

**EFFECT OF BIODEGRADATION PERIOD ON THE CHEMICAL
COMPOSITION AND IN-VITRO FERMENTATION CHARACTERISTICS
OF RICE STRAW WITH 60% PALM OIL SLUDGE INCLUSION IN
RUMINANT FEEDING**

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ABSTRACT

Ruminants depend mostly on all year round grazing on natural pastures and crop residue. Most of this areas face seasonal dry periods in which the availability of these pasture decreases and also its quality by a reduction in the content of digestible energy and nitrogen. Ruminant livestock raised in Nigeria tend to reflect the cyclical variation in quality and quantity of these available forages. Although ruminants are endowed with the ability to convert low quality feed into high quality animal 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, fiber content, digestibility, vitamin and minerals. Biodegradation of crop residues using edible mushroom has been reported to give rise to products with enhanced nutritive values that promote farm animal performance. This study was carried out to determine the effect of biodegradation period on the chemical composition and in vitro fermentation of rice straw with 60% palm oil sludge (POS) in ruminant feeding. Different inclusion rate of palm oil sludge (POS) and rice straw such as 0%RS, 40%RS and 100% RS at different biodegradation periods; 0, 2, 4 and 6 weeks. The substrates used were dried, mixed to inclusion levels (40% rice straw and 60% palm oil sludge), inoculated with fungal (*Pleurotus tuber-regium*) to aid biodegradation and further milled to uniform power. Chemical composition and in *vitro* fermentation study were carried out and data analyzed. However, there was no significant difference between the chemical composition, gas volume production and shown by the data analyzed but the post in *vitro* gas production has significant difference. Thus, this results shows that it can be included in ruminant diets as an increase in crude protein and decrease in crude fiber and methane gas production.

CERTIFICATION

This is to certify that this project work carried out by Uyoyou Annabel EVIVIE, Department of Animal science, Faculty of Agriculture, University of Benin, Benin City, Nigeria, under the supervision of Professor M. A. Bamikole and Mrs. B. O. Isaac

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DEDICATION

I dedicate this project work to Almighty **God** who through His love has saved me and helped me to complete this phase of my Education and to my supervisors **Prof. M. A. Bamikole** and **Mrs. Blessing Isaac**, also to my loving and caring parents, **Mr. and Mrs. Austin Evivie**, for their relentless effort and support.

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TABLE OF CONTENT

Title pages	-	-	-	-	-	-	-	-	-	i
Abstract	-	-	-	-	-	-	-	-	-	ii
Certification	-	-	-	-	-	-	-	-	-	iii
Dedication	-	-	-	-	-	-	-	-	-	iii
Acknowledgement	-	-	-	-	-	-	-	-	-	iv
Table of Content	-	-	-	-	-	-	-	-	-	v
List of Table	-	-	-	-	-	-	-	-	-	vii
CHAPTER ONE										
INTRODUCTION	-	-	-	-	-	-	-	-	-	1
Justification	-	-	-	-	-	-	-	-	-	3
Objectives	-	-	-	-	-	-	-	-	-	3
CHAPTER TWO										
2.0 LITERATURE REVIEW	-	-	-	-	-	-	-	-	-	5
2.1 Rice and Palm Oil	-	-	-	-	-	-	-	-	-	6
2.2 Rice Straw	-	-	-	-	-	-	-	-	-	6
2.2.1 Chemical composition and Nutritive value of Rice Straw	-	-	-	-	-	-	-	-	-	8
2.3 Palm Oil Sludge	-	-	-	-	-	-	-	-	-	9
2.3.1 Chemical Composition and Nutritive value of palm oil sludge	-	-	-	-	-	-	-	-	-	12

2.4 Biodegradation	-	-	-	-	-	-	-	-	14
2.4.1 White rot fungi	-	-	-	-	-	-	-	-	15
2.5 In Vitro Fermentation	-	-	-	-	-	-	-	-	15
CHAPTER THREE									
3.0 MATERIAL AND METHODS	-	-	-	-	-	-	-	-	17
3.1 Location of experiment	-	-	-	-	-	-	-	-	17
3.2 Collection of samples	-	-	-	-	-	-	-	-	17
3.2.1 Collection of rice straw	-	-	-	-	-	-	-	-	17
3.2.1 Collection of palm oils sludge	-	-	-	-	-	-	-	-	17
3.2.3 Collection of fungal samples	-	-	-	-	-	-	-	-	17
3.3 Preparation of Culture and Fungi spores	-	-	-	-	-	-	-	-	17
3.4 Preparation and inoculation of samples	-	-	-	-	-	-	-	-	18
3.4.1 Preparation of 100% rice straw culture	-	-	-	-	-	-	-	-	18
3.4.2 Preparation of palm oil sludge and rice straw mixture	-	-	-	-	-	-	-	-	18
3.4.3 Preparation of 100% palm oil sludge	-	-	-	-	-	-	-	-	19
3.5 Inoculation of samples	-	-	-	-	-	-	-	-	19
3.6 Experimental design	-	-	-	-	-	-	-	-	19
3.6.1 Experimental Treatment	-	-	-	-	-	-	-	-	19

3.7 In-vitro fermentation study	-	-	-	-	-	-	-	-	19
3.7.1 Buffer preparation	-	-	-	-	-	-	-	-	20
3.7.2 Collection of rumen liquor	-	-	-	-	-	-	-	-	20
3.7.3 Incubation of samples	-	-	-	-	-	-	-	-	20
3.7.4 Determination of methane and dry matter digestibility	-	-	-	-	-	-	-	-	21
3.7.5 Determination of organic matter digestibility and other post in vitro parameters	-	-	-	-	-	-	-	-	21
3.8 Chemical analysis	-	-	-	-	-	-	-	-	22
3.8.1 Crude protein determination	-	-	-	-	-	-	-	-	22
3.8.2 Neutral detergent fibre	-	-	-	-	-	-	-	-	23
3.8.3 Acid detergent fibre	-	-	-	-	-	-	-	-	24
3.8.4 Ash and organic matter determination	-	-	-	-	-	-	-	-	25
3.8.5 Crude fiber	-	-	-	-	-	-	-	-	25
3.8.6 Ether Extract	-	-	-	-	-	-	-	-	27
3.9 Statistical analysis	-	-	-	-	-	-	-	-	27
CHAPTER FOUR									
4.0 RESULTS	-	-	-	-	-	-	-	-	-
4.1 Chemical composition of different inclusion levels of rice straw	-	-	-	-	-	-	-	-	-
4.1.1 Dry matter content	-	-	-	-	-	-	-	-	-

4.1.2 Crude protein content	-	-	-	-	-	-	-
4.1.3 Ether extract content	-	-	-	-	-	-	-
4.1.4 Crude fibre content -	-	-	-	-	-	-	-
4.1.5 Ash content -	-	-	-	-	-	-	-

4.1.6 Organic matter

4.1.7 Neutral detergent fibre

4.1.8 Acid detergent fibre

4.1.9 Hemicellulose content

4.2 In vitro gas volume production of different inclusion levels of rice straws

4.2.1 At six hours

4.2.2 At nine hours

4.2.3 At twelve hours

4.2.4 At fifteen hours

4.2.5 At eighteen hours

4.2.6 At twenty-one hours

4.2.7 At twenty-four hours

4.3 Post in vitro gas production of different inclusion levels of rice straw

4.3.1 Methane production

4.3.2 Methane gas volume

4.3.3 Dry matter digestibility

4.3.4 Fermentation efficiency

4.3.5 Short chain fatty acid

4.3.6 Metabolisable energy

4.3.7 Organic matter digestibility

CHAPTER FIVE

5.0 DISCUSSION

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

6.2 Recommendation

REFERENCES - - - - - 28

LIST OF TABLES

Table 2.2.2: Chemical composition of rice straw	8
Table 2.3.2: Chemical composition of palm oil sludge	13

CHAPTER ONE

1.0 INTRODUCTION

In developing countries around the world example Nigeria, ruminant livestock production is underdeveloped resulting from the fact that livestock production is still very much at the subsistence level. For the few who are into commercial livestock production are faced with the problem of raising their animals predominantly on forages which are inherently poor in nutritive values. Ruminants depend mostly on the all year grazing on natural pastures and crop residue. Most of this areas face seasonal dry periods in which the availability of these pasture decreases and also its quality by a reduction in the content of digestible energy and nitrogen. Ruminants livestock raised here tend to reflect the cyclical variation in quality and quantity of these available forages. Although ruminants are endowed with the ability to convert low quality feed into high quality animal 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, fiber content, digestibility, vitamin and minerals. (Akinfemi and Ogunwole, 2012)

Rice straw is a by-product from rice farming; it is the residue of the rice plant left after the grains have been harvested. (Wuanor and Ayoade, 2017). Rice straws are either burned and left on the field before the next ploughing, ploughed down as soil improver or used as a feed for livestock. Although rice straw produced every year is plentiful, the amount a ruminant can consume is not sufficient to sustain a reasonable level of production (Kampla *et al.*, 2015) as it is constrained by low digestibility due to high silica and lignin as well as low protein and energy contents when given as the only feed to animals (Emtenan *et al* 2012).

