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Bioconversion of wheat straw by *Lentinus tuber regium* and its potential utilization as food, medicine and animal feed

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ABSTRACT: Bioconversion of wheat straw supplemented or not with ammonium nitrate by *Lentinus tuber regium* (Fr) was investigated by long term solid state fermentation using fungal growth, sclerotium and sporophore production, lignin degradation and the *in vitro* digestibility as indices of bioconversion capability. Wheat straw supported vigorous fungal growth producing sclerotium and fruiting body initials within 50 days. *Lentinus tuber regium* demonstrated high lignolytic capability with high lignin degradation (Org. matter/lignin degradation coefficient = 2). Fungal growth mediated specific physicochemical transformation of straw substrate including degradation of lignin and increase of *in vitro* digestibility of straw substrate, and production of high quality sclerotium and fruiting body. Ammonium nitrate supplement, although, played little or no role in sclerotium and fruiting body initiation, induced very high increase in fungal metabolic rate, sustained greater sclerotium and fruiting body yield, lignin degradation, enhanced lignin degradation coefficient, breakdown of organic matter, and greater efficiency of degraded organics. It however delayed and depressed the increase in the *in vitro* digestibility of straw substrate. Protein analysis of the sclerotium showed high values of protein with a high quality amino acid profile for humans. Regression analysis showed proportionality between a) lignin degradation and the *in vitro* digestibility b) degradation of organic matter and sclerotium/fruiting body production c) lignin degradation and degradation of organic matter. The above underscore the capability of *Lentinus tuber regium* to selectively biodegrade lignin and efficiently mediate conversion of wheat straw to high quality mushroom for humans and, upgraded high quality animal feed.

1 INTRODUCTION

Lentinus tuber regium (syn. *Pleurotus tuber regium)* is an edible tropical fast growing, sclerotium forming, rapid wood degrading fungus, used in Nigeria in various food and medicinal preparations (Okhuoya & Ajerio 1988, Oso 1977). The fungus produces sporosphores and large spherical to ovoid sclerotium, rich in protein and in minerals within dry wood of certain tropical tree species (Zoberi 1972, Kadiri & Fasidi 1990). Both the sclerotium and the sporosphores can serve as food and inoculum in mushroom production. The sclerotium owing to its peculiarity as an organ of survival during drought (Oso 1977, Okhuoya & Ajerio 1988), can serve useful socio-ecological and economical purpose in West Africa. For instance, it can provide much needed food during dry season, which is normally the period of food scarcity in West Africa, particularly so, since it can easily be stored under field conditions.

Presently, however, *Lentinus tuber regium* is a threatened species in West Africa. Increasing deforestation as a result of increasing population pressure threatens the natural habitat of edible mushrooms in West Africa. Most people still depend on collection of wild edible mushrooms, a practice fraught with the danger of collecting poisonous mushrooms (Okhuoya & Okogbo 1990). For the above reasons and in view of rapid agricultural and urban development, which are destroying the natural habitats of these mushrooms, commercial cultivation will not only

ensure the provision of mushroom delicacy regularly but also help in preserving their germplasm.

So far however, relatively few studies have been carried out to elucidate the growth characteristics of edible mushrooms in West Africa, even though cultivable mushrooms abound there (Okhuoya & Etugo 1993). It is therefore urgent and necessary to intensify research on these mushrooms in order to elucidate their growth characteristics, which are indispensable for the development of appropriate technology to optimize their cultivation and industrial commercialization (Braun 1998).

This study examines the bioconversion of wheat straw in solid state fermentation by *Lentinus tuber regium,* using fungal growth, production of sclerotium and sporosphorocarp, lignin degradation, degradation of organic matter and the in vitro digestibility of straw substrate as indices of bioconversion to judge the capability of *Lentinus tuber regium* to convert wheat straw into food for humans and into high quality animal feed.

