

INTRODUCTION

Despite large demand of livestock and their products in developing countries (Thornton, 2010), the productivity per head of livestock is usually low mainly because of various factors. The most important cause of poor livestock productivity in tropical regions of the world is inadequate nutrition (MacMillan, 1996; Sere et al., 2008). The major feed resources in the tropics including Ethiopia are crop residues such as straws which are high in fiber (Van Soest, 2006; McDonald et al., 2010). Moreover, these feed resources are characterized by for animal feeding is their low digestibility, due mainly to non-polysaccharide components such as phenolic acids (Kuhad et al., 2013; Elghandour et al., 2014). To improve the nutritional quality of straws and agricultural byproducts, different strategies have been used to disrupt carbohydrate-lignin complexes, facilitate the access of cellulolytic microorganisms to the structural carbohydrates and improve the quality and nutritive value of straw (Valdes et al., 2015; Alsersy et al., 2015). In the past decades, many efforts have been employed to remove the lignin and/or to break up the linkages between lignin and carbohydrates and to increase their feeding values by biological treatments (Liu and Orskov, 2000; Zhu et al., 2005; Eun, 2006).

Biological agents such as fungi are a potential treatment agent which can be used to remove lignin and increase the digestibility of low-quality forages (Khattab et al., 2011; Sharma and Arora, 2015). The basidiomycetes fungi have the capability to degrade lignin in cell walls (Khattab et al., 2013). Among these are white- rot fungi, which are capable of decomposing and mineralizing plant cell components because, during fungal colonization of a suitable substrate, the easily digestible carbohydrates are converted into simpler sugars (Khattab et al., 2013; Kholif et al., 2014). This is known as the fungus' primary metabolism. These sugars are

totally consumed by the fungus and afterward secondary metabolism is initiated, which consists of the breakdown of structural carbohydrates and lignin from substrates by extracellular enzymes like laccase, manganese peroxidase and peroxidase (Khattab et al., 2013). Because of the high annual production of agricultural residues and their low nutritional quality (Khattab et al., 2013), it is essential to seek new techniques to increase their nutritive value, at low energy cost and in an environmentally safe manner. The objective of this paper was to review role of white rot fungi as a biological treatment of low-quality animal feeds.

The white rot fungi

Microorganisms such as the brown, white and soft-rot fungi have been used to breakdown lignin and hemicellulose crop residues (Seker et al., 2006). Attempts had been made to identify species of white-rot fungi for their ability to grow on straws that improved their nutritive value (Yamakawa et al., 1992). White rot fungi are known to degrade lignin to a great extent and at a fast pace when compared to any other group of organisms (De Koker et al., 2000). They are the only fungi that can take the complete lignin mineralization (Moore Landecker, 1996). These organisms are also able of delignifying lingocellulosic substrate selectively, modifying or degrading the lignin and transforming the lignocelluloses substrate of the decomposition to high quality feed for ruminants (Chaudhary, 1998), or utilizing the polysaccharides liberated by hydrolysis and fermentation, in order to produce fuels and other chemicals (Puniya and Singh, 1998).

Fungi that are active in the biodegradation of wood can be classified into three main groups according to their methods of degrading biomass, specifically white-rot, brown-rot, and soft-rot fungi. White- rot and brown-rot fungi belong to Basidiomycetes, whereas soft-rot fungi belong to Ascomycetes (Hatakka, 2001). Brown-rot fungi preferentially attack cellulose and hemi-cellulose, leaving lignin intact, thus, decaying residue turning brown and causes only limited changes in lignin. These results in lower *in vitro* digestibility compared to untreated substrate (Mahesh and Mohini, 2013). Soft-rot fungi leave the White-rot fungi, belonging to the wood-decaying *basidiomycetes*, as lignocellulolytic microorganisms can decompose and metabolize all plant cell constituents (cellulose, hemicellulose and lignin) by their enzymes (Eriksson et al., 1990). Many species of whiterot fungi which are effective lignin degraders have been used to assess their ability to improve the nutritive value of fodder for ruminant nutrition (Howard et al., 2003) (Figure 1).

Their extracellular lignin-modifying enzymes consist of lignin- peroxidase, manganese-dependent *peroxidase laccase* (phenol oxidase) and H₂O₂-producing oxidase (aryl-alcohol oxidase (Arora and Gill, 2005; Lechner and Papinutti, 2006). Some white- rot fungi are able to decompose free phenolic monomers and to break the bonds with which lignin is cross-linked to the polysaccharides in straw thereby enhance digestibility (Fazaeli et al., 2006). The bio-conversion of straw is circumscribed to the group of white-rot fungi, which are capable to colonize on cereal straw and liberate water soluble substrates from the polymers during SSF and thus improve the digestibility (Fazaeli et al., 2003). Among the edible white-rot fungi, the *Pleurotus* species have been shown to be more efficient (Taniguchi et al., 2005). The potential of *Pleurotus* fungi such as *P. ostreatus* and *P. eryngii* to reduce indigestible cell wall components and increase the dry mater digestibility (DMD) of straw has been reported (Fazaeli et al., 2004). The *Pleurotus* fungi have different ability to grow on straw and decompose its structural carbohydrate because of the variation in culture behaviour and culturing conditions (Fazaeli et al., 2002).

