Studies on Dietary Fermented Mixture of Cassava and Palm Kernel Cake on Carcass Characterisitcs of Broilers and Pigs

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CERTIFICATION

I certify that this work "**Studies on Dietary Fermented Mixture of Cassava and Palm Kernel Cake on Carcass Characteristics of Broilers and Pigs**" was carried out by Aladi, Nnanyere Okwunna (20094833908) in partial fulfilment for the award of the degree of Doctor of Philosophy in Animal Products Technology in the Department of Animal Science and Technology of the Federal University of Technology, Owerri.

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DEDICATION

To the greater glory of God, this work is dedicated to all my teachers especially

Rev. Fr. Nathaniel Ndiokwere, PhD

&

Engr. Nathaniel C. Onwuagha

in appreciation for all the formation I received from you.

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ABSTRACT

Five experiments were carried out to determine the effect of replacing maize with solid state fermented mixture of cassava root pulp and palm kernel cake on performance, carcass and meat quality of broiler chicks and pigs. First, 3 inoculation techniques were evaluated for their efficacy in solid state fermentation of the mixture namely; direct inoculation with *Aspergillus niger*, batch inoculation with previously inoculated samples, and spontaneous inoculation. In study 2, sundried spontaneously fermented samples (FEMCARPP) were used to replace maize in broiler chicks diets, whereas in study 3, the performance, carcass characteristics and meat quality of broiler finishers fed diets containing wet or sundried FEMCARPP were compared to the controls (maize based diet) and another diet containing a mixture of cassava root meal and palm kernel cake (CSM-PKC mix) as replacement for maize. In study 4, FEMCARPP was used to replace maize in diet of weaner pig whereas study 5 evaluated the performance, carcass and meat quality of pigs fed diets in which maize was replaced FEMCARPP and CSM-PKC mix. Results show that, all inoculation techniques were efficient in improving the physicochemical characteristics of the mixture for inclusion in poultry ration. Dustiness of cassava meals was completely removed, protein content increased significantly, while crude fibre levels reduced in treatments relative to the control. Broiler chicks fed sundried FERMCARPP had significantly ($p<0.05$) lower live weight gains, feed intake, feed conversion ratio and cost per kg weight gain than those fed the control diet. Carcass characteristics were similar ($p>0.05$) while meat quality of chicks fed diets containing FEMCARPP was better ($p<0.05$) than the control and CSM-PKC mix diets. Chicks fed wet FEMCARPP had lower live weight gains and feed intake ($p<0.05$). Their feed conversion ratio was similar ($p>0.05$) to the control but superior to chicks fed diets containing sundried FEMCARPP and CSM-PKC mix. Cost per kg weight gained was better among chicks fed FEMCARPP without sun drying. Pigs at both weaner (study 4) and grower-finisher (study 5) stages fed diets on FEMCARPP was high ($p<0.05$) in live weight, weight gain, lower feed intake, feed conversion ratio and lower cost per kg weight gained. No significant differences were found for carcass characteristics of pigs. Meat of pigs fed maize based diets was significantly ($p<0.05$) higher water holding capacity and cooking loss; but with lower tenderness score when compared to those fed FEMCARPP based diets. Both were tenderer than those fed CSM-PKC diet. It is therefore concluded that solid state fermentation of spontaneously inoculated mixture of cassava root pulp and palm kernel cake is an effective tool for improving the nutritive value of the mixture for use in poultry and pig diets. The product can be used without further drying to replace maize in poultry and swine diets without detrimental effects on production, health, and carcass and meat quality of broilers and pigs. Solid state fermentation of spontaneously inoculated mixture of cassava root pulp and palm kernel cake is therefore recommended for poultry and pig farmers for efficient productivities and profit.

Keywords: solid state fermentation, cassava meal, palm kernel cake, carcass characteristics, meat quality, broilers chicks, pigs.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

It is predicted severally that, the consumption of meat and other livestock products would rather triple worldwide by the year 2020 due to increase in population, per capita income and urbanization as well as improvement in education, standard of living, technological development, trade and communication (Delgado *et al*., 1998; FAO, 1999). These increases are expected in the developing countries of Africa, Asia and Pacific regions, more than expected in developed countries. However in Nigeria and most sub-Saharan Africa countries, total meat consumption has increased when compared to total meat production, and hence these regions import large amount of meat to keep up with its growing domestic demand (FAOSTAT, 2012).

Information on meat consumption of the major animal foods showed that, consumption of beef in Nigeria is 10 g against 40 g global mean, sheep and goat meat is 10 g against 11 g global mean while pork and poultry stand at 12 g and 4 g against the global means of 117 g and 48 g respectively (FAOSTAT, 2012). Pig meat makes the highest contribution to world meat production, but suffers the highest prejudice globally. The first is religious prejudice; pork and pork products are not consumed by Muslims, Jews and some Christian fundamentalist groups. The animal is regarded as dirty by many cultures and tribes (Aladi, 2006). Pork is very high in energy and lipid concentrations and this makes it to suffer discrimination by health dieticians; who link pork meat consumption to the development of atherosclerosis and related problems (Hu *et al*., 1999). Nonetheless, this bias is usually counterbalanced by the high organoleptic

quality of pork and pork products. Pork is processed into various products with extremely wide organoleptic and attractive characteristics (Ikeme, 1990).

Second to pork in global meat production and consumption is poultry meat. In sub-Saharan Africa, poultry production is an important component of agricultural economy; where it provides animal protein to the populace as well as employment to a considerable percentage of the people. Poultry production is attractive because birds are able to adapt easily, have economic value, rapid generation time and high rate of productivity. In Nigeria, there has been a rapid expansion of commercial poultry production in recent years. Intensive non-ruminant production is becoming unsustainable due to its high demand on grains, since this creates inflationary pressure on this vital human food ingredient (Okoli *et al*., 2012).

The rising demand for maize, soybean and groundnut meals for alternative uses has driven the cost of these important animal feed materials upwards in the region that has never produced enough of these to meet the demand for human consumption, as well as for beverage industries and livestock production (Uchegbu *et al*., 2011). Several authors have suggested that, the solution is to search for alternative feed ingredients, such as the abundant agro industrial by-products which are exploited by the farmers (Uchegbu *et al*., 2011; Udedibie *et al*., 2004). Among the alternative feedstuffs are palm kernel cake (PKC), abattoir blood, rumen wastes, maize/sorghum based brewers spent grain and cassava root meal (Udedibie *et al*., 2004; Uchegbu, 2005; Iroegbu *et al*., 2008). Over the years, researchers have tried to develop balanced diets with various blends of alternative raw materials, and have recorded modest results (Udedibie *et al.,* 2004; Uchegbu, 2005; Anyanwu *et al*., 2008; Uchegbu *et al*., 2011a and 2011b). Most

attempts did not succeed in reducing pressure on the use of maize, soybean or groundnut. Thus, there appeared to be the need for continued search for novel feedstuffs that would bridge the supply deficit in farm animal production.

High fibre contents, low metabolizable energy, low crude proteins and poor essential amino acid profiles as well as other essential nutrients have been blamed as the principal factors limiting the use of alternative feed materials in poultry and pig feeding (Choct, 2001; Hutagalund, 2001). In addition, many of these are laden with several anti-nutritional agents. For example, cassava is limited by cyanogenic glucosides, which on hydrolysis yield hydrogen cyanide which is poisonous to animals. Though cassava and its by-products contain low to moderate levels of fibre, the crude protein contents are very low (≤ 3.0%), and meals are very floury (Agumbiade *et al*., 2001). The low crude protein content implies that, higher levels of protein supplements will be needed, if cassava is incorporated into livestock diets. This often affects the economic benefits of feeding cassava based diets. The flouriness on the other hand makes consumption of cassava based diets difficult for poultry, and may create respiratory difficulties for pigs when fed in mash form (Garcia & Dale, 1999; Enyenihi *et al*., 2013).

The cyanide content of cassava meals is easily removed using processing techniques such as soaking, grating, boiling and fermentation (Tewe, 1996), as well as wetting (Bradbury, 2004; Udedibie, 2004). Flouriness can be controlled by blending with vegetable oils (Udedibie *et al*., 2012), or gelatinization (Udedibie *et al*., 2008). Addition of vegetable oils to livestock feeds often leads to rancidity problems, thus, limiting shelf life and feed intake in animals (Ezeokeke *et al*., 2008). Gelatinization

produces very stable products but increases cost of feed production because, the processing technique is associated with high fuel demand (Okoli *et al*., 2012).

Palm Kernel Cake (PKC) has been reported to be a relatively cheap and abundant ingredient that can be utilized in ration formulation (Zahari & Alimon, 2006; Adesehinwa, 2007). However, it has found limited use in poultry and swine feeding because of low nutrient availability, grittiness and the presence of the highly indigestible β-mannan (Sundu *et al*., 2006). Mannan is s structural polysaccharide which constitutes up to 35 % of the cell wall of PKC, and can only be broken down when the enzyme β-mannanase has degraded it into short chain polysaccharides (Ademark, 2000). Most vertebrates do not elaborate β-mannanase in their digestive systems.

Mannan degradation has been attempted through the use of feed additives and/or modification of intestinal flora by incorporation of mannanase producing microbes, which have yielded marginal results (Chong, 1999; Okorie *et al*., 2011; Lawal *et al*., 2010). In recent years, some researchers have used solid state fermentation to improve the nutritive value of PKC through the inoculation of *Aspergillus niger*. Other authors have also demonstrated that *Aspergillus niger* in single or mixed culture can improve the protein content of cassava root meals and its by-products (Raimbault *et al.,* 1977; Kompaiang *et al*., 1992; Soccol *et al*., 1994).

1.2 Problem Statement

The maize supply deficit and the consequent high price has continued to necessitate the search for alternative feedstuffs for animal feeding. A blend of cassava root meal and

PKC in a ratio of 1:1 will yield a product that is similar to maize in proximate composition. However, the twin problems of detoxification of cassava root meal and the digestibility of the non starch polysaccharides in PKC are issues that must be resolved for the product to be profitably used by farmers. This product if made safe with high nutrient availability would become a feasible alternative to maize.

1.3 Objectives

The main objective of this work was to develop a novel feedstuff for poultry and pigs from a mixture of cassava root pulp and palm kernel cake, using solid state fermentation technique. The specific objectives are to:

i. determine the effects of solid state fermentation on the physicochemical characteristics and fungal ecology of mixture of cassava root pulp and palm kernel cake,
ii. determine the effect of dietary inclusion of this product on growth performance, haematology, carcass and meat quality) of broilers chicks and pigs,
iii. make recommendations to stakeholders (feed millers, farmer, meat industries, researchers, policy makers, etc.

1.4 Justification

The findings of this study will be very relevant to many stake holders in the animal industry. First, it will provide farmers with an alternative to cushion the effect of high dependence on maize and other cereal products, which is currently insufficient. It will help reduce competition and create alternative feeds for farmers. Since cassava is relatively more abundant and may be cheaper than maize, this may translate to cheaper

meat and eggs. Consumers will benefit from lower retail prices and be better disposed to meet their nutritional needs from quality animal protein sources. The product is expected to add value to cassava and palm kernel. This will be beneficial to the farmers and agro industry in the long run. In addition, the study will generate data and organize reasonable body of literature for researchers not only in the agricultural industry, but in environmental and sustainable technological development. This will further equip agricultural policy makers with relevant information needed in formulating programmes and policies for the industry.

1.5 Scope of the work

This work was targeted at developing a low cost alternative technology for enhancing the nutritive value of cassava root pulp and palm kernel cake in poultry and pig diets using solid state fermentation. The effect of replacing maize with the product on growth performance, haematology, serum biochemical indices, carcass characteristics and meat quality of broilers and pigs will be evaluated.

CHAPTER TWO

LITERATURE REVIEW

2.1 CASSAVA

2.1.1 Origin and Distribution of Cassava

Cassava (*Manihot esculenta,* Crantz), is a shrub of family *Euphorbiaceae*, native to South America. It is cultivated as an annual crop in tropical and sub tropical regions for its edible starchy tuberous root and has been grown extensively as an important economic root crop in Southeast Asia, Tropical Africa and Central America (Fauquet & Fargette, 1990). Cassava is the third largest source of carbohydrates for human food in the world after rice and maize but a poor source of protein (FAO, 2000). Cassava is a major staple food in the developing world, providing a basic diet for over half a billion people (Claude & Dennis, 1990). According to Nweke *et al*. (2002), cassava plays five important roles in African development: famine-reserve crop, rural staple food, cash crop for both rural and urban households and, to a minor extent, raw material for feed and chemical industries. Cassava has the ability to withstand poor soils and drought (FAO, 1995) and the plant can yield 25 to 60 tonnes per hectare depending on variety and cultivation practices (Chauynarong *et al.*, 2009). Cassava is tolerant to drought; it is productive in poor soil where other staple crops cannot grow without intensive inputs (Bradbury and Holloway, 1988; Leihner, 2002).

According to FAO (2012), the total world cassava production in 2010 is estimated to be 230 million tonnes which represent an increase of 25% over that of 2000. Table 2.1

summarises the most important cassava producing countries. In 2010, Nigeria produced 37 million tonnes, making it the world's largest producer.

Table 2.1: The major cassava producing countries in 2010

Countries	Root production (x 1000 tons)		Yield (tons/ha)		Production per capita (kg fresh weight)
	Fresh roots	Dry weight*	Fresh roots	Dry weight*	
Nigeria	37500	13100	12	4.2	230
Brazil	24500	8600	14	4.9	126
Indonesia	23900	8400	20	7.0	100
Thailand	22000	7700	19	6.7	314
D. R. Congo	15000	5300	8	2.8	231
Angola	13800	4800	15	5.3	728
Ghana	13500	4700	13	4.6	563
Vietnam	8500	3000	17	12.0	97
India	8000	3000	34	12.0	67
Mozambique	5700	2000	6	2.1	248
Uganda	5300	1900	12	4.2	161
China	4700	1600	17	5.9	34
Tanzania	4400	1500	6	2.1	64
Cambodia	4200	1500	20	7.0	300
Malawi	4000	1400	21	7.4	267

Adapted from FAO, 2012

*Estimated dry matter in fresh cassava root was 35 %

Though Nigeria is the largest producing country, Thailand is the major exporting country of dried cassava and its products, with a total of 77% of world's export in 2005 (Chauynarong *et al*., 2009). In South America, it is used mainly for animal feed (about one-third) followed by human consumption and thereafter starch production. In Asia, consumption of fresh roots and exportation to the European Union for use in animal feed are important, but its use for bio-fuel production is increasing (Almeida, 1995; Westby, 2002; Jansson *et al*., 2009).

Cassava varieties are of two types, sweet type which is used for human consumption is soft to touch or tender texture, with no bitterness and low hydrocyanic acid content while the bitter cassava contains higher hydrocyanic acid and is most suitable for tapioca industries, such as tapioca tables, tapioca flour, and alcohol (Falusi & Adeleye, 1998). Degree of bitterness in cassava partly correlates with cyanogen concentrations (Chiwona-Karltun *et al*., 2004). Thus, bitter varieties are associated with high concentrations of cyanogenic glycosides (> 100 mg/kg fresh weight) (Sundaresan *et al*., 1987; Nambisan & Sundersan, 1994; Chiwona-Karltun *et al*., 2004). Sweet varieties have a high concentration of free sugars though it does not always follow that they have low concentrations of cyanogenic glycoside (Borges & Fukuda, 1989; King & Bradbury, 1995). However, bitter taste and high level of cyanogens can also be related to environmental stress conditions, such as drought, low soil fertility and pest attack (Bruijn, 1971).

2.1.2 Nutrient Composition of Cassava

A comparative summary of the chemical and nutrient composition of cassava roots and maize grains are shown Tables 2.2a and 2.2b. Whole fresh cassava roots contain approximately 65 % water, 1 – 2 % Crude Protein, 0.2 – 0.5 % Ether Extract, 0.8 – 1.0 % crude fibre, and 1 – 2 % ash and 30 -35 % nitrogen free extract (Gomez, 1979) and so it is essentially a carbohydrates source. Tewe (2004) reported that its composition shows 60 - 65 % moisture, 20-31 % carbohydrates, 1-2 % crude protein and a comparatively low content of vitamins and mineral. However, the roots are rich in calcium and vitamins C and contain nutritionally significant quantity of thiamine, riboflavin and nicotinic acid (Onwueme, 1978).

The predominant constituent of dry cassava is starch, which may account for 70 to 80 percent of its composition (Gomez, 1979). Cassava starch granules are mainly composed of two polysaccharides, amylose (20%) and amylopectin (80%) (Sandoval, 2008). There is a large variation in sucrose content between cassava genotypes. In certain sweet varieties, sucrose constitutes up to 17% of total carbohydrates (Hendershot, 1972). Generally, cassava roots have less than 1% free sugars (Bradbury & Holloway, 1988). The metabolizable energy value of cassava root meal (CRM) for poultry varies from 12.0 to 14.6 MJ/kg dry matter (Olson *et al*., 1969; Maust *et al*., 1972; Hutagalung *et al*., 1974; Muller *et al*., 1975; Fetuga and Oluyemi, 1976; Khajarern *et al*., 1982).

Table 2.2a: Chemical and Nutrient composition of Cassava root meals and maize

Parameters	Unit	Cassava Root [a]		Maize [b]
		Dehydrated	Fresh	
Dry matter	% as fed	87.60	37.60	90.00
Crude protein	% DM	2.90	2.60	8.00
Crude fibre	% DM	3.90	3.70	2.40
NDF	% DM	8.00	7.80	15.50
ADF	% DM	5.40	5.30	3.20
Lignin	% DM	1.70	1.60	0.50
Ether extract	% DM	0.70	0.80	4.50
Ash	% DM	3.90	2.80	4.30
Starch (polarimetry)	% DM	80.40	80.80	73.30
Total sugars	% DM	2.40		1.70
Gross energy	MJ/kg DM	16.80	17.10	18.80
Minerals				
Calcium	g/kg DM	1.70	1.60	0.40
Phosphorus	g/kg DM	1.10	1.20	2.90
Potassium	g/kg DM	9.90	7.70	3.60
Sodium	g/kg DM	0.30		0.50
Magnesium	g/kg DM	0.90	1.10	1.30
Manganese	mg/kg DM	23.00		140
Zinc	mg/kg DM	33.00		240
Copper	mg/kg DM	5.00		10.00
Iron	mg/kg DM	24.00		116.00

[a]Heuze *et al*. (2014), [b] Heuze *et al*. (2013)

Table 2.2b Nutritive values of Cassava Root Meals and Maize

Parameters	Unit	Dehydrated Cassava	Fresh Cassava	maize
Energy digestibility, growing pig	%	90.80	92.10	87.9
DE growing pig	MJ/kg DM	15.30	15.70	16.6
MEn growing pig	MJ/kg DM	15.00	15.40	16.2
NE growing pig	MJ/kg DM	12.20	12.60	12.9
Nitrogen digestibility, growing pig	%	52.30		79.7
Poultry nutritive values				
AMEn cockerel	MJ/kg DM	15.10	15.20	15.2
AMEn broiler	MJ/kg DM	15.10	15.20	14.8

[a]Heuze *et al*. (2014), [b] Heuze *et al*. (2013)
DE – digestible energy;
MEn – metabolizable energy,
NE – net energy;
AMEn – apparent metabolizable energy

A comparison of the amino acid composition of cassava roots and maize is shown in Table 2.3. Cassava root has less than the recommended minimum limit of almost all essential amino acids, except tryptophan and should be eaten along with other crops rich in essential amino acids to supplement the deficiency, such as vegetables, cereals, fish and meat.(Tuvana, 2012). Cassava is also very low in fat or lipids. According to Hudson & Ogunsua (1974), the lipids are mainly polar galactosyl diglycerides and the fatty acids in cassava are mainly saturated. They also noted that cassava is poor in vitamins such as vitamins A, B_1, B_2 and niacin, though higher level of vitamin C as well as minerals and calcium have been reported by Onwueme (1978). Cassava root products are deficient in carotene and other carotenoids, making it necessary for supplementation of these pigments for the maintenance of normal egg yolk and broilers skin pigmentation (Kanto & Hutagalung, 2005).

2.1.3 Cassava Products in Poultry Diet

The apparent metabolizable energy (AME) of cassava root meals for broiler chickens ranges from 13.4 to 15.8 MJ/kg DM with a mean value of 15.1 MJ/kg DM (FAO Feedipedia). Earlier attempts to replace cereals with cassava root products in poultry rations resulted in generally depressed performances of cassava fed chickens (Tabayayong, 1935; McMilllian & Dudley, 1941; Vogt, 1966). This was probably due to presence of HCN in the cassava used and composition of the diets. Later, Enriques and Ross (1967) overcame the growth depression associated with cassava based diets by supplementing methionine at 0.15 and 0.20 percent of 50 percent cassava diets. A similar report by Olson *et al.* (1969a) showed that the supplementation of cystine at

0.40 % of a 45 % cassava diet gave comparable results to the 0.20 percent supplement of methionine. Subsequently, efforts were redirected at establishing optimum levels of

Table 2.3: Amino acid composition of cassava root and maize

Amino acids (% crude Protein)	**Cassava** [a]		**Maize** [b]
	Dehydrated	Fresh	
Alanine	5.30		7.2
Arginine	5.00	7.70	6.6
Aspartic acid	6.60		6.6
Cystine	1.60		2.1
Glutamic acid	12.50		18.8
Glycine	3.40		4
Histidine	3.60	1.50	3.4
Isoleucine	2.70	5.30	4.4
Leucine	5.10	5.60	12.6
Lysine	3.90	6.20	2.9
Methionine	1.60	0.60	1.7
Phenylalanine	2.90	3.50	5.7
Proline	3.30		9.5
Serine	3.20		5
Threonine	2.90	3.80	4.2
Tryptophan	0.80	0.50	1.8
Tyrosine	1.70		5.4
Valine	4.50	4.50	5.3

[a]Heuze *et al*. (2014), [b] Heuze *et al*. (2013) www.feedipedia.org

inclusion and types / levels of supplementation of cassava based diets to make them meet precisely the nutritional needs of poultry at the most economical cost (Khajarern & Khajarern, 1992). Many of these authors observed that part of the methionine was needed to make up for nutrient deficiencies, as soybean meal was used as the main source of protein and the rest was for HCN detoxification. It was however questioned whether the extra methionine is needed for HCN detoxification (Adegbola, 1977). Khajarern & Khajarern (1992) concluded that for effective use of cassava roots in poultry diets, it is important to use products with the lowest possible HCN contents. Rations should be balanced in all nutrients, particularly in energy, sulphur containing amino acids, phosphorus, zinc, iodine and vitamin B_{12}). Dustiness and bulkiness should be controlled so as to make feed more palatable by pelleting or addition of molasses or fats. Finally cassava based rations should be supplemented with carotene and carotenoid rich feedstuffs for normal egg yolk colour and broiler meat pigmentation.

Studies by Aderemi *et al*. (2000), Akinfala *et al*. (2003) and Udedibie *et al*. (2004) have reported positive results due to better nutrients balance that meet requirements of broilers and layers. Eshiett & Ademosun (1980) reported that there was no significant difference in birds fed cassava meal at 0, 100, 300 or 450 g/kg diet. In a study by Gomez *et al*. (1983), the performance of chicken on control diets was similar to that of chickens fed cassava root meal of cultivars low or high in cyanide contents up to 200 g/kg diet. Waldroup *et al*. (1984) observed that replacement of one third of the maize with cassava will have no adverse effects on the body weight gains of broilers but there was a reduction in weight gain at higher levels. Broiler chicks fed cassava based diets with zinc (50 or 100mg/kg) for 56 days had body weight gain that did not differ

between the groups except that chickens given cassava at 500g/kg cassava with 50mg Zn/kg weighed significantly more than others (Ekpeyong & Obi, 1986).

When sundried cassava peel meal was included in broiler ration at 50, 100 and 150g/kg, it was observed that there was no significant treatment influence on feed consumption, live weight gain and the efficiency of feed utilization but feed intake tended to increase with increasing levels (Osei, 1992). Replacement of maize with fermented whole cassava meal at 0, 20, 40, 60, 80 and 100 percent resulted to lower weight gains only on total replacement of maize (Onjoro *et al*., 1998). Obikaonu & Udedibie (2006) reported that birds fed ensiled cassava peel meal diet had similar feed intake and body weight gain as the control group whereas the feed conversion ration of birds on sun dried cassava peel meal was poor. In a study by Oyebimpe *et al*., (2006), cassava peel meal at 200 g/kg diet could replace maize in broiler diets with no reduction in growth performance. Ervbetine *et al.* (2003) reported that at 100 g/kg diet, cassava products (50:50 cassava root meal and leaf meal) in broiler diet had no effects on growth rates, feed conversion and carcass characteristics.

Adeyemi *et al*. (2008) studied the effects of enrichment by fermentation and by form of feed presentation the value of cassava meal as livestock feed. They formulated six dietary treatments in which 0, 12.5 and 25 % of maize was replaced on a weight for weight basis with cassava enhanced with dried cage layer waste and fermented with rumen filtrate (CCLW). Each inclusion level was fed as mash and pellet form to broilers for duration of 6 weeks. Increasing the level of CCLW significantly reduced average daily weight gain and average final weight when fed in mash form. The retention of crude protein (CP), crude fibre (CF) and ether extract (EE) were depressed

significantly with increasing level of CCLW in broiler diet. Pelleting significantly improved nutrient retention compared to mash form (68.70 vs. 60.52 % for CP, 72.03 vs. 60.41 % EE and 71.64 vs. 63.33 % for CF). Abdominal fat pad weight was significantly, reduced with increasing concentration of CCLW in the diet when fed in mash form, however pelleting significantly increased abdominal fat weight on all dietary treatments. However, the level of CCLW in the diet did not significantly affect breast and thigh weights but the form of feed presentation significantly influenced the weight of these two choice retail cuts.

2.1.4 Cassava as Swine Feedstuff

Gomez *et al*. (1976) reported that pigs do not readily consume fresh bitter cassava roots and that if protein supplement was supplied *ad libitum* with fresh chopped bitter roots, the pigs consumed an excess of the supplement to compensate for a reduced intake of the bitter roots. When fresh bitter roots were mixed with the supplement, pigs did not consume enough feed and lost weight during the experimental period. However, Ochetin (1993) reported that pigs fed fresh cassava based diets grew as fast (0.78 vs. 0.77 kg/day) and were as efficient (3.74 vs. 3.77) in converting feed into body weight as those fed commercial control diets. Nnadi and Omeke (2010) reported that replacing maize with cassava root meal at 25% level in the diet of grower pigs had no significant effect on performance but at 50 % and above performance of the pigs were generally inferior to those of the control. Tada *et al*. (2004) evaluated the effect of replacing maize with cassava meal at 0, 25, 50, 75 and 100 percent, and concluded that though cassava meal effectively replaced maize at all levels of inclusion, profit margins at higher levels does not justify their cost of inclusion. Gomez *et al*., (1983) observed no

enefit from 0.2 to 0.3 % Methionine supplementation of high cassava (65 %) fed to all classes of pigs.

Jimenez *et al*. (2005) studied the effect of the introduction of graded levels of cassava root meal (CRM) at 0, 20 and 40% of the diet) on performance and carcass traits. The experimental diets contained 20% of a mixed foliage meal made from equal weights of cassava leaves and *Trichanthera gigantea* leaves compared to a control diet with neither cassava roots nor foliage meal. There were no significant differences for performance traits of the animals, between the control treatment and the others containing the mixed foliage meal and up to 40% CRM. They concluded that pigs fed *ad libitum* diets formulated to contain 40% of CRM and 20% from mixed cassava and *trichanthera* leaf meals have similar performance and carcass traits as those fed a conventional diet.

2.1.5 Limitations to the Use of Cassava in Livestock Diets

The use of cassava root meals as livestock feed ingredient is limited by three principal factors, namely high contents of cyanogenic glucosides (linamarin and lotaustralin), low crude protein content with poor essential amino acid profile and dustiness leading to low palatability.

2.1.5.1 Cyanogenic glucosides

Varieties of cassava plants produce amino derived compounds termed cyanogenic glycosides as a defence biomolecules (Montagnac *et al*., 2009). All tissues of the plant except the seeds contain 4 to 5 cyanogenic glycosides (McMahon *et al*., 1995) The principal ones are linamarin (2–β–D glycophranosyloxyl isobutyronitrils) and

lotaustralin in a ratio of 93:7 (Teles, 1995). Cassava tissues also contain the enzyme linamarase, which can hydrolyse cyanogenic glycoside but the enzyme is not located in the same cell compartments as the cyanogenic glycosides (Bruijn, 1971; Nweke, 1994; Teles, 1995). Cyanogenic glycosides are located inside vacuoles and the enzyme linamarase (a β-glucosidase) are found in the cell wall (Conn, 1994).

When cassava tissues are disrupted, cyanogenic glycosides are leached from vacuoles and come into contact with linamarase resulting to the production cyanohydrin from linamarin and 2-butanone cyanohydrin from lotaustralin (Conn, 1994). These cyanohydrins are unstable and decompose spontaneously to the corresponding ketones and hydrogen cyanide (HCN) at pH values above 5 and temperatures above 30 ^{o}C. Cyanohydrin degradation can also be catalysed by α-hydroxynitrile lyase, located in apoplastic space (White *et al*., 1994). This is illustrated in figure 2.1. The concentrations of cyanogens vary in different varieties, between tissues in the same plant and even between compartments of the same tissue (Barrios and Bressani, 1967; Bruijn, 1971; Nambisan and Sundaresan, 1994; Wheatley and Chuzel, 1993; Burns *et al*., 2012). Longitudinally, cyanogen concentration in cassava roots increases from insertion point on the plant to the root terminal, and in the transverse direction, cyanogenic glycosides levels decrease from the external area to centre of the root (Bruijn, 1971).

Hydrogen cyanide is very toxic to livestock due to its competitive and irreversible bonding to oxygen binding sites of the cytochrome oxidase complex (Garret & Grisham, 2005). In 1887, the crew of Stanley's remarkable expedition through

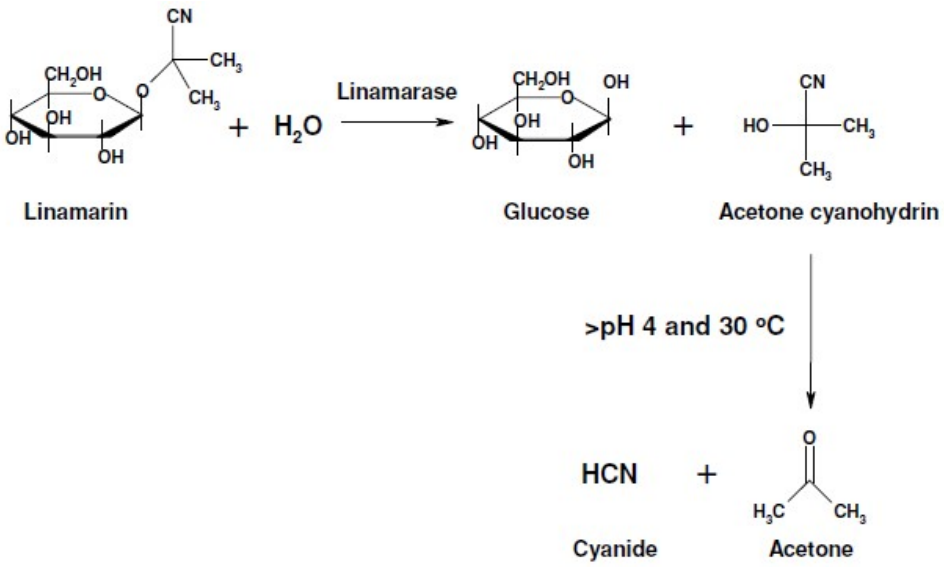

Figure 2.1: Hydrolysis of linamarin, 1 is β-glucosidase (linamarase)(pH=5.5), 2 is α-hydroxynitrile lyase (pH≥5, Temp. ≥30). Adapted from Conn (1994)

Congo (present-day Zaire), suffered from sometimes fatal acute poisonings when bitter cassava roots were consumed without the extensive soaking as applied by the local inhabitants (Manning, 1985). Most common illness related to cassava consumption is due to prolonged exposure to comparable low concentrations of cyanogens in ingested cassava products (Rosling, 1988). Chronic ingestion of fresh or processed cassava based diets containing sub lethal dietary cyanide has reportedly caused impaired thyroid function and growth, neonatal death and lower birth rates in animals (Fatufe *et al*., 2007; Ernesto *et al*., 2000). Odukwe (1994) reported that consumption of cassava and its products causes cyanide poisoning with symptoms of vomiting, nausea, dizziness, stomach pains, weakness, headache and diarrhoea and occasionally death. Moreover, high dietary cyanogens exposure from poorly processed cassava roots may be associated with the occurrence of a neurological disorder 'Konzo' and irreversible paralysis of the legs (Udedible *et al*., 2004). Thiocyanate, the main metabolite of cyanide in humans, had been identified as aggravating iodine deficiency and increasing the prevalence of goitre in a Zairian population consuming insufficiently processed cassava (Ermans *et al*., 1980). In Nigeria, Osuntokun (1981) found an association between a neurologic degenerative disease, Tropical Ataxic Neuropathy (TAN) and long-term moderate cyanide exposure from cassava consumption.

