# THE EFFECT OF BIODEGRADATION PERIOD ON THE CHEMICAL COMPOSITION AND *IN VITRO* FERMENTATION CHARACTERISTICS OF RICE STRAW WITH 15% PALM OIL SLUDGE INCLUSION IN RUMINANT FEEDING

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**BENIN CITY, NIGERIA**

**JANUARY, 2023.**

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF AGRICULTURE HONORS (B. AGRIC.) IN ANIMAL SCIENCE**

**JANUARY, 2023.**

## CERTIFICATION

This is to certify that this project titled **"The Effect of Biodegradation Period on the Chemical Composition and *in vitro* Fermentation Characteristics of Rice Straw with 15% Palm Oil Sludge Inclusion in Ruminant Feeding"** was carried out by **Esther Osariemen ESELE** with Matriculation Number **AGR1600181** of the Department of Animal Science, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria.

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| **MRS. B. O. ISAAC** | **PROF. J. A. IMASUEN** |
| ***Project Supervisor*** | ***Head of Department*** |
| **DATE:_____** | **DATE:_____** |

## DEDICATION

This project report is dedicated to God Almighty for a successful completion of my programme in the University of Benin. To YOU ALONE LORD be all the glory, honor and adoration, yesterday, today and (always) forevermore, Amen.

## ACKNOWLEDGEMENTS

My sincere and profound gratitude goes to God Almighty who gave me the grace and wisdom to carry out this research.

My humble gratitude goes to my project supervisor, Mrs. B. O. Isaac for her selfless guidance and correction that has led to the successful completion of my project. Special thanks go to my H. O. D. Prof. J. A. Imasuen and all the lecturers in Animal Science Department and Faculty of Agriculture at large. Thanks to you all for the knowledge impacted in me. May God richly bless you all.

Special thanks to my loving parents: Mr. and Mrs. P. F. Esele, my wonderful siblings, Richard, Elizabeth, Favour and Uyi for their extra ordinary love and support shown to me towards the successful completion of my undergraduate program.

My sincere gratitude also goes to my friends and course mates, Uwa, Silver, Osamudiamen, Sonia, Diamond, Berry and others too numerous to be mentioned, for their various contributions, you all are simple the best.

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## ABSTRACT

The nutritive value of rice straw biodegraded with *Pleurotus tuberregium* (PTR) were studied through analysis of their chemical composition, cell wall components, and in vitro fermentation characteristics. Chemical analysis showed an increase in the crude protein from 4.375 at (0% for 8 weeks) to 9.625 (at 0 % for 8 weeks), and increases from 3.500 (at 15% POS for 4 weeks) to 9.625 (at 15% POS for 6 weeks). Fungal treatment decreases crude fiber from 37.00 % in 0% POS rice straw treatment at 8 weeks to 27.00% in 0 weeks of biodegradation. The neutral detergent fiber, acid detergent fiber and hemicellulose was significantly different ($p<0.05$). A significant higher (80.33) OMD was obtained in rice straw after 8 weeks of biodegradation, and the gas volume produced was not significantly different after 24 hours of Biodegradation. It is therefore concluded from this study that *P. tuberregium* treatment on rice straw with 0% and 15% POS improved the potential feeding value of the resultant substrate. Therefore, the product of fungal treatment has a good potential as feed resources for ruminants.

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background of the Study

Poor feeding decreases productivity of the animal. A vast array of literature on nutrition-reproduction interactions shows that good feeding increases milk production of lactating animals. It also increases growth rate of meat producing animals, giving more meat. Good nutrition increases reproductive efficiency: higher cyclicity, lower age at first calving, lower inter-calving interval, higher productive life and higher profitability to farmers (FAO/IAEA, 2002). Furthermore, now a good body of evidence exists showing that *in-utero* nutrition has impact on productivity and health of off-springs later in life (Bell and Greenwood, 2013, 2016).

Rice straw is the vegetative part of the rice plant (*Oryza sativa* L.), cut at grain harvest or after. Rice straw is a versatile by-product of rice cultivation as it is used in many ways including fodder for livestock and even as a building material. However, the increase in productivity and size of paddy areas, among other things, has led to a huge excess of rice straw where the most cost-effective way of disposing of the residue is seen as burning the biomass in the paddy field (Kadam *et al*., 2000).

Now-a-days, there is global concern on human activities such as burning of wastes or refuse with the view of reducing impact of burning on ozone layer depletion. Such global concern, therefore, necessitated alternative option or method for recycling of waste or residues into beneficial products. The possibility of recycling rice straw into value added products then comes into view (Akinfemi and Ogunwole, 2012). It may be burned and left on the field before the next ploughing, ploughed down as soil improver or used as a feed for livestock (Kadam *et al.,* 2000).

Huge amounts of agricultural wastes are produced from crop farms all over the world annually (Akinyemi, 2012) one of which is rice straw. Rice straw, a residue arising from rice farming, is produced in copious quantities worldwide annually. It is a residue of the rice plant left after the grains have been harvested (Drake*et al.,* 2002). It is reported to be low in nitrogen, rich in polysaccharides and has a high lignin and silica content, limiting voluntary intake and reducing degradability by ruminal micro-organisms all of which serve as a hindrance to its effective utility as a livestock feed (Drake*et al.,* 2002 and Sarklong*et al.,* 2010).

Biodegradation of crop residues using edible mushroom has been reported to give rise to products with enhanced nutritive value that promote farm animal performance (Akinyemi*et al.,* 2010). Thus rice straw could also be converted to a useful animal feed resource through biological treatment. This is particularly

useful to ensure provision of animal protein sources for the teeming world population.

The palm oil sludge is the large liquid waste that comes from the clarification and sterilization processes in milling oil palm. It improves the nutritive value and digestibility of rice straw as well as its palatability. Palm oil sludge is commonly referred to as Palm Oil Mill Effluent (POME), which is brown slurry composed of 4-5% solids, mainly organic, 0.5-1% residual oil, and about 95% water. The effluent also contains high concentrations of organic nitrogen (Heuzé *et al.,* 2015). Bamikole and Ikhatua (2009) reported that POS has good potential as ruminant feed as an energy source and could be directly used in ruminant feeding as an energy source.

The *Pleurotus tuberregium* is an edible gilled fungus which is native to the tropics. It's a saprotroph found on dead wood. This mushroom has the ability to degrade cellulose and lignin. In this research, the *Pleurotus tuberregium* is used to break down the linking bond between lignin and cellulose which is responsible for the high level of high fibre in rice straw and in turn the low digestibility of the straw (Isikhuemhen, and LeBauer,2004). Kuforiji and Fasidi (2009) reported that hemicelluloses and cellulose a reduced when *Pleurotus tuberregium* was used during biodegradation of agro-industrial wastes.

## 1.2 Justification of the Study

Nigeria's population is growing at a faster rate than the increase in animal products in the country whose population is expected to reach 402 million people by the year 2050. Only an optimum animal production level will be able to help alleviate poverty, provide food security and meet other needs of such a growing population (Bamaiyi, 2013).

A large number of animals are suffering from a supply of adequate feeds both in quality and quantity. The available roughage and concentrate for feeding livestock can meet only 50 and 10% respectively of the requirement. Animal production as an important sector of the economy of any nation is crucial in ensuring food security. Livestock has been known for ages to meet the animal protein requirement of man and many other benefits they provide for farmers and the national economy.