Palm oil sludge, is the by-product of the oil palm processing industry. Palm oil sludge (POS) is the wastewater that is produced from the processing and extraction of palm oil. In oil palm plantations, the POS is used as a fertilizer and soil cover, while its utilization in animal feed is limited. Palm oil sludge has considerable potential as animal feed with its variability in chemical composition i.e., high mineral, medium crude protein contents and high fat, also utilization as feed can also help in pollution reduction. Bamikole and Ikatua (2009) reported that POS has considerable potential as ruminant energy source feed. (Zain *et al.*, 2012) Because POS is a waste product of the crude palm oil industry it is relatively cheap in Nigeria. (Adebiyi *et al.*, 2020)

Rice straws are difficult to use in ruminant diet because of their high crude fiber content (lignin and cellulose) (Adebiyi *et al.*, 2020). Lignin is a complex and heterogeneous aromatic biopolymer which provides strength to the wood structure and protect it against microbial attacks. It has been said to be removed with chemical or biological methods.

Biodegradation of crop residues using edible mushroom has been reported to give rise to products with enhanced nutritive values that promote farm animal performance. Thus rice straws could be converted to a more useful animal feed source through biological treatment. This is very useful as continuous evaluating of forages and other feed material to provide solutions to feeding problems are being sourced by animal scientist worldwide. The *in vitro* gas production technique of accessing nutritive value of feed and feedstuff is somewhat convenient due to less requirement of time, less cost and ability to analyze many samples almost simultaneously. Usage of chemical composition in combination with *in vitro* digestibility and degradability can be a useful tool for evaluation of nutritive value of feed resource. (Wuanor and Ayoade, 2017)

1.1 JUSTIFICATION

Feeding is major constraint in the production of ruminant livestock due to high cost and seasonal of conventional foodstuff availability, thus various other methods like the use of crop residue are now being incorporated into the feeding system.

Rice straws, a by-product of rice production are currently being used as feed sources for ruminant animal in rice-producing areas. It is high in lignin and silica content, limiting voluntary intake and reducing degradability by rumen microbes (Kamla *et al.*, 2015). Palm oil sludge (POS) represents an alternative to poultry feed but its utilization is limited due to the high content of crude fiber (lignin and cellulose) and low crude protein. (Nuraini *et al.*, 2017).

Combination and treatments of these agricultural wastes can help to improve nutritive quality and digestibility as well as protein content. The use of ligninolytic fungi and their extracellular ligninolytic enzymes for treatments results in degrading cellulose and hemicelluloses contents which improve its nutritional value. The use of fungi pre-treatment is expected to be practical, cost effective and environmental-friendly approach for enhancing the nutritive value of rice (Kamla *et al.*,2015) for ruminant feeding. Hence the need for this study in order to investigate the effects of this biodegradation on rice straws and palm oil sludge on its chemical composition & in vitro fermentation characteristics.

1.2 OBJECTIVES OF THE STUDY

The main objectives of this study is to determine the effect of biodegradation period on the chemical composition and in vitro fermentation of rice straw with 60% palm oil sludge (POS) in ruminant feeding. The specific objectives of this study are:

1. The effect of biodegradation period on the chemical composition of rice straw, palm oil sludge and their mixture (40% rice straw and 60% palm oil sludge).
2. The in vitro gas volume and dry matter digestibility of raw and biodegraded rice straw, palm oil sludge and their mixture.
3. The post in vitro fermentation parameters of raw and biodegraded rice straw, palm oil sludge and their mixture.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rice and Palm oil.

Rice (*Oryza Sativa* L.) is the staple food of over half the world's population and the most important among all the cereal crops (Dogara et Jumare, 2014). In Nigeria, rice has consumption per capita of 32kg indicating 4.7% increase in the past decade making the total consumption to be 6.4 million tonnes in 2017 as against 3.7 million tonnes produced per year (Erhie *et al.*, 2018).

The oil palm (*Elaeisis guineensis*) is regarded as a stabilizing crop to global food security especially in developing countries and has become an increasingly important driver of economic development and poverty reduction in the major producing countries of the south-east Asia, Central and West Africa (Cheng Hai Teoh, 2010). Oil palm is an important agricultural crop which yields three important sources of food, namely palm oil, palm kernel oil and palm kernel cake all of which are very important both locally and internationally in the world market. An average for 3.7 tonnes of palm oil, 0.4 tonnes of palm kernel oil and 0.6 tonnes of palm kernel cake is obtainable form one hectare of land (Basiron, 2010).

2.2 Rice Straw.

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 paddy field. Thus most of the rice straws generated from

farming in Nigeria are either fed to livestock, burnt or allowed to rot. 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). Most commonly the use of rice straws as a main source of roughages to animals.

Feeding of only rice straw does not provide enough nutrient to the ruminants to maintain high production levels due to the low nutritive value of this highly lignified material. The amount a ruminant can consume is not sufficient to sustain a reasonable level of production due to its low nutritive value therefore, rice straw to bovines is limited by low voluntary intake, slow rate of digestion and low voluntary intake, slow rate digestion and low content of available energy, protein, minerals and vitamin (Van Soest, 2006).

In the past years, several studies have been reported on the physical and chemical characterization and utilization of rice straw as ruminant feed. In addition, numerous methods of physical, chemical and biological treatments have been investigated, including supplementation with other feed stuffs or components in order to improve the utilization of rice straws by ruminants. (Sarnklong *et al*, 2010).

2.2.1 Chemical composition and Nutritive Value Rice Straw.

Rice straw is one of the most abundant lignocellulosic waste materials in the world. It contains 32-43% cellulose, 19-25% hemicellulose. 5-12% lignin, 18.8% ash (14-16%) and extractives (10-12%) (Yoswathana and phuriphapat, 2010). The structural complexity of rice straw due to

the presence of lignin is a major constraint for enzymatic and microbial attacks (Balasubramanian, 2013). The lignin component acts as a physical barrier and must be removed to make the carbohydrates available for further hydrolysis process. Lignin provide structural support to the plant, impermeability and resistance to degradation. To break down these components cellulase, hemicellulase and ligninase enzymes are required (Kamla *et al.*, 2015). However, lignin cannot be broken down in the rumen due to the lack of ligninase. Even if lignin could be degraded in the rumen it would not provide much energy for the animals. Lignin, however, has important effects on livestock production through effects on degradability and feed intake.

Silica, one element of the rice cell walls, can be present in high concentration ranging from 5% to 15% depending on the rice variety and the availability of this mineral in the soil. Silica reduces palatability and degradability of rice straw in the rumen due to its direct action in preventing colonization by ruminant microorganisms. The role of silica on the quality of rice straws was also reviewed by Van Soest (2006), in an attempt to put into perspective, the problems of silicon metabolism (Sarnklong *et al.*, 2010).

