DEVELOPMENT OF FEED RESOURCE USING REJECTED OR WASTE BREAD MATERIALS AND CASSAVA OFFALS

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

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ABSTRACT

This study was carried out to develop a feed resource using rejected or waste bread materials and cassava offals. A survey on bread waste in Being City was carried out using a questionnaire. Results showed that the apparent mean shelf-life of bread wasted in Benin City was 3.14 days. Plain and banana bread waste were analysed for microbial and proximate analyses for 1, 3, 5 ,8 and 12 days. Plain Bread was mixed with cassava offals at different ratio with 600ml of water and stored for 2, 6 and 10 days to age. The developed feed were analysed for Proximate Composition in tripheate. The developed feed Treatments were: T1 (100%Bread: 0%Cassava Offals), T2 (75% Bread: 25% Cassava Offals), T3 (50%Bread: 50%Casssava Offals), T4 (25% Bread: 75% Cassava Offals),T5 (0%Bread: 100%Cassava Offals). Results showed that treatment T1 of the developed feed had higher significant ($p<0.05$) difference on DM, CP and EE than other treatments. Also ageing had no significant effect ($p>0.05$) on the developed feed. This study showed that T4 can be incorporated in animal feed to reduce cost of production and serve as an alternative feed resource rather than wasting the by-products.

CERTIFICATION

This is to certify that this Project work was carried out by Onyema, Cynthia CHIKAODI with Matriculation Number AGR1600240 of the Department of Animal Science, Faculty of Agriculture, University of Benin, under the supervision of:

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DEDICATION

I dedicate this Project work to God Almighty, who has been my source of strength and inspiration; and also to my beloved parents, Mr. and Mrs. Onyema for their support.

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CHAPTER ONE

1.0 INTRODUCTION

The year round availability of quality and quantity of feed for livestock as well as its feeding is the main basis of livestock production. In addition, the primary source of income of about one billion people globally is dependent on livestock production (Salmon *et al.,* 2020).

Feed resources are residues and by-products of food crops obtained from agro-industrial by-products and/or non-conventional feed resources. Formulating a feed using these feed resources in regions of very high population density where crop production is the predominant activity and the major feed resources are the residues and by-products of food crops will encourage “creation of feed from waste”. Additionally, the major driven force of livestock production is the feed, animal health, animal breeding and productivity, and 70% of the overall expenses of maintaining livestock goes to feeding (Makkar, 2018). The utilization of non-conventional feed sources would help to reduce food insecurity in majority of developing countries and balance the struggle between humans and animals for conventional feed sources.

Accordingly, the search for alternative feed year round sources for livestock has led to exploitation of cassava by-products. Apart from the perennial nature of cassava, all the parts could be harnessed for livestock feeding purpose; the leaves, roots, cassava peels, cassava offals, cassava seviate e.t.c. (Nwokoro *et al.,* 2005). Cassava offals are the by-

product of the production of processed fermented cassava “Fufu”, a popular West African meal. Cassava tubers are peeled, washed and then fermented. The resulting product is then sieved and the waste collected is the Cassava Offals. This by-product contains ash, low cyanide, starch, crude protein and crude fibre resulting from the variability in nutrients arising from climatic and edaphic factors (Nwokoro *et al.,* 2005). Cassava Offals tends to pose an environmental problem with increase in production of cassava products. Hence, the cassava offals generated from cassava processing sites can be used as a potential feed source to reduce production cost in livestock diet. In addition, cassava offals contains substantial level of energy, if harnessed could be a good energy source in livestock diet (Nwokoro *et al.,* 2005). Thus, the need to improve the nutritional value of the waste as energy source becomes essential and to improve it nutritional value of this feed resource alternative as a suitable and acceptable feed ingredients, several feed technologies, such as fermentation (Oloruntola *et al.,* 2016), enzyme supplementation (Ogunsipe *et al.,* 2015, Oloruntola *et al.,* 2018), and many others had to be employed as just a few studies and research has been done on cassava offals.

Bread is a common food produced from the dough of flour and water, usually by baking. It is one of the oldest artificial foods which is popular around the world (Onoja *et al.,* 2011). Nutritionally, bread is an ample source of nutrition for the grain’s category. Also, it is considered a good source of carbohydrates through whole grains, nutrients such as iron, magnesium, vitamins, selenium and dietary fiber (Christiana,

2019). Apart from being readily available and cheap, both cassava offals and bread waste can be used as an alternative feed resource by farmers as they are unaware of the nutritional benefits of these by-products. Hence, the need for the development of a feed resource using cassava offals and bread waste.

1.1 Justification of this Study

A significant constraint for maintaining a sustainable food production system is the unstable ratio between food and feed, as well as the different climatic changes, degradation of land, and water scarcity in various regions. There is the need to develop a feed resource using waste from some staple and conventional food which are commonly dispose as there is lack of knowledge on their nutritional value in livestock production. The prevailing demand of the cassava crop and bread as a staple food in Nigeria is relatively high, the byproducts cassava offals and bread waste which has a substantial amount of energy and protein respectively are largely available but under-utilized. Hence, this study was carried out to develop a feed resource using rejected or waste bread materials and cassava offals to maximize products and improve feedstuff with high nutrients and cost effectiveness for livestock and feed processing industries.

1.2 Objective of the Study

The broad objective of this study was to develop a feed resource using bread waste and cassava offals.

