FUNGAL-DEGRADED MAIZE BY-PRODUCTS AS FEED FOR WEST AFRICAN DWARF RAM

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ABSTRACT

Maize by-products are potential feed resources for ruminants if properly harnessed. Their uses are however limited by high fibre content and low digestibility which can be enhanced by fungal degradation. Information on the use of fungal-degradation of maize by-products as feed for ram is scanty. The nutritive value of biodegraded maize by-products in West African Dwarf (WAD) ram was therefore assessed. Maize Cob (MC), Maize Husk, Maize Stover, and Maize Straw were degraded for 40 days using four different fungi: *Pleurotus sajor-caju, Pleurotus pulmonarius, Lentinus subnudus, and Pleurotus tuber-regium*. The substrates were analyzed for changes in the proximate composition and fibre fractions. In-Vitro Gas Production (IVGP) was used to predict the Metabolizable Energy (ME), Organic Matter Digestibility (OMD) and Short Chain Fatty Acids (SCFA). The MC and Pt were thereafter selected for *in-vivo* studies using twenty rams allotted to five groups of four rams per treatment in a completely randomized design. Each group was fed of the diets in which MC (g/100g) treated with Pt replaced wheat offal at 0(A), 25 (B), 50 (C), 75 (D) and 100 (E) as supplement to basal *Panicum maximum* in an experiment lasting 114 days. Parameters measured were Voluntary Dry Matter Intake (VDMI), Average Daily Weight Gain (ADWG), Feed Conversion Ratio (FCR), ruminal-pH, Total Volatile Fatty Acids (TVFA) and Ammonia-Nitrogen (NH_3-N). The rams were sacrificed and data were obtained on Hot Carcass Weight (HCW) and Rib-Eye-Area (REA). Data collected were analyzed using ANOVA ($p = 0.05$). Fungal-degradation increased the crude protein from 6.8 to 9.4% while crude fibre, acid detergent fibre and acid detergent lignin were significantly reduced from 29.5 to 14.5% to 14.5, 49.9 to 40.7% and 15.8 to 11.2% respectively in MC. The IVGP, OMD, ME and SCF also improved significantly from 8.3 to 32.8ml, 38 to 52.6%, 5.2 to 7.7MJ/kg DM and 0.4 to 0.7µM respectively. Fungal- degradation significantly improved the VDMI (g) which increased consistently from 676.8 in rams on control diet to 709.4 for rams on diet E. The ADWG (g/d) recorded for rams ranged from 67.7 for rams on control diet to 88.8 for animals on diets E. The FCR and ruminat- pH for rams on diets A (9.99, 6.76), B (8.83, 6.74), C (8.34, 6.17), D (8.28, 6.70) and E (7.99, 6.45) were significantly lower compared with those on control diet. The TVFA (meq/L) increased from 10.1 to 12.8 in

diets A to E. The NH_3-N (mg/mL) also increased from 18.2 to 26.4 in diet A to E. Treatment effects on REA were significantly higher for those on bio-degraded diets compared with the control diet. The HCW (Kg) were 9.6, 12.0, 10.5, 10.2 and 10.7 for rams on treatments A, B, C, D and E respectively and was improved by fungal-degradation. Inclusion of maize cob treated with *Pleurotus tuber-regium* in the diet of rams improved the voluntary feed intake, digestibility, hot carcass weight and rib-eye-area. Biodegraded maize cob completely replaced wheat offal in the diet of West African Dwarf rams.

Keywords: Fungal-degradation, Maize Cob, WAD rams, Rib-eye-area
Word count: 494

CERTIFICATION

I certify that this study was carried out under my supervision by Abayomi AKINFEMI in the Department of Animal Science, University of Ibadan, Ibadan.

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DEDICATION

TO GOD ALMIGHTY

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

Among the constraint facing livestock production in developing countries, poor animal nutrition and productivity arising from inadequate feed supplies, stands at the fore (Williams *et al.*, 1995). Availability of pasture, legumes and browse plants is believed to be dependent on the season of the year. Most ruminant livestock, especially cattle, sheep and goats obtain most of their nutrients from herbages, growing on poor soils. These herbages often grow fibrous and are of low nutritive value and digestibility, resulting in low productivity of animals. They gain weight slowly in the rainy season and lose it rapidly in the dry season (Babayemi and Bamikole, 2006).

Quality of forage and supply vary greatly with seasons (Steel, 1996). At the onset of dry season, grasses become scarce. They become abundant during the raining season. The animals gain weight appreciably but shed it quickly in the dry season (Babayemi and Bamikole, 2006). Grass is complete in nutrients but may be low in crude protein and high in crude fibre as to seriously support ruminants (Bamikole *et al.*, 2004c). The nutritional quality of forages declines with advancing age due to lignification (Mako, 2009). Ruminants cannot meet the nutrient requirement for growth, maintenance and production when they are totally reared on grass alone (Adegbola, 1985).

The increase in human population coupled with shrinking land area is causing apparent shortage of essential food items (Makkar, 1994). A constant threat to human survival today is the remarkable difference between the rate of livestock production and growth of human population (Atinmo, 1993). Without exemption, Nigeria, like most other nations of the world, especially the developing countries, is faced with competition between man and his livestock for available feed resources.Cereals like maize, guinea corn constitute the bulk of the components of livestock rations as sources of dietary energy. They constitute part of staple food of man. The need therefore, to increase feed supply and quality through an improved production and utilization of pasture and fodder shrubs is imminent (Mako, 2009). Makkar (1994) reported that a large area of the land is degraded, and so limit available space for herbage production. The remaining little land for herbage establishment and management involves some expenditure in the form of labour, and

other inputs. It is therefore worthy of note that planted pastures would be of limited application to the small livestock farmer sector because of the huge capital requirements and management (Mako, 2009).

Growing concern about this problem has prompted animal production scientists to search for ways to promote more efficient utilization of available feed resources. Crop residues – the fibrous by-products which result from the cultivation of cereals, pulses, oil palm and tubers – represent important feed resources for animal production in developing countries. They are important adjuncts to natural pastures and planted forages, and are often used to fill feed gaps during period of acute shortage of other feed resources (Williams *et al.*, 1976). Nevertheless, very little efforts have been directed to improving animal production by feeding crop residues in communal areas by extension workers.They should translate research findings into the language understood by farmers. Nevertheless, there is still a perception that the potential of crop residues as livestock feed has not been fully exploited. This is partly due to the fact that crop residues have low nutritive value, such as its content of metabolizable energy and crude protein. Consequently, many governments in both developed and developing countries have launched research programmes to improve the nutritive value and utilization of crop residues. Emphasis in much of this work has been on improving crop residue intake and digestibility by ruminants through upgrading and/or supplementation (Sundstol and Owen, 1984; Doyle *et al*., 1986; Little and Said, 1987; Kiran Singh and Schiere, 1993).

A huge tonnage of maize residues and by-product is produced annually in Nigeria (Isikhuemhen *et al*., 1996). A large percentage of this is set on fire while a fraction is utilized as animal feed, and on many occasions, left in the farm to rot. Digestibility of lignocellulosics (fibrous crop residues) is known to be correlated with lignin content (Isikhuemhen *et al*., 1996). In order to improve the digestibility of lignocellulosics, physical, chemical and biological methods of delignification can be used (Baker *et al.*, 1975 and Jackson, 1978). White-rot fungi, such as edible mushrooms, can degrade fibrous crop residues and by-products, whereby, not only the digestibility of lignocellulose but the nutritional value, also increases.

Edible mushrooms are able to bioconvert a wide variety of lignocellulosic material due to the secretion of extra cellular enzymes (Chang and Buswell, 1996 and Rajarathman *et al.*, 1998). Evidence (Belewu and Belewu, 2005) showed that the bioconverted materials have higher content of protein and a decrease in fiber and can be used as ruminant feed supplement (Mahrous, 2005).

The natural microbial delignification of wood dust (Zadrazil *et al*., 1990) testifies to the great potential of white-rot fungi (edible mushroom) in degrading lignocelluloses, leading to enhance utilization of animal feed. The incorporation of fungal treated wastes along with other feeds in the diet of small ruminants can offset the dry season shortages of pastures.

1. 1 OBJECTIVES OF THE STUDY

The broad objective of this study is to increase the nutritive value of maize residues and by-products through biodegradation with edible fungi (mushroom).

The specific objectives were to determine:

a. Changes in nutrient value of maize residue and by-products after fungal treatment.
b. *In vitro* gas production of fungal treated maize residue and by-products.
c. Performance of West African Dwarf rams fed the treated maize by-product basal diet in terms of nutrient intake, nitrogen utilization and carcass quality.

1.2 JUSTIFICATION OF THE STUDY

1. Maize residues and by-products are available in Nigeria in large quantities
2. There is the need to reduce environmental pollution associated with burning of maize residues and by-products.
2. Maize residues could be used as a source of lignocellulosic biomas for animals if treated with fungi
3. Encouraging the use of locally available fungi rather than synthetic enzymes which are more expensive and require storage conditions with short shelf life
4. Improved performance of ruminants resulting from biodegradation of maize residues

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Voluntary Intake

The digestibility of a feed is most accurately defined as the proportion which is not excreted in the feaces and which therefore is assumed to be absorbed by animal (McDonald *et al.*, 1988). Digestibility is affected by the chemical composition and stage of maturity of forage and also by processing and chemical treatments. Voluntary feed intake and digestibility of energy decreases as crude protein content of forages decreases.

Goat utilizes high fiber feeds more than sheep (Adebowale, 1983). Goat required minimum fiber content in its feed for efficient utilization of nutrient. When crude fibre content of feed dropped from 15 to 5%, digestibility dry matter and crude protein intake, digestibility, nitrogen utilization and live-weight gain declined despite an increase in a more nutritious and acceptable concentrate supplement (Adebowale, 1983). Increasing crude fibre content in feed did not affect dry matter intake in goats whereas crude fibre level greater or equal to 15% in feed reduced dry matter intake in sheep (Adebowale and Ademosun, 1981).

In a digestibility trial, the feed under investigation is given to the animal in known amount and the output of faeces measured. It should be thoroughly mixed before hand to obtain uniform composition. It is then given to the animal for at least a week before collection of faeces begins in order to accustom the animals to the diet and to clear from the tract the residues of previous feeds. This preliminary period is followed by a period when feed intake and faecal output are recorded. It is highly desirable that diet should be given at the same time each day and that the amount of feed should not vary from day to day (McDonald *et al.*, 1988).

Ruminants have a four compartment stomach. The rumen is the largest compartment, where millions of bacteria grow under anaerobic (low oxygen) conditions. These bacteria are responsible for the digestion of fiber (cellulose) and are the reason why ruminant can consume a wide variety of by-product feedstuffs derived from the processing of plants for human food. Livestock industries in the developed countries utilize the majority of these

highly fibrous by-products by including them in feed for cattle, sheep and goats (McDonald *et al.*, 1988).. The nutritive value, or energy content, of an animal affects intake, or how much the animal will eat. Digestibility and intake, in turn, determine the feed's productive performance, such as to support milk synthesis or muscle growth. However, studies with live animals (*in vivo*) to determine the digestibility of feeds are time consuming, laborious, expensive and require large quantities of feed. Such experiments are not suited for the rapid and routine feed evaluation undertaken by commercial laboratories that provide feed information to livestock producers and feed manufacturers.

2.2 The Rumen Environment

The rumen environment appears to be controlled by the type and quantity of feeds eaten. There is periodic mixing through contraction of the rumen with rumination, diffusion or secretion into the rumen, absorption of nutrients from the rumen and passage of material down the digestive tract (Preston and Leng, 1987). Under abnormal circumstances, the rumen environment is drastically disorganized. Suddenly, introduction of diet not included into the feed offered like grain, lacticacidaemia may occur (Preston and Leng, 1987). This could be due to a drop in ruminal pH, growth of *Streptococcus bovis* and the accumulation of lactic acid. Saliva help to maintain fluid state of the rumen environment and so facilitate access of micro-organisms to the plant materials. The quatity of saliva secreted by ruminants depends on the diet.

Saliva, a buffered solution of about pH 8, contains high concentration of sodium and phosphate ions. Both the saliva and bicarbonate movement across the rumen epithelium maintained the pH within narrow limits (Preston and Leng, 1987).

The buffered rumen liquor favored the growth of anaerobic bacteria, fungi and protozoa with accumulation of VFA's in the fluid (up to 0.2molar). For continuous fermentation however, the ruminal pH must be constantly maintained at neutral level and to ensure VFAs absorption. The biomass of microbes in the rumen is also maintained at a constant level by the passage of microbes down the digestive. Methane and carbon dioxide are produced as the end products of fermentation. At low rumen pH, carbodioxide comes out of solution and accumulates in a pocket of dorsal sac. Methane and carbon dioxide are

largely eliminated by eructation (Dougherty *et al.,* 1964). At high pH most of the carbon dioxide produced by fermentation or entering the saliva, is absorbed and excreted via the lungs (Preston and Leng, 1987).

2.3 Importance of rumen microbes

1. They provide enzymes that can digest fibre component of feed whereas animals do not produce such enzymes.
2. They can utilize simple forms of nitrogen such as urea to synthesize their cellular proteins. This reduces the dependence of ruminant on high quality dietary proteins.
3. They synthesise the B-complex vitamins as a result the animal is not fully dependent upon dietary proteins.

2.4 Rumen Microbial Ecosystem

The microbial ecosystem in the rumen is complex and highly dependent on diet. The vast majority of ruminant consume a mixture of carbohydrates of which cellulose and hemicelluloses are the largest components. However, at times the diet can contain large amounts of soluble carbohydrate or starch (e.g. molasses or grain).

Plants have developed molecular structure in their cell walls specifically to deter invasion by micro-organisms. In the rumen, the main agents that breakdown carbohydrate are anaerobic bacteria, protozoa and fungi. The anaerobic bacteria are the principal agents for fermenting plant cell-wall carbohydrates but the anaerobic phycomycetous fungi, (Bauchop, 1981) may at times be extremely important. There is close relationship between fungi and other microbes in the rumen since the fungi appear to be the first organism to invade plant cell wall, which allows bacteria fermentation to start and to continue (Preston and Leng, 1987). Some bacteria in the rumen assume a symtopic association, where one organism uses the products of fermentation of another and the removal of the end-product allows further fermentation of the primary feeds source by the first organism (Preston and Leng, 1987).

2.5 Role of ammonia in rumen fermentation

Between 40 and 60% of the dry matter content of microbial cells are protein, and therefore the synthesis of amino acid and proteins are the reactions that require ATP. The pathways of synthesis of amino acids in rumen microbes are not clearly defined. It is however; abundantly clear that ammonia-N is highly important for the efficient synthesis of amino acids and therefore microbial protein (Satter and Slyter, 1974). At low ammonia level in rumen fluid, reactions that fix ammonia into acids require ATP, whereas, when ammonia level is high above a certain optimum, the ammonia is incorporated into amino acids without using ATP (Satter and Slyter, 1974).

It has been suggested (Satter and Slyter, 1974) that maximum microbial synthesis rate occured at ammonia-N concentrations between 5 and 8 mg N/ml. Different options have been found by other researchers, suggesting that diet influences the optimum ammonia level. Another study (Schaefar *et al.*, 1980) suggests the value may be as high as 14 mg N/100 ml depending on diet. The high ammonia concentration needed for maximum cell growth suggests that the rumen micro-organisms probably have similar mechanism for incorporation of ammonia via glutamate dehydrogenase.

2.6 Volatile fatty acids

Volatile fatty acids (VFAs) are the end products of fermentations of carbohydrate by micro organisms. The predominant VFAs in the rumen fluid are acetic, propionic and butyric acid with isobutyric, isovaleric, valeric and other acids generally present in small amount. The rate and extent to which these acids are produced is indicative of microbial activity in the rumen. Bergman (1990) reported that the concentration of individual VFAs is related to the nature of the feed. Similarly, Firkins *et al.*, (1986); Robinson *et al.*, (1986) reported that the amount of VFA produced depend on the extent (effective degradability) of the feed ingested.

2.7 Methane production from feeds

Ruminants depend on micro-organisms to digest and ferment plant cell wall and polysaccharides into energy sources, such as volatile fatty acids (VFAs) and other organic acids. This is an energetically wasteful process since it resulten in the portion of

the animal feed being converted to CH_4 which is eructated as gas. Approximately 6% of dietary gross intake energy is lost to the atmosphere as CH_4 (Holter and Young, 1992; De Ramus *et al.,* 2003). Emission of CH_4 and other volatile organic compounds from ruminants, and their effect on air quality attracted the attention of air regulatory agencies in many parts of the world. Methane is perceived as contributing to climate change and global warming (Johnson and Johnson, 1995). It traps outgoing terrestrial infrared radiation 20 times more effectively than CO_2, which leads to increased surface temperature.This and it directly affects atmospheric oxidation reactions that produce CO_2 in animal agriculture.

There may be a potential for reducing the extent of CH_4 production by manipulating the diet and management practice that influence ruminant microbial fermentation (Johnson and Johnson, 1995). Environmental pollution from dairy farms can be caused by overfeeding and/or poor synchronization of release of nutrients in the rumen. An attempt therefore, has been made to manipulate rumen fermentation using ionosphores, fats and yeast cultures (Johnson and Johnson, 1995). For example, addition of monensin to dairy cattle rations decreased CH_4 production, decreased feed intake and increased milk yield (Saur *et al.,* 1998), suggesting that reduction in CH_4 production per unit of ingested feed is associated with improvement of feed utilization efficiency. A suppressing influence of ration fat content on CH_4 production has been reported (Saur *et al.,* 1998). It is not only the total amount of fat, but also its composition that exerts biologically important influences on rumen fermentation (Getachew *et al.,* 2001 and Fievez *et al.,* 2003). Getachew *et al.,* 2005 observed differences in methane produced from incubation of commercial total mixed rations (TMR) for lactating cow. The proportion of CH_4 in total gas did not differ among TMR at 6 and 24 hr of incubations but differences did not occur at 48 and 78 hr giving an average of 33.8ml CH_4/gDM was produced at 24 hr of incubation. Approximately 0.80 of total CH_4 was produced during the first 24 hrs of fermentation.

2.8 *In vitro* gas production method of assessing ruminant feeds

An animal feed intake, and how well the feed is digested, reflect the feed's production performance. The *in vitro* gas production technique is relatively simple method for

evaluating feeds, as large numbers of samples can be incubated and analyzed at the same time. This method has been applied successfully for a variety of purposes, feed evaluation, including calculating organic matter digestibility, the metabolizable energy of feds and kinetics of their fermentation: determining how feed value is affected by added fat, antinutritive factors and rumen modifiers; quantifying the energy value of feed mixtures (rations); monitoring microbial changes in the rumen; synchronizing nutrient digestion and selecting forage nutrient targets for agricultural biotechnology. More than half of the nutrient consumed by ruminant animals leave the animal unutilized and undigested, and are excreted in faeces, urine and gases. The *in vitro* gas production method can be used to examine animal waste components that impact the environment and develop appropriate mitigations.

The digestibility of feeds can also be estimated by biological methods known as *in vitro* techniques, which are conducted outside of the animal system but simulate the digestion process. Generally, *in vitro* techniques are those based on measuring either fermentation residues or products. The former measures the unfermented residue remaining after *in vitro* incubation of a feed with rumen fluid. This approach involves collecting fluid from the rumen of a ruminant that is fitted with a permanent cannula. This method for forage evaluation was first reported in by Tilley and Terry, (1963), using ruminal fluid obtained from sheep with a rumen fistula. A rumen fistula is formed by surgically transecting the skin and the rumen, suturing the rumen to the skin and allowing the rumen to heal, creating a permanent opening into the rumen. The cannula fits in the fistula to close the rumen in order to collect the fluid containing bacteria needed for the *in vitro* incubations.

Materials not recovered in the residue following incubation are assumed to be fermented, providing estimates of the extent of digestion for various feeds. More recent methods (Getachew *et al*., 2000) measure the products of anaerobic fermentation. Rumen fermentation by anaerobic microbes results in production of short chain fatty acid (SCFA), gases [carbon dioxide (CO_4) and methane (CH_4)] and microbial mass. The amount of gas produced is proportional to acid production; thereby serving as an indicator of acids produced by fermentation (Getachew *et al*., 2000). The amount of gas produced during incubation is measured to predict the extent and rate of feed digestion.

In addition to quantifying the chemical composition of feeds, some commercial laboratories in the developed countries offer *in vitro* feed digestibility as a component of their feed analytical packages. These data can be used in new ration-evaluation computer models with the goal of optimizing nutrient utilization and animal production performance, thereby minimizing the environmental impacts of nutrient excretion in the animal's urine and faeces.