The chemical composition of rice straws varies between varieties and growing seasons, with higher nitrogen and cellulose contents in early-season rice compared to others. The level of phosphorus in rice straw is less than the level of about 0.3 percent that animals need growth and normal fertility. A level of about 0.4 percent of calcium in the diet is usually considered adequate for livestock, and many samples of rice straws have this amount. Rice straw contains high levels of oxalates (1-2% dry matter (DM)). Rice straw also differs from other straws in having a high (1-2%) content of oxalates. These are broken down in the rumen to carbonates and bicarbonates,

absorbed, then excreted in the urine. Below is a table on the chemical composition of rice straw (Kamla *et al.*, 2015).

Table 2.2.2: Chemical composition of Rice Straw.

Components	Percent dry weight
Crude fiber	29.8
Crude Protein	4.5
Ash	16
Silica	15.8
Lignin	12
Hemicellulose	25
Cellulose	43
Total Nitrogen	0.67
Phosphorus	0.08
Digestible energy, mcal/kg	1.9

Source: Kamla *et al.*, (2015); Sarnklong *et al.*, (2010).

2.3 Palm Oil Sludge

Palm oil sludge (POS) is also known as palm oil effluent [POE], palm oil slurry [POS], palm oil mill effluent [POME], oil palm slurry [OPS], decanter cake [DC] (Mosunmola and Olatunde, 2020). It is the voluminous liquid waste that originates from the sterilization and clarification forms in milling oil palm (Heuze *et al.*, 2015) which large amount and steam of hot water are used. When fresh, it is a thick brownish liquid that is complied with high concentrations of total solids oil and grease, chemical oxygen demand (COD) and biological oxygen demand (BOD) (Pogaku and Sarbatly, 2013). The raw effluent contains about 90-95% water and includes residual oil, soil particles and suspended solids. An industrial oil palm mill produces about 2.5 t of sludge per ton of palm oil, or 0.5 ton of sludge per ton of fresh fruit (Heuze *et al.*, 2015). POS is non-toxic as no chemicals are added to the extraction process and also acidic with a p^H of around 4.5 as it contains organic acids in complex forms that are suitable to be used as carbon sources. However, Ho and Tan (1983) reported that the suspended solids or particulate fraction of the effluent contribute with less than 50% to the total pollutant level (Mohammadreza and Sohelia, 2014)

Palm oil sludge is a highly polluting material and much research has been dedicated to means of alleviating its threat to the environment. Since the 1980-1990s (depending on the country), development have taken place in the crude palm oil industry to make it environmentally more sustainable, and wastewater systems are now commonplace in the main areas of production (Heuze *et al.*, 2015). Biological treatment depends enormously on consortium of microorganism's activities, which operate the organic substances present in the POS as supplements and eventually degrade these organic matters into simple by-products such as methane, carbon dioxide and hydrogen supplied, and water. The biological treatment process

requires a large pond to hold POS in place for the effective biodegradation, which regularly takes a few days relying upon the sort and native of the microorganisms (Mohammadreza and Sohelia, 2014). Still for the palm oil industry treatment of POS is considered a burden rather than a part of the production process. For obvious reasons, raw POS or partially treated POS is still being discharged into nearby rivers or land, as this is the cheapest and easiest method for disposal. However, excessive quantities of untreated POS deplete a water body of its oxygen and suffocate aquatic life (Madaki and Seng, 2013)

Utilization of POS as a feed can also be beneficial through reduced pollution by increasing the consumption by livestock, if possible near the production site to avoid wastage, palatability issues and transportation though this is only a partial solution to the problem of its pollution (Heuze *et al.*, 2015). Bamikole and Ikatua (2009) reported that POS had potential as ruminant feed energy source. POS is a cost effective by product of palm oil which can be used as an alternative energy source to ruminant as it is readily available and represent sustainable feed resources.

Palm oil sludge (POS) inclusion in diets can be an effective measure in overcoming lack of grazing pasture for smaller ruminants (Abubakr *et al.*, 2015). According to Hristov *et al* (2013) the inclusion of lipids in the diets of ruminants is an effective strategy for reducing enteric methane emission, but the applicability of this practice will depend on its cost effectiveness and its effect on feed intake, productivity and milk production. Hence the inclusion of POS in ruminant diets may have a good potential in reducing enteric methane emission since it contains more phyto-nutrients [vitamin E, vitamin K and alpha-carotene, beta-carotene, lycopene and 20 other carotenes] than any other dietary oil. These nutrients are powerful antioxidants that protect the oil from oxidation (Ugwuowo *et al.*, 2015)

There have been numerous attempts to convert POS into a viable animal feed resource; however, most methods have been discontinued due to the large initial capital investment required, and particularly to the cost of fuel for dehydration. In Malaysia, one method used to convert fresh palm oil sludge into a potential feedstuff involved concentration by centrifugation or decantation, followed by absorption on other dry feeds like tapioca chips, grass meal or palm kernel meal. Perhaps, one idea would be to promote the use of the fresh centrifuged sludge solids (15-20% dry matter) which has only been used in dry, concentrated rations. This approach however, might require supplementation to increase the crude protein content to that of a cereal, as well as some molasses to improve palatability (Heuze *et al.*, 2015).

However, the by-products of POS (palm oil sludge) can be problematic as base materials for feed since it has high crude fiber and low – medium crude protein content, as this combination can reduce digestibility. To improve the nutrition value of palm oil sludge, leaf blades can be used in the fermentation process. Fermentation is a biological feed processing technology that utilized microorganism activity to reconstruct the nutrition of low quality food materials. It is expected that this fermentation process will increase its digestibility (Muntarudin *et al.*, 2018). Palm Oil Sludge can be used as components in compound feeds, combining Palm kernel cake [PKC] and POS can partly provide complete-based diets for low cost feeding systems, particularly fattening rations beef cattle, field trials with cattle and pigs in estates have shown improved lean weight gain [LWG] with PKC-POS feeding. Similarly, combining POS and sago meal (40%;45%) has successfully been used for feeding local sheep (Heuze *et al.*, 2015).

2.3.1 Chemical Composition and Nutritive Value of Palm Oil Sludge.

Composition of Palm Oil Sludge (POS) depends mainly on raw material quality, season and the particular operations being used at any given time. As stated earlier, POS when fresh is a thick brownish colloidal mixture of water, oil and fine suspended solids. It is hot (80-90°C) (Yahaya and Seng, 2013) and possesses large amount of total solids (40,500-75000 mg/L) and oil grease (2000-8300mg/L). It is suspended solids content in the range of 18,000-47,000 mg/L, total nitrogen in the range of 400-800mg/L while the ash content is between 3000-42000 mg/L (Madaki and Seng, 2013). POS has very height biochemical oxygen demand (BOD) and chemical oxygen demand (COD) in the range of 25,000 and 54,000mg/L and 50,000 to > 100,000mg/L, respectively. These values are 100 times more than that of municipal sewage (Iwuagwu and Ugwuanyi, 2014)

Khalid and Wan Mustafa (1992) and Ma *et al.* (1993) all reported that POS is non-toxic. It is however low in p^H because of the organic acids produced in the fermentation process; it is acidic with levels of about 4.5 as it contains organic acids in complex forms that are suitable to be used as carbon source (Yahaya and Seng, 2013)

According to Habib *et al.* (1997), toxic materials such as lead can also be found in palm oil sludge, but Jmae et al (1996) argued that levels are usually below sub lethal levels (> 17.5 ug/g) and their view lead is found in POS as result of contamination from plastic and metal pipes, tanks and containers where lead is widely used in paints and glazing materials.

Fresh palm oil effluent contains mostly water (90-95%). Decanted effluent contains 60-80% DM and dried effluent contains more than 90% DM. The contents of the dry matter are characterized by considerable variability with particularly, the amount of residual oil varying from 5% to more

than 70% DM. Effluent from palm oil mills using wet process are lower than fat effluents from mills using dry process (Heuze *et al.*, 2015). The material is characterized by high content of ether extract (29.7%), ash (8.7%) and medium CP content (11.0%) (**Table 2.3.2**). Wide variability in ash content and CP digestibility in POS results in widely different feeding values. The content of CF, cellulose, NDF and gross energy (GE) are 12.7%, 20%, 66.6% and 23.1MJ/kg respectively.