The specific objectives were to:

1. carry out a survey on bread waste in Benin City;
2. carry out microbial and proximate analyses on bread waste;
3. determine the proximate composition of the feed developed using cassava offals and bread waste.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Origin of Cassava

Cassava is a perennial woody shrub that belongs to the Euphorbiaceae family, one of the largest within the dicots. It is known as *yucca, rumu, manioc* in Spanish speaking and Central America; *cassada* (Haiti), *ubi katella* or *kaspe* (Indonesia); *tupi* (India); *ege, gbaguda, akpu, rogo* (Nigeria), also known as *tapioca*, Brazilian arrowroot originated from tropical America and was first introduced to the Congo basin, Africa, by the Portuguese around 1558 (Akoroda and Ikpi, 1992). Portuguese explorers introduced cassava to Africa during the 16th and 17th centuries through their trade with the Africa coasts and nearby islands. Africans then spread cassava further, and it is now found in almost all parts of tropical Africa. Over the past five (5) years (2008-2012 inclusive), cassava production on the African continent (approximately 54% of global production) as a whole has been growing at a faster rate (approximately 4%) compared with other major regions (worldwide average growth rate, +1.2%). African cassava production has surpassed 145 million tonne in 2011, approximately 57% of the global crop that year (256 million tonne, FAO 2013). Nigeria alone contributed 36% of all African production, which is approximately 52 million tonne (FAO, 2013).

Recently, cassava production stands at 278 million metric tons worldwide, in which Africa produces about 61% (192 million metric tons) with Nigeria leading with a

production of over 60 million metric tons in 2020, and continuous production has increased in the phase of climatic change (FAO, 2020). Today, Nigeria and Congo-Kinshasa AE are the biggest producers of cassava (Rey, 2016).

2.2 Scientific Classification of Cassava

Kingdom:	*Plantea*
Subkingdom:	*Tracheobionta*
Super-Division:	*Spermatophyta*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Sub-Class:	*Rosidae*
Order:	*Euphorbiales*
Family:	*Euphorbiaceae*
Genus:	*Manihot mill*
Species:	*M. esculenta*
Botanical Name:	*Manihot esculenta* Crantz.

Source: Word Checklist of Selected Plant Families (WCSP, 2017).

2.3. Cassava Plant

Cassava is an extensively cultivated annualS crop in tropical and subtropical regions for its edible underground tuberous root, recognized as one of the highest yielder starches and the third most important source of calories in the tropics after rice and maize (Food Safety Network, 2014). It is a major staple food in the developing world, providing a basic diet for over 800 million people (Lebot 2009; Ecocorp, 2011). It is an important staple in many developing countries of Africa, South and Central America, India and Southeast Asia (Al Afas *et al.,* 2006).

Primarily, the methods are subsistence in nature and maybe unable to support industrial level demands (FAO, 2013). It is one of most easily cultivated crop plant due to its hardiness and drought adaptation as it does well on poor soils and low rainfall. It also acts as a famine reserve because of its wide harvesting duration. Cassava offers flexibility to resource-poor farmers because it serves as either subsistence or a cash crop (Stone, 2002). It shelf life that is generally accepted is within 24 to 48hrs after harvest (Andrew, 2002). Cassava utilization patterns vary considerably in different parts of the world. The whole cassava plants are beneficial to man. The roots and leaves are consumed and its stems are used for propagation. Cassava varieties have been classified as bitter or sweet depending on their cyanogenic glucoside contents. The major drawbacks of the cassava crop are the low tuber protein content, rapid tuber perishability following harvest, and high content of

the cyanogenic glucosides which is the main toxic substance in the cassava roots. Processing of cassava roots into dry form reduces the moisture content; convert it into more durable and stable product with less volume, which makes it more transportable. Processing is also necessary to improve palatability, eliminate or reduce the level of cassava cyanide contents (Cardoso *et al.,* 2005). Also, only a reported 5% of cassava is currently used as livestock feed (Apata and Babalola, 2012).

2.4 Cassava Offals

Cassava offal is a by-product or waste obtained from the processing of fermented cassava "Fufu", a popular West African meal. It is presently produced by mainly rural processers at both household and in a small scale in eastern and south Western Nigeria. The processing of cassava offals involves the combination of unit of activities such as; peeling, washing, slicing, grating and soaking, boiling, steaming, drying, pounding and milling. In the processing of cassava offals, the roots are usually fermented, which normally involves peeling to get rid of two outer coverings: a thin brown outer covering, and a thicker leathery parenchymatous inner covering (Nwokoro *et al.,* 2005).

Cassava offals is produced by manually peeling of cassava roots with hand knives, washed and made ready for soaking. The peeled and washed cassava roots are cut manually into chunks of different sizes and soaked in water for 3-4 to undergo lactic acid fermentation. During soaking, the pH value decreases and the roots soften,

facilitating the reduction in potentially toxic cyanogenic compounds (Ayornor, 1985; Oyewole and Odunfa, 1992; Westby and Choo, 1994). When sufficiently soft, the roots are taken out, broken by hand and fibres removed by sieving. The starch suspension is allowed to sediment for about 24hrs and after sedimentation, the cassava offals is produced.