2 MATERIAL AND METHODS

2.1 *Fungus*

The culture of *Lentinus tuber regium* (syn. *Pleurotus tuber regium)* was obtained from the culture collection of the Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Center, Braunschweig, Germany (Dr. Zadrazil). An inoculum was prepared by growing the fungus for 7 days on malt extract agar at 25°C. Two agar plugs (diameter: 9 mm) were used as inoculum.

2.2 *Wheat Straw Substrate Treatment*

Forty gram aliquots of milled wheat straw *(Triticum sativum)* particle size < 1 mm, mixed with 120 ml of water or of 0.25% ammonium nitrate were sterilized in 500 ml Erlenmeyer flasks for 30 min. at 121°C. The flasks were inoculated with two agar plugs in triplicate and were incubated for 25, 50, 75, 100, 125, 150, 175, 201, 225, 250, 275, 301, and 324 days in the dark at 30°C at constant moisture level. Similarly prepared flasks without fungal inoculum were incubated as control. Fungal mycelial growth and formation of sporocarp and sclerotium were visually monitored during incubation.

2.3 *Analytical Methods*

At the expiration of each incubation period, sclerotia and sporocarps were harvested from the substrate, dried at 105°C and the loss of organic matter was estimated by weighing. The substrates were then milled, and aliquots were taken for analysis. Lignin was analyzed as described by Zadrazil (1977). The percentage loss of lignin was computed according to Zadrazil & Brunnert (1980). The in vitro digestibility was determined by the method of Tilley and Terry (1963), slightly modified as described by Zadrazil (1977). For the determination of the content of water soluble substances 1 g of substrate was milled, added to 50 ml distilled water and heated for 3 h at 80°C. After cooling to room temperature the solution was filled up to 100 ml and filtered. The filtration cake was dried for 17 h at 105°C and weighed. The filtrates were used for the determination of the pH-Value, the sodium and potassium contents by AAS (model ELEX6361, Eppendorf, Germany) and the determination of extractable organic carbon by a Liqui-TOC-Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). The amino acids were determined by oxidizing and non-oxidizing hydrolysis of substrate, centrifugation and chromatographical quantification (Aminosaure-Analysator 6300, Beckmann, Germany).

3 RESULTS

3.1 *Fungal growth on wheat straw and formation of sclerotium, and sporocarp*

Wheat straw treated or not with NH4NO3 similarly supported vigorous mycelial growth, producing small spherical sclerotia and sporocarp initials within 50 days (Fig.l). Although ammonium nitrate supplement played little or no role on mycelial growth, sclerotium and sporocarp initiation, it hastened sclerotium and sporocarp production and induced a high yield. Sclerotium and sporocarp production started after 50 days incubation on ammonium nitrate treated straw (N+ straw) 50 days earlier than on untreated straw (No straw). Within 125 days, NH4NC>3 induced a 10-fold relative increase in sclerotium and sporocarp production. Accordingly yield rose from 3.8% unit (= initial substrate dry weight) on day 50, peaked at 5.5% unit on day 150, maintaining a relatively high productivity, on the average, above 4.6% unit for 225 days. On NO straw, on the other hand, yield rose from 2.8% unit only after 150 days incubation, and then increased at a constant rate, peaked at 5.8% unit on day 301 and remained constant at this level till day 324.

Protein analysis of sclerotium and sporocarp showed high value of protein with high quality amino acid profile for humans (Table 1). Crude protein level was 10%. Valin, isoleucin, phenylalanin and threonin levels far exceeded the normal level requirement, while methionin, leucin, and tyrosin levels fell a little short. Lysin level was about 25% of the required standard for humans.

3.2 *Degradation of organic matter*

Degradation of wheat straw organics proceeded at a relatively constant rate over time on both straw treatments (Fig. 1). Degradation time course was fairly linear on NQ straw. Organic matter degraded at an average rate of 0.165% unit day~l over time, reached 27% unit on day 150 and peaked at 42% on day 324. Ammonium nitrate supplement induced a relative increase of 65% on degradation rate within the first 150 days. Degradation rate was also linear on N+ straw, about 0.273% unit day'l. Accordingly degradation rose to 41% on day 150, peaked at 44% unit on day 275 and then declined thereafter.