It is rich in mineral and therefore suitable to be used as an organic fertilizer in crop cultivation. The average concentration of Ca, P, K and Mg are 0.8, 0.3, 2.5 and 0.7 percent, respectively. Ammonia N, B, Fe, Mn, Cu and Zn are 35, 7.6, 46.5, 2.0, 0.89 and 2.3 mg/liter, respectively (Heuze *et al.*, 2015).

Table 2.3.2: Chemical composition of Palm oil mill effluent, decanted.

MAIN ANALYSIS	UNIT	AVG	SD	MIN	MAX	Nb
Dry Matter	% as fed	68.4	6.7	57.1	75.0	6
Crude protein	% DM	11.0	1.8	9.0	14.4	7
Crude fiber	% DM	7.9	4.3	3.2	12.7	6
NDF	% DM	28.2	19.6	15.2	66.6	5
ADF	% DM	22.1	19.8	9.6	56.8	5
Lignin	% DM	7.6	4.7	4.4	15.8	5
Ether extract EE	% DM	29.2	10.3	10.9	38.8	7
Ash	% DM	8.7	2.9	5.2	12.6	6
Gross energy	MJ/kg DM	23.1		20.3	23.1	2

Source: Onibi et al 2011, Heuze et al 2015

2.4 Biodegradation

Biodegradation is the process by which complex organic molecules are converted into its simpler forms by the action of enzymes secreted by fungi, bacteria or any other living microorganisms. The digestibility of various types of straw and other plant material by ruminants is very low because of the high contents of cellulose and lignin. Lignin is a complex and heterogeneous aromatic biopolymer, which provides strength to the wood structure and protects it against microbial attack. Lignin degradation is an oxidative process and the mechanism is quite non-specific as indicated by the many different products that have been isolated from many different substrates degraded by lignolytic cultures (Adenipekun and Fasidi, 2005).

Biodegradation of crop residue using edible mushroom has been reported to give rise to products with enhanced nutritive value that promote farm animal performance (Akinfemi *et al.*, 2010). Thus rice straws and palm oil sludge could also be converted to useful animal feed resources through biological treatment. This is particularly useful to ensure provision of animal protein sources for the teeming world population. Animal nutritionist are continuously evaluating forages and other feed resources so as to provide solutions to problems arising from practical feeding of livestock in specific situations and on a year round basis (Wuanor and Ayoade, 2017).

The feed value of mushroom is limited by the low polysaccharide degradation achieved during digestion within the rumen as a result of lignin, which acts as a barrier depriving the cellulolytic and hemicellulolytic enzymes access to the polysaccharide components. They also noted that several white-rot fungi exhibit a capacity to increase the in-vitro digestibility of rice straws for the production of ruminant feedstuff.

2.4.1 White rot fungi

White rot fungi, belonging to the wood-decaying *basidiomycetes*, as lignocellulolytic microorganisms are able to decompose and metabolize all plant cell constituents (cellulose, hemicellulose and lignin) by their enzymes. Many species of white-rot fungi which are effective lignin degraders have been used to assess their ability to improve the nutritive value of fodder for ruminant nutrition. Their extracellular lignin-modifying enzymes consist of lignin-peroxidase (LiP), manganese-dependant peroxidase (MnP), laccase (phenol oxidase) and H₂O₂-producing oxidase (aryl-alcohol oxidase; AAO and glyoxaloxidase) (Sarnklong *et al.*, 2010). White rot fungi are well known for their ability to produce hydrolytic and oxidative extracellular enzymes and decompose lignocellulosic substrates (Philippousis *et al.*, 2011). In this regard, the genera *Pleurotus* and *Lentinula* belong to a group of widely distributed edible mushrooms with the ability to degrade several lignocellulosic substrates (Bento *et al.*, 2014). Although several studies have been conducted on solid state fermentation of agricultural wastes by temperate exotic mushrooms, little study has been done on tropical mushrooms species especially Nigerian ones such as *Pleurotus tuber-regium* (Adenipekun and Fasidi, 2005)

2.5 In Vitro Fermentation

In vitro fermentation is a laboratory experiment done to mimic the situation in the rumen of the live animal. *In vitro* is done to create the environment in the living animal to study the digestibility and gas production in the rumen of the animal. *In vitro* fermentation has been carried out to evaluate digestibility can be used often to estimate nutritive quality of different grass specie. There are various biological methods / techniques currently available to determine the nutritive value of ruminant feed. *In vitro* techniques are routinely used to evaluate the nutritional evaluation of feeds due to their convenience, adaptability and efficiency. In recent

years, research on nutritional evaluation of feeds using *in vitro* techniques has gained wider acceptance due to its ease of adoption, repeatability, minimized use of animals and decrease in funding for *in vivo* evaluation of feeds (Getachew *et al.*, 2005).

In vitro gas production method provides a quick assessment of nutritional value (Tona *et al* 2013). The *in vitro* gas production has been widely used to estimate the nutritive quality of different class of forages (Njidda and Nasiru, 2010). This gas production technique of assessing nutritive value of feeds and feedstuffs is convenient due to less requirement of time, less cost and ability to analyze many samples almost simultaneously (Wuanor and Ayoade, 2017). Gas production is determined by the procedure of Menke and Steingass (1988). The ground samples (200mg) are weighed into 100ml calibrated glass syringes (HaberleLabortechnik, Lonsee-Ettlenschieb Germany) with pistons lubricated with Vaseline. Buffered mineral solution (Menke and Steingass, 1988) is prepared and placed in a water bath at 39⁰C under continuous flushing with CO₂. The gas production test is routinely used in feed research in relating gas volume to both the extent and rate of degradation of substrate (Blummel and Becker, 1977). The *in vitro* gas production method has been successfully used to evaluate the energy value of several classes of feed (Getachew *et al*, 2005). It was suggested that the *in vitro* gas technique is precise in measuring both rate and extent of fermentation in addition to the residue of fermentation at different time of incubation (Wuanor and Ayoade, 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of experiment

The experiment was conducted in the Crop Science laboratory at the faculty of Agriculture University of Benin, Ugbowo campus, Benin city. The in vitro fermentation and chemical composition study was carried out in the Department of Animal Science Laboratory Faculty of Agriculture, University of Benin.

3.2 Collection of samples

3.2.1 Collection of Rice straw samples

The rice straws were obtained from Weppa Wano farm, Aghenebode, Edo state. The rice straw samples were sun dried in order to reduce moisture content and avoid deterioration.

3.2.2 Collection of palm oil sludge samples

Palm oil sludge was obtained from Nigerian Institute for Oil Palm Research (NIFOR). The sample was sun dried to reduce its moisture content and reduce spoilage caused by micro-organisms.

3.2.3 Collection of Fungal samples

The *Pleurotus Tuber-regium* samples were obtained from Oliha market, Benin city, Edo state.

3.3 Preparation of Culture and Fungi spore

Eight (8) grams of Potato Dextrose Agar (PDA) was weighed and put in a measuring cylinder. It was then mixed with 200 ml of distilled water until a uniform homogenized solution was formed and then transferred into a conical flask. Gentamycin was added to the sample in order to inhibit the growth of unwanted bacteria and encourage growth of the desired fungi.

The conical flask used was properly covered with foil paper and cotton wool. And thereafter sterilized using a pressure pot and gas cylinder for 15 minutes at 121⁰C. After sterilization, the solution was cooled to ambient temperature. The inoculation chamber was thoroughly cleaned and sterilized using dilute ethanol and cotton wool and then the spirit lamp was lit in order to keep it warm and prevent growth of microorganism, the prepared PDA was poured at the base of ten (10) petri dishes.

The Pleurotus species was then chopped into smaller pieces using a sterilized razor and carefully transferred into the petri dishes, covered and left to grow for one to two weeks.

3.4 Preparation and Inoculation of samples

3.4.1 Preparation of 100% Rice straw culture

The rice straw samples were cut to a length of 3-4 cm and washed to remove sand and other debris. The moisture content of the rice straw was adjusted to 75%, then fifty (50) grams weighed into a 60 ml sterile bottle corked with cotton wool and covered with foil paper. The bottles were then loaded into a pressure pot and heated for 30 minutes at 121⁰C. The bottles were allowed to cool at room temperature before inoculation.

