Evaluation of the potentials of cowpea seedhulls using fungi mixed culture

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Abstract
An experiment was conducted to determine the nutrient composition of cowpea seedhull subjected to three different white rot fungus (Aspergillus niger. Rhizopus stolonifer and Trichoderma viride) at different fermentation periods for possibility of inclusion in poultry diets. 30 grams of the hull was inoculated with 10^7 spores of A. niger. R. stolonifer and T. viride separately at 30^0C for periods of 0. 7 and 14 days. The substrates were analyzed for proximate and mineral compositions before and after fermentation at end of each period. Fermentation with the inoculum of A. niger caused an increase of 110.34% (14.11% to 29.68%) in Crude Protein (CP) content of the seedhull after 14 days compared to 52.01% (14.11% to 21.45%) and 99.14% (14.11 to 28.10%) with the spores of T. viride and R. stolonifer respectively. A corresponding decrease in Crude fibre (CF) content was observed as the CP content increases. the CF content decreased from 30.00% to 18.00% (40% decrease) in day 14 when the hull was fermented with the inoculum of A. niger. while 15.58% (30.00% to 26.00%) and 33.33% (30.00% to 20.00%) decrease were observed with the inoculum of R. stolonifer and T. viride respectively. Fermentation with inoculum of A. niger resulted into 22.15%. 23.45% and 26.15% reductions in Acid Detergent Fibre (ADF). Neutral Detergent Fibre (NDF) and Acid Detergent Lignin (ADL) contents respectively in day 14. compared to 18.86%. 22.22% and 38.46% when inoculum of R. stolonifer was used. The result also showed significant ($p < 0.05$) reductions in the cellulose and hemicellulose contents of the hull. The results indicated that cowpea seedhull could serve as a supplement in poultry diet. The level would however need to be determined in further studies.

Key words: Cowpea seedhull. Fermentation. inoculum

Introduction
Cowpea seedhull is a crop residue which is available in Nigeria in large quantities. It is a post threshing residue which though high in fibre, is finding use in ruminant nutrition. The utilization of cowpea hulls for monogastric livestock feeding has not been realized. Various research workers have reported the hulls to be high in fibre. As is the case with other crop residues and Agro-industrial by- products, Cowpea hulls are characterized by low energy and protein. low digestibility/bioavailability and low acceptability (Faniyi, 1998). An approach to circumventing these limitations is microbial enrichment process through fungal fermentation. which Balagopalan (1996) described as an inexpensive tool for breaking down the fibre and increasing the protein level.

Upgrading of these residues. improves their nutritive value. (digestibility). sterilizes. detoxifies. concentrates and by biological means produces utilizable commodity from a substance of little initial feeding value (Wiseman and Cole. 1983).

Aspergillus niger. Trichoderma viride and *Rhizopus stolonifer* are fungi species that have been identified to possess cellulase system which breaks starch and non-starch polysaccharides to monomer sugars which are utilized to produce single cell protein (Balagopalan. 1996; Singh *et al.*, 1990).

In a bid to harness more unconventional feedstuffs to resolve the shortage of livestock feeds and products and environmental pollution. this experiment was designed to assess the nutritional potentials locked up in this crop residue after solid state fermentation with the fungi species.

Material and methods
Experimental materials
The cowpea seedhulls used in this experiment was obtained from Bodija Foodstuff Market. Ibadan. Nigeria.

Preparation of Sample and Inoculation
Pure cultures of *Trichoderma viride*, *Aspergillus niger* and *Rhizopus stolonifer* were obtained from the culture bank of the Department of Pharmaceutical microbiology and cultivated on PDA slant at 30°c. A 7 days old slant of each of the fungi was used for the fermentation process.

10mls of distilled water was used to harvest the spores of the fungi, the spore count was done using haemocytometer to obtain 10^7 spores which was used for the Solid State Fermentation.

Solid state fermentation
Solid state fermentations were carried out in 250mL Erlenmeyer flasks, in a controlled temperature chambers at 30 °C. The solid substrate contained 30g of milled cowpea seedhull. Sterilisation was done at 121°C for 20 minutes. The moisture content was adjusted by addition of sterile distilled water prior to inoculation to 53 %. Three mL of each of the inoculums was used to inoculate the substrate. The flask for each fungus was duplicated for each periods of 0, 7 and 14 days harvest.

Chemical Analysis: The samples were analyzed for proximate composition using the procedure of A.O.A.C. (1990). The fibre fractions Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF), Acid Detergent Lignin (ADL), Cellulose and Hemicellulose were determined using Van Soest and Mason, (1991). The digests were read on flame photometry for Iron (Fe) and Copper (Cu) compositions.

Statistical Analysis: The data obtained were subjected to statistical analysis of variance (ANOVA) of SAS (1999) while significant means were separated using Duncan Multiple Range test of the same package.