Gas measuring technique has been routine in feed evaluation (Getachew *et al*., 2001), when a high correlation was found between metabolizable energy (ME) measured in live animals and that production from gas production. The *in vitro* gas technique has several advantages over other *in vitro* method that is based on measuring residues. Gas production reflects all nutrients fermented. Soluble as well as insoluble and fractions that are not fermentable do not contribute to gas production.

Furthermore, the kinetics of fermentation can be obtained from a single incubation, allowing the rate of fermentation to be calculated. Gas measurement is a direct measure of microbial activity and can be a better index of forage ME content than an indirect *in vitro* measure, based on nutrients fermented. The gas technique is relatively simple and does not require sophisticated equipment, making it easy to conduct for research and commercial purposes (Getachew *et al*., 2000). Rumen fluid is collected from a cow with a rumen fistula. Fermentation are conducted in large (100 milliliter, ml) calibrated glass syringes in an anaerobic medium inoculated with rumen fluid. Incubations can be carried out either in an incubator with a rotating disc or in a thermostatically controlled water bath (102^0F). The volume of gas produced in 24 hrs from incubating 200 milligrams (mg) of feed, together with the concentration of crude protein and crude fat, is used to predict ME. Large numbers of samples can be analyzed during a single 24 hour incubation run.

In Department of Animal Science University of Ibadan, three non-lactating West African dwarf female goats are used as donor animals.

The *in vitro* gas method has been applied successfully in many developing countries for a variety of purposes in feed evaluation.

2.9 Estimation of gas production parameters

Gas volume produced *in vitro* experiments can be used in estimating organic matter digestibility, metabolisable energy and kinetics of fermentation

2.9.1 Organic Matter Digestibility

The digestibility of measured organic matter is closely correlated with that predicted from gas production and the crude protein and ash contents of feeds. Therefore, the method can be used to predict the extent of digestion for various feeds.

2.9.2 Energy content of feeds

The gas method has also been used to predict the ME content of feeds. A model equation has been developed with data generated by *in vivo* studies conducted with a variety of feeds and *in vitro* gas production. The gas measurement provides a better estimate of the ME level of feeds, when combined with some chemical constituents, compared with calculations based on chemical constituents only. Recently, seven laboratories around the world that use a gas method- including UC Davis carried out a comparative test to assess the reputability of the technique in predicting the energy value of feeds, and found that the gas method was repeatable among laboratories (Getachew *et al.*, 2002).

2.9.3 Kinetics of Fermentation

In assessing nutritive value, the rate at which a feed or its chemical constituents are digested in the rumen is as important as the extent of digestion. The pattern of feed fermentation (Kinetics of fermentation) is one of several factors that influence voluntary feed intake by ruminants. The rate at which different chemical constituents are fermented is a reflection of microbial growth and accessibility of the feed to microbial enzymes. By describing gas production mathematically, kinetic data can be analyzed to evaluate substrate and media-related differences as well as the ferment ability of soluble and slowly fermentable component of feeds. The gas method is an ideal technique to generate kinetics of fermentation, as it allows recording of gas produced at several times in the incubation period, which is used to predict the rate at which feed is digested.

The gas method has been used to evaluate the effects of grain processing on the rate and extent of gas production (De Peters *et al.*, 2003).

2.10 Effects of added fat on feed degradation

Fallow and Yellow grease (YG), both rendering by-products, are typical fat used in the diets of lactating dairy cows. The gas technique was used at to examine the effect of source and levels of added fat on gas production and rumen fermentation of a total mixed ration (Getachew *et al.*, 2000). Fatty acids in the form of triglyceride (YG) has no effect (when comprising up to 25% of the diet) on gas production, but fatty acids in the form of potassium salts (YG soap) significantly depressed gas production in the animal. However, there is a limit to the amount of fatty acids that can be successfully fed, and this is lower than *in vitro*. The fatty acids in potassium salts are quickly available to microbes as free fatty acids in ruminal fluid, and have detrimental effects on microbial growth. In contrast, the fatty acids in the triglyceride form must be released through hydrolysis of the ester bond and therefore are available at a slower rate. Hydrolysis refers to breaking the chemical bond between the individual fatty acid and the glycerol backbone of the triglyceride. The effects of fatty acids on rumen fermentation are important because feeds with high levels of residual fat, for example rice bran created in the production of white rice are commonly fed to ruminants.

2.11 Antinutritive factors

The gas method can be used to measure how microbial activity lowers feed digestibility. Some feeds, such as forage legumes and cotton seed, contain phenolics, alkaloids and saponins that have negative biological effects on microbes and reduce microbial growth in rumen. Tannins are naturally occurring polyphenolic compounds found in plants, which form complexes with feed and microbial proteins and can depress feed digestibility in the rumen. The effect of tannins on the nutritive value of feds can be studied using tannin-binding agents, such as polyethylene glycol (PEG), which strongly binds the tannins and inhibits their biological effects. The present increase in gas production when PEG is present indicates the rate at which tannins depress rumen fermentation of feeds.

After adding PEG to limit tannin effects, gas production increased by 22%, 71% and 211% in apple ring acacia (*Acacia albida*), beach acacia (*Acacia cyanophylla)* and red calliandra (*Calliandra thyrsus)* respectively, which are browse plants (Getachew *et al.*, 2000).

2.12 Rumen modifiers

The gas method is also utilized to study feed additive and rumen fermentation modifiers such as monensin sodium, by incubating feed in the presence or absence of these compounds. Rumen modifiers are compounds that are added to the diet to modify the populations of bacteria in the rumen. For example, some compounds are fed to reduce methanogenic bacteria to reduce methane production in the rumen. Previous studies have shown that the addition of saponins and tannins in an *in vitro* system increases microbial protein synthesis (Makkar *et al.*, 1999). Yeast and yeast fermentation products are routinely added to the diets of lactating dairy cows although their mode of action has not been clearly identified. By studying the impact of various rumen modifiers on microbial fermentation, effects important to milk production in commercial dairy farms can be quantified feed associative effects.

The *in vitro* gas production method is currently being used to assess "associative" effects of feeds used in rations. Rations are mixture of individual feeds, with a multitude of possible combinations. The energy value of a ration is generally calculated by adding up the energy values of the individual feeds in the ration, on the assumption that the individual energy value of any particular feed is the same in every possible combination with other feeds (Makkar *et al.*, 1995). However, this is not always true. For example when poor-quality forage such as wheat straw – is fed to a ruminants, its digestibility is low, but adding nitrogen in the form of urea or protein, the digestibility of the straw will be increased and in turn, the energy derived from straw organic matter in the diet will be increased. Recent studies indicate that positive associative effects on *in vitro* gas production occurred when rice straw was incubated in mixtures with hay or mulberry leaves (Liu *et al.*, 2002).

2.13 Monitoring Rumen Microbial Change

In addition to rate and extent of digestion, the gas production method can be used to study substrate related factors that influence microbial population in the rumen. This enables manipulation of microflora to increase the utilization of feeds through degradation of fiber and lignin, increasing the efficiency of nitrogen utilization or allowing the degradation of antinutritional and toxic components of feeds. Such controlled fermentation system could potentially be used with genetic engineering of plants to solve animal productivity problems. The technique is suitable for application of molecular bases assays, such as polymerise chain reaction (PCR) and ribonucleic acid (RNA) – targeted oligonucleotide probes, to study and measure rumen microbial growth, with the goal of increasing the efficient utilization of feeds and reducing environmental impacts. Recently, Muetzel and Becker (2003) used the gas technique, in combination with ribosomel RNA targeted probes to measure the efficiency of microbial growth when barley straw was supplemented with legume leaves.

Nutrient synchronization carbohydrate and nitrogen sources must be available simultaneously in order to maximize microbial growth. Ruminal ammonia concentrations can be influenced by the degradation rates of carbohydrates and nitrogen-containing compounds. For a given level of dietary protein, an increased rate of protein degradation enhances the runimal ammonia concentration while an increased rate of carbohydrate degradation decreases. Increased carbohydrate availability for fermentation promotes microbial growth and as a result less nitrogen is lost from the rumen in the form of ammonia-nitrogen (Getachew *et al.,* 2000). The gas method offers an opportunity to study microbial requirement for nitrogen and carbohydrate to enable efficient fermentation activity and accumulation in the rumen. Using this technique, studies have been conducted to assess rumen microbial requirements for nitrogen when different types of carbohydrate sources are incubated.

2.14 Environmental degradation

More than half of the nutrients consumed by ruminants leave the animal unutilized and undigested, and are excreted in faeces, urine and gases. This increases animal production costs as well as environmental impacts, by contaminating surface and ground water and

contributing to air pollution. The nitrogenous and organic compounds excreted are further decomposed and can cause odors in residential areas. Increasing the efficiency of feed utilization reduces the amount of utilized nutrients leaving the animal. Significant reductions in nitrogenous compounds (Kuelling *et al.*, 2003) and in methane production (Johnson and Johnson, 1995) can be achieved by manipulating animal diets. The *in vitro* gas method can be used to study the efficiency of feed utilization and to examine animal waste components that impact the environment in order to develop appropriate mitigation strategies.

2.15 Animal factors on microbial fiber digestion

Animal and feeding systems can have a significant effect on the digestion of fibre. Notably, intake, dietary interactions, feeding strategies and feed additives will, to some degree, influence microbial growth and subsequent fiber digestion.

2.15.1 Intake

The extent of fibre digestion is the result of competition between the rates of digestion and passage and, as such, is not a static value. Rumen liquid and particulate turnover rates are positively correlated with intake. Thus, as intake increases, the digest flowing from the rumen will contain feed particles at earlier stages of digestion, and this will result in a lower dry matter digestibility (Russell *et al.*, 1992). Because the rate of degradation of structural carbohydrate is of the same order as passage rate, at high levels of intake the depression in digestibility of structural carbohydrate can be two to three times greater than that of the faster degrading, nonstructural carbohydrate. Although a high level of intake may depress ruminal fibre digestion, compensation occurs through increases in gross energy intake and hindgut digestion (Bourquin *et al.*, 1990).

2.15.2 Composition of dietary fiber

Rumen available energy normally limits growth of bacteria, and any additional organic matter fermented in the rumen as a result of changing the forage: concentrate ratio will probably increase microbial protein synthesis by providing more energy. (Sniffen *et al.*, 1992) suggested that the yield of bacteria was maximized with a forage content of 70% in the diet dry matter. Because structural carbohydrate-fermenting microbes are usually

limited by a ruminal pH less than 6 (Hoover, 1986), the depression in fiber digestibility at higher inclusion rates of concentrate can most likely be explained by the rapid degradation of nonstructural carbohydrate. It is likely that fiber digestion will not be maximized at single forage: concentrate ratio; rather, it will depend on the various rates of digestion of structural and nonstructural carbohydrate supplied by the forage and the concentrate. This may be shown indirectly by the studies of Tamminga (1981), who reported no relationship between forage: concentrate ratio and bacterial yield.

2.15.3 Particle size chemical and biological treatments

Although physical processing of forages by grinding and pelleting does provide a greater surface area for attack by enzymes, utilization of structural carbohydrate is not increased; rather, improvements in animal performance arise primarily from an increased digestible energy intake (Bourquin *et al.,* 1990). In fact, fiber digestibility is reduced by 3.3% as a result of reduced residency time in the rumen. Chemical treatments such as sodium hydroxide, potassium hydroxide and ammonia will partially solubilize hemicellulose and lignin, as well as hydrolyze acetic, phenolic and ureic acid esters. Oxidative treatment of forage with sulfur dioxide or peroxide results in the degradation of lignin and extensive solubilization of structural carbohydrate (Fahey *et al.,* 1993). Potential improvements in fiber digestion could result from the use of alkaline hydrogen peroxide, which is a combined hydrolytic and oxidative process. The use of white-rot fungi for converting lignocellulosic materials to more digestible feedstuffs for ruminants has also been intensively investigated. *In vitro* dry matter digestibility was increased 30% and 13% for rice straw leaf and stem, respectively (Karunanandaa *et al.,* 1995). Fungal treatment enhanced digestion of the mesophyll tissue and improved access for ruminal microorganisms by collapsing the vascular bundles.

2.15.4 Effective fiber

Recent use of the term effective fiber (eNDF) acknowledges the different functionality of dietary fiber. Milk fat, chewing rate, and particle size have all been used as an index of effective fiber. Currently, the Cornell Net Carbohydrate and Protein System (CNCPS) uses eNDF to adjust ruminal pH and passage rate (Sniffen *et al.,* 1992). Factors other than particle size that influence eNDF include the degree of lignifications of the fiber,

degree of hydration, and bulk density. The importance of eNDF can be seen in the reduced growth rate of structural carbohydrate-fermentating microorganisms and the reduction in total microbial yield when ruminal pH is lower than 6.2 (this being related to a dietary eNDF of 20%). Research to further quantify the value of eNDF for a range of feeds is much needed.

2.15.5 Feeding strategies

Robinson (1989) indicated that fiber digestion may be limited by the order and frequency of substrate presentation to the rumen. A total mixed diet provides an optimal balance of nutrients to the microorganisms, thereby stabilizing fermentation. The potential to modify the ruminal environment is perhaps greater when separate, twice-daily feeding of forage and concentrate is practiced. Feeding diets, especially those that are highly fermentable, more frequently than twice a day is generally thought to stabilize the ruminal environment.

This reduction in diurnal variation of fermentation end products, in conjunction with improved coupling of protein and energy, and energy release in the rumen, can increase the rate of fiber digestion.

2.15.6 Additives

The addition of buffers and alkalinizing products (sodium bicarbonate, sodium sesquicarbonate, magnesium oxide, sodium bentonite) to the diet of lactating diary cows can improve fiber digestion by reducing the period of time during the day that ruminal pH is less than 6. A buffer may overcome limitations to fiber digestion in diets that have a high proportion of low pH silages, fermented feeds with a moisture content greater than 50%, an acid detergent fiber < 19%, finely chopped haylage, a high proportion of concentrate, irregular feeding of high levels of concentrate, or finely ground concentrate (Hutjens, 1992). The ionophore monensin can improve cellulose digestion of diets high in readily available pH (Russell *et al.*, 1992). Ruminal fill and rate of passage are also influenced. Other ionosphores reported to produce similar results include lasalocid, salinomycin, lysocellin, narasin and tetronasin (Wallace, 1994). Further research is required to determine if these function as an antibacterial or an antifungal agent in the

rumen and if the efficiency responses in the animal race are a result of ruminal or post-ruminal effects.

Yeast culture and their extracts, particularly *Aspergillus oryzae* and *Saccharomyces cereviciae*, have a highly variable effect on animal performance and efficiency.

Directly fed microbials, or probiotics, are organisms with the ability to maintain a bacterial balance in the host animals' digestive tract during stressful or disease situations. It is currently thought that these additives remove oxygen from the ruminal environment, thereby increasing bacteria viability, and result in pH stability and increased rate (but not extent) of cellulolysis (Wallace, 1994). The further development of strains of probiotics to stimulate the growth of specific types of ruminal bacteria may result in diet-specific additives. Recently, the use of extracellular enzymes that have been protected from the digestive process has been proposed as a method to improve fiber digestion (Wallace, 1994).

2.16 Agricultural by-products and their characteristics

Only a part of agricultural products can be utilized by man himself. The amount of by-products for feeding farm animals can be considerable. There is a considerable variation in quantities and qualities of by-products between crops, influenced by species, varieties, climate, season, region and stage at harvest. The most important parts of roughage are the aerial parts (stems, leaves). These can be utilized fresh or dry, cut or grazed, in the field or in the stable/barn.

Human does consume considerable amounts of crop by-products. These by-products, which contain high amount of fibrous material, are not easy to be disposed because it may cause environmental pollution. Also by-products usually contain quite high amount of fibrous substances and with increasing commodity yields, the amount of crop residues will certainly increase.

Statistic on production and utilization of fibrous residues in Nigeria are inadequate. However, the production of roughage could be fairly estimated accurately from crop production, if reliable data are available.

Low quality roughage is found in poor range grazing lands. It also include enormous amount of cereal crop residues such as rice straw, wheat straw, bean straw, maize stover, corn cobs and rice hulls. These by-products are all characterized by their high fiber contents. Analysis of roughages by detergent procedures (Goering and Van Soest, 1970) showed that they are high in lignin, cellulose and hemicelluloses.

Also must cereal waste characterized as low crude protein, low available energy and deficient in certain minerals. These low quality roughages are inefficiently utilized by ruminants. This is due to low digestibility and poor nutritive value associated particularly with cereal straw. Their utilization is also limited because of low voluntary intake of the animals and their huge bulk, which makes transportation more costly. These materials supply no more energy than poor quality hay, TDN is less than 50% and starch value (SV) is less than 29% (Balch, 1976).

The chemical composition of roughages varies with the variety of plant (Kharat, 1974 and, Salem and Jackson, 1975), location (Van Soest, 1988) and agricultural practices employed in the growing of the crop and handling of the residue from which they are obtained. Chahal (1985) referred those differences between crop residues and wood residues to be due to their chemical composition. He found that crop residues contain 30-40% cellulose, 16-27% hemicelluloses, 3-13% lignin, and 3.6-7.2% crude protein, while the wood residues contain 45-56% cellulose, 10-25% hemicelluloses and 18-30% lignin.

2.16.1 Determining nutritive value

The nutrient composition of feeds is commonly determined primarily by chemical analyses. However, this does not provide sufficient information to determine the feed true nutritive value. The efficiency by which an animal utilizes feed nutrients has significant impact in its productive performance and waste production.

The *in vitro* gas production system helps to better quantity nutrient utilization and its accuracy in describing digestibility in animal has been validated in numerous experiments. Animal experiments will continue to add information to our understanding of nutrient metabolism. However, where applicable the *in vitro* gas production system can be used to predict animal performance at a much lower cost.

Based on the strong relationship between measured digestibility and that predicted from gas production, regression equation has been developed and the method has been standardized. In addition, the method can evaluate the impact of biotechnological changes in plants on their nutritive value and other factors that affect rumen fermentation.

2.16.2 Chemical composition of low quality roughages

They are characterized by:

- High level of crude fibre i.e. high lingo-cellulose content (lignin, cellulose and hemicellulose).
- Low nitrogen content i.e. low in crude protein.
- Low in some minerals like calcium and phosphorus.
- Low in nitrogen free extracts (soluble carbohydrates)
- Some antinutritional factors such as tannins, phytic acids etc.

2.16.3 Utilization by ruminants

Their utilization is limited by the above listed characteristics and also the following

- low voluntary intake and digestibility by animals
- Bulkiness which makes their transportation costly.

2.16.4 Improvement of their nutritive value

This means increasing their utilization i.e.

- Increase quality
- Increase intake by animals
- Increase animal production (meat, milk and wool)

2.16.5 Biological treatment to up-grade feeding value of the roughages

The use of various chemical and biochemical methods has been advocated for many years (Beckham, 1922; Kaufmann *et al.*, 1978; Kristensen *et al.*, 1978 and Anon 1984) to enhance digestibility and nutritional qualities of straws. However, the use of chemicals can be tedious and costly, and further treatments to eliminate side effects of the chemicals can make the process uneconomic.

Thus, Han (1978); Han and Anderson (1975); Zadrazil (1977); (1978); Kahlon *et al.*, (1990) tried microbiological methods for improving the nutrition quality of plant residues. The feed produced by this way was better than that produced by chemical methods and no side effects were noticed on the cattle.

The new developments in biotechnology in the field of animal production involve at least four major areas:

1. Improvement of livestock species through recombinant DNA technology and gene transfer technology.
2. The management of animal health and welfare.
3. Improvement of crop residues to be used as feeds for animal production and upgrading of feedstuffs.
4. Prospects for manipulation of animal physiology and biochemistry (Teller and Vanbelle, 1993).

2.17 Improving utilization of poor quality roughages

In recent years, considerable attention has been given to improve utilization of low quality roughages through mechanical, chemical and biological treatments. The importance of such improvements in animal feeding system warrants even more attention in the developing countries (Wanapat, 1981).

2.18 Degradation of lignocelluloses

Lignocellulose consists of lignin, hemicellulose and cellulose. Because of the difficulty in dissolving lignin without destroying it and some of its subunits, its exact chemical structure is difficult to ascertain (Howard *et al.*, 2003). In general, lignin contains three aromatic alcohols (Coniferyl alcohol, Sinapyl and P- Coumaryl). Lignin is further linked to both hemicellulose and cellulose forming a physical seal around the latter two components that is an impenetrable barrier preventing penetration of solution and enzymes.