Rice straw alone, which contributes a huge amount of about 87% of the roughage feed of animals (Haque*et al*., 2007) has been shown to have poor nutritive value due to the high fibrous nature so also palm oil sludge. Palm oil sludge is used to improve the palatability, digestibility and nutritive content of the rice straw. Biodegradation breaks down the cellulose and lignin content of rice straw and palm oil sludge, therefore improving their digestibility and as well as its nutritional value. *Pleurotus tuberregium* is used to break down the components of

the rice straw and palm sludge, thereby improving the rice straw as a feed for the animals.

Rice straw meets the criteria as non-conventional feedstuff, such as having a lot of availability, low competition with human's need as food and lower price. The utilization of rice straw as a ruminant feed is constrained by low level of protein and high ligno-cellulosic properties.

Biodegradation of rice straw is an avenue to reduce the cell wall properties and improve the crude protein content for better feeding value of ruminant livestock.

## 1.3 Objective of the study

### 1.3.1 Main objective

To determine the effect of biodegradation period on the chemical composition and *in vitro* fermentation characteristics of rice straw with 15% palm oil sludge inclusion in ruminant feeding.

### 1.3.2 Specific objectives are to determine the:

1. effect of biodegradation on the chemical composition (CP, Ash, OM, NDF, ADF and Hemicellulose) of rice straw and palm oil sludge mixture (85% rice straw and 15% oil palm sludge);
2. effect of biodegradation on the *in vitro* fermentation parameters of rice straw, palm oil sludge and their mixture;

3. dry matter intake, digestibility and nitrogen utilization of rice straw, palm oil sludge and their mixtures at 2, 4, 6 and 8 weeks inoculation *in vitro*.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The Utilisation of Rice Straw in Ruminant Feeding

Ruminants are endowed with the ability to convert low quality feed into high quality protein and utilize feeds from land not suitable for cultivation of crops, however, the utilization of these low quality crop residues is hampered by its low protein content, fibre, digestibility, vitamin and minerals (Akinfemi, 2010).

Most of the rice straws generated from rice farming in Nigeria are either fed to livestock, burnt or allowed to rot. Apart from the inherent problem of air pollution caused by burning, it also releases particle matter into the atmosphere. In recent time, there is global concern on human activities such as burning of wastes or refuse with the view of reducing impact of burning on ozone layer depletion. Such global concern, therefore, necessitated alternative option or method of recycling of waste or residues into beneficial products.

There is the need for increased food production from livestock through more efficient use of the available feeds, including rice straw. However, the relationship between available grazing land and animal populations in Africa, when compared to the rest of the world, shows a marked imbalance indicating the need for better use of crop residues if animal populations are to increase. Incomplete utilization

of the feed resources from the land, rather than limitations in the availability of land perse, represents the principal constraint to productivity from livestock as well as the viability of small farm systems in Asia (Devendra, 1983). Thirdly, the utilization of rice straw by ruminants is possibly the most efficient means of conversion of this residue to overcome problems of pollution through slow breakdown or burning. This means of disposal overcomes problems of collection and transportation as the straw is used on the farm and useful products such as draught power, meat, milk and manure are produced.

The voluntary intake of low quality roughage, such as straw, is regulated by interactions between metabolic processes in the tissues and the transactions that occur in the reticulo-rumen (Weston, 1979, 1982, 1984). With straw, the level of intake is governed by the amount of material in the reticulo-rumen, its rate of digestion and the rate of passage of digesta out of the reticulo-rumen. Thus, mechanical processes associated with digestion, namely; particle size reduction during eating and rumination, and rates of microbial fermentation are important in relation to the level of intake. These aspects have received very little investigation with rice straw, although it is known that the rate of digestion is depressed by nutrient deficiencies, particularly of nitrogen, which restrict microbial activity in the reticulo-rumen. In many cases, rectifying such deficiencies results in a significantly increased intake.

Where rice straw constitutes 100% of the diet, then all its limitations, in terms of physical characteristics, chemical characteristics and low contents of essential nutrients will manifest themselves and the animals will lose weight. However, sometimes even small contributions from other feeds in a mixed diet can produce markedly improved results through effects at the rumen and/or tissue level. Knowledge of the specific limiting nutrients in a particular batch of straw can enable the selection of the supplements that will make up those deficiencies effectively and there have been many examples in which this has been demonstrated.

### 2.1.1 Alternative Uses for Rice Straw

Several reviews (e.g. Han, 1978; Devendra, 1982; Castillo, 1983) have considered the alternative uses of rice straw. Consequently, these will only be dealt with briefly here. The more important uses of rice straw can be listed as:

- Burning.
- Fertilizer for paddy fields.
- Mulch for vegetable production.
- Substrate for mushroom growth.
- Fibre subjected to acid hydrolysis and the resulting sugars used for single cell protein production.
- Bedding for livestock and poultry.

- Fibre for paper manufacture or use in construction materials.
- Fuel to produce heat.
- Feed for livestock.

### 2.1.2 The utilization of Palm Oil Sludge (POS) in Ruminant Feeding

Palm oil sludge (POS) refers to a general description of the discharge from palm oil extraction. It includes various liquids, dirt, residual oil and suspended solids. The residual oil and suspended solids constitute an important source of animal feed for ruminants (buffaloes, cattle, goats and sheep) and non-ruminants (poultry and pigs). It is a waste of crude palm oil industry and relatively cheap to come by in Nigeria. Hence, it is reasonable to take the advantage of the ability of animals to turn this unconventional animal feed ingredient into useful animal products for human consumption and assess its potential in reducing lipid deposition in ruminants.

A diet based on oil palm by-products (palm kernel cake 30%, palm press fibre 15%, palm oil mill effluent 18%, molasses 35% plus urea and minerals) met the nutritional requirements of dairy cattle (Shibata *et al.,* 1988a; Shibata *et al.,* 1988b). In feedlot cattle fed a 40:60 mixture of dried palm effluent and palm kernel expeller meal, growth was lower (650-690 vs. 750-760 g/d) than in animals fed only palm kernel meal, but performance was still considered satisfactory because of the high price differential between palm effluent and palm kernel meal

(Yusoff*et al.,* 1989). In Thai native cattle fed *Plicatulum* hay as a roughage source, decanted palm oil effluent included at 40% in the concentrate affected intake, digestibility, rumen fermentation and blood metabolites and it was concluded that the optimum level should not exceed 30% (Seephueak *et al.,* 2011). In goats fed a basal diet of rice straw, 3:1 and 1:1 ratios of palm effluent. Palm kernel meal were equally good supplements in terms of live-weight gain (Phang*et al.,* 1992).