Residual cassava cyanogens, when ingested, are hydrolysed in the human digestive system. It is assumed that intestinal microbes hydrolyse the cyanogens (Teles, 1995). The HCN in the human body is metabolised to thiocyanate (McMahnon & Birnbaum, 1990). Figure 2.2 shows a scheme of the metabolism of cyanide to thiocyanate in the human body. The conversion of HCN to thiocyanate is catalysed by rhodanase and 3-

mercaptopyruvate sulphur transferase (Westley, 1981; Vazques *et al*., 1987). These enzymes require sulphur, which is supplied from sulphur amino acids. The thiocyanate (SCN-) is eliminated from the human body through urine and saliva. Deficiency in essential sulphur amino acids may enhance the risks in illnesses such as Tropical Ataxic Neuropathy, epidemic spastic paraparesis, also known as konzo (Rosling, 1986; Rosling, 1988; Casadei *et al*., 1990; Nzwalo & Cliff, 2011). Excessive levels of SCN may under a long term exposure lead to reduced iodine uptake, which in an iodine-deficient region may contribute to endemic goitre and cretinism (Bradbury & Holloway, 1988; Delange *et al*., 1994).

2.1.5.1.1 Cyanide Reduction in Cassava

Cyanide in cassava can be found as bound glucosides, cyanohydrins, and free cyanide (Cooke, 1983). Each of the 3 forms has different toxicity and reacts differently to processing techniques that remove cyanide (Cooke and Maduagwu, 1978). The residual level of cyanogens in cassava products vary depending on the nature and duration of the processing method(s) employed. Common among these are peeling, chipping, grating, soaking in water, roasting, cooking, fermentation, gelatinization, etc. A summary of the effect of processing techniques on cyanide removal is shown in Table 2.4

2.1.5.1.2 Boiling

Table 2.4 shows that most of the processing methods except boiling were very effective in reducing cyanide levels in cassava. Montagnac *et al*. (2008) attributed the inefficiency of boiling to the high temperature (100 ^{0}C) which denature the heat labile

linamarase (β-glucosidase). Cooke & Maduagwu (1978) reported that bound glucosides were reduced to 45 to 50 % after 25 min of boiling while free cyanide and

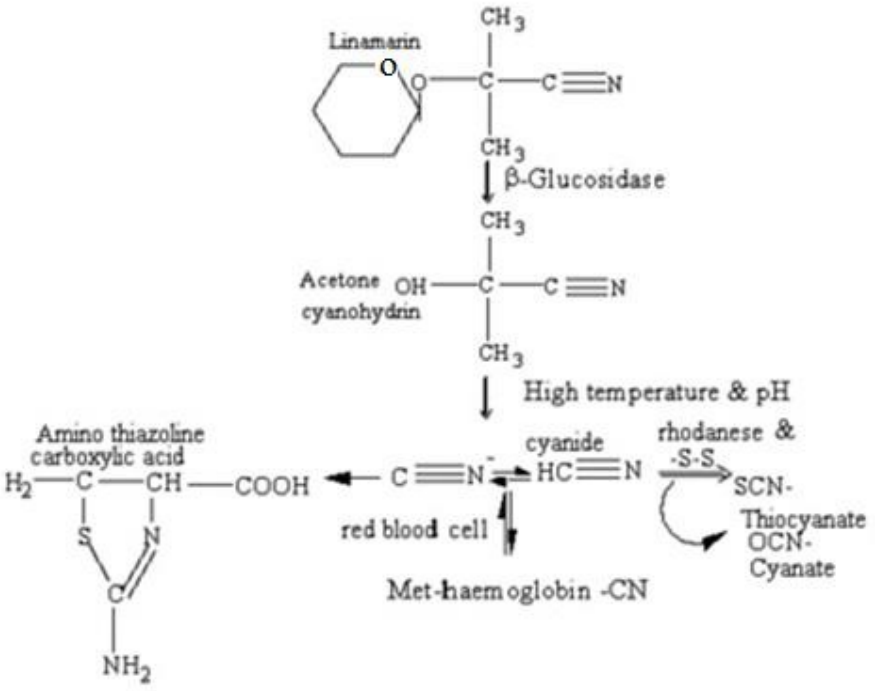

Figure 2.2 Metabolism of cyanide to thiocyanate in the human body (source: Rosling, 1994)

Table 2.4 Common Processing Methods for Cassava

Processing method	Countries of use	Estimate Cyanohydrin removal (%)	Sources
Boiling of fresh roots	All countries that use cassava as food	25 – 65	Nambisan & Sundaresan (1985)
Sun drying after chipping	Mostly African countries	65 – 75	Cardoso *et a*l. (2005) Mlingi & Bainbridge (1994)
Soaking in water (fermentation) / sun drying	Malawi, Tanzania, Zambia, Uganda, DR Congo	97 – 98	Nyirenda *et al*. (2011) Cardoso *et al*. (2005)
Heap fermentation / sun drying	Uganda, Tanzania, Mozambique	83 – 95	Essers *et al*. (1995) Zvanya *et al*. (2002)
Grating / fermenting / roasting	West African countries, Mozambique	97 – 98	Cardoso *et al*. (2005) Westby & Choo, (1994

Source: Tivana (2012)

cyanohydrin in boiled cassava roots are found at very low concentrations. Nambisan & Sundaressan (1985) showed that the residual cyanogens glucoside increase with particle size and decrease with proportion of water relative to the fresh roots after 30 minutes of boiling. Their results further buttressed the fact that the final cyanide level in cassava products is largely dependent on the initial cyanogenic glucoside concentration. High temperature is as well responsible for very low efficiency of steaming, baking and frying as techniques for HCN reduction in cassava and its products (Montagnac *et al*., 2008).

2.1.5.1.3 Drying

The effectiveness of drying in HCN reduction in cassava is dependent on the drying temperature and particle size. At temperatures above 55 ˚C, linamarase activity is inhibited and therefore linamarin becomes concentrated in dried cassava (Montagnac *et al*., 2008). Cooke & Maduagwu (1978) observed a cyanide reduction of 29 % at 46 ˚C and of 10 % at 80 ˚C. In 10 mm thick chips, Nambisan (1994) observed similar cyanide reductions of 45 % to 50 % and 53 % to 60 % at 50 and 70 ˚C, respectively. Nambisan (1994) showed that at equal temperatures, a decrease in cassava size will lead to an increase in cyanide retention in the oven-drying processes. This is because thin chips dry faster allowing less time for linamarase to act on the glucosides. Sun drying therefore is more effective than oven drying in removing HCN. Free cyanide contents of 30 % total cyanogens in oven-dried and 60% in sun-dried cassava have been reported (Gomez *et al*., others 1984; Gomez & Valdivieso, 1984). Chip thickness may still be an important factor in cyanogen removal during sun-drying because thin chips dry faster. Nambisan & Sundaresan (1985) reported a 52 % to 58 % cyanogen glucoside retention in 3-mm-thick chips, and 27 % to 33 % cyanogen glucoside

retention in 10-mm-thick chips. Cyanogenic glucoside breakdown during sun drying depends on enzymatic hydrolysis and on gradual root cell disintegration. Thinner cassava pieces dry faster and at low moisture content levels (13 %), linamarase is inactivated and cyanogen glucoside breakdown ceases (Mlingi & Bainbridge, 1994). Cyanohydrin removal is increased with complete sun-drying. A possible explanation would be that dehydration of the roots and moisture losses result in pH changes, which affect cyanohydrins stability (Mlingi & Bainbridge, 1994). Sun-drying is not very effective in linamarin removal, and speeding up the drying process, e.g. by reducing the size of the pieces, aggravates this. There is a potential for increasing the effectiveness of cyanogen removal by reducing the initial drying rate, followed by thorough final drying. (Essers *et al*., 1995)

2.1.5.1.4 Fermentation

To a microbiologist, the term fermentation describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidised, and an organic carbohydrate acts as the electron acceptor (Adams, 1990). Campbell-Platt (1987) however defined fermented foods as those foods which have been subjected to the action of micro-organisms or enzymes so that desirable biochemical changes cause significant modification to the food. The fermentation of grated cassava roots is efficient at removing cyanogenic glucosides.

Fermentation of cassava occurs in three major forms, soaking in water, fermentation of grated roots and heap fermentation. A summary of these techniques was presented in Table 2.4 above. The fermentation of soaked roots in water is much more effective than that of grated roots in terms of cyanogen reduction (Muzanila *et al*., 2000; Wesby,

2002; Cardoso *et al*., 2005). According to Westby & Choo (1994), more than 90% of total cyanogens were removed after 3 days of fermentation and about one-third of initial linamarin was found in the water and no significant accumulation of cyanohydrin or free cyanide was noted. In soaked roots, microbial growth is essential because it disrupt the cellular tissues of roots, which has the combined effect of allowing cyanogenic glycosides to come in contact with linamarase (Westby & Choo, 1994) Soaking for long periods can introduce fungi (Thambirajah, 1989), mold spores, and undesirable bacteria into the final products (Hakimjee & Lindgren, 1988). The mold is typically nontoxic and contributes to reduced viscosity in weaning foods. The undesirable bacteria are thought to be destroyed during the cooking process (Hakimjee & Lindgren 1988).

However, Vasconcelos *et al*. (1990) showed that microorganisms played only a small role in cyanogen reduction and that grating was mainly responsible for linamarin hydrolysis. They reported that high concentrations of cyanohydrins and free cyanide were left in the fermented paste. This might be explained by the stability of cyanohydrins at acidic pH (Cooke, 1978). Thus, post fermentation operations are important and need to be effective for reducing cyanohydrins and free cyanide levels in such final products (Montagnac *et al*., 2008).

A combined process of grating (shredding) and fermentation of cassava roots is practised in many parts of West Africa (Westby & Choo, 1994; Westby, 2002; Obilie *et al*., 2004). The shredded cassava roots are allowed to ferment in sacks for 1-7 days, which encourages lactic acid fermentation. The pH after 3 days decreases from 6 to 4. The fermentation is dominated by lactic acid bacteria (Westby & Twiddy, 1992). The

massive disruption of cassava tissues maximizes the hydrolysis of cyanogenic glycosides as they come in contact with linamarase. Lactic acid bacteria play only a limited role in cyanogen reduction (Westby & Choo, 1994). This method often leaves behind significant residual concentrations of cyanohydrin because pH decreases during fermentation and cyanohydrins are stable at low pH (Vasconcelos *et al.*, 1990). Essers *et al*. (1995) found that though microbial growth resulted in additional reduction of the cyanogenic glucoside levels to 29.8 % (+ 18.9) of the ones which were obtained after non-inoculated incubation, the reduction was better correlated (r = 0.86) with the extent of root softening than microbial growth. It was therefore concluded that microorganisms played only a small role in cyanogen reduction and that grating was mainly responsible for linamarin hydrolysis in previous reports.

In a related study designed to clarify the mechanism(s) of breakdown of cyanogenic glucosides during the solid-substrate fermentation, (Essers, 1995a) reported that the most important contribution of microorganisms to linamarin reduction in solid-substrate fermentation of cassava is the cell-wall-degrading activity which enhanced the contact between endogenous linamarase and linamarin. Cyanide retention ranging from 12.5% to 16.5% in cassava roots that have undergone heap fermentation has been reported (Essers *et al*., 1995a; Cardoso *et al*., 1998; Ernesto *et al*., 2000, 2002a,b). The process involves peeling of cassava roots, sun drying for 1 to 3 days, heaping and covering, fermentation, scraping off the mould mycelia, crushing into crumbs, sun drying, pounding and sieving into flour. These processed underscore the role of the ancillary processes.

2.1.5.2 Low Protein Content of cassava products

Another serious limitation of cassava and its products in monogastric nutrition is its protein content which is low in quantity and quality compared to cereal grain feedstuffs. When utilized in replacing cereals in diets for monogastric animals, it becomes imperative to supplement to avoid protein deficiencies (Agunbiade *et al*., 2001) and these supplements are usually very expensive. There is thus the need to identify means of improving the protein quality of cassava, especially those that can be easily adapted on the farm (Adeyemi *et al*., 2008). Noomhorm *et al*. (1992) reported that the conversion of a part of the starch in cassava root meal (CRM) to protein by microbes during the process of solid-state fermentation has great potential as a means of improving the feeding value of cassava root meal. Adeyemi & Sipe (2004) reported an improvement in crude protein concentration of cassava root when fermented with rumen filtrate with or without ammonium sulphate as the source of nitrogen. Adeyemi *et al*. (2008) obtained a 237.8 % increase in the crude protein value of whole cassava root meal fermented with rumen filtrate using caged layer waste as source of nitrogen. Azoulay *et al*. (1980) reported that *Candida tropicalis* can be grown directly on corn or cassava powders so that the resultant mixture of biomass and residual corn or cassava contains about 20 % protein, which represents a balanced diet for either animal or humans.

Perhaps the most significant contribution of solid state fermentation in cassava processing is protein enrichment of the biomass. Tai & Mbongo (1994) evaluated the utilization of cassava peels as substrate for crude protein formation. Effects of pH, incubation temperature, moisture levels and inorganic supplementation of mash on

crude protein formation were studied. They found that protein production was highest in mash supplemented with urea, and temperature of 30°C, pH of 5.5 and moisture content of 13.0 % were found as the optimum conditions. Single cell protein production and organic substance reduction in the effluent are the aims of most bioconversion processes. Candida species as *C. utilis, C. arborea and C. tropicalis* are most successful for cell mass production (Balagopalan & Podjama, 1988; De Mot, 1990). Though many reports have demonstrated an increased biomass yield using solid state fermentation, the protein yield was still not high enough, thus requiring that modification of conditions for fermentation. For microbial biomass protein production, Symba process has been proposed (Daubresse *et al*., 1987). This process which has been used at industrial scale involves a combination of an amylolytic strain and *Candida utilis* in mixed culture condition. The latter hydrolysis starch to glucose and *C. utilis* grows on this substrate.

In recent years, there has been increasing interest in the use of solid state fermentation processes as alternatives to submerged fermentation such as batch, continuous and fed-batch fermentation. This is because it has lower energy requirements and produces less waste water and hence incures little environmental concerns (Lonsane & Ramash, 1990). Solid state fermentation (SFF) has been widely used in the production of traditional oriental foods and alcoholic beverage: Khao-mak in Thai, Tempeh in Indonesia, Soy sauce and Sake in Japan etc (Raimbault, 1998). The advantages of SSF as compared with submerged fermentation include: SSF is relatively not amenable to bacterial contamination since bacterial growth is restricted by low water activity. Therefore, serious contamination on a solid medium rarely occurs. Secondly, handling of the fermented residue is very simple. Since the moisture content of the fermented

residue is very low, it can be dried easily and used as animal feed. It also results in minimal waste water management and reduced energy cost (Sato & Sudo, 1999; Hosobuchi & Yoshikawa, 1999).

Supplementation of natural nitrogenous material like chicken dung, pine apple peel, groundnut waste, etc. for enhancing the fermentation of cassava has been attempted. Balagopalan & Podjama (1988) reported that among the nitrogenous supplements tested, pineapple peel at 25 % elevated the protein to 4 to 5 % while mixture of 12.5 % pineapple peel and 12.5 % chicken dung elevated the protein to 7 %. Soybean and groundnut meals were found to be better additives to facilitate protein enrichment. Yuthavong & Gibbons (1994) reported that using urea as nitrogen source, maximum growth of *Candida eichhorniae* in solid state process was observed. In their study, corncobs were mixed in fermentation to enhance aeration. After one week of incubation, 12-19% of protein yield were obtained. Reade & Gregory (1975) found that with urea as the nitrogen source, no pH control was necessary in simple, non aseptic and low cost process for the conversion of cassava by using *Aspergillus fumigatus* in submerged fermentation. Conditions for the production were optimized for both liquid and solid cultures, but solid state proved to be more efficient than liquid culture (Tani *et al*., 1986).

Fermentation of cassava with *Aspergillus, Neurospora*, and *Rhizopus* could increase the protein content (Khor *et al*., 1977). Protein enrichment of cassava by *A. oryzae* in solid state fermentation has been studied (Zvauya & Muzondo, 1993). During fermentation there was an initial increase in pH to 5 after 10 hr followed by a gradual decrease to 3.1. Amylolytic enzyme were active in the stationary phase reaching peak after 50 hr of

fermentation, while yeasts and lactic acid bacteria increased during the early stages of fermentation and then levelled off. After 50 hr of fermentation protein content had increased from less than 2% to 19%, while starch content had decreased from 80 to 4g/100g substrate. Solid state fermentation for protein enrichment of cassava were tested on pilot units in Burundi (Central Africa), and the product contained 10.7 % of dry matter protein versus 1% before fermentation (Daubresse *et al*., 1986).

In a solid state fermentation of cassava using *Rhizopus spp*., Soccol (1994) reported that glucoamylase production was higher on raw cassava than on cooked cassava and after 48 hr of fermentation, the protein content was increased from 1.75 % to 11.3 %. Charoensiri *et al.* (1990) isolated *Cephalosporium eichhorniae* 152, a filamentous soil fungus and used it to enhance the protein quality of cassava for animal feed production. This thermophilic, obligately acidophilic fungus grew well at 45 °C and pH 3.8 and could use cassava as substrate for biomass production. Effects of initial moisture content (400, 450, 550, and 600 g/kg), fermentation temperature (30, 35, 40, and 45°C), and inoculum concentration (2×10^6, 2×10^7 and 2×10^8 spores/g) on protein enrichment of cassava meal were studied using 3 *Aspergillus* species (*A. niger, A. oryzae var. Oryzae* CBS102.07, and *A. hennbergii* CBS118.35) (Zvauya & Muzondo, 1994). Optimum conditions for protein enrichment was found at 550 g/kg initial moisture, 40 °C and inoculum concentration of 2×10^7 spores/g substrate.

2.1.5.3 Palatability Problems

Physical properties such as dustiness and bulkiness are related to palatability and may limit feed intake. Thus, the processing of cassava-based diets usually includes pelleting and the addition of molasses or fat to eliminate dust and improve the texture of the feed

(Garcia & Dale, 1999). Miiller *et al*. (1974) analysed several experiments in Singapore and Malaysia on broilers given various levels of cassava and concluded that at all levels, when cassava based feeds were provided as mash, poorer growth and feed conversion were obtained than with corn-based diets. However, similar performance was obtained when the diets were pelleted which ensured optimum feed intake. Chou & Muller (1972) found no deleterious effects when the cassava level in the ration was increased to 58% in a pelleted diet. Pelleting decreases the volume of cassava based feeds by about a third, thereby increasing their bulk densities, overcomes the problem of dustiness, and ensures an optimum feed intake. Though pelleting of cassava based diets generated high temperature, it did not significantly alter the HCN concentration (Pangrahi *et al*., 1992).

When dry starch is heated in excess water, it starts swelling as the water is absorbed by the granules. After a certain period, the process is irreversible and is called gelatinization. This occurs within a temperature range in which the starch granules lose their birefringence when observed under the microscope (Adejumo *et al*., 2011). Gelatinization is commonly used to remove or reduce the flouriness in cassava meal based foods. It is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing hydrogen bonding sites (the hydroxyl, hydrogen and oxygen) to engage more water. Boiling, toasting and frying enable cassava meals to become gelatinized in various degrees thereby making the food more palatable. Udedibie *et al*. (2013) reported that drying gelatinized fermented cassava meals (peeled and unpeeled) produced a shelf stable product. When this product was fed to broilers and laying hens, it replaced maize without any deleterious effects on performance and products quality (except egg yolk colour and pigmentation

of broiler skins). Though gelatinization produces very stable products it increases cost of feed production due to its high demand and dependence on fuel (Okoli *et al*., 2012). Molasses can be added to improve palatability and consumption of cassava based livestock feeds. Flouriness is controlled by blending with vegetable oils (Udedibie *et al*., 2009). However, addition of vegetable oil to livestock feeds often leads to rancidity problems and reduction in shelf life and feed intake in animals (Ezeokeke *et al*., 2008).

2.2 Palm Kernel Cake

Palm kernel cake (PKC) is the residue obtained after the extraction of palm kernel oil from the seeds of oil palm tree. PKC results after two stages of oil extraction from palm fruits. The first is the primary extraction of palm oil from the pericarp portion of the fruit which produces the kernel, palm oil sludge (POS) and palm pressed fibre (PPF) as by-products (FAO, 2002). Separation and extraction of oil from the endocarp (kernel) yields Palm Kernel cake as the principal by-product. Two methods are used for the extraction of oil from the crushed kernels. The first is the conventional mechanical screw press method, which produces the expeller products known as Palm Kernel Cake (PKC). The other is solvent (usually hexane) extraction method, which results in a type known as the palm kernel meal (PKM). Both names are often used interchangeably (Chim, 2002; Okeudo *et al*, 2005).

2.2.1 Nutrient Composition of Palm Kernel Cake (Meal)

The nutrient composition of PKC is largely dependent on the method and efficiency of oil extraction (Sundu, *et al*., 2006). Expeller-pressed PKC has higher oil contents than solvent extracted meal. According to Okeudo *et al*. (2005) and McDonald *et al.* (2010), the by-product from the mechanical expeller procedure is referred to as Palm Kernel

cake, while that from the solvent extraction technique is called palm kernel meal. O'mara *et al*. (1999) reported values of 5 – 12 percent ether extract for expeller pressed PKC and 0.5 – 3 percent for solvent extracted PKC. Although PKC supplies both protein and energy, it is more of a protein source (Fetuga *et al*., 1977; Nwokolo *et al*., 1977; FAO, 2002). Palm kernel meals are nutritionally inferior to most oil meals, namely soybean meal and groundnut meal (Tables 2.5 and 2.6) due to the high proportion of cell wall constituents (crude fibre) which is not affected by the extraction process. Chin (2002) reported that PKC is a medium grade protein feed with high fibre content and limited amino acid contents. Palm kernel cake is high in sulphur containing amino acids (Table 2.7). Of all the essential amino acids, the available lysine, histidine, threonine and methionine are the only limiting ones (Nwokolo, *et al*., 1976). PKC is relatively high in some minerals like calcium, phosphorus, magnesium, manganese, iron but low in zinc and copper (Nwokolo *et al*., 1976). The ratio of calcium to phosphorus is more favourable than in many other oilseed cakes (Madonald *et al*., 2010). PKC is palatable, gritty in nature and has a lower biological value than groundnut cake (Onwudike, 1986b). Its high content of cell wall constituents (crude fibre 14-28%; NDF 60-80%; ADF 35-50%; Lignin 10-18% DM) make palm kernel cake dry and gritty and so is not readily accepted by ruminants and pigs (Göhl, 1982). The resistance of dietary fibre in PKC to monogastric digestive enzymes has been reported by Sundu & Dinge (2003)

Table 2.5 Nutrient composition of palm kernel cake, groundnut cake and soybean meal

Composition	Unit	Palm Kernel Cake	Groundnut Cake	Soybean Meal
Dry matter	% as fed	91.20	92.3	90.7
Crude protein	% DM	16.70	49	49.3
Crude fibre	% DM	19.80	7	4.9
NDF	% DM	73.00	14	11.1
ADF	% DM	44.80	9.4	5.9
Lignin	% DM	13.40	2.4	0.5
Ether extract	% DM	9.20	10.1	7.7
Ash	% DM	4.70	5.8	6.8
Total sugars	% DM	2.40	10.3	9.3
Gross energy	MJ/kg DM	20.10	21.7	20.8
Minerals				
Calcium	g/kg DM	2.80	1.2	4.60
Phosphorus	g/kg DM	6.00	6.5	7.20
Potassium	g/kg DM	6.50	13.5	21.00
Sodium	g/kg DM	0.20	0.2	0.20
Magnesium	g/kg DM	3.10	3.4	3.20
Manganese	mg/kg DM	181.00	45	
Zinc	mg/kg DM	68.00	57	
Copper	mg/kg DM	28.00	15	
Iron	mg/kg DM	589.00	1009	129
Secondary metabolites				
Tannins (eq. tannic acid)	g/kg DM	4.50	2.1	0.8

Source: FAO Feedipedia

Table 2.6 Nutritive values of Palm Kernel Cake

Parameter	**Unit**	Palm Kernel Cake	Groundnut Cake	Soybean Meal	
Energy digestibility, growing pig	%	42.00	79.2	87.1	*
DE growing pig	MJ/kg DM	8.40	17.2	18.1	*
MEn growing pig	MJ/kg DM	7.80	16	16.5	*
NE growing pig	MJ/kg DM	5.40	10.6	10	*
Nitrogen digestibility, growing pig	%	63.10	83	89.5	*
Poultry nutritive values					
AMEn broiler	MJ/kg DM	11.90	11.70	10.90	

Source: FAO Feedipedia (2015)

Table 2.7: Amino acid composition and nutritive value of Palm Kernel cake, groundnut cake and Soybean meal

Amino acids (% Crude protein)	**Palm Kernel Cake**	**Groundnut Cake**	**Soybean Meal**
Alanine	4.00	4.2	3.40
Arginine	12.70	12	7.80
Aspartic acid	7.70	12.6	4.50
Cystine	1.20	1	0.70
Glutamic acid	18.60	20.9	15.70
Glycine	4.50	6.1	3.90
Histidine	2.10	2.4	1.20
Isoleucine	3.50	3.3	2.90
Leucine	6.20	6.6	5.30
Lysine	2.90	3.5	2.00
Methionine	1.80	0.9	1.00
Phenylalanine	3.90	4.9	3.40
Proline	3.10	3.8	2.60
Serine	4.30	5.1	3.30
Threonine	3.10	2.9	2.60
Tryptophan	1.20	1.1	0.70
Tyrosine	2.50	65	2.00
Valine	5.00	4.1	4.30

Source: FAO Feedipedia (2015)

2.2.2 Palm Kernel Cake as Poultry Feedstuff

Osei and Amo (1987) evaluated palm kernel cake as a broiler feed ingredient. PKC was used to replace maize partially at levels of 0, 5, 7.5 12.5 and 15 percent. These levels did not significantly affect feed consumption and body weight up to 8 weeks of age. For PKC to be used in poultry diets, it needs to be combined with other protein sources in order to increase dietary levels of some deficient amino acids (Onwudike *et al.,* 1986b). Armas *et al*. (1977) reported that 45 percent PKC in broiler diets even without lysine and methionine supplementation was effective. Babatunde *et al*. (1975) observed significant improvement in the growth of chicks by supplementing PKC with Methionine. Longe (1984) reported that layers fed 20% PKC consumed significantly more feed but produced less eggs than the control. In another study, Onwudike (1986b) demonstrated that starter pullets can be raised on diets containing up to 34% PKC while the grower pullets can be raised on 38% PKC without any deleterious effect on rate of egg production, egg weight, weight of first egg dropped and feed intake. The prolonged exposure of the pullets in the later study may have given them advantage over the layers used by Longe (1984). When the levels of PKC was increased beyond 40% in hens' diets, egg production, egg weight, feed intake and efficiency of feed utilization dropped significantly, yolks became paler, body weight of hens reduced and birds produced watery droppings (Onwudike, 1988; Panigrahi & White, 1998). Broilers can be fed diets containing 30% fermented PKC without any adverse effect on performance (Noraini *et al.*, 2008; Akpodiete *et al.*, 2006).

2.2.3 Palm Kernel Cake as a Swine Feedstuff

Whereas the gross energy of PKC is 20.1 MJ/Kg DM, its metabolizable energy for growing pigs is only 7.8 MJ/Kg DM. This has been attributed to its high fibre content

and to the Maillard reactions occurring during processing of palm kernel. According to Gohl (1982), the digestibility of most amino acids in PKC is about 20 to 30 percentage points lower than that of maize grain. The standardised ileal lysine digestibility is 37 % for the palm kernel meal but 80% for maize (Février *et al*., 2001; Sauvant *et al*., 2004). In a digestibility trial conducted by Amaefule *et al*. (2009), it was reported that inclusion of 40 % PKC in diet of growing pigs did not significantly affect digestibility of crude protein, protein utilization and nitrogen balance while the energy utilization indices were superior to those fed the maize based diet except ME as percentage of gross energy. Drawing an inference from a rat based study; Boateng *et al*. (2013) concluded that PKC had the potential to be included in maize starter diets for pigs at up to 35% with no adverse effects on growth. However, an optimal level of 30% was recorded when they placed growing pigs on PKC based diets with exogenous enzyme (Mannanase PLT^{TM}) supplementation. Using PKC from two different sources, Rhule (1998) had earlier reported that 30% inclusion of PKC produced average daily gains of 0.46 and 0.49 kg/day against the control (0.57 kg/day) during the grower period and corresponding values of 0.63, and 0.65 kg/day against (0.60 kg/day) during the finisher period. Feed conversion ratios (kg feed/kg live weight gain) 3.87, 3.94 and 3.44 (grower period) and 5.63, 4.91 and 4.53 (finisher period).

The reduction in growth performance among pigs fed palm kernel cake based diets is attributed to the high non-starch polysaccharides content (mannan), to the low palatability of the meal and its low amino acids and energy digestibility (Kim *et al*., 2001). In West Africa or Southern Asia, utilization of palm kernel meal as livestock can be economically viable and up to 40 % may be included in diets for growing-finishing pigs to replace cereal grains without deleterious effects on growth performance (400 to

500 g/d in average) and meat quality (Jegede *et al*., 1994; Rhule, 1996; Fatufe *et al*., 2007). Adehesinwa (2007) reported that PKC can effectively replace maize, weight for weight, in diets of growing pigs as an energy source, even up to the total replacement of the maize fraction (30 kg/100 kg of diet) without depressing the performance of growing pigs and efficient utilization of the diet.

Supplementations with enzyme complexes have been reported to enhance the nutritional value of palm kernel cake diets for growing pigs. Its energy value can be improved by the application of an enzyme complex to the diet that facilitates the breakdown of non-starch polysaccharides (Ao *et al*., 2011). The success recorded by Amaefule *et al*. (2009) in performance of pigs fed high levels of PKC could be attributed to inclusion of up to 35 – 40 % brewers dried grains (BDG) in the pigs' rations. BDG is a by product of the brewing industry and is believed to be laden with microbial proteins and other unidentified growth promoting factors especially when fed in fresh wet form. According to Adehesinwa (2009) PKC supplemented with cassava flour waste in diets of growing crossbred pigs can be used to replace the maize fraction without depressing the performance of the animals. In a similar manner, Anyaegbu, *et al*. (2012) demonstrated that different blends of fermented cassava meal, palm kernel cake and brewers' dried grain could completely replace maize in ration of growing-finishing pigs.

2.2.4 Limitations of Palm Kernel Cake as Livestock Feed Ingredient

The major factor responsible for the grittiness and very low digestibility of PKC has been identified as mannan. Ariff omar *et al.* (1998) reported that PKC contains 30 % β-mannan which cause depression in feed conversion efficiency and reduce weight gain

by 20 -25 % in poultry. Mannans are linear chains of β-(1→4) mannan and found in the seed endosperms of certain plant species. It believed to provide the molecular basis for the hardness characteristic of palm kernels (Per Hagglund, 2002). In nature, mannan exists in two forms: acetylated galactoglucomannan, which has heterogeneous backbone of β-1, 4-linked mannose and is usually found in softwood. The other form is galactomannan, which has a homogeneous backbone of β-1, 4-linked mannose and is found in seeds of leguminous plants (Ethier *et al.*, 1998). Generally the later polysaccharide is a linear polymer of mannose (Stoll *et al.*, 1999). Pure mannans may be defined to include those polysaccharides that contain less than 10% non-mannose sugar (glucose) residues (Chong, 1999). They form the major part of endosperm (kernels) of palm seeds where they form massive wall thickenings in the endosperm and are clearly the molecular basis of the palm kernel's characteristic hardness (Meier & Reid, 1982). All endospermic legumes such as guar and locust bean, contains galactomannans in the endosperm during seed development (Meier & Reid, 1977). Glucomannans represent a minor component in cereal grains (Mares & Stone, 1973).