Of the three components, lignin is the most recalcitrant to degradation whereas cellulose, because of its highly ordered crystalline structure, is more resistant to hydrolysis than hemicellulose. Alkaline (Chahal, 1992) and acid (Nguyen 1993) hydrolysis methods have

been used to degrade lignocellulose. Weak acids tend to remove lignin but result in poor hydrolysis of cellulose whereas strong acid treatment occurs under relatively extreme corrosive conditions of high temperature and pH which necessitate the use of expensive equipment. Also, unspecific side reactions occurred which yield non-specific, by-products other than glucose, promote glucose degradation and therefore reduce its yield.

Some of the unspecific products can be deleterious to subsequent fermentation unless removed. There are also environmental concerns associated with the disposal of spent acid and alkaline. For many processes enzymes are preferred to acid or alkaline since they are specific biocatalysts, can operate under much milder reaction conditions, do not produce undesirable products and are environmentally friendly.

In a recent review, Malherbe and Cloete, (2003) reiterated that the primary objective of lignocellulose potential of the cellulose is encrusted by lignin within the lignocelulose matrix. They expressed the opinion that a combination of solid state fermentation (SSF) technology with the ability of an appropriate fungus to selectively degrade lignin will make possible industrial scale implementation of lignocellulose-based biotechnologies.

2.19 Crop residues

Crop residues are materials, which are generated after the crop has been harvested while agro-industrial by-products are derived from the processing of a particular crop or animal product usually by an agricultural firm (Dixon and Egan, 1987). The nutritional composition and nature of crop residues produced depends on the amount and types of crops grown in that area.

Research to date has concentrated on determining biological value of residues as they occur rather than on methods of increasing their value. Attempts to improve the nutritional quality of fibrous residues have been confined mainly to physical treatments such as grinding. There is a need to explore and, where possible, apply other methods of treatment if their potential value as animal feed is to be fully realized.

2.20 Concept of upgrading agricultural wastes

Approximately 2 billion tons of cereal grains and 140 million tons of legume and oil seeds are produced throughout the world each year, which yield an estimated 230 million tons of fibrous material as part of a variety of by-products (Choct, 1998). In legumes, NSP also play a role as an energy storage material. The role of fibre in monogastric diets has attracted much attention in recent year due to the fact that the soluble NSP elicit anti-nutritive effects and the utilization of NSP as a feed material in monogastric is very poor. More efficient utilization of potentially utilizable nutrients for food production is therefore of paramount importance to sustainability of agriculture in the future.

Longe (1988) and Dierick, (1989) advocated the increased utilization of non-convectional feed resources in non-ruminant feed. They suggested processing techniques, which are simple and inexpensive, and do not significantly increase costs but still make it worthwhile in term of nutrient availability.

Attempts to modify their nutritive value have involved alteration of the physical structure as well as modifications of the lingo-cellulose complex through the following treatments

- Biological treatment
- Physical treatment
- Chemical treatment

2.20.1 Biological treatment

The possibility of biological methods of agricultural wastes treatment has a great appeal as an alternative to the use of expensive (in terms of money and energy) chemicals, pollution would also be reduced. It must be remembered, however, that whatever organism is grown on the straw must obtain its energy from the straw itself. Organisms which degrade cellulose and hemicellulose are of no use since they only deplete the straw of nutrients which the ruminant itself can digest even without any treatment.

Successful biological treatment must be based upon the use of organism which degrades lignin. While there is no organism that degrades only lignin, there are some, notably the *white-rot* fungi, which degrade more lignin than they do cellulose, thus leaving a residue with a lower percentage of lignin that the original material.

Lee *et al.*, (1983) reported a significant effect of enzyme activity on the degree of polymerization of crystalline are peeled of progressively. The new cellulose chains exposed at the adsorption of enzyme in the early reaction period is restricted by the structural limitation; more enzyme molecules can be adsorbed as more new surfaces are exposed in parallel with the progress of hydrolysis of crystalline cellulose.

White-rot fungi and basidomycetes fungi are probably the most efficient terrestrial microorganisms capable of utilizing all of the polymers of lignocelluloses residue, which is not available for fungal growth in their macromolecular form (Kirk, 1983). Wood (1995) reported that a rate of both hydrolytic and oxidizing reaction are excited into the lignocelluloses substrate, acting the polymerize (the lignocelluloses polymers) into compounds of lower molecular weight which can be assimilated by the fungus.

Also Kirk (1975) added that, *white-rot* fungi decompose wood by converting the lignin eventually to CO_2 and H_2O. The *white-rot* fungi and basidomycetes fungi are able to produce a range of lignocelullolytic enzyme when grown on various suitable media (Augusto and Vassili, 1972; Paice *et al.*, 1978, Erikson, 1981; Fan *et al.*, 1982 and Priest, 1983).

Various works on pretreatment mechanism ranging from delignification, saccharrification, irradiation with high electron, subdivision into micro size particles and steeping in alkali which provide enhanced utilization of food carbohydrate by bacterial enzymes have been reviewed (Millet *et al.*, 1970**,** Benvink and Mulder, 1989).
Biological treatments include the use of microbial proteins, antibiotics, probiotics, enzymes, and ensiling etcetera. These constitute the most recent methods of enrichment of non digestible feedstuffs or those imbued with the well known anti-nutrients. Dierick (1989), emphasized that polyphenols such as tannins are not removed by physical or chemical treatments but by fermentation or germination. Even, the nutritive value of maize in form of lysine and tryptophan contents leading to improvement in biological value and utilizable protein was achieved through germination, (Ram *et al.*, 1979). Besides ensiling, the most recent additive for improving silage quality is the biological aid. This involves microbial inoculants and cellulolytic enzymes with easy and safer

handling and application to its credit. It is neither volatile nor corrosive, and is aimed at breaking down cell walls to provide a wealth of readily available substrates (Dutton, 1987). Cowan *et al.,* (1996) advocated that addition of enzymes to feed ingredients results in an improved energy availability that reduces the difference between gross and metabolisable energy of raw material. The level of improvement seen is related to energy type and to dosage and correlated well with the substrate specificity of the various enzymes present. The microbial enzyme sources, which account for 90% of commercial enzymes, use around the world (Underkoffler, 1972) is more advantageous than the commercially prepared enzymes. The following advantages are found in the microbial enzymes;

i. Microbial enzymes do not compete for glandular tissues of animals with other more expensive products made from a limited supply of the same glandular tissues.

ii. There is scanty of non microbial sources.

iii. There is irregularity and non predictability of supplies from non-microbial sources which may be subjected to seasonal, climatic and other agriculturally related uncontrollable variables

iv. Production from non-microbial sources cannot be expended at will in response to increased demand. Microorganisms both aerobic and anaerobic are able to produce extracellular enzymes to degrade macromolecules like starch, cellulose, hemicellulose, lignin and pectin of the plant cell (Priest, 1984) as well as proteins and other membrane constituents.

It has been reported that the solid state fermentation (SSF) is an alternative process to produce fungal microbal enzymes using lignocellulose materials from agricultural wastes due to its low capital investment and lower operating cost (Haltrich *et al.,* 1996; Jecu, 2000)

2.20.2 Physical treatment

In smallholder livestock systems most physical treatments of crop residues are either too expensive, the equipment is not available or too labour intensive. However, there are

benefits in reducing particle size (not necessarily grinding), for ensiling and in stall-feeding. Reduction of particle size can be achieved by using a power driven chopper.

There are other advantages, in that the surface area of non-lignin material exposed to microbial attack increased the rate of digestion, thereby reducing a possible limitation to intake (Van Soest, 1982**).**

2.20.3 Chemical treatment

The potential for increasing digestibility and intake of fibrous residues through treatment with alkali has been widely researched and comprehensively reviewed (Sundstol and Owen, 1984). Urea treatment is of most practical significance in the tropics, acting both as an alkali and a source of supplementary nitrogen (N) to materials inherently low in crude protein.

Treatment procedure will vary according to circumstances. Smith *et al.,* (1989) found that five percent urea in solution, added at a rate of at least 20%, weight for weight, solution to dry stover, followed by an incubation period of five weeks gave the greatest improvement. The stover had been rotor slashed before treatment. Urea treatment is relatively easy to apply and is effective. However, its uptake at small holder farm level has been slow. Cost is often cited as a reason for this. Cost of urea prohibits adequate usage and there is environmental concern over disposal of spent urea.

2.21 Ecological Background for Cultivation of White Rot Fungi in Solid State Fermentation

2.21.1 Influence of fungal species

Ligninolytic microorganisms are mainly wood inhabiting fungi which are able to colonize different plant residues and increase the digestibility of the substrate. The influence of fugal species on the decomposition of wheat straw and the *in vitro* digestibility and decomposition of lignin has been comprehensively discussed by Zadrazil (1985). The physiological behaviour of these fungi with respect to lignocellulose degradation can be used to divide them into groups, a, b, c and d. Fungi in the first group, (a), decompose the substrate without lignin degradation (*brown-rot* fungi). The *in vitro* digestibility in this case is negative in comparison to untreated straw. Examples are:

Agrocybe aegerita and *Flammulina velutipes.* Similar results can be obtained by the cultivation of lower fungi, bacteria or yeasts on cereal straw.

The second group, (b), includes fungi, which decompose the lignin well, but other substrate components are decomposed only partially. The *in vitro* digestibility in this case increases. Examples are: *Dichomitus squalens, Abortiporus biennis, Stropharia rugosoannulata, Pleurotus eryngii* and *P. sajor caju.*

The third group, (c), includes fungi which decompose lignin and other substrate components, but then *in vitro* digestibility decreases. This may be due to toxicity of substrate extracts for the rumen microorganisms used for determining digestibility.

Fourth group, (d), consists of fungi which decompose lignin and other components very rapidly and change the in vitro digestibility only partially. An example is *Sporotrichum pulverulentum* which decomposes approximately 3% of the organic matter daily but causes no significant change in digestibility of cereal straw (Zadrazil and Brunnert, 1982; Zadrazil, 1985).

Selection of proper fungi for the biodegradation is an important step towards an effective utilization of SSF technology. An ideal fungus should have high saprophytic and colonization ability and selective lignolytic activity. It should be non-pathogenic and non-toxic to animals and human beings. It must be genetically stable. Among the promising white rot fungi found for improving the degradability or in vitro digestibility of wheat straw are strains of *Cyathus stercoreus, Dichomitus squalens, Pleurotus spp, Phlebia radiate and Pycnoporus cinnnabarinus* (Zadrazil and Brunnert, 1982).

2.21.2 Influence of substrate composition and quality

Different substrates, including beech sawdust, rice husks, rape, reed and sunflower, have been tested with different fungi for the decomposition of organic matter, lignin decomposition and *in vitro* digestibility (Zadrazil, 1980). Each substrate was subjected to 60 days of SSF by each fungus. All these factors were strongly dependent on fungal species and the kind of plant waste substrate. *Pleurotus spp., Dichomitus squalens, Abortiporus biennis* and *Stropharia rugosoannulata* showed good lignin decomposition

and increased *in vitro* digestibility of all substrates except rice husks. *Agrocybe aegerita*, *Flammulina velutipes, Volvariella volvaceae* and other *brown-rot* fungi decomposed the modified lignin only to a small extent and decreased the *in-vitro* digestibility. However, rice husk treatment with *white-rot* fungi did not improve its digestibility; this effect is probably caused by the high incrustation of rice husks with SiO_2.

2.21.3 Influence of fermentation temperature

The temperature of fermentation influences not only the decomposition rate of the organic matter but also the sequence of decomposition of the substrate components. With all the fungi studied, the raising of temperature between 22^0 and 30^0C increased the decomposition rate of the organic matter (Zadrazil, 1977; Moo Young *et al.*, 1978). Large differences in the rates of straw decomposition caused by the temperature were observed for *Pleurotus cornicopiae* and *Stropharia rugosonnulata.* A positive correlation between increase of temperature and lignin decomposition or *in vitro* digestibility was only visible for *Stropharia rugosoannulata.*

2.21.4 Influence of fermentation period

The growth and life cycles of fungi in substrate can be distinguished into the periods of colonization of the substrate, maturation of fungus, induction of fruiting bodies and autolysis. During the first stage of fungal growth and colonization of the substrate, the content of the digestible substances for ruminants decreases (Zadrazil, 1977). Immediately after colonization, the in vitro digestibility of fungal substrate increases, but it decreases thereafter in old substrate which have a relatively high content of accumulated minerals. Under favourable conditions, some fungi can totally mineralize cereal straw during a 80-100 day period of fermentation (Zadrazil, 1985).

2.21.5 Influence of nitrogen supplementation

The supplementation of substrate with NH_4NO_3 has been found to change the decomposition rate and the sequence of decomposition of substrate components. *Stropharia rugosoannulata, Agrocybe aegerita* and *Pleurotus* sp. *Florida* were stimulated during the decomposition of substrate by the addition of low concentration of NH_4NO_3 concentration (Zadrazil and Brunnert, 1982). A decrease in *in vitro* digestibility of

substrate mixture was observed in all the fungi when the substrate received higher concentrations of NH_4NO_3.

2.21.6 Influence of the ratio of liquid to gaseous phase of substrate

With increasing water content and constant substrate volume, the air content of the substrate decreases. This results in increased water tension and swelling of the substrate. All the fungi investigated have shown good growth on substrates with varying water contents (from 25 to 15 mL water/25g straw). With both the lowest and the highest water contents, the decomposition rate of the total organic matter has been found to decrease. Similarly, the decomposition of lignin and accumulation of digestible substances also decrease. The fungi have shown specific growth optima for various air and water contents of the substrate (Zadrazil and Brunnert, 1982).

2.21.7 Influence of composition of gas in the gaseous phase

The losses of organic matter and lignin after fermentation of wheat straw with *Pleurotus sajor caju* have been found to be highest in the 100% oxygen atmosphere (Kamra and Zadrazil, 1986), followed by those in air in the case of *P. eryngii*. Carbondioxide at 1-20% in the atmosphere influences neither organic matter loss nor lignin degradation, but at 30% concentration the organic matter loss slightly increases and lignin degradation is considerably decreased. Lignin is degraded at a much lower rate with less than 20% oxygen in the atmosphere. The increase in the *in vitro* digestibility is highest in pure oxygen, followed by that in the air. Carbondioxide at 1-10% positively influences the increase in digestibility, but at a higher concentration the digestibility is reduced (Kamra and Zadrazil, 1986).

Gaseous metabolites of fungal degradation of straw have strong influence on mineralization of organic matter, loss of lignin and the in vitro digestibility. For large scale process the composition of gaseous phase proposed to be the key-factor. Influence on the composition of substrate after different treatments with gaseous metabolites is reported in the literature (Buta *et al.*, 1989; Chiavari *et al.*, 1988).

2.22 Solid state fermentation

Aerobic microbial transformation of solid materials or "Solid Substrate Fermentation" (SSF) can be defined as the application of living organisms and their component to industrial products and the process not an industrial in itself, but an improvement technology that will have a large impact on many different sector (Hamlyn, 1998). Aderolu (2000) considered SSF as a process in which solid substrate are decomposed by known mono or mixed cultures of micro organisms under controlled environmental conditions, with the aim of producing high quality product. The substrate is characterized by relatively low water content (Zadrazil *et al*., 1990).

It has been reported that solid state fermentation (SSF) is an attractive alternative process to produce fungal microbial enzymes using lignocellulosic materials from agricultural wastes due to its lower capital investment and lower operating cost (Haltrich *et al.*, 1996; Jecu, 2000). SSF process, for the reasons stated, will be ideal for developing countries. Solid-state fermentations are characterized by the complete or almost complete absence of free liquid. Water, which is essential for microbial activities, is present in an absorbed or in complexed-form within the solid matrix and the substrate (Cannel and Moo-Young, 1980). These cultivation conditions are especially suitable for the growth of fungi, known to grow at relatively low water activities. As the microorganisms in SSF grow under conditions closer to their natural habitats, they are more capable of producing enzymes and metabolites which will not be produced or will be produced only in low yield in submerge conditions (Jecu, 2000). SSFs are practical for complex substrates including agricultural, forestry and food-processing residues and wastes which are used as carbon sources for the production of lignocellulolytic enzymes (Haltrich *et al.*, 1996). Compared with the two-stage hydrolysis-fermentation process during ethanol production from lignocellulosics, Sun and Cheng (2002), reported that SSF has the following advantages: (1) increase in hydrolysis rate by conversion of sugars that inhibit the enzyme (cellulase) activity; (2) lower enzyme requirement; (3) higher product yield; (4) lower requirement for sterile conditions since glucose is removed immediately and ethanol is produced; (5) shorter process time; and (6) less reactor volume. Malherbe and Cloete, 2003 reiterated that the primary objective of lignocellulose treatment by the various industries is to access the potential of the cellulose encrusted by lignin within the lignocellulose matrix.

They expressed the opinion that a combination of SSF technology with the ability of an appropriate fungus to selectively degrade lignin will make possible industrial-scale implementation of lignocellulose-based biotechnologies. New applications of SSF have been suggested for the production of antibiotics (Barrios-Gonzalez *et al*., 1994), secondaries metabolites (Trejo-Hernandez *et al*., 1992, 1993) or enriched foodstuffs (Senez *et al.*, 1980). SSF is a batch process using natural heterogeneous materials (Raimbault, 1998 and Tengerdy, 1985), containing complex polymers like lignin (Agosin *et al*, 1989), pectin (Kumar, 1987**;** Oriol, *et al.*, 1988) and lignocellulose (Roussos, 1985). SSF has been focused mainly to the production of feed, hydrolytic enzymes, organic acids, gibberelins, flavours and biopesticides.

Bacteria, yeasts and fungi can grow on solid substrates, and find application in SSF processes. Bacteria's are mainly involved in composting, ensiling and some other food processes (Doelle *et al*., 1992). Yeasts can be used for ethanol and food or feed production (Saucedo-Castañeda *et al*., 1992a, 1992b). But filamentous fungi are the most important group of microorganisms used in SSF process owing to their physiological, enzymological and biochemical properties. The hyphal mode of fungal growth and their good tolerance to low water activity (A_w) and high osmotic pressure conditions make fungi efficient and competitive in natural microflora for bioconversion of solid substrates (Raimbault,1998).

Microorganisms are currently the primary source of industrial enzymes: 50% originates from fungi and yeast, 35% from bacteria, while the remaining15 % is either from plant and animal origin. Microbial enzymes are either produced through submerged fermentation (SMF), or solid substrate fermentation (SSF) techniques. According to the Central Food Technological Research Institute (CFTRI) in India, enzymes production by SSF accomplishes high productivity per unit volume of fermented space than SMF technique. Processing waste such as soybean hulls and Cassava peels (Ofuya and Nwajuba, 1990) has been upgraded through production of enzymes by SSF technique. The work of authors like Iyayi and Losel (2001), Belewu and Banjo, (1999), Iyayi and Aderolu (2004), Yang *et al.*, (1980), Onilude (1994), Balagopalan (1996), among others clearly showed the use of microorganism for upgrading lignocelluloses into animal feeds.

Like all technologies, SSF has its disadvantages and these have received the attention by Mudgett (1986). Problems commonly associated with SSF are heat buildup, bacteria contamination, scale-up, biomass growth estimation and control of substrate content.

2.23 Cultivation of *white-rot* fungi in solid state fermentation

A huge tonnage of agricultural wastes is produced annually in the world. A large percentage of this is burnt, especially in developing countries (Fasidi and Ekuere, 1993), while a fraction is utilized as animal feed. Digestibility of lignocellulosics has been known to be correlated with lignin content. In order to improve the digestibility of lignocellulosics, physical, chemical and biological method of delignification can be used (Jackson, 1978). *White-rot* fungi can degrade lignin selectively (Zadrazil, 1985), whereby not only the digestibility of lignocellulose but the nutritional value also increase.

The natural microbial delignification of wood reported in literature (Phillipi, 1893; Knoche *et al.*, 1929; Zadrazil *et al.*, 1982) testifies the great potential of *white-rot* fungi in upgrading of lignocellulosics. Various *white-rot* fungi have been studied for the selective delignification of agricultural wastes as an alternative to chemical and physical pretreatments (Kirk and Moore, 1972; Zadrazil, 1980; Zadrazil and Brunnert, 1981).

The major problems of biological upgrading of lignocellulose include finding suitable organisms (whose metabolic patterns differ from those of rumen flora and fauna), favourable cultivation conditions and cheap large-scale process. The proposed process, therefore, should be simple and implemented by low-cost technology. Solid state fermentation (SSF has been known for centuries and used successfully for food process in different countries (Reade and Gregory, 1975; Zadrazil, 1977; Moo-Young *et al.*, 1979; Kahlon *et al.*, 1990; Bau *et al.*, 1994). Solid state fermentation of lignocellulosics leading to the production of animal feed, human food and spent compost for soil remendiation is economical and can be practiced particularly in developing countries. In this article, fungal activities on lignocellulosics and biological possibilities of upgrading them to animal feed using SSF technology are presented.