3.3 *Lignin degradation*

Lignin degradation (LD) followed a similar trend as organic matter degradation. However, lignin degradation was, 2-fold the organic matter degradation rate and therefore indicating that *Lentinus tuber regium* is selectively lignolytic (Fig. 2). Initially within the first 25 days, lignin degraded similarly on both straw treatments at a rate of 0.56% unit day~l. Rate declined on NQ straw, however, after 50 days over time to 0.25% dayl. Lignin degradation increased accordingly from 14% to 51% unit and 75% unit on day 25, 150, and day 301 respectively. On N+ straw, on the other hand, LD rate increased 2-fold relatively after the initial 25 days. Accordingly LD increased and peaked at 78% on day 150 and declined later thereafter to 60% unit. Lignin degradation time course clearly indicated the beneficial effect of ammonium

Table 1 Comparison of the contents of essential amino acids in sclerotia of *Lentinus tuber—regium* with reference to the essential amino acids for humans.

Essentiell amino acid (EAA)	Amino acid content in Sclerotia (g EAA per kg nutrient-protein)	Demand (g EAA per kg nutient-protein)
Leucin	45	48
Lysin	10	42
Isoleucin	46	42
Valin	53	42
Phenylalanin	147	28
Tyrosin	21	28
Threonin	46	28
Methionin	16	22

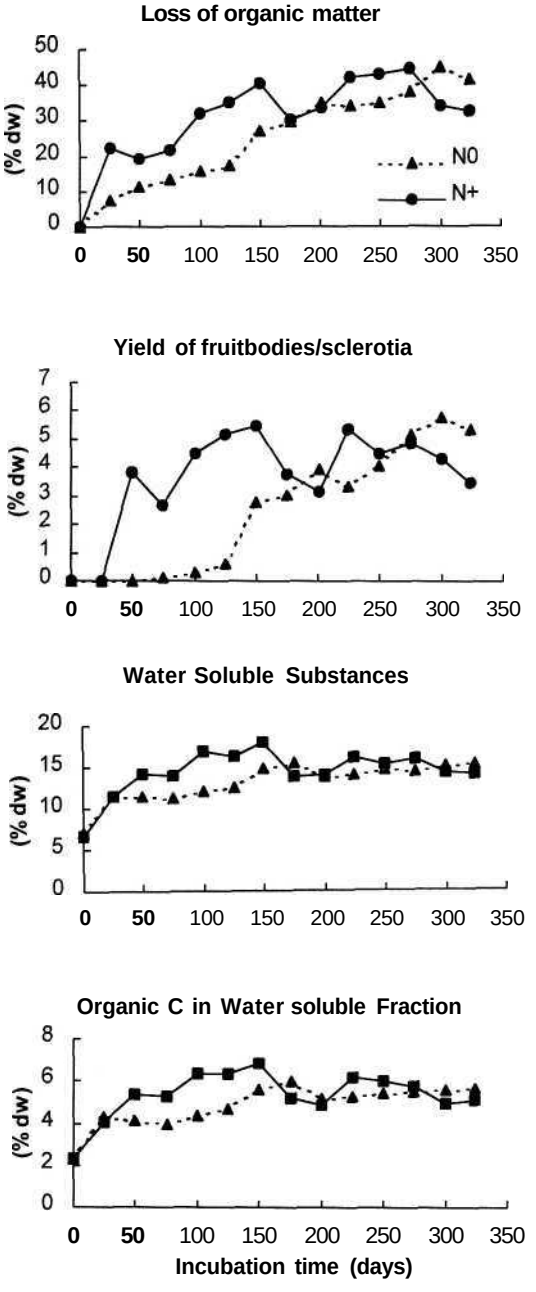

Fig. 1 Loss of organic matter, yield of sclerotia / fruitbodies, release of water soluble substances and organic carbon during SSF of wheat straw for 350 days using *Lentinus tuber regium* without and with supplementation of MfyNC^. Values are given as percentage of initial substrate (dw).

nitrate supplement. Ammonium nitrate induced a 2-fold increase both on fungal lignolytic capacity and on lignin degradation rate.