Dusterhoff and Voragen (1991) characterized the polysaccharide component of PKC. They found it to contain negligible amount of starch (1 g/kg) and some of the protein was not digested even after pronase treatment implying that the residual protein was either structurally bound in the cell wall or present as inaccessible cytoplasmic material. There were about 726 g of cell wall materials per kg of PKC containing approximately 7.3% protein, 17.5% lignin, 5% ash and 74.6% non-starch polysaccharides. Further analysis by Dusterhoft *et al*. (1992) confirmed that the major polysaccharide in PKC is linear mannans with very low galactose substitution (78% of total non-starch polysaccharides). Daud & Jarvis (1992) also showed that highly crystalline linear

β(1,4)-D-mannan is the principal component of the non-starch polysaccharide found in the cell wall of PKC. The value of PKC as feed is further reduced by presence of shells that accompany local processing.

2.2.5. Improving the Nutritive Value of Palm Kernel Cake

The highly crystalline linear glucomannans found in PKC are resistant to the normal enzymatic action of monogastric animals particularly poultry (Daud & Jarvis, 1992). To digest mannan, a group of enzymes complex known as β-mannanases are needed to break it down into oligosaccharides (Ademark, 2000). Mannanase activity is found in endosperms and its activity increases from a negligible value shortly after imbibitions to a high value and then decreases (Matheson, 1990). Though endogenous mannanase activity was found in palm kernel (endosperm), heat treatments during processing (oil extraction) denature it. As a result, mannanase and other enzyme activities, if any, are not expected to be found in PKC (Chong, 1999). Using *in vitro* enzyme studies, Dusterhoft *et al.,* (1993a) found that reducing the particle size, thereby increasing the surface area available for enzymatic degradation enhanced the solubilisation of PKC. By comparing the release of glucose (cellulose) and mannose (mannan) from PKC, Dusterhoft *et al.*, (1993b) concluded that cellulose hydrolysis does not enhance mannan degradation nor vice versa. Rather, in the degradation of PKC cell wall, a kind of synergistic action of mannanases and glucanases is involved (Dusterhoft *et al.*, 1993c). Daud *et al*. (1997) found no synergistic action, instead they reported an increase of 8.5 % (or 230 kcal/kg) in apparent metabolizable energy (AME) of PKC-based broiler starters diet treated with 0.1% mannanase (Alltech Inc., USA). Cellulase supplementation of the same diet did not increase AME significantly.

β-mannanase, also known as 1, 4-β-D mannan mannanohydrolase (EC 3.2.1.78), belongs to the glycosyl hydrolyase families (Stoll *et al*., 1999). This enzyme hydrolyses the 1-4 linkage of mannan to produce simple sugar mannose, and thus reduce the mannan content in PKC. β-mannanase can be isolated from bacteria, fungi and even plants and an increasing number of β-mannanase genes are being cloned from these sources (Ethier *et al*., 1998). Microbes that play important roles in the degradation of mannan in PKC and other plant by-products *in vivo* include *Aspergillus tamarii* (Civas *et al*., 1984), *A. niger* (Ademark *et al*., *1998*; Siti Norita *et al*., *2010*), *Sporotrichum cellulophilum* (Araujo & Ward, 1991), *Scopulariosis candida* (Mudau & Setati, 2008), *Aspergillus fumigatus* (Puchart *et al*., 2004) and *Trichoderma reesei* (Arisan Atac *et al*., 1993). Consequently enzymatic or microbial degradation of PKC has become veritable biotechnological options in improving digestibility and hence nutrient availability of PKC (Zahari & Alimon, 2006). Enzymic depolymerization of PKC releases digestible sugars that will be fully absorbed and metabolized by poultry. Supplementation with specific enzymes can improve nutrient digestibility and worked efficiently to breakdown mannans in PKC (Iyayi & Davies, 2005). *Aspergillus niger* can be used as inoculum for fermentation of PKC (Abdul Rahman *et al.*, 2010). The fermentation with *Aspergillus niger* was reported to increase the true metabolizable energy (TME) of PKC from 5.5 MJ/kg to 8.1 MJ/kg (Ramin *et al.*, 2011; Alimon, 2004).

2.2.5.1 Enzymatic Degradation of palm kernel cake

The ß-mannanases cleave the backbone of galacto(gluco)mannans, releasing mannooligosaccharides. This type of mannan degradation is performed by the action of ß-endomannanases (ß-mannanases) and ß-mannosidases, commonly expressed by *Aspergilli* (de Vries, 2001). Several structural features in the polymer determine the

ability of ß-mannanases to hydrolyse the mannan backbone, such as the ratio of glucose to mannose and the number and distribution of substituents on the backbone (McCleary, 1991). It has been shown that ß-mannanase is most active on galactomannans with a low substitution of the backbone (Civas *et al*., 1984), and the presence of galactose residues on the mannan backbone significantly prevents ß-mannanase activity (McCleary & Matheson, 1983). The main products of ß-mannanase activity on mannan are mannobiose and mannotriose. ß-mannosidases act on the non reducing ends of mannooligosaccharides, releasing mannose. Substrate specificity studies show that ß-mannosidase is able to completely release terminal mannose residues when one or more adjacent unsubstituted mannose residues are present (Ademark *et al*., 2000).

The benefits of exogenous enzyme addition to cereal-based diets for poultry have been widely reported in literature (Bedford & Classen, 1992; Campbell & Bedford, 1992), but the supplementation of exogenous enzymes to improve the digestion of non-conventional feedstuffs such as PKC is not well established (Chong, 1999). Iyayi & Davies (2005) reported that weight gain and feed intake were significantly ($p<0.05$) higher on the enzyme supplemented BDG and PKM diets at the starter phase, whereas at the finisher phase, while feed intake was significantly ($p<0.05$) increased with enzyme supplementation, the weight gain was not significantly affected. The FCR also did not significantly change with enzyme supplementation at the starter phase, but at the finisher phase, feed conversion ratio was significantly ($p<0.05$) higher.

In a study to determine whether enzyme supplementation could improve the nutritive quality of PKC, Chong (1999) reported that Altech PKCase (a mixture of mannanase,

α-galactosidase and protease) was effective for PKC saccharification. According to him, PKCase supplementation increased the release of reducing sugars by 26.8 – 67.4 percent, reduced the fibre content and increased the apparent metabolizable energy and true metabolizable energy in PKC. Okorie *et al*. (2011) found that PKC with exogenous enzyme (Nutri-zym) supplementation at the rate of 100 mg/Kg could replace up to 60 % of maize in broilers rations without any deleterious effect on performance, carcass characteristics and haematological indices.

Lawal *et al,* (2010) used Palm Kernel Cake as a substrate to elicit the production of polysaccharidases from *Aspergillus niger*, *Trichoderma viride*, *Rhizopus stolonifer* and *Mucor mucedo*. The extracted enzymes produced were purified and used to ferment PKC in solid state at the rate of 250 ml/kg of the material for 7 days. They found that cellulose and hemicellulose fractions were significantly ($p<0.05$) reduced in the biodegraded PKC. This was better than the PKC supplemented with Roxazyme G2G. The level of soluble sugars increased in a similar trend. Crude protein, phosphorus and energy increased significantly ($p<0.05$) in the biodegraded PKC. In addition the apparent digestibility of nutrients was significantly improved ($p<0.05$) in birds that received the diets based on the biodegraded PKC. Feed conversion and weight gain of the birds fed the biodegraded PKC were significantly ($p<0.05$) higher.

2.4.5.2 Microbial Degradation / Enrichment of Palm Kernel Cake

The use of microorganisms capable of producing mannan degrading enzymes in the bioconversion of agricultural waste containing mannan-based polysaccharides into valuable products such as animal feeds has been advocated by several authors (Raimbault, 1998; Iluyemi *et al*., 2011; Iyayi & Davies, 2005; Othman *et al*., 2013 and.

Ramin *et al*., 2010). β-endomannanases commonly produced by *Aspergilli* are responsible for degradation of mannan component of plant polysaccharides. The ability of *A. niger* to grow in palm kernel cake waste, for mannanase production has been reported by Iluyemi *et al*. (2011). Solid state fermentation of Palm kernel cake by *Rhizopus oryzae* ME01 showed modest improvements in protein and ash contents though the value was still low compared to PKC fermented by other fungal strain with sufficient aeration (Othman *et al*., 2013). Ramin *et al*. (2010) found that after 10 days of SSF both *Aspergillus niger* and *Rhizopus oryzae* treatments increased the CP concentration in PKC from approximately 18 to 27 % and decreased the concentrations of NDF and ADF from approximately 74 and 43 % to 56 and 37 %, respectively. Khin (2004) reported the same increase in CP when *Aspergillus niger* was used for the fermentation of PKC, while Iluyemi *et al.* (2006) reported a greater increase (33%) when PKC was cultured under SSF. The amount of gas released when PKC was fermented for 10 days with *Aspergillus niger* and *Rhizopus oryzae* was lower than that for control fresh PKC. This is probably due to production of statins by the fungi during fermentation of PKC. Wolin & Miller (2006) reported inhibition of growth of methanogens in the rumen when hydroxymethylglutaryl-SCoA (statins) was used as an inhibitor, while there was no effect on growth of cellulolytic bacteria.

In a bid to understand the nature of mycelial interactions and corresponding production of mannan-degrading enzymes of PKC, Iluyemi & Hanafi (2009) co-cultured *Aspergillus niger* with three *Trichoderma* strains (*T. harzianum, T. longiobrachiatum and T. koningii*) on potato dextrose agar (PDA). About 57% of observed interactions on PDA were deadlock, 29% replacement and 14% intermingling. In *Trichoderma sp. / A. niger* mixed cultures, there was an overall significant enhancement of enzyme: 2 to 200

fold (β-D-mannanase), 8 to 25 fold (β-mannosidase) and from no change to 15 fold increase (α-galactosidase). Though there was no obvious relationship between enzyme production and protein yield, co-culturing of *A. niger* with the *Trichoderma* strains showed an enhancement of mannan-degrading enzyme activities without reducing biomass yield.

Increased protein in the biomass and enhanced protein digestibility are obvious advantages of microbial degradation to enzymatic degradation. However, improvements in the value of PKC using microbes under SSF are not without cost. Muangekeow & Chinajariyawong (2009) studied true amino acid digestibility and metabolizable energy of PKC fermented with *Aspergillus wentii TISTR* 3075 using adult meat type crossbred chickens. They found an increase in the entire true amino acids digestibility except for arginine. They found that *Aspergillus wentii* may use up nitrogen-free extract (NFE) in PKM as an energy source during the fermentation process, resulting in a lower metabolizable energy of fermented PKM when compared to normal PKM (without fermentation). In a related study, Marini *et al*. (2008) found that crude protein of fermented PKC (24.7%) increased significantly compared to the value in untreated PKC (17.5%). The total essential amino acid in fermented PKC was significantly increased (6.3%) compared to the value in untreated PKC. The fermented PKC contained 15.7% of total amino acid, accounted for 63.4% of the crude protein. Rat bioassay on protein quality showed that when fermented PKC was fed as the only protein source, the diet did not support growth rate. Hence, it is not advisable to feed fermented PKC as the sole protein source in the diet of animals.

Siti Norita *et al*. (2009) evaluated activities of mannan-degrading enzymes produced by *Aspergillus niger*. The optimum pH for mannanase, endoglucanase and -galactosidase were obtained at pH 3.5 while pH optimum for mannosidase was occurred at pH 3.0. The mannanase, endoglucanase, mannosidase and galactosidase were stable at pH 3.5 to 7, pH 3.5 to 6.5, pH 4 to 7 and pH 3.5 to 5.0, respectively. The mannanases from *A. niger* had two optimum temperatures (at 50 °C and 60 °C) and its half-life was 6 h and 4 h at 60 °C and 70 °C, respectively. The mannosidase, endoglucanase and galactosidase displayed optimal activity at 70 °C, 60 °C and 50 – 60 °C, respectively. The mannosidase had half-life of 1.5 h at 70 °C, while -galactosidase had a half-life of 2.5 h at 60 °C and endoglucanase had a half-life of 6 h at 60 °C and 45 min at 70 °C. Using a laboratory column bioreactor, Peyman *et al*. (2010) reported that the highest level of β-mannanase (2117.89 U/g) was obtained when SSF process was performed at incubation temperature, initial moisture level and aeration rate of 32.5C, 60% and 0.5 l/min, respectively. Their statistical model suggested that the optimal conditions for attaining the highest level of β-mannanase were incubation temperature of 32 ^{0}C, initial moisture level of 59% and aeration rate of 0.5 l/min. This will give a β-mannanase yield of 2231.26 U/g.

2.3 Carcass Composition and Meat Quality

2.3.1 Factors affecting Carcass characteristics of broiler chickens

Carcass analysis involves the measurement of different body parts of broilers chicken including the organs immediately after slaughter. This is often done in order to determine weight gain and response to feed given; that is the ability of the bird to convert feed to meat. Genetic variation is known to influence carcass and meat quality

of broilers. Kokszynski & Bernacki (2008) reported that the highest body weight (1892.5 g), eviscerated carcass weight with neck (1406.9 g) and slaughter yield (74.5%) were found in Ross 308 chickens, whilst the lowest values (1753.3; 1288.2 g; 73.3 % respectively) of these traits occurred in JV chickens. The highest muscle contents (45.4% breast muscles and leg muscles in carcass) and the lowest fattiness (7.9% skin with subcutaneous fat and 1.5% abdominal fat) were found in Hubbard Evolution chickens. However, Chen *et al*. (1987) reported that across Arbor Acres, Hubbard, Indian River and Ross × Arbor Acres hybrids, sex and age rather than breed significantly influenced the weight and slaughter yields of male and female carcasses. Males had significantly bigger carcasses than females at all ages and slaughter yields were higher in the males in all age groups. The reason was found to be significantly greater slaughter weight of male broiler chickens. Feijen (1997) in four experiments examined slaughter properties of broiler carcass in ten genotypes. The highest dressing percentage of "ready for the grill" was recorded by Cobb 500 chickens, while Hybro was the fourth in the range of chickens. Renden *et al*. (1992) and Acar *et al*. (1991) did not find significant differences in the dressing percentage between two commercial genotypes of broiler chickens.

Carcass yield is an indication of the quality and utilization of the ration (Bamgbose & Niba, 1998). Poor utilization of feed reduce dressed weight including breast and thigh muscles. Energy and protein levels as well as the ratio of energy to good quality protein in broilers diets influence carcass composition (Babatunde & Fetuga, 1976). Numerous works has been done to determine energy level that would meet the broiler chick requirement and ensure positive nitrogen balance (Sell *et al*., 1985 and 1989; Waldroup *et al*., 1993). The need to feed broiler chicken to obtain optimal carcass and meat

quality yields is now well appreciated by processors and researchers alike. 24 % dietary protein with 12.13MJ/kg metabolizable energy is recommended for good carcass composition in broiler starter chickens (Ojewola & Lange, 1999). Acar *et al*. (1991) and Holsheimer (1981) found that the weight of carcass was not under the influence of the energy-protein level of food in the 43rd day of age, while on the 53rd day of age the carcass weight increased significantly in chickens fed with meals with low energy-protein ratio as well as in the chickens fed with high energy-protein meals.

Higher weight values of proventriculus and intestine have been attributed to additional bulk and greater volume of digesta staying in the gastro-intestinal tract during enzymatic digestion (Savory & Gentle, 1976; Longe & Fagbenro-Byron, 1990; Ander, 1992). Furthermore, it has been suggested that structural carbohydrates in monogastric diet caused the intestinal wall and the gastro-intestinal tract to increase and thicken (Thorburn & Wilcox, 1985). Increased shank weight indicates poor growth and performance while high values of the liver, heart, spleen on broilers had been attributed to higher physiological activities by these organs triggered by the presence of some anti-nutritional factors (Uchegbu *et al*., 2004). Mardhati *et al*. (2011) replaced about 40 % of the maize in broiler rations and reported that though the performance and eviscerated weight of broilers on control diets were superior to those fed PKC supplemented diets, no significant differences were found for dressing percentage and proportions of breast muscle meat, drumstick, wings and abdominal fats. Okorie *et al* (2011) also found that including mechanically pressed PKC fortified with exogenous enzyme (Nutri-zym) at 20, 25 and 30 % of the diet did not have any effect on the carcass composition and characteristics of Marshall broiler finishers. Okon & Ogunmodede (1996) demonstrated that broilers reared on 25 % PKC diets have smaller

heads and intestines than those on control. Okeudo *et al*. (2006) also reported that the percent head and shank decreased significantly in broilers raised on 30 percent PKC when compared to the control. George and Sese (2012) reported that though there were significant differences among birds in feed intake, feed conversion ratio and feed efficiency when whole cassava root meal was used to replace white maize in broiler rations, the average weekly body weight and carcass weight were not affected.

2.3.2 Meat quality of broiler chickens

Quality is the composite of those characteristics that differentiate individual units of a product which have significance in determining the degree of acceptability of that unit to the user. Water-holding capacity, colour, pH and sensory acceptability are commonly used to evaluate meat quality. Consumers prefer meat that is juicy, tender, and not pale (McKee & Sams, 1997; Van Laack *et al*., 2000; Schilling *et al*., 2003; Fletcher & Smith, 2006). The effect of dietary protein and energy concentration on meat quality of chickens has been the target of a number of studies. Decrease in dietary energy level from 13.5 to 9.5 MJ/kg resulted in meats becoming less tender and less juicy (Ristic *et al*., 1990; Arafa *et al*., 1985). Decreasing the protein content of the diets from 21 % also leads to less tender, juicy and flavour, and sometimes higher cooking losses (Ristic *et al*., 1990). Indeed when both the protein and the energy concentration of the birds' diet are decreased, meat appears more tender in fast growing chickens but tougher in slow-growing counterparts. It appears there is still no documented evidence on the effect of inclusion of cassava meal and palm kernel cake on meat quality of broiler chicken and hence most inferences about them are based on the interplay of energy and protein levels (i.e. energy protein ratio) and fatty acid profile of the diets fed.

The fatty acids profile of the muscle lipids reflects the composition of the dietary fat (Lin *et al*., 1989; Sheehy *et al*., 1993). Some fatty acids from feed are deposited directly in the muscle and other body tissues. Thus, diet directly affects the composition of broiler meat, especially thigh meat due to higher fat percentages when compared with breast meat (Wood & Enser, 1997). Therefore, increased concentration of polyunsaturated fatty acids in the feed, in free form or in triglycerides, can lead to higher concentrations of polyunsaturated fatty acids in the muscle tissue. This increases the oxidation potential and potentially decreases the shelf-life of meat (Adams *et al*., 1994; Cortinas *et al*., 2004; Suksombat *et al*., 2007). Although, it is desirable from a dietary point of view, meat with a high unsaturated fatty acid content especially Ω-3 poly unsaturated fatty acids (PUFA) is more susceptible to lipid oxidation (Gray and Pearson, 1989; Lin *et al*., 1989; Sheehy *et al*., 1993). This may result in altered sensory and processing qualities such as off-odours and flavours and poor water retention (Ajuyah *et al*., 1993).

Problems like these can be overcome by incorporating anti-oxidants into the poultry diets. α -Tocopherol and tocopheryl acetate (Vitamin E) are the major and most efficient anti-oxidants for this purpose (Sheldon *et al*., 1997). Dietary supplementation with vitamin E significantly delays lipid oxidation, prevents the development of off-odours and flavours during storage, and decreases water losses from chicken meat (Blum *et al*., 1997; De Winne & Dirinck, 1996). Vitamin E also improves the tenderness, juiciness and flavour of chicken meat (Ristic, 1991). Meat from turkey fed a diet supplemented with vitamin E has a more stable colour and better processing qualities (Sheldon *et al*., 1997). The inclusion of vitamin E in the diet should reduce the

incidence of Pale soft exudative (PSE) condition probably by preventing oxidation damage to cell membrane (Anon, 1995; Ferket *et al*., 1995).

2.5.2 Carcass Composition and Meat Quality of Pigs

The value of a pig carcass is largely determined by its weight, fatness level and muscularity (Irshad *et al.,* 2012), Carcass evaluation is essential in determining carcass value at the market place and the pork producer must be concerned about production of edible pork which is acceptable by the consumer Meat animal carcasses vary in composition through genetic, age and sex of animal, nutritional, and environmental factors. As an animal matures, it undergoes an increase in the ratio of muscle to bone, followed by a decrease in muscle growth rate and an increase in the ratio of fat to muscle (Lawrie, 1998). Consequently physiological maturity seems to be the most important factor influencing the carcass composition of animals. Breeds may partition fat and muscle differently between body depots. During growth and development, intermuscular fat is deposited before subcutaneous fat, which is deposited before intramuscular fat (Warriss, 2000). Differences in body composition are more pronounced when pigs of different mature weights are compared at the same weight, rather than at the same age (Miller, 2002).

The efficiency of meat animals in converting feed into meat is generally related to the level of feed intake, but the relationship is rather complex. The relationship between plane of nutrition and development of the different tissues of the body has been demonstrated by Hammond (1932, 1944) and Butterfield & Berg (1966). The brain and the nervous system have priority over bone, muscle and fat in that order. Although fast rates of growth caused by a high plane of nutrition can lead to an earlier onset of the

fattening phase of growth, when the protein/energy ratio is increased, the fastest-growing animals may become leaner (Campbell & King, 1982). Indeed, when the ratio is very high the growth rate may be diminished (Campbell *et al*., 1984). Since males have a higher protein/energy requirement than females, this factor can cause differences between the sexes in the composition of the carcasses when the energy intake, at a given ratio, is altered (Campbell & King, 1982).

The highest efficiency in converting feed energy into body weight is achieved when animals are fed *ad libitum*. But, if feed energy intake exceeds the amount needed for lean tissue growth, the excess is used for fat deposition. Thus, animals fed high-concentrate diets *ad-lib* usually produce more carcass fat, and consequently, are less efficient in converting feed to lean meat than are animals fed slightly below *ad libitum* energy intake, even though the *ad libitum* fed animals would be more efficient in total feed energy retention. This is particularly evident in the later growth stages, as muscles and bone approach their mature sizes. An adequate and continuous supply of protein is required in animal diets for growth and maintenance of tissues. All meat animals are able to synthesize fatty acids and/or adipose tissue from carbohydrates and proteins, and the fat which is deposited is characteristic of the species.

Several methods are available for evaluation of the expected lean yield in a pork carcass. The method selected will often be dictated by the facilities and manpower that are available for collecting the needed information. Though the most accurate method of lean determination is complete physical separation of the lean, fat and bone in the carcass, the most practical method of evaluating carcass leanness appears to be through the use of a regression equation similar to that which has been used for several years to

establish beef yield grades. These equations depend largely on accurate determination of hot carcass weight, loin eye area and fat depth to predict lean composition and percentages. The American Meat Science Association (AMSA) in conjunction with the National Pork Producers Council has developed six procedures and consequent equations to determine the standardized fat free lean of pork carcasses (Burson, 2001).

Boateng *et al*. (2013) reported that mean dressing percentage for pigs fed diets containing 20, 30 and 40 % PKC levels were 62.23, 63.09 and 63.56 respectively, while the mean back fat thickness were 1.10, 1.36 and 1.61 cm, respectively. Earlier Rhule (1996) reported that palm kernel cake with high level of residual fat induced higher average daily gain, better feed conversion efficiency, increased carcass fatness with reduced leanness in pigs. The incorporation of mannanases by Boating *et al* (2013) may be responsible for this reverse, indicating that mannanase inclusion in the diets must have had a positive effect on digestibility, and hence higher growth rate. It has been reported that dietary fats affect the average daily gain, feed conversion efficiency, carcass fat, and keeping quality of pork products (Myer *et al*., 1992).

Aro and Akinjokun (2012) fed growing pigs with cassava peels fermented with a mixture of *Aspergillus fumigaues* and *Lactobacillus coryneformis* and *Lactobaccilus delbrueckii*, and reported that though female animals had thicker back fat at the 1st and 10th ribs and at the 1st lumbar vertebra whilst animals fed 40 % fermented cassava peel meal diets had the leanest carcasses. The highest muscle protein percentage (15.09 %) was found among pigs raised on 0 % inclusion level. Ochetim (1993) reported there were no differences in dressing percentage (78.1 vs. 78), back fat thickness (2.76 vs. 2.78), rib eye muscle area (29.2 vs. 29.1 cm^2) and relative proportions of the different

carcass cuts between pigs raised on commercial pig ration and those fed fresh unpeeled cassava root fortified with copra cake, meat and bone meal and a local vitamin premix from 15 to 85 kg live weight.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Location of the study

The study was carried out at the Teaching and Research Farm of the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri (FUTO). Owerri is located in the South Eastern agro ecological zone of Nigeria, with the mean annual rainfall, temperature and relative humidity of 2500 mm, 26.5-27 °C and 70-80 % respectively. The duration of the dry season (number of months with less than 65mm of rainfall) is 3 months and the annual evapo-transpiration is 1450 mm. The soil is sandy loan with an average pH of 5.5 (Adeyemi, 2011).

3.2 Broad Description of the Study

Five experiments were conducted to evaluate the efficacy of solid state fermentation as a tool for enhancing the nutritive value of mixture of cassava root pulp (CRP) and palm kernel cake for feeding broiler chickens and pigs. The experiments were:

1. Production, physicochemical characteristics and fungal ecology of fermented mixture of cassava root pulp and palm kernel cake (FEMCARPP)
2. Performance of broilers fed diets containing sundried fermented mixture of cassava root pulp and palm kernel cake as replacement for maize.
3. Effects of dietary inclusion of wet or dry fermented mixture of cassava root pulp and palm kernel cake on live weight gains, carcass characteristics and meat quality of broiler finishers
4. Live weight gains and haematological profile of pigs fed diets containing varying levels of fermented mixture of cassava root pulp and palm kernel cake.

5. Live weight gains, carcass characteristics and meat quality of growing pigs fed fermented mixture of cassava root pulp and palm kernel cake as replacement for maize.

3.2 Study 1:

Physicochemical characteristics and fungal ecology of differently fermented mixture of cassava root pulp and palm kernel cake (FEMCARRP)

3.2.1 Study Sites

The isolation and determination of the microorganisms involved in this study were carried out at the Biotechnology Laboratory of the Department of Biotechnology, FUTO. All fermentation and physical parameters (particle size, bulk density, specific gravity and water holding capacity) were carried out at the Animal Science laboratory, FUTO.

3.2.2 Collection and Processing of Experimental Materials

3.2.2.1 Procurement of Cassava and Processing into Pulp

Fresh cassava tubers were procured from local markets at Ihiagwa, Eziobodo, Obinze and Oforola. The tubers were peeled, washed and grated using a conventional cassava grating machine.

3.2.2.2 Palm Kernel Cake

Expeller palm kernel cake was sourced from a local dealer in Owerri. It was run on hammer mill to pulverize the crumbs and to ensure more homogeneous particle size.

3.2.2.3 Preparation of garri agar

Garri agar was prepared following the method described by Okorondu *et al*. (2013). Garri (a popular staple of sub-Saharan Africa) was procured from dealers in Owerri. It is produced grated cassava roots. After grating, pulp is packed into jute bags under manual press for 1 – 3 days during which fermentation and partial drying takes place by draining of juice and moisture evaporation. Thereafter, it is sieved and pan fried over an open fire usually with addition of little palm oil. The products hue is light yellow and is crispy to touch. However it is creamy white if palm oil was not added. Garri may be consumed directly, that without further processing, and in various ways including soaking in water like corn flakes.

The garri was ground and sieved with a sterile muslin cloth to make the particles fine and smooth. A 28 g of the garri powder, 14 g of agar powder and 8 g of *Hibiscus rabdriffa* (Zobo leaf) powder were measured and poured into a 1 litre conical flask containing 500 ml of freshly prepared distilled water. The flask was covered with a sterile plastic cork and shaken to allow the contents mix up and distribute uniformly in the water. The contents were later made up with distilled water to the 1 L mark. The flask was aseptically covered with non-absorbent cotton wool and aluminium foil. The mixture was swirled properly and then autoclaved at 121 ^{0}C for 15 minutes and allowed to cool to 45 ^{0}C. It was then dispensed into Petri dishes and allowed to solidify.

3.2.2.4 Nutrient Broth

A nutrient broth was prepared dissolving an equivalent of 18 g of $(NH_4)_2SO_4$, 5.4 g of urea and 10 g of KH_2PO_4 in 100 g of distilled water.

3.2.2.5 Isolation of *Aspergillus niger*

Agricultural soil collected from FUTO Crop Farm was used as source of inoculum for isolation of *Aspergillus niger*. A 1 g of the soil was introduced into 9 ml of sterile water and 10 fold serial dilution of up to 10^{-3} to 10^{-6} was inoculated onto Sabround Dextrose Agar (SDA) at room temperature (25 ± 3 °C). After 4 days, discrete colonies of *A. niger* were collected and sub-cultured on the garri agar. Cultures of pure colonies were then maintained in garri agar (Okorondu *et al*., 2013) after which they were kept on refrigeration at 4 °C till they are used.

3.2.3 Preliminary Study

A preliminary study was carried out to determine the proportion of milled cassava root pulp and palm kernel cake that will yield a 1:1 ratio of cassava root pulp and PKC on dry matter basis. This was necessary because a 1:1 mixture (w/w) of cassava meal and PKC will yield a product similar in composition to maize in dry matter and crude protein. Four 100 g samples of freshly grated root pulp were oven dried at 75 °C to a constant weight. The final weights were determined as the average dry matter composition which ranged from 32 to 35 % with a mean of 33.4 %. This means that, 1 kg of grated cassava root pulp will yield about 334 g of dry matter and so will mix with 334 g of PKC.

3.2.4 Experimental Setup

Four experimental treatments (TA, TB, TC and TD) were used to evaluate the effect of different fermentation techniques on the physicochemical characteristics of the mixture

of the experimental materials. These treatments are summarized as presented in Table 3.1.

Treatment A:

Treatment A was designed to evaluate the effect of direct inoculation with *Aspergillus niger* on solid state mixtures of cassava pulp and PKC. A two kilogram (2 kg) blend was produced which contained fresh cassava root pulp (CRP), ground palm kernel cake (PKC) and nutrient broth in the ratio of 10: 3: 1. These were weighed with a digital scale (Kenwood Electronic Scale Model 6S49). The nutrient broth contained 10^7 spores of *Aspergillus niger* per cm^3. The substrate mixture was transferred into a 5 litre capacity plastic container and covered loosely to create a solid state aerobic fermentation environment. The setup was replicated twelve times and allowed to ferment for 19 days.

On days 7, 11, 15 and 19 of the experiment, three replicates were selected at random, and were thinly (< 1 cm thick) spread on a black plastic mat placed on a concrete floor. After drying to a crispy and friable mass, the samples were weighed with an electronic scale (Silvano, model BS-2508). This weight was recorded as final weight of the sample. The differences in weight were recorded as moisture lost. The samples were then labelled and stored in air tight plastic containers until needed for analyses.

Treatment B:

Treatment B was designed to study spontaneous fermentation techniques on the sample mixture. The substrate setup as described previously was repeated, but the media solution was replaced with distilled water.

Table 3.1: Set up of the fermentation experiments

Treatment	Design
A	Evaluation of the effect of direct inoculation of *Aspergillus niger* on solid state mixtures of cassava root pulp and palm kernel cake under aerobic conditions
B	Evaluation of the effect of spontaneous microbial fermentation on solid state mixtures of cassava root pulp and palm kernel cake under micro aerobic conditions
C	Evaluation of the effect of batch inoculations of *A. niger* on solid state mixtures of cassava pulp and palm kernel cake using already fermented samples from Treatment A as inoculants under aerobic conditions.
D	Solid state mixtures of cassava root pulp and palm kernel cake without fungal inoculation and fermentation, and serving as control.

Each of the 12 replicates were spread on plastic tray (the conventional chick tray) and allowed to stand under shade at ambient temperature (27 ˚C) and humidity (70 – 80 %) for 24 hours. Each sample was reweighed and moisture loss determined. The equivalent loss in moisture was replenished with distilled water to restore the substrate to its initial weight of 2 kg. Afterwards each sample was transferred into a 3.25 litres plastic container and then covered tightly to create a micro anaerobic environment. On days 7, 11, 15 and 19 of the experiment, three replicates were selected at random, and sundried and stored as previously described.

Treatment C:

Treatment C evaluated the effect of batch inoculation using already fermented samples from treatment A. 300 g of the 19 days old solid state fermented substrates from TA was collected and divided into four equal parts. These were transferred to Petri dishes and kept on a laboratory work bench at room temperature (28 °C). The dishes were opened every 2 days and the samples moistened by spraying with the nutrient broth until visible fungal mycelia growth became evident on the 14th day. The four samples were pooled and mixed to achieve a uniform distribution of fungal spores. It was left to incubate for 2 days on a sterilized plastic tray covered with muslin cloth.