Results and discussion

The result of the Proximate analysis, Cellulose, Hemicellulose and Crude fibre fractions of biodegraded cowpea seedhull using different white rot fungus are presented in the tables below.

From the result, *A. niger* inoculum resulted in an increase of 110.34% (14.11% to 29.68%), while *R. stolonifer* inoculated samples gave 99.14% (14.11% to 21.45%) increase in CP after 14 days of fermentation.

The CP content of the substrates increased with periods of fermentation because as the day increases, mycelia growth increases, spore formation and germination takes place and this will increase the enzyme secreted by the fungi for extracellular digestion, thus resulting in the accumulation of mycelia protein which ultimately increase the CP of the substrate. Ofuya and Nwajiuba, (1990) reported an increase in CP to be due to fungal mycelia or mycelia protein being influenced by carbon to nitrogen protein ratio. Murata *et al.*, (1967) reported that changes in protein of fermented product could have resulted from slight protein synthesis by the proliferation of the microorganism used and a synthesis of enzyme protein, or from a rearrangement of the different proportions following the degradation of other constituents. Iyayi and Losel (1999) concluded that between day 12 and 15 are the best period for fungi activity, however Aderolu (2000) submitted that between day 0 and 10, none of the growth limiting factors of micro-organism like lack of oxygen, moisture and heat requirement were lacking when using different *Trichoderma sp* on agro- industrial product. Abu *et al.*, (1997) biodegrading sweet potato with *A. niger* obtained an increase of 134.98% (4.95% to 11.83%) and 35.15% when *A. Oryzae* was used (4.97% to 6.69%).

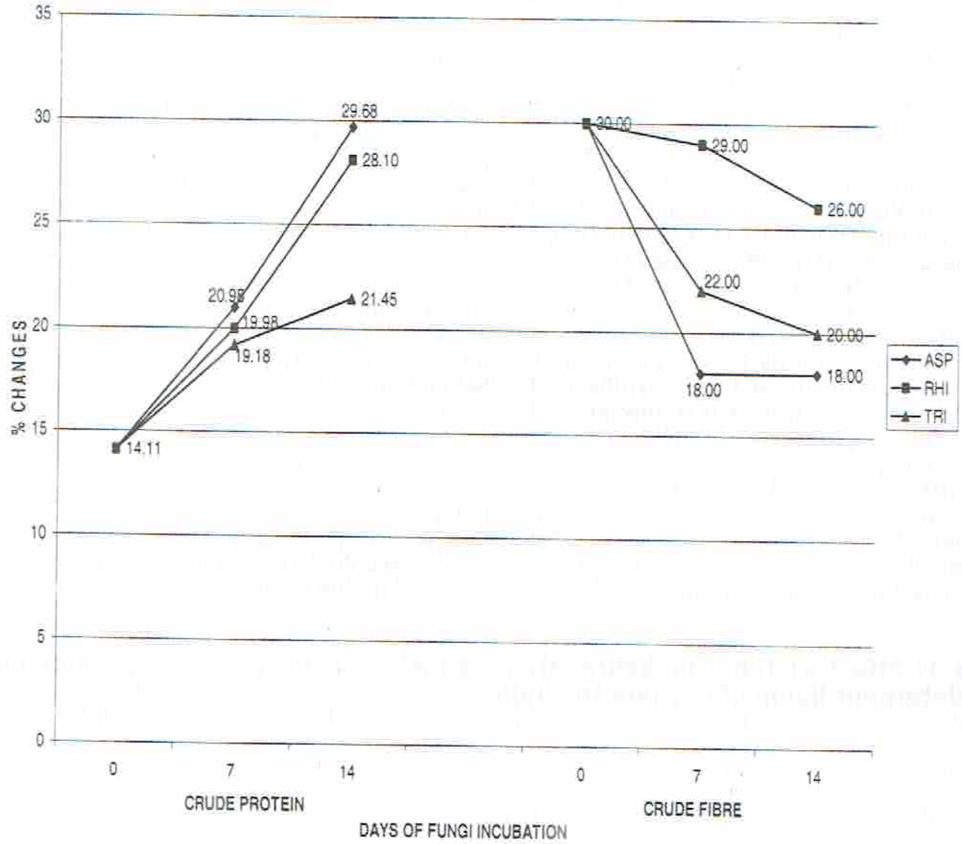

Figure 1: Changes In Crude Protein and Crude Fibre Composition Of Cowpea SeedHull At Different Fermentation Periods Using Different White Rot Fungi