Table 2. Correlation between different factors influencing solid state fermentation of straw from *Lentinus tuber regium* during incubation. Values given as percentage of initial dry weight.

	y = mx + b	r^
LOM / Fruitbody Sclerotium (No)	5.32x+ 13.49	0.98
LOM / Fruitbody Sclerotium (N+)	5.67x+ 10.06	0.70
Loss of Lignin / LOM (No)	1.65x + 4.01	0.99
LOM / IVD (No)	1.12x+8.13	0.45
Loss of Lignin / IVD (No)	2.91x + 9.93	0.89
Loss of Lignin / Fruitbodies / Sclerotium (No)	0.96x + 24.83	0.96

3.4 *Changes in* in vitro *digestibility (IVD)*

The time course of changes in the in vitro digestibility on both straw treatments differed radically from those of organic matter and lignin degradation (Fig. 2). On NQ straw, IVD increased at a fairly constant rate of 0.134% unit day" 1 over time, peaked at 27% unit on day 175 and remained constant over time till day 324. Ammonium nitrate supplement, on the other hand, reduced and impeded IVD initially. Subsequently, however, it induced a 3-fold relative rate increase of about 0.36% unit dayl from day 50 to day 100, and sustained a fairly high IVD of 25% unit till day 150, and then oscillated thereafter overtime about that level. The time course of IVD on both straw treatment indicated that IVD attained the maximum level 25 days earlier with ammonium nitrate treatment relative to untreated straw. Despite the later induced increases with ammonium nitrate, on the average ammonium nitrate delayed and reduced IVD of straw substrate virtually.

3.5 *Correlation between organic matter degradation, sclerotium andsporocarpproduction, lignin degradation and the in vitro digestibility*

The relationship between organic matter degradation and sclerotium and sporocarp production was highlighted by the regression analysis (Table 2), which indicated direct proportionality between these two parameters and elucidated the difference in time lag of sclerotium production shown in the yield time course with NQ straw and with N+ straw treatment respectively. Fungal degradation of organic matter supplied the essential soluble substances required for fungal metabolism and for the formation/production of sclerotium. Accordingly sclerotium production started at the expiration if the time period needed to release the essential primary substances. Reference to the regression curve (Table 2), 13% unit of organic matter degradation was needed to start sclerotium production on NQ straw, while only 10% unit was needed to start sclerotium production on N+ straw. This indicated that ammonium nitrate supplement increased the efficacy of degraded organics to produce sclerotium. As was reported above, organic matter degradation was 1.65-fold faster on N+ straw. It therefore became evident that ammonium nitrate hastened sclerotium production and increased the efficacy of degraded organics to produce sclerotium, inducing about 20% greater amount of sclerotium per unit organic matter degraded relative to untreated straw.

3.6 *Correlation between organic matter degradation (LOM), lignin degradation (LD),and the in vitro digestibility (IVD)*

Fungal degradation of lignin played major roles in bioconversion of straw. Firstly, lignin degradation facilitated the degradation of other organics encrusted and bound up by lignin; secondly, LD reduced the relative level of indigestible organic matter, thereby increasing IVD relatively; thirdly, LD released soluble substances thereby increasing the IVD absolutely. It is also however, evident that the released soluble substances, the by-products of lignolysis, enhanced sclerotium formation, which in turn, involved transformation of soluble substances into indigestible hyphal and sclerotium saccharides and polysaccharides. This implied a reduction of IVD, some how.