## 2.2 Chemical Composition of Rice Straw Biodebgraded by White-Rot Fungi

The nutritive value of rice straw treated with three different edible mushrooms: *Pleurotusostreatus* (POR), *Pleurotuspulmonarius* (PPR) and *Pleurotus tuber-regium* (PTR) were studied through analysis of their proximate composition, mineral composition, crude fibre fractions and in vitro digestibility. Results of the proximate analysis showed an increase in the crude protein from 4.69 % in control to 7.69 % for PTR. Fungal treatment decreased crude fibre from 32.89 % in control to 19.96 % in PTR. Treatment effect on cellulose, neutral detergent fibre, acid detergent fibre and acid detergent lignin was significant. The estimated metabolisable energy (ME) (MJ.kg-1 DM), organic matter digestibility (OMD %) and short chain fatty acid (SCFA) (µm) ranged from 6.47 (control) to 7.54 (POR), 51.17 (control) to 57.02 (POR) and 0.657 (control) to 0.848 (POR). Treatment effect on the insoluble but degradable fraction (b) was significant ranging from 22 mL in control to 28.33 mL in PTR. It is therefore concluded from this study that

treatment of rice straw with different edible mushrooms improved the potential feeding value of the resultant substrate. Therefore, the product of fungal treatment has a good potential as feed resources for ruminants (Akinfemi A. and Ogunwole O. A., 2012).

**Table 2.1: Proximate composition and cell wall contents (g.kg$^{-1}$dm) of fungal treated rice straw.**

| Component | Control | POR | PPR | PTR | SEM |
|---|---|---|---|---|---|
| Dry matter | 93.00$^{a}$ | 86.75$^{b}$ | 84.21$^{c}$ | 86.00$^{b}$ | 0.03 |
| Crude protein | 4.69$^{b}$ | 7.39$^{a}$ | 7.18$^{a}$ | 7.69$^{a}$ | 0.12 |
| Crude fibre | 32.89$^{a}$ | 20.96$^{b}$ | 21.59$^{b}$ | 19.96$^{c}$ | 0.17 |
| Ether extract | 1.66$^{b}$ | 2.09$^{a}$ | 2.13$^{a}$ | 2.33$^{c}$ | 0.07 |
| Ash | 11.95$^{a}$ | 8.26$^{d}$ | 8.31$^{c}$ | 9.26$^{b}$ | 0.003 |
| Nitrogen free extract | 48.81$^{b}$ | 61.30$^{a}$ | 60.79$^{a}$ | 61.38$^{a}$ | 0.13 |
| Neutral detergent fibre | 69.96$^{a}$ | 61.67$^{c}$ | 62.79$^{b}$ | 61.38$^{d}$ | 0.003 |
| Acid detergent fibre | 56.28$^{a}$ | 48.12$^{c}$ | 49.78$^{b}$ | 47.12$^{d}$ | 0.003 |
| Acid detergent lignin | 12.54$^{a}$ | 10.06$^{c}$ | 10.15$^{b}$ | 9.68$^{d}$ | 0.003 |
| Cellulose | 43.74$^{a}$ | 38.06$^{c}$ | 39.63$^{b}$ | 37.44$^{d}$ | 0.005 |
| Hemicellulose | 13.68$^{b}$ | 13.55$^{c}$ | 13.01$^{d}$ | 14.26$^{a}$ | 0.005 |

Row means with different superscripts differ significantly at ($P<0.05$), n=3
POR = *Pleurotusostreatus* treated rice straw.
PPR = *Pleurotuspulmonarius* treated rice straw.

The proximate composition of fungal treated rice straw presented in this study showed that changes in the CP contents compared favourably with those reported for some fungal treated residues favourably with those reported for some fungal treated residues (Akinfemi *et al.,* 2010c). Such apparent increase could be due to the proliferation of fungi during degradation (Farkas, 1979; Belewu and Belewu, 2005). This agrees with the report published by Farkas (1979) and Jacqueline and Viser (1996), who noted that the extracellular enzymes secreting fungus contain amorphous home and heteropolysaccharides, which are associated with fungal protein. Some authors (Zadrazil, 1993; Belewu and Okhawere, 1998, and Akinfemi *et al.,* 2010b) reported that colonization of substrates by fungal mycelia results in increase in their nutritional values.

The variations in the CP content as affected by the fungi used may be attributed to strain differences, length of fermentation and the physiological behaviour of the fungi. 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 straw. This means that fungi not only grow 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. This trend is consistent with decrease in NDF, ADF and ADL (Albores *et al.,* 2006).

The mineral contents (g.kg-1 DM) showed that PPR had the highest concentration of Ca (11.04) and Mg (5.00), and a significantly highest gas volume was obtained in PPR and the gas production rate constant (C) was not significant.

**Table 2.2: Mineral compositions (mg.kg$^{-1}$) of major minerals and trace minerals (ppm) of fungal treated rice straw.**

| Component | Control | POR | PPR | PTR | SEM |
|---|---|---|---|---|---|
| **Major minerals:** | | | | | |
| Na | 0.0609$^{a}$ | 0.0501$^{c}$ | 0.0350$^{b}$ | 0.0400$^{d}$ | 0.010 |
| K | 0.957$^{a}$ | 0.789$^{c}$ | 0.817$^{b}$ | 0.760$^{d}$ | 0.002 |
| Ca | 2.24$^{d}$ | 9.20$^{c}$ | 11.04$^{a}$ | 9.60$^{b}$ | 0.020 |
| P | 0.39$^{c}$ | 1.57$^{a}$ | 0.61$^{b}$ | 0.400$^{c}$ | 0.017 |
| Mg | 2.35$^{b}$ | 4.23$^{a}$ | 5.00$^{a}$ | 4.30$^{a}$ | 0.170 |
| **Trace minerals:** | | | | | |
| Cu | 0.005 | 0.012 | 0.014 | 0.017 | 0.001 |
| Fe | 0.45$^{d}$ | 0.64$^{a}$ | 0.60$^{b}$ | 0.47$^{c}$ | 0.002 |
| Zn | 0.021$^{b}$ | 0.053$^{a}$ | 0.050$^{a}$ | 0.030$^{b}$ | 0.002 |
| Mg | 0.29$^{d}$ | 0.43$^{a}$ | 0.41$^{b}$ | 0.32$^{c}$ | 0.002 |

Row means with different superscripts differ significantly at ($P<0.05$), n=3.
POR = *Pleurotusostreatus* treated rice straw.
PPR = *Pleurotuspulmonarius* treated rice straw.
PTR = *Pleurotus tuberreguim* treated rice straw, SEM = Standard error of mean.

## 2.3 Chemical Composition of Palm Oil Sludge Biodebgraded by White-Rot Fungi

The nutritive values of POS are variable depending on oil palm varieties (Bamikole and Ikhatua, 2009) and oil extraction process. POS from the palm oil mill with a wet processing is low in fat. In contrast, the POS from the palm oil mill with a dry processing with a high fat content, which is a POS were used in this experiment from dry processing.

In Nigeria, EE content of POS 33.9-38.84% was recorded (Bamikole and Ikhatua, 2009; Bamikole and Babayemi, 2008) while in Malaysia, EE content of POS6.33-8.8% was recorded (ShibataandOsman,1988; Vadiveloo, 1986). This is indicative of variation in the efficiency of oil extraction methods (Bamikole and Babayemi, 2008).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Experimental Sites

The biodegradation process was carried out at the Department of Crop Science Laboratory University of Benin. The chemical composition and in-vitro study were carried out at the Department of Animal Science Laboratory.

#### 3.1.1 Collection of Fresh Rice Straw and Palm Oil Sludge

Fresh rice straw and palm oil sludge was obtained from Weppa Wanofarm, Agene-bode, and Nigeria institute for palm oil Research (NIFOR) Benin City, Edo State respectively. The rice Straw samples were washed to remove sand and othewrdebri. Palm oil sludge (pos) and rice straw were thereafter sun dried in order to reduce moisture and avoid deterioration and spoilage. The rice straw sample were chopped to 3-4cm, both samples were therefore kept in air tight containers for further analysis.