3.4.2 Preparation of palm oil sludge and rice straw mixture

The ratio of palm oil sludge to rice straw was 6:4. The bottles were filled and sterilized at 121°C for 15 minutes and left to cool to ambient temperature. The laminar flow chamber was thoroughly cleaned and sterilized and the spirit lamp lit to cool the place. The bottles were inoculated with the fungi culture and were sampled at 0,2,4 and 6 weeks respectively.

3.4.3 Preparation of 100% palm oil sludge.

A known weight of Palm oil sludge was weighed and filled into 20 bottles. The samples were sterilized for an hour in a pressure pot to 121°C for 30 minutes and left to cool. The laminar flow wood was cleaned and sterilized, the bottles inoculated with the fungi culture and were sampled at 0,2,4 and 6 weeks.

3.5 Inoculation of samples

All samples were inoculated with two disc of spores using an ewe bearer. The bottles were kept at room temperature and monitored for biodegradation every 2 weeks i.e. 2,4 and 6 weeks.

3.6 Experimental design

The study was conducted using Completely Randomized Design (CRD). The factorial arrangement was 3x4 i.e. (3 treatments: 0% POS, 60%POS and 100% POS. With four biodegraded periods/replicates :0, 2, 4 and 6 weeks).

3.6.1 Experimental Treatments

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

3.7 In vitro fermentation study

3.7.1 Buffer preparation

Buffer was prepared according to standard procedures of Navaro villa *et al* (2011). It was prepared a day before collection of rumen liquor and maintained at a p^H of 6.2 at 39⁰C. The inoculum was made up of buffer solution and rumen liquor at a ratio of 2:1.

3.7.2 Collection of rumen liquor

Rumen liquor was collected from West African Dwarf (WAD) goats exclusively on a medium quality diet reared at the university of Benin Farm project, Benin city. This was collected early in the morning before feeding using a stomach tube. The liquor was collected into pre-warmed thermostat flasks and taken to the laboratory where it was strained through a four layered cheese cloth after which it was placed in a water bath and gassed with CO₂ to maintain anaerobic conditions.

3.7.3 Incubation of samples

The milled samples into inoculation darken bags, sealed and put into 100ml calibrated syringes to be used for inoculation. Into the syringes containing the biodegraded samples and fitted with clipped silicon tubes was dispensed 30ml of inoculum. Three syringes containing just inoculum served as blank. In vitro incubation was carried out for 24 hours and monitored at 3-hour interval. The head space of the syringes containing the incubated syringes was read and recorded at each interval and at the end of 24 hours, incubation was terminated by putting the syringes in ice cold water to prevent further gas production.

3.7.4 Determination of methane and dry matter digestibility

40% NaOH was prepared and four (4) ml injected into the incubation syringes at the end for 24-hour incubation period. Carbon(IV) oxide was absorbed by NaOH leaving methane behind. Hence, after the injection of NaOH the volume of gas produced was methane.

Dry matter digestibility (DMD), the sealed bags containing the samples were removed from the syringes and washed under running tap water until they became clean. The bags were then oven dried at a temperature of 100°C until constant weight was attained. The dry matter digestibility was calculated as follows:

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

$$\frac{\text{Weight of sample before incubation}}{\text{Weight of sample before incubation}} \quad 1$$

The formulas used for the calculated the Fermentation Efficiency (FE) and effect of methane reduction (CH₄red %) are given below:

$$\text{CH}_4\text{red (\%)} = \frac{\text{Average CH}_4 \text{ of the control} - \text{CH}_4 \text{ of treated sample}}{\text{Average CH}_4 \text{ of the control}} \times 100$$

$$\frac{\text{Average CH}_4 \text{ of the control}}{\text{Average CH}_4 \text{ of the control}} \quad 1$$

$$\text{Fermentation Efficiency (FE)} = \frac{\text{Dry matter digestibility (g/kg)}}{\text{Total Gas Volume (Ml/g)}}$$

$$\frac{\text{Dry matter digestibility (g/kg)}}{\text{Total Gas Volume (Ml/g)}}$$

3.7.5 Determination of organic matter digestibility and other post in vitro parameters.

The following parameters which included short chain fatty acids (SCFA), organic matter digestibility (OMD) and metabolisable energy (ME) were estimated using the following equations.

$$\text{ME} = 2.20 + 0.136\text{GV} + 0.057\text{CP} + 0.00029\text{CF} \text{ (Menke and Steingass, 1988).}$$

$$\text{SCFA} = 0.0239\text{GV} - 0.0601 \text{ (Getachew *et al.*, 1999).}$$

$$\text{OMD} = 14.88 + 0.088\text{GV} + 0.45\text{CP} + 0.651\text{XA} \text{ (Menke and Steingass, 1988).}$$

Where GV = Total gas volume

CP= Crude protein

CF= Crude fibre; XA = Ash.

3.8 Chemical analysis

Samples were analyzed for dry matter (DM), crude protein (CP), ash and ether extract (EE) contents, using the standard procedure of the Association of Official Analytical Chemists (AOAC 2000) while the cell wall components i.e. acid detergent fibre (ADF) and Neutral detergent fibre (NDF) were determined using standard method of Van Soest *et al.*, (1991).

3.8.1 Crude protein determination

Crude protein was determined using Kjeldahl procedure 0.5g of the sample was weighed into a digestion tube thereafter the sample was transferred into a digestion tube. 10ml of 98% concentrated Sulphuric acid (H₂SO₄) and 2g of catalyst mixture (1:9; CuSO₄: NaSO₄) was added to the sample. The digestion tube was placed in a standard automated digester and heated at 420°C for 1 hour. The digest was put into a universal bottle (120ml) and topped with distilled water up to 50ml mark. After cooling the digestion tub, distillation was carried out by adding

5ml of digest to 5ml of 60% NaOH in a Kjeldahl flask. This was distilled (using an automated distilling machine) into 5ml of boric indicator. About 150ml of the distillate was collected and titrated with 0.1N HCL. This was done for the different crop residue and each sample was replicated two times. Crude protein content was calculated using the formula:

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

$$1000 \times 5 \times W$$

Where NA = Normality of acid

VA = Volume of acid used

W= Weight of sample

3.8.2 Neutral detergent fibre

0.5g of sample was weighed and placed in a beaker and 50ml of neutral detergent solution was added, the mixture was then placed on a heating mantle which had a temperature of 50-70°C. The sample upon boiling was heated for one (1) hour while the 50 ml mark was maintained by topping with hot water. After heating, the mixture was filtered using a muslin cloth and the residue was washed with boiling water. After filtering, the residue was placed in an oven at temperature of 70°C and was dried for 24 hours and more until constant weight was attained. Two more replicates were carried out. Neutral detergent fibre (NDF) can be calculated using the formula:

$$NDF (\%) = \frac{(Wt \text{ of plate with cloth} + Wt \text{ of residue after drying}) - Wt \text{ of plate with cloth before drying}}{Wt \text{ of samples}} \times 100/1$$

Wt of samples

Component of neutral detergent solution/ liter of distilled water includes

- Sodium lauryl sulphate
- Ethylenediaminetetra-acetic acid (Disodium salt)
- Sodium borate decahydrate (Borax)
- Disodium hydrogen phosphate anhydrous
- 2-Ethoxyl ethanol (Ethyleneglycol monoethyl ether)

3.8.3 Acid detergent fibre

A known weight (0.5g) of the sample was weighed and placed into a beaker and 50ml of acid detergent solution was added the mixture was placed on a heating mantle and brought to a temperature of 50-70°C. The sample upon boiling was heated for one hour while the 50 ml mark was maintained by topping with hot water. After heating, the mixture was then filtered using a muslin cloth and the residue was washed with boiling water. After filtering the residue was placed in an oven at a temperature of 70°C and was dried for 24 hours until a constant weight was attained. This was replicated two more times. The Acid detergent fibre (ADF) can be calculated using the formula:

$$\text{ADF (\%)} = \frac{(\text{Wt of plate with cloth + wt. of residue after drying}) - \text{Wt of plate with cloth before drying}}{\text{Wt of sample}} \times 100/1$$

Components of acid detergent solution includes the following

- Concentrated sulphuric Acid (H₂SO₄)
- Cetyl trimethyl ammonium bromide (CTAB).