3.7 *Relationship between lignin degradation and organic matter degradation*

The time course of organic matter degradation and of lignin degradation showed similar trend (Fig.l, 2). Regression analysis further indicated the direct proportionality between the two parameters, and elucidated their relationship (Table 2). Both increased linearly over time. However, lignin degradation rate was 2-fold to 1.8-fold the organic matter degradation rate. This further demonstrated the selective lignolytic capacity *ofLentihus tuber regium.* Reference to the regression curves lignin degradation of 4.01% unit and of 3.74% unit were needed to elicite increase in organic matter degradation on NO straw and on N+ straw respectively (Table 2). This implied that NH4NO3 increased the efficacy of lignolysis to induce decomposition of organic matter during fungal fermentation. Lignin encrustation of cellulosics constitute effective barrier to their bioconversion and to their biodegradation. It is only through lignolysis that these organics could become accessible to bioconversion. The relationship between lignin degradation and organic matter degradation can be expressed by the „Lignin Coefficient", which may be defined as the ratio of degraded lignin/initial lignin or degraded organic material.

The greater this lignin coefficient, the less the organic matter loss per unit degraded lignin mass, and consequently the more efficient the lignolytic capacity of the white rot fungus. Lignin coefficient greater than unity implies that quantitatively greater lignin mass relative to other organics is degraded and that the white rot fungus is selectively lignolytic. Bioconversion of straw lignocellulosics into high quality animal feed aims at the least loss of other organics and at greatest lignin degradation. On both straw treatments, *Lentinus tuber regium* exhibited high lignin coefficient, which was greater than 2 at the initial stage of fermentation. Ammonium nitrate induced greater lignin coefficient thereby greater fungal selective lignolytic capacity.

3.8 *Lignin degradation and the* in vitro *digestibility*

The time course of lignin degradation and the in vitro digestibility (IVD) highlighted the relationship between these two parameters. The IVD exhibited 25 day time lag relative to lignin degradation. On both straw treatments, the IVD increased linearly at a rate which is one third that of lignin degradation rate. It then followed, that for optimization of straw substrate for high quality animal feed production, an incubation period of 100 days and 75 days was sufficient with N+ straw and with NO straw respectively. It is therefore evident that NH4NO3 shortened the period needed in order to upgrade wheat straw into high quality animal feed, but reduced by 5% unit the IVD of the straw.

The relationship between lignin degradation and IVD was further elucidated by the regression analysis on table 2 which showed a direct proportionality between the 2 parameters. Reference to regression curve, lignin degradation of 10% unit was needed in order to initiate increases in IVD on both straw treatments. However, in order to produce an increase of, for instance, 5% unit, a further 10% unit of lignin degradation was needed on NQ straw treatment, while 30% unit was needed on N+ straw treatment. Ammonium nitrate therefore reduced by 20% the lignin degradation efficacy to produce increase in the IVD relatively, although the supplement shortened the incubation period required for the attainment of maximum the *in vitro* digestibility.

3.9 *Water soluble substances*

During incubation the content of water soluble substances increased from 7 % to values between 10 and 15 %. The N+ showed a faster increase of release of water soluble substances. After day 175 both NQ and N+ showed nearly the same values. The same trends were determined for the content of organic C in the water soluble fraction. After day 175 values between 4 and 7 % were obtained.

pH-value

The pH-value of sterile substrate was 6.2. This value decreased during the first 25 days to 5.0 and was constant at this level during the following incubation period. Due to the dissoziation of $NH4^+$ the pH was lower in the N+ substrate.