#### 3.1.2 Collection of *Pleurotus tuberregium* Culture

Pure culture of *Pleurotus tuberregium* (Fr. Singer) was obtained from Plant Biology and Biotechnology (PBB) Laboratory Department University of Benin, Benin City, Edo State.

### 3.1.3 Preparation and Inoculation of Rice Straw

The moisture content of the Rice Straw (RS) was adjusted to 75% using sterile $H_2O$. Fifty(50) grames(g) of rice straw was thereafter loaded into a 35CL of sterile bottle cocked with cotton wool and covered with foil paper , this was done in replicated 10 times, the bottles were loaded into a pressure pot and heated for 30-mins. at 12$^oC$ the bottles were allowed to cool at room temperature before innoculation. Thereafter they were loaded into an inoculation chamber .the bottles substrates were then inoculated with pure culture of *Pleurotus tuberregium* (Fr. Singer). Termination was done after full colonization of substrates**.**

### 3.1.4 Preparation and Inoculation of Rice Straw and Palm Oil Sludge Mixture

The moisture content of rice straw and palm oil sludge (POS) was adjusted to 75%using sterile $H_2O$, fifty (50) grams of rice straw and palm oil sludge mixture in 85:15 respectively were loaded into a 35CL sterile bottle , cocked with cotton wool and covered with foil paper. This was done in 10 replicate each. The bottles samples were then loaded into a pressure pot and heated for 30 minutes at $121^0C$, and were allowed to cool at room temperature before inoculation.

Thereafter the bottles were loaded into the innoculation chamber. The bottle subtracts were innoculated at 5% level of substrate. Sampling was done at every two weeks interval for eight consecutive weeks.

Plate 1: The growth of *Pleurotus tuberregium*.


Plate 2: Inoculation of rice straw and palm oil sludge mixture.

## 3.2 Experimental Design

The study was carried out in a Completely Randomized Design (CRD)**.**

### 3.2.1 Experimental Treatments

Sampling was done at different biodegraded periods: 0, 2, 4, 6 and 8 weeks with 100% rice straw (0% POS) and 100% POS (0% rice straw) as control.

### 3.2.2 Chemical Composition

The standard methods of analysis of Association of Official Analytical chemist (AOAC, 2000) were used to determine the moisture content, crude protein, ash content, ether extract and nitrogen free extract of the samples, while that of Van Soest*et al.,* (1991) was used for the determination of the cell wall components which are Acid detergent fiber (ADF) and Neutral detergent fiber (NDF).

### 3.2.3 Determination of Dry Matter Digestibility

For the dry matter digestibility, samples of rice straw from each treatment were taken and weighed. The samples were then dried in oven at 100$^{o}$C until constant weight was attained and dry matter determined as expressed as the percentage of original weight.

$$DMD\% = \frac{\text{Weight of sample before incubation-weight of sample after incubation}}{\text{Weight of sample before incubation}} \times 100$$

### 3.2.4 Determination of Crude Protein

The determination of the Crude Protein was carried out using Kjedahl's procedure. One gram (1g) of the samples was measured into the digestion tube and then transferred to the digestion tube. 20 mL of 72% concentrated Sulphuric acid ($H_2SO_4$) and 4g of catalyst mixture (1:9 $CuSo_4$: $NaSo_4$) was added to the sample. The digestion tube was placed in a standard automated digester and heated to $42^0$C for 1 hour. The digest was put in a universal bottle (120 mL) and topped with distilled water to 50 mL mark.

After digestion, distillation was carried out by adding 5 mL if the digest to 5 mL of 60% NaOH in Kjeldahl's flask. This was distilled using automated distilling machine into 5 mL of boric indicator. About 150 mL of distillate was collected and titrated using 0.1N HCL. This was done for each treatment and replicated twice. The crude protein was calculated using the formula below.

$$CP = \frac{NA \times 14 \times VA \times 100 \times 100 \times 6.25}{1000 \times w \times 5}$$

Where NA = Normality of acid

VA = Volume of acid

W = Weight of the sample.

### 3.2.5 Determination of crude fiber

A known weight (0.5g) of the sample was weighed into a beaker and 50 mL of acid was added. The mixture was placed in the heating mantle with a temperature of 70-100$^{o}$C. The sample upon boiling was heated for 30 minutes while the 50 mL mark was maintained by topping with hot water. After heating, the mixture was filtered with a fiber cloth and the residue was washed with boiling water until a clear solution was attained. The residue was then placed back into the beaker and 50 mL of base was added. The mixture was placed back on the heating mantle with same temperature and upon boiling, was heated for 30 minutes while maintaining the 50 mL mark by topping with hot water. After heating, the mixture was filtered with fiber cloth and the residue was washed with boiling water until a clear solution was attained. The sample was placed in a lab oven of temperature 70$^{o}$C and dried for 30 minutes. After drying, the samples were placed in crucibles and weighed before heating in the muffle furnace at 550$^{o}$C for 30 minutes. The crucibles were left to cool in a desiccator after removal from the furnace and then weighed after.

The Crude fiber was calculated as thus:

$$\%CF = \frac{\text{Wt. of crude fiber}}{\text{Wt. of sample}} \times \frac{100}{1}$$

### 3.2.6 Determination of Ether Extract

1 gram of the milled samples were measured into a filter paper and then placed in the soxhlet extractor with the petroleum ether. The process was done until the water in the sample was clear. It was replicated three times.

$$\%EE = \frac{(\text{Wt. of filter paper+sample})-\text{Wt. of filter paper}}{\text{Wt. of Sample}} \times \frac{100}{1}$$

### 3.2.7 Ash and Organic Matter Determination

1 gram of the samples were weighed into crucible of known weight and heated in the muffle furnace at $550^0$C for 6 hours. The residue was left to cool in a dessicator after removal from the furnace and then weighed after cooling.

Organic matter was determined by subtracting the value of ash from 100.

Organic matter (%) = 100 - ash (%).

### 3.2.8 Acid Detergent Fiber

A known weight (1g) of the sample was weighed into a beaker and 100 mL of acid detergent solution was added, the mixture was placed in the heating mantle which had a temperature of 70 - $100^0$C. The sample upon boiling was heated for 1 hour while the 100mL mark was maintained by topping with hot water. After hearing, the mixture was filtered with darcon cloth and the residue was washed

with boiling water until a clear solution was attained. The sample was placed in the oven of temperature of $70^0$Cand dried for 24 hours and more until a constant weight was attained. This was carried out for the various treatments. The Acid Detergent Fiber (ADF) was calculated using the following formula;

$$\% \text{ ADF} = \frac{\text{Wt. of residue}}{\text{Wt. of sample}} \times \frac{100}{1}$$

### 3.2.9 Neutral Detergent Fiber

A known weight (1g) of the sample was weighed into a beaker and 100 mL of Neutral Detergent Solution was added, the mixture was placed in the heating mantle which had a temperature of 70 - $100^0$ C. The sample upon boiling was heated for 1 hour while the 100 mL mark was maintained by topping with hot water. After hearing, the mixture was filtered with darcon cloth and the residue was washed with boiling water. After filtering, the sample was placed in an oven for $70^0$C and dried for 24 hours and more until a constant weight was attained. This was carried out for the various treatments.