2.24 The non starch polysaccharides and plant cell wall constituents

The carbohydrates which include the low molecular weight (LMW) sugar, starch and various cell wall and storage non-starch polysaccharides (NS) are the most important energy sources for non-ruminant and ruminant animals (Bach Knudsen, 1997). The NSP and lignin are the principal components of cell wall and are commonly referred to as dietary fibre. (Theander *et al.*, 1993, 1994). Dusterhoft *et al.*, (1991) observed that the intracellular NSPs such as fructan and mannans may also be constituents of some plant materials.

The plant cell walls consist of a series of polysaccharides often associated and /or substituted with protein and phenolic compounds in some cells together with the phenolic polymer lignin (Selvendran 1984, Theander *et al.*, 1993). Bach Knudsen (1997) reported the building blocks of the cell wall polysaccharide to include: pentose; arabinose and xylose, the hexoses; glucose, galactose and mannose, the 6 deoxyhexoses; rhamnose and fucose and the uronic acid, glucoronic and galacturonic acids. The main polysaccharides of plant cell wall according to Selvendran (1984), and Theander *et al.*, (1993) are cellulose, arabinoxylans, mixed linked (1-3, 1-4) D-glucan (β-glucans), Xyloglucan, Xylan, rhamnogalcturonans and arabinogalactan.

Among the numerous types of cell wall polymer (Chesson, 1995), five major classes of fibre can be selected according to their chemical structure and their properties: 4 classes of water insoluble polymer (Lignin, cellulose, hemicellulose, pectic substances) and one class of various water soluble NSP and oligosaccharide.

Oldale (1996), concluded that NSP of plant cell wall-cellulose, hemicellulose, pectin, lignin and protein, are also found in the plant cell wall, however, the author observed that the NSP fraction accounts for 70-95% of the cell wall.

2.24.1 Lignin

Lignin can be described as a branched network of phenylpropane units (Bach Knudsen, 1997). Selvendran (1984) described lignin as an amorphous high molecular weight, aromatic polymer composed of phenylpropane residues. Lignins are partly linked to cell wall cellulose and non-cellulose polysaccharides (Liyama *et al.*, 1994). It is formed at the

site of lignifications by the enzymatic dehydrogenation and subsequent polymerization of coniferyl, sinapyl and *P*-coumaric alcohols, the monomeric units derived from these alcohols are called guiacyl (3-methoxyl 4-hydroxyl phenyl propane) and *P*-coumaryl (4-hydroxyl phenyl propane) residues respectively (Selvendran, 1984).

In live plants, lignifications physiologically serve two main functions. It cements and anchors the cellulose microfibrills and other matrix polysaccharides and this may stiffen the wall thus preventing biochemical degradation and physical damage of the wall (Bach-Knudsen, 1997). These properties of lignified wall are important in dietary fibre content because they minimize the bacteria degradation of the walls in the intestine of animals and man. Van Soest and McQueen (1973) reported that lignin is generally regarded as the main non-carbohydrate fraction in plant cell wall and the source of such resistance to microbial degradation. Gordon *et al.,* (1983) however, reported that lignin is not only a component of the lignocellulose matrix; it also forms part of the lignin carbohydrate complex stabilized by phenolic acid (Ferulic, 4-coumaric acid) and acetyl constituent of cell wall. The lignin-carbohydrate complexes are usually chemically modified during microbial attack and are not recovered in acid detergent fibre (Gordon *et al.,* 1983).

Anderson and Chen (1979) indicated that lignin is virtually insoluble in strong acid or alkali and it is not digested or absorbed in the colon. Lignin can bind bile salts and other organic materials and may delay or impair small intestinal absorption of associated nutrients.

Identifying lignin degrading microorganism has been hampered because of the lack of reliable assays, but significant progress has been made through the use of a ^{14}C-labelled lignin assay (Freer and Detroy, 1982).

Two families of lignolytic enzymes are widely considered to play a key role in the enzymatic degradation: Phenol oxidase (Laccase) and Peroxidases (lignin Peroxidase (Lip) and Manganese peroxidase (MnP) (Krause *et al.*, 2003, Malherbe and Cloete, 2003). Other enzymes whose roles have not been fully elucidated include H_20_2-producing enzymes: glyoxal oxidase (Kerstern and Kirk 1987), glucose oxidase (Bourbonnaise and

Paice 1988), methanol oxidase (Nishida and Eriksson, 1987) and oxido – reductase (Bao and Renganathan, 1991).

2.24.2 Hemicellulose

According to Eastwood and Passmore (1983), hemicelluloses are group of several polysaccharides, with lower degree of polymerization than cellulose. They have a β 1-4 linked backbone of xylose, mannose or glucose residues that can form extensive hydrogen bond with cellulose. The authors also reported that xyloglucan is the major hemicellulose of primary cell wall in dicotyledonous plants while mixed linked glucan (β 1→3, 4) and arabinoxylans are the predominant hemicelluloses in the cereal seed.

Hemicelluloses include other branched heteropolymer (units linked in β 1→3, β 1→6, α 1→4, α 1→3) such as highly branched arabinogalactan (in soyabean), galactomannans (seeds of legumes) or glucomannan.

Quantitatively, hemicellulose constitutes among 10 to 25%DM in forage and agro-industrial by-products (bran, oil seed and legume seed, hulls and pulps) and about 2 – 12% DM of grains and roots. They are closely associated with lignin in the cell wall and along with cellulose they both encrust the cell wall and form the secondary thickened tissues (Van Soest and McQueen, 1973). Anderson and Chen (1979) concluded that hemicellulose exhibit water-holding capacity and can bind cation. The principal sugar components of these hemicellulose heteropolysaccharides (Howard *et al.*, 2003) are: D-xylose, D-mannose, D-glucose, D-galactose L-arabinose D-lucoronic acid, 4-0 methyl-D-glucuronic acid, D-galacturonic acid and to a lesser extent, L-rhamnose, L-fucose and various O-methylated sugars.

Based on the amino acid or nucleic acid sequence of their catalytic modules, hemicellulases are either glycoside hydrolases (GHs) which hydrolyse glycosidic bonds or carbohydrate esterases (CEs) which hydrolyse ester linkages of acetate or ferulic acid side groups (Howard *et al.*, 2003) and according to their primary sequence homology, they have been grouped into various families (Henrissat and Bairoch, 1996; Rabinovich *et al.*, 2002 a, b).

Xylan is the most abundant hemicellulose and xylanases are one of the major hemicellulases which hydrolyse the β – 1,4 bond in the xylan backbone yielding short xylooligomers which are further hydrolysed into single xylose units by β – xylosidase . Other xylanases are α -D glucuronidases which hydrolyse the α, 1 –2 glycosidic bond of the 4-0 methy l- D- glucuronic acid side chain of xylans (Howard *et al.*, 2003).

2.24.3 Cellulose

It is the major structural polysaccharide of the plant cell wall, and the most widespread polymer on the earth (Anderson and Chen, 1979). It is homopolymer (contrary to hemicellulose and pectin), formed from linear chain of β (1→4) linked D-glucopyranosyl unit (whereas starch is formed of α (1→4) linked D glucopyranosyl chain). Cellulose molecules are arranged within the microfibrils in a highly ordered crystalline state in chains 4000-6000mm long and possibly 4mm in diameter each consisting of several thousands of glucose units (Eastwood and Passmore, 1983). Cellulose is soluble and partially hydrolysed in strong acid solution (i.e. 72% $H_2$504).

Quantitatively, cellulose represent 40-50% of dry matter in hull of legumes and oilseeds, 10-30% in forage and beet pulps, 3-15% in oil seeds or legume seed (Annison and Choct, 1993). Most cereal grains contain small quantities of cellulose 1-5% DM except in oats (10%). Mares and Stone, (1973) reported that cellulose in cereal grain cell wall can be recovered from the insoluble residue left after vigorous extraction of cell wall material matrix component with alkali.

Cellulases can be divided into three major enzymes activity classes (Goyal *et al.*, 1991; Rabinovich *et al.*, 2002 a, b).

These are:

(1) Endoglucanases or endo 1 –4, β glucanase (EC.3.21.4)

(2) Cellebiohydrolases (E.C 3.21.91)

(3) β – glucosidase (E.C.3.2. 1.21)

Endoglucanases are often called Carboxymethyl cellulose (CM). Cellulases are proposed to initiate attack randomly at multiple internal sites in the amorphous region of the

cellulose fibre opening up sites for subsequent attack by Cellobiohydrolases (Wood, 1991).

Cellobiohydrolases often called Exoglucanases, are the major component of the fungal cellulose system accounting for 40 – 70% of the total crystalline cellulose. Cellobiohydrolases remove mono and dimers from the end of the glucose chain.

β -glucosidase hydrolyse glucose dimers and in some cases cello-oligosaccharides to glucose. Generally, endoglucanases and cellobiohydrolases work synergistically in the hydrolysis of cellulose but the details of the mechanism involved are still unclear (Rabinovich *et al.*, 2001 b).

CHAPTER THREE

3.0 EXPERIMENT 1

CHANGES IN THE PROXIMATE AND CRUDE FIBER FRACTIONS OF MAIZE RESIDUES AND BY-PRODUCTS AFTER TREATMENT WITH FOUR EDIBLE MUSHROOMS

3.1 INTRODUCTION

One of the major problems of ruminant production in Nigeria is the scarcity of forages all year round. Livestock have abundance of pasture to consume in the first six months of the raining season during which growth is relatively enhanced. The other six months are always followed by scarcity of forages as a consequence of the dry period, resulting in standing hay and low quality feed that can culminate in loss of weight by grazing animals (Babayemi *et al.,* 2006).

Agricultural residues are sources of protein and energy, and can be abundant throughout the year (Destroy *et al.,* 1978). Such agricultural crop wastes such as maize cobs, maize husk, maize straw and maize stover, consist mainly of cellulose and hemicellulose, with lower content of lignin, nitrogenous compounds, and ash (Abdullah and Zafar, 1999). Even though maize wastes contain cellulose its main shortcomings as animal feed are; (i) low digestibility; (ii) low protein content; (iii) poor acceptability; and (iv) bulkiness (Abdullah *et al.,* 2006)

It is apparent; therefore that bioconversion is a possible intervention that will help overcome these limitations before it can be used as ruminant feed. Therefore the aim of this study is to enhance utilization of this vast bulk of maize residue most of which is so burnt as fuel or left on the farm to rot. The crop residue are incubated with white-rot fungi (edible mushroom).

3.2 MATERIALS AND METHODS

3.2.1 Sample collection

Dried samples of maize cobs, maize stover, maize straw and maize husk were collected from the Teaching and Research Farm, University of Ibadan, Nigeria. The materials were milled and oven-cured at 65^0C until a constant weight to obtaine the dry matter (DM).

3.2.2 Fungus

The sporophores of *Pleurotus tuber-regium, Lentinus subnudus, Pleurotus pulmonarius* and *Pleurotus sajor caju* growing in the wild were collected from Ibadan University botanical garden. These were tissue cultured to obtain fungal mycelia (Jonathan and Fasidi, 2001). The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

3.2.3 Degradation of maize cobs, maize stover, maize straw and maize husk by *Pleurotus tuber-regium, Lentinus subnudus, Pleurotus pulmonarius* and *Pleurotus sajor caju*

3.2.3.1 Preparation of substrate

The jam bottles used for this study were thoroughly washed, dried for 10 min. at 100^0C. 25.00g of the dried milled substrate was weighed induvidually into each jam bottle and 70ml distilled water was added. The bottle was immediately covered with tin foil and sterilized at 121^0C for 15 min. Each treatment was done in triplicate.

3.2.3.2 Inoculation

Each bottle was inoculated at the centre of the substrate with 2, 10.00mm mycelia disc and covered immediately. They were kept in the dark cupboard in the laboratory at 30^0C and 100% relative humidity (RH). At day 21 of inoculation, the experimental bottles were autoclaved to terminate the mycelia growth. Samples of biodegradations were oven dried to constant weight for chemical analysis and *in vitro* digestibility.

3.3 CHEMICAL ANALYSIS

3.3.1 PROXIMATE ANALYSIS

Proximate analysis of all samples on dry matter basis was conducted according to the procedure of the Association of Official Analytical Chemists (A.O.A C., 1995). The Nitrogen free extract (NFE) was estimated as follows: NFE = 100 - (% Crude Protein + % Crude fibre + % Ether Extract + %Ash).

3.3.2 CELL WALL COMPONENTS

Cell wall components consisting of Neutral Detergent Fibre (NDF), Acid Detergent fibre (ADF) and Acid Detergent Lignin (ADL) were determined by the method of Van Soest *et al* (1991). Hemicellulose was estimated as the difference between NDF and ADF and Cellulose as the difference between ADF and ADL

3.4 STATISTICAL ANALYSIS

The samples replicated three times in a completely randomized design were subjected to analysis of variance using SAS (1999). Significant means were separated using Duncan multiple range test of the same software.

3.5 RESULTS

The effect of fungal treatments on the proximate composition and crude fiber fractions of maize straw are presented in Tables 1 and 2. Treatment effects on DM, CP, Ash, NFE of maize straw were significant ($p<0.05$). Values of Crude protein (CP) ranged from 7.37% in UMS to 16.41% in LSM-treated residue. A corresponding drop in the crude fiber (CF) was obtained with values ranging from 29.75% for UMS to 20.61% in PTM-treated sample. The results obtained also showed that DM and ash contents differed significantly ($P < 0.05$). The ADF was significantly ($P < 0.05$) affected by the fungal treatment decreasing from 47.04% for UMS to 41.74% for PTM. Similar trends of progressive decline was also recorded for NDF and ADF with values ranging from 68.35% (UMS) to 60.89% (LSM), and 13.79% (UMS) to 11.48% (PSM) respectively. Apparent variations in ADL was however significant.

Tables 3 and 4 showed the proximate composition and crude fiber fractions of fungal treated maize husk. Fugal treatment significantly influenced all the parameters measured. Treatment effects on DM, CP, CF, Ash, NFE of maize straw were significant ($p<0.05$). Values obtained for CP ranged from 7.44% (UMS) to 9.90% PPM. However, the increased CP contents of treated substrates were not significant ($p<0.05$). The CF on the other hand, were influenced by fungal treatment. The values recorded decreased from 30.45% (UMS) to 14.15% (PTM).The NFE ranged from 42.48% for UMS to 36.43% for LSM.treated samples. Biodegradation brought about decline in the crude fiber fractions. The ADF decreased from 49.15% (UMS) to 39.27% (LSM), NDF from 71.14% (UMS) to 58.96% (LSM), ADL from 14.87% (UMS) to 11.27% (PSM), cellulose from 34.25 (UMS) to 27.73% (LSM) and hemicellulose from 21.99% (UMS) to 18.46% (PSM).

Tables 5 and 6 represent the changes in proximate composition and crude fiber fractions of maize stover as affected by fungal treatment. All parameters measured differed significantly ($P<0.05$). The highest value of 88.85% and 31.84% was recorded for DM and CF respectively in the control (UMS) while the treated substrates recorded lower values. The value obtained for CP was highest (19.63%) in PPM-treated substrate and lowest (3.72%) in UMS. The EE values ranged from 0.83% for UMS to 1.81% for PPM-treated. Similarly, PPM-treated residue also recorded the highest values (10.37% and

56.95%) in the ash and NFE contents respectively. The variations observed in crude fiber fractions after fungal treatments were significant ($p<0.05$).Wide variations were also obtained in ADF declining from 46.53% (UMS) to 41.76% (PSM), NDF decreased from 67.86% (UMS) to 61.24% (LSM) while ADL declined from 13.63% (UMS) to 11.54% (PSM). Cellulose and hemicellulose fractions differed significantly ($P<0.05$).

Shown in Tables 7 and 8 are the results of fungal treatment on the proximate composition and crude fiber fractions of maize cob. Generally, the CP and EE were higher in the fungal treated maize cobs compared with the untreated. The highest values recorded for CP and EE were obtained in LSM (10.64%) and PPM (1.66%) respectively. Significant increase was also obtained in the ash contents after fungal treatment with values ranging from 2.87% (UMS) to 3.32% (PPM).

Table 1: Proximate composition (g/100gDM) of fungal treated maize straw

	TREATMENT					
PARAMETERS	**UMS**	**LSM**	**PTM**	**PSM**	**PPM**	**SEM**
Dry matter	88.77[b]	86.75[c]	86.74[c]	86.37[d]	87.66[b]	3.33
Crude protein	7.36[e]	16.41[a]	14.49[b]	12.62[c]	9.66[d]	3.30
Ether extract	1.58[e]	3.01[b]	2.24[d]	3.21[a]	2.86[c]	2.97
Ash	8.78[d]	8.95[c]	8.96[c]	9.14[b]	9.78[a]	0.01
Crude fibre	29.75[a]	24.46[c]	20.61[e]	22.54[d]	26.70[b]	0.02
NFE	73.44[a]	62.68[e]	65.30[d]	65.68[c]	67.91[b]	0.06

a-e, means on the same row with different superscripts are significant (P<0.05). UMS = untreated maize straw, LSM = *Lentinus subnudus* treated maize straw, PTM = *Pleurotus tuber regium* treated maize straw, PSM = *P. tuber regium* treated maize straw, PSM = *P. sajor caju* treated maize straw, NFE = Nitrogen free extract, SEM = standard error of mean.

Table 2: Cell wall fractions (g/100g DM) of fungal treated maize straw

	TREATMENT					
PARAMETERS	**UMS**	**LSM**	**PTM**	**PSM**	**PPM**	**SEM**
ADF	47.67[a]	43.71[d]	41.26[e]	44.27[b]	43.85[c]	3.33
NDF	69.05[a]	61.68[d]	61.49[e]	63.01[c]	63.72[b]	0.00
ADL	13.59[a]	11.98[d]	11.94[e]	12.16[c]	12.26[b]	3.33
Cellulose	34.08[a]	31.73[c]	29.32[e]	32.11[b]	31.59[d]	0.01
Hemicellulose	21.38[a]	11.97[e]	20.23[b]	18.74[d]	19.37[c]	0.01

a-e, means on the same row with different superscripts are significant ($P<0.05$). UMS = untreated maize straw, LSM = *Lentinus subnudus* treated maize straw, PTM = *Pleurotus tuber regium* treated maize straw, PSM = *P. tuber regium* treated maize straw, PSM = *P. sajor caju* treated maize straw, ADF = Acid detergent fiber, NDF = Neutral detergent fiber, ADL = Acid detergent lignin, SEM = standard error of mean.

Table 3: Proximate composition (g/100g DM) fugal treated maize husk

	TREATMENT					
PARAMETERS	**UMS**	**LSM**	**PTM**	**PSM**	**PPM**	**SEM**
Dry matter	88.85[a]	86.78[b]	86.89[b]	86.44[b]	87.70[a]	0.01
Crude protein	7.44[a]	9.89[a]	9.67[a]	9.55[a]	9.90[a]	0.30
Ether extract	1.27[b]	2.52[a]	1.75[a]	2.78[a]	2.82[a]	0.21
Ash	3.32[c]	3.53[b]	3.90[a]	3.86[a]	10.37[a]	0.26
Crude fiber	30.45[a]	22.27[c]	24.29[b]	14.15[e]	19.07[d]	0.33
NFE	42.48[a]	36.43[b]	39.49[ab]	39.49[ab]	42.18[a]	0.63

a-d, means on the same row with different superscripts are significant (P<0.05). UMS = untreated maize husk, LSM = *Lentinus subnudus* treated maize husk, PTM = *Pleurotus tuber regium* treated maize husk, PSM = *P. tuber regium* treated maize husk, PSM = *P. sajor caju* treated maize husk, NFE = Nitrogen free extract, SEM = standard error of mean.

Table 4: Cell wall fractions (g/100g DM) of fungal treated maize husk

	TREATMENT					
PARAMETERS	**UMS**	**LSM**	**PTM**	**PSM**	**PPM**	**SEM**
ADF	49.15[a]	39.27[d]	41.05[c]	44.09[b]	43.94[b]	0.30
NDF	71.14[a]	58.96[c]	60.58[c]	62.55[b]	63.13[b]	0.33
ADL	14.87[a]	11.54[b]	11.89[b]	11.27[b]	12.14[b]	0.28
Cellulose	34.25[a]	27.73[d]	29.43[c]	32.82[b]	31.80[b]	0.24
Hemicellulose	21.99[a]	19.69[b]	19.53[b]	18.46[b]	19.90[b]	0.33

a-d, means on the same row with different superscripts are significant ($P<0.05$). UMS = untreated maize husk, LSM = *Lentinus subnudus* treated maize husk, PTM = *Pleurotus tuber regium* treated maize husk, PSM = *P. tuber regium* treated maize husk, PSM = *P. sajor caju* treated maize husk, NFE = Nitrogen free extract, ADF = Acid detergent fiber, NDF = Neutral detergent fiber, ADL = Acid detergent lignin, SEM = standard error of mean.