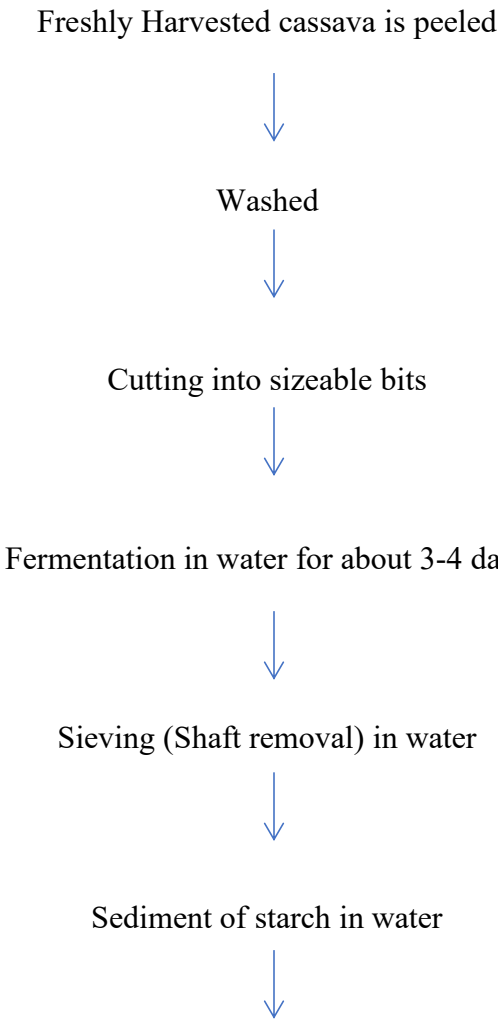

Figure 1: Derivation Procedure for Cassava Offals

Source: Nwokoro *et al.*, 2005

2.5 Chemical and Nutritional Composition of Cassava Offals

It is indicated that proximate dry matter, starch yield, and cyanide content are some of the most important quality indicators in the food sector when selecting raw materials. Proximate composition (proteins, lipids, fiber, ash, and moisture), starch yield, and dry matter content are examples of such parameters (Mubanga *et al.,* 2019). Cultivar, geographical location, plant maturity stage, and environmental variables are elements that influence chemical composition in plants (Burns *et al.,* 2012; Agiriga and Owe, 2016). As a result of this, determining the starch and other chemical components of cassava cultivars might be helpful in selecting cassava cultivars for various foods and feed formulations, processing, and ultimately industrial applications (Manano *et al.,* 2018). Hence, Cassava Offals has been reported by Nwokoro *et al.* (2005) to contain low cyanide, with starch ranging between 70.50% to 77.52%, crude protein 1.72% to 2.21% and crude fibre 1.26% to 3.20% and this is as a result of the variability in nutrients arising from climatic and edaphic factors.

The biochemical composition of cassava roots varies based on cultural methods such as root pruning, age at harvest, maturity at harvest, storage environment, area of growth, and postharvest practices (Manano *et al.,* 2018). And Cassava's nutritional value is determined by the plant portion (root or leaves), cultivar, age, geographical location, and environmental conditions (Agiriga and Owe, 2016; Salvador *et al.,* 2014).

Table 2.1: Nutritional and Cyanide composition of Cassava Offals in Benin City

S/N	Nutrients	Cassava Offals
1.	Dry Matter (%).	85.02
2.	Crude Protein (%)	2.21
3.	Ether Extract (%)	0.45
4.	Crude Fiber (%)	1.82
5.	Ash(%)	2.22
6.	Starch(%)	76.10
7.	Cyanide (mg/kg)	1.02

Source: Nwokoro *et al.*, 2005

2.6 Physical Properties of Cassava Offals

Cassava processing involves combination of unit activities such as; peeling, slicing, grating, soaking, boiling, steaming, roasting, drying, pounding and milling. In the processing of cassava offals, the roots are usually fermented which normally involves peeling to get rid of two outer coverings: a thin brown outer covering, and a thicker leathery parenchymatous inner covering (Nwokoro *et al.,* 2005). Physical deterioration of cassava occurs only a few days after harvest and this may contribute to the variable quality of cassava products such as cassava offals.

Some of the physical properties of cassava offals include their bulk density, particle size distribution, moisture content, porosity, and color.

Bulk density: Cassava offals have a low bulk density, which makes them easy to handle and transport. It ranges from about 0.2 g/cm to 30.8 g/cm^3 (Adetunji and Adedeji, 2018).

Particle size: The particle size of cassava offals affects their nutritional value and digestibility. Finely ground cassava offals are more digestible than coarse ones because they have a larger surface area, which allows for better microbial activity (Kudi *et al.,* 2014).

Moisture content: The moisture content of cassava offals varies depending on the processing method and storage. It varies from 10 to 20%, while the ash content ranges from 3 to 10% (Chinma *et al.,* 2016). The high moisture content of cassava offals makes them susceptible to microbial growth and spoilage, which can affect their quality and shelf life. However, the high ash content of cassava offals makes them a good source of minerals and nutrients, which can be used in animal feed formulations (Oyewole *et al.,* 2019).

Porosity: Cassava offals are highly porous, which means they have a large surface area and can absorb water and nutrients. This property makes them useful as a soil conditioner and fertilizer.

Color: The color of cassava offals varies depending on the processing method from which they are derived.

2.7. Uses of Cassava Offals

Cassava offals as a by-product derived from processing of cassava roots have few uses in various applications. It can be used as a source of organic fertilizer to crops which improve soil fertility, increase crop yield, and reduce soil erosion (Nguyen *et al.,* 2012). Cassava offals can be used in the production of several industrial products such as bioethanol, biodegradable plastics, and enzymes (Oboh *et al.,* 2012; Sutivisedsak *et al.,* 2019). It also been used as an energy source in animal feed, and study found that cassava offals could be used to replace maize in the diets of growing pigs without affecting their growth performance (Ogunade *et al.,* 2020).

2.8 Bread

Bread is the most popular and common food in the human diet; which is widely consumed with a steady increasing rate throughout the world (Edama *et al.,* 2015). It is made from flour dough; mainly wheat flour and water and often baked. It is one of the oldest meals produced by humans for many years. Various countries have their distinct way and recipes of making bread. Baked dough which comprises of flour, water, yeast, and other components like salt and fats is referred to as bread (Foodie, 2022).

Bread was usually baked in clay or wood ovens in past years, but recently, technology has completely changed how we make and eat bread. Modern baking are now implored which makes the techniques more efficient, quicker and yield reliable results. Bread may now be produced by automated bakeries with little manual labor in large

quantities. Also, commercial ways have been employed in baking of bread frequently in many countries by adding additives to improve the shelf life, flavour, texture, nutrition, colour and ease of production (Wikipedia, 2022).

Some of the local breads in Nigeria includes;

***Agege* bread**

This is one of the popularly known bread in Nigeria which is liked by many. It name "agege" was derived from the well-known Agege in Lagos State, Nigeria. Agege bread is fluffy and smooth. Its ingredients include; Dry yeast, flour, eggs, milk, granulated sugar, salt, water and melted sugar (Foodie in Lagos, 2022).

Whole wheat bread

This type of bread is mostly consumed by diabetic patients, elderly people and people watching their weight. The major ingredients are the wheat flour and other ingredients are; yeast, milk, salt etc. Wheat bread is often more expensive than a normal white bread (Foodie in Lagos, 2022).