Utilization of white rot fungi

Fungal strains can be collected from the surrounding and maintained on solid media (fore example Potato Dextrose Agar, Formedium, Hunstanton- UK) and stored at room temperature. The dose of application of fungus to feeds varies. Montañez-Valdez et al. (2008) added 250 g of the *Pleurotus djamor* strain to a 10 kg of maize stover packed by polyethylene bag. The wheat grain spawn of two *Pleurotus* fungi including *P. florida* (*PF*) and *P. ostreatus* (*PO*), were used to inoculate the straw, at the rate of 3.5 kg spawn per 100 kg straw fresh weight basis (Fazaeli, 2007). The nutritive value of low-quality feeds, which has been widely, reported using rape straw (Tripathi et al., 2008), wheat straw (Tuyen, 2012), rice straw (Sharma and Arora, 2010), and corn Stover and sugarcane bagasse (Tuyen et al., 2013).



Figure 1. White rot fungi

Effect of fungal treatment on crop residues

Impact on chemical constituents

Fungal cultures used in ruminant diets include yeasts, generally *S. cerevisiae*, and a mold, generally *Aspergillus oryzae*. In adult cattle, dietary addition of yeasts and *Aspergillus oryzae* (AO) extract have been shown to increase feed efficiency and weight gain, and slightly increase milk production in lactating dairy cows. Among ruminal bacteria, two functional groups, the fiber digesting and lactate utilizing bacteria, are stimulated by addition of fungal cultures (Martin and Nisbet, 1992). One approach that has recently been widely investigated is the application of live microbial preparations, in order to promote digestion and intestinal hygiene, enhance animal performance and reduce usage of antibiotics (Jouany and Morgavi, 2007; Guedes et al., 2008; Wallace et al., 2008). It is indicated that microbial additives may benefit ruminant nutrition in terms of live weight gain and milk production by a magnitude of 7 to 8% (Wallace, 1994). Yeast cells promote growth of rumen bacteria, and cellulolytic and lactate-utilizing bacteria can be preferentially stimulated (Chaucheyras et al., 2008).

Degradation of Bermuda grass stems was improved by 29-32%, after 6 weeks, using *Ceriporiopsis subvermispora* and by 63-77% using *Cyathus stercoreus* (Okano et al., 2005). Masayuki et al. (2005) reported three white rot fungi *Pleurotus ostreatus*, *Phanerochaete*

chrysosporium and Trametes versicolor that cause 41, 21 and 37% lignin loss when grown on rice straw for 60 days at 250c. (Sirlene et al., 2004) reported white rot fungus Ceriporiopsis subvermispora caused higher loss of dry weight (32%) in bagasse when incubated for 30 days with 1% inoculum under solid state fermentation. Strophoria rugosoannulata and Pleurotus cornucopiae degraded wheat straw 60 to 65%; P. florida degraded 45% and A.aegerita degraded wheat straw up to 25% only in 17 weeks at 300c incubation. P.chrysosporium when tested individually caused 26.45% weight loss of the substrate and 28.95% lignin loss when grown on wheat straw for six weeks, where as caused lignin loss upto 36% when combination of P. chrysosprium and D. flavida were used (Zadražil and Brunnert, 1980).