Thereafter, 12 replicates of a 500 g substrate were prepared as previously described in treatment 1. Each of the substrates were mixed with about 20 g of the fermented samples, and transferred into 3.5 l plastic containers, and then covered loosely as

described in treatment A. On days 7, 11, 15 and 19 of the fermentation, 3 samples were selected at random, dried and stored as previously described.

Treatment D:

Treatment D served as control and was not subjected to any fermentation. Three 2 Kg samples of the substrates were prepared following the procedures previously described in treatment B (TB). They were dried on a black plastic mat without fermentation and stored as previously described. On days 7, 11, 15 and 19 of storage period, 100 g of the samples were taken from each container, and analysed for their physicochemical compositions.

3.2.5 Determination of the Physical Characteristics

The physical characteristics of the products of A,B, C and D were evaluated for their final weight (air dry), substrate particle size distribution, bulk density, specific gravity and water holding capacity.

3.2.5.1 Final Weight

The weight of the samples after drying were expressed as a percencentage of the the initial weight as follows;

Final weight =

3.2.5.2 Particle Size Distribution

Two laboratory sieves of 2.00 mm and 0.85 mm was used to separate the feed samples into 3 particle size groups namely; coarse (> 2.00 mm), fine (≤ 2.0 ≥ 0.85 mm) and smooth (< 0.85 mm). About 100 g of each sample was first sieved with the 2.00 mm

sieve to separate coarse feed particles greater than 2.00mm. The sievates were later passed through a 0.85 mm sieve to remove the smooth particles less than 0.85 mm. The weight of each category was expressed as a percentage of the original weight of the sample. This was repeated 3 times for each experimental sample.

3.2.5.3 Bulk Density

The bulk density was determined as weight of a fermented sample divided by its volume as expressed with the formula.

$$\text{Bulk density} = \frac{weight\ of\ a\ given\ sample\ of\ test\ material}{volume\ of\ the\ sample}\ X\ 1000$$

The volume of the beaker was determined by filling it with water from a measuring cylinder. The beaker was then emptied and dried. Test materials were then filled into the beaker and carefully cut to level with a knife edge and weighed. This was repeated three times for each replicate of the experiment. The mean weight for each material was then divided by the volume of the cylinder and then multiplied by 1000 to convert the value to the S.I unit equivalent i.e. g/dm^3.

3.2.5.4 Specific Gravity (SG)

The specific gravity (SG) of the samples were determined as the weight of the sample used for the determination of bulk density divided by the weight of equivalent of volume water as expressed by the formula

$$\text{Specific gravity} = \frac{weight\ of\ a\ given\ volume\ of\ test\ material}{weight\ of\ equivalent\ volume\ of\ water}$$

3.2.4.5 Water holding capacity (WHC)

A modification of the filtration method described by Makinde and Sonaiya (2007) was used for the determination of the water holding capacity. A pyrex glass funnel was plugged with moistened non absorbent cotton wool and then placed on a conical glass as stand. The set up was weighed using a laboratory digital scale (Silvano, model BS-2508). A sample of the feed material was poured into the funnel and levelled off to the brim without pressing. The set up reweighted to determine the weight of the feed material. The feed material was then covered by a moistened filter paper (Whatman NO. 1) which was previously trimmed to fit into the outer circumference of funnel. The funnel and its content were set up below a burette filled with water

Water was dropped from the burette (70 drops per minute) through this feed material. At the first drop of water from the funnel, the burette was stopped and the whole set up was reweighed. The difference between the final weight and that of the set up with dry feed was calculated and recorded as weight of water absorbed by the feed material. The ratio of the weight of the water held by the sample material to the weight of the dry feed was given as the water holding capacity of the sample in g water / g dry feed as expressed by the formula

$$\text{Water Holding Capacity} - \frac{weight\ of\ a\ water\ retained\ by\ the\ feed\ sample}{weight\ of\ feed\ sample}$$

This was replicated three times for each sample of the experiments

3.2.6 Proximate Analysis of Experimental Materials

The proximate biochemical compositions of the experimental materials (dried cassava root pulp, palm kernel cake, fermented mixture of cassava root pulp and palm kernel

cake) were determined using the procedures of AOAC (1990). The determination was done in triplicate.

3.2.6.1 Moisture content determination

1.0 g of each sample was placed in an oven and dried at 105 ˚C for three hours. The sample was allowed to cool in a desiccator and then re-weighed. The percentage moisture content was calculated by expressing the loss in weight on drying as a fraction of the initial weight of sample used and multiplied by 100, as expressed by the formula

$$MC\ (\%) = W_0 / W_i \times 100$$

Where

MC = Moisture content,

W_o = loss in weight (g) on drying and

W_i = initial weight of sample (g).

3.2.6.2 Ash content determination

The ash content was determined using the ignition method. The crucibles used were thoroughly washed and pre-heated in a muffle furnace to about 500 ˚C. One gram of the sample weighed in triplicate and placed in weighed crucible and then re-weighed. The crucible was covered with its lid. The number noted and then placed in a cold muffle furnace. The temperature was allowed to rise to 500 ˚C and the ashing carried on for 3 hours at the same temperature. The crucible was removed from the furnace, allowed to cool in a desiccator and re-weighed. The percentage ash content was calculated using the formula:

$$Ash\ (\%) = (M_a / M_s) \times 100$$

Where:

M_a = weight of ash (g)

M_s = weight of sample used (g)

3.2.6.3 Crude protein (CP) determination

Determination of crude protein was done by determining the total nitrogen using the macro - Kjeldhal method. This involved digestion, distillation and titration. One gram of the sample was weighed in triplicate and placed in digestion flasks. Few granules of anti - bumps and about 3.0 g of copper catalyst mixture (96 % anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide) were added to each of the flasks. Digestion was then commenced by adding 20 cm^3 concentrated sulphuric acid, and continued until a clear solution was obtained and the flask allowed to cool. The digest was filtered and made up to 100 cm^3 with distilled water, and 20 cm^3 of the diluted digest was pipetted into round-bottomed flasks and used in the distillation step. For distillation, the round-bottomed flask was set on a heating mantle and connected, using a Liebig condenser, to a beaker (receiver flask) containing 20 cm^3 of 2 % boric acid, with screened methyl red indicator. The condenser was submerged in the boric acid by the use of a Buchner funnel. Thirty (30) cm^3 of 40 % sodium hydroxide was injected into the flask and distillation of the ammonia formed commenced by heating the flask. The distillation was continued until the boric acid solution completely changed from purple to greenish – yellow. The boric acid mixture (containing the ammonium borate complex formed) was titrated with 0.1N HCl to colourless end point and the titre recorded. The total organic nitrogen was calculated using the formula:

$$\% \text{ TON} = (TV \times NE \times TV_d) / M_s \times V_d \text{ x } 100$$

Where

TON = Total Nitrogen,

TV = Titre value,

NE = Nitrogen equivalent to molarity of acid,

TV_d = Total volume to which digest was diluted,

M_s = Mass of sample (g)

V_d = Volume of digest distilled.

$$\% \text{ crude protein} = \% \text{ TON} \times 6.25$$

3.2.6.4 Ether Extract (EE) determination

Determination of ether extract content of the samples was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40 – 60 ˚C). Three (3.0) g of the sample was weighed in triplicate and secured in Soxhlet extraction thimble. The thimble was put into 20 cm^3 capacity soxhlet extractor. A washed, oven - dried 100 cm^3 round - bottomed flask was weighed and approximately 60 cm^3 of the 40 – 60 ˚C boiling range petroleum ether added to it. The flask was mounted on the heating mantle and connected to the extractor (with condenser). The condenser and heating mantle were then activated and extraction carried on for 4 hours. At the end of extraction, the solvent was evaporated and the flask dried in the oven (at 60 ˚C). The flask was cooled and re – weighed. The percentage crude lipid was calculated using the formula:

$$\% \text{ EE} = M_{ex} / M_s \times 100$$

Where

M_{ex} = mass of extract (g)

M_s = Mass of sample used (g)

3.2.6.5 Nitrogen Free Extract Determination

Nitrogen free extract of each sample was estimated by difference. The sum of percentages of all the other proximate components was subtracted from 100 as expressed by the formula

Nitrogen free extract (%) = [100 – (% moisture + % CP + % EE + % ash)].

3.2.6.6 Calculation of Metabolizable Energy

This was calculated based on proximate composition using the prediction equations described by Pauzenga (1985):

ME = 37 x CP % + 81.8 x EE% + 35.5 x NFE %.

Where; ME = metabolizable energy (kcal/kg)

CP = crude protein,

EE = ether extract,

NFE = nitrogen free extract,

3.2.7 Identification of Fungal Organisms

On days 7, 15 and 19, samples were taken from each of treatments A, B C and D selected for drying using sterile knife tips. The tip was sterilised after each collection by heating over a non luminous blue flame for 5 seconds and then cleaned with sterile cotton wool moistened with ethanol. The samples were cultured on garri agar for 5 days

and fungal colonies were identified using the standard guides described by Alexopoulos & Beneke (1952) and Dungan & Dungan (2006) as shown in Table 3.2.

Table 3.2 Fungal morphology and microscopy for Identification

	Colonal morphology	Microscopy	Colour	Texture	Maximum growth rate	Suspected microrganisms
W	Whitish dense cottony aerial mycelia, up to 20 mm high, white spores and yellow base. Diameter of 8 cm within 4 days	Irregularly shaped unbranched sporangiosphore with rhizoid, non septate, many oval spores.	White	Wooly	Within 3 days	*Rhizopus stolonifer*
X	Compact white surface, which later turns black, attaining a diameter of 4 to 5 cm within 7 days. Black spores and short aerial mycelium.	Dense layer of dark brown to black conidiosphores, septate hyphae.	White or yellow base. Dark brown to black conidiosphore	Wooly	4 days	*Aspergillus niger*
Y	White but later turned yellow within 3 days and later turned dull brown after 7 days. Diameter of 4 to 5 cm	Septate hyphae, radiate conidia head, pale yellow later become dull brown	Greenish yellow later became dull brown.	Powdery	4 days	*Aspergillus oryzae*
Z	Cottony appearance from the front.	Non septate hyphae, sporangiosphores and spores are visible	Initially white becomes greyish brown in time.	Cottony	4 days	*Mucor spp*.

3.2.8 Experimental Design and Analysis

The physical characteristics (bulk density, specific gravity, particle size distribution and water holding capacity) were analysed using a Completely Randomized Block Design (CRBD). The inoculation methods TA, TB, TC and TD served as treatments, whereas periods of fermentation served as blocks. The proximate composition was analyzed using a 4 x 4 factorial. The factors are the four method of inoculation and four periods of fermentation. Fungal counts were analysed using a 3 x 4 factorial, which means 3 fermentation periods and 4 inoculation methods. Significantly different means were separated using the least significant difference as described by Little and Hills (1978).

3.2.9 Selection of Fermentation Technique

Choice was made between the 3 techniques based on the following criteria; physicochemical characteristics of the end products, cost and ease of adaptation on farm. Based on the results of the 3 fermentation techniques A, B and C, treatment B (spontaneous inoculation) was selected for scale up.

3.2.10 Scale-up of the spontaneous fermentation technique

Scaling up of the method involved the mixing of freshly grated cassava root pulp and palm kernel cake in a ratio of 3:1 (w/w). This was done in the evening, spread on a plastic mat and left over night. The following morning the mixture was packed into plastic containers (50 litres capacity) without any compression and closed tightly. The mixture was allowed to ferment for 5 more days. After fermentation, the mixture was sundried on polyethene sheets spread on a concrete slab until a friable crispy mass was produced. This was packed into plastic containers and stored in a cool dry place until

use. The fermented mass was named Fermented Mixture of Cassava Root Pulp and Palm Kernel Cake (FEMCARPP).

3.3 Study 2:

Performance of broilers fed diets containing sundried fermented mixture of cassava root pulp and palm kernel cake (FEMCARPP) as replacement for maize

3.3.1 Feed Raw Material and Diets Formulations

FEMCARRP was produced as previously described and other feed materials as in Tables 3.3 and 3.4 were purchased from local dealers in Owerri. Three experimental broiler starter and 3 broiler finisher diets were formulated such that FEMCARPP replaced maize in diet B, while a 1:1 mixture ratio of cassava root meal and palm kernel cake replaced maize in diet C. The ingredient composition and calculated nutrient composition of the experimental diets are shown in Tables 3.3 and 3.4. The control diet (Treatment A) contained maize as the test material. Dietary levels of FEMCARRP and soybean were adjusted in treatment B to maintain crude protein level within the recommendation for broiler chicks. The inclusion of the other of the ingredients were maintained at the same level.

3.3.2 Experimental chicks and design

A total of ninety (90) Arbor Acre day old broiler chicks were used for the experiment. The chicks were weighed on arrival and placed on commercial broiler starter feed formulated to provide 21% crude protein, 10% fat, 9% crude fibre,1% calcium, 0.45% available phosphorus, 2800 Kcal/Kg metabolizable energy for one week. On the 8^{th} day (beginning of the second week) the chicks were re-weighed and randomly assigned to 3 treatment groups of 30 chicks and each with 3 replicates, giving 10 chicks per replicate. The design was a completely randomised design (CRD).

Table 3.3: Ingredients and Calculated Chemical Composition of the experimental broiler starter diets

Ingredients	MAIZE (control)	FEMCARPP	CSM-PKC mix
Maize	56.00	0.00	0.00
FEMCARRP	0.00	62.00	0.00
Cassava root meal	0.00	0.00	28.00
Palm kernel cake	0.00	0.00	28.00
Soybean meal	30.00	24.00	30.00
Wheat offal	6.00	6.00	6.00
Fish meal	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00
Lysine	0.25	o.25	0.25
Methionine	0.25	0.25	0.25
Vitamin & mineral premix*	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated chemical composition			
Crude Protein	21.66	22.51	21.75
Crude Fibre	4.14	9.40	14.77
Crude Fat	4.11	8.70	9.11
Calcium	1.28	1.74	1.74
Phosphorous	1.00	1.41	1.40
Ash	3.59	5.54	5.54
Metabolizable energy (Kcal/kg)	2839	2487	2633

CRM – Cassava Root Meal, PKC – Palm kernel meal

*Formulated to supplied per kg diet Vit A, 10,000 I.U; Vit D3 2000 I.U; Vit E 40mg; Vit K3, 2mg; Vit B1 2.4mg; Vit B2 4.8mg; Vit B6 4.8, Niacin, 32mg; Pantothenic acid, 8mg; Biotin 0.1mg, Vit B12, 0.02mg; Folic Acid, 0.8mg; Cholin Chloride, 40mg; Manganese, 80mg; Iron, 40mg; Zinc, 36mg; Copper, 1.6mg; Iodine, 1.24mg; Cobalt, 0.2mg; Selenium, 0.08mg.

Table 3.4: Ingredients and Calculated Chemical Composition of the experimental broiler finisher diets

Ingredients	MAIZE (Control)	FEMCARPP	CSM-PKC mix
Maize	60.00	0.00	0.00
FEMCARRP	0.00	68.00	0.00
CRM/PKC blend	0.00	0.00	30.00
Palm kernel cake	0.00	0.00	30.00
Soybean meal	22.00	14.00	22.00
Wheat offal	10.00	10.00	10.00
Fish meal	3.00	3.00	3.00
Bone meal	4.00	4.00	4.00
Lysine	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Vit. & mineral premix*	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated Chemical Composition			
Crude Protein	18.47	19.53	19.07
Crude Fibre	4.10	9.87	15.52
Crude Fat	3.19	8.17	9.06
Calcium	1.60	1.74	1.74
Phosphorous	1.16	1.42	1.43
Ash	3.25	5.25	5.35
Metabolizable energy	2804.58	2464	2614

CRM – Cassava Root Meal, PKC – Palm kernel meal

*Formulated to supply per kg diet: Vit A, 900 I.U; Vit D3, 1440 I.U, Vit E, 21.6mg; Vit K, 2.7mg; Vit B1, 1.8mg; Vit B2, 3.6mg; Vit B6, 2.7mg; Niacin, 21.6mg; Pantothenic acid, 9.0mg; Biotin, 0.036mg; Vit B12, 0.018mg; Folic acid, 0.54mg; Cholin chloride, 270mg; Manganese, 108mg; Iron, 18mg; Zinc, 27mg; Copper, 1.44mg; Iodine, 0.72mg; Cobalt, 0.1mg; Selenium, 0.1mg; Growth promoter, 14.4mg; Anti- Oxidant, 720.

Each group was fed each of the experimental starter diets for the next twenty-one (21) days. From 29th day of age, each treatment group was fed the corresponding finisher diet until slaughter at 52 days of age. Feed and water were given *ad-libitum* while routine vaccinations and medications were done according to the conventional practice.

3.3.3 Data Collection

3.3.3.1 Growth Performance

The chicks were weighed at the beginning of the experiment and subsequently each week whle feed intake was determined daily as the difference between the quantity given and the left over. At the end of the experiments (28th day for starter and 49th day for finisher periods), the difference between the final live weight and initial live weight were determined and recorded as weight gain. The weight gained was further divided by the period of experiment to determine the average daily gain. To determine the feed conversion ratio, average daily feed intake was divided by the average daily weight gain. Quantity of protein consumed was calculated as the quantity of feed consumed multiplied by the percent crude protein content of the feed. Mortality was recorded on daily basis and summed up at the end of the experiment. Cost of feed and other inputs were estimated using the prevailing market prices..

3.3.3.2 Carcass and organ evaluation

All the chicks were fasted on the 52nd day of the experiment. Water was provided *ad lib*. The following day, 2 birds with live weights closest to the mean of each replicate were selected. They were carefully labelled and transferred to slaughter pen. Each bird was slaughtered by severing the jugular vein and allowing it to bleed to death in a

vertical position (head down). After bleeding, they were individually weighed and scalded in hot water, defeathered and eviscerated. The carcasses were cut into parts according to the methods described by USDA (1998). The offal (the head, neck, shank, wings) and organs (heart, liver, gizzard, gastrointestinal tract and abdominal fat) were carefully separated and weighed. The weight of each part was expressed as percentage of the live weight of the bird.

3.3.3.3 Meat Quality Evaluation

The left thighs of birds from each treatment were individually weighed, and kept in air tight polythene bags. These samples were properly labelled and left in a refrigerator at 7 ^{0}C for 24 hours. The following day, the samples were carefully removed from the polyethene bags, dried with filter paper and reweighed. The difference in weight were expressed as a percentage of the initial weight and recorded as the drip loss for each sample.

The water holding capacity was measured according to the procedures described by Kauffman *et al*. (1992) and Honikel (1998). A portion weighing approximately 3.5 g was removed from each drumstick and wrapped in filter papers of equivalent weight and reweighed. Each of the samples was subjected to mechanical pressure for 3 minutes using a screw jack. The meat residues were then recovered and reweighed. The difference in weight of meat sample was recorded as the weight of expelled fluid. This was expressed as a percentage of the weight of initial sample and recorded as the water holding capacity of the meat.

To determine the cooking loss, samples weighing approximately 10 g were also cut from each drumstick, weighed and wrapped individually in polythene bags. The samples were carefully labelled and heated under steam for 20 minutes. Afterwards, samples were allowed to cool to room temperature and later dried with filter paper and reweighed. The decrease in weight was expressed as a percentage of the fresh weight and recorded as percentage cooking loss.

3.3.3.4 Haematological Analysis

3.3.3.4.1 Blood Collection

As each bird was bled, 2 ml of blood was collected into bijou bottles treated with ethylene diamine tetra acetic acid (EDTA) for haematological analysis. Another 10 ml of blood was collected and allowed to coagulate in sterile sample bottles for serum biochemical evaluation. The time of sampling was usually between 9.00 and 10.00 am, while analysis took place within 12 hours of sampling.

3.3.3.4.2 Haematological assay

Red blood cell count: The red blood cell count was determined via the improved Neubauer ruled chamber after diluting with 0.02 ml of blood mixed with EDTA. A 4 ml of formaldehyde citrate solution was used as the diluting liquid at 1 : 200 using a Pasteur pipette. The two ruled areas of the improved Neubauer chamber were filled with diluted blood and allowed to stand for 3 minutes for the cells to settle. The cells were counted in accordance with the procedures of WHO (1980) using the formula

$$\text{Cell count } (10^{12}/\text{ L}) = N \times (D/A) \times 10 \times 10^{9}$$

Where

N = Total number of cells counted

D = Dilution factor of blood

A = Total counted area (mm^3)

10 = Factor to convert area to volume (iμ)

10^9 = Factor to convert count per iμ to count per litre

Packed cell volume (PCV): The packed cell volume was determined by the Wintrobe-microhematocrit method (Schalm *et al.*, 1975). The Wintrobe-microhematocrit tube was filled with blood and spun for 5 minutes in a centrifuge at 3000 rpm and the packed cell volume read as a percentage using the designed scale reader.

%PCV = Height of red cell column / Height of total blood column X 100

Haemoglobin concentration: This was determined using the haemoglobin cyanide method formerly called cyanmethemoglobin method. Blood was mixed with Drabkin's solution, a solution that contains ferrocyanide and cyanide. The ferrocyanide oxidizes the iron in the hemoglobin thereby changing hemoglobin to methemoglobin. Methemoglobin then unites with the cyanide to form cyanmethemoglobin. Cyanmethemoglobin produces colour which is measured in a colorimeter. The hemoglobin concentration was finally estimated as described by Cole (1986).

Red cell indices*:* The Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were expressed in picogram (pg), femtolitre (fl) and grams / 100 ml respectively. The MCH, MCV and MCHC were determined from RBC, PCV and hemoglobin (Hb). These hematological constant were calculated using the appropriate formulae as described by Jain (1986).

$$MCV\ (fl) = PCV\ (L / L) / RBC \times 10^{12} / L;$$

$$MCH\ (pg) = Hb \times 10\ (g / l) / RBC \times 10^{12} / L \text{ and}$$

$$MCHC\ (g / l) = Hb \times 10\ (g / l) / PCV\ (L / L))$$

White blood cell count*:* The white blood cell count was obtained using a haemocytometer with Natt and Hendricks diluent to obtain a 1: 200 blood dilutions. The diluents and samples were mixed and carefully loaded into a counting chamber. This was left for 2 – 3 minutes for the cells to settle before they were counted using improved Neubauer haemocytometer at magnification of × 40. All the cells in the entire central square (1 mm^2) were thereafter estimated in accordance with method of Schalm *et al.* (1975).

$$\text{Cell count } (109 / L) = N \times (D/A) \times 10 \times 10^9$$

Where

N = Total number of cells counted

D = Dilution factor of blood

A = Total counted area (mm3)

10 = Factor to convert area to volume (iμ)

10^9 = Factor to convert count per iμ to count per litre

Differential white blood cell count: A blood smear was prepared by placing a drop of blood on a glass slide with a Pasteur pipette and neatly covered to make a thin film of blood sample. After drying, the sample was stained with Leishman's stain and covered with phosphate buffer and allowed to stand for 8 to 10 minutes before washing off the stain. The sample was treated with cedar wood oil before placing under x100 lens 92

magnification of Olympus microscope. The number of neutrophils, lymphocytes, basophils, monocytes and eosinophils were determined and expressed as percentages of the total white blood cell count.

3.3.3.3 Serum Biochemical Assay

Serum urea: Serum urea was determined by the Berthelot method (Tiez, 1976). A 0.02 ml of serum sample was added to each test tube and distilled water to other tube (the blank). Four (4) ml of freshly prepared urea colour reagent was pipetted into each tube and mixed vigorously. The content of each tube was incubated at 100 °C for 15 minutes. The absorbance was read and recorded at 530 nm wavelength using spectrophotometer (model and manufacturer).

Serum glucose: Blank, standard and treatment tubes were labelled, and 2 ml of glucose reagent was pipetted into each tube and placed for 5 minutes in a water bath set at 37 °C. A 0.02 ml of serum sample was added to each tube, mixed and incubated at 37 °C for 10 minutes. Spectrophotometer was zeroed with blank sample and absorbance of all tubes read at 520 nm. The glucose level was thereafter calculated as;

$$\text{Concentration of glucose (mg / dl)}$$

$$= [\text{ Abs. of treatment / Abs. of std }] \times \text{concentration of std.}$$

Serum creatinine: Creatinine was determined quantitatively by the modified end point method (Heinegard & Tiderstrom, 1973). Equal volumes of sodium dodecylsulphate reagent, phosphate borate buffer and picric acid reagent were mixed thoroughly and 5 ml was pipetted into test tubes labelled reagent blank, standard, control or treatments. A

0.2 ml of serum was pipetted into the test tube and distilled water pipetted into reagent blank and mixed. All test samples were heated in a water bath at 37 °C for 15 minutes. Absorbance was read and recorded from a spectrophotometer at 490 nm zeroed with reagent blank. The creatinine value of the treatment sample was calculated using the following general formula:

Creatinine (μmol / L) =

[(Abs of serum samples / Abs of 200 μmol) / creatinine standard] × 200.

***Serum lipids*:** The serum lipids were determined by ferric chloride - sulphuric acid reaction (Elleston & Caraway, 1970). 5.0 ml isopropyl alcohol was added to 0.2 ml of blood and spun vigorously. Standard 0.2 ml of cholesterol and 0.2 ml of triglycerides were also put in the appropriate tubes. Also, a 3.0 ml of glacial acetic acid was pipetted into all the tubes and mixed properly and thereafter 0.3 ml of iron was added and mixed. 0.5 ml of H_2SO_4 was added to one tube at a time and allowed to cool. Cholesterol and triglyceride absorbances were read at 450 nm and 570 nm, respectively, after zeroing the spectrophotometer with reagent blank. The cholesterol value of the treatment sample was calculated using the following general formula:

Cholesterol in sample (mg / dl)

= [Abs of serum samples / Abs of std] × Cholesterol std × 200.

Triglycerides (TG) sample (mg / dl)

= [Abs of serum samples / Abs of std] × TG std × 200.

Serum sodium: A 1.0 ml of filtrate reagent was added to test tubes labelled blank, standard or treatment groups according to the colorimeter method of Tiez (1976). Thereafter, 0.05 ml of serum sample each was added to all the test tubes and distilled water to the blank and shaken vigorously. The test tubes were spun at high speed (1000 rpm) for 10 minutes. 0.05 ml of colour reagent was added to all tubes and mixed. Finally, the spectrophotometer was zeroed with distilled water and absorbance of all the tubes were read at 550 nm. The sodium content of samples were estimated from readings using standard solutions.

Serum potassium: A 1.0 ml of potassium reagent was added to standard, control and treatment tubes according to the procedures of Tiez (1976). 0.01 ml of samples each was added to the tubes mixed and left at room temperature for 3 minutes. Absorbance for all tubes were read and recorded at 500 nm wavelength after zeroing the spectrophotometer with reagent blank. Sodium potassium ratio was determined by dividing the sodium value by the potassium value.

Serum calcium: The serum calcium values were determined by the complex metric procedures of Gitelman (1967). This involved the use of calcium reagent, calcium buffer and calcium standard. The test tubes were labelled blank, standard, control and treatments. 1.0 ml of reagents was pipetted into each tube and 0.02 ml of serum sample added to the respective tubes, mixed and thereafter allowed to stand for 60 seconds at room temperature. Absorbance was read at 570 nm wavelengths after zeroing the spectrophotometer with reagent blank.

Serum phosphorus: The modified colorimetric method of Skeggs & Hochestrasser (1964) was used for phosphorus analysis. In the first stage which is deproteinization, 7.6 ml of tricarboxylic acid (TCA) reagent and 0.4 ml of serum were added into a centrifuge tube. The contents were well mixed, allowed to stand for 5 minutes and then centrifuged for 5 minutes. In the second stage, 1.0 ml each of water, ammonium molybdate and methol were added into 3 test tubes labelled test, standard and blank. The test tube labelled test was also added 3.0 ml of supernant, and in the test tube labeled standard was added 2.5 ml of TCA reagent, 0.5 ml of the standard phosphorus solution, and in the blank was added 2.0 ml of TCA. The content was mixed, stood at room temperature for 20 minutes and absorbance read at 680 nm wavelengths, after zeroing the spectrophotometer with reagent blank. The concentration of phosphorus in the serum was then calculated using the formula

Concentration of phosphorus in serum

= [Abs of test x concentration of std] / Abs of std]

Serum chloride: The modified colorimetric method of Skeggs & Hochestrasser (1964) was used to determine chloride content of serum. 0.01 ml of blank, standard, control and treatment tubes and 3 drops of K_2CrO_3 indicator was pipetted. The tubes were subsequently titrated and end point marked by brown colouration.

Serum bicarbonate: Serum bicarbonate was determined by the colorimetric method. 0.1 ml of sodium tetraphenyl boron was added to standard, control and treatment tubes. Thereafter, 0.01 ml of samples each was added to the tubes mixed and left at room temperature for 3 minutes. Absorbance for all tubes was read at 500 nm after zeroing

the spectrophotometer. Absorbance of standard solutions were used to calculate the bicarbonate contents in samples..

Serum liver enzymes: Alanine aminotransferase (ALT), Aspartate transferase (AST) and alkaline phosphatase (ALP) were estimated. Serum ALT, AST and ALP were determined by colorimetric end point method of Reitman & Frankel (1953) and Roschlan *et al*. (1974).

Alanine aminotransferase: Test tubes were labelled treatment, standard and blank. Thereafter, 0.5 ml of ALT substrate reagent was added to the standard, blank and treatment tubes. 0.1 ml of sample was added to the treatment tube whereas 0.1 ml each of standard reagent and distilled water were added to the standard and blank tubes. These were mixed and left in a warm bath (37 ˚C) for 25 minutes. 0.5 ml of 2, 4 - DNPH was added to all the tubes (treatment, standard and blank) and all the tubes were subsequently incubated for 10 minutes at room temperature. 0.8 ml of 0.5N NaOH was added to each test tube and mixed thoroughly. Absorbance of every tube was read and recorded from a spectrophotometer at 510 nm wavelength zeroed with reagent blank. The ALT value of the treatment sample was determined by comparing it's absorbance reading with that of standard. The ALT of the treatment sample was calculated as ALT (IU / L) = [Abs of treatment / Abs of std] × conc. of std

Aspartate aminotransferase (AST): A 0.5 ml of AST substrate reagent was added to the standard, blank and treatment tubes. Thereafter, 0.5 ml of serum was added to the treatment tube, whereas 0.1 ml each of substrate reagent and distilled water was added to the standard tube and blank tubes respectively. These were mixed and left in a warm

bath (37 ˚C) for 25 minutes. Subsequently, 0.5 ml of 2, 4 - DNPH was added to all the tubes (treatment, standard and blank) and all the tubes were subsequently incubated for 15 - 20 minutes at room temperature. 0.5 ml of 0.5N NaOH solution was then added to each of the tubes and mixed thoroughly. Absorbance of every tube was read and recorded from a spectrophotometer at 510 nm wavelength zeroed with reagent blank. The AST value of the treatment samples were determined by comparing absorbance reading with that of a known standard. The AST of the treatment sample were calculated as:

$$\text{AST (IU / L)} = [\text{Abs of treatment / Abs of std}] \times \text{conc. of std.}$$

ALT: AST **r**atio was determined by dividing the ALT value by the AST value.

***Serum alkaline phosphatase (ALP)*:** Test tubes were labelled treatment, standard and blank. 0.5 ml of ALP solution and ALP buffer reagent was added to the standard, blank and treatment tubes and incubated for 5 minutes at 37 ˚C. Thereafter 0.1 ml each of serum sample, ALP standard and distilled water were added to the treatment tubes, standard tube and blank tube, respectively, and left at 37 ˚C for 15 - 20 minutes. 0.8 ml of 0.5N NaOH, 1.2 ml of $NaHCO_3$, 1.0 ml of 4 - aminoantipyrene and 1.0 ml of potassium ferrocyanide solutions were each measured into the treatment, standard and blank tubes respectively. The tubes were properly mixed and read with a spectrophotometer at 500 nm wavelength. The serum ALP of the treatment samples were calculated as:

$$\text{ALP (IU / L)} = [\text{Abs of treatment / Abs of std}] \times \text{concentration of std}$$

***Serum bilirubin*:** Total bilirubin and conjugated bilirubin were determined by colorimetric end point method of Reitman & Frankel (1953) and Roschlan *et al*. (1974). Test tubes were labelled treatment or blank. 0.2 ml of serum sample, 0.5 ml of mixed Diazo reagent, 2.5 ml of methanol and 2.0 ml of distilled water each were pipetted into the treatment and blank tubes. The tubes were properly mixed and incubated in a dark compartment for 20 minutes, and read at 540 nm with a spectrophotometer. The serum bilirubin of the treatment sample were calculated as

Conc. of bilirubin (mg / dl)

= [Abs. of treatment – Abs. of blank / Abs of std – Abs. of blank] × conc. std

3.3.4 Statistical Analysis

Data collected on the experiment were subjected to analysis of variance (ANOVA) in a completetely randomised design (CRD). Significantly different means were separated using the LSD as described by Little & Hills (1974).