Banana bread

Banana bread is very soft and moist. Its main ingredient is banana puree. And the banana is responsible the moist nature of the bread and gives it a delightful flavour and texture (Foodie in Lagos, 2022).

White Bread

White bread is the normal bread that is sold over the counter at shops and supermarkets. It is the top item in every bakery in Nigeria. It is a type of bread that is relatively available irrespective of one's background and socioeconomic level. Ingredients are; Sugar Salt, yeast, softened butter, flour and other bread ingredients (Foodie in Lagos, 2022).

Coconut bread

Coconut bread is another type of bread in Nigeria. Because of its exceptional and fantastic flavor, it is regarded as luxury bread. An actual coconut provides the rich coconut flavor of coconut bread. The bread is made using grated or shredded coconut and coconut cream. Sweet, golden, and crunchy bread is the result of the baking process (Foodie in Lagos, 2022).

2.9 Bread Waste

Food waste is generally a pressing issue in today's world, with substantial economic, environmental and social implications. And a large portion of food wastes are leftover bread, which is the most commonly eaten and most wasted food in the world. Millions

of tonnes of bread are wasted each year in the world. This is as a result of the quick deterioration of bread and bread goods. Also, due to a lot of bread that are uneaten in homes and not yet sold out in shops, thereby leading to surplus bread that eventually goes to waste (Evans *et al.,* 2012). People often purchase more bread than they can consume before it goes stale or moldy, leading to discard (Quested *et al.,* 2013).

Bread waste has significant economic implications, as it represents a loss of resources invested in bread production and distribution (Parfitt *et al.,* 2010). It environmental consequences are profound as bread production involves the consumption of water, energy, and agricultural resources. So when bread is discarded, it contributes to greenhouse gas emissions in landfills (Gustavsson *et al.,* 2011). And resources used to produce wasted bread could be redirected to address hunger and malnutrition (Buzby *et al.,* 2014). Alternatively, leftover bread could be utilized as a resource for feeding livestock.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Location

The experiment was carried out in the Department of Animal science Laboratory of the Faculty of Agriculture and Department of Microbiology Laboratory of Faculty of Life Science, University of Benin, Ugbowo Campus Benin City Edo State Nigeria. University of Benin is located between latitude 620N of the equator and longitude 54^0 and 6^0E of the Greenwich meridian in the forest zone with an average temperature of 27.6^0C (NAA, 2014). Benin City has an average annual rainfall, relatively humidity and daily sunshine of 2162mm, 72.5% and 6.68%hours respectively (Google earth, 2022).

3.2 Experimental Materials

The materials used for this experiment include questionnaires which were shared to bakeries and bread sellers. This was used to determine the amount of bread waste in areas around Benin City, Edo State. Fresh cassava offals were obtained, procured and sun-dried. Fresh baked bread was procured for the experiment.

3.3 Bread Waste Survey

A survey was carried out to know the quantity of bread wasted in Benin City, Edo State. Questionnaires were distributed to various bakeries and retailers who sold bread in areas such as Ekosodin, BDPA, Textile Mile road, Oluku, Isihior, and UNIBEN. A total of 190 questionnaires were shared, 15 were given to bread bakeries and 175 were given to bread wholesalers and retailers and data were collected. These data were coded and analyzed.

3.4 Feed Development

Feed development was performed using cassava offals and bread waste at varying levels. Microbial and proximate analyses were done also on two bread types; banana bread and white (plain) bread. These breads were left for 5 different days (i.e. 1, 3, 5, 8 and 12) to deteriorate and samples were collected. Fresh cassava offals were collected from a cassava processing centre in Eyaen village, Edo State. The cassava offals which were thoroughly washed with clean water to remove any form of contaminants, was spread on a flat surface and sun-dried. The cassava offals were left to dry for about 10 to 14 days, due to the weather conditions until it was properly dried. After it was dried, it was milled. White (plain) bread was purchased from Nadia bakery, Ugbowo, Benin City, Edo State. It was left for 12 days to deteriorate and spoil. Then, the white (plain) bread was mixed with cassava offals at different ratio (i.e. 0%, 25%, 50%, 75%, and 100%) with 600ml of water and stored for 2,6 and 10 days to age. For

each of these days, samples were collected and dried, thereafter; proximate analysis was done on the treatments.

3.5 Experimental Design

The experiment was carried out using a randomized complete block design.

3.6 Microbial Analysis

3.6.1 Sterilization of Material

Materials such as Petri-dishes, pipette, glass containers (conical flask, round bottom flask) and bottles were washed, drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160oC for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol.

3.6.2 Preparation and sterilization of media

All media used were obtained from Oxoid and were prepared according to manufacturers' instruction. The media used in this study include Plate count agar, Bacillus cereus agar (BCA), Eosin methylene blue agar (EMB), Mannitol salt agar (MSA), Salmonella Shigella agar (SSA), Pseudomonas cetrimide agar (PCA), Triple sugar iron agar (TSI), Simmons citrate agar (SCA) and Mueller Hinton agar (MHA).

3.6.3 Enumeration and isolation of total heterotrophic bacterial and fungal count

One-thousand-fold serial dilution of the samples was prepared aseptically in sterile physiological saline. An aliquot of 0.1 ml was inoculated using the pour plating technique. Appropriate media were used for fungal and bacterial enumeration. Tryptone soy agar (supplemented with fluconazole) for bacteria and potato dextrose agar (supplemented with chloramphenicol) for fungi. Plates were cultured at 28 ± 2^{0}C for 24 hours. The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula below:

$$\text{Cfu/ml} = \frac{\text{Number of colonies x dilution fold/series)}}{\text{Volume of inoculum}}$$

(Willey *et al.,* 2008).

3.6.4 Phenotypic identification of bacteria from samples

Following successful pour plate technique, isolation and culture was made from a single colony and characterized using cultural, morphological and biochemical methods using the Bergey's manual. Several tests such as Gram reaction, catalase, urease, indole, oxidase, sugar fermentation, citrate utilization, respective reaction on triple sugar iron agar tests were carried out to presumptively identify bacterial isolates (Holt *et al.,* 1994).