3.8.4 Ash and Organic matter determination

1g of sample was weighed into a crucible of known weight and heated in a muffle furnace at 550°C for 6 hours. The residue was placed in desiccator to cool 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 - weight of ash (%)

3.8.5 Crude fiber

Crude fiber is made up of insoluble polysaccharides such as cellulose, hemicellulose, xylems, lignin, and chitin and other fibrous carbohydrates.

Determination of CF;

- 2g of the sample was weighed into a beaker
- The sample was dried in the oven for a period of time to remove moisture
- Fat was extracted from the sample
- Sample was then boiled in 100mL of 1.25% sulphuric acid for 30 minutes and filtered using warm water and boiled again in 100mL of 1.25% sodium hydroxide for 30 minutes and also filtered
- Sample was transferred into a crucible and was oven dried
- After drying, the sample was weighed

- Sample residue was then ash in a muffle furnace to burn off the crude fiber
- After ashing, the ash was allowed to cool and was place in a desiccator to further cool and weighed using a sensitive scale.
- The weight of the CF in the sample was calculated by subtracting the weight of ash obtained from the dried weight obtained above.
- The weight obtained was fitted into the formula;

$$\%CF = \frac{\text{WT of CF}}{\text{WT of sample}} \times 100$$

3.8.6 Ether extracts EE (Crude fat)

Ether extracts fraction consists of Triglycerides, phospholipids, steroids, alcohols, fat, soluble vitamins, pigments etc.

The EE of my experimental samples were obtained by continuous extraction of the materials with petroleum ether.

2g of the sample was wrapped properly in a fat free filter paper and put in a soxhlet apparatus. A flask containing the petroleum ether was placed on a heating mantle maintained at a low temperature of 50°C. As the solvent gets heated up, the hot solvent rises and drips through the samples and extracts the fat and discharges back into the flask. The process was allowed to continue until all the fat was extracted from the samples. This took about 3 hours. At the end of the extraction the filter paper containing the samples were placed in the laboratory oven where it

was evened dried to constant weight and was place in a desiccator to cool and weighed using a sensitive scale. The weight of the samples was fitted into the formula;

$$\%EE = \frac{\text{WT of filter paper + sample} - \text{WT of filter paper}}{\text{WT of sample}}$$

3.9 Statistical analysis

Data collected at different period were analyzed using GLM procedure of SAS (2013) and separation of means was done using the Duncan Multiple Range Test (DMRT) in the same SAS (2013) software.

CHAPTER FOUR

4.0 RESULTS

4.1 Chemical composition of different levels of rice straw inclusion

Table 4.1, 4.2 and 4.3 shows the chemical composition of rice straw at different inclusion levels.

4.1.1 Dry matter content

The dry matter content (%) ranged from 83.15 (100%RS at 0 weeks) to 62.73 (40%RS at 4 weeks)..

4.1.2 Crude protein content

The crude protein content was recorded highest in 40%RS at 4 weeks (11.375%) and the lowest in 100%RS at 6 weeks (3.500%).

4.1.3 Ether extract content

The highest ether extract content was recorded in 0%RS at 6 weeks (67.5%) and the lowest recorded in 100%RS at 2 weeks (3.850%).

4.1.4 Crude fibre content

The lowest crude fibre content was in 0%RS at 6 weeks (2.000%) and the highest in 100%RS at 0 weeks (29.000%).

4.1.5 Ash content

100%RS at 6weeks (25.000%) had the highest ash content and 0%RS at 0 weeks (2.000%) having the lowest.

4.1.6 Organic matter

The lowest organic matter content was in 100%RS at 6 weeks (75.000%) and the highest being in 0%RS at 0 weeks (98.000%).

4.1.7 Neutral detergent fibre

The neutral detergent fibre highest values was recorded in 100%Rs at 0 weeks (67.500%) and the lowest was recorded in 0%RS at 0 weeks (21.250%). There was no significant difference ($P > 0.05$).

4.1.8 Acid detergent fibre

100%RS at 2 weeks (49.000%) recorded the highest acid detergent fibre value while 0%RS at 4 weeks (10.000%) recorded the lowest.

4.1.9 Hemicellulose content

The highest value for hemicellulose content was in 100%RS at 6 weeks (26.000%) and the lowest at 0%RS at 6weeks (7.800%).

Table 4.1: Chemical Composition of rice straw and palm oil sludge mixture at 2, 4 and 6 weeks periods of biodegradation

Chemical composition (%)	Biodegradation period (%)	Inclusion levels of rice straws		
		0%RS	40%RS	100%RS
DM	0	70.96	73.48	83.15
	2	71.84	63.42	66.27
	4	65.90	62.73	66.49
	6	69.36	65.92	67.28
OM	0	98.00	89.50	81.00
	2	97.50	89.50	79.00
	4	97.00	90.00	76.00
	6	95.00	90.00	75.00
CP	0	8.75	8.75	8.75
	2	5.25	10.50	9.13
	4	7.25	11.38	5.25
	6	5.25	5.50	3.50
EE	0	56.50	32.00	5.00
	2	63.00	32.50	3.85
	4	63.50	27.00	5.00
	6	67.50	21.50	9.50

Means with different superscripts along the same row are significantly different ($P < 0.05$)

0%RS= 0% Rice straw + 100% palm oil sludge

40%RS = 40% rice straw + 60% palm oil sludge

100%RS = 100%Rice straw + 0% palm oil sludge.

DM- Dry matter; OM- Organic matter; CP- Crude Protein; EE- Ether Extract

Table 4.2: Chemical composition of rice straw and palm oil sludge mixture at 2,4 and 6 weeks periods of biodegradation

Chemical composition (%)	Biodegradation period (weeks)	Inclusion levels of rice straws		
		0%RS	40%RS	100%RS
CF	0	9.00	17.00	29.00
	2	7.00	20.00	19.00
	4	6.00	12.00	23.00
	6	2.00	15.00	21.00
ASH	0	2.00	10.50	19.00
	2	2.50	10.50	21.00
	4	3.00	10.00	24.00
	6	5.00	10.00	25.00
ADF	0	11.50	21.00	47.00
	2	22.00	12.00	49.00
	4	10.00	21.00	33.00
	6	15.50	12.00	14.50
NDF	0	21.25	34.00	67.50
	2	30.00	32.00	61.00
	4	23.00	35.00	53.00
	6	23.30	30.20	40.50
HEMI	0	9.75	12.00	20.50
	2	8.00	20.00	12.00
	4	13.00	12.00	20.00
	6	7.80	18.20	26.00

Means with different superscripts along the same row are significantly different ($P < 0.05$)

0%RS= 0% Rice straw + 100% palm oil sludge

40%RS = 40% rice straw + 60% palm oil sludge

100%RS = 100%Rice straw + 0% palm oil sludge.

CF- Crude Fibre; ADF- Acid detergent fibre; NDF- Neutral detergent fibre;

4.2 *In vitro* gas volume production of different levels of rice straw inclusion

4.2.1 At six hours

At six hours of incubation, there was no significant difference ($P > 0.05$) as all levels of inclusion recorded the same gas volume (2.000 mL/200 mgDM).

4.2.2 At nine hours

The gas volume (mL/200 mg DM) recorded in 0% RS at 2 weeks (3.333) and 40%RS at 2 weeks (3.333) was the highest at nine hours of incubation while 0%RS at 0 weeks and 4 weeks, 40% at 0 weeks and 6 weeks, 100%RS at all biodegradation levels recorded the lowest gas volume (2.000mL/ 200 mgDM) respectively. There was no significant difference ($P > 0.05$) recorded.

4.2.3 At twelve hours

The 0%RS at 6 weeks and 40%RS at 2 weeks of biodegradation recorded the highest gas volume (4.000 mL/200mg DM) at 12 hours of incubation and 100%RS at 4 and 6 weeks recorded the lowest gas volume (2.000 mL/200mg DM). There was no significant difference ($P > 0.05$) observed at 12 hours of incubation.