Fungal treated straw contained higher CP, EE and ash contents and lower OM, CF, NFE, NDF, ADF, ADL, hemicellulose and cellulose contents than untreated straw. Authors indicated that fungal treatment of straw increased crude protein from 3.20 to 11.62% (El-Rahman et al., 2014). Treatment of rice straw by P. pulmonarius increases CP contents from 4.50% recorded in the control to 4.60% at day 10, 4.78% at day 20 and 9.36% after forty days of fermentation (Jonathan et al., 2012). Biological treatment of mixed straw (wheat and cotton) by three strains of fungus: Pleorotus Ostreatus, Pleorotus Corniucopia and Pleorotus Salmoneos, degraded 52.1%, 59.3% and 39.4% lignin and improved in vitro organic matter digestibility from 33.0% to 60.1%, 51.9% and 50.6%, respectively (Yosef et al., 2011). Corn stover inoculated with 15% Trichoderma viride and incubated for 21 days had increased protein from 6.52% to 10.28%, but decreased NDF from 64.27% to 55.39%, ADF from 44.49% to 37.77%, hemicellulose from 19.78 to 18.02% (Islamiyati et al., 2013). Fungal treatment of rice straw decreased crude fibre from 32.89% in control to 19.96% (Akinfemi and Ogunwole, 2012). The crude protein of cacao shell inoculated with P. chrysosporium and incubated for 15 days increased from 8.57% to 11.52%, with decreased crude fiber from 44.21% to 29.94%, and increase ash content from 6.79% to 7.12%. The increased protein content was due to bioconversion of organic materials that had been broken down into one of the fungi body components or due to the addition of microbial protein during fermentation process (Belewu, 2008). Bagasse inoculated with Trichoderma viride has an increase in protein, cellulose, hemicellulose, and ash content, and decrease in ADF, NDF, and acid detergent lignin (ADL) content (Azim et al., 2011). The nutritive value of rice straw treated with Pleurotusostreatus (POR), Pleurotus pulmonarius (PPR) and Pleurotus tuber-regium (PTR) were studied by (Oziel Dante Montañez et al., 2015; Nasehi et al., 2014) (Table 1).

Table 1. Comparison of composition of fungal treated and untreated straws of different crops (%)

Parameter	*Untreated straw	*Treated straw	**Untreated	**Treated straw
DM	92	92.6	91.13	93.38
OM	82.5	82.6	83.43	79.19
CP	4.8	4.4	3.2	11.62
NDF	55.9a	47.65b	67.73	61.23
ADF	25.7	27.2	46.77	42.17
C	33.7	32.4	29.88	28.01
HC	29.8a	20.4b	20.96	19.06
Lignin	12.2	11.9	16.89	14.16
Ash	9.4	9.6	16.57	20.81

Source: *Oziel Dante Montañez et al. (2015), **El-Rahman et al. (2014).

Note: DM=dry matter; OM=organic matter; CP=crude protein; NDF=neutral detergent fiber; ADF=acid detergent fiber; C=cellulose; HC=hemicelluloses

The losses of NDF ADF and ADL contents from the rice straw suggested that the ability of Fungi to solubilise and utilize the cell walls as carbon sources and thus changed the ratio of insoluble to soluble carbohydrates in the straw. Similarly, Samsudin et al. (2013) reported that inoculation of effective microorganisms (EM) on the fungal- treated rice straw reduced the NDF and ADF content from 79.54 to 74.02% for NDF and 63.69 to 59.74% for ADF, respectively indicating the beneficial effect of EM in reducing hemicelluloses and cellulose content of rice straw. 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%). Shrivastava et al. (2012) 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. ersicolor* fermented straw. Adenipekun and Dada (2013), carried out studies on the degradation of cotton waste, rice straw and cocoa pod husks using *Pleurotus* pulmonarius in cultures incubated for 0-60 days. Crude protein increased significantly throughout the incubation period from 1.27% in the control to 12.63% in cotton waste, 6.65% to 14.82% in rice straw and in cocoa pod husk from 7.04% to 13.82%. Crude fibre decreased significantly in cotton waste and cocoa husk from 5.88% to 5.31% and from 39.88% to 34.95% respectively but an increase was observed in rice straw from 18.42% in control to 28.08% after 60 days of incubation period.

Effect fungal treated feeds on animal performance

Intake and digestibility

alves fed ration contained fungal treated straw had higher in all nutrients digestibility (El-Rahman et al., 2014). Akinfemi and Ogunwole (2012), point out that fungal treatment of rice straw not only improved the CP contents but also enhanced digestibility. 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). Safa et al. (2011), studied the effect of solid state fermentation by *Trichoderma Viride* on nutritive values rice straw (RS) and corn stalks (CS). Total feed intake and total dry mater (TDM) of rations containing either treated rice straw or treated corn straw was higher than intake of either untreated rice straw or untreated corn straw. The digestibility of DM and OM were 34.8 and 35.0%, respectively, in the initial straw, whereas there were 45.2 and 44.8% in FTWS; 41.0 and 41.5% in SPWS respectively (Fazaeli, 2007)

Effect on performances of animals

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 (Fazaeli et al., 2002; Mahesh, 2012; Omer et al., 2012) although it is not consistent with all types of white rot fungi. 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). Ramirez-Bribiesca et al. (2010) evaluated the influence of *P. ostreatus* spent corn straw on the performance of feedlot 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. Omer et al. (2012) had shown that biologically treated corn stalks (using Trichoderma ressi) can com- pletely 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. 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 (Fazaeli et al., 2004). Mahesh (2012) observed a linear reduction in CH4 (%) from fungal treated wheat straws which contained lesser fibre fractions (NDF and ADF) than untreated straw. Enteric CH4 emissions are highest when the animal is fed with poor quality forages. Thus, by fungal treatment, 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 CH4 emissions relative to nutrients digestibility, in ruminants (Mahesh, 2012). Safa et al. (2011), also reported positive effects of solid state fermentation on rice straw (RS) and corn stalks (CS) by *Trichoderma Viride* in terms of feed intake and body weight gain by sheep. The improvement in daily gain as a result of adding biological treatments may be due to its effect on microbial efficiency and organic matter digestibility.