3.6.5 Morphology identification

The morphological identity of each bacteria isolate was obtained by Gram staining so as to know the gram reaction, cell morphology and arrangement by viewing under the microscope. The gram stain procedure is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1min then washed with distilled water.

Subsequently the slides were flooded with Lugol's iodine solution for 30sec and then washed off with distilled water. 95% alcohol was used for decolorization for 10sec and immediately washed off with distilled water.

Finally, the smear was counter stained with safranin for 1min and washed off.

The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of ×100 magnifications to view the slides.

3.6.6 Biochemical identification

Biochemical test was carried out so as to help in the identification of the bacteria isolates as a phenotypic (cultural) characteristic is not sufficient. The various biochemical test carried out are shown below;

3.6.6.1 Oxidase test

This is mainly used to differentiate between pseudomonas from other gram-negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochrome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are gram positive and gram negative respectively were employed as control. A piece of filter paper using sterilizes wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 seconds.

3.6.6.2 Urease test

This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like Proteus mirabilis from other non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 degrees centigrade for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

3.6.6.3 Indole production test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The best is usually used in differentiating gram-negative bacilli especially those of Enterobacteriaceae. Five grams of

commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degrees centigrade for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacteria isolates was inoculated into the peptone broth. The inoculated media was incubated 37 degrees centigrade for 24 hours after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150ml of amyl alcohol, 10g dimethylamino-benzaldehyde and 150ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

3.6.6.4 Citrate utilization test

This test is used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is Simon`s citrate agar. In the preparation, 22 grams of commercially available Simon`s citrate agar was dissolved in litre of distilled water and sterilized by autoclaving at 121 degrees centigrade for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stabling the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 degrees centigrade for about 24 hours. Positive result is indicated by a change in colour from green to bright blue colouration.

3.6.6.5 Catalase test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$2H_2O_2 \rightarrow 2H_2O + O_2$

3.6.7 Sugar fermentation and production of gases using Triple sugar iron agar (TSI)

TSI was prepared following manufacturer's instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hr. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presentence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

3.7. Proximate Analysis

The standard methods of analysis of Association of Official Analytic Chemist (AOAC, 2000) were adopted in the determine of dry matter, moisture content, crude protein, ash content, ether extract, crude fiber, and NFE of the samples.

3.7.1 Moisture content determination

Moisture content was determined by drying the sample in an oven.

Procedure: 10g of the sample was weighed into a silica dish previously dried and weighed. The sample was then dried in an oven for 75°C for 24 hours. The sample was cooled in a desiccator after drying and then weighed. The drying and weighing continued until a constant weight was achieved.

3.7.2 Dry matter content determination

Since the water content of feed varies very widely, ingredients and feed are usually compared for their nutrient content on moisture free or dry matter (DM) basis. However, the sample was expressed on dry matter basis. Thus, DM was calculated as:

% DM = 100 - % Moisture.

3.7.3 Determination of crude protein

The determination of Crude protein was carried out using kjedahl's procedure. 0.5gram of the samples was measured into a conical flask, 10ml of 72% concentrated sulphuric acid and 2gram of mixed catalyst (CuSO4:NaSO4 at ratio1:9) was added to

the sample. The samples were heated to 420oC until a light green digest is attained. The digest was put into a universal reagent bottle (120ml) and diluted with distilled water to 50ml mark.

After digestion, distillation was carried out by adding 5ml of the digest to 10ml of 40% NaOH and 30ml of distilled water in kjeldahl's flask. This was distilled into 5ml of boric acid indicator. About 20ml of distillate was collected and titrated using 0.1N HCL. This was done for each treatment. The Crude protein was calculated using the formulae 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

3.7.4 Determination of crude fibre

A known weight (1g) of the sample was weighed into a beaker and 100ml of 1.25% H2SO4 was added. The mixture was placed in the heating mantle with a temperature of 70^0C- 100^0C. The sample upon boiling was heated for 30 minutes while the 100ml 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 100ml of 1.25% NaOH 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 100ml mark by topping with hot water. After heating, the mixture was 3.6.5 Determination of Ash

2gram of the samples was weighed into crucible of known weight and heated in the muffle furnace at 550^0C for 6 hours. The crucible was left to cool in a desiccator 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

3.7.5 Determination of ash

2gram of the samples was weighed into crucible of known weight and heated in the muffle furnace at 550^0C for 6 hours. The crucible was left to cool in a desiccator 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

3.7.6 Determination of ether extract

A spatula was used to collect 1g of the sample into a fat free filter paper of known weight, the fat free filter paper containing the sample was carefully folded and labelled and put in a 250ml flask containing the solvent (petroleum ether). The flask was

placed on a heating mantle with temperature ranging from 50oC and 70oC. During heating, ether vapour raises through the free fat paper and is collected and cooled in a condensing unit of the apparatus. After a time, lapse of 4hrs, sample is collected and dried in an oven for 3hrs at 80^{o}C. Dried sample is put in a desiccator before weight.

$$\%\text{Ether Extract} = \frac{\text{Total weight} - \text{Tare weight}}{\text{Weight of the sample}} \times \frac{100}{1}$$

3.8. Statistical Analysis

The data in the figures and tables are expressed as mean ± standard deviation of three repetitions. Analysis of Variance (ANOVA) and Duncan's multiple comparison tests were performed using SPSS statistical software (IBM SPSS Statistics 22). Significance was determined at $p = 0.05$ for all statistical analyses.

Plate 1: Developed feed products

Plate 2: Crushing of developed feed products

CHAPTER FOUR

RESULTS

4.1 Survey of Bread waste in Benin City Environs

The apparent shelf-life of bread waste surveyed in Benin City was wasted at the mean of 3.31 days.