3.4 Study 3:

Effects of dietary inclusion of wet or dry fermented mixture of cassava root pulp and palm kernel cake on liveweight gain, carcass characteristics and meat quality of broiler finishers

A second broiler feeding experiment was carried out to evaluate the effects of dietary FEMCARRP in wet or dry forms on the performance of broilers.

3.4.1 Experimental Site

This experiment was carried out in the Poultry Unit of the Teaching and Research Farm of the School of Agriculture and Agricultural Technology, FUTO.

3.4.2 Feed Raw Material and Diet Formulation

Fresh and sundried FEMCARRP were produced as previously described. Four experimental diets (T1, T2, T3 and T4) were formulated as in Table 3.5. T1 (control) contained 60 % maize as the principal energy source. The maize in T1 was replaced 60 % wet FEMCARPP in T2 (dry matter basis) and the dried FEMCARPP in T3, while T4 had a 50:50 mixture of cassava meal/PKC to replace maize. The ingredient and calculated nutrient composition are shown. Due to the moist nature of T2 diets, fresh samples were prepared and fed to the birds each morning and leftovers were weighed and discarded at the end of the day to prevent the development of rancid flavours and consumption of mouldy feed.

Table 3.5: Ingredient and calculated chemical composition of the experimental diets

INGREDIENTS (%)	MAIZE	FEMCARPP		CSM-PKC mix
		WET	DRY	
Maize	60.00	-	-	-
Wet FEMCARPP	-	60.00	-	-
Dried FEMCARPP	-	-	60.00	-
Cassava meal	-	-	-	30.00
Palm Kernel Cake	-	-	-	30.00
Soybean meal	22.00	22.00	22.00	22.00
Wheat offal	5.00	5.00	5.00	5.00
Fish meal	3.00	3.00	3.00	3.00
Bone meal	4.00	4.00	4.00	4.00
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Vitamin / mineral premix*	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated nutrient composition				
Crude protein	18.49	19.19	19.19	19.19
ME(Kcal/kg)	2804.00	2604.00	2604.00	2604.00
Crude Fibre (max)	4.09	9.07	9.07	9.07
Ash	3.25	5.35	5.35	5.35
Calcium	1.60	1.74	1.74	1.74
Phosphorus	1.16	1.43	1.43	1.43
Methionine	0.23	0.49	0.49	0.49
Lysine	0.82	1.81	1.81	1.81

*Supplied per kg diet: Vit A, 900 I.U; Vit D3, 1440 I.U, Vit E, 21.6mg; Vit K, 2.7mg; Vit B1, 1.8mg; Vit B2, 3.6mg; Vit B6, 2.7mg; Niacin, 21.6mg; Pantothenic acid, 9.0mg; Biotin, 0.036mg; Vit B12, 0.018mg; Folic acid, 0.54mg; Cholin chloride, 270mg; Manganese, 108mg; Iron, 18mg; Zinc, 27mg; Copper, 1.44mg; Iodine, 0.72mg; Cobalt, 0.1mg; Selenium, 0.1mg; Growth promoter, 14.4mg; Anti- Oxidant, 720.

3.4.3 Experimental birds and design

120 Abor Acres broiler chicks aged 3 weeks were used for the experiment. The chicks were purchased at day old and raised in the farm on a commercial broiler starter diet. The label on the feed indicated that it contained 21 % crude protein (min.), 10 % fat (max.) 9 % crude fibre (max.) 1 % calcium (min.), 0.45 % available phosphorus (min.) and 2800 Kcal/Kg metabolizable energy (min.). All vaccines and other medications were administered according to the conventional practice. At 21 days of age, the chicks were randomly assigned to four treatment groups of 30 birds with 3 replicates in a completely randomized design (CRD). Each replicate had a total of 10 chicks. Each group was randomly assigned to 1 of the 4 experimental diets. Feed and water were offered *ad libitum* throughout the experimental period.

3.4.4 Data Collection

3.4.4.1 Growth performance

All chicks were weighed on replicate basis at the beginning of the experiment and at the end of each week. Weight gain was determined as the difference between the weight of that replicate from that of the previous week. This was further divided by the duration (7) to determine the average daily weight gain (ADWG) for the week. Feed intake was determined daily as the difference between quantity of feed offered and the leftover the following morning. Feed conversion ratio was calculated as the quotient of ADFI and ADWG. Mortalities were recorded daily and summed up for the replicates at the end of experiment. Cost of feed was determined based on the prevailing market price.

3.4.4.2 Carcass and Organ Evaluation

At the end of the experiment (7^{th} week of age), the birds were starved for feed for 24 hours, while water was provided *ad libitum*. Eight birds weighing approximately 2.0 kg were randomly selected from each of the treatments (2 per replicate) for carcass evaluation. They were individually weighed, labelled and bled to death by severing the jugular vein. They were dressed and the carcass and internal organ parameters determined as previously described (USDA, 1989). Internal organs were carefully separated and weighed individually. The weight of each component was expressed as a percentage live weight of the birds.

3.4.4.3 Meat Quality Evaluation

The left thighs of each of the dressed carcasses were deboned and the skin completely removed. Each was labelled, weighed and transferred into an air tight polythene bag (Ziploc) and stored in a refrigerator and maintained at 7 - 10 ^{0}C. After 24 hours, the samples were evaluated for drip loss, water holding capacity and cooking loss following the methods previously described as in study 2.

3.4.4.4 Organoleptic Evaluation

The left drumstick of each bird was deboned completely and cut into 2 or 3 samples weighing approximately 50 g each. Each sample was dipped into saline solution (30 g/L) for 15 seconds, wrapped in a polythene bag and labelled according to its treatment group. The samples from each treatment group were cooked separately for 20 minutes under steam, cooled to about 40 ˚C and kept in a clean stainless steel food flask. After about 30 minutes, the samples were distributed to a 30 member trained assessors drawn

from the 500 level students of Department of Animal Science and Technology, FUTO. The panellists were instructed to masticate the meat samples and score them for tenderness, juiciness, flavour intensity, connective tissue amount, off flavour and degree of likeness using an eight points category rating scale as described by Miller (1998).

3.4.5 Statistical Analysis

Data generated were evaluated using analysis of variance (ANOVA) of completely randomised design and significantly different means were separated using the Least Significant Difference (LSD) as described by Little & Hills (1974).

Study 4:

Live weight gains and haematological profile of pigs fed diets containing fermented mixture of cassava root pulp and palm kernel cake

3.5.1 Experimental Site

This experiment was carried out at the Piggery Unit of the Teaching and Research Farm of the Federal University of Technology, Owerri.

3.5.2 Experimental Pigs and Design

A total of 40 piglets farrowed at the FUTO Teaching and Research Farm were used for this experiment. They were weaned at 40 days of age and were given subcutaneous injection of I-vermectin to control both ecto and endo parasites. They were randomly assigned to the five treatments groups (4 males and 4 females per treatment). Because of space constraint, all animals within the same treatment were housed in one pen and fed together. The pens were of similar dimension (2 x 4 m). The piglets were weighed individually at the beginning of each week and each treatment group was fed 4 % of their body weights.

3.5.3 Feed Raw Material and Diets Formulation

Wet FEMCARRP was produced as previously described in study 1. Other feed ingredients were sourced from local dealers in Owerri. Five experimental weaner rations were formulated as in Table 3.6. Diet A contained 44 % maize and served as control. . In diet B, 50 % of the maize was replaced with FEMCAPP while in diet C it was completely replaced by FEMCARPP. In diet D, maize was partially (50 %)

replaced with a 1:1 blend of cassava root meal and PKC while in diet E, it was completely replaced by the blend. The details are shown.

3.5.4 Data Collection

3.5.4.1 Growth performance

The piglets were weighed individually at the beginning of each week until the end of the experiment. Each treatment group was allowed a daily supply of feed equivalent to 5 % of their body weights. Feed left over the following morning was collected and weighed, and the difference between the feed given and the weight of feed leftover is recorded as quantity of feed consumed. The live weights obtained and the various quantities of feed consumed by each treatment group were used to estimate the following parameters:

- Average Daily Weight Gain (ADWG) estimated by dividing the mean weight gain (WG) of each treatment group by the number of days taken to acquire such weight.

$$\text{ADWG} = \text{WG/period}$$

- Average Daily Feed Intake (ADFI) was gotten by dividing the mean feed intake of each pen by the number of animals in that pen

$$\text{ADFI} = \text{mean feed intake / number of animals)}$$

- Feed Conversion Ratio (FCR) is gotten from dividing ADFI by ADWG.

Table 3.6: Ingredients and calculated chemical composition of swine weaners' diets

Ingredients (%)	TA(%)	TB(%)	TC(%)	TD(%)	TE(%)
Maize	44.00	22.00	0.00	22.00	0.00
Cassava meal	0.00	0.00	0.00	11.00	22.00
Palm Kernel Cake	0.00	0.00	0.00	11.00	22.00
FECARPP	0.00	22.00	44.00	0.00	0.00
Soya bean meal	20.00	20.00	20.00	20.00	20.00
Wheat offal	28.00	28.00	28.00	28.00	28.00
Fish meal	4.00	4.00	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Lysine	0.25	0.25	0.25	0.25	0.25
Methimine	0.25	0.25	0.25	0.25	0.25
Min / Vitamin premix*	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated chemical composition					
Crude protein (%)	19.64	20.96	22.15	19.86	20.08
Metabolizable energy(kcal/me)	2461.04	2262.45	2063.85	2391.74	2322.44
Crude fibre	5.34	7.17	8.99	9.53	13.72
Calcium	1.29	1.34	1.39	1.34	1.39
Phosphorus	1.17	1.27	1.37	1.27	1.37
Lysine	0.95	1.08	1.22	1.08	1.22
Methionine	0.27	0.37	0.47	0.37	0.47

*Formulated to provide per kg of feed, Vit A 10,000,000IU; Vit D3 2,000,000IU; Vit E8,000IU; Vit K 2,000,mg; Vit B1 2,000mg; Vit B2 5,500mg; Vit B6 1,2000mg; Vit B12 12 mg; Biotin 30mg; Folic Acid 600mg; Niacin 10,000mg; Pantothenic Acid 7,000mg; Choline Chloride 500 mg; Vit 10,000mg; Iron

3.5.4.2 Haematological Assessment

At the end of the weaners' stage (13th week of age), 2 ml of blood was collected from the jugular vein of four piglets (2 males and 2 females) from each treatment. The samples were transferred (< 45 sec) into EDTA treated sample bottles and stored under ice for haematological analysis. The samples were collected between the hours of 9.00 and 10.00 am while analysis was done within 4 hours of sampling. The samples were analyzed for total red blood cell (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), total white blood cell (WBC) and percentage neutrophil, lymphocytes and eosinophils using methods previously described as in study 2. The mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were also calculated as previously described.

3.5.5 Experimental Design and Data Analyses

All data collected from the experiment was subjected to analysis of variance (ANOVA) in a Completely Randomized Design (CRD), while significant difference of the means were separated using Least Significant Difference method (LSD).as described by Little & Hills (1974).

Study 5:

Live weight gains, carcass characteristics and meat quality of grower pigs fed fermented mixture of cassava root pulp and palm kernel cake as replacement for maize

3.6.1 Experimental Animals and Design

After study 4, 24 grower pigs (12 males and 12 females) were selected from the pool and each identified with ear tags. The pigs were dewormed orally with Piperazine. Each sex was divided into three groups of 4 animals each and housed in pens of similar dimension (2 x 3 m). Each group was randomly assigned to either of the three treatment diets. At 20 weeks of age, all boars were transferred to pens measuring 3 by 4 m to create more space and minimize aggression, while the gilts were retained in the same pens. The pigs were weighed at the beginning of the experiment and every other week. Daily feed allowance for the first week was based on 4 % of body weight and on the first day of the other weeks, feed allowance was increased by 20 %. The leftover on the previous day was used to adjust the feed allowance for the week.

3.6.2 Experimental Diets

Three experimental grower and 3 finisher pig rations were formulated and fed from 14 to 20 and 21 to 25 weeks of age, respectively and are as in Tables 3.7 and 3.8. Diet A contains 50 % maize and served as control. In diet B, the maize was completely replaced with FEMCAPP while in diet C it was replaced by a 50:50 blend cassava meal and palm kernel cake. The details are shown

Table 3.7: Ingredients and calculated chemical composition of swine growers' diets

	Diets		
Ingredients	TA	TB	TC
Maize	50.00	-	-
FEMCARPP	-	50.00	-
Cassava meal	-	-	25.00
Palm kernel cake	-	-	25.00
Soybean meal	10.00	10.00	10.00
Wheat offal	32.00	32.00	32.00
Fish meal	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00
Vitamin/mineral premix*	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Lysine	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated chemical composition			
Crude protein. (%)	16.40	19.25	16.90
Metaboliable energy (Kcal/kg)	2475.00	2224.00	2318.00
Crude fibre (%)	5.25	9.71	14.78
Calcium (%)	1,28	1.31	1.39
Phosphorus (%)	1.17	1.40	1.39
Lysine	0.72	1.02	1.02
Methionine	0.22	0.45	0.45
Total ash	3.98	5.73	5.73

*Grower premix formulated to supply per kg diet: Vit. A 10,000,000 IU; Vit. D3 2,000,000 IU; Vit. E 8,000 IU; Vit. K 2,000 mg; Vit. B1 2,000 mg; Vit. B2 5,500 mg; Vit. B6 1,200 mg; Vit. B12 12 mg; biotin 30 mg; folic acid 600 mg; niacin 10,000 mg; pantothenic acid 7,000 mg; choline chloride 500,000 mg; Vit. C 10,000 mg; Iron 60,000 mg; Mn 80,000 mg; Cu 8,00 mg; Zn 50,000 mg; iodine 2,000 mg; cobalt 450 mg; Selenium 100 mg; mg 100,000 mg and Anti oxidant 6,000 mg.

Table 3.8: Ingredients and calculated chemical composition of swine finishers' diets

Ingredients	Treatment Diets		
	TA	TB	TC
Maize	50.00	-	-
FEMCARPP	-	50.00	-
Cassava meal	-	-	25.00
Palm kernel cake	-	-	25.00
Soybean meal	4.00	4.00	4.00
Wheat offal	38.00	38.00	38.00
Fish meal	4.00	4.00	4.00
Bone meal	3.00	3.00	3.00
Vitamin/mineral premix*	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Methionine	0.25	0.25	0.25
Lysine	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated chemical composition (%)			
Crude protein	14.90	15.35	15.35
Metabolizable energy (kcal/kg)	2405	1954	2248
Crude fibre	5.46	9.92	14.99
Calcium	1.27	1.39	1.39
Phosphorus	1.21	1.43	1.44
Lysine	0.60	0.90	0.90
Methionine	0.20	0.43	0.43
Total ash	4.00	5.75	5.75

*Premix formulated to supply per kg diet: Vit. A 10,000,000 IU; Vit. D3 2,000,000 IU; Vit. E 8,000 IU; Vit. K 2,000 mg; Vit. B1 2,000 mg; Vit. B2 5,500 mg; Vit. B6 1,200 mg; Vit. B12 12 mg; biotin 30 mg; folic acid 600 mg; niacin 10,000 mg; pantothenic acid 7,000 mg; choline chloride 500,000 mg; Vit. C 10,000 mg; Iron 60,000 mg; Mn 80,000 mg; Cu 8,00 mg; Zn 50,000 mg; iodine 2,000 mg; cobalt 450 mg; Selenium 100 mg; mg 100,000 mg and Anti oxidant 6,000 mg.

3.6.3 Data Collection

3.6.3.1 Performance Parameters

Data on live weight, weight gain, feed intake and feed conversion ration were collected as described in study 4.

3.6.3.2 Carcass characteristics

At the end of the finisher stage of the experiment (25 weeks), all the boars were fasted for 24 hours with unrestricted access to water. The following day, they were weighed and mechanically stunned and bled to death by severing the jugular vein while the carcass hung in a vertical position (head down). The carcasses were dressed and weighed. The carcass length, rib-eye area and back fat thickness were measured by tracing with acetate paper according to the method described by Burson (2001). Thereafter, the carcass was cut into the primal cuts following the standard guidelines as described by FAO (1991). The exact description of the cuts is given in appendix 1. Each joint was weighed and recorded. The Carcass weight and length were used to determine dressing percentage and percentage lean cuts following the procedures described by Burson (2001). The offal (head, trotters, tail and internal organs) were also carefully separated and weighed. The weights were expressed as percentages of the live-weight of the animals.

3.6.3.3 Meat Quality Assessment

The water holding capacity and drip loss were measured according to the procedures described by Kauffman *et al*. (1992) and Honikel (1998) as previously described in study 3 for broilers. In this case, the *Longissimus dorsi* (LD) muscle from both sides at

the 10^{th} and 11^{th} ribs were used for the drip loss and water holding capacity measurements. Cooking loss was also determined as described in study 3 using section of loins from 11^{th} rib to the last lumbar vertebra.

3.6.3.4 Sensory Evaluation

After 48 hours of refrigerated storage, the *Semitendinosus* muscle of each carcass was carefully removed and chopped into pieces weighing approximately 50 g. The samples were soaked briefly (30 seconds) in a saline solution (30 g/l) and then wrapped individually in a polythene bag. Each bag was well labelled and boiled in a pot containing water for 30 minutes. A panel of 24 trained assessors drawn from the final year students of the Department of Animal Science and Technology were used as the sensory panel. The panellists were instructed to wholly masticate the sample and evaluate them for tenderness, juiciness, flavour intensity, connective tissue amounts and hedonic rating using the 8 point category rating scale as described by Miller (1998).

3.6.4 Experimental Design and Data analyses

Analysis of variance (ANOVA) was carried out on all performance, carcass characteristics and meat quality parameters using completely randomized design (CRD). Significantly different means were separated using Least Significant Difference method as described by Little & Hills (1974).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Study 1

Physicochemical characteristics and fungal ecology of differently fermented mixture of cassava root pulp and palm kernel cake

4.1.1 Physical Characteristics of fermented materials

4.1.1.1 Final weight

The airdried weights of the experimental samples after air drying are shown as in Table 4.1.1. The dry weights of treatments A, B and C were all significantly higher than the control on the 7th day of fermentation. This can be attributed to a possible increase in biomass and / or chemical changes in the nature of the fermented substrates. Until the 19th day of fermentation, the dry weights of treatment C was significantly higher ($p<0.05$) those of treatment A (*A. niger* inoculation) and the spontaneously inoculated samples (TB). Of particular note is the trend of increases across the treatment groups. Though, there were no significant block (period of fermentation) effect ($P>0.05$) in *A. niger* inoculated samples, TA increased from 59.30 g on day 7 to 60.62 g on day 19, whereas the was decrease in TC from 69.06 g to 67.75 g within the same period. On the other hand, spontaneously inoculated samples increased significantly ($P<0.05$) from 55.43 g to 63.33 g for the same period. This suggests that the agents and / or the products of fermentation may vary between the *A. niger* inoculated samples and the spontaneously inoculated ones. This obviously requires further investigation.

Table 4.1.1 Final weight (per 100g) of the test materials (substrates) after sun drying

Period of Fermentation	Method of Inoculation			TD	SEM
	TA	TB	TC		
7	59.30^{a}	55.43^{ad}	69.06^{e}	52.64^{ad}	1.470
11	57.56^{a}	57.33^{a}	65.00^{e}	50.97^{d}	
15	58.22^{ab}	56.05^{a}	65.25^{e}	50.25^{d}	
19	60.62^{ac}	63.33^{ce}	67.75^{e}	49.78^{d}	

$^{a, b, c, d}$, means in the same row and column with different superscript are significantly different ($p<0.05$). TA – *Aspergillus niger; TB* – Spontaneous, TC – Batch inoculation, TD- No fermentation (Control)

4.1.1.2 Particle Size Distribution

The particle size distributions of the fermented materials are shown in Table 4.1.2. There were no significant differences ($P > 0.05$) in particle size distribution among TA, TB and TC. However, it is obvious that all the treatments improved in particle size distribution of the test material up to 7th day of fermentation. This is because cassava meals are very floury, and could pass through 0.02 mm sieves. On the other hand, processors mill the kernels to particle sizes less than 0.5 mm to ensure optimal oil extraction from palm kernels, The results show that the dustiness characteristic of cassava meals was completely eliminated by the experiments. It is possible that *A. niger* must served as a binding agent in the samples they grew (A and C). However, judging from the aroma of the fermented substrates some proportion of the fatty acids in the fermented products may have been short chain volatile fatty acids. It is as well possible that the heat energy generated during fermentation may have initiated a partial gelling effect on the starch granules. This as well needs further investigation.

4.1.1.3 Bulk Density, Specific Gravity and Water Holding Capacity

The results for the bulk density, specific gravity and water holding capacity of the fermented samples are as shown in Table 4.1.3. The bulk density of the fermented samples decreased progressively with period of fermentation. However, the decrease was not significant until 19th day of fermentation except for the TA which became significant on the 15th day of fermentation. There were significant treatment differences ($P<0.05$) at all the stages of fermentation except at the 11th day, when the values for *A. niger* inoculated samples (369.33) was similar ($P>0.05$) to the spontaneously inoculated treatment (381.10). The result is worthy of note; 53.15 for TA, 22.98 for TB and 37.50 for TC.

Table 4.1.2 Particle distribution of experimental samples

Size (%)	Period of Fermentation (days)	Method of Inoculation			TD (control)	SEM
		TA	TB	TC		
> 2.0mm (%)	7	22.31	18.33	16.41		5.520
	11	19.08	19.00	18.28		
	15	22.78	14.67	18.50		
	19	24.87	20.67	18.64		
< 2.0≥ 0.85mm	7	34.41	35.00	28.16		9.320
(%)	11	32.96	35.33	29.37		
	15	29.96	32.00	28.91		
	19	40.15	35.00	28.91		
< 0.85mm (%)	7	43.28	46.67	55.43		13.75
	11	47.96	45.67	52.35		
	15	47.26	53.33	52.59		
	19	34.98	44.33	52.45		

TA – *Aspergillus niger; TB* – Spontaneous, TC – Batch inoculation

Table 4.1.3 Bulk density, Specific gravity and Water holding capacity of experimental materials

Parameter	Period of Fermentation in days	Method of Inoculation			TD (control)	SEM
		TA	TB	TC		
Bulk Density	7	373.70^{a}	396.07^{b}	350.00^{c}	382.86^{ab}	7.884
(g/dm^3)	11	369.33^{a}	381.10^{ac}	316.63^{e}	382.87^{ac}	
	15	321.31^{b}	393.00^{c}	300.00^{e}	382.85^{ac}	
	19	320.45^{b}	366.09^{d}	312.50^{be}	382.78^{a}	
	Range	53.25	22.98	37.50	0.09	
Specific gravity	7	0.39^{a}	0.39^{a}	0.38^{a}	0.42^{c}	0.108
	11	0.35^{b}	0.37^{abd}	0.35^{d}	0.42^{c}	
	15	0.30^{c}	0.38^{ab}	0.33^{d}	0.42^{c}	
	19	0.29^{c}	0.36^{bd}	0.34^{d}	0.42^{c}	
Water Holding Capacity (g/g of feed)	7	1.6^{a}	1.96^{c}	2.65^{d}	1.83^{ac}	0.109
	11	1.84^{a}	1.99^{ac}	2.04^{a}	1.79^{a}	
	15	1.39^{ab}	2.04^{c}	1.94^{c}	1.85^{c}	
	19	1.21^{ab}	2.04^{c}	1.91^{c}	1.84^{c}	

^{a,b,c,d}, means in the same row and column with different superscript are significantly different ($p<0.05$). TA – *Aspergillus niger,* TB – Spontaneous, TC – Batch inoculation, TD – No fermentation (control)

The specific gravity of control samples did no vary with period of fermentation (storage) and were consistently higher ($p<0.05$) than those of treatments A, B and C. Specific gravity of the samples fermented with *A. niger* (A and C) at 11th day of fermentation were significantly lower ($p<0.05$) than those fermented for 7 days. After 11 days of fermentation, no further decrease was observed among the batch inoculated samples (treatment C), but the decrease continued until 15 days of fermentation among samples inoculated directly with *A. niger* (treatment A). The decrease among spontaneously inoculated samples became significant ($p<0.05$) only at 19th day of fermentation.

The water holding capacity (WHC) of treatments A and C decreased progressively with period of fermentation. The values however were not significant ($P>0.05$) among samples as in TA whereas the values of TC at 7th day of fermentation (2.65) was significantly lower than those at 19th day (1.91). The WHC in spontaneously inoculated samples increased progressively and show no significant ($p>0.05$) result after 19 days of fermentation

4.1.2 Proximate Composition

4.1.2.1 Effect on inoculation method on proximate composition

The effect of inoculation method on the proximate composition of experimental materials is shown in Table 4.1.4a. The table shows that the proximate compositions of the materials were highly affected ($p < 0.001$) by inoculation method except for the ash contents. The dry matter content of the samples inoculated with *A. niger* (89.04 %) did not vary significantly ($p>0.05$) from the control (89.44 %). The spontaneously inoculated samples

and those inoculated with samples that were previously fermented with *A. niger* had significantly ($p < 0.001$) lower dry matter content. This suggests that the dynamics of the fermentation processes as well as the organisms implicated in the inoculation methods vary from each other. Omoikhoje *et al.* (2014) reported reductions in dry matter similar to those implicated in spontaneous and batch inoculation, when other agro industrial by-products such as rice bran, cassava by-products, saw dust and oil palm sludge were subjected to solid state fermentation.

The crude protein contents of all fermented samples were significantly higher ($p < 0.001$) than the unfermented samples. The crude protein content of batch inoculated samples (15.37 %) were higher ($p < 0.001$) than the spontaneously inoculated samples (14.67 %), which in turn was higher than the *A. niger* fermented samples (14.16 %). These represent an increment of 57.6 %, 50.4 % and 45.2 % in crude protein content over the unfermented samples by the batch, spontaneously and *A. niger* inoculated samples respectively. Iyayi and Aderolu (2004) reported a 32 % increase in crude protein content of palm kernel meal fermented with *Trichoderma viride* while Iluyemi *et al*. (2006) reported an increase of 33 %. Soccol *et al*. (1995) had reported a 1000 % increase in crude protein of cassava bagasse subjected to solid state fermentation using *Rhizopus*.

Table 4.1.4a: Effect of treatment on proximate composition of fermented mixture of cassava root pulp and palm kernel cake

Parameters (%)	A	B	C	D	SEM
Dry Matter	89.04[a]	87.11[b]	85.79[c]	89.44a	0.275
Crude Protein	14.16[c]	14.67[b]	15.37[a]	9.75[d]	0.062
Ether Extract	4.09[c]	4.83[b]	6.04[a]	6.27[a]	0.141
Crude Fibre (effective)	11.53[b]	13.14[a]	13.17[a]	12.55[a]	0.181
Ash	4.57	4.4	4.6	4.39	0.089
Nitrogen free extractives	54.68[a]	50.89[b]	47.44[c]	56.49[a]	0.952
Metabolizable energy (Kcal/Kg)	2800.14[ab]	2744.82[b]	2747.16[b]	2878.87[a]	31.791

[a,b,c] Means with different superscripts within a row are significantly different ($p < 0.001$). A- *Aspergillus niger*, B- spontaneous, C – Batch, D – No inoculation (control)

Iyayi *et al*. (2004) reported an increase in crude protein content of 41 % when wheat offal was fermented with *A. niger* under similar conditions. Though, Sukaryana *et al*. (2010) reported an increase of 29.58 when a mixture of palm kernel cake and rice bran was fermented with *Trichoderma viride*.

The crude fibre contents of the spontaneously and batch inoculated samples were similar ($p>0.05$) to the control whereas those of *A. niger* inoculated samples were significantly lower than the rest ($p < 0.001$). This suggests that the *A. niger* was more efficient in converting crude fibre to effective fibre than the other fungi implicated in the other treatments. Sukaryana *et al*. (2004) reported a decrease of 22.53 % in crude fibre while Ramin *et al*. (2010) reported a decrease in concentrations of NDF and ADF from 74 % and 43 % to 56 % and 37 %, respectively; when palm kernel cake was fermented with *A. niger* or *Rhizopus oryzae*. The ether extract content of A. niger inoculated samples (4.09 %) was significantly lower ($p < 0.001$) than the spontaneously inoculated ones (4.83 %) which were in turn lower than the control and batch inoculated samples (6.04 % and 6.27 % respectively).

4.1.2.2 Effect of period of fermentation on proximate composition

The effects of period of fermentation on proximate composition of the fermented materials are shown in table 4.1.4b. Very highly significant effect of period of fermentation ($p < 0.001$) was found for all parameters except for the ash contents. The dry matter contents decreased with period of fermentation while crude protein contents tended to increase. The dry matter contents of the samples at 7 and 11 days of fermentation (88.41 and 88.19) were significantly higher ($p < 0.001$) that those at 15 and 19 days of fermentation (87.52 and

Table 4.1.4b: Effect of period of fermentation on proximate composition of fermented mixture of cassava root pulp and palm kernel cake

	Period of fermentation (days)					
Parameters (%)	0	7	11	15	19	SEM
Dry Matter	89.44[a]	88.41[b]	88.19[b]	87.52[c]	87.26[c]	0.2754
Crude Protein	9.75[c]	13.33[b]	13.37[b]	13.62[a]	13.64[a]	0.062
Ether Extract	6.27[a]	5.28[b]	5.15[b]	5.35[b]	5.45[b]	0.141
Crude Fibre	12.55[b]	12.2[a]	12.67[b]	13.39[ab]	12.13[a]	0.181
ASH	4.39	4.35	4.67	4.35	4.59	0.089
Nitrogen free extractives	56.49[a]	54.08[ab]	53.16[b]	50.8[c]	51.46[bc]	0.952
Metabolizable energy (Kcal/Kg)	2878.87[a]	2845.33[a]	2803.12[ab]	2745.36[b]	2777.18[b]	31.791

[a,b,c] Means with different superscripts within a row are significantly different ($p < 0.001$)

87.26). The crude protein content of samples at 15 and 19 days of fermentation were higher than those of 7 and 11 days of fermentation.

Mean crude fibre levels were highest ($p<0.001$) at 15 days of fermentation whereas no significant differences ($p < 0.05$) were found between samples at 7 and 19 days of fermentation. NFE values varied significantly ($p < 0.05$). The samples fermented for 7 and 11 days were significantly lower in NFE ($p < 0.05$) than those fermented for 15 and 19 days. NFE values were lowest ($p < 0.05$) at 15 days of fermentation. This may be attributed to higher contents of fibre and proteins and lower dry matter. Compared to the unfermented samples, there was decrease in dry matter content, ether extract, and NFE while protein contents increase among fermented samples. These cumulatively led to decrease in metabolizable energy of fermented samples. As contents remained relatively unaffected while the crude fibre content was not consistent with period of fermentation.

4.1.2.3 Interactions between inoculation method and period of fermentation on proximate composition of fermented materials

Table 4.1.4c shows the interaction between inoculation method and period of fermentation on the proximate composition of the fermented materials. Significant interaction effect on dry matter content was found for only the spontaneously fermented samples ($p < 0.001$). The DM contents of spontaneously inoculated samples after 15 and 19 days (85.83 % and 85.70 %) were significantly ($p < 0.001$) lower than the corresponding values at 7 and 11 days of fermentation (88.53 % and 88.31 %). Little interaction effect was found for crude protein contents of the fermented samples; instead the inoculation method had more dominant effect on crude protein contents.