The study by El-Rahman et al. (2014) studied the effect of Phanerochaete chrysoporium treatment on nutritional value of rice straw in which chopped rice straw was treated with fungi under aerobic condition 14 days as fermentation period. The treated straw were fed to calves with concentrate mixture and found that addition of treated straw in growing calves ration, improved nutrient digestibility, body weight gain and economic efficiency. The DMI as (kg/h/d) of calves was insignificant higher for calves fed treated than those fed untreated rice straw (8.96 vs. 9.10 kg/h/d), respectively. The feed conversion (kg DM/ kg gain) showed that the fungus treatment of rice straw recorded the best value (6.87) compared to untreated rice straw (7.16). Rice straw that has been fermented using white rot fungi can be used to substitute elephant grass up to 70% in the ration of goats (Mustabi et al., 2013). The average body weights gain of cows fed with fungal (Pleurotus ostreatus) treated wheat straw in a total mixed ration was 743 g per day (Fazaeli et al., 2004).

Factors affecting broader utilization of fungal treatments

The use of fungi and/or their enzymes that metabolize lignocelluloses is a potential biological treatment to improve the nutritional value of straw by selective delignification (Sarnklong et al., 2010). Nevertheless, its utilization may be hindered due to difficulties and lack of technology to produce large quantities of fungi or their enzymes to meet the requirements particularly in developing countries. There are also a number of serious problems to consider and overcome. Fungi may produce toxic substances. It is also difficult to control the optimal conditions for fungal growth, such as pH, temperature, pressure, O2 and CO2 concentration when treating the fodder. With recent developments in fermentation technology and alternative enzyme production system, the costs of these materials are expected to decline in the future. Hence, new commercial products could play important roles in future ruminant production systems (Zadrazil et al., 1995). The effect of white rot fungi on the lignocellulose matrix is a complex phenomenon controlled by many variables and their interactions.

Inconsistent digestibility and utilization of nutrients

Many studies indicated that the biological treatments tend to increase *in vitro* digestibility of treated materials as (Zadrazil et al., 1995; Sharma and Arora, 2010) and *in vivo* digestibility

(Chen et al., 1995; Akinfemi et al., 2009; Arora and Sharma, 2009). While the insignificant effect in nutrients digestibility recorded in this study may be attributed to that neither untreated rice straw nor treated rice straw was the only roughage source in the animal ration, hence the experimental rations contained about 19% high quality roughage material (clover hay). These results disagree with the findings obtained by many workers (Mahesh and Mohini, 2013; Fazaeli et al., 2004, Kabirifard et al., 2007; Omer et al., 2012; Shrivastava et al., 2012) who found that utilization of biological treated crop residues in animal feeding resulted in a positive response in terms of nitrogen balance.

Dry matter loss

Loss of weight of the substrate is one disadvantage of fungal treatments of low-quality feeds. 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). Jonathan et al. (2012) reported that dry matter reduced significantly from 88.74% in control to 86.80% in *Lentinus subnudus* and 86.55% in *Pleurotus tuber- regium treatments*. Weight loss caused by *Oxyporus latemarginatus* and *Rigidoporus vinctus* fungi were reported to be 27.6%, and 13.7% respectively (Mohamed, 2014). High degradation rate of wheat straw was observed with the fungus which achieved a 43% loss of dry matter long time incubation period and relatively short period of fermentation time been recommended in order to reduce DM loss (Owen, 2012).

Conclusion

Even though several treatments have been used to improve the degradability and voluntary intake of roughages, such as physical or chemical treatments, the practical use of such applications is limited due to societal and environmental concerns. The biological treatment of roughages is untouched avenue to improve roughage diets if appropriate procedure is applied. One of the biological roughage treatment methods is the use of fungal strains. Using ligninolytic fungi, including their enzymes, may be one potential alternative to provide a more practical and environmental-friendly approach for enhancing the nutritive value of roughages. However, there should be a means to identify suitable white-rot species that have no side effects and optimally used to improve the characteristics of crop residues. Moreover, the optimal conditions to incubate crop residues with a fungus have to be well researched and documented for the purpose of achieving optimal feeding quality of the remaining roughage-fungi mixture. As a general remark the application of ligninolytic fungi or their enzymes combined with pre-treatments to rice straw may be an alternative way to improve the nutritional quality of crop residues such as straws and enhance livestock productivity in the tropics.

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