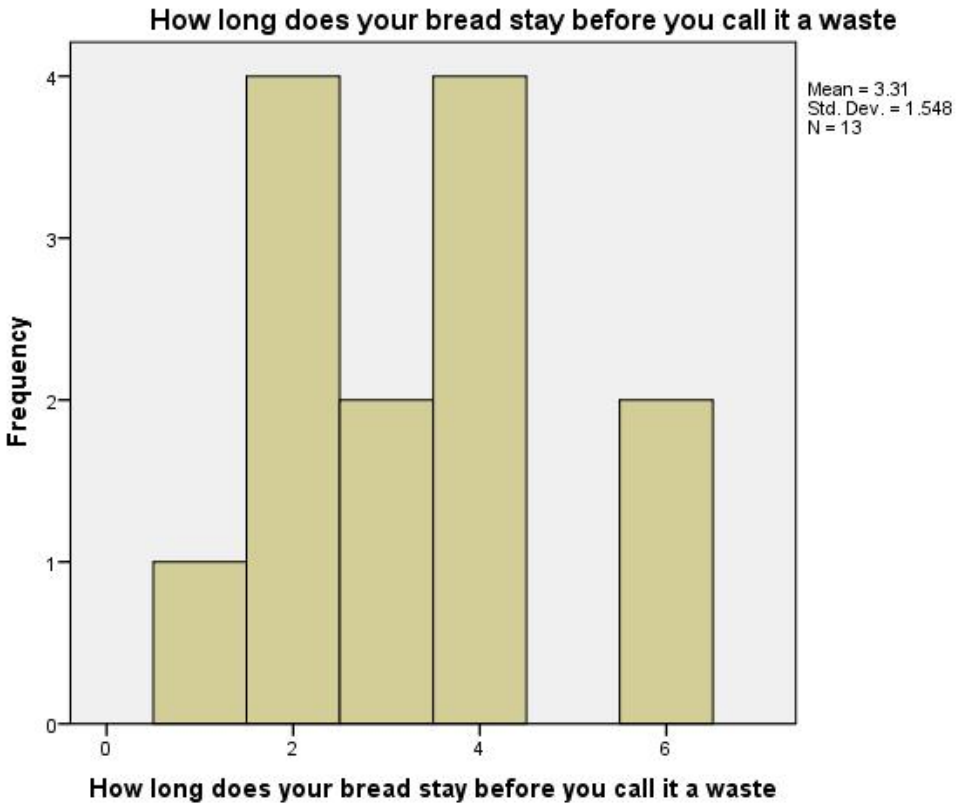

Figure 4.1: Apparent shelf-life of bread wasted in Benin City

4.2. Survey of Bread wasted during Production in Benin City Environs

The apparent shelf-life of bread wasted during production surveyed in Benin City was at the mean of 1.57.

Figure 4.2: Apparent shelf-life of bread wasted during production in Benin City

The average microbial count of white and banana breads stored for 1-12days is presented in Table 4.1. Results showed that there was no significant difference ($p>0.05$) between the bacteria count of white (plain) and banana bread. In addition, there was no significant difference ($p>0.05$) between the fungi count of white (plain) and banana.

Table 4.1: Average microbial count of white and banana bread stored between 1 and 12 days

Bread	**Bacteria Count(cfu/G)**	**Fungi Count(cfu/G)**
White	6325333	5554667
Banana	3366000	5598000
LSD	10486796.2	11830147.8

The study showed that bacteria growth from day 1 to 5 was more in banana bread, but from day 8 to 12, bacteria growth was more in white bread as shown in the Figure 4.3.

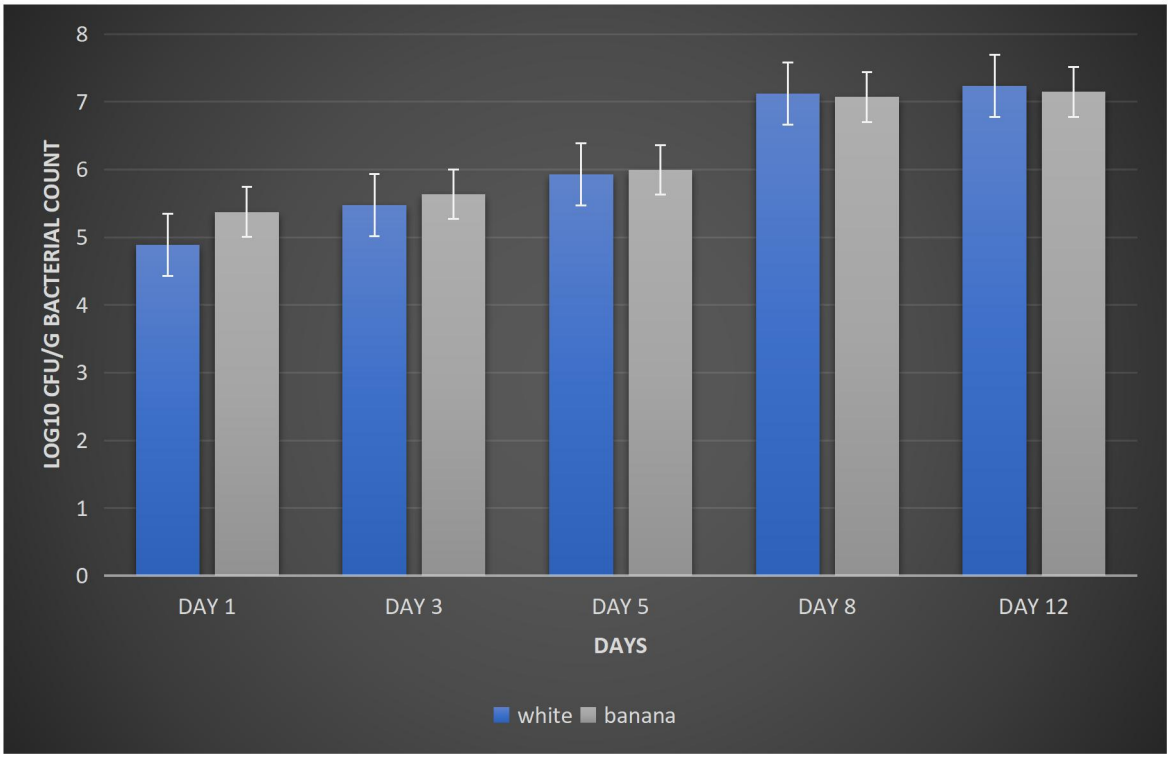

Figure 4.3: Total heterotrophic bacteria count for white and banana bread (log10 cfu/g)

The study showed that fungi growth was more in banana bread, but had a uniform growth as the day progresses (day 12) in Figure 4.4.