Table 5: Proximate composition (g/100g DM) fugal treated maize stover

	TREATMENT					
PARAMETERS	**UMS**	**LSM**	**PTM**	**PSM**	**PPM**	**SEM**
Dry matter	88.85[a]	86.56[d]	86.80[c]	36.47[e]	87.72[b]	0.02
Crude protein	3.72[e]	14.34[b]	8.45[d]	12.17[c]	19.63[a]	3.33
Ether extract	0.83[e]	1.62[c]	1.23[d]	1.67[b]	1.81[a]	3.33
Ash	9.45[e]	10.15[b]	9.90[c]	9.65[d]	10.37[a]	2.97
Crude fiber	31.84[a]	14.42[e]	19.06[c]	18.14[d]	25.24[b]	0.01
NFE	45.84[b]	40.53[d]	61.36[e]	41.63[c]	56.95[a]	0.01

a-e, means on the same row with different superscripts are significant (P<0.05). UMS = untreated maize stover, LSM = *Lentinus subnudus* treated maize stover, PTM = *Pleurotus tuber regium* treated maize stover, PSM = *P. tuber regium* treated maize stover, PSM = *P. sajor caju* treated maize stover, NFE = Nitrogen free extract, SEM = standard error of mean.

Table 6: Cell wall fractions (g/100g DM) of fungal treated maize stover

	TREATMENT					
PARAMETERS	**UMS**	**LSM**	**PTM**	**PSM**	**PPM**	**SEM**
ADF	46.53[a]	42.73[c]	41.81[d]	41.76[e]	44.27[b]	0.01
NDF	67.86[a]	61.24[e]	62.04[d]	62.52[c]	63.73[b]	3.33
ADL	13.63[a]	11.84[d]	12.04[c]	11.54[e]	12.64[b]	3.33
Cellulose	32.90[a]	30.90[c]	29.80[e]	30.23[d]	31.63[b]	0.01
Hemicellulose	21.33[a]	18.51[e]	20.21[c]	20.76[b]	19.46[d]	0.01

a-e, means on the same row with different superscripts are significant (P<0.05). UMS = untreated maize stover, LSM = *Lentinus subnudus* treated maize stover, PTM = *Pleurotus tuber regium* treated maize stover, PSM = *P. tuber regium* treated maize stover, PSM = *P. sajor caju* treated maize stover, ADF = Acid detergent fiber, NDF = Neutral detergent fiber, ADL = Acid detergent lignin, SEM = standard error of mean.

Table 7: Proximate composition (g/100g DM) fugal treated maize cob

	TREATMENT					
PARAMETERS	**UMS**	**LSM**	**PTM**	**PSM**	**PPM**	**SEM**
Dry matter	88.57^{b}	86.66^{d}	86.80^{c}	86.40^{e}	88.72^{a}	0.02
Crude protein	6.82^{e}	10.64^{a}	9.45^{d}	10.37^{b}	10.05^{c}	3.33
Ether extract	0.38^{de}	1.38^{c}	0.65^{d}	1.49^{b}	1.66^{a}	3.33
Ash	2.87^{d}	3.16^{b}	2.98^{c}	3.20^{b}	3.32^{a}	3.33
Crude fiber	32.68^{b}	17.44^{e}	26.80^{c}	24.14^{d}	32.89^{a}	0.02
NFE	42.75^{b}	32.62^{e}	39.88^{c}	39.20	47.92	0.02

a-e, means on the same row with different superscripts are significant (P<0.05). UMS = untreated maize cob, LSM = *Lentinus subnudus* treated maize cob, PTM = *Pleurotus tuber regium* treated maize cob, PSM = *P. tuber regium* treated maize cob, PSM = *P. sajor caju* treated maize cob, NFE = Nitrogen free extract, SEM = standard error of mean.

Table 8: Cell wall fractions (g/100g DM) of fungal treated maize cob

	TREATMENT					
PARAMETERS	**UMS**	**LSM**	**PTM**	**PSM**	**PPM**	**SEM**
ADF	47.04[a]	41.98[e]	41.74[e]	44.56[c]	44.70[b]	0.02
NDF	68.35[a]	60.85[e]	61.09[d]	62.52[c]	63.73[b]	3.33
ADL	13.79[a]	13.79[a]	11.77[c]	11.48[d]	12.46[b]	0.02
Cellulose	33.35[a]	30.48[d]	29.97[e]	33.08[b]	32.24[c]	0.02
Hemicellulose	21.31[a]	18.87[d]	19.35[b]	17.96[e]	19.03[c]	0.01

a-e, means on the same row with different superscripts are significant (P<0.05). UMS = untreated maize cob, LSM = *Lentinus subnudus* treated maize cob, PTM = *Pleurotus tuber regium* treated maize cob, PSM = *P. tuber regium* treated maize cob, PSM = *P. sajor caju* treated maize cob, ADF = Acid detergent fiber, NDF = Neutral detergent fiber, ADL = Acid detergent lignin, SEM = standard error of mean.

3.6 DISCUSSION

Changes in the proximate composition and crude fiber fractions of maize crop residues treated with fungi

Fungal treatments increased ($p<0.05$) the CP and ash contents of the different crop residues and by-products compared with the control. Such apparent increase could be due to the proliferation of fungi during degradation (Belewu and Belewu, 2005 and Farkas (1979) and Jacqueline and Viser (1996)). This agrees with the report published by Farkas (1979); Jacqueline and Viser (1996) of which noted that the extracellular enzymes secreting fungus contain amorphous, homo and heteropolysaccharides which are associated with fungal protein. Increase in the CP content of the treated crop residues could be due to the secretion of certain extracellular enzymes which are proteineous in nature during their breakdown of cell wall and its subsequent metabolism (Kadiri, 1990). Crude protein increase is also associated with the hydrolysis of starch to glucose and its subsequent use by some organism as a carbon source to synthesize fungal biomass rich in protein (Bender, 1970; Hammond and Wood, 1985). The increase in ash contents on the other hand, may be a reflection of decreasing CF and NFE.

Some authors (Zadrazil, 1993; Belewu and Okhawere, 1998) reported that colonization of substrates by fungal mycelia results in increase in their nutritional values. All the fungi used were effective in degradation of CF because the hyphae of these fungi were capable of penetrating deep into the cells of the residues and by-products. This means that the fungi not only grew on the surface of the substrate but also penetrated deep into the substrates. This observation is consistent with such findings (Shoukry *et al.*, 1985) in which CF decreased while CP increased. A trend which was consistent with decreas e in NDF, ADF, and ADL (Albores *et al.*, 2006). Earlier reports (Karunanada *et al.*, 1995) concluded that lignifications of structural polysaccharides not only inhibited ruminal microbial digestion of polysaccharides by forming 3-D matrix, but also depicted highly liquefied tissues which formed a physical barrier. This prevented accessibility of highly digestible tissues to the action of hydrolytic enzyme of the rumen microorganisms. Increased digestibility was also associated with the degradation of structural carbohydrates (Mukherjee and Nandi, 2004). Fungal treatment of cotton stalks also

resulted in decreased NDF, ADF, ADL, Cellulose and hemicellulose (Mahrous, 2005). Furthermore, the decrease in hemicellulose and cellulose of the crop residues in this study suggested the substrate is acceptable to the degrading fungi. It provides the fungi with the energy source for growth as reported elsewhere (Rolz *et al.*, 1986). Utilization of cellulose and hemicellulose has been associated with the duration of formation (Chen *et al.*, 1995). In this study, the substrates were fermented for 40days.Extension of the period may be examined in future. It's however conclusive in this study that treatment of maize wastes and by-products with edible mushrooms initiated a positive change in the proximate composition and crude fiber fractions for better utilization. Ranked in order of fungal improvement are: LSM, PSM, PPM, and USM.

CHAPTER FOUR

4.0 EXPERIMENT 2

ASSESSMENT OF NUTRITIONAL QUALITY OF MAIZE RESIDUES AND BY-PRODUCTS AFTER TREATMENT WITH FOUR EDIBLE MUSHROOMS

4.1 INTRODUCTION

The nutrient composition of feeds is commonly determined by chemical analysis. However, this does not provide sufficient information for determination of the true nutritive value. In addition, the determination of intake and digestibility of feedstuffs *in vivo* is time consuming, laborious, expensive which requires large qualities of feed and may be unsuitable for large scale feed evaluation (Coelho *et al.*, 1988; Carro *et al.*, 1994). The *in vitro* gas production technique is used for evaluation of nutritive value particularly to estimate agro-industry by-products (Krishna and Gurther 1987), various types of tropical plants (Krishnamorthy *et al.*, 1995 and Sallam, 2005), compound feeds (Aiple *et al.*, 1996), different feed classes (Getachew *et al.*, (1998), fermentation kinetics (Groot *et al.*, 1998) and energy value of straw (Makkar *et al.*, 1999). Gas measurement also provides a useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs. It is less expensive, less time-consuming and allows incubation to be maintained more precisely than in *in-vivo*. The *in vitro* (Tilley and Terry 1963), (Mehrez and Orskov, 1997), and enzymatic (Jones and Hayward, 1975) methods have been widely used to predict digestibility of feeds, and as selection tool for screening feeds for nutritional quality. The *in vitro* gas methods based on syringes (Menke *et al.*, 1979; Blummel *et al.*, 1997) appears to be the most suitable for use in developing countries. Other *in vitro* methods (Tilley and Terry, 1963) and nylon bag methods are based on gravimetric measurement which depicts disappearance of the substrate (the components which may not necessarily contribute to fermentation), whereas, gas measurement focuses on the appearances of fermentation products. In the gas method, kinetics of fermentation can be studied on single sample and therefore a relatively small amount of sample is required or a larger number of samples can be evaluated at a time. In this experiment, *in vitro* gas production technique of Menke is applied to assess the nutritional value of fungal treated maize wastes and by-products.

4.2 MATERIALS AND METHODS

Dried samples of maize residues (maize husk, maize stover, maize straw and maize cob) were collected from the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. The materials were milled and oven-treated at 65^0C to constant weight for dry matter determination.

4.2.1 The fungus

The sporophores of *Pleurotus tuber-ragium, Pleurotus pulmonarius, Pleurotus sajor caju and Lentinus subnudus* growing in the wild were collected from Ibadan University botanical garden. These were tissue cultured to obtain fungal mycelia (Jonathan and Fasidi, 2001). The pure culture obtained was maintained on plate of potato dextrose agar (PDA).

4.2.2 Degradation of maize husk, maize stover,maize cob and maize straw by *P. tuber-regium, P. pulmonarius, P. sajor caju* and *L. subnudus*.

4.2.2.1 Preparation of substrate

The jam bottles used for this study were thoroughly washed, dried for 10min. at 100^0C. 25.00g of the dried milled substrates were weighed separately into a jam bottle and 70ml distilled water were added. The bottle was immediately covered with aluminum foil and sterilized in the autoclave at 121^0C for 15 min. Each treatment was in triplicates.

4.2.2.2 Inoculation

Each bottle was inoculated at the center of the substrate with 2, 10.00mm mycelia disc and covered immediately. They were kept in the dark cupboard in the laboratory at 30^0C and 100% relative humidity (RH). At day 40 day of inoculation, the experimental bottles were autoclaved to terminate the mycelia growth. Samples of biodegradation were oven dried to constant weight for chemical analysis and *in vitro* digestibility.

4.2.2.3 *In vitro* gas production

Rumen fluid was obtained from three West African Dwarf female goats through suction tube via the oesophagus before morning feed. The animals were fed with 40% concentrate (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fishmeal) and 60% Guinea grass. Incubation was carried out (Menke and Steingass, 1998) in 120ml calibrated syringes in three batches at 39^0C. To 200mg sample in the syringe was added 30ml inoculums containing cheese cloth strained rumen liquor and buffer (9.8g $NaHCO_3$ + 2.77g Na_2HPO_4 + 0.57gKCL + 0.47gNaCl + 0.12g$MgSO_4.7H_2O$ + 0.16g$CaCl_2$. $2H_2O$ in a ratio (1:4 v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24 hrs. After 24hr of incubation, 4ml of NaOH (10M) was introduced to estimate the amount of methane produced (Fievez *et al.*, 2005). The average volume of gas produced from the blanks was deducted from the total volume of gas produced. Fermentation characteristics were estimated using the equation $Y = a + b\ (1 - e^{ct})$ (Orskov and McDonald, 1979), where Y = volume of gas produced at time 't', a = intercept (gas produced from the soluble fraction), b = gas production rate constant for the insoluble fraction, (a + b) = final gas produced, C = gas production rate constant for the insoluble fraction (b), t = incubation time. Metabolizable energy (ME, MJ/Kg DM) and organic matter digestibility (OMD %) were estimated (Menke and Steingass, 1998) and short chain fatty acids (SCFA) was calculated Getachew *et al.*, (1999).

ME MJ/kg DM = 2.20 + 0.136 *Gv + 0.057^* + 0.0029^*CF

OMD = 14.88 + 0.88Gv + 0.45CP + 0.651XA;

SCFA = 0.0239^*Gv – 0.0601;

Where Gv, CP, CF and XA are net gas production (ml/200mg DM) crude protein, crude fiber and ash of the incubated sample respectively.

4.3 STATISTICAL ANALYSIS

Data obtained were subjected to analysis of variance (ANOVA) and where significant difference occurred means were separated by Duncan (1955) using Statistical Analysis System (SAS) 1999 package.

4.4 RESULTS

The *in vitro* gas production characteristics, gas volume, estimated metabolizable energy (ME), short chain fatty acid (SCFA) and organic matter digestibility (OMD) of fungal treated maize stover, maize cob and maize husk are presented in Tables 9, 10, 11 and 12 respectively. Table 1 showed that the variation was significant ($P<0.05$). The value for degradation of the insoluble but degradable fraction (b mL) increased from 12.34mL in UM to 14.34mL in LSM. The value obtained in LSM (14.34mL) however were similar ($P>0.05$) compared with that obtained for PPM. The gas production rate constant (Ch^{-1}) as obtained in this study ranged from, 0.016 h^{-1} (UMS) to 0.026 h^{-1} (PTM). Although the slowest rate was recorded for UMS, nevertheless this is no significantly different from the value for LSM (0.020h^{-1}), PSM (0.024h^{-1}) and PPM (0.024h^{-1}). Gas volume at 24hr of incubation (Gv 24) was significantly ($P<0.05$) influenced by fungal treatments. The lowest gas volume was produced by the control (UM) (20.67mL) while the highest volume was produced in LSM (28.67mL). The apparent variation in gas volume obtained in PSM (27.67mL) and PPM (26.33mL), and also in LSM (28.67mL) and PSM (27.67mL) were not significant.Variations in the estimated metabolizable energy (ME), short chain fatty acid (SCFA) and organic matter digestibility (OMD) were significant ($P<0.05$). The result recorded for ME ranged from 5.52 MJ/kg DM in UM to 7.11MJ/kg DM in LSM, SCFA ranged from 0.434µm in UM to 0.625µm in LSM while OMD ranged from 42.09% in UM to 53.31% in LSM.

In Table 10, the insoluble but degradable fraction (b, mL) increased with fungal treatment from 10.34 (UM) to 13 (LSM). The values obtained in LSM (13) and PTM (12.30) were not significantly different ($P>0.05$) but differed significantly ($P<0.05$) from UM (10.34) and PPM (11.00). There were also no significant difference ($P>0.05$) in UM (10.34), PSM (11.17) and PPM (11.00). Gas rates production constant (Ch^{-1}) obtained in UM (0.019h^{-1}), PTM (0.022h^{-1}) PSM (0.023h^{-1}) and (0.027h^{-1}) were not significantly ($P>0.05$) different. Gas volume at 24hr of incubation (Gv 24) increased from 20.67mL (UM) to 25.33mL (PTM). The estimated ME, SCFA and OMD consistently increased with fungal treatment. The highest ME was estimated for PTM (6.26 MJ/kg DM) and the least, 5.49 MJ/kg DM was estimated for UM. Similar trend was observed for the SCFA and OMD

with values ranging from 0.433µm (UM) to 0.545µm (PTM), and 38.01% (UM) to 43.36% PTM.

From Table 11, the insoluble but degradable (b, mL) increased from 11.66mL in UM to 11.67mL in PTM. With the exception of PSM, faster rates of gas production were obtained in the control (UM). Gas volume after 24hr of incubation varied significantly ($P<0.05$) across the treatments. The highest volume was obtained in LSM (29mL) and the least volume was recorded in the control (UM, 20.33mL). Gas volumes recorded in PSM (26.33mL) and PPM (26mL) were not significantly ($P>0.05$) different. The Estimated ME was highest in LSM (6.75 MJ/kg DM) and least in UM (5.45 MJ/kg DM).The Estimated SCFA ranged from 0.550µm to 0.750µm for UM and LSM respectively. Estimated OMD increased from 38.28% to 48.97% in UM and PPM respectively.

In Table 12, the apparent variation in the insoluble but degradable fraction (b, mL) was highest in PSM (15.67mL) followed by LSM (14.34mL) and PPM (13.66mL) while the least value was recorded in both UM (12.34mL) and PTM (11.50mL). Faster rates (Ch^{-1}) were generally higher in all the fungal treated straw compared with the control. A Similar trend of progressive increased was recorded for Gv 24.The treatment effects on ME, SCFA and OMD were significant ($p<0.05$).Values of ME ranged from 5.52 to 7.11 MJ/kg DM, SCFA from 0.434 to 0.514µm and OMD from 42.09% to 53.31%. The highest values in ME, SCFA and OMD were recorded for LSM. Shown in Figures 1 to 4 are the graphs of *in vitro* gas production pattern of maize stover, maize cob, maize husk and maize straw as affected by different fungal treatments while the graphs of methane (CH_4) production as affected by fungal treatments are presented in Figures 5 to 8. Generally low CH_4 (ml/200mg DM) were produced in the *Pleurotus pulmonarius* treated maize stover, maize husk and maize straw.

Table 9: *In vitro* gas production characteristics, gas volume, estimated metabolisable energy (ME), short chain fatty acid (SC FA) and organic matter digestibility (OMD) of fungal treated maize stover.

Parameter	UM	LSM	PTM	PSM	PPM	SEM
b (mL)	12.7^{d}	22.33^{a}	12.84^{d}	15.50^{c}	16.15^{b}	0.26
Ch^{-1}	0.007^{d}	0.015^{c}	0.052^{a}	0.022^{b}	0.002^{b}	3.33
Gv 24	20.00^{c}	34.33^{c}	24.67^{b}	26.00^{b}	26.00	0.41
ME (MJ/kg DM)	5.22^{e}	7.73^{a}	6.09^{d}	6.48^{c}	6.95^{b}	0.04
SCFA (µm)	0.418^{e}	0.760^{a}	0.530^{d}	0.648^{c}	0.693^{b}	0.01
OMD (%)	40.49^{e}	58.46^{a}	47.06^{a}	49.75^{c}	53.58^{b}	0.00

a-e, means on the same row with different superscripts are significant ($P<0.05$). UMS = untreated maize straw, LSM = *Lentinus subnudus* treated maize straw, PTM = *Pleurotus tuber regium* treated maize straw, PSM = *P. tuber regium* treated maize straw, PSM = *P. sajor caju* treated maize straw, C = Gas production rate constant, Gv24 = Gas volume at 24hr of gas production, ME = Metabolizable energy, SCFA =Short chain fatty acid, OMD = Organic matter digestibility, SEM = standard error of mean.

Table 10: *In vitro* gas production characteristics, gas volume, estimated metabolisable energy (ME), short chain fatty acid (SC FA) and organic matter digestibility (OMD) of fungal treated maize cob.

Parameter	UM	LSM	PTM	PSM	PPM	SEM
b (mL)	10.34^{c}	13.00^{a}	12.30^{ab}	11.17b^{c}	11.00^{c}	0.21
Ch^{-1}	0.019^{b}	0.030^{a}	0.022^{ab}	0.023^{ab}	0.027^{ab}	1.52
Gv 24	20.67^{c}	24.00^{ab}	25.33^{a}	22.67^{bc}	23.33^{ab}	0.40
ME (MJ/kg DM)	5.49^{d}	6.12^{b}	6.26^{a}	5.94^{c}	6.04b^{c}	0.02
SCFA (µm)	0.433^{d}	0.5135^{b}	0.545^{a}	0.482^{c}	0.497b^{c}	0.01
OMD (%)	38.01^{c}	42.85^{a}	43.36^{a}	41.58^{b}	42.09^{b}	0.13

a-d, means on the same row with different superscripts are significant (P<0.05). UMS = untreated maize straw, LSM = *Lentinus subnudus* treated maize straw, PTM = *Pleurotus tuber regium* treated maize straw, PSM = *P. tuber regium* treated maize straw, PSM = *P. sajor caju* treated maize straw, C = Gas production rate constant, Gv24 = Gas volume at 24hr of gas production, ME = Metabolizable energy, SCFA =Short chain fatty acid, OMD = Organic matter digestibility, SEM = standard error of mean.