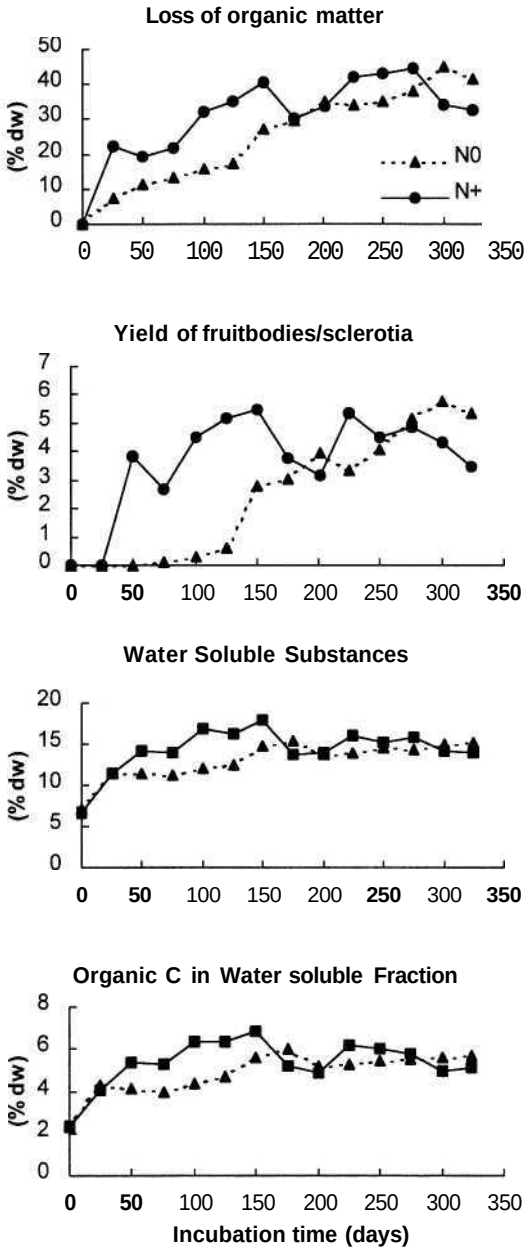

Fig. 2 Lignin degradation, changes of in vitro digestibility and fluctuation of pH value during SSF of wheat straw by *Pleurotus tuber regium* at 25 °C during 350 days.

3.10 *Release of sodium and potassium*

The content of sodium was nearly constant during incubation time at values between 0,5 and 0,8 g / kg dw (Fig.3). In contrast to that the potassium value increased from values of ca. 7 g / kg dw to values of 1.1 (N+) and 1.4 g / kg dw (No).

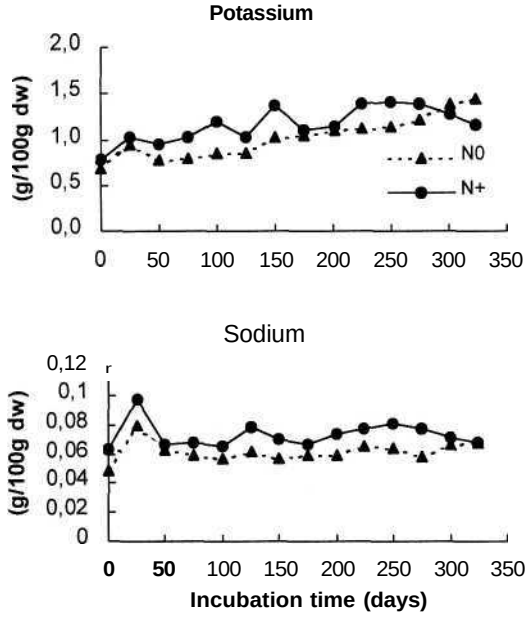

Fig. 3 Release of Potassium and Sodium from Straw Substrate by *Pleurotus tuber regium* during 350 days of SSF fermentation.