The Neutral Detergent Fiber was calculated using the following formula:

$$\% \text{ NDF} = \frac{\text{Wt. of residue}}{\text{Wt. of sample}} \times \frac{100}{1}$$

#### 3.2.10.1 *In vitro* Fermentation Study

#### 3.2.10.2 Sample Preparation

100g of substrate was put in a darcon bag, weighed, sealed and appropriately labeled.

#### 3.2.10.3 Buffer Preparation

Preparation of buffer was done a day before collection of rumen liquor and maintained at a temperature of $39^0$C and pH of 6.2. The modified *in vitro* fermentation procedure by Navaro-Villa *et al*. (2011) was adopted, a phosphate-bicarbonate buffer (Mould*et al*., 2005).

Used (g/LO) consists of:

- Disodium hydrogen phosphate anhydrous (Na2HPO4), $12H_20$ (1.985g).
- Potassiumdihydrogen phosphate ($KH_2PO_4$) (1.302g).
- Sodium hydroxide (NaOH) (0.100g).
- Sodium bicarbonate (NaHCO3) (5.418g).
- Magnesium chloride (with water) ($MgCL.6H_2O$) (0.105g).
- Ammonium hydrogen chloride ($NH_4HCO_3$) (1.407g).

#### 3.2.10.4 Collection and Preparation of Rumen Liquor

Rumen liquor was collected from goats housed at the University of Benin, Farm project, Ugbowo campus, Benin City. The rumen liquor was collected in the early hours of the morning before the animals were fed, into a pre-warmed flask. The collection was through a stomach tube the rumen liquor in the pre-warmed flask was taken to the laboratory where it was strained through fur layers of cheese cloth. The strained liquor was mixed with a buffer solution in a ratio of 1:2. The mixture was put in a water bath and gassed with $CO_2$ to maintain anaerobic condition and a temperature of $39^0$C to keep the microorganisms alive.

Plate 3: Collection of Rumen liquor.

Plate 4: Preparation of Rumen liquor.

#### 3.2.10.5 Incubation

The *In vitro* incubation was carried out using 100 mL calibrated syringes containing 30ml of the lnoculums (Rumen liquor: buffer, 1:2). The weighed darcon bags containing the substrates were place inside the syringes before the innoculum was introduced into the syringes. The syringes were fitted with silicon tube and clipped before placing them in the incubator at $39^0$C. The syringes containing only inoculum served as the blank while the bags containing only the substrate served as the control. The time for the commencement of incubation was noted and the syringes were monitored at three hour intervals for the next 24 hours. For each incubation time, the head space of the syringes were measured and recorded. At 24 hours of incubation, the final readings were taken and the syringes put on ice to stop further gas production.

Plate 5: Syringe containing Inoculum and Darcon bag of sample.

Plate 6: Clipping Syringe before putting in the incubator.

### 3.2.10.6 Determination of Methane and Dry Matter Digestibility

Forty percent (40%) NaOH was prepared and four (4) mL was injected into the incubation syringes after the 24th hour. The NaOH absorbs $CO_2$ to leave $CH_4$. The volume of grass remaining in the syringes gave the volume of methane produced. Thereafter, the bags containing the samples and were removed from each syringes and washed under running tap water until the water became clear.

The bags were then dried at $100^{0C}$ to constant weight. Dry matter digestibility (DMD) was calculated as follows:

$$DMD\% = \frac{\text{Weight of sample before incubation-weight of sample after incubation}}{\text{Weight of sample before incubation}} \times \frac{100}{1}$$

The Fermentation Efficiency (FE) and effect of methane reduction ($CH_4$) is calculated as:

$$FE = \frac{\text{Dry matter digestibility (mg/kg)}}{\text{Total gas volume (mL/g)}}$$

$$CH_4red\ (\%) = \frac{\text{Average } CH_4 \text{ of the control- } CH_4 \text{ of treated sample}}{\text{Average } CH_4 \text{ of the control}} \times \frac{100}{1}$$

### 3.2.10.7 Estimation of post in vitro fermentation parameters

The post incubation parameters such as short chain fatty acids (SCFA) and organic matter digestibility (OMD%) were estimated at 24 hours post gas production using the equation established by Menke and Stengass (1988) and Metabolizable Energy (ME) by Getachew *et al.* (1999) as stated below:

SCFA = 0.0239 GV – 0.0601

OMD% = 14.88 + 0.88 GV + 0.45 CP + 0.651XA

ME = 2.20 + 0.136 GV + 0.057 CP

Where: GV = net gas produced (mL/mg DM) at 24 hour incubation time,

CP = crude protein sample at 24 hour incubation time.

XA = ash of incubated sample

### 3.3 Statistical Analysis

Data were analyzed using analysis of variance in a completely randomized design of SAS (2014) procedure. Separation of means was done using Duncan Multiple Range Test (DMRT).

# CHAPTER FOUR

## 4.0 RESULTS

## 4.1 Chemical Composition of Raw and Biodegraded Rice Straw with 15% Palm Oil Sludge

Table 1 captured the dry matter, crude protein, ether extract, ash and nitrogen free extract of rice straw with 15% palm oil sludge.

### 4.1.1 Dry Matter

The highest dry matter content (g/100g DM) of rice straw (RS) with 0% palm oil sludge (POS) (93.50) was recorded at 6 weeks and the lowest (91.75) at 4 weeks of biodegradation, there were significantly different ($p<0.05$) from one another. At 15% POS, no significant different ($p>0.05$) was observed after 8 weeks of biodegradation.

### 4.1.2 Crude protein

There was no significant difference ($p>0.05$) observed for RS after 8 weeks of biodegradation. Rice straw with 15% POS for 2 (4.375) and 6 weeks (9.625) recorded the lowest and highest CP content (g/100g DM) respectively. The CP content at 6 weeks and 8 weeks (8.750) were not significantly different ($p>0.05$). However the aforementioned was significantly different ($p<0.05$) from raw RS with 15% POS (at 0 week).

### 4.1.3 Ether Extract

Ether Extract (EE) content (g/100g DM) ranges from 12.50 to 16.50 at 8 and 0 weeks of biodegradation for rice straw with 0% POS respectively, These were significantly different ($p<0.05$) from each other. At 15% POS, the highest and lowest EE content were obtained at 0 (16.00) and 2, 6 (13.00) weeks of biodegradation respectively. These were significantly different ($p<0.05$) from one another. The EE content at 0 weeks was not significantly different ($p>0.05$) from 4 (15.50) and 8 (14.00) weeks.

### 4.1.4 Ash

The ash content (g/100g DM) of rice straw with 0% POS for 8 weeks of biodegradation was significantly ($p<0.05$) higher than 17.50 and 20.00 for 2 and 4 weeks of biodegradation respectively. The ash content of RS with 15% POS ranges from 1600 - 22.50 in 4 and 6 weeks respectively. No significant different ($p>0.05$) was observed in the ash content of 0, 8, (19.00) and 2 (18.00) weeks from each other.

### 4.1.5 Nitrogen Free Extract

No significant difference ($p>0.05$) was observed In the nitrogen free extract value of RS with 0% and 15% POS after 8 weeks of biodegradation and control samples (0 week).