4.2.4 At fifteen hours

At 15 hours of incubation 40%RS at 6 weeks recorded the highest gas volume (10.667 mL/ 200mg DM) which was significantly higher than 100%RS at 0, 4 and 6 weeks (3.333m L/ 200mg DM) which recorded the lowest gas volume. There was no significant difference ($P > 0.05$) observed.

4.2.5 At eighteen hours

40%RS recorded the highest gas volume at 2 and 4 weeks period of biodegradation (5.333 mL/200mg DM) while (4.000mL/ 200mg DM) the lowest gas volume was recorded at 0%RS, 40%RS at 0, 6 weeks and 100%RS at 4, 6 weeks respectively. At 18 hours of incubation there was no significant difference ($P > 0.05$) observed among treatments.

4.2.6 At twenty-one hours

The gas volume (mL/200mg DM) ranged from 6.667 (in 40%RS at 4 weeks) to 4.000 (100%RS at 6 weeks). No significant difference ($P > 0.05$) was observed at 21 hours of incubation.

4.2.7 At twenty-four hours

The highest gas volume recorded (mL/200mg DM) was at 40%RS at 4 weeks (8.000mL/200mg DM) and the lowest recorded at 0%RS at 0 weeks (4.000mL/200mg DM). While there was no significant difference ($P > 0.05$) recorded at this hour of incubation.

Table 4.3: In-vitro gas production of biodegraded rice straw and palm oil sludge mixture

Incubation hours	Biodegradation period (Weeks)	Inclusion levels of rice straws			SEM
		0%RS	40%RS	100%RS	
6h	0	2.000	2.000	2.000	0.000
	2	2.000	2.000	2.000	0.000
	4	2.000	2.000	2.000	0.000
	6	2.000	2.000	2.000	0.000
	SEM	0.000	0.000	0.000	
9h	0	2.000	2.000	2.000	0.000
	2	3.333	3.333	2.000	0.770
	4	2.000	2.667	2.000	0.544
	6	2.667	2.000	2.000	0.544
	SEM	0.667	0.667	0.000	
12h	0	2.667	3.333	2.667	0.943
	2	3.333	4.000	2.667	0.770
	4	2.667	2.667	2.000	0.770
	6	4.000	3.333	2.000	0.544
	SEM	0.817	0.817	0.667	

Means with different superscripts along the same row are significantly different ($P < 0.05$)

0%RS= 0% Rice straw + 100% palm oil sludge

40%RS = 40% rice straw + 60% palm oil sludge

100%RS = 100%Rice straw + 0% palm oil sludge.

SEM = Standard Error of Mean

Table 4.4: In-vitro gas production of biodegraded rice straw and palm oil mixture

Incubation hours	Biodegradation period (Weeks)	Inclusion levels of rice straws			SEM
		0%RS	40%RS	100%RS	
15h	0	4.000	4.000	3.333	0.544
	2	4.000	4.000	4.000	0.000
	4	4.000	4.000	3.333	0.544
	6	4.000	10.667	3.333	5.470
	SEM	0.000	4.714	0.817	
18h	0	4.000	4.000	4.667	0.544
	2	4.000	5.333	4.667	0.770
	4	4.000	5.333	4.000	0.544
	6	4.000	4.000	4.000	0.000
	SEM	0.000	0.667	0.667	
21h	0	4.000	4.000	5.333	1.089
	2	4.000	6.000	4.667	1.089
	4	4.000	6.667	4.667	1.540
	6	4.667	4.667	4.000	0.770
	SEM	0.471	1.564	1.155	
24h	0	4.000	4.667	6.667	1.540
	2	4.667	6.667	5.333	1.633
	4	5.333	8.000	5.333	2.611
	6	5.333	6.000	5.333	0.770
	SEM	0.817	2.539	1.491	

Means with different superscripts along the same row are significantly different ($P < 0.05$)

0%RS= 0% Rice straw + 100% palm oil sludge

40%RS = 40% rice straw + 60% palm oil sludge

100%RS = 100%Rice straw + 0% palm oil sludge.

SEM = Standard Error of Mean

4.3 Post in vitro production of different levels of rice straw inclusions

Post in vitro gas production is presented in Table

4.3.1 Methane production

Methane production (CH₄) was recorded as highest in 40%RS at 4 weeks (6.000mL) while the lowest in 0%RS at 6 weeks (3.333mL) and 100%RS at 2 weeks (3.333mL). However, there was no significant difference ($P < 0.05$) between treatments.

4.3.2 Methane gas volume

The highest methane gas volume (CH₄GV) was recorded in 40%RS at 4 weeks (54.670) and the lowest methane gas volume recorded in 40%RS at 0 weeks (14.670). There was no significant difference ($P > 0.05$) observed between treatments.

4.3.3 Dry matter digestibility

The DMD (%) value recorded highest in 0%RS at 4 weeks (49.700) and lowest in 40%RS at 0 weeks (11.767). 0%RS at 4 weeks (49.700) was significantly different ($P < 0.05$) from 40%RS at 0 weeks (11.767) but was not significantly different ($P > 0.05$) from the other samples.

4.3.4 Fermentation efficiency

The highest record for fermentation efficiency (FE) was in 0%RS at 6 weeks (10.472) and the lowest in 40%RS at 0 weeks (2.661). 0%RS at 6 weeks was significantly different ($P < 0.05$) from 40%RS at 0 weeks (2.661) and 100%RS at 0 weeks (5.861) but no significant different ($P > 0.05$) between the former and the other samples. There was significant difference ($P < 0.05$) between 40%RS at 0 weeks (2.661) and 100%RS at 0 weeks (5.861).

4.3.5 Short fatty chain acid

The value for short fatty chain acid (SFCA) ranged from 0.131 (in 40%RS at 4 weeks) to 0.036 (in 0%RS at 0 weeks). No significant difference ($P > 0.05$) was observed among samples.

4.3.6 Metabolisable energy

The metabolisable energy (ME) value was recorded highest in 40%RS at 4 weeks (3.940) and the lowest in 0%RS at 2 weeks (3.136). 40%RS at 0,2,4,6 weeks (3.338, 3.711, 3.940, 3.491), 0%RS at 0,4 weeks (3.245, 3.340) and 100%RS at 0,6 weeks (3.614, 3.246) were significantly different ($P < 0.05$) from the other samples. 0%RS at 2 weeks (3.136) and 100%RS at 6 weeks (3.169) was significantly different ($P < 0.05$) from 0%RS at 6 weeks (3.225) and 100%RS at 2 weeks (3.451).

4.3.7 Organic matter digestibility

The organic matter digestibility (OMD) was recorded highest in 100%RS at 4 weeks (37.672) and the lowest in 0%RS at 2 weeks (22.977). 100%RS was not significantly different ($P > 0.05$) from 40%RS at 4 weeks but was significantly different ($P < 0.05$) from 40%RS at 0,2,6 weeks (29.434, 31.982, 30.386) and 0%RS samples. 0%RS at 0,2,6 weeks (23.640, 22.977, 25.191) were significantly different ($P < 0.05$) from 40%RS at 0,2,6 weeks and 0%RS at 4 weeks. 0%RS at 4 weeks (24.789) was not significantly different ($P > 0.05$) from 40%RS at 0,2,6 weeks.

Table 4.5: Post in vitro parameters of biodegraded rice straw and palm oil sludge mixture at 0,2,4 and 6 weeks of biodegradation.