Table 11: *In vitro* gas production characteristics, gas volume, estimated metabolisable energy (ME), short chain fatty acid (SC FA) and organic matter digestibility (OMD) of fungal treated maize husk.

Parameter	UM	LSM	PTM	PSM	PPM	SEM
b (mL)	11.66^{d}	16.00^{a}	11.67^{d}	13.83^{b}	13.50^{c}	1.78
Ch^{-1}	0.029^{b}	0.016^{e}	0.025^{c}	0.032^{a}	0.022^{d}	3.33
Gv 24	20.33^{d}	29.00^{a}	23.67^{c}	26.33^{b}	26.00^{b}	1.20
ME (MJ/kg DM)	5.45^{d}	6.75^{a}	6.37^{b}	6.37^{b}	6.34^{b}	0.06
SCFA (µm)	0.550^{d}	0.750^{a}	0.690^{b}	0.690^{b}	0.630^{c}	0.03
OMD (%)	38.28^{d}	45.99^{b}	42.60^{c}	44.89^{bc}	48.97^{a}	0.47

a-e, means on the same row with different superscripts are significant (P<0.05). UMS = untreated maize straw, LSM = *Lentinus subnudus* treated maize straw, PTM = *Pleurotus tuber regium* treated maize straw, PSM = *P. tuber regium* treated maize straw, PSM = *P. sajor caju* treated maize straw, C = Gas production rate constant, Gv24 = Gas volume at 24hr of gas production, ME = Metabolizable energy, SCFA =Short chain fatty acid, OMD = Organic matter digestibility, SEM = standard error of mean.

Table 12: *In vitro* gas production characteristics, gas volume, estimated metabolizable energy (ME), short chain fatty acid (SC FA) and organic matter digestibility (OMD) of fungal treated maize straw.

Parameter	UM	LSM	PTM	PSM	PPM	SEM
b (mL)	12.34^{c}	14.34^{b}	11.50^{c}	15.67^{a}	13.66^{b}	0.02
C h^{-1}	0.0016^{c}	0.020^{b}	0.026^{a}	0.024^{a}	0.024^{a}	0.01
Gv 24	20.67^{d}	28.67^{a}	24.00^{c}	27.67^{ab}	26.33^{b}	0.31
ME (MJ/kg DM)	5.52^{d}	7.11^{a}	6.35^{c}	6.75^{b}	6.45^{c}	0.02
SCFA (µm)	0.434^{e}	0.625^{a}	0.514^{d}	0.601^{b}	0.577^{c}	0.01
OMD (%)	42.09^{d}	53.31^{a}	48.34	50.55^{b}	49.05^{c}	0.15

a-e, means on the same row with different superscripts are significant ($P<0.05$). UMS = untreated maize straw, LSM = *Lentinus subnudus* treated maize straw, PTM = *Pleurotus tuber regium* treated maize straw, PSM = *P. tuber regium* treated maize straw, PSM = *P. sajor caju* treated maize straw, C = Gas production rate constant, Gv24 = Gas volume at 24hr of gas production, ME = Metabolizable energy, SCFA =Short chain fatty acid, OMD = Organic matter digestibility, SEM = standard error of mean.

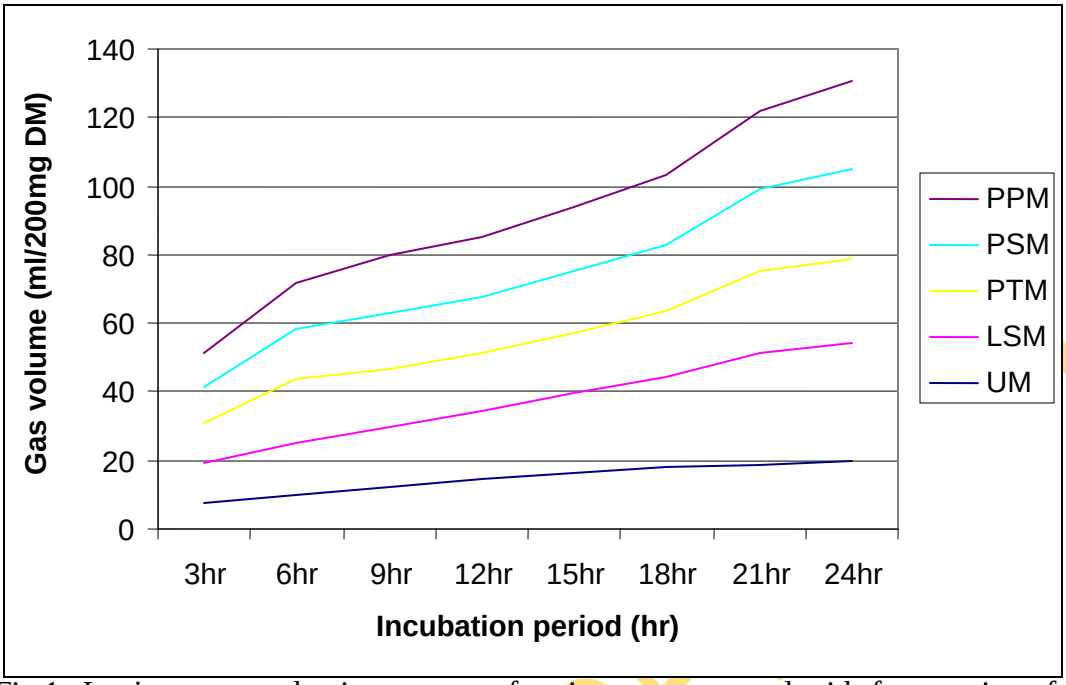

Fig.1: *In vitro* gas production pattern of maize stover treated with four strains of edible mushroom

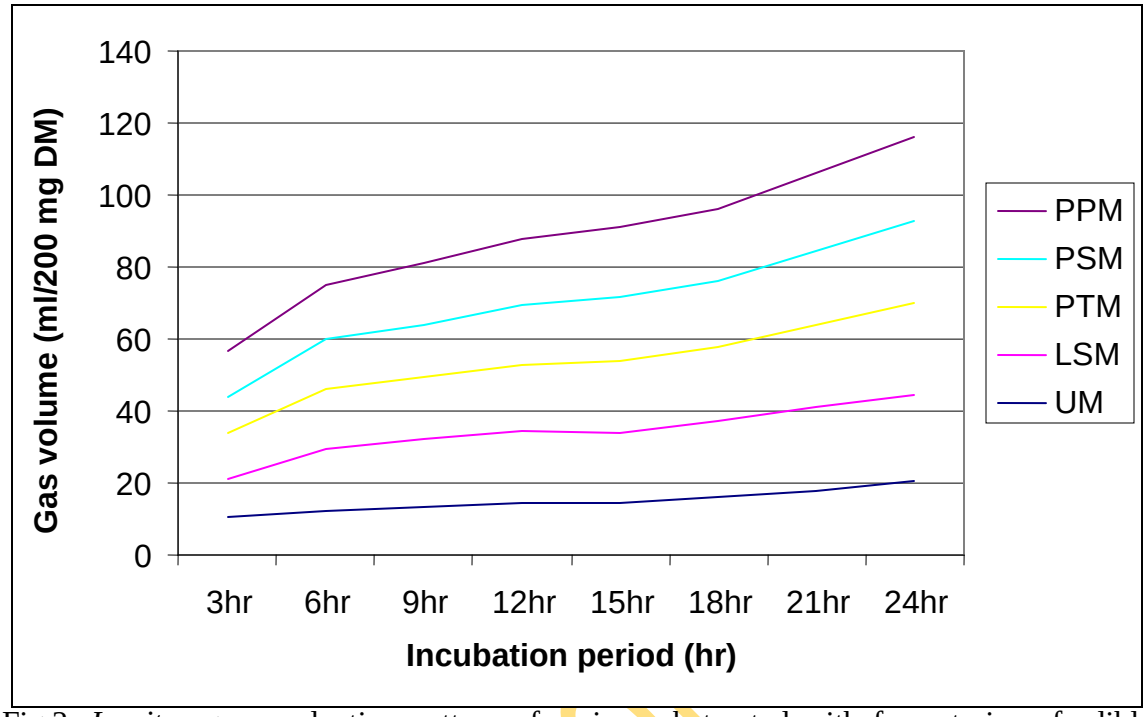

Fig.2: *In vitro* gas production pattern of maize cob treated with four strains of edible mushroom

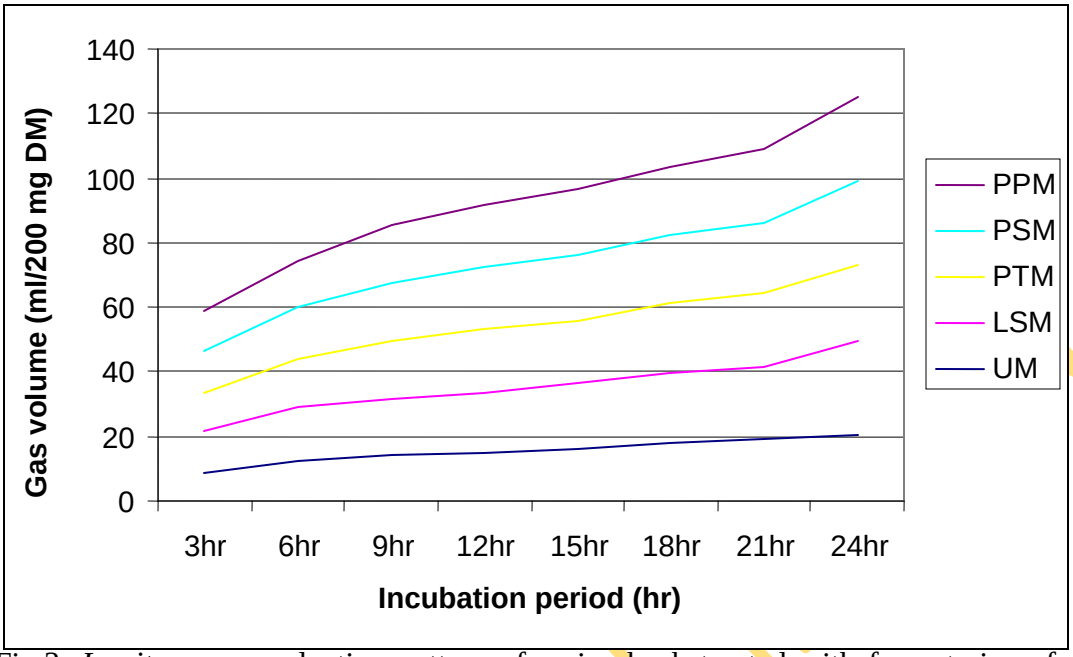

Fig.3: *In vitro* gas production pattern of maize husk treated with four strains of edible mushroom

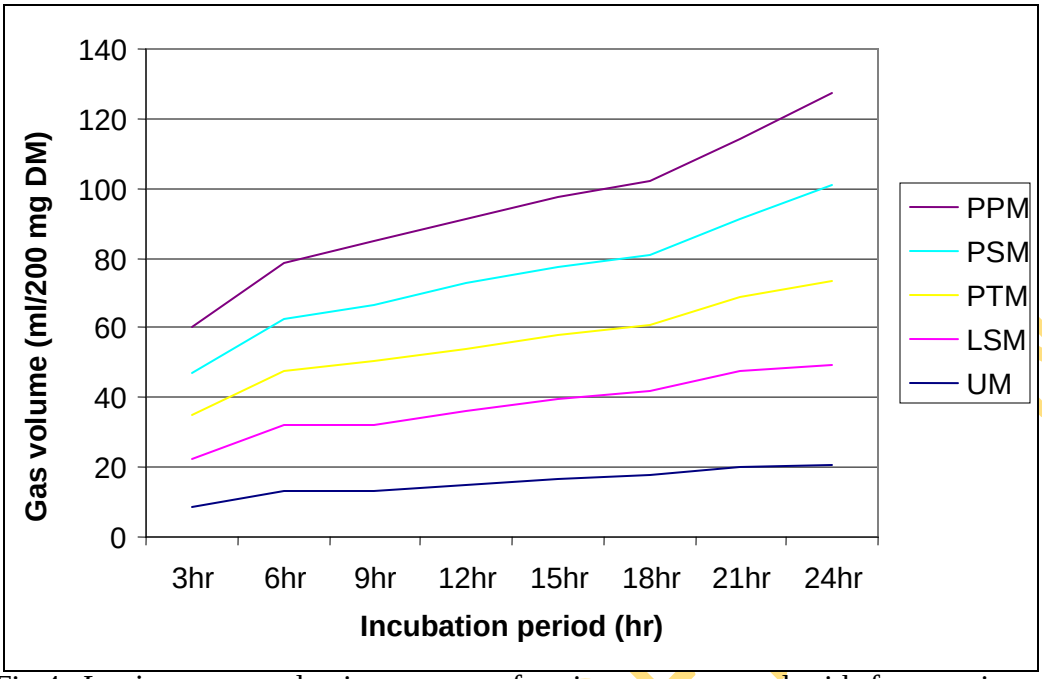

Fig.4: *In vitro* gas production pattern of maize straw treated with four strains of edible mushroom

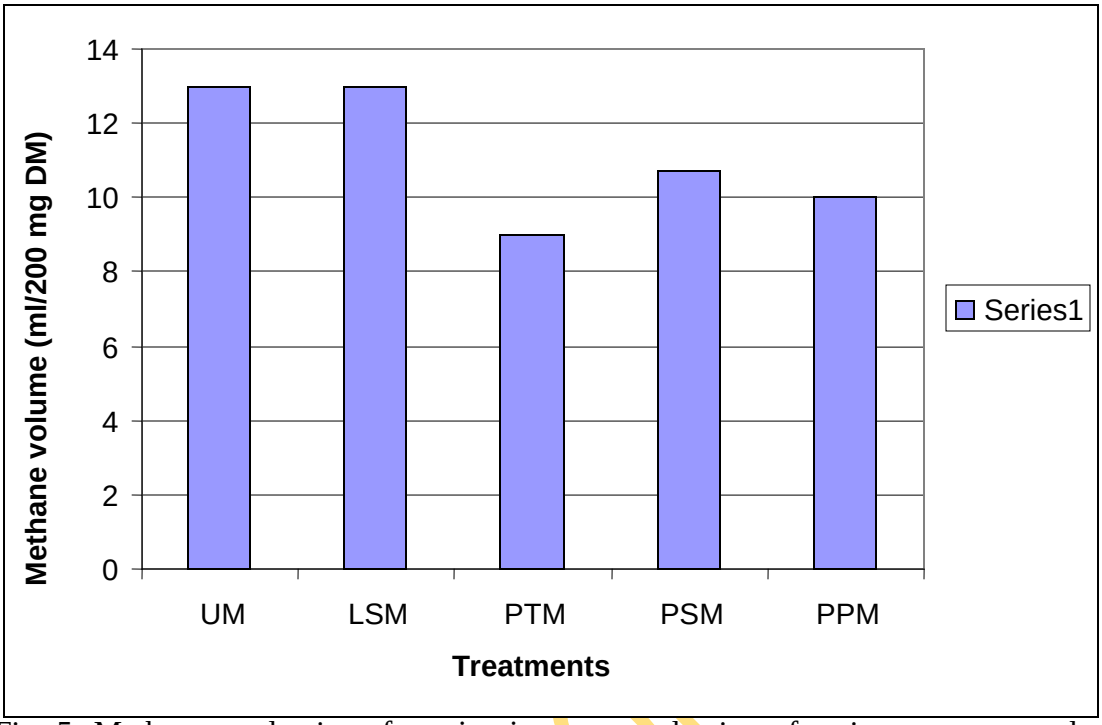

Fig. 5: Methane productions from *in vitro* gas production of maize stover treated with four strains of edible mushroom

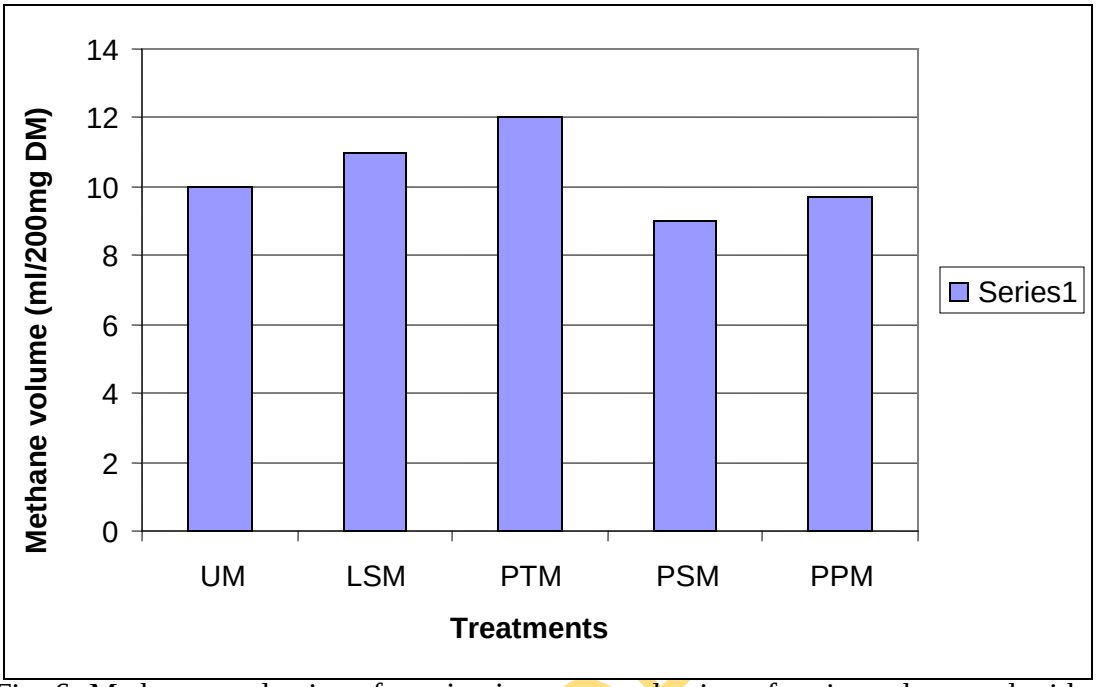

Fig. 6: Methane productions from *in vitro* gas production of maize cob treated with four strains of edible mushroom

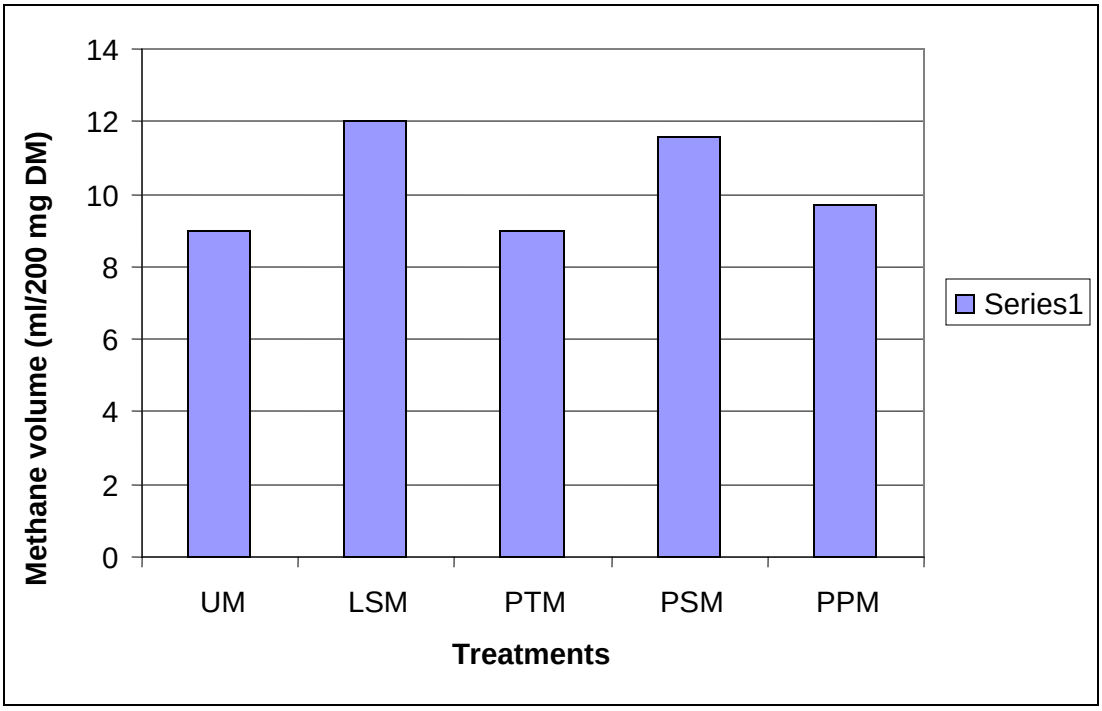

Fig. 7: Methane productions from *in vitro* gas production of maize husk treated with four strains of edible mushroom