The CP content of the substrates increased with periods of fermentation because as the day increases, mycelia growth increases, spore formation and germination takes place and this will increase the enzyme secreted by the fungi for extracellular digestion, thus resulting in the accumulation of mycelia protein which ultimately increase the CP of the substrate. Ofuya and Nwajiuba, (1990) reported an increase in CP to be due to fungal mycelia or mycelia protein being influenced by carbon to nitrogen protein ratio. Murata *et al.*, (1967) reported that changes in protein of fermented product could have resulted from slight protein synthesis by the proliferation of the microorganism used and a synthesis of enzyme protein, or from a rearrangement of the different proportions following the degradation of other constituents. Iyayi and Losel (1999) concluded that between day 12 and 15 are the best period for fungi activity, however Aderolu (2000) submitted that between day 0 and 10, none of the growth limiting factors of micro-organism like lack of oxygen, moisture and heat requirement were lacking when using different *Trichoderma sp* on agro- industrial product. Abu *et al.*, (1997) biodegrading sweet potato with *A. niger* obtained an increase of 134.98% (4.95% to 11.83%) and 35.15% when *A. Oryzae* was used (4.97% to 6.69%). Among the three fungus (*A. niger R. stolonifer* and *T. viride*), inoculum of *A.* niger was observed to degrade cowpea seedhull better, this is in agreement with the work of Afolabi (1999), who observed an increase of 100.30% after 10 days of biodegrading cassava peal (3.5% to 7%) and 45.68% (4.38% to 6.38%) yam peel respectively. The ability of *A. niger* to perform better than other fungi could have resulted from the fast growth rate of *A. niger* and ability produce a multienzymes compared to others. According to de Vries and Visser, (2001) most of commercial enzymes produced today are from Aspergillies.

A corresponding decrease in CF content of the seedhull with increase in CP was observed in this study (Figure. 1).

Table 1: Effect of fungi biobegradation at different fermentation periods on acid detergent lignin of cowpea seedhull

Organism	DAY 0	DAY 7	DAY 14	SEM
A. niger	13.00^{a}	11.00^{b}	9.60^{b}	1.29
Rhiz. stolonifer	13.00^{a}	10.00^{ab}	8.00^{b}	1.00
Tri. viride	13.00	12.00	10.00	1.73
SEM	1.00	1.29	1.73	

a.b.c.. means in the same row with different superscript are significantly different ($p<0.05$)
A. niger… Aspergillus niger, Rhiz. stolonifer…..Rhizopus stolonifer
Tri. viride…….. Trichoderma viride, SEM…… Standard Error of Means

The CF content decreased from 30.00 to 18.00% (40% decreases) after 7 and 14 days with *A. niger* inoculated substrates, while 15.58% (30.00% to 26.00%) and 33.33% (30.00% to 20.00%) decrease were observed after 14 days of fermentation with the inoculum of *R. stolonifer* and *T. viride* respectively. Spores of *A. niger* and *T. viride* shows no significant difference in degrading CF content of the seedhull. The reduction in CF content of all the substrates in this study was due to the the microbes, the lower will the CF composition as observed with the *A. niger* degraded substrates. Theodorous *et al.*, (1989) activities of the fungal enzyme which degraded the non starch polysaccharides. The effects of the microbes include the disruption of the large molecular weight substrates, reduction of viscosity and encapsulation (Campbell *et al.*, 1986). Belewu and Okhawere (1998) working with rice husk observed a decrease from 30% (day 0) to 7.55% (day 40). The higher the hydrolyzing and saccharifying ability of

concluded that variation in the quantity and quality of the hydrolytic enzyme along with other variation among the microbes could affect the values obtained. Iyayi and Aderolu. (2004) reduced the fibre level of agro-industrial waste by between 35% and 40% using *T. viride* for the fermentation

Table 2: Effect of fungi biodegradation at different fermentation periods on acid detergent fibre and nneutral detergent fibre of cowpea seedhull

Organism	DAY 0	DAY 7	DAY 14	SEM
A. niger	53.00^{a} (81.00^{a})	45.00^{bxy} (68.00^{b})	40.00^{b} (62.00^{b})	1.41 (1.4)
Rhiz. stolonifer	53.00^{a} (81.00^{a})	47.00^{abx} (69.00^{b})	43.00^{b} (63.00^{c})	2.16 (1.0)
Tri. Viride	53.00^{a} (81.00^{a})	41.00^{by} (64.00^{b})	44.00^{b} (62.00^{b})	0.81 (1.0)
SEM	1.00 (1.00)	1.29 (1.4)	2.16 (1.4)	

Neutral Detergent fibre values are in the Parenthesis
a.b.c.. means in the same row with different superscript are significantly different ($p<0.05$)
x.y... means in the same column with different superscript are significantly different ($P<0.05$)
A. niger... Aspergillus niger. Rhiz. stolonifer.....Rhizopus stolonifer
Tri. viride........ Trichoderma viride; SEM...... Standard Error of Means