Table 4.1.4c: Interactions between inoculation method and period of fermentation on the proximate composition of the experimental material

Parameters (%)	Period of fermentation	Method of inoculation				SEM	P – value
		A	B	C	D		
Dry matter	7	88.88^{a}	88.58^{a}	86.17^{b}	89.99^{a}	0.551	0.001
	11	89.15^{a}	88.31^{a}	85.71^{b}	89.59^{a}		
	15	88.94^{a}	85.83^{b}	85.79^{b}	89.50^{a}		
	19	89.20^{a}	85.70^{b}	85.47^{b}	88.68^{a}		
Crude protein	7	13.77^{c}	14.59^{b}	15.17^{a}	9.80^{d}	0.125	0.001
	11	14.24^{bd}	14.24^{b}	15.40^{a}	9.60^{cd}		
	15	14.23^{e}	14.93^{c}	15.46^{a}	9.85^{d}		
	19	14.41^{e}	14.94^{c}	15.46^{a}	9.74^{d}		
Ether extract	7	3.95^{be}	4.45^{b}	5.91^{a}	6.82^{a}	0.281	0.01
	11	3.91^{ce}	4.74^{b}	5.97^{a}	5.97^{a}		
	15	4.23^{be}	4.92^{b}	6.13^{a}	6.13^{a}		
	19	4.29^{ce}	5.21^{b}	6.15^{a}	6.15^{a}		
Effective fibre	7	11.28^{a}	11.63^{a}	13.13^{b}	12.75^{b}	0.361	0.01
	11	12.30^{b}	12.87^{b}	12.91^{b}	12.60^{b}		
	15	11.59^{ab}	14.86^{c}	14.24^{c}	12.85^{b}		
	19	10.95^{a}	13.19^{b}	12.39^{b}	11.98^{b}		
Ash	7	4.55	4.12	4.37	4.36	0.178	Ns
	11	4.43	5.41	4.49	4.35		
	15	4.51	3.90	4.65	4.35		
	19	4.80	4.19	4.87	4.50		

$^{a, b, c, d}$, means in the same row or column with different superscript are significantly different. A – *Aspergillus niger;* B - spontaneous, C – Batch and D – Nil (Control)

Though the crude protein content of *A. niger* inoculated samples at 7 days of fermentation was significantly ($p < 0.001$) lower that the values at 11 days of fermentation, further increment in days of fermentation did not influence crude protein levels significantly ($p < 0.05$).

The work suggests that, the fungi in the spontaneously inoculated samples were more effective in increasing the level of crude protein than the pure cultures of *A. niger* inoculation. These fungi must have been well adapted to the substrate either individually or through multiplicity of interactions. On the other hand, screened pure cultures of *A. niger* needed time to adjust to the substrate. Crude Protein content of batch treatment was higher than treatments TA and TB. It is clear that the microbes in batch cultures have adapted better to the substrates and incubation environment, which resulted in the higher crude protein level. This is in line with the earlier findings of Vorachinda *et al.* (2011) who observed that the rate of protein and biomass turnover in batch or scale up experiments were higher than the controls (pure cultures). These results are in agreement with the findings of Ademafio *et al* (2010) who reported a 10 fold increment in crude protein content of cassava peels, whereas Sukaryana *et al*. (2010) reported a 29.58% increment in crude protein when a mixture of palm kernel cake and rice bran on a ratio of 1:1 was inoculated with *Trichoderma viride*. Vorachinda *et al*. (2011) reported a 14.25% increment in crude protein of cassava pulp / soymilk residue using *A. niger*.

It is noted that, ether extract fraction did not vary significantly ($p < 0.05$) among batch inoculated and control samples throughout the experimental period. Their values were consistently higher ($p < 0.001$) than the spontaneously inoculated which were higher (p

< 0.01) than the corresponding values as observd with *A. niger* inoculated samples at 11, 15 and 19 days of fermentation. After 7 days of fermentation, the fat contents of the experimental samples stood at 3.95, 4.45, 5.91 and 6.81 for TA, TB, TC and TC, respectively. It is possible that some equilibrium mechanism was set up which controlled evolution, utilization or retention of fat within the system. However, the values within each method of inoculation did not vary with period ($p < 0.05$).

The residual crude fibre content of *A. niger* inoculated samples was high at 11 days of fermentation, and low at 19 days. It was high at 15 days of fermentation among spontaneous inoculated and batch inoculated samples. The lowest value for spontaneously inoculated samples was recorded at 7 days of fermentation, and at 19 days for batch inoculated samples. Treatment A gave low values throughout the period of experiment. This indicates that *Aspergillus niger* was able to utilize or breakdown the crude fibre of test material. This is expected, considering the fact that *A. niger* elaborate mannanase and other cellulolytic enzymes (Abdeshahian *et al*, 2010: Keing & Omar, 2004). Sukaryana *et al.* (2010) reported a decrease in crude fibre content of 22.53 %.

Table 4.1.4d shows the interactions between inoculation methods and period of fermentation. Significant interaction effects were found for NFE values on spontaneously inoculated and batch inoculated samples ($p < 0.01$) over the *A. niger* inoculated as well as on the control samples. The NFE values for spontaneously inoculated samples at 15 and 19 days of fermentation were significantly ($p < 0.01$) higher than those at 7 and 11 days of fermentation period. On the other hand, the value for batch inoculated samples at 7 days of fermentation (50.93) was higher ($p < 0.05$)

Table 4.1.4.d: Interactions between inoculation methods and period of fermentation on calculated NFE and ME of fermented materials

Parameters	Period (days)	Inoculation method				SEM
		A	B	C	D	
NFE (%)	7	55.33^{ab}	53.79^{ab}	50.93^{a}	56.27^{ab}	1.904
	11	54.28^{ab}	54.38^{b}	46.93^{a}	57.07^{ab}	
	15	54.37^{a}	47.22^{c}	45.31^{bc}	56.32^{a}	
	19	54.76^{a}	48.18^{c}	46.59^{bc}	56.31^{a}	
ME (Kcal/Kg)	7	2797.09^{a}	2813.65^{a}	2852.77^{a}	2917.79^{a}	63.581
	11	2773.14^{a}	2845.10^{a}	2724.56^{b}	2869.69^{a}	
	15	2802.63^{a}	2631.30^{b}	2682.11^{b}	2865.40^{a}	
	19	2827.68^{a}	2689.22^{b}	2729.19^{b}	2862.61^{a}	

$^{a, b, c, d}$, means in the same row or column with different superscript are significantly different. A – *Aspergillus niger;* B - spontaneous, C – Batch and D – Nil (Control)

better than those at 11 days (46.93), 15 days (45.34) and 19 days (46.58) of fermentation period. No significant differences ($p < 0.05$) were found for the metabolizable energy of all the samples after 7 days of fermentation. Batch inoculated samples had lower ME values at 11 days of fermentation while both spontaneous and batch inoculated samples had lower ($p < 0.05$) values at 15 and 19 days of fermentation. The non significant drop in ME values of *A. niger* inoculated samples must have been due to the ability of the fungi to elaborate mannanase and other non starch polysaccharidases (de Vries, 2001, Daud *et al*., 1997). This must have increased the soluble carbohydrate fractions of the substrate fermented by *A. niger*). Inoculation x period of fermentation did not significantly ($p > 0.05$) affect ash content of the samples. Synthesis of biomass protein also requires energy while Raimbault (1999) reported that, evolution of metabolic heat is characteristic of solid state fermentation processes.

This could lead to drop in ME values except a concomitant increase is made in ether extract and digestible carbohydrate fractions of the fermented mass. The results of this work are in line with those of Manilal *et al.* (1987), who postulated that fermentation by microbial inoculation is probably the most plausible way by which bulky wastes could be transformed into utilizable feed ingredient for livestock. Microbial fermentation has played a significant role in the nutritional enhancement of the underexploited agro-industrial by-products, generated through the harvesting and processing of cassava root (Soccol, *et al*., 1994). The spontaneous inoculation technique is therefore judged suitable for improving the feed value of CRP-PKC blend for 7 days and is cost effective. Extending the period of fermentation to 19 days neither improves nor leads to any deterioration of product quality.

4.1.3 Fungal counts after fermentation

The fungal colonies identified in each culture plate and their respective counts are listed in Tables 4.1.5a, 4.1.5b and 4.1.5c. No fungal colonies were found among the samples in treatment D and so it is not likely that any fermentation took place among them. All samples in Treatment A had monoclonal cultures of *A. niger* whereas, those of Treatments B and C had mixed cultures of *A. niger, A. oryzae, Mucor spp. and Rhizopus spp*. The effects of inoculation method and the period of fermentation on the total fungal counts of fermented samples are shown in Tables 4.1.6a and 4.1.6b, respectively. Total fungal counts varied significantly ($p < 0.001$) with the method of inoculation whereas no significant effect of period of fermentation was found ($p > 0.05$). *A. niger* inoculated samples had significantly ($p < 0.001$) lower fungal counts (6.89 cfu) than the spontaneous and batch inoculated samples (12.00 cfu and 11.56 cfu respectively).

Table 4.1.6c shows the interactions between inoculation method and period of fermentation. *A. niger* inoculated samples had significantly ($p < 0.001$) lower counts than the spontaneously and batch inoculated samples. Table 4.1.7a shows the mean counts per plate of the different fungal species isolated in Treatments B and C. The results showed that the method of inoculation was highly significant ($p < 0.001$) effect on the population of the different distribution of fungal species after fermentation. Among the spontaneously inoculated samples, *A. oryzae* was significantly ($p < 0.001$) higher on mean count per plate (5.00 cfu) than the *A. niger*, *Rhizopus* and the *Mucor* species. No differences were found among the *A. niger*, *Rhizopus* and *Mucor spp*

counts within the treatment.

Table 4.1.5a: Fungi isolates after 6 days of fermentation

Sample	Dilution Factor	Colony code	Isolates identified	Counts
A1	6×10^5		*Aspergillus niger*	6
A2	5×10^5		*Aspergillus niger*	7
A3	5×10^5		*Aspergillus niger*	6
B1	9×10^5	B_{1b}	*Aspergillus niger*	2
		B_{1c}	*Aspergillus oryzae*	7
		B_{1d}	*Mucor spp.*	2
B2	8×10^5	B_{2a}	*Rhizopus spp.*	2
		B_{2b}	*Aspergillus niger*	1
		B_{2c}	*Aspergillus oryzae*	3
		B_{2d}	*Mucor spp.*	2
B3	9×10^5	B_{3a}	*Rhizopus spp.*	2
		B_{3b}	*Aspergillus niger*	1
		B_{3c}	*Aspergillus oryzae*	6
		B_{3d}	*Mucor spp.*	2
C1	5×10^5	$C1^a$	*Aspergillus niger*	7
		$C1^b$	*Aspergillus oryzae*	1
C2	5×10^5	$C2^a$	*Aspergillus niger*	9
C3	$5 \times 10_5$	$C3^a$	*Aspergillus niger*	9
	5×10^5	$C3^b$	*Aspergillus oryzae*	2
	5×10^5	$C3^c$	*Mucor spp.*	1
D1			*Nil*	
D2			*Nil*	
D3			*Nil*	

Note: No fungal colonies were found in treatment D (control)

Table 4.1.5b: Fungi isolates after 14 days of fermentation

Sample	Dilution Factor	Colony code	Isolates identified	Counts
A4	5×10^5		*Aspergillus niger*	7
A5	7×10^5		*Aspergillus niger*	8
A6	6×10^5		*Aspergillus niger*	6
B4	7×10^5	B_{4a}	*Rhizopus spp.*	6
		B_{4b}	*Aspergillus niger*	3
		B_{4c}	*Aspergillus oryzae*	4
		B_{4d}	*Mucor spp.*	3
B5	8×10^5	B_{5a}	*Rhizopus spp.*	4
		B_{5b}	*Aspergillus niger*	3
		B_{5c}	*Aspergillus oryzae*	1
		B_{5d}	*Mucor spp.*	2
B6	8×10^5	B_{6a}	*Rhizopus spp.*	5
		B_{6b}	*Aspergillus niger*	3
		B_{6c}	*Aspergillus oryzae*	2
		B_{6d}	*Mucor spp.*	2
C4	5×10^5	C_{4a}	*Aspergillus niger*	10
	5×10^5	C_{4b}	*Aspergillus oryzae*	1
	5×10^5	C_{4c}	*Rhizopus spp.*	2
C5	5×10^5	C_{5a}	*Aspergillus niger*	9
C6	5×10^5	C_{6a}	*Aspergillus niger*	8
			Aspergillus oryzae	3
	5×10^5	C_{6b}	*Rhizopus spp.*	2
D1			*Nil*	
D2			*Nil*	
D3			*Nil*	

Note: No fungal colonies were found in treatment D (control)

Table 4.1.5c: Fungi isolates after 19 days of fermentation

Sample	Dilution Factor	Colony code	Isolates identified	Counts
A7	6×10^5		*Aspergillus niger*	7
A8	7×10^5		*Aspergillus niger*	7
A9	7×10^5		*Aspergillus niger*	8
B7	9.5×10^5	B_{7b}	*Aspergillus niger*	3
		B_{7c}	*Aspergillus oryzae*	4
		B_{7d}	*Mucor spp.*	2
B8	8.5×10^5	B_{8a}	*Rhizopus spp.*	2
		B_{8b}	*Aspergillus niger*	2
		B_{8c}	*Aspergillus oryzae*	10
		B_{8d}	*Mucor spp.*	2
B9	9×10^5	B_{9a}	*Rhizopus spp.*	2
		B_{9b}	*Aspergillus niger*	2
		B_{9c}	*Aspergillus oryzae*	8
		B_{9d}	*Mucor spp.*	2
C7	6×10^5	$C7^a$	*Aspergillus niger*	5
		$C7^b$	*Aspergillus oryzae*	5
		$C7^c$	*Mucor spp.*	2
C8	6×10^5	$C8^a$	*Aspergillus niger*	4
		$C8^b$	*Aspergillus oryzae*	6
		$C8^c$	*Mucor spp.*	1
C9	6×10^5	$C9^a$	*Rhizopus spp.*	2
		$C9^b$	*Aspergillus niger*	4
		$C9^c$	*Aspergillus oryzae*	7
		C9d	*Mucor spp.*	2
D1			*Nil*	
D2			*Nil*	
D3			*Nil*	

Note: No fungal colonies were found in treatment D (control)

Table 4.1.6a: Effect of method of inoculation on total fungal counts of fermented materials

Parameter	Inoculation method			SEM	p-value
	A. niger	Spontaneous	batch		
Total fungal counts (cfu)	6.89[a]	12.00[b]	11.56[b]	1.0344	0.001

Table 4.1.6b: Effect of period of fermentation on the total fungal counts of fermented samples

Parameter	Period of fermentation				SEM	P - value
	0 (control)	7	15	19		
Total fungal counts (cfu)	Nil	8.78	10.67	11.00	1.0344	0.05

Table 4.1.6c: Interactions between inoculation methods and period of fermentation on the total fungal counts

Period of fermentation (days)	Inoculation method			SEM	P-value
	A	B	C		
17	6.33[a]	10.33[b]	9.67[b]	1.034	0.001
15	7.00[a]	12.67[b]	12.33[b]		
19	7.33[a]	13.00[b]	12.67[b]		

Among the batch inoculated samples, the mean count of *A. niger* was very significantly higher ($p < 0.001$) than *A. oryzae*, which in turn was higher than those of the *Rhizopus* and the *Mucor* species. No differences were found between the counts of *Rhizopus* and *Mucor* species. This suggests that, the characteristics of spontaneously inoculated samples were largely influenced by the *A. oryzae,* while those of batch inoculated samples were influenced more by *A. niger*.

The relationship between period of fermentation and the distribution of the different fungal species in the fermented samples are shown in Table 4.1.7. Among the spontaneously inoculated samples, the population of the *A. oryzae* was higher than other fungi at 7 and 19 days of fermentation. At 15 days of fermentation, the population of *Rhizopus spp* was higher ($p < 0.001$) than that of *A. oryzae*. The later was not significantly ($p > 0.05$) higher than those of *Rhizopus* and *Mucor* species. Among the batch inoculated samples, colonies of *A. niger* were significantly higher ($p < 0.001$) in number at 7 days (8.33 cfu) and 15 days (9.00 cfu) than the *A. oryzae* (1.00 cfu and 1.33 cfu respectively). However, among samples fermented for 19 days, the *A. oryzae* had significant ($p < 0.001$) higher count (6.00 cfu). Interestingly, the sum of *A. niger* and *A. oryzae* counts at 15 and 19 days of fermentation was constant (10.00 cfu), which suggests that, the population of *A. niger* may be inversely related to that of *A. oryzae* in such as manner that *A. niger* was linearly giving way for *A. oryzae* as period of fermentation increases.

Table 4.1.7: Effect of period of fermentation on the distribution of fungi species

Inoculation method	Period of fermentation	Mean fungal counts (cfu)				SEM	Level
		A. niger	*A. oryzae*	*Rhizopus*	*Mucor*		
A. niger	7	6.67				0.608	Ns
	15	7.00					
	19	7.33					
Spontaneous	7	1.33^{a}	5.33^{b}	1.33^{a}	2.00^{a}	0.536	0.001
	15	3.00^{a}	2.33^{a}	5.00^{b}	2.33^{a}		
	19	2.33^{a}	7.33^{b}	1.33^{a}	2.00^{a}		
Batch	7	8.33^{a}	1.00^{b}	nil	0.33^{bc}	0.379	0.001
	15	9.00^{a}	1.33^{b}	1.33^{bc}	nil		
	19	4.33^{b}	6.00^{a}	0.67^{c}	1.67^{c}		

^{a,b,c} Means within a row with different superscripts are significantly different ($p<0.001$)

It is possible that some synergistic interactions between the fungi species, the substrate and their metabolic products took place during the fermentation. The initial condition of the substrate in this experiment may have favoured the growth of *Aspergillus oryzae* more than the other fungi species. By the second week, the accumulation of metabolic products, accompanied by changes in the pH, ionic potential, water activity and nutrient concentrations may have favoured the growth of *Rhizopus spp*. At 15^{th} day of fermentation where *Rhizopus spp.* dominated, the mean number of colonies of *A. niger* were similar to *A. oryzae*. The population of *Mucor spp*. however remained constant in most samples and across all the period of experiment. For the batch inoculated samples, (treatment C), the number of spores of *A. niger* may have been so high that spontaneously seeded spores of *A. oryzae* could not effectively compete until the growth of *A. niger* had reached its lag phase. It is possible that these fungi have different growth characteristics and hence more research is however needed to evaluate these hypotheses.

The zero fungal growth found among control samples (Treatment D) must have been due to very low moisture contents of their substrates. Filamentous fungi such as *A. niger, A. oryzae, Rhizopus spp. and Mucor spp.* implicated in this experiment require moisture for germination and growth (Madigan *et al*., 2003). Low moisture content reduce solubility of nutrients in substrates (Perez-Guerra *et al*., 2003), it is therefore logical that even if the samples of Treatment D had accumulated fungal spores after autoclaving, low water activity (a_w) of their substrates prevented them from germinating. Hence, it could be inferred that no fungal fermentation occurred in these samples. In most fermentation involving fungi, water is one of the end products and if

not evacuated would accumulate and increase the moisture content of the end products with obvious implications for the fungi and the system. Higher levels of moisture usually give impact to the inner particles of the substrate, reduce porosity of the substrate which in turn reduce oxygen transportation to the fungal cells (Sandhya *et al*., 2005).

4.2 Study 2

Performance of broiler chicks fed diets containing sundried feremented mixture of cassava root pulp and palm kernel cake as replacement for maize

4.2.1 Performance parameters

4.2.1.1 Live Weight

The performances of the experimental chicks are shown in Table 4.2.1 below. The FEMCARRP used here has been described in section 3.2.9. There was a significant difference in the final live weight among the chicks fed the different diets. The chicks fed the control diet was significantly high ($p<0.05$) final live weight (2052.27g) when compared to those fed the diets containing FEMCARPP (1524.43) and CSM-PKC (1565.50).There were no significant differences ($p>0.05$) in live weight between birds fed diets B and C. This implies that, fermentation did not significantly improve the nutritional value of the test material (FEMCARPP) in this trial, which is not in line with the earlier findings of Chukwukaelo (2016).

4.2.1.2 Body weight gain.

In line with the final live weights, Treatments B and C recorded significantly ($P < 0.05$) lower values for body weight gain than as noted in Treatment A. The crude fibre levels were as well high for FEMCARPP (9.87%) and CSM-PKC mix (15.52) based diets. Ramin *et al.,* (2008) reported that treatment with microbes such as fungus and bacteria have shown positive effects on cellulose and fibre digestion utilization but the results of this work did not reflect such improvement. Neither the body weight nor the weight gain improved significantly among birds on FEMCARPP based diets over those on CSM-PKC mix based diets.

Table 4.2.1 Performance of broiler chickens fed FEMCARPP and CSM-PKC mix as replacements for maize

Parameters	Maize	FEMCARPP	CSM-PKC mix	SEM
Initial live weight (g)	77.72	80.73	77.23	2.02
Final live weight (g)	2052.27[a]	1524.43[b]	1565.50[b]	91.35
Weight gain (g)	1964.55[a]	1443.70[b]	1448.27[b]	89.79
Growth rate (g/day)	46.78[a]	34.38[b]	35.44[b]	2.14
Feed intake (g/day)	111.30[a]	98.97[b]	105.78[ab]	2.24
Feed conversion ratio	2.38[a]	2.89[b]	2.99[b]	0.11
Protein efficiency ratio (%)	46.04[c]	58.91[b]	61.47[c]	2.41
Feed cost (₦/kg)	89.13	64.44	78.10	
Feed cost (₦/Kg wt gain)	454.23[a]	446.30[a]	539.33[b]	10.22
Mortality	0	0	0.6	-

[ab] means within the same row with different superscripts are significantly different ($p < 0.05$).

4.2.1.3 Feed Intake

The feed intake of the experimental birds differed significantly ($p<0.05$). The average daily feed intake of chicks fed FEMCARPP (98.97 g) was not different ($p > 0.05$) to birds fed CSM-PKC mix (105.78g), but significantly lower ($p < 0.05$) than those fed maize based diets (111.3 g). The lower feed intake recorded on chicks fed FEMCARPP based rations may have resulted from palatability problems of their feed. Off flavours may have developed during fermentation and drying period which impacted negatively on feed intake.

4.2.1.4 Feed Conversion Ratio

Chicks fed maize based diets had significantly ($P < 0.05$) superior feed conversion ratio (2.38) to those fed FEMCARPP (2.89) and CSM-PKC mix (2.99) respectively. The superior feed conversion ratio among chicks fed the control diet may be related to the lower fibre contents of the rations. This suggests that chicks on Treatment B were better able to utilize the fibre in their diets. Ramin *et al.,* (2008) had reported that treatment of fibrous feed materials with microbes such as fungus and bacteria have positive effects on cellulose and fibre digestion. This further suggests that the lower weight gain among chicks in Treatment B must have been due to poor feed intake due to poor palatability of the feed.

4.2.1.5 Protein Efficiency Ratio (PER)

Chicks placed on the control diet were significantly efficient ($p < 0.05$) in utilizing the proteins in their diet (46.04 %) than those fed FEMCARPP based diet (58.91 %) which in turn was superior to those fed CSM-PKC diet (61.47 %). The poor efficiency of

FEMCARPP must be due to low metabolizable energy of the diets. Adequate protein:energy ratio is needed for efficient utilization of proteins (NRC, 1994). On this basis as well the chicks fed CSM-PKC based ration is expected to have yielded superior PER values, but this was not the case here. The possible explanation is that the protein in FEMCARPP based rations must have had superior quality when compared to those in CSM-PKC based diets, which resulted in superior PER and similar FCR values. It can therefore be concluded that, the fungal solid state fermentation improved protein quality even though energy values were reduced.

4.2.1.6 Feed Cost per Kg Weight Gain

The mean cost per kg weight gain of chicks fed the control diet (N 454.23/kg) was higher than those of FEMCARPP diet (N 446.30/kg), which was lower than that of CSM-PKC diets (539.33/kg). This implies that, it is economically viable to feed FEMCARPP based ration to broilers due to its cost. FEMCARPP products would be a viable alternative if maize and other cereals are needed to be replaced in broiler rations.

4.2.2 Carcass Characteristics

The results of the carcass analysis of the broiler chickens used in this experiment are shown in Table 4.2.2. There were no significant differences ($p > 0.05$) for the dressing percentage and the entire cut-up carcasses across the treatment groups, except for the back, head and neck. The chicks on FEMCARPP and CSM-PKC mix had significantly higher percentage of head (2.45 and 2.33) when compared to chicks fed the Control (2.14). This is in agreement with the finding of Okeudo *et al*. (2004) who reported that birds fed high proportions of PKC had higher proportion of head in the carcass.

Table 4.2.2 Carcass characteristics of broilers fed FEMCARPP and CSM-PKC mix as replacements for maize

Parameter (% live weight)	A	B	C	SEM
Live weight (g)	2650.37[a]	1905.12[b]	2086.10[b]	185.48
Dressing percentage	69.99	71.12	68.20	2.699
Breast muscle	37.37	26.76	26.18	1.035
Drumstick	9.54	10.51	9.83	0.651
Thigh	27.69	28.55	26.77	0.707
Wings	3.75	4.14	4.05	0.157
Back	5.39[a]	5.31[a]	4.86[b]	0.141
Head	2.13[a]	2.45[b]	2.33[b]	0.059
Neck	3.55[a]	4.01[b]	3.46[a]	0.158
Shank	3.58	4.09	4.12	0.270
Abdominal fat pad	2.53[a]	0.27[b]	0.08[c]	0.349

a,b,c Means within a row with different superscripts are significantly different ($p < 0.05$)

The proportion of neck was higher among chicks fed FEMCARPP based rations. Chicks fed Control diet had significantly ($p < 0.05$) higher proportion of back (5.39 versus 5.31 and 4.86) and abdominal fat (2.53 versus 0.27 and 0.08). The reason for the differences in proportion of neck and back is not very clear but the higher proportion of abdominal fat is expected. Abdominal fat is known to increase with live weight and metabolizable energy content of rations. This is an indication that the control diet provided nutrients in excess of the basic requirement of the chicks for growth and maintenance. The higher abdominal fat value for birds on FEMCARPP over those fed CSM-PKC mix implies that nutrients were better utilized despite the very low metabolizable energy values of the diets. The results generally indicate that, chicks placed on control diet had poor carcass characteristics over chicks the chicks fed FEMCARPP and CSM-PKC based diets which produced leaner carcasses.

Results of the internal organs weights of the experimental chicks are shown in Table 4.2.3. The proportion of viscera among chicks on Treatment C (13.74) were higher than those of Treatments A (9.46) and B (10.39). Though, there were no significant differences ($p < 0.05$) in actual values for length of GIT per bird, when the length is expressed as a proportion of the live weight of the chicks, those on Treatment C (CSM-PKC mix) had significant ($p < 0.05$) higher mean value (11.29) than those fed maize (7.74) and FEMCARPP (8.89) based diets. The weight of gizzards of chicks in Treatments B and C were significantly higher ($p < 0.05$) when compared to the control. Chicks on control diet were lower in percentage gizzard content than those fed diets B and C. Though, the size of the spleen among chicks fed CSM-PKC mix (0.15) were

significantly higher than those of control (0.10) and FEMCARPP (0.10), the heart and liver weights were similar across treatment groups. It can therefore be inferred that the

Table 4.2.3: Internal organ characteristics of broilers fed FEMCARPP and CSM-PKC mix as replacements for maize

Parameter	A	B	C	SEM
Total viscera	9.46[a]	10.38[a]	13.74[b]	0.818
Length of intestine (cm)	204.33	187.83	211.5	15.188
Length of intestine (per g body weight)	7.74[a]	8.89[a]	11.29[b]	0.881
Full gizzard weight	2.36[a]	3.17[b]	3.85[b]	0.362
Empty gizzard weight	1.65[a]	2.38[b]	2.56[b]	0.256
Liver	1.61	1.60	1.81	0.183
Spleen	0.10[a]	0.10[a]	0.15[b]	0.018
Heart	0.40	0.29	0.35	0.042

[a,b,c] Means within a row with different superscripts are significantly different ($p < 0.05$)

chicks were apparently healthy and were not clinically challenged by the inclusion of FEMCARPP in their diets.

4.2.3 Meat Quality

Results of some meat quality assessments are shown in Table 4.2.4. Meat from chicks fed maize based diets was significantly higher ($p < 0.05$) in drip loss (2.24) and cooking loss (29.86) but lower in water holding capacity (41.97) when compared to those fed FEMCARPP based diets (2.04, 21.61 and 48.03, respectively). The cooking loss and water holding capacity of chicks fed CSM-PKC mix based diet were similar to those fed FEMCARPP. Differences in water holding capacity may be due to differences in fat contents because moisture and fat contents are negatively related. The higher fat content in TA is expected to positively influence flavour, juiciness and tenderness whereas the higher water holding capacity would influence technological quality.

3.3.4 Haematology and biochemical indices

The result of the haematology of the experimental chicks is shown in Table 4.2.5. No significant differences ($p>0.05$) were found for most of the haematological parameters. All the parameters were within the normal range for chicks raised in similar environments (Udedibie *et al*., 2008; Enyenihi *et al*., 2009 and Okere, 2011), and these fall within the normal range as established by Mitruka and Rawnsley (1977) for broiler chickens. Chicks fed the control diet had significantly ($p < 0.05$) higher neutrophil and lower lymphocyte values when compared to birds on the other diets. Though, chicks fed control diet had significantly ($p<0.05$) lower values of percentage lymphocytes, the

values were within the normal range as reported by Enyenihi *et al*., (2013); Mitruka & Rawnsley (1977).

Table 4.2.4: Meat quality characteristics of broilers fed FEMCARP and CSM-PKC mix as replacements for maize

Parameter	Maize	FEMCARPP	CSM-PKC mix	SEM
Drip loss	2.24^{b}	2.04^{a}	2.75^{c}	0.082
Cooking loss	29.86^{a}	21.61^{b}	22.79^{b}	1.377
Water holding capacity	41.97^{a}	48.03^{b}	47.04^{b}	2.319

^{a,b,c} Means within a row with different superscripts are significantly different ($p < 0.05$)

Table 4.2.5: Haematology of broilers fed FEMCARPP or cassava meal and palm kernel mixture as replacements for maize

	diets containing			
Parameters	**Maize**	FEMCARP	CSM-PKC	SEM
Haemoglobin	9.97	10.25	10.05	0.553
Packed cell volume	34.08	35.10	34.37	1.828
RBC ($x10^6/\mu L$)	2.56	2.69	2.65	0.137
MCV (pg)	133.10	130.73	133.88	3.717
MCH	38.88	38.07	37.92	0.644
MCHC. (g/dl)	29.03	29.13	29.03	0.536
Platelet count ($x\ 10^3/\mu L$	25.67^a	19.83^{ab}	16.50^b	3.161
Total white blood cells ($X10^3/\mu L$)	84.25^a	84.08^a	75.17^b	1.651
*Lymphosites (%)	77.67^a	90.67^b	87.50^b	4.433
*Neutrophil (%)	22.33^a	9.33^b	12.50^b	4.433
Basophils	-	-	-	
Eosinophils	-	-	-	

a, b, c – means with different superscripts within a row are significantly different ($p < 0.05$)
RBC – total red cell count: PCV – packed cell volume: MCV – mean corpuscular volume: MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration,

Emenalom *et al.*, 2009 and Ogbuewu *et al.,* 2008) had showed that, the number of erythrocytes in animals for good health varies with species, age, sex, diet and clinical condition. The PCV, erythrocytes and haemoglobin are known to be positively correlated with quality and quantity of protein consumed. Brown & Clime (1972) reported that, decrease in red blood cell count is usually associated with low quality feeds and protein deficiency. The PCV is an indicator of blood dilution (Wilson and Brigstoke, 1981) while haemoglobin measure is the ability of an animal to withstand some level of respiratory stress (Sainsbury, 1983).

The serum chemistry and other biochemical indices of the experimental birds are shown in Table 4.2.6. Most of the serum biochemical components were not affected by the treatments ($P > 0.05$) except Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP). The chicks fed CSM-PKC mix had significantly lower ALT values (18.00 iu/L) when compared to the control (25.00 iu/L). The reason for this is not quite clear. In cinical cases, the most important causes of raised ALT activity are hepatocellular injury (Cheesbrough, 2000). Chicks fed the CSM-PKC based diets had significantly ($p < 0.05$) higher serum alkaline phosphatase (ALP) activity. The ALP consists of a group of heterogeneous isoenzymes that catalyze the hydrolysis of monophospate esters at alkaline pH (Syakalima *et al.*, 1998). Mclung *et al*. (1972) reported a positive correlation between serum alkaline phosphatase and fertility in laying hens. Neem leaf meal based diets has been reported to enhance ALP activities in rabbits and birds (Ogbuewu, 2008; Obikaonu *et al.*, 2011).