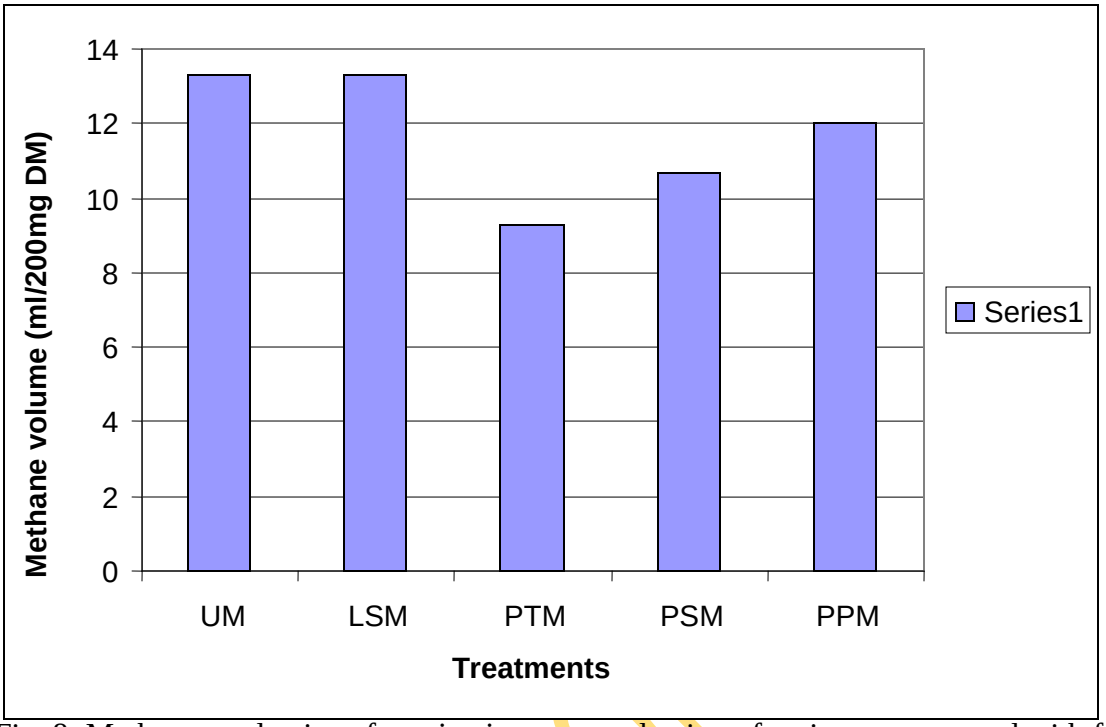

Fig. 8: Methane productions from *in vitro* gas production of maize straw treated with four strains of edible mushroom

4.5 DISCUSSION

Gas production can be regarded as an indicator of carbohydrate degradation and the low gas production in the untreated cob, husk, stover and straw could be explained by lignin binding to the carbohydrate. The inhibition of enzymes or microorganisms (Griffiths, 1986), complexing with lignocellulose, and preventing the microbial digestion could also be implicated. Tropical feeds contain considerable amounts of phenolic compounds that can reduce *in vitro* gas production (Sallam, 2005). However, since gas production on incubation of feeds in buffered rumen fluid is associated with fermentation, the low gas production from maize cob, maize husk, maize stover and maize straw could be related to the low nutritive value of these wastes and by-products. The higher gas production in the fungal treated substrates is further explained by the improvement in the CP and CF. In addition, cell wall contents (NDF and ADF) were negatively correlated with gas production at all incubation times. This may tend to reduce the microbial activity through increase in adverse environmental conditions as incubation time progress. This is consistent with De Boever *et al.* (2005), who reported that gas production was negatively related with NDF content and positively with starch. Also, the relatively higher level of ADL in all the untreated substrates explained in part the limited *in vitro* degradation and therefore the lower amount of gas produced. However, since gas production on incubation of feeds in buffered rumen fluid is associated with feed fermentation and carbohydrate fractions, the low gas production from the untreated substrates could be related to low feeding value of these feeds. On the other hand, the main reason for the higher degradability of the fungal treated substrates is the reduction in the lignin which protects carbohydrates from attack by rumen microbes. The current findings agree with such report (Melaku *et al.,* 2003) in which fibrous constituents, especially lignin negatively influenced *in vitro* gas production. Incubation of feedstuff with buffered rumen fluid *in vitro*, the carbohydrates are fermented to short chain fatty acids (SCFA), gases mainly CO_2 and CH_4, and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Wolin, 1960; Steingass and Menke, 1986) and substantial changes in carbohydrate fractions were reflected by total gas produced (Deaville and Givens, 2001). Gas production from protein

fermentation is relatively small as compared to carbohydrate fermentation while, contribution of fat to gas production is negligible (Wolin, 1960).

High fermentation of the insoluble but degradable fraction (b), obtained for the fungal treated substrates, especially maize stover could possibly be influenced by the carbohydrate fractions readily available to the microbial population (Chumpawadee, *et al.*, 2007). However, the case is slightly different for some fungi used in treatment of maize cob, maize husk and maize straw. This could be due to differences in physiological behaviour of the fungi used.

The fast rates of gas production (C), observed for the treated maize stover, maize husk, maize straw and maze cob, were likely influenced by soluble carbohydrate fractions readily availability to the microbial population. Slower rate of gas production in the untreated suggests that those feedstuffs were less depreciable by microbes in the rumen. The high rate of fermentation of treated substrates could also be related to its high CP content and low NDF, ADF and ADL. Reports (Kamalak, (2005) and Abdulrazak *et al.*, 2000) suggested that gas production and estimated parameters are negatively correlated with NDF and ADF. The present study estimated ME of fungal treated and untreated maize cob, maize husk, maize straw and maize stover were similar to those reported elsewhere (Nitipot and Sommart, 2003). In addition, with the exception of *Lentinus subnudus* treated maize stover and maize straw, other estimated ME values were observed to be lower than that reported by NRC (2001). Menke and Steingass (1988) reported a strong correlation between ME values measured *in vivo* and predicted from 24hr *in vitro* gas production and chemical composition of feed. The *in vitro* gas production method has been widely used to evaluate the energy value of several classes of feed (Aiple *et al*, 1996, Getachew *et al*., 1998; Getachew *et al*, 2002,). Krishamoothy *et al*., (1995) also suggested *in vitro* gas production technique should be considered for estimating ME in tropical feedstuffs, because evaluation of ME by other technique required labour, cost, time and complexity. There was a positive correlation between metabolizable energy calculated from *in vitro* gas production together with CP and fat content with metabolizable energy value of conventional feeds measured *in vivo* (Menke and Steingass, 1998). Menke and Steingass (1998) and Chenost *et al.*, (1997) concluded

that the estimation of ME is more accurate when based on gas and chemical constituents measurements as compared to calculations based on chemical constituents only. Also, there are significant correlation between *in vitro* gas measurement and *in vivo* digestibility.

The higher SCFA predicted from gas production in the treated maize cob, maize straw, maize husk and maize stover is probably due to higher absolute gas production, which was most evident in the 24hr of incubation (Fig. 1 – 4). The gas production from cereal straws (Blummel and Orskov, 1993), cereal grains (Opatpatanakit *et al.*, 1994) and different classes of feeds (Blummel and Orskov, 1990) incubated *in vitro* in buffered rumen fluid was closely, related to the production of SCFA. This was based on carbohydrate fermentation. Getachew *et al* (2002) reported the close association between SCFA and gas production *in vitro.*The use of this relationship between SCFA and gas production to estimate the SCFA production from gas values, which is an indicator of energy availability to the animal (Sallam *et al.*, 2007). High OMD obtained in the treated substrates may be due to the major carbohydrate of the substrate which is mainly starch, fermented by amylolytic bacteria and protozoa (Kotarski *et al.,* 1992). This result implies that the microbes in the rumen and animal have high nutrient uptake. The higher fiber content of the untreated wastes probably resulted in lower OMD since high NDF and ADL content in feedstuffs result in lower fiber degradation (Van Soest, 1988). In general, the tropical forages and concentrate feedstuff have a large proportion of lignified cell walls with low fermentation rates and digestibility, leading to low digestibility rates and limited intake (Ibrahim *et al.,* 1995; Hindrichsen *et al.,* 2001). However, fungal treatment greatly improved the CF and CP hence, high rates of digestibility and predicted OMD.

The *in vitro* gas production technique can be used to determine the nutritive value of fungal treated agricultural wastes and by-products, and to identify differences among their potential digestibility.

The high methane volume exhibited by *Lentinus subnudus* treated maize husk is indicative of energy loss to the animal. Babayemi, (2006) reported that methane production has negative effect on the animal in one hand as it is energy loss to the animal and on the other hand, when accumulates in the rumen results in bloat. However, the

reduction in methane in *Pleurotus pulmonarius* and *Pleurotus sajor caju* treated wastes could be due to the conversion of CO_2 and H_2 to acetate instead of methane. Ranke in order of improved gas production are : PTM, LSM, PPM, PSM and UMS

CHAPTER FIVE

5.0 EXPERIMENT THREE

PERFORMANCE CHARACTERISTICS OF THE WAD RAMS FED GRADED LEVELS OF PLEUROTUS TUBEREGIUM TREATED MAIZE COB AND GUINEA GRASS (*Panicum maximum*)

5.1 INTRODUCTION

In developing countries of the world, natural pastures make up the bulk of the feed consumed by grazing animals. This is because ruminants are endowed with the ability to degrade and utilize forages with the help of microbes in their rumen. However the major constraint to livestock production in Nigeria, is inadequate all year-round supply of pasures. There is therefore, a need to search for more available roughage particularly agricultural by-product such as maize cob. Such agricultural by-product may be bulky with high fibre, low protein, vitamins and minerals. Therefore sustainability of livestock on grasses and crop residues alone become difficult, hence the need for alternatives. Fungal treatment is preferable to other treatment, such as application of chemical (Salman *et al.,* 2008). Burning of crop residues and by-product leads to environmental pollution, consequently health hazards.

Recently, the production of microbial protein from agricultural wastes and by-products has received the attention of several workers (Bellany, 1975; Dunlop, 1975; Han and Anderson, 1975; Moo-Young *et al.,* 1978; Garg and Neolantan, 1981). Much interest has been evinced in new biotechniques for improving the nutritive value of lignocellulose using biological treatment in solid substrate fermentation under non-sterile conditions (Gupta *et al.*, 1985). Such scientists (Shoukry *et al.,* 1985; Khorshed 2000; El-Ashry *et al.,* 2003, and El-Kady *et al.,* 2006), used biological treatments like *Trichoderma viride* to improve the nutritive value and digestibility of poor quality roughages.

Fungal treatment will lead to the beneficial effects in ruminant performance and such treatments are most likely to be of great value for livestock in positive nitrogen balance. *Pleurotus tuber-reguim* was used to improve the nutritive value of maize cobs. This study was conducted to investigate effects of dietary levels of *Pleurotus tuberregium* treated maize cob on the performance of West African dwarf (WAD) rams grazing *Panicum*

maximum. Nutrient digestibility, nitrogen balance, NH_3-N, TVFA and pH were the criteria for evaluation

5.2 MATERIALS AND METHOD

5.2.1 Fungal treatments of maize cob in large scale (on-farm condition)

The experiment was carried out at the small ruminant unit of Research Farm National Cereal Research Institute (NCRI) Moore Plantation, Ibadan between the period of August and December, 2009.

A heap of 500kg of milled maize cob was moistened with water on a concrete floor, covered with cellophane sheet and allowed to ferment for two weeks. The fermenting heap of milled maize cob was turned every third interval to allow even distribution of heat. After the completion of the composting process, the fermented substrate was then transferred to 3-tier inoculation trays (2ft x 6ft) and allowed to cool before inoculating with active fungal culture (spawn). The mixture of active fungal culture prepared in bags was used at 10% w/w, mixed well into the cool fermented maize cobs and allowed to ferment for 40 days.

At the end of the fermentation period, the treated maize cob was sun dried until the substrate attained less than 10% moisture content.It was then bagged and stored until required for feeding trials with West African Dwarf (WAD) rams.

5.2.2 Preparation of active fungal culture for on-farm inoculation of maize cob.

The active fungal culture of *Pleurotus tuber-reguim* obtained from the culture bank of Department of Botany and Microbiology, University of Ibadan was reproduced in bags for on-farm inoculation. Each 5kg bag of sterilized guinea corn grains was inoculated at 5% w/w, and immediately the bag was sealed and kept in a dark room for two weeks to allow total ramification of the guinea corn grains by the active fungal culture. The treated substrates were subsequently used for inoculation on large scale.

Table 13: Gross composition (%) of experimental diets

Ingredient	T_1	T_2	T_3	T_4	T_5
Maize bran	30	30	30	30	30
PKC	10	10	10	10	10
GNC	10	10	10	10	10
Wheat offal	40	30	20	10	-
FTMC	-	10	20	30	40
Common salt	1.0	1.0	1.0	1.0	1.0
DCP	3.75	3.75	3.75	3.75	3.75
SBM	3.75	3.75	3.75	3.75	3.75
Vit. Premix	1.50	1.50	1.50	1.50	1.50
Total	100	100	100	100	100

T_1 = 0% Fugal treated maize cob, T_2 = 10% fungal treated maize cob, T_3 = 20% fungal treated maize cob, T_4 = 30% fugal treated maize cob, T_5 = 40% fungal treated maize cob, PKC = Palm kernel cake, GNC = groundnut cake, FTMC = Fungal treated maize cob, DCP = dicalcium phosphate, SBM = soybean meal.

5.2.3 Formulation and composition of the experimental diets

Five experimental diet supplements feed mixtures were formulated as follows:

T_1 = the first mixture contained 0% fungal treated maize cob

T_2 = the second mixture contained 10% fungal treated maize cob

T_3 = the third mixture contained 20% fugal treated maize cob

T_4 = the fourth mixture contained 30% fungal treated maize cob

T_5 = the fifth mixture contained 40% treated maize cob.

The dietary ingredients were mixed fortnightly and packed in sacks lined with polythene sheets to avoid rancidity and loss of palatability. Formulation of diets, proximate composition and gross energy of diets, and proximate composition of guinea grass (*Panicum maximum)*, wheat offal and fungal treated maize cob (FTMC) are presented in Tables 13, 14, and 15.

Table 14: Proximate composition and gross energy of experimental diets

Parameters	T_1	T_2	T_3	T_4	T_5
DM	89.88	89.66	89.83	89.76	89.21
CP	16.56	16.74	16.38	15.96	16.14
CF	22.14	17.89	19.21	20.69	21.76
Ash	8.21	10.28	11.26	9.28	10.81
EE	3.87	3.64	3.61	3.76	3.58
NFE	49.22	51.00	49.54	50.31	48.21
GE Kcal/kg	3985	3978	3876	3787	3724

DM = Dry matter, CP = Crude protein, CF = Crude fibre, EE = ether extract, NFE = Nitrogen free extract, GE = Gross energy, T_1 = 0% Fugal treated maize cob, T_2 = 10% fungal treated maize cob, T_3 = 20% fungal treated maize cob, T_4 = 30% fugal treated maize cob, T_5 = 40% fungal treated maize cob,

Table 15: Proximate composition of Guinea grass, wheat offal and fungal treated maize cob (FTMC)

Parameters	Guinea grass	**Wheat offal**	**FTMC**
Dry matter	42.03	90.00	92.50
Crude protein	6.93	17.13	18.80
Crude fibre	43.49	12.17	19.36
Ether extract	3.21	5.56	8.73
Ash	10.15	6.30	7.85
Nitrogen free extract	36.22	31.16	37.41

5.2.4 Experimental animals

Twenty (20) West African dwarf (WAD) – rams that were growers aged 5-6 month weighing 15.6-16kg were used for the experiment. They were purchased from the sheep market in Iwo, Oyo state. On arrival, the WAD-rams were given prophylactic treatments, which consisted of intramuscular application of oxytetracycline and vitamin B complex, at the dosage of 1ml/10kg body weight of the animal. They were also drenched with 10% lavasol to control endoparasite, and treated for mange and other ectoparasites using ectopore. A preliminary period of 14 days was allowed to acclimatize the animals to their new environment and feed.

5.2.5 Housing

Each WAD-ram was housed individually in a paired well ventilated metabolic cage made of wood and partitioned. Each metabolic cage was equipped with wooden feed troughs and plastic drinkers.

5.2.6 Feeding of WAD-rams

On arrival, the rams were allowed six weeks to acclimatized to the experimental diets with gradual withdrawal of their usual diets which they were used to. The twenty (20) WAD rams were weighed and randomly allotted to five groups of four replicates in a completely randomized design (CRD). For easy identification and separation, labeled tag was placed on each animal corresponding to the pen number. The supplemental feed diet (Table1) was fed at 5% body weight and thereafter released to zero graze on *Panicum maximum*. Refusals were weighed the following morning and deducted from the total amount served, for the determination of feed intake. Daily feeds were served to meet 5% of rams body weight and were frequently adjusted to ensure that each animal received about 20% of feed above its previous days consumption. Samples from refusal were taken for proximate composition. Fresh water was served daily; Salt licks was placed permanently in each pen. Weights of the rams were taken before the commencement of the experiment and subsequently once a weekly. The rams were weighed in the morning before feeding.

A one hundred and five days (105) feeding trial was initiated between December 2008 and March 2009.

5.2.7 Digestibility trials

Twenty WAD-rams used for the growth studies were randomly selected for determining the digestibility and N-balance of the diets. The rams were confined in individual modified metabolism cages (Akinsoyinu, 1974) for separate collection of faeces and urine in a completely randomized designed. The rams were offered the experimental diets prior to 7 days digestibility period. The animals were weighed at the beginning and end of the digestibility trials. During 7 days of collection period, total faeces were weighed daily. A 10% sample of total faeces was stored in a freeze at -4^0C. The sample of each day was bulked at day 7of collection. The bulk for each animal was mixed and dried in the oven at 60^0C for chemical analysis. Individual urine sample was collected and weighed daily in the morning using measuring plastic container. At collection, 2ml of 10 Tetraoxosulphate VI acids (H_2SO_4) was added to each container to prevent microbial growth and loss of nitrogen. Ten percent of total urine was taken daily and stored at -4^0C for nitrogen analysis. Daily feed was served at 5% body weight. Refusals were taken and mixed for the entire collection period on individual basis using an air tight plastic bag. On the last day of digestibility trial, samples of ruminal contents were taken at 3hr post feeding via stomach tube and strained through four layer of cheese cloth. Samples were separated into 2 portions, the first was used for immediate determination of ruminal-pH using digital pH meter and ammonia-nitrogen (NH_3-N) according to AOAC,(1995), while the second portion was stored at -20 ^{O}C after adding two drops of toluene and a thin layer of paraffin oil till analysis for TVFA's according to Warner (1964).

5.3 CHEMICAL ANALYSIS

The supplemental feeds refusals and dried faeces for each animal were ground through a 1mm mesh screen for analysis. Two gm of milled samples in duplicate was taken proximate analysis. Nitrogen (N) content of each milled sample was determined by the standard Kjeldhal method (AOAC, 1991) and the amount of crude protein (CP) was (N x 6.25). Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and crude fiber (CF) were assessed using the methods proposed by Van Soest *et al.,* (1991).

5.4 STATISTICAL ANALYSIS

Data were subjected to analysis of variance using procedure of SAS (1999). Significant means were separated using the Duncan Multiple range test of the same package. Experimental model of the design is:

$Yij = \mu + \alpha_i + \Sigma ij$; Σij = Composite Error

Yij = Individual observation

μ = General mean of the population

α_i = Treatment effect

5.5 RESULTS

5.5.1 Feed intake

Dry matter intake (DMI), final body weight, means weight gain (MWG), average daily gain (AVDG) and feed conversion ratio (FCR) of the experimental rations are presented in Table 16. The results showed that treatment effects on DMI, MWG and AVDG were significant ($P<0.05$) and higher for WAD rams on T_5 followed by T_4, T_3 and T_2 with the least value obtained for animals on the control T_1. Animals on T_5 which is 100% replacement of wheat offal had the highest DMI, MWG and AVDG. It was observed that DMI increased with increasing levels of supplementation and with corresponding increased MWG and AVDG. The average FCR values were 9.99, 8.33, 8.34, 8.28 and 7.99 for animals on T_1, T_2, T_3, T_4 and T_5 respectively. The WAD rams on group T_5 had the lowest FCR (7.99). The values for FCR for group T_5 were significantly ($P < 0.05$) higher than others. However, variations in the FCR values for rams on T_2, T_3, T_4 and T_5 were not significant. The total DMI/BW % values were 3.03, 3.08, 3.04, 3.05, and 3.08 for rams on T_1, T_2, T_3, T_4 and T_5 respectively. Also rams on T_3 and T_4 were not significant ($P > 0.05$), such variations however, were significantly ($P < 0.05$) higher for rams on T_5.