Parameters	Periods (weeks)	Inclusion levels of rice straws			SEM
		0%RS	40%RS	100RS%	
OMD	0	23.640 ^C	29.434 ^b	37.053 ^a	1.510
	2	22.977 ^c	31.982 ^b	37.351 ^a	1.950
	4	24.789 ^b	33.550 ^a	37.672 ^a	2.551
	6	25.191 ^c	30.383 ^b	37.423 ^a	0.582
	SEM	1.203	2.518	1.680	
ME	0	3.245 ^a	3.338 ^a	3.614 ^a	0.662
	2	3.136 ^b	3.711 ^a	3.451 ^{ab}	0.225
	4	3.340 ^a	3.490 ^a	3.246 ^a	0.378
	6	3.225 ^{ab}	3.491 ^a	3.169 ^b	0.121
	SEM	0.175	0.362	0.212	
SCFA	0	0.036 ^a	0.051 ^a	0.099 ^a	0.037
	2	0.051 ^a	0.099 ^a	0.067 ^a	1.732
	4	0.067 ^a	0.131 ^a	0.067 ^a	0.062
	6	0.067 ^a	0.083 ^a	0.067 ^a	0.018
	SEM	0.031	0.061	0.025	

Means with different superscripts along the same row are significantly different (P < 0.05)

0%RS= 0% Rice straw + 100% palm oil sludge

40%RS = 40% rice straw + 60% palm oil sludge

100%RS = 100%Rice straw + 0% palm oil sludge.

SEM = Standard Error of Mean OMD- Organic matter digestibility; ME- metabolisable energy;

SCFA- short chain fatty acid

Table 4.6: Post in vitro parameters of biodegraded rice straw and palm oil sludge mixture at 0,2,4 and 6 weeks of biodegradation.

Parameters	Periods (wks)	Inclusion levels of rice straws			SEM
		0%RS	40%RS	100RS%	
FE	0	7.033 ^a	2.661 ^b	5.861 ^{ab}	1.570
	2	5.869 ^a	6.217 ^a	6.153 ^a	3.047
	4	9.625 ^a	6.815 ^a	4.817 ^a	2.337
	6	10.472 ^a	6.628 ^a	6.656 ^a	4.631
	SEM	1.815	1.969	2.049	
DMD	0	28.133 ^a	11.767 ^{ab}	33.767 ^a	4.096
	2	27.367 ^a	35.633 ^a	30.767 ^a	1.732
	4	49.700 ^a	42.167 ^a	27.767 ^a	9.493
	6	48.470 ^a	39.770 ^a	34.570 ^a	16.194
	SEM	11.843	3.838	8.868	
CH ₄ GV	0	16.000 ^a	14.670 ^a	33.330 ^a	11.102
	2	18.670 ^a	33.330 ^a	17.330 ^a	11.624
	4	21.330 ^a	54.670 ^a	25.330 ^a	24.440
	6	18.667 ^a	28.000 ^a	21.333 ^a	5.863
	SEM	8.379	22.978	10.872	
CH ₄	0	4.000 ^a	3.333 ^a	4.667 ^a	0.770
	2	4.000 ^a	4.667 ^a	3.333 ^a	0.770
	4	4.000 ^a	6.000 ^a	4.667 ^a	1.087
	6	3.333 ^a	4.667 ^a	4.000 ^a	0.770
	SEM	0.471	1.155	0.816	

Means with different superscripts along the same row are significantly different (P < 0.05)

0%RS= 0% Rice straw + 100% palm oil sludge

40%RS = 40% rice straw + 60% palm oil sludge

100%RS = 100%Rice straw + 0% palm oil sludge.

SEM = Standard Error of Mean DMD- Dry matter digestibility; CH₄- Methane; CH₄GV- Methane gas volume; FE- Fermentation energy

CHAPTER 5

5.0 DISCUSSION

The CF content for 40%RS at 6 weeks was low which could be as a result of cellulosic enzymes secreted by cellulolytic fungi. As observed by Belewu and Belewu (2005), the degradation of banana leaves decreased in fibre contents and fractions due to the production of various enzymes during the vegetative and reproductive phases with lignocellulose degrading properties but that of 100%RS was slightly higher than that value.

The increased level of CP in 40%RS at 4 weeks implies that the fungus effectively degraded the rice straw and colonized it causing the CP levels to rise due to CP of the fungal biomass and enzymes (Wuanor and Ayoade, 2017). According to Akinfemi et al (2009), increase in CP levels of fungal treated materials was caused by secretion of certain extra cellular enzymes which are proteinous in nature during the materials breakdown and its subsequent metabolism. The 40%RS reduced in hemicellulose from the second to four weeks as did 100%RS from the first to second week of biodegradation. Kuforiji and Fasidi (2009) reported that hemicellulose and celluloses reduced when *Pleurotus tuber-regium* was used during biodegradation of agro-industrial wastes. They stated that it showed the extent to which the substrates were able to utilize the mushroom.

Akinfemi *et al.* (2009) were of the view that decreases in the value of the neutral detergent fibre and acid detergent fibre were as a result of extra cellular enzymes produced by *Pleurotus* species. There was a noticeable difference in the levels of ADF for 40%RS at 0 weeks and that of 6 weeks of biodegradation and a slight change of the same treatments in regards to NDF. The hemicellulose factions generally increased due to fungal treatment means the fungi secreted

enzymes less effective in breaking down the components. The high EE value of 0%RS at 6 weeks implies the fungi contributed more EE and the lower EE value of 40%RS and 100%RS at 6 weeks than at 0 weeks implies the EE was used as nutrient for fungi development (Wuanor and Ayoade, 2017). According to Kholif *et al.* (2001), some micro-organisms consume some fatty acids as suitable energy for growth. The ash content for 100%RS and 0% RS increased slightly in the 0 to 6 weeks' period and a slight decreased in 40%RS. According to Wuanor and Ayoade (2017) general increment in ash values of the treated straw means more minerals and vitamins were available after the degradation.

In vitro gas production

According to Sommart (2006), the production of methane gas best describes palatability, digestibility, fermentation end product and microbial synthesis of the substrate by the microbes in the *in vitro* system. Gas produced at 6 hours did not significantly differ. The total gas increment in treated samples results were agreement with results reported by Akinfemi *et al.*(2009) and Akinfemi and Muktar (2012). The improved rate of gas production in fungal treated materials signified more carbohydrate availability per unit time and also how fast the treated materials would release nutrient for animal use (Wuanor and Ayoade, 2017). It has been reported that rate of gas production determines digestion time and consequently how long a potentially disgestiable material would occupy space (Areghore and Abdulrazak, 2005). Thus treated materials would make them easily digested. In general, carbohydrates degradation is indicated by gas production and low gas production can be explained by lignin binding to the carbohydrate (Akinfemi, 2011).

The increase in metabolisable energy in 40%RS at 0 - 6 weeks signifies that fungal treatment produced more nutritive products as ME estimation makes use of gas volume, protein and crude

fibre fractions of the feeds which is in accordance to values gotten by Wuanor and Ayoade (2017). Organic matter digestibility improved due to fungal treatment as seen in difference in 40%RS, 0%RS and 100%RS at 0 - 6 weeks. Due to reduction in lignin which inhibits digestibility and the high ash value and gas volume used in estimating the organic matter digestibility (Wuanor and Ayoade, 2017).

Generally, methane represents source of energy waste or loss, as the animal have to eructate or belch it to prevent bloat (Babayemi *et al.*, 2006). Methane production decreased due to fungal treatment as observed in 40%RS 4 - 6 weeks, 0%RS 0-6 weeks and 100%RS at 0 - 6weeks. The reduction in methane production makes the treated materials more environmentally friendly, as they would contribute less to global warming (Mastrandrea and Schneider, 2008).

The short chain fatty acid production increase in the samples is reasoned to be due to higher absolute gas production. Close association between SCFA and gas production in *in vitro* was reported.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study shows that an increase in crude protein and digestibility, and decrease in methane gas volume production and crude fiber as a result of the biodegradation caused by the fungal *Pleurotus tuberregium*. The high values for DMD obtained implies high natural availability for rumen microbes. This study validates that pre-treatment of crop residue to increase the nutritive values and aiding digestibility by ruminant animals. Biological treatment of crop residue with fungal has potential for improving feeding values of rice straw for ruminant animals. However, Further studies can be carried out.

6.2 Recommendation

Pre-treatment of rice straw and palm oil sludge helps increase the nutritive value in ruminants. However, inclusion of palm oil sludge in ruminant diet should carefully done as increased lipids in ruminant diets do not favor the growth of rumen microbes.

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