**Table 4.1: Chemical composition of rice straw with 15% palm oil sludge at 2, 4, 6, and 8 weeks of biodegradation.**

| Chemical Components | Biodegradation period (weeks) | Different inclusion level of POS | |
|---|---|---|---|
| | | 0% | 15% |
| **DM** | 0 | 92.25$^{ab}$ | 93.75 |
| | 2 | 92.25$^{ab}$ | 94.00 |
| | 4 | 91.75$^{b}$ | 93.25 |
| | 6 | 93.50$^{a}$ | 93.25 |
| | 8 | 92.00$^{ab}$ | 93.75 |
| | **SEM** | **0.395** | **0.559** |
| **Cp** | 0 | 7.875 | 6.125$^{ab}$ |
| | 2 | 8.750 | 4.375$^{c}$ |
| | 4 | 7.000 | 3.500$^{c}$ |
| | 6 | 4.375 | 9.625$^{a}$ |
| | 8 | 9.625 | 8.750$^{ab}$ |
| | **SEM** | **1.438** | **0.732** |
| **EE** | 0 | 16.50$^{a}$ | 16.00$^{a}$ |
| | 2 | 14.50$^{ab}$ | 13.00$^{b}$ |
| | 4 | 14.50$^{ab}$ | 15.50$^{ab}$ |
| | 6 | 16.00$^{ab}$ | 13.00$^{b}$ |
| | 8 | 12.50$^{b}$ | 14.00$^{ab}$ |
| | **SEM** | **0.975** | **0.707** |
| **ASH** | 0 | 21.50$^{ab}$ | 19.00$^{ab}$ |
| | 2 | 17.50$^{b}$ | 18.00$^{ab}$ |
| | 4 | 20.00$^{b}$ | 16.00$^{b}$ |
| | 6 | 23.00$^{ab}$ | 22.50$^{a}$ |
| | 8 | 29.00$^{a}$ | 19.00$^{ab}$ |
| | **SEM** | **2.019** | **1.432** |
| **NFE** | 0 | 9.380 | 19.13 |
| | 2 | 19.00 | 31.13 |
| | 4 | 17.75 | 25.75 |
| | 6 | 19.63 | 26.13 |
| | 8 | 13.87 | 27.50 |
| | **SEM** | **4.45** | **4.90** |

Means on the same volume with different superscript (a, b, c) are significantly ($p<0.05$) different.

SEM= standard error of mean, DM = Dry matter, CP= Crude protein, EE= Ether extract, NFE= Nitrogen free extract.

0% POS = Rice straw with 0% POS, 15% POS= Rice straw with 15% POS.

Table 2 presents the crude fiber, Neutral detergent fiber, Acid detergent fiber and Hemicellulose content of rice straw with 15% palm oil sludge**.**

### 4.1.6 Crude Fiber

There was no significant difference ($p>0.05$) observed In the crude fiber values of RS with 0% and 15% POS after 8 weeks of biodegradation and control (0 week).

### 4.1.7 Neutral Detergent Fiber

There was no significant difference ($p>0.05$) at 0% rice straw after 8 weeks of biodegradation. Neutral detergent fiber (NDF),which is a cell wall fraction were also presented in the table 4.2, the NDF value range from 54.00 (at 0 week) to 89.00 (at 4 weeks) for raw rice straw with 15% POS. The aforementioned was significantly different ($p<0.05$) from other weeks of biodegradation.

### 4.1.8 Acid Detergent Fiber

The acid detergent fiber (g/100gDM) value range from 71.50 - 81.00 for RS with 0% POS, however no significant difference ($p>0.05$) was observed within treatments. The lowest ADF value was recorded at 0 weeks (42.50) and the highest was at 8 weeks (74.00) for rice straw at 15% POS. The ADF value were significantly increases ($p<0.05$) after 8 weeks of biodegradation.

### 4.1.9 Hemicellulose

Hemicellulose value (g/100g DM) ranges from 16.50 to 8.50 at 2 and 4 weeks for rice straw with 0% POS respectively. These were significantly different ($p<0.05$) from each other. At 15% POS, the lowest and highest hemicellulose value were recorded at 0 (11.50) and 2 (18.00) weeks respectively. There was no significantly different ($p>0.05$) between control (0 week) and 8 weeks (12.50) of biodegradation.

**Table 4.2: Chemical composition of rice straw with 15% palm oil sludge at 2, 4, 6, and 8 weeks of biodegradation.**

| Chemical Components | Biodegradation period (weeks) | Different inclusion level of POS | |
|---|---|---|---|
| | | **0%** | **15%** |
| **CF** | 0 | 37.00 | 33.50 |
| | 2 | 32.50 | 27.50 |
| | 4 | 32.50 | 32.50 |
| | 6 | 30.50 | 22.00 |
| | 8 | 27.00 | 24.50 |
| | **SEM** | **3.49** | **3.36** |
| **NDF** | 0 | 85.50 | $54.00^{d}$ |
| | 2 | 86.50 | $84.50^{c}$ |
| | 4 | 86.00 | $89.00^{a}$ |
| | 6 | 93.50 | $88.00^{ab}$ |
| | 8 | 89.00 | $86.50^{bc}$ |
| | **SEM** | **5.47** | **0.570** |
| **ADF** | 0 | 71.50 | $42.50^{c}$ |
| | 2 | 70.00 | $66.50^{b}$ |
| | 4 | 77.50 | $73.00^{a}$ |
| | 6 | 81.00 | $73.50^{a}$ |
| | 8 | 77.50 | $74.00^{a}$ |
| | **SEM** | **5.42** | **1.294** |
| **HEMI** | 0 | $14.00^{ab}$ | $11.50^{b}$ |
| | 2 | $16.50^{a}$ | $18.00^{a}$ |
| | 4 | $8.50^{b}$ | $16.00^{ab}$ |
| | 6 | $12.50^{ab}$ | $14.50^{ab}$ |
| | 8 | $11.50^{ab}$ | $12.50^{b}$ |
| | **SEM** | **1.483** | **1.194** |

Means on the same volume with different superscript (a,b,c) are significantly ($p<0.05$) different.
SEM= standard error of mean
CF = Crude fiber
NDF= Neutral detergent fiber
ADF= Acid detergent fiber
HEMI = Hemicellulose
0% POS= Rice straw with 0% POS
15% POS= Rice straw with 15% POS

## 4.2 *In vitro* Volume of Gas Produced (mL/200 mg DM) at Different Incubation Time (Hours)

In Table 3 below shows the volume of gas (ml/200 mg DM) produced every 3 hours interval for 24 hours.

At 3 hours of incubation, gas volume (ml/200g DM) produced ranges from 2.000 (at 2 weeks ) to 6.667 (at 8 weeks) and 2.000 (at 6 weeks) to 5.333 (at 4 and 6 weeks) for Rice straw at 0% and 15% POS respectively. No significant difference ($p>0.05$) was observed for RS with 15% POS after 8 weeks of biodegradation.

At 6 hours, the lowest volume of gas was 4.000 (at 0 week) and the highest was 9.333 (at 8weeks) for rice straw with 0% POS. After 8 weeks of biodegradation, gas Volume of RS at 8 weeks differs significantly ($p<0.05$) from other weeks. At 15% POS, the gas produced was not significantly difference ($p>0.05$) after 8 weeks of biodegradation.