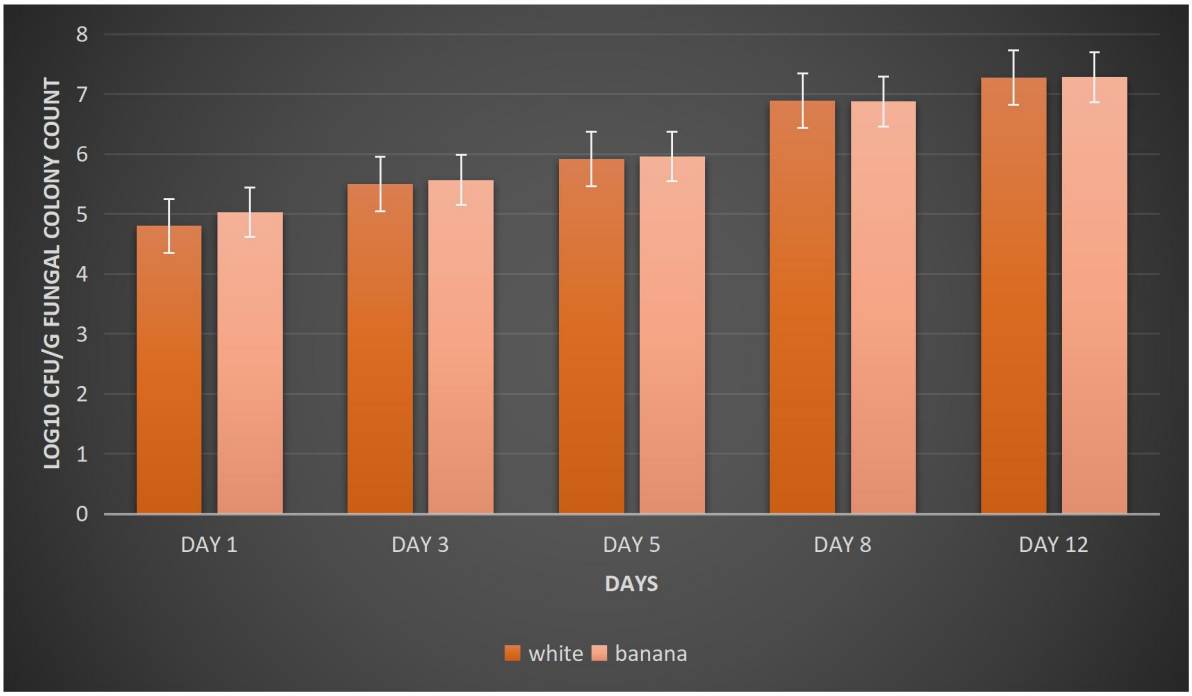

Figure 4.4: Total fungal colony count for white and banana bread (log10 cfu/g)

The proximate composition of the feed resource developed using Bread and Cassava Offals at varying levels was shown in Table 4.2. The dry matter values among the treatments are significantly different ($p>0.05$) with T1 being the highest value of 35.10% and T5 being the lowest value of 28.98. The crude protein obtained varied with T1 having the highest value of 10.14% and T5 having the least value of 2.07%. T1 was not significantly different ($p>0.05$) from T3, however, T2 varied significantly ($p>0.05$) from T1, T3 and T5. The Crude fibre mean also varied with T5 having the highest fibre value of 1.09% and T1 the lowest value of 0.21%. T3 was not significantly different ($p>0.05$) from T4. However, T3 showed significant ($p>0.05$) variation from T1, T2 and T5. The ether extract values among the treatments are significantly different ($p>0.05$) with T1 being the highest value with 9.89% and T2 being the lowest with 7.89%. Ash treatments are significantly different ($p>0.05$) with T5 having the highest value of 2.15% and T1 being the lowest value of 1.85%. Nitrogen free extract are significantly different ($p>0.05$) with T5 being the highest value with 85.93% and T1 with the lowest value of 77.7%.

Table 4.2: Proximate Composition of feed resource developed using bread waste and cassava offals at varying Levels

PARAMETER	T1	T2	T3	T4	T5	SEM
DM	35.10^{d}	31.05^{b}	32.46^{bc}	33.05^{c}	28.98^{a}	0.521
CP	10.14^{a}	6.45^{c}	6.28^{c}	7.18^{b}	2.07^{d}	0.1731
CF	0.21^{c}	0.54^{b}	0.94^{a}	0.94^{a}	1.09^{a}	0.0666
EE	9.89^{a}	7.89^{b}	8.22^{ab}	8.67^{ab}	8.78^{ab}	0.598
ASH	1.85^{c}	2.02^{ab}	2.05^{ab}	1.96^{bc}	2.13a	0.0470
NFE	77.90^{a}	83.09^{c}	82.51^{bc}	81.24^{b}	85.93^{d}	0.579

^{a,b,c,d} =Treatment mean on the row with different superscript differ significantly(p>0.05)

T1 = 100%Bread: 0%Cassava Offals (Control)

T2 = 75%Bread: 25%Cassava Offals

T3 = 50%Bread: 50%Casssava Offals

T4 = 25%Bread : 75%Cassava Offals

T5 = 0%Bread : 100%Cassava Offals

SEM Standard Error Mean.