4 DISCUSSION

Lentinus tuber regium mediated specific physicochemical transformation of wheat straw including degradation of lignin, increase in the in vitro digestibility of straw substrate, production of high quality sclerotium. Despite fast and vigorous mycelial growth and timely production of sclerotium and sporocarp initials, production of matured sporocarp and sclerotium started relatively late: after 150 days on NQ straw, compared to 30 days period observed with *Lentinus tuber regium* on rice straw, maize straw and Andropogon straw during similar solid state fermentation (Fasidi & Ekuere 1993). Ammoniun nitrate supplement hastened up production to 50 days, suggesting that too high C/N might be the constraint. Simultaneous production of sclerotium and sporocarps seems to be unique to wheat straw substrate among other agricultural wastes tested under solid state fermentation, where only sporocarps at a time were produced. Oso (1977) and Okhuoya (1993) following comparative nutritional investigations on sclerotium and sporocarps observed that sclerotium was not a storage organ but constituted an essential means of survival in dry season, and so was probably produced because of drought environmental stress. In this study, however, fermentation was carried out at high relative humidity and moisture level. Simultaneous production of sclerotium and sporocarp could be an asset, however, in commercial mushroom enterprise, particularly in West Africa, since sclerotium can be easily harvested, preserved and transported at room temperature, far more so than the sporocarp. Besides simultaneous production of the two different mushroom products with the same input would imply diversity of production. This could be also commercially desirable. Nevertheless this simultaneous production of sclerotium and sporocarp should be a subject for which future studies are needed in order to harness the resources of wheat straw in fungal fermentation.

Protein analysis of sclerotium and sporocarps showed high value of protein with high quality amino acid profile for humans. The particularly high level of phenylalanin- about 5 times greater than the required standard level for humans, seems to suggest that *Lentinus tuber regium* could constitute a valuable source of this essential amino acid.

The vigorous mycelial growth which mediated specific physiochemical transformation of wheat straw, particularly degradation of lignin and increase in the in vitro digestibility of the

straw could be attributed to the fungal highly selective lignolytic capacity prominently greatest at the initial fermentation stage, exhibiting a lignin coefficient greater than 2. The highly selective lignolytic capacity of *Lentinus tuber regium*,- a valuable asset in environmental management could also be even harnessed to address the biodetoxiflcation of recalcitrant aromatic pollutants, particularly the PAHs which exhibit similar molecular structure with lignin. For other white rot fungi the potential of degradation of organic pollutants is already known (Bumpus et al., 1985, Wolter et al., 1997).

The spectacular fungal induced increase in the in vitro digestibility of wheat straw indicated the potential resourcefulness of *Lentinus tuber regium* to upgrade straw into high quality animal feed. This potential is particularly needed in order to upgrade the abundantly produced straw wastes into valuable animal feed in humid tropics, and enhance production of high quality ruminant animal protein.

The ammonium nitrate beneficial effect was seemingly not linked with increase in mycelial production or the production of sporocarp and sclerotium initials, since both treated and untreated straw equally supported vigorous mycelial growth, producing sclerotium and sporocarp initials within 50 days incubation period. The beneficial effect could be attributed to the lowered C/N in treated straw which hastened fungal lignolysis and thereby further orchestrated the reduction of C/N, to NH4NO3 enhanced lignin and other organics biodegradation and also to the ammonium nitrate induced increase in the efficacy of degraded lignin and other by-products to produce greater sclerotium per unit degraded lignin mass. This same effect might have increased the fungal lignin coefficient. Domsch & Zadrazil (1982), observed that release of CC>2 during decomposition of organics reduced the substrate C/N. Fasidi & Olorunmaiye (1994) observed that C/N ratios of 4:1 and 1:4 were optimal for fungal mushroom production.

The delay and the depressive effect of NFfyNC^ on the in vitro digestibility despite its above mentioned beneficial contribution could be attributed to the NH4NC>3 enhanced production of sclerotium and sporocarp saccharides and polysaccharides which were produced from the by products of lignolysis. These saccharides are not readily digestible. *Zadrazil &* Brunnert (1980), also observed a reduction in the in vitro digestibility of wheat straw treated with NH4NC>3 supplement during solid state fermentation with *Pleurotus eryngii, Lentinus edodes* and *Pleurotus salmoneo stramineus.*

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