The gas produced at 9 hours of incubation ranges from 8.670 to 15.330 for rice straw at 0 and 8 weeks respectively. There was no significant difference ($p>0.05$) in the volume of gas produced for 15% POS after 8 weeks of biodegradation.

At 12 hours the volume of gas produced was high at 18.000(at 0% 8 weeks) and low at 8.000 (at 0% 2 weeks) , these were significantly different ($p<0.05$)from

each other. At 15% POS There was no significant difference ($p>0.05$) after 8 weeks of biodegradation.

At 15 hours of incubation the gas produced ranges from 10.67 to 22.67 for RS at 2 and 8 weeks respectively. No significant difference ($p>0.05$) observed at 15% POS after 8werks of biodegradation.

At 18 hours of incubation, RS with 0% POS at 2 weeks had the lowest gas volume (12.00) and RS with 0% POS at 8weeks has the highest volume, (26.67). Rice straw with 0% POS at 2 and 8 weeks were significantly different ($p<0.05$) from each other. At 15% POS, there was significant difference ($p>0.05$) in the gas produced after 8 weeks of biodegradation.

At 21 hours of incubation, it was presented that RS with 0% POS at 8 weeks (33.3) had the highest and the lowest was presented at 2 weeks (14.67) respectively. They were significantly difference ($p<0.05$) from each other. RS with 15% POS, there was no significant difference ($p>0.05$) after 8 weeks.

24 hours, the volume of gas produced ranges from 38.67 to 18.67 for rice straw with 0% POS at 4 weeks and 2 weeks respectively. These were significantly different ($p<0.05$) from each other. At 15% POS, no significant difference ($p>0.05$) was observed after 8 weeks of biodegradation. .

**Table 4.3: Volume of gas produced at different incubation time by 0% and 15% treatment over the period of 0, 2, 4, 6 and 8weeks.**

| Incubation time (Hours) | Biodegradation period (weeks) | Different inclusion level of POS | |
|---|---|---|---|
| | | 0% | 15% |
| **H3** | 0 | $4.000^{bc}$ | $4.667^{ab}$ |
| | 2 | $2.000^{c}$ | $4.667^{ab}$ |
| | 4 | $6.667^{ab}$ | $5.333^{ab}$ |
| | 6 | $4.667^{abc}$ | $2.000^{b}$ |
| | 8 | $6.667^{ab}$ | $5.333^{ab}$ |
| | **S E M** | **1.311** | **1.223** |
| **H6** | 0 | $7.333^{b}$ | $9.333^{ab}$ |
| | 2 | $4.000^{b}$ | $6.667^{b}$ |
| | 4 | $8.667^{b}$ | $8.667^{b}$ |
| | 6 | $8.000^{b}$ | $5.333^{b}$ |
| | 8 | $9.333^{ab}$ | $9.333^{ab}$ |
| | **SEM** | **1.738** | **1.700** |
| **H9** | 0 | $8.67^{bc}$ | 12.00 |
| | 2 | $6.00^{c}$ | 9.33 |
| | 4 | $10.67^{bc}$ | 13.33 |
| | 6 | $12.67^{bc}$ | 8.00 |
| | 8 | $15.33^{b}$ | 12.00 |
| | **SEM** | **2.329** | **1.682** |
| **H12** | 0 | $15.33^{bc}$ | 15.33 |
| | 2 | $8.00^{c}$ | 13.33 |
| | 4 | $12.67^{bc}$ | 15.33 |
| | 6 | $16.67^{bc}$ | 11.33 |
| | 8 | $18.00^{b}$ | 14.67 |
| | **SEM** | **2.90** | **1.361** |

SEM = Standard error of mean.

Means on the same column with different superscript (a,b,c) are significantly ($p<0.05$) different.

0% POS = Rice straw with 0% POS, 15% POS= Rice straw with 15% POS.

**Table 4.4: Volume of gas produced at different incubation time by 0% and 15% treatment over the period of 0, 2, 4, 6 and 8 weeks.**

| Incubation time (Hours) | Biodegradation Period (weeks) | Different inclusion level of POS | |
|---|---|---|---|
| | | 0% | 15% |
| **H15** | 0 | $20.00^{b}$ | 18.00 |
| | 2 | $10.67^{b}$ | 14.67 |
| | 4 | $15.33^{b}$ | 18.67 |
| | 6 | $19.33^{b}$ | 14.67 |
| | 8 | $22.67^{ab}$ | 18.00 |
| | **SEM** | **3.69** | **1.738** |
| **H18** | 0 | $22.00^{bc}$ | 22.00 |
| | 2 | $12.00^{c}$ | 18.00 |
| | 4 | $19.33^{bc}$ | 21.33 |
| | 6 | $22.67^{bc}$ | 17.33 |
| | 8 | $26.67^{ab}$ | 20.00 |
| | **SEM** | **3.98** | **2.68** |
| **H21** | 0 | $27.33^{bc}$ | 26.00 |
| | 2 | $14.67^{c}$ | 19.33 |
| | 4 | $23.33^{bc}$ | 25.33 |
| | 6 | $28.67^{bc}$ | 23.33 |
| | 8 | $33.33^{ab}$ | 24.00 |
| | **SEM** | **4.30** | **2.47** |
| **H24** | 0 | $31.33^{ab}$ | 28.67 |
| | 2 | $18.67^{b}$ | 24.00 |
| | 4 | $38.67^{a}$ | 28.67 |
| | 6 | $36.67^{ab}$ | 31.33 |
| | 8 | $35.33^{ab}$ | 30.66 |
| | **SEM** | **5.55** | **2.93** |

SEM = Standard error of mean.

Means on the same colum with different superscript (a,b,c) are significantly ($p<0.05$) different.

0% POS = Rice straw with 0% POS, 15% POS= Rice straw with 15% POS.

## 4.3 Post *in vitro* Fermentation Parameters of Raw and Biodegraded Rice Straw and Mixture

Post *in vitro* fermentation parameters of raw and biodegraded rice straw and it's mixture is presented in the table below.

### 4.3.1 Methane

The highest (24.00) and lowest (9.33) volume of gas produced (mL/200 mg DM) was recorded at RS with 0% POS for 8 and 2 weeks respectively. No significant difference ($p>0.05$) was observed in the methane produced at RS with 15% POS after 8 weeks of biodegradation.

### 4.3.2 Methane Gas Volume

No significant difference ($p>0.05$) was observed for methane gas volume of RS with 0% and 15% POS after 8 weeks of biodegradation and control (0 week).

### 4.3.3 Organic Matter Digestibility

The organic matter digestibility (OMD) ranges from 42.05 to 80.33 for RS at 2 and 8 weeks respectively, these were significantly different ($p<0.05$) from each other. At 15% POS, The highest and lowest OMD values were obtained at 6 (71.37) and 2 (56.01) weeks of biodegradation respectively. No significant different ($p>0.05$) was observed between 2 and 4 weeks of biodegradation.

### 4.3.4 Fermentable Energy

The fermentable energy (FE) values range from 1.024 to 2.127 for RS with 0% POS at 6 and 0 weeks respectively. After 8 weeks of biodegradation control (0 week) differs significantly ($p<0.05$) from other weeks. At RS with 15% POS, ,no significant difference ($p>0.05$) was recorded after 8 weeks of biodegradation.

