

Biological treatment of crop residues as an option for feed improvement in the tropics: A review

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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 of 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.

Introduction

Despite large demand of livestock and their products in developing countries [1], 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 [2,3]. The major feed resources in the tropics including Ethiopia are crop residues such as straws which are high in fiber [4,5]. Moreover, these feed resources are characterized by for animal feeding is their low digestibility, due mainly to non-polysaccharide components such as phenolic acids [6,7]. 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 [8,9]. 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 [10-12].

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 [13,14]. The basidiomycetes fungi have the capability to degrade lignin in cell walls [15]. 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 [15,16]. 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 extra-cellular enzymes like laccase, manganese peroxidase and peroxidase [15]. Because of the high annual production of agricultural residues and their low nutritional quality [15], 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 [17]. Attempts had been made to identify species of white-rot fungi for their ability to grow on straws that improved their nutritive value [18]. 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 [19]. They are the only fungi that can take the complete lignin mineralization [20]. These organisms are also able of delignifying lignocellulosic substrate selectively, modifying or degrading the lignin and transforming the lignocelluloses substrate of the decomposition to high quality feed for ruminants [21], or utilizing the polysaccharides liberated by hydrolysis and fermentation, in order to produce fuels and other chemicals [22].

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 [23]. Brown-rot fungi preferentially

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attack cellulose and hemicellulose, 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 [24]. 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 [25]. 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 [26] (Figure 1).

Their extracellular lignin-modifying enzymes consist of lignin-peroxidase, manganese-dependent *peroxidase laccase* (phenol oxidase) and H₂O₂-producing oxidase (aryl-alcohol oxidase [27,28]). 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 [29]. 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 [30]. Among the edible white-rot fungi, the *Pleurotus* species have been shown to be more efficient [31]. 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 [32]. 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 [33].

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.* [34] 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 *Postreatus* (PO), were used to inoculate the straw, at the rate of 3.5 kg spawn per 100 kg straw fresh weight basis [35]. The nutritive value of low-quality feeds, which has been widely, reported using rape straw [36], wheat straw [37], rice straw [38], and corn Stover and sugarcane bagasse [39].



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 [40]. 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 [41-43]. 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% [44]. Yeast cells promote growth of rumen bacteria, and cellulolytic and lactate-utilizing bacteria can be preferentially stimulated [45].

Degradation of Bermuda grass stems was improved by 29-32%, after 6 weeks, using *Ceriporiopsis subvermispota* and by 63-77% using *Cyathus stercoreus* [46]. Masayuki *et al.* [47] 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. [48] reported white rot fungus *Ceriporiopsis subvermispota* 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. chrysosporium* and *D. flavida* were used [49].

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% [50]. 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 [51]. Biological treatment of mixed straw (wheat and cotton) by three strains of fungus: *Pleurotus Ostreatus*, *Pleurotus Cornucopia* and *Pleurotus Salmoensis*, 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 [52]. 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% [53]. Fungal treatment of rice straw decreased crude fibre from 32.89% in control to 19.96% [54]. 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 [55]. 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 [56]. The nutritive value of rice straw treated with *Pleurotusostreatus* (POR), *Pleurotus pulmonarius* (PPR) and *Pleurotus tuber-regium* (PTR) were studied by [57,58] (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 | 55.9 ^a | 47.65 ^b | 67.73 | 61.23 |
| ADF | 25.7 | 27.2 | 46.77 | 42.17 |
| C | 33.7 | 32.4 | 29.88 | 28.01 |
| HC | 29.8 ^a | 20.4 ^b | 20.96 | 19.06 |
| Lignin | 12.2 | 11.9 | 16.89 | 14.16 |
| Ash | 9.4 | 9.6 | 16.57 | 20.81 |

Source: *[57], **[50].

Key: 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.* [59] 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.* [60] 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.* [61] 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 [62], 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

Calves fed ration contained fungal treated straw had higher in all nutrients digestibility [50]. Akinfemi and Ogunwale [54], 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 [33] and palm leaves treated with *Pleurotus florida* for sheep [63]. Safa *et al.* [64], 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 matter (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 [35].

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 [33,65,66] 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 [33] and palm leaves treated with *Pleurotus florida* for sheep [63]. Ramirez-Bribiesca *et al.* [60] 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 *Postreatus*. A significantly increased DM intake and growth rates were noted by Akinfemi and Ladipo [67] in West African dwarf lambs fed with biologically treated maize cobs replacing wheat offal in guinea grass (*Panicum maximum*) based diets. Omer *et al.* [66] 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. 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 [32]. Mahesh [65] 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 [65]. Safa *et al.* [64], 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.* [50] studied the effect of Phanerochaete chrysosporium 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 [68]. 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 [32].

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 [69]. 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 [70]. 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 [70,71] and *in vivo* digestibility [72-74]. 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 [24,32,61,63,66,75] 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 [76]. Jonathan *et al.* [77] 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 vincinus* fungi were reported to be 27.6%, and 13.7% respectively [78]. 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 [79].

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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