Table 4.2.6: Serum chemistry and biochemical indices of broilers fed FEMCARPP or cassava meal and palm kernel mixture as replacements for maize

Chemistry	A	B	C	SEM
Cl (m**mol/L)**	118.40	103.95	117.98	11.331
Na (mmol/L)	144.98	137.13	149.83	10.761
K (mmol/L)	5.40	4.55	4.60	0.530
HCO_3(mmol/L)	28.83	28.17	27.83	1.386
Urea (mg/dL)	10.40	11.83	12.23	0.885
Creatinine (mg/dL)	0.24	0.28	0.32	0.031
Total bilirubin (mg/dL)	0.11	0.05	0.08	0.100
Conjugated bilirubin (mg/dL)	0.05	0.03	0.04	0.009
ALT (iu/L)	25.00^a	23.33^a	18.00^b	2.277
AST (iu/L)	352.17	272.67	282.50	39.557
ALP (iu/L)	1750.50^a	1474.50^a	2572.33^b	283.858
Calcium (mg/dL)	11.15^a	9.80^b	10.05^b	0.261
Phosphate (mg/dl)	2.75	2.65	2.67	0.228
Glucose (mg/dL)	143.07	149.18	149.98	14.813
Uric acid (mg/dL)	2.80	2.30	2.63	0.225
Cholesterol (mg/dL)	92.52	95.87	104.88	7.200
Triglyceride (mg/dL)	20.42	21.05	23.30	2.683

a, b, c – means with different superscripts within a row are significantly different ($p < 0.05$)
A – Maize, B – FEMCARPP and C- cassava and palm kernel mixtures; ALT – Alanine aminotransferase; AST – Aspartate transferase; ALP – Alkaline phoaphatase,

4.3 Study 3

Effect of dietary inclusion of wet or dry fermented mixture of cassava root pulp and palm kernel cake on liveweight gains, carcass and meat quality of broiler finishers

4.3.1 Performance of Experimental Chicks

The performance of the chicks fed the experimental rations is shown in Table 4.3.1. The results showed that there were significant differences ($p < 0.05$) in final live weight, weight gain, feed intake and feed conversion ratio of the chicks. The live weight of the chicks on control diet (2272.2 g) was superior ($p < 0.05$) to those fed sun dried FEMCARPP (2177.8 g) and CSM-PKC mix (2146.3 g). These were in turn significantly higher than chicks on wet FEMCARPP diet. The average daily weight gain followed similar pattern observed for the live weight. The weekly live-weight of the chicks plotted against age is shown in Figure 4.1. The chart showed that the growth pattern was similar in all the treatment groups. Figure 4.2 shows the weekly average daily weight gain of experimental chicks plotted against age (4 to 7 weeks of age). The average daily weight gain increased in all treatments groups at 6 weeks of age, and took a declined curve at 7 weeks of age. Cumulative average daily weight gain had similar trend (Figure 4.3). These suggested that, weight gain was optimal at 6 weeks of age. The feed intake of chicks on the four treatment diets increased progressively with age (figure 4.4). There was no differences ($P > 0.05$) in feed intake among chicks on maize based (control diet), dried FEMCARPP and CSM-PKC throughout the experiment (Table 4.3.1). As from 5 weeks of age, chicks on wet FEMCARPP consumed less feed than other treatment groups.

Table 4.3.1: Growth performance of broilers finishers fed wet and dried FEMCARPP as replacements for maize

Parameters	Maize	FEMCARPP		CSM-PKC	SEM
		Wet	Dried		
Initial live weight @ 3 weeks (g)	825.9	819.2	818.8	820.4	3.112
Final live weight @ 7 weeks (g)	2272.2[a]	2005.6[c]	2177.8[b]	2146.3[b]	31.518
Weight gain (g)	1446.6	1186.4	1359.0	1325.9	7.213
Av. daily weight gain (g/day)	51.8[c]	42.4[a]	48.5[b]	47.4[b]	1.097
Av. daily feed intake (g/day)	121.92[b]	99.73[a]	121.71[b]	127.04[b]	3.229
Feed conversion ratio	2.35	2.36	2.51	2.68	0.1856
Feed cost (N/kg)	102.63	81.60	87.63	95.13	
Feed cost per kg weight gain (N)	311.43[b]	244.66[d]	277.19[c]	328.26[a]	5.85
Margin (N/kg meat)	0.00	66.77	34.24	- 16.81	
Mortality (%)	3.33	0.00	0.00	13.33	

[a,b,c] Means with different superscripts within a row are significantly different ($p < 0.05$)
CSM-PKC - sundried blend of Cassava meal and Palm Kernel Cake

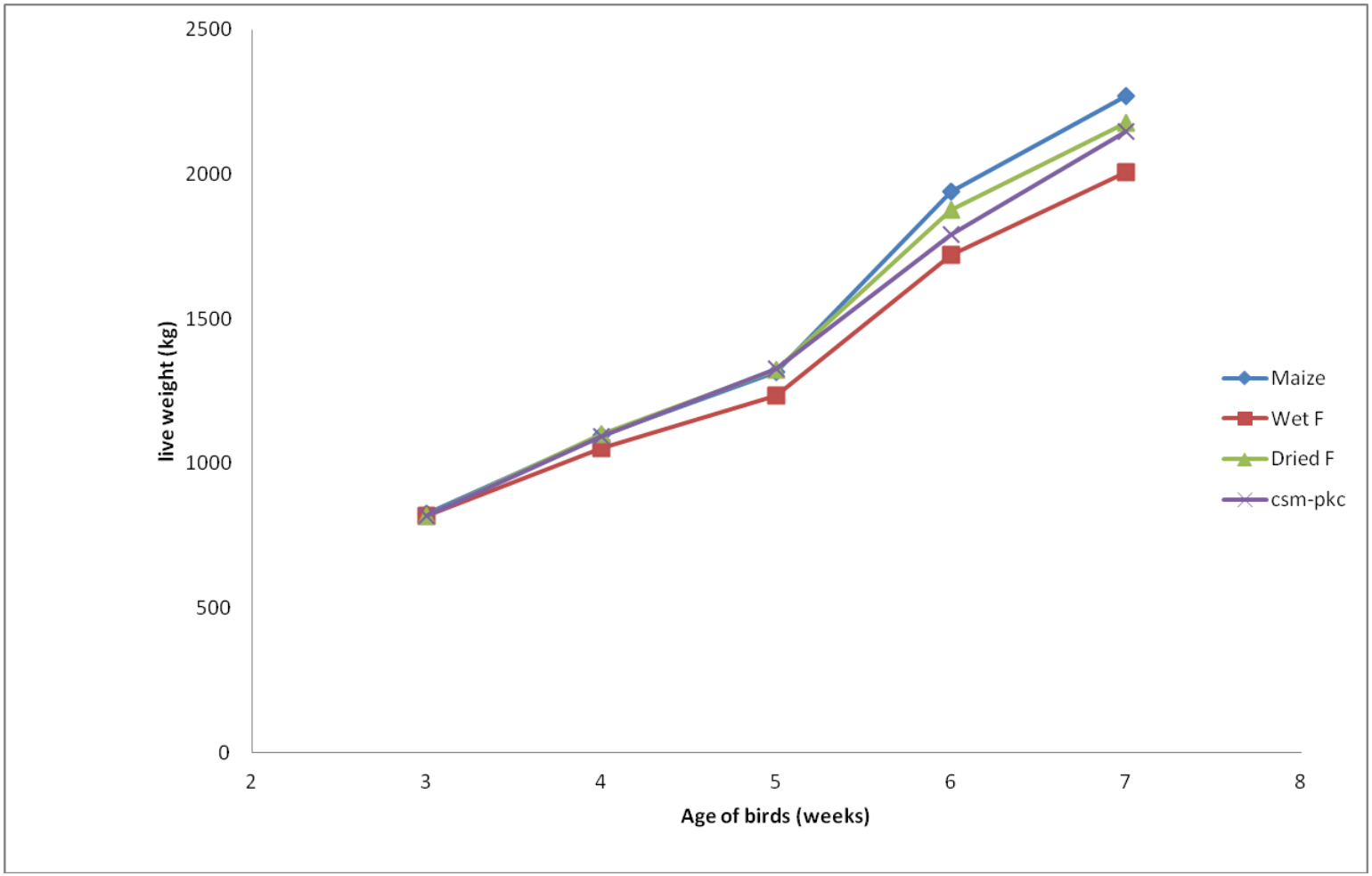

Figure 4.1: Graph of live weights of experimental broiler finishers plotted against age (weeks)

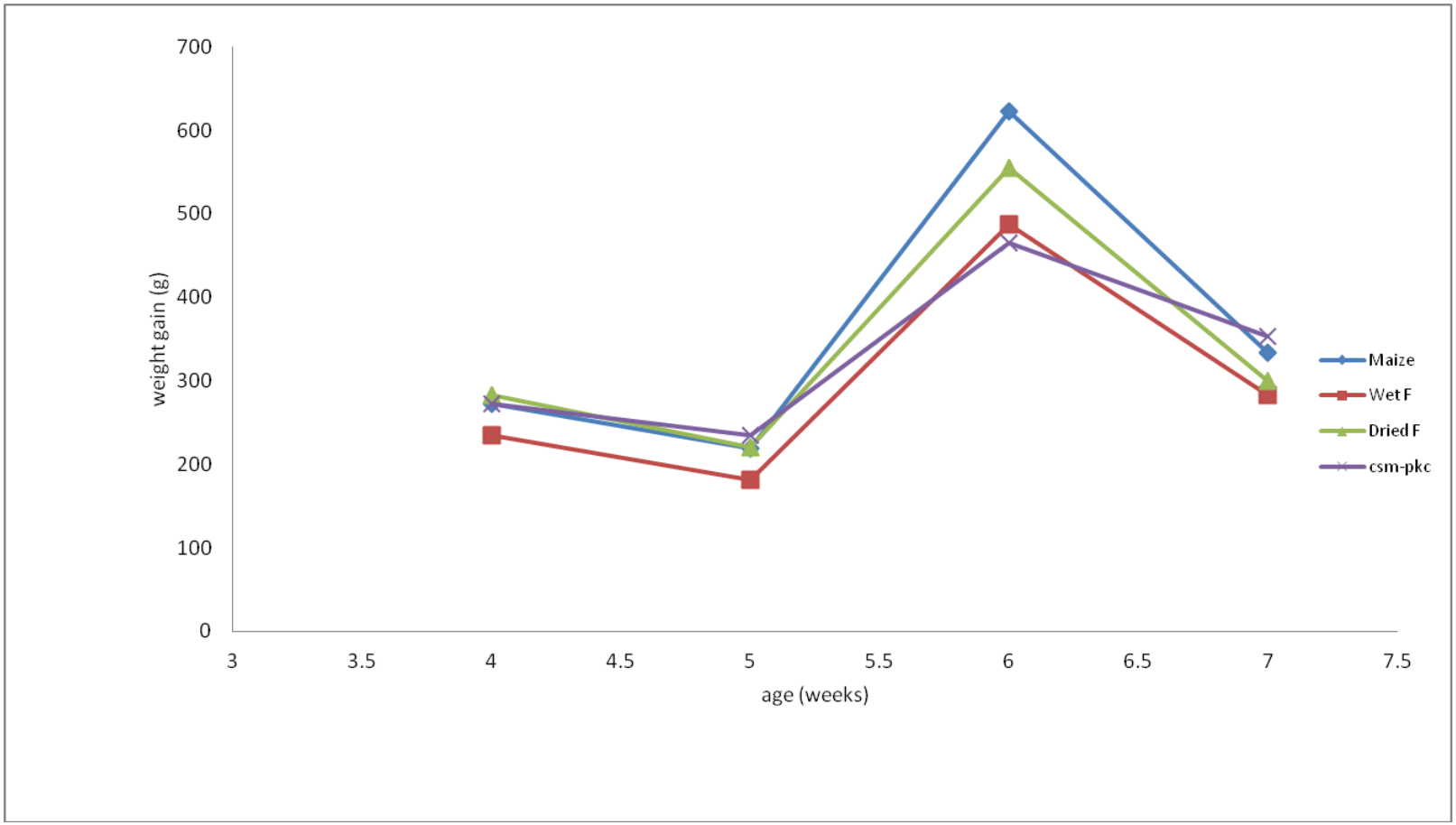

Figure 4.2: Graph of weight gain of broilers finishers fed experimental diets against age

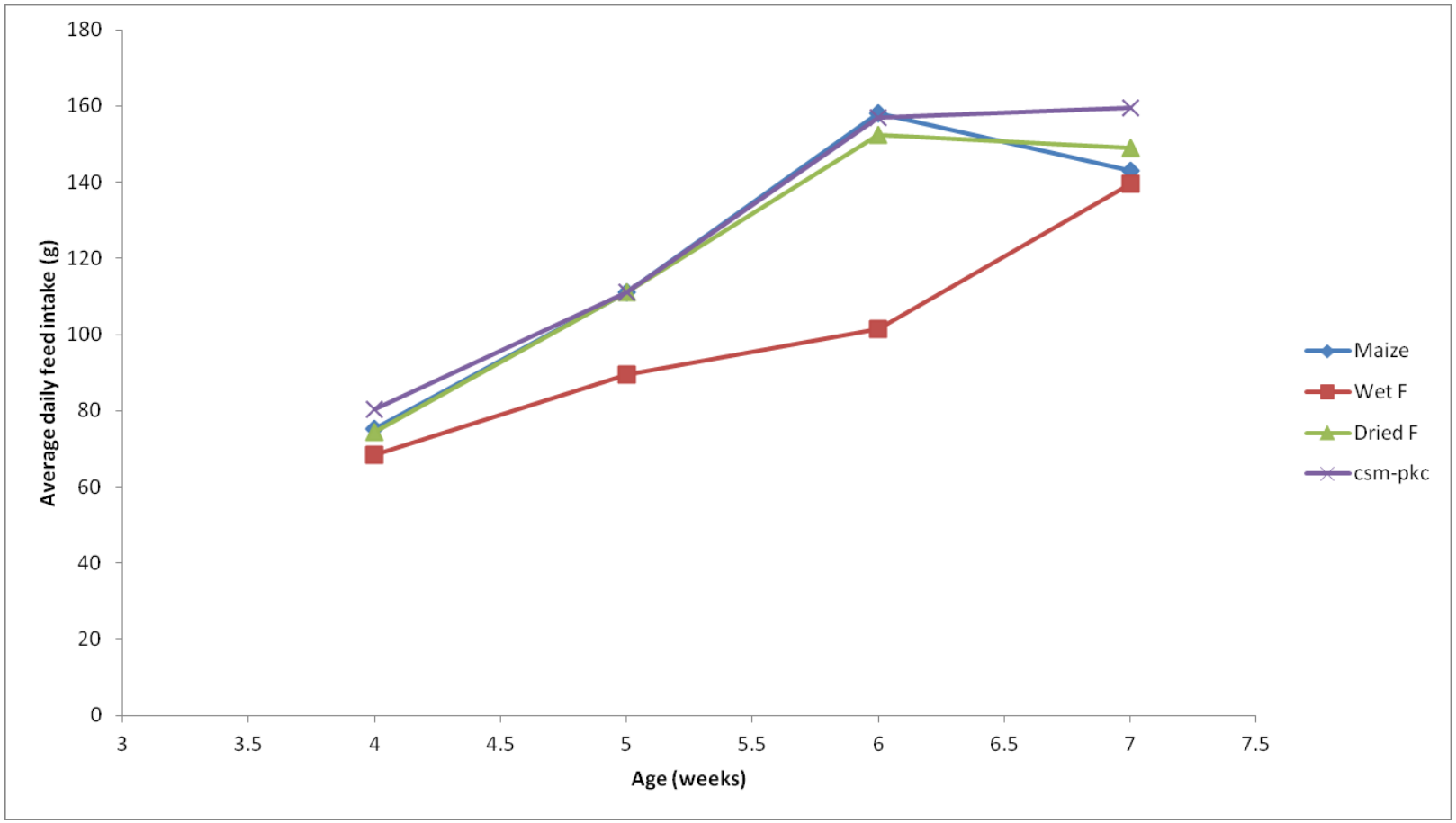

Figure 4.3: Graph of average daily weight gain of broiler finishers fed the experimental diets plotted against age

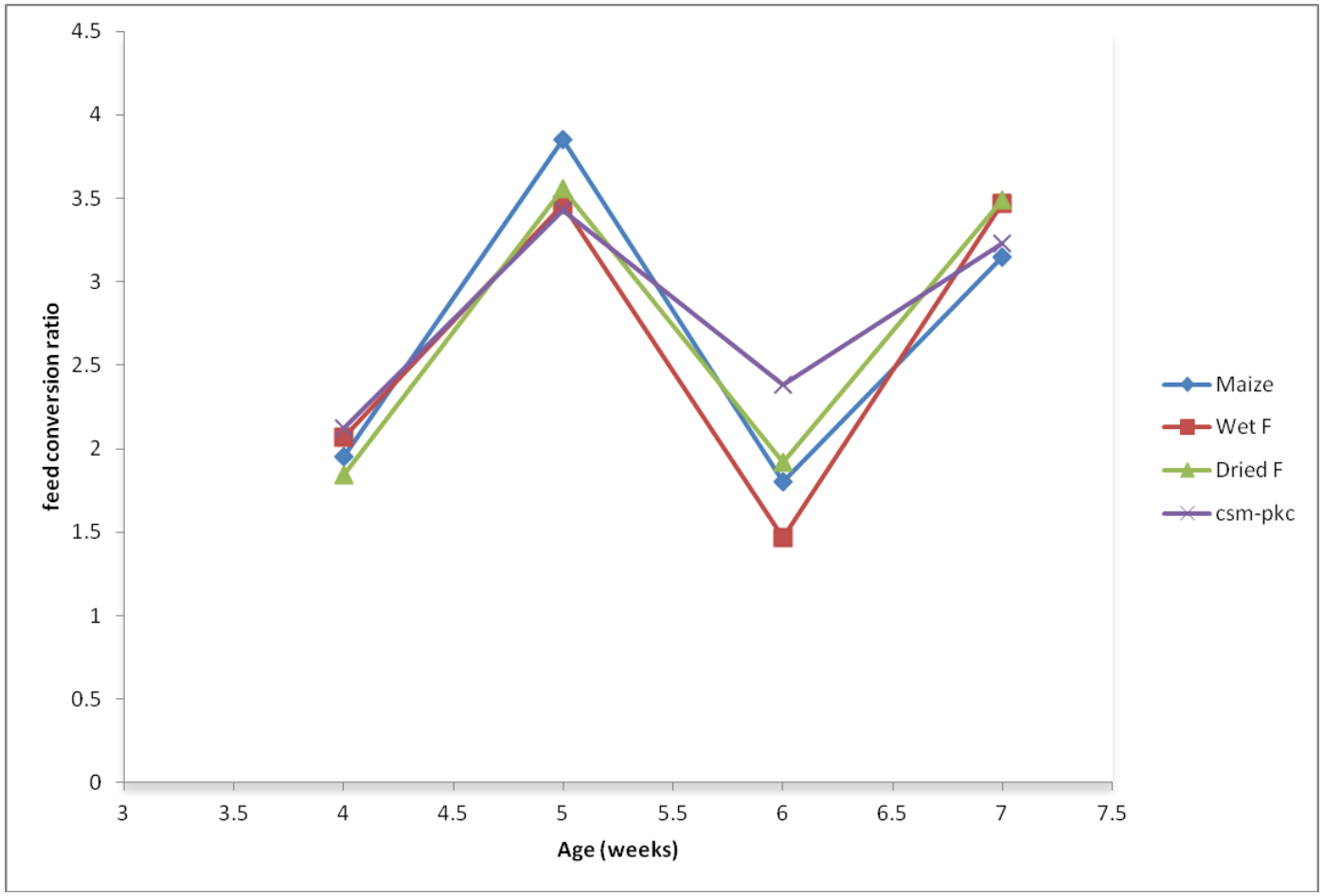

Figure 4.4: Gragp of feed conversion ratio of broiler finishers fed experimental diets

However, there was a significantly ($p < 0.05$) lower feed intake among chicks fed diets based on wet FEMCARPP. Two factors seem to have negatively affected feed intake among the chicks fed wet FEMCARPP; the first is sudden change from dry to moist feed, coupled with possible off flavours from the wet FEMCARPP. The other is physical characteristics such as volume, bulk density and specific gravity of FEMCARPP based ration. Figure 4.4 shows that among chicks on FEMCARPP, feed intake increased progressively until the end of the experiment whereas in the other treatments, it increased up to 6 weeks of age and then stabilized. This implies that physical nature of FERMCARPP based diets impacted negatively on dry matter and energy intake.

The feed conversion ratio revealed non significant ($p > 0.05$) relation across treatment groups. The results indicated that, the lower live weights of broilers on wet FEMCARPP were principally due to lower dry matter intake, owing to the physical (nutrient density) and flavour characteristics of the feed. It is therefore noted that, carcass and economic considerations are important factors to be considered in judging the performance of the experimental chicks. The economic assessment of the treatment diets showed that chicks fed wet FEMCARPP would make a better return for the farmer on investments. The margin per kg live weight gain at the prevailing market price was N 66.77 for the chicks fed wet FEMCARPP and N 34.24 for those fed sundried FEMCARPP. The replacement of maize with CSM-PKC mix increased the cost of feed and this led to a loss of N 16.81 per kg wet gained by the chicks. This meant that, when compared to the CSM-PKC based diet, feeding wet FEMCARPP will result to a good profit margin of N 83.60 per Kg weight.

4.3.2 Carcass and Internal Organ characteristics

The results of the carcass and internal organ characteristics of the experiment are shown in Table 4.3.2. The results showed that, there are no significant differences ($p > 0.05$) in all the parameters studied except for gizzard weight. This further supports the proposition that the lower live weight gain recorded by chickss fed wet FEMCARPP was principally due to lower dry matter intake, rather than poor nutritive value of the feed materials. High proportion of dietary fibre among the chicks fed diets containing FEMCARPP and the CSM-PKC mix may have been responsible for the the significantly ($p<0.05$) higher full gizzard weights compared to their counterparts fed the control diets. High dietary fibre have been reported by authors to increase gizzard weight (Udedibie *et al*., 2009; Okeudo *et al*., 2006)

4.3.3 Meat Quality Characteristics

No significant differences ($p < 0.05$) were recorded for water holding capacity, drip loss and cooking loss among the meats from the experimental chicks (Table 4.3.3). Meat from broilers fed wet FEMCARPP was lowest ($p < 0.05$) tenderness score (5.00 out 8) while those fed CSM-PKC mix had the highest tenderness score (6.00 out 8) among all treatment groups. Flavour score for chicks on CSM-PKC (5.67) was superior to other treatments while chicks on maize based diet had significantly ($p < 0.05$) the lowest score (4.0). These results are in line with the findings of Okeudo *et al*. (2005) who found that meat from broilers fed diets supplemented with high levels of palm kernel cake were highly flavoured and liked by consumers.

Table 4.3.2: Carcass characteristics (% live weight) of broiler finishers fed FEMCARPP as replacement for maize

Parameters	Maize	FEMCARPP		CSM-PKC	SEM
		Wet	Dried		
Live weight (kg)	2.25	2.23	2.21	2.23	0.051
Dressing percentage	65.48	67.75	66.58	64.11	1.605
Breast	22.97	29.39	30.74	26.78	1.611
Thigh	24.47	17.67	19.79	20.76	2.643
Drumstick	10.96	10.84	10.64	10.68	0.445
Back	6.17	6.29	6.32	6.20	0.308
Wings	4.06	4.05	3.91	3.93	0.190
Head	2.30	2.19	2.21	2.28	0.191
Neck	3.21	3.16	3.17	3.09	0.316
Shank	3.77	3.48	3.46	3.57	0.311
Gastrointestinal tract	4.72	4.88	5.33	4.98	0.527
Gizzard (full)	1.92[a]	2.85[b]	2.93[b]	2.69[b]	0.200
Gizzard (dressed)	1.73	1.74	2.00	1.86	0.217
Liver	1.83	1.57	1.57	1.58	0.256
Heart	0.55	0.34	0.44	0.40	0.076
Abdominal fat	2.25	1.86	1.79	1.83	0.281

[a,b,c..f] Means with different superscripts within a row are significantly different ($p < 0.05$) CSM-PKC - sundried blend of Cassava Root meal and Palm Kernel Cake

Table 4.3.3: Meat quality characteristics of broiler finisher birds fed FEMCARPP as replacement of maize

Parameters	Maize	FEMCARPP		CSM-PKC	SEM
		Wet	Dry		
Water holding capacity (g/100g)	41.25	44.37	46.63	31.93	2.837
Cooking loss (g/100g)	26.10	27.31	26.58	21.94	3.273
Drip loss (g/100g)	1.99	2.34	1.75	2.27	0.438
Organoleptic assessment					
a) Tenderness	5.38^{bc}	5.00^{c}	5.63^{ab}	6.00^{a}	0.263
b) Juiciness	3.75	4.5	4.63	4.87	0.569
c) Flavour intensity	4.00^{c}	4.88^{b}	5.25^{ab}	5.63^{a}	0.381
d) Connective tissue amount	5.13	4.63	6.25	6.63	1.061
e) Hedonic (degree of likeness)	4.57^{a}	4.75^{ab}	5.44^{ab}	5.78^{ab}	0.488

$^{a,b,c..f}$means with different superscripts within a row are significantly different ($p < 0.05$)

CSM-PKC - sundried blend of Cassava Root meal and Palm Kernel Cake

4.4 Study 4

Liveweight gains and haematology of weaner pigs diets containing fermented mixture of cassava root pulp and palm kernel cake

4.4.1 Performance Parameters

4.4.1.1 Live Weight

The performance of experimental weaner pigs fed diets containing maize; FEMCARPP and CSM-PKC mix are shown in table 4.4.1. The results show that there were significant differences ($p < 0.05$) in live weight, average daily gain, feed intake and feed conversion ratio. The live weights of pigs increased progressively with age irrespective of the treatment diets (Figure 4.5). At the end of the experiment, the live weights of weaner pigs fed diet (TB) in which 50 % of the maize was replaced by FEMCARPP (9.86 kg) was significantly higher ($p < 0.05$) than those of treatments TA, TC, TD and TE (8.56, 9.42, 7.92 and 8.03 respectively). The live weight of pigs on 100 % maize replacement level (9.42 kg) were significantly ($p < 0.05$) higher than those on control (maize base diet). Pigs on CSM-PKC mix (TD and TE) were significantly lower ($p<0.05$) than the control. This is in agreement with the findings of Fetuga *et a*l. (1977) and Yeong *et al*. (1981) which showed that; with proper balancing of dietary ingredients, monogastric animal could tolerate high levels of PKC and subsequently increase growth performance.

4.4.1.2 Weight Gain

The average daily weight gain of pigs on 50 % replacement level (TB) were superior ($p < 0.05$) than those on 100 % replacement level (TC). Pigs on the two treatments were significantly higher in daily weight gain ($p < 0.05$) than those on control diet (TA).

Table 4.4.1: Live weights of weaner pigs fed FEMCARPP or mixture of PKC and cassava root meal as Replacement for Maize.

Parameter	MAIZE	FEMCARPP		CSM-PKC mix		SEM
	TA	TB (50 %)	TC (100 %)	TD (50 %)	TE (100 %)	
Live Weight (Kg)	4.66	4.80	4.72	4.66	4.91	0.154
Final live weight (kg)	8.57[c]	9.86[a]	9.42[b]	7.92[e]	8.03[d]	0.133
Average daily weight gain (g)	79.80[c]	103.27[a]	95.92[b]	66.53[d]	63.67[d]	4.219
Average daily feed intake	246.33	276.33	222.45	294.49	283.06	
Feed conversion ratio	3.09	2.68	2.32	4.43	4.45	

[a, b, c,] and [d] -values with different superscript are significantly different

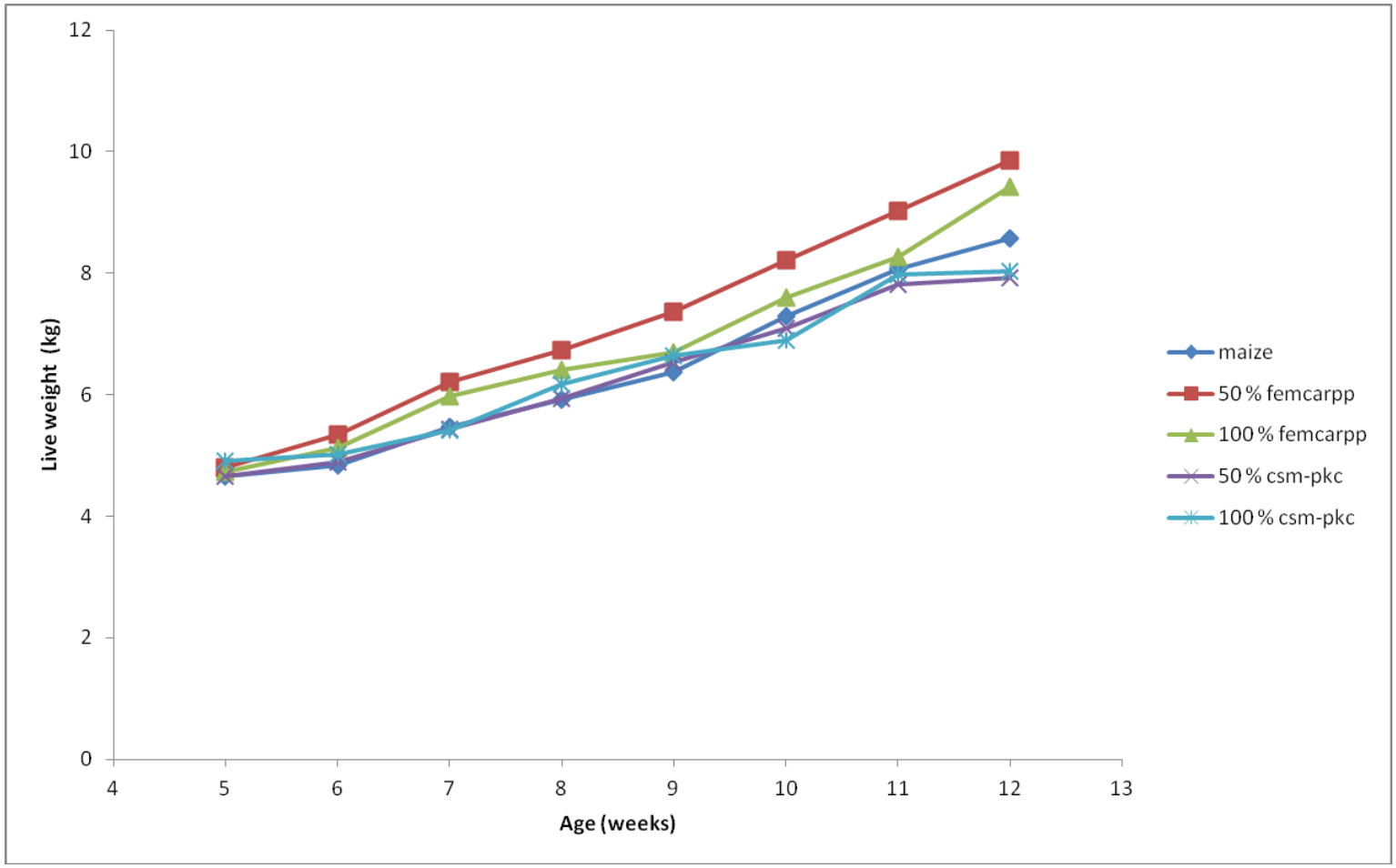

Figure 4.5: Graph of live weight of weaner pigs fed experimental diets against age

The average daily weight gains of pigs on CSM-PKC based diets were inferior to those on control and FEMCARPP based diets. This superior performance of pigs on FEMCARPP implies that, the animals were better able to utilize nutrients in FEMCARPP based rations irrespective of the apparently lower metabolizable energy content of these rations. This improved performance must have resulted from both reduction in antinutritional factors and from enhanced physicochemical characteristics of cassava and palm kernel cake. Both factors would lead to increased digestibility and nutrient uptake from FEMCARPP based rations. Figure 4.6 shows the average weekly weight gain of weaner pigs fed the experimental rations. It shows that, the weight gains of weaner pigs fed FEMCARPP (50 and 100 %) were consistently higher than those on control and CSM-PKC based diets.

Three reasons could be proffered for this. One is the higher crude protein values of the rations which may be of superior quality, and next is increased nutrient availability from the FEMCARPP due to increased digestibility of the non-starch polysaccharide fractions, and possible reduction of anti-nutritive factors by fermentation. Svanberg & Lorri (1997) had reported that solid state fermentation (SSF) had potentials to increase nutrient digestibility by solubilisation of non starch polysaccharides and to reduce anti nutritive effects. The third is a possible probiotic effect of the fermentative microbes implicited in the fermentation process. Fermented diets have been reported to reduce scouring in animals and diarrhoea in small children (Mensa, 1997; Adams & Nicolaides, 1997).