### 4.3.5 Metabolizable Energy

The highest metabolizable energy (MJ/kg DM)) value was recorded at rice straw with 0% POS for 4 weeks (8.022) and the lowest records at rice straw with 0% POS for 2 weeks (4.546). The results shows that at rice straw with 0% POS (2 weeks) differs significantly ($p<0.50$) from other weeks. At 15% POS, the highest value was recorded 7.319 and lowest 5.824 at 6 and 2 weeks respectively, however these were significantly different ($p<0.05$) from each other.

### 4.3.6 Dry Matter Digestibility

The highest dry matter digestibility of rice straw with 0% POS was observed at 0 week (66.03) and lowest at 2 weeks (13.23) of biodegradation. They were not significantly different ($p<0.05$) from each other. At 15% POS, no significant different ($p>0.05$) was observed after 8 weeks of biodegradation.

### 4.3.7 Short Chain Fatty Acid

The SCFA had the highest value recorded with 0.8640 at 0% for 4 weeks and lowest recorded with 0.4061 at 0% for 2 weeks. These were significantly different ($p<0.05$) from each other. At 15% POS no significant difference ($p>0.05$) was observed after 8 weeks of biodegradation.

**Table 4.5: Results of post in vitro fermentation parameters of the different Components at different period of 0, 2, 4, 6, and 8 weeks.**

| Post in vitro Parameters | Biodegradation Period (weeks) | Different inclusion level of POS | |
|---|---|---|---|
| | | 0% | 15% |
| $CH_4$(200mL/mg DM) | 0 | 21.33$^{ab}$ | 14.67 |
| | 2 | 9.33$^{b}$ | 15.33 |
| | 4 | 22.67$^{ab}$ | 20.67 |
| | 6 | 20.00$^{ab}$ | 20.00 |
| | 8 | 24.00$^{ab}$ | 17.33 |
| | **SEM** | **4.71** | **4.98** |
| $CH_4$ Gv | 0 | 0.6847 | 0.5176 |
| | 2 | 0.3630 | 0.6487 |
| | 4 | 0.6025 | 0.7181 |
| | 6 | 0.5509 | 0.6487 |
| | 8 | 0.6784 | 0.5536 |
| | **SEM** | **0.1267** | **0.1101** |
| OMD (%) | 0 | 69.77$^{ab}$ | 62.60$^{bc}$ |
| | 2 | 42.05$^{b}$ | 56.01$^{c}$ |
| | 4 | 72.77$^{ab}$ | 57.77$^{c}$ |
| | 6 | 73.34$^{ab}$ | 71.93$^{a}$ |
| | 8 | 80.33$^{a}$ | 66.33$^{ab}$ |
| | **SEM** | **9.30** | **2.58** |
| FE | 0 | 2.127 | 1.186 |
| | 2 | 0.505 | 1.572 |
| | 4 | 0.817 | 0.949 |
| | 6 | 1.024 | 0.832 |
| | 8 | 1.042 | 0.737 |
| | **SEM** | **0.299** | **0.478** |

SEM= Standard error of mean.
Means on the same column with different superscript (a, b, c) are significantly ($p<0.05$) different.
CH4 = Methane
CH4Gv = Methane gas volume
OMD = Organic matter digestibility
FE = Fermentable energy.
0% POS = Rice straw with 0% POS.
15% POS = Rice straw with 15% POS.

**Table 4.6: Results of post in vitro fermentation parameters of the different Components at different period of 0, 2, 4, 6 and 8 weeks.**

| Post in vitro Parameters | Biodegradation Period (weeks) | Different inclusion level of POS | |
|---|---|---|---|
| | | 0% | 15% |
| M E (MJ/kg DM) | 0 | 7.176$^{ab}$ | 6.612$^{bc}$ |
| | 2 | 4.546$^{b}$ | 5.824$^{c}$ |
| | 4 | 8.022$^{ab}$ | 6.412$^{bc}$ |
| | 6 | 7.549$^{ab}$ | 7.319$^{b}$ |
| | 8 | 7.865$^{ab}$ | 7.130$^{bc}$ |
| | **SEM** | **1.079** | **0.399** |
| DMD (g/100g DM) | 0 | 66.03$^{a}$ | 36.20 |
| | 2 | 13.23$^{b}$ | 34.93 |
| | 4 | 28.07$^{b}$ | 6.47 |
| | 6 | 37.37$^{b}$ | 24.33 |
| | 8 | 35.80$^{b}$ | 21.67 |
| | **SEM** | **7.43** | **12.13** |
| SCFA (200ml DM) | 0 | 0.6888$^{ab}$ | 0.6250 |
| | 2 | 0.4061$^{b}$ | 0.5135 |
| | 4 | 0.8640$^{a}$ | 0.6250 |
| | 6 | 0.8162$^{ab}$ | 0.6888 |
| | 8 | 0.7844$^{ab}$ | 0.6728 |
| | **SEM** | **0.1271** | **0.0701** |

SEM = Standard Error of mean
Means on the same column with different super script (a,b,c) are significantly ($p<0.05$) different.
ME = Metabolizable energy
DMD = Dry matter digestibility
SCFA = Short chain fatty acid
0% POS = Rice straw with 0% POS
15% POS = Rice straw with 15% POS

# CHAPTER FIVE

## 5.0 DISCUSSION

The CP content for Rice straw of 0% and 15% POS at 8 weeks of biodegradation was above 6-7% CP range required in ruminant diet which is effective for rumen function (Millford and Hsydock, 1965). Fungal treatment increase the CP and ash content of rice straw with 0% POS after 8weeks of biodegradation due to the increase in proliferation of fungal during biodegradation. (Farkas, 1979 and Belewu 2005) Fungi is very active when the hypheae of the fungi which is rich in protein penetrate deep into the cell of the straw this implies that fungal not only grow on surface but also penetrate deep into the cell.

The NDF, ADF and HEMI which are the cell wall components increase in 0% raw rice straw and decrease in 15% POS control, NDF values were lowest only in control period at both 0% and 15% POS, High ADF content can affect digestibility of feed so additives can be added to improve the quality.

The *in vitro* gas production test data shows that gas produced at 3 Hour was lower than others, therefore rate of gas production increases as the period of hour of incubation increases, perhaps this resulted from carbohydrate present.

Although gas production is a nutritionally wasteful product (Mauricio et al., 1999), but provides a useful basic from which metabolisable energy (ME), organic

matter digestibility (OMD) and short chain fatty acid (SCFA) may be predicted. However, since the treated rice straw yield better SCFA than the control suggest a potential to make energy available to the ruminants (Babayemi *et al.,* 2006).There was an increase in OMD, of rice straw with 0% and 15% POS. The High OMD observed suggest high nutrient uptake by microbes (Chumpawade *et al.,* 2007).

# CHAPTER SIX

## 6.0 CONCLUSION AND RECOMMENDATION

## 6.1 CONCLUSION

This study shows that rice straw and it's mixture with POS biodegraded with Pleurotus tuber-regium improve crude protein and systematically increases after 8 weeks of biodegradation. The OMD of rice straw with 0 and 15% POS was significantly improved after 8 weeks of biodegradation.

## 6.2 RECOMMENDATION

Rice straw with 0% and 15% POS at 8 weeks of biodegradation can be fed to ruminant as supplement or alternative feed.

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