5.5.2 Apparent digestibility

Apparent digestibility by WAD rams fed graded levels of fungal treated maize cobs (FTMC) are shown in Table 17. Treatment effects on apparent digestibility of CP, CF, EE, Ash, NFE, ADF, NDF, cellulose and hemicellulose were significant ($P < 0.05$) for animals on T_5 followed by T_4, T_3 and T_2. However, the least value was recorded for animals fed the comtrol diet (T_1). It was observed that variations in apparent digestibility increased with increasing levels of supplementation with FTMC compared with the control diet. Variations in NFE and cellulose apparent digestibility were not significant ($P > 0.05$) in T_2 and T_3.

5.5.3 Nitrogen utilization and rumen fluid parameters

Data of nitrogen utilization and rumen fluid parameters are presented in Table 18. Treatments effects on N-intake of FTMC were significant ($P<0.05$). Faecal-N was higher for animals on T_1 (1.99 g/d kgW $^{0.75}$), T_2 (1.65 g/d kgW $^{0.75}$) and T_4 (1.55 g/d kgW $^{0.75}$) compared with those on T_3 (0.94 g/d kgW $^{0.75}$) and T_5 (0.99 g/d kgW $^{0.75}$). Variation in

urinary nitrogen as affected by treatment was not significant (P > 0.05) for T_1, T_2, T_3 and T_5. The highest (4.18g/d kgW $^{0.75}$) and least (2.49g/d kgW $^{0.75}$) Nitrogen balance were obseverd for animals on T_5, which is 100% replacement of wheat offal and conrol diet.

Data illustrated in Table 18 indicated that ruminal-pH as affected by treatments were significant (P<0.05) for rams on T_3 and T_4 compared with those T_1 and T_3 after 3hrs post feeding. The pH values recorded ranged from 6.76 to 9.08. The TVFA's obtained in this study were 10.10meg/mL, 10.30 meq/mL, 11.92 meg/mL, 12.37 meq/mL and 12.84 meg/mL for T_1, T_2, T_3, T_4 and T_5 respectively. Variations in TVFA were generally higher in all the fungal supplemented treatments compared with control. Similarly, NH_3-N increased significantly (P > 0.05) with increasing levels of supplementation. The values recorded were 18.20 mg/mL, 19.60 mg/ml, 23.75mg/ml, 24.20 mg/ml and 26.40 mg/mL for T_1, T_2, T_3, T_4 and T_5 respectively.

Table 16: Growth Performance of WAD rams fed the experimental diets

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
DMI g/d $W^{0.75}$	132.68^{e}	134.48^{d}	136.46^{c}	139.41^{b}	141.45^{a}	0.003
Initial body weight, kg	13.00	12.75	13.00	13.00	12.75	-
Final body weight, kg	21.13^{e}	22.33^{d}	23.57^{c}	26.01^{b}	27.33^{a}	0.003
Mean weight gain, kg	8.13^{e}	9.58^{d}	9.67^{c}	10.33^{b}	11.58^{a}	0.003
Total DMI/BW%	3.03^{c}	3.08^{a}	3.04^{bc}	3.05^{b}	3.08^{a}	0.020
Feed conversation ratio	9.99^{a}	8.83^{b}	8.34^{b}	8.28^{b}	7.79^{b}	0.210

abcd means along the same row with different superscripts are significant ($P < 0.005$), T_1 = 0% Fugal treated maize cobs, T_2 = 10% fugal treated maize cobs, T_3 = 20% fungal treated maize cobs, T_4 = 40% fungal treated maize cobs, SEM = Standard error of mean.

Table 17: Apparent digestibility by WAD rams fed graded levels of Fungal treated maize cob (FTMC)

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
CP	60.00^{e}	65.90^{d}	67.21^{c}	69.47^{b}	71.83^{a}	0.21
CF	60.08^{e}	62.32^{d}	64.49^{c}	66.23^{b}	67.34^{a}	0.003
EE	66.55^{e}	67.12^{d}	68.72^{c}	71.01^{b}	73.91^{a}	0.003
Ash	68.16^{e}	70.42^{d}	72.38^{c}	76.50^{b}	77.50^{a}	0.02
NFE	72.75^{d}	78.98^{c}	79.11^{c}	80.47^{b}	82.13^{a}	0.15
DM	64.57^{e}	66.03^{d}	67.67^{c}	69.47^{b}	70.58^{a}	0.003
ADF	71.45^{e}	76.86^{d}	77.28^{c}	78.62^{b}	81.81^{a}	0.003
NDF	70.10^{e}	73.60^{d}	77.76^{c}	81.00^{b}	81.91^{a}	0.15
Cellulose	66.97^{d}	75.20^{c}	75.44^{c}	78.59^{b}	81.83^{a}	0.02
Hemicellulose	62.40^{e}	69.25^{d}	70.33^{c}	72.85^{b}	74.19^{a}	0.02

abcd means along the same row with different superscripts are significant ($P < 0.005$), T_1 = 0% Fugal treated maize cobs, T_2 = 10% fugal treated maize cobs, T_3 = 20% fungal treated maize cobs, T_4 = 40% fungal treated maize cobs, DM = Dry matter, CP = Crude protein, CF = Crude fibre, EE = ether extract, NFE = Nitrogen free extract,ADF = Acid detergent fibre, NDF = Neutral detergent fibre, SEM = Standard error of mean.

Table 18: Nitrogen utilization and rumen fluid parameters of WAD rams fed experimental diet

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
N – intake $gd^{-1}/kgW^{0.75}$	8.71^{d}	8.76^{b}	8.76^{b}	8.55^{c}	9.08^{a}	0.003
Faecal-N (g/d^{1}) $kgW^{0.75}$	1.99^{a}	1.65^{ab}	0.94^{b}	1.55^{ab}	0.99^{e}	0.15
Urinary-N (g/d^{-1}) $KgW^{0.75}$	4.25^{a}	3.99^{ab}	4.20^{ab}	3.00^{b}	4.45^{a}	0.21
N-balance (g/d^{-1}) $KgW^{0.75}$	2.49^{b}	3.46^{a}	3.62^{a}	4.00^{a}	4.18^{a}	0.21
Rumen fluid parameters at 6hrs post feeding						
pH (g/d^{-1})	6.76^{a}	7.74^{b}	8.76^{b}	8.55^{c}	9.08^{a}	0.003
TVFA meq mL^{-1}	10.10^{e}	10.30^{d}	11.92^{c}	12.37^{b}	12.84^{a}	0.02
NH_3–N mg/ mL^{-1}	18.20^{e}	19.60^{d}	23.75^{c}	24.20^{b}	26.40^{a}	0.03

abcd means along the same row with different superscripts are significant ($P < 0.005$), T_1= 0% Fugal treated maize cobs, T_2 = 10% fugal treated maize cobs, T_3 = 20% fungal treated maize cobs, T_4 = 40% fungal treated maize cobs, TVFA = Total volatile fatty acid, N = nitrogen, SEM = Standard error of mean.

5.6 DISCUSSION

The DMI which increased with the increasing levels of fungal inclusion may be due to the beneficial effects of the fermentation. The DMI is a basic limiting factor in feed utilization since this will affect the overall performance of farm animals (Mako, 2009). These results were in agreement with other reports (Mahrous and Abou-Ammou, 2005; Bassuny *et al.,* 2005; and El-Kady *et al.,* 2006). They repeated that DMI of biologically treated roughage improved compared with untreated roughage. The higher values of MWG and AVDG obtained for rams in this study may be due to the higher DMI and utilization which affect overall performance. On the contrary, the rams on control (T_1) recorded the lowest MWG and AVDG, which might be due to 0% FTMC having high lignocelluloses bonding which limit digestibility. The higher AVDG of rams on treated ration could also be attributed to the higher and rapid by-pass of protein from the rumen and subsequently digestion and absorption in the abomasum and duodenum. Chen *et al.*, (1995) reported that microbial colonization of highly lignified particles is limited. Rams on treatments 2, 3, 4 and 5 consumed above 3% of their body weight, which agrees with the value of 3 to 5% body as DMI recommended for ruminants (ARC, 1980; Devendra, 1991).

The FCR is the quantity of feed required to produce a unit increase in body weight. Thus the lower the FCR, the better the FCR recorded for the WAD rams. The lowest FCR for rams on T_5 may be the influence of the DMI and more so, the relationship between total DMI and total weight gain of the rams on FTMC was proportional and highly significant ($P < 0.05$) indicating that DMI for rams on FTMC directly influenced the growth of rams. The high apparent digestibility of all the measured parameters obtained in the entire supplemented ration, especially T_5 may be indicative of proper utilization of the feedstuffs. Additionally, the increasing levels of FTMC showed beneficial effects of the fungal preparation used. On the same trend several authors (Titi and Lubbadeh, 2004; Wang *et al.,* 2004; Dean *et al.,* 2005; Eun and Beauchemin, 2005; Yu *et al.,* 2005; Mohammed *et al.,* 2005; El-Kady *et al.,* 2006) showed an increase in DM, CP, CF and NFE digestibility when fungal treated beet bulp were supplemented in animal diets. Deraz and Ismail (2001) reported that Trichoderma treatments had the effect of loosening lignocellulotic bonds and sulubilizing some of the hemicelluloses content. The highest

apparent digestibility of CP, CF, Ash, NFE, Cellulose, hemicelluloses, ADF and NDF could be an indication of increased microorganism's biomass, while the highest digestibility of CF may be due to increase in the activity of enzymes produced by microorganisms (Gado *et al.,* (2007a). Also Colombatto *et al.,* (2003) reported that enzymes were more efficient in degrading fibre without increasing methane production in the rumen of the animal.

It is observed in this study that T_5 (100% replacement of wheat offal) is the most suitable of the test diets.

The improved positive nitrogen is in agreement with other reports (Langer *et al.,* 1982; Marwaha *et al.,* 1990 and Bakshi and Jander, 1991) who observed when conducted feeding trial on growing Jersey calves fed fungal treated wheat. They found that retained nitrogen was 29% of N-intake. However, Walli *et al.,* (1991) noticed a positive N-balance when they fed calves on fungal treated wheat straw. The present observation is also consistent with the findings of other scientists (Kakkar *et al.,* 1990; El-Ashry *et al.,* 1997 and Gado *et al.,* 2007b).

Generally, the superiority of N-retention in a specific ration is affected by several factors. These include possible production of microbial protein synthesis and increased presence of fermentable energy (Hagemeister, *et al.,* 1981), differences in availability of fermentable energy (Tagari *et al.,* 1976), variability in nitrogen which might escape fermentation from the rumen and increased utilization of ammonia in the rumen (Holzer *et al.,* 1986) and the effect of free fats in protein synthesis (Sutton *et al.,* 1983).This may further explain the variations in nitrogen retention as affected by treatment

The lower pH obtained in T_3 and T_5 may be related to fermentation process of both non-structural and structural carbohydrate, and production of volatile fatty acids, which affected the pH to some limit until they are proportionally and relatively absorbed from the rumen wall. This assumption is in agreement with report elsewhere (Reddy and Reddy 1985) which stated that pH values were inversely related to TVFA's concentration in the rumen. Kay (1983) reported that the proportion of the individual fatty acids depends on the acidity of the rumen. The anaerobic fermentation of fungal treatment (T_4 and T_5) was more efficiently faster yielding more TVFA's than T_2, T_3 and control (T_1). Also, it may be due to the increase digestibility of organic matter (El-Ashry *et al.,* 2003).

The TVFA's concentration in the rumen may also be affected by other factor such as DM digestibility, rate of absorption, rumen pH, transportation of the digestion from the rumen to other parts of the digestive tract and the microbial population in their activities (Salman *et al.,* 2008). One factor or more of these cases could change its pattern with proceeding time and might affect the total concentration of TVFA's found in the rumen media. Higher values of rumen liquor ammonia-nitrogen observed with the fungal treated based rations especially T_5 indicated that the release of ammonia-nitrogen from those rations were easier (Pujszo, 1964) than the control or that the treated ration were well utilized by rumen microbes.

Other investigators attributed the increase in ammonia-nitrogen concentration in the rumen to reduction of ammonia-nitrogen absorption by rumen epithelium or to a decrease in the efficiency of microbial protein synthesis (Smith *et al.*, 1980; Ikwuegbu and Sulton, 1980). Moreover, others (Salman *et al.,* 2008; Khorshed (2000) and El-Ashry *et al.*, 1997) observed significant increases in rumen ammonia-nitrogen concentration with fungal treated residues. Yadav and Yadav (1988) observed that increased ruminal ammonia nitrogen concentration might be due to higher intake of nitrogen and higher crude protein digestibility. Their findings are consistent with the present result.

CHAPTER SIX

6.0 EVALUATION OF MEAT CHARACTERISTICS OF WEST AFRICAN DWARF RAMS FED GRADED LEVELS OF FUNGAL TREATED MAIZE COB AND GUINEA GRASS

6.1 INTRODUCTION

The production of sheep mutton (especially rams) is carried out in a wide range of environment using various different production systems throughout the world (Koyancu, 2008). Meat from sheep accounts for approximately 18.0% of the total red meat production in Turkey (TURKSTAT, 2005). The present meat production of West African dwarf rams is far from optimal.

There is therefore the need to continue to research on the performance of West African dwarf rams on different feeding regime using locally available feeding stuffs treating that can improve their performance. For this reason the carcass quality, mainly the prime cuts of West African dwarf rams previously on graded levels of fungal treated maize cob was evaluated.

6.2 MATRIALS AND METHODS

Four (4) grower rams were randomly selected per treatment for carcass evaluation. Prior to slaughtering, the animals were starved overnight, weighed, slaughtered by severing the jugular vein and allowed to bleed. The animals were then dressed. Each dressed carcass was split down along the dorsal mid line. The left and right were divided into prime cuts: neck, shoulder, rack, loin, flank and leg (Colomer-Rocher *et al.,* 1987).

6.2.1 Measurements

$$\text{Dressing \%} = \frac{\text{Hot carcass weight}}{\text{Live weight at slaughter}} \times \frac{100}{1}$$

Rib eye area – This is the area of the surface of the *Longissimus dorsi* at the ribbing site between 12^{th} and 13^{th} ribs perpendicular to the back line.

The rib-eye muscle was traced on an acetate paper and its area calculated in square centimeter by using graph sheet.

The offal was weighed.

6.3 STATISTICAL ANALYSIS

Data were subjected to analysis of variance using procedure of SAS (1999). Significant means were separated using the Duncan Multiple range test of the same package. Experimental model of the design is:

$Yij = \mu + \alpha_i + \Sigma ij$; Σij = Composite Error

Yij = Individual observation

μ = General mean of the population

α_i = Treatment effect

6.4 RESULTS

Table 19 shows the internal and external offal of West African dwarf rams fed graded levels of fungal treated corn cobs. There were wide variations in the different internal and external organs measured with the exception of live weight at slaughter. The hot carcass weight was highest for rams on T_2 followed by T_3, T_4 and T_5. However, the variations between T_1 (control) and T_4 was not significant ($P>0.05$). Dressing percentage (50.11%) and rib-eye-area (38.30cm^2) was significantly high for ram on T_5 compared with other treatments. The least values were obtained for rams on T_1, that is, the control diet. The various weights obtained for neck weight, loin weight, flank weight and leg weight were consistently high for rams on T_5 , when compared with other treatments followed closely by T_4 in most of the cases. Variations in rack weight and kidney weight were not significant ($P>0.05$). The heart and lung/trachea weight increased with increase in fungal inclusion. The variations observed for the skin were not significant between T_1 and T_2, and between T_4 and T_5. The weight of the head increased from 5.76kg in T_2 to 7.67kg in T_5. However, the different values obtained in T_2 to T_5 were not significant.

Table 19: Internal/External Offals of West African Dwarf (WAD) rams fed fungal treated corn cobs as percentage of slaughtered weight

Parameters	T_1	T_2	T_3	T_4	T_5	SEM
Live weight at slaughter (kg)	21.00	22.20	21.12	21.35	21.35	0.170
Hot carcass weight (kg)	9.55^{c}	11.99^{a}	10.47^{b}	10.20^{bc}	10.70^{b}	0.150
Dressing %	45.47^{e}	49.50^{b}	48.47^{c}	48.30^{d}	50.11^{a}	0.020
Rib-eye-area (cm^2)	28.55^{e}	30.70^{d}	32.35^{c}	36.50^{b}	38.30^{a}	0.260
Neck weight (kg)	0.75^{c}	0.76^{c}	0.78^{b}	0.79^{b}	0.82^{a}	0.003
Shoulder weight (kg)	0.65^{b}	0.68^{ab}	0.70^{ab}	0.72^{ab}	0.76^{a}	0.020
Rack weight (Kg)	0.40	0.43	0.44	0.47	0.50	0.020
Loin weight (kg)	0.25^{d}	0.27c	0.27^{c}	0.29^{b}	0.32^{a}	0.003
Flank weight (kg)	0.52^{d}	0.58^{e}	0.58^{c}	0.61^{b}	0.63^{a}	0.003
Leg weight (kg)	0.65^{d}	0.75^{c}	0.77^{b}	0.78^{b}	0.81^{a}	0.003
Head (kg)	5.76^{b}	6.14^{a}	7.07^{a}	7.25^{a}	7.67^{a}	1.280
Heart (kg)	0.28^{d}	0.27^{c}	0.27^{c}	0.29^{b}	0.32^{a}	0.003
Skin (kg)	11.67^{c}	11.99^{bc}	12.62^{ab}	12.94^{a}	13.02^{a}	0.150
Lung/Trachea (kg)	1.19^{e}	1.30^{d}	1.46^{c}	1.84^{b}	1.98^{a}	0.020
Kidney (kg)	0.14	0.14	0.14	0.14	0.14	0.003

abcd means along the same row with different superscripts are significant different ($P < 0.005$), T_1 = 0% Fugal treated maize cobs, T_2 = 10% fugal treated maize cobs, T_3 = 20% fungal treated maize cobs, T_4 = 40% fungal treated maize cobs, SEM = Standard error of mean.

6.5 DISCUSSION

The dressing percentage obtained in this study agrees with findings of other researchers (Akeapinar, 1981; Akgunduz *et al.,* 1994 and Ogan 2001) who reported a range in values from 47.60 to 53.20%. These results were also consistent with those measured by Macit (2002); Perez *et al.* (2002) and Shadnoush *et al*, (2004). The higher values obtained for those on treated diet could mean a beneficial effect of the fungal inclusion. It could also be deduced that the dressing percentage was influenced by differences in daily gain (Table 16). This observation agrees with those reported elsewhere (Omojola, 2006) who observed higher carcass weight with goat slaughtered with higher slaughter weight. This however disagrees with findings elsewhere (Wood *et al.,* 1980) who suggested that when slaughter weight increased, carcass consumption changed with a decrease in muscle proportion and an increase kidney pelvic and internal fat, which correspond to the standard lamb growth pattern. In this study there was no noticeable internal fat.

The rib-eye-area is an indication of meatiness. In the present study, rams on treated feeds were more meaty that the control. There are some reports that suggest that rib-eye-area decreased with the increased metabolic energy levels (Shiran, 1995).

Chestnutt (1994) reported that plain of nutrition had no effect on *Longissimus dorsi* muscle. Kirton *et al.* (1995) further stressed that breed and plain of nutrition did not influence *Longissimus dorsi* muscle and its depth. Similar result was reported by Shiran (1995) in Lori-Bakhtiari rams' lambs.

Several authors (Wood *et al.,* 1980; Akgunduz *et al.,* 1994 and Ogan 2001) noted that the effect of slaughter weight is associated with the offal. However, in this study, the effects are small and insignificant. The internal organs (Heart and lung/trachea) which were heavier for rams on treated diets could be probably due to the demand for oxygen in the blood myoglobin for respiration. This may also be attributed to better multiplication of rumen microbes and increase by-pass protein for small intestine enzymatic digestion and formation of body tissues.

6.6 CONCLUSION

From the results obtained in this study, it can be concluded that rams on fungal treatments influenced the carcass composition and dressing percentage of West African Dwarf rams. The best result was however observed at 100% level of inclusion in their diets.

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