The age effect on proximate composition of the feed resource developed using Bread and Cassava Offals at varying levels was shown in Table 4.3. The ageing effect on dry matter and crude protein content of the feed resource developed showed significant difference ($p>0.05$) between 2, 6 and 10 days. . The crude fibre and ether extract showed no significant difference ($p>0.05$) between 2, 6 and 10 days. Also, ash mean varied with T5 having the highest value of 2.15% and T1 being the lowest value of 1.85%.showed no significant difference ($p>0.05$) between 2 and 10 days, except day 6 which showed a significant difference ($p>0.05$) between 2 and 10 days. And NFE showed no significant difference ($p>0.05$) between 2, 6 and 10 days.

Table 4.3: Aging Effect on Proximate Composition of Feed Resource Developed Using Bread and Cassava Offals at Varying Levels.

Proximate	DAY 2	DAY 6	DAY 10
Dry Matter	36.77^{a}	29.61^{c}	30.00^{b}
Crude Protein	6.86a	5.71^{c}	6.7^{b}
Crude Fiber	0.83^{a}	0.75^{b}	0.65^{c}
Ether Extract	8.20^{b}	10.33^{a}	7.53^{c}
Ash	2.01^{a}	2.00^{b}	2.01^{a}
NFE	82.28^{b}	81.21^{c}	82.93^{a}

a,b,c,d =Treatment mean on the row with different superscript differ significantly (p>0.05)

NFE=Nitrogen Free Extract

CHAPTER FIVE

DISCUSSION

5.1. Bread Waste Survey Evaluation

Bread waste is a major part of the global food wasted in domestic households and is wasted in the whole supply chain (Brancoli *et al.,* 2019). The apparent shelf-life of bread waste and bread wasted during production surveyed in Benin City was wasted at the mean of 3.31 days and 1.57 respectively. As reported by Evans *et al.* (2012), due to a lot of bread that are uneaten in homes and not yet sold out in shops, has led to surplus bread that goes to waste. And People often purchase more bread than they can consume before it goes stale or moldy, leading to discard (Quested *et al.,* 2013).

5.2. Microbial Analysis of White (plain) Bread

Results showed that there was no significant difference ($p>0.05$) between the bacteria count of white (plain) and banana bread. In addition, there was no significant difference ($p>0.05$) between the fungi count of white (plain) and banana.

5.3 Proximate Composition

The dry matter values among the treatments are significantly different ($p>0.05$) with T1 being the highest value of 35.10% and T5 being the lowest value of 28.98. The lower moisture content of bread waste was as the rate of changes in proton mobility (Nouri *et al.,* 2018). The crude protein content of T1 was not significantly different

(p>0.05) from T2, however, but there were significant difference (p>0.05) in T1, T4 and T5. However, T4 had crude protein level of 7.18%, which is more than the normal level of cassava offals, so it should be implored in livestock feeding as the Crude protein level was increased due to the combination. Cassava Offals has low cyanide with starch ranging between 70.50% to 77.52% and crude protein 1.72% to 2.21% (Nwokoro *et al.,* 2005), thereby making it nutritionally acceptable and available for livestock consumption. The crude fibre mean also varied with T5 having the highest fibre value of 1.09% and T1 the lowest value of 0.21%. T1 and T2 showed no significant different (p>0.05), however, there was no significant variation (p>0.05) between T3, T4 and T5. The ether extract values among the treatments showed significant different (p>0.05) in T1 and T2. And there was no significant different (p>0.05) in T3, T4 and T5. Ash treatments varied with T5 having the highest value of 2.15% and T1 being the lowest value of 1.85%. There was significantly different (p>0.05) in T1, T4 and T5, however there was no significantly different (p>0.05) in T2 and T3 with T5 having the highest value of 2.15% and T1 being the lowest value of 1.85%. Nitrogen free extract are significantly different (p>0.05) with T5 being the highest value with 85.93% and T1 with the lowest value of 77.7%.

5.4. Aging Effect on the Proximate Composition of the feed resource developed.

The ageing effect on dry matter and crude protein content of the feed resource developed showed significant difference ($p>0.05$) between 2, 6 and 10 days. Ageing of the bread waste matrix attributed to the reduced mobility and caused loss of water from the crumb (Curti *et al.,* 2011; Ronda *et al.,* 2017). The crude fibre and ether extract showed no significant difference ($p>0.05$) between 2, 6 and 10 days. Also ash showed no significant difference ($p>0.05$) between 2 and 10 days, except day 6 which showed a significant difference ($p>0.05$) between 2 and 10 days. And NFE showed no significant difference ($p>0.05$) between 2, 6 and 10 days.

The feed resource developed products of this study is a new feed with DM of 28.98% to 35.10%, CP of 2.07% to 10.14%, CF of 0.21% to 1.09%, EE of 7.89% to 9.89%, ASH of 1.85% to 2.18% and NFE of 77.90% to 85.93%. The low DM levels of the developed feed resource maybe connected to the difference in the by-products used. Similarly, the CP levels is substantial and the CF level is low, this indicates the feed resource if properly developed maybe useful for livestock feeding especially for monogastric, Tewe as reported by Nwokoro *et al.* (2005). Also, some of the products in these products with substantial amount of crude protein level like T1 and T4 may be utilized as a protein supplement to low quality feed such as crop residues to increase productivity of livestock in tropical regions.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

There is a continuous demand for alternative feed resource in feed processing industries due to unstable balance, struggle between humans and animals for conventional feed sources. This study has shown that bread waste and cassava offals can be developed as an acceptable feed resource if incorporated at a ratio of 25% bread waste and 75% cassava offals, as it readily available all year round and helps to reduce production cost in livestock diet as there is a continuous demand in alternative feed resource in feed processing industries due to insufficient supply of feed resource, high prices and competition with the human food. Also, the developed feed resource must be formulated with care, particularly with regards in balancing vitamins, minerals and other essential nutrients.

6.2 Recommendation

The addition of bread waste and cassava offals improved the crude protein content of cassava offals: hence, rather than wasting cassava offals, it could be incorporated with bread waste in developing new feed for livestock production. In addition, bread waste should also be tried in animal feed as it nutrients composition was improved and the developed products could be considered to be fully adopted in the commercial livestock feed industry.

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