The crude fibre fractions of fungus biodegraded cowpea seedhulls were shown in Tables 1 and 2. The Acid detergent lignin (ADL) (Table 1) of *A. niger* fermented substrate decreased with increase in the period of fermentation. Although, there was no significant ($p>0.05$) difference between day 7 and 14, the ADL content decreased from 11% (day 7) to 9.6% (day 14). The effect of the inoculum of *R. stolonifer* on ADL content revealed a decreased from 13% (day 0) to 8% day (14) which represent 38.46% reduction. This result correlated with the work of Belewu and Banjo. (1999) who observed a decrease of 31.86% and 39.05% in the lignin content when rice husk and Sorghum Stover were degraded with *Pleurotus sojar caju*. The result obtained in this study is justified by the fact that fungus used can produce lignin degrading enzymes such as Lignin peroxidase and Manganese peroxidase (de Vries *et al*., 1997) which are able to cause the reductions observed in the lignin contents. Neutral detergent (NDF) and Acid detergent fibre (ADF) (Table 2) were also observed to decrease with increase in the periods of fermentation. The ADF content decreased significantly from 53% to 40%, 53% to 43% and 53% to 41% in *A. niger*, *R. stolonifer* and *T. viride* fermented substrates respectively. While NDF content also decreased by 23.45%, 22.22% and 23.45% with the inoculums of *A. niger*, *R. stolonifer and T. viride* respectively.

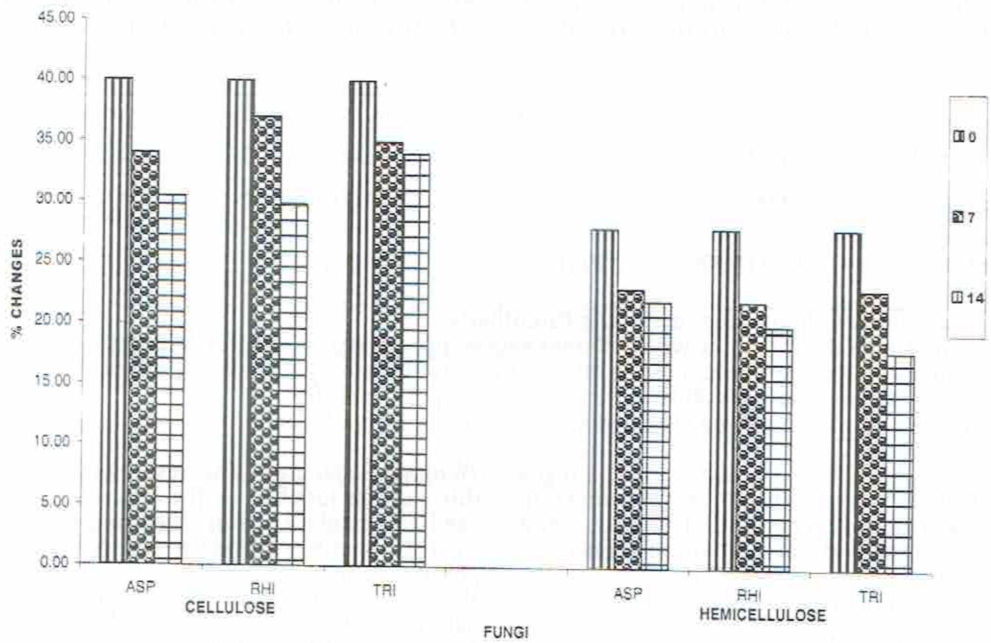

Figure 2: Changes In Cellulose and Hemicellulose Composition Of Cowpea Seedhull After Biodegradation With Fungi At Different Fermentation Periods

Figure 2, shows the variations in hemicellulose and cellulose content of cowpea seedhull as a result of fungus biodegradation. The loss in cellulose content was observed to be more in the *A. niger* degraded substrate, the reduction was from 40% (day 0) to 30.4% (day 14) compared to values observed in *R. stolonifer and T. viride* fermented substrates. This is possible because endoglucanase, cellobiohydrolase, β-glycosidase and exoglucanase which are involved in the biodegrading of cellulose have been identified in aspergilli (de Vries and Visser, 2001) thus loss of 24% of cellulose by spore of *A. niger* compared to 12.50% and 15.00% obtained with spores of *R. stolonifer and T. viride* respectively were observed.

Conclusion

The degradation of fibrous agro-industrial waste like cowpea seedhull by fungi in SSF will serve as a cheap source of nutrients in livestock ration, reduce competition for grains (cereals and pulses) between man and livestock, provide new and additional source of income to agro-processors and reduce environmental pollution hazards due to its disposal.

The decrease in CF components with a simultaneous increase in the proportion of CP suggests that degradation using *Aspergillus niger* in a solid state fermentation (SSF) compared to *Rhizopus stolonifer* and *Trichoderma viride* can improve the nutritional value of cowpea seedhull thus making it useful as monogastric feed ingredient.

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