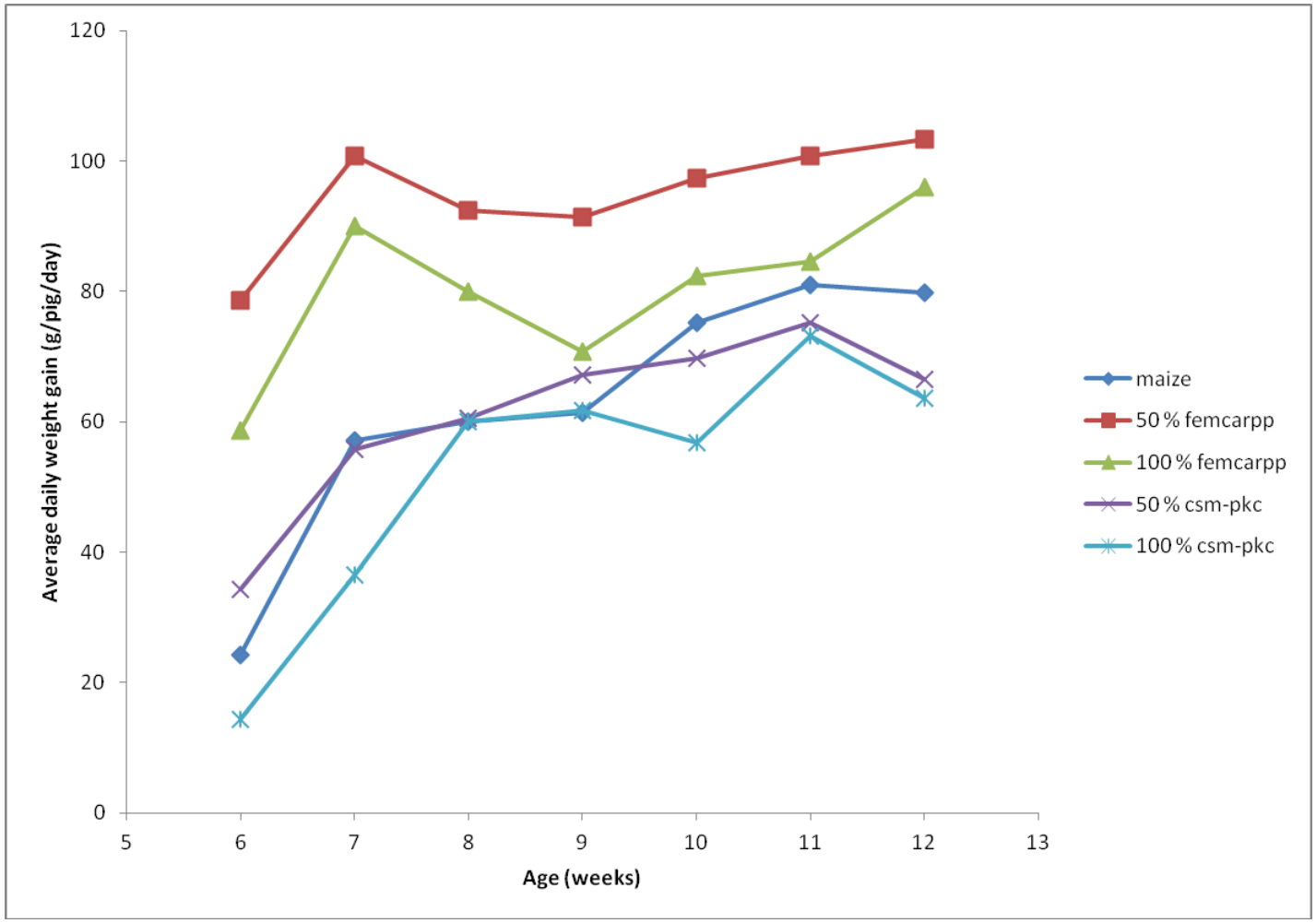
120
100
80
60
40
20
0
Average daily weight gain (g/pig/day)
5
6
7
8
9
10
11
12
13
Age (weeks)
maize
50 % femcarpp
100 % femcarpp
50 % csm-pkc
100 % csm-pkc

Figure 4.6: Average daily weight of pigs fed experimental rations with age

4.4.1.3 Feed Intake

Feed intake was significantly higher ($p < 0.05$) among pigs on the CSM-PKC based diet (283.06 g) over the control (246.33 g) which in turn was higher than those fed FEMCARPP (222.45 g) based diets. Since the diets containing maize and CSM-PKC were moistened similar to those found in FEMCARPP before feeding, feed intake must have been influenced by physicochemical characteristics intrinsic to the fermentation process. Pigs on 50 % replacement level consumed significantly ($P < 0.05$) more feed than those on maize replaced FEMCARPP. This means increasing levels of FEMCARPP suppressed feed intake. The graph of average feed intake against age (Figure 4.7) showed that pigs on FEMCARPP (50 and 100 %) consistently consumed lower amount of feed than the control and CSM-PKC based rations.

4.4.1.4 Feed Utilization

The feed conversion ratio of pigs on control diet (3.09) was similar ($p > 0.05$) to those fed FEMCARPP based diets (2.68 and 2.32), which were superior to those fed the CSM-PKC based diets ($p < 0.05$). The weekly feed conversion ratio of the experimental pigs is shown in Figure 4.8. The superior FCR values shown by pigs on FEMCARPP during the first week of the experiment suggested that, they adapted faster to the rations containing the fermented product (FEMCARPP). This improvement may have come from enhanced fibre digestion among pig fed diets containing FEMCARPP. Ramin *et al*. (2008) have reported that, treatment with microbes such as fungus and bacteria have positive effects on digestion of fibre. It is possible that the process of fermentation or the organism implicated conferred a probiotic effect on the FEMCARPP.

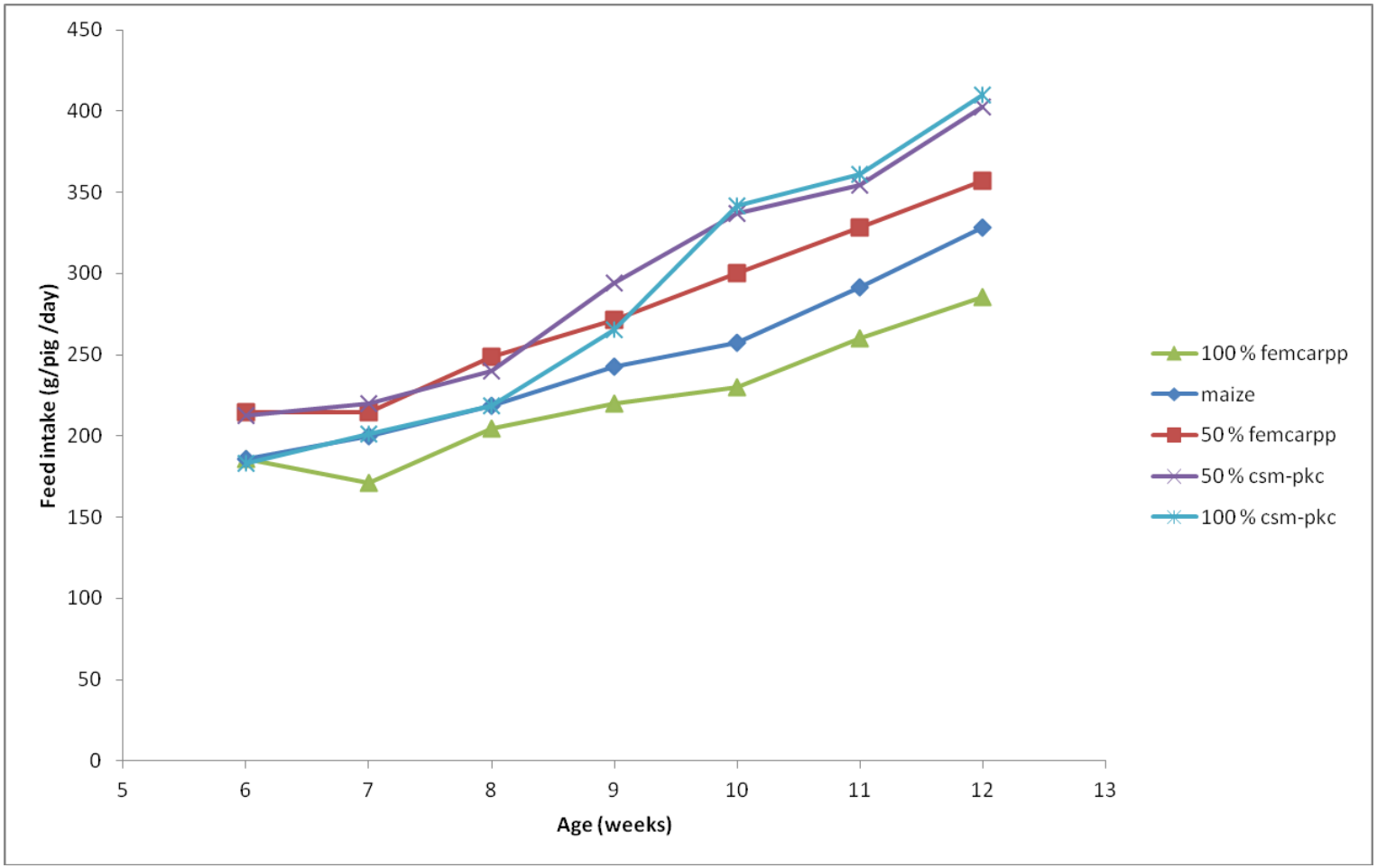

Figure 4.7: Graph of average daily feed intake of weaner pigs fed experimental rations plotted against age

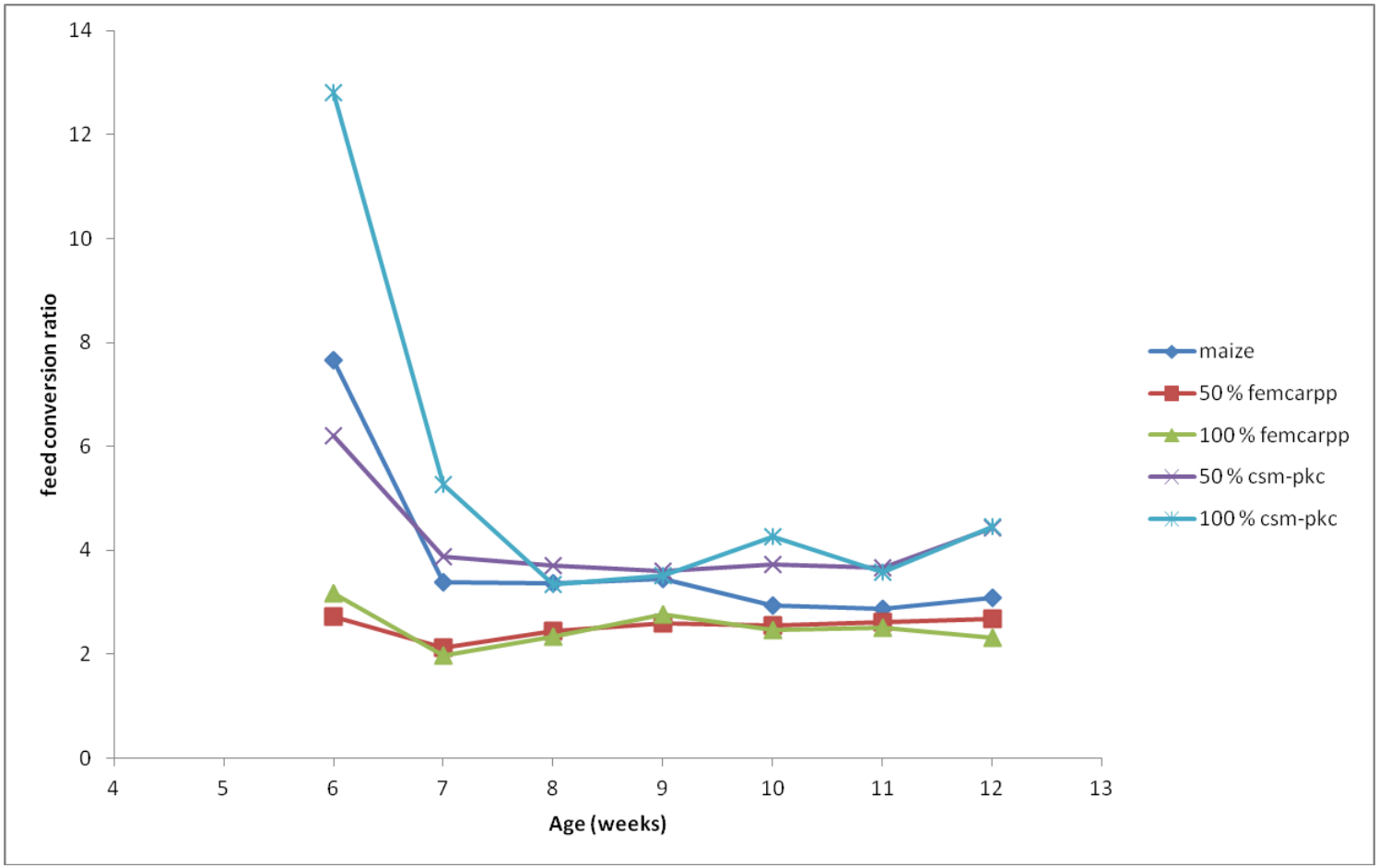
14
12
10
8
6
4
2
0
feed conversion ratio
4
5
6
7
8
9
10
11
12
13
Age (weeks)
maize
50 % femcarpp
100 % femcarpp
50 % csm-pkc
100 % csm-pkc

Figure 4.8: Graph of feed conversion ratio of weaner pigs fed experimental diets plotted against age

4.4.2 Haematology

The results of the haematological evaluation are shown in Table 4.4.2. No significant differences were found for all the haematological parameters evaluated in this study. The findings of the present study are in line with those of Akintunde *et al.* (2001) who fed growing pigs palm kernel based diets supplemented with a commercial enzyme Allzyme$^{(R)}$. In a related study in which palm kernel cake was supplemented with cassava flour waste, no significant differences were also found for total serum protein and glucose levels whereas urea nitrogen, creatinine and cholesterol levels were significantly lower ($p < 0.05$) among pigs fed control (maize based) diets. Nevertheless, values reported in this study are within the normal range for pigs of similar age (Radostitis *et al.,* 1994). It is evident therefore that the piglets were healthy and were not clinically affected by the treatment diets.

Table 4.4.2: Haematological indices of weaner pigs fed diets containing fermented mixture of cassava root pulp and palm kernel cake as replacement for maize

Parameter		FEMCARPP		CSM-PKC mix		SEM
	Control	50 %	100 %	50 %	100 %	
RBC (x 10^6/µl)	5.30	5.05	4.95	5.10	4.75	0.41
Hemoglobin (g/µl)	12.85	11.5	11.75	12.80	11.70	1.84
Packed cell volume (%)	38.45	34.40	35.50	38.7	35.05	3.43
Mean corpuscular volume (fl)	7.45	7.20	7.25	7.6	7.60	0.32
Mean corpuscular haemoglobin (*p*g)	2.45	2.25	2.50	2.5	2.50	0.13
Mean corpuscular haemoglobin concentration (g/dl)	33.30	33.35	33.35	33.20	32.95	0.49
Total leucocytes (x 10^3 /µl)	13.00	13.75	10.20	7.50	13.00	2.11
Neutrophil (%)	26.10	29.25	29.30	26.70	28.25	3.79
Lymphocyte (%)	73.15	70.55	69.85	72.50	71.40	3.77
Eosinophils (%)	0.70	0.20	0.85	0.80	0.35	0.45

4.5 Study 5

Live-weight gain, carcass characteristics and meat meat quality of grower pigs fed fermented mixture of cassava root pulp and palm kernel cake as replacement for maize

4.5.1 Performance parameters

4.5.1.1 Live Weight and Weight Gain

The performances of grower pigs fed the experimental diets are shown in Table 4.5.1. The results showed that there are significant differences in live weight, average daily gain, feed intake and feed conversion ratio. The average live weight of pigs fed FEMCARPP was similar to those fed the control diet ($p > 0.05$) whereas both were superior to those fed the CSM-PKC based diet. The results also showed that sex did not significantly affect the growth performance of the animals. The live weight of boars and gilts fed FEMCARPP based diets (36.07 and 37.86 kg) were higher than those on diets formulated with CSM-PKC mix (33.22 and 31.92). This showed that nutritive value of FEMCARPP was improved by the spontaneous solid state fermentation. These results are in agreement with the findings of Dairo *et al.* (2008), who reported that, solid state fermentation improved nutritive value of palm kernel cake for laying hens. Similar results were reported by Oboh (2006) on cassava peels, where average daily weight gain followed similar trend. No significant sex effect was found for liveweight, weight gain and feed conversion ratio on the experiment studied.

Table 4.5.1: Performance of Grower-Finisher pigs fed FEMCARP and CSM-PKC mix as replacement for maize

PARAMETER	Maize		FEMCARPP		CSM-PKC MIX		SEM
(Kg)	Male	Female	Male	Female	male	Female	
Initial live weight (kg)	15.96	16.50	15.70	16.90	16.27	15.97	0.701
Final live weight (kg)	37.94[a]	36.93[a]	36.07[b]	37.86[a]	33.22[c]	31.92[c]	0.639
Average daily weight gain (kg/pig/day)	0.25[a]	0.23[a]	0.25[a]	0.25[a]	0.21[b]	0.19[b]	0.041
Feed intake (kg/pig/day)	0.86[a]	0.84[a]	0.87[a]	0.92[a]	0.87[a]	0.83[b]	0.039
Feed conversion ratio	3.44[b]	3.60[b]	3.5[b]	3.72[b]	4.05[a]	4.3[a]	0.505
Cost of feed (N/kg)	89.17	89.17	79.24	79.24	89.20	89.20	
Cost of feed per kg weight gain	293.17[a]	308.07[a]	284.14[a]	292.01[a]	395.07[b]	390.39[b]	15.119

[a, b, c] Means within the same row with different superscript are significantly different($p<0.05$).

4.6.1.2 Feed Intake and Utilization

The feed intakes of pigs on the treatment diets were similar except for the females on CSM-PKC based diets, which consumed significantly ($p < 0.05$) smaller quantity of feed. This must have resulted from the relatively smaller live weights, since daily feed allowance was based on 4 % of body weight till the end of the previous week. During the trial, pigs on FEMCARPP rarely finished their daily feed allowance, but consumed their feed gradually. The characteristics of the feed enabled them fill without compromising on performance. Maize based rations had higher bulk density, less volume and must have been more palatable. It can be concluded that, solid state fermentation enhanced the physical characteristics of FEMCARPP which led to optimal utilization of feed. It is likely that if the animals were fed *ad libitum*, feed intake will have been higher among pigs fed rations based on maize and CSM-PKC mix. These have obvious negative consequences for the environment. Pigs on CSM-PKC mix had significantly ($p < 0.05$) higher feed conversion ratio than those on control and FEMCARPP based diets. The FCR of grower pigs on FEMCARPP (3.50 and 3.72) were similar to those found among pigs on control diets (3.44 and 3.60). Earlier reports by Ao *et al* (2011) and Aro & Akinjokun (2012) are in agreement with these findings.

The weekly live weights, weight gains, feed intakes and feed conversion ratios are shown as in Figures 4.9, 4.11 and 4.12, respectively. They showed that, live weight, weight gain and feed intake increased significantly with period of treatment (age of the pigs), while feed conversion ratios were not affected by age. Average daily weight gain increases progressively in all treatment groups until the 20^{th} week of the experiment. This period coincided with the grower phase of the project. The values however dropped in all treatment groups at the 24^{th} week and the rate of drop was similar in all

groups. This suggests that most pigs achieved optimal muscle size (growth) around 18 to 20 weeks of age (i.e. 6 - 8th week of the experiment) and so growth must have shifted to more fat deposition rather than increase in actual cell mass (protoplasm). Fat accretion requires more energy and hence low FCR at 24th week.

4.5.2 Carcass and Internal Organ Characteristics

The carcass and internal organ characteristics are shown in Table 4.5.2. No significant differences were found for carcass and internal organs of the experimental animals except for the proportion on tail. This implies that the nutrients were well assimilated in all treatment diets. The lower calculated metabolizable energy of the test diets and higher crude fibre did not affect the significantly affect the carcass cuts and internal organ weights. The significantly higher proportion of tail among animal on control diet could have been due to precission errors during cutting instead of treatment effect. Proportion of gastrointestinal tract increased numerically from treatment A to C though was not significant. The increased proportion of crude fibre must have been responsible for the increase.

4.6.3 Meat Quality Characteristics

The meat quality and panel sensory scores for meat from pigs fed experimental rations are shown in Table 4.5.3. There were no significant differences in drip loss among treatments The water holding capacity of pigs fed maize based rations (49.93 %) were significantly higher ($p < 0.05$) than those fed FEMCARPP (46.82 %) which in turn was higher than those fed CSM-PKC mix (43.55 %). Cooking loss also followed the same trend.

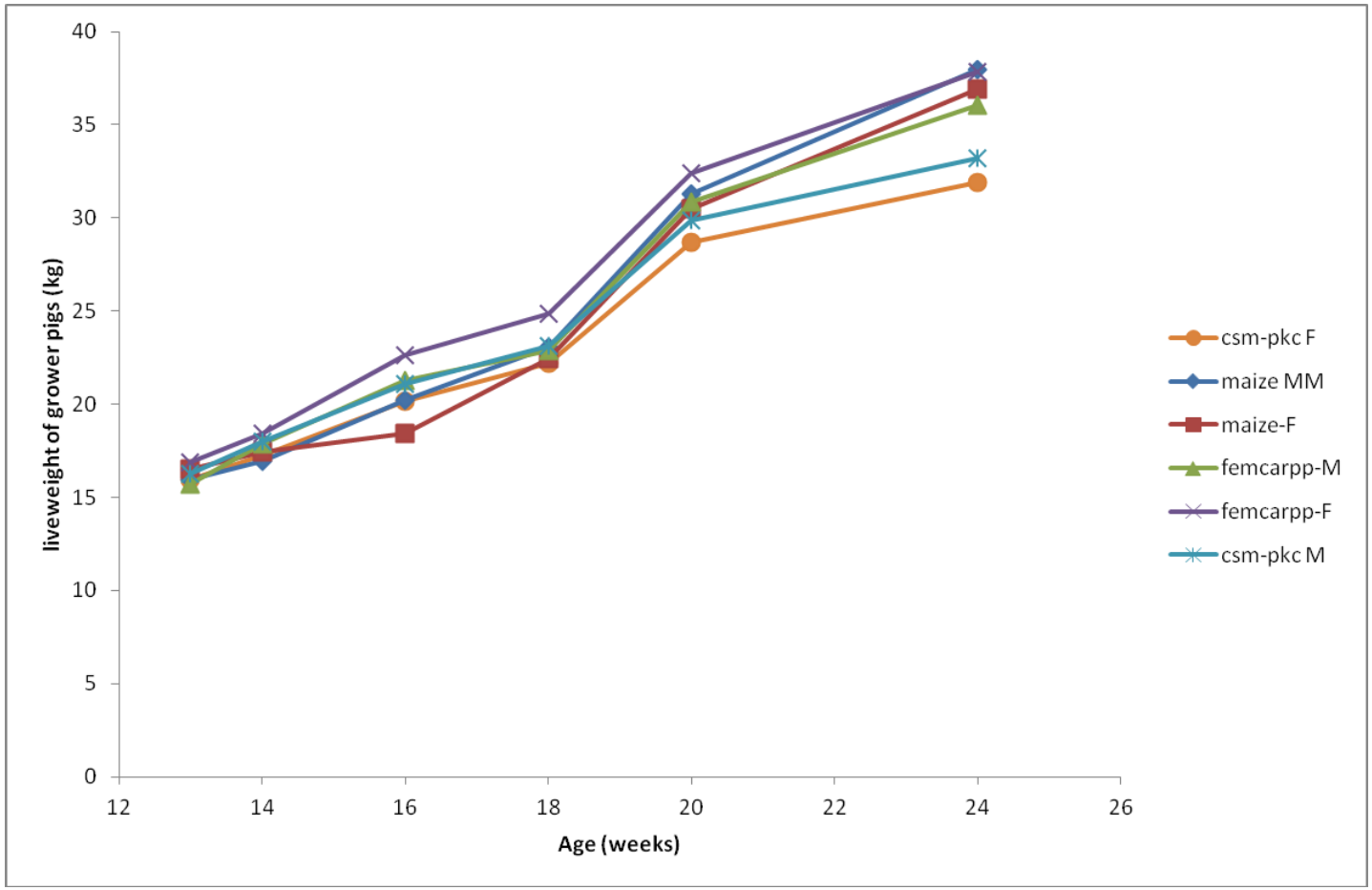

Figure 4.9: Graph of live weight of grower pigs fed experimental diets against age

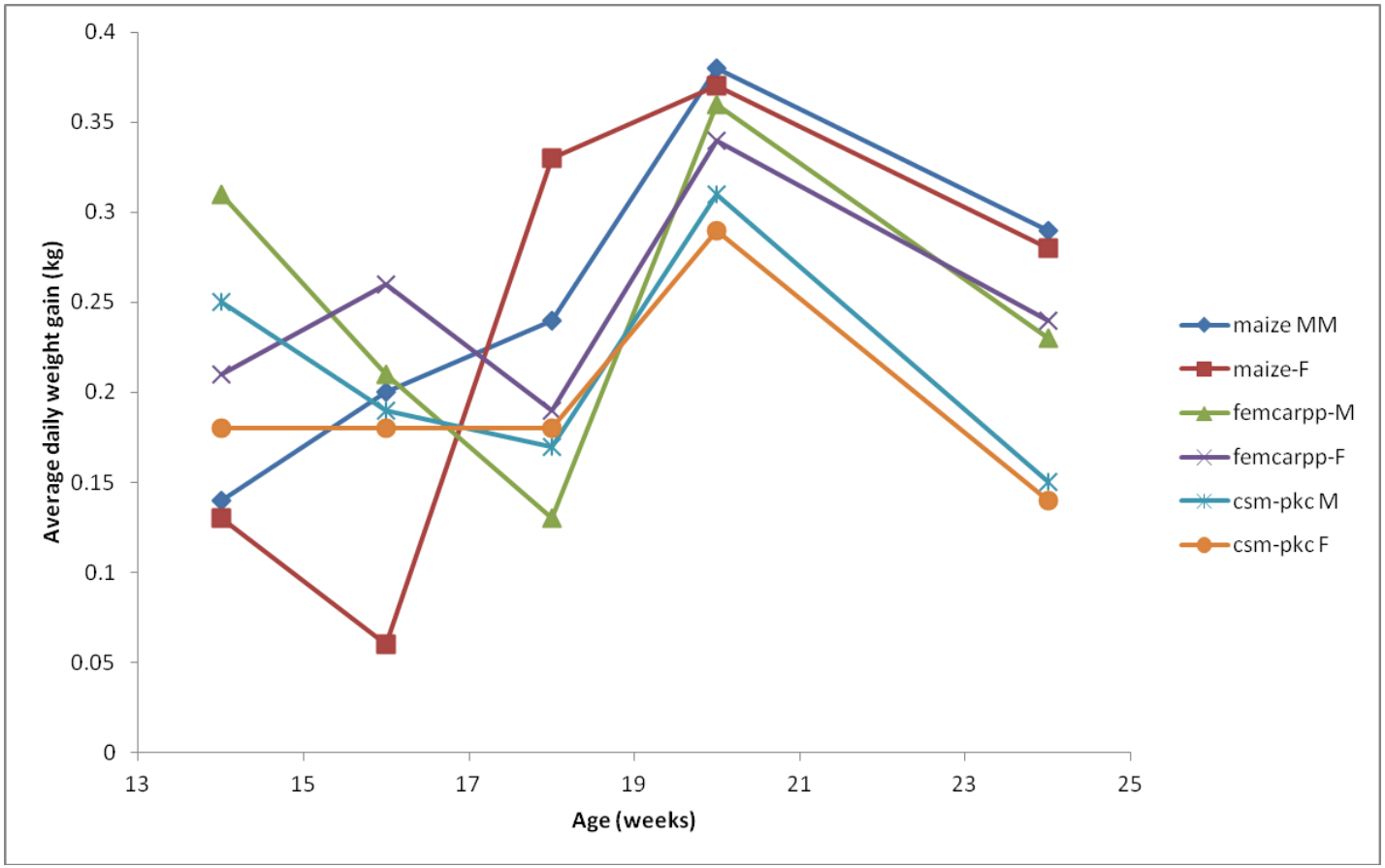

Figure 4.10: Graph of average daily weight gain of grower pigs fed experimental diets against age

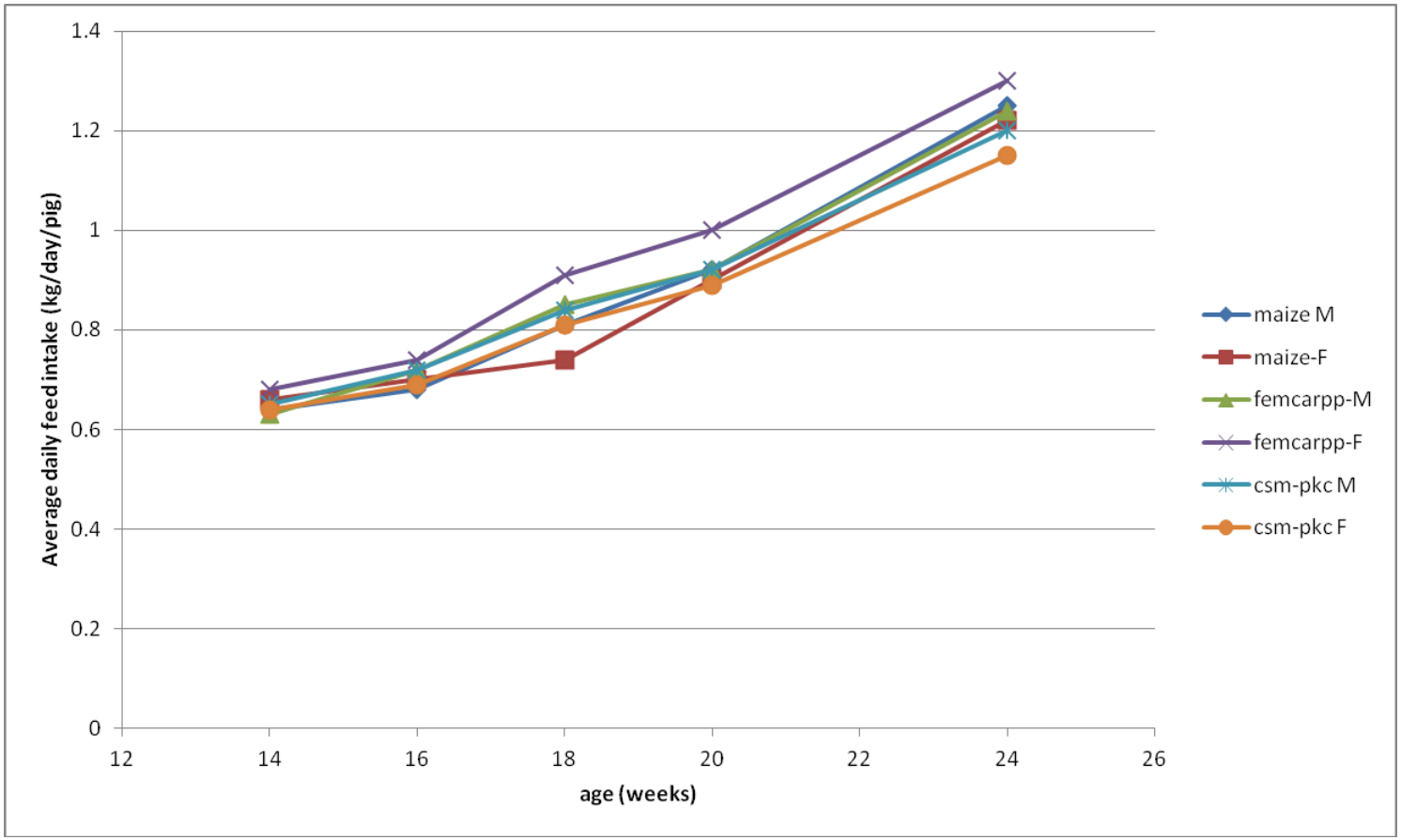

Figure 4.11: Graph of average daily feed intake of grower pigs fed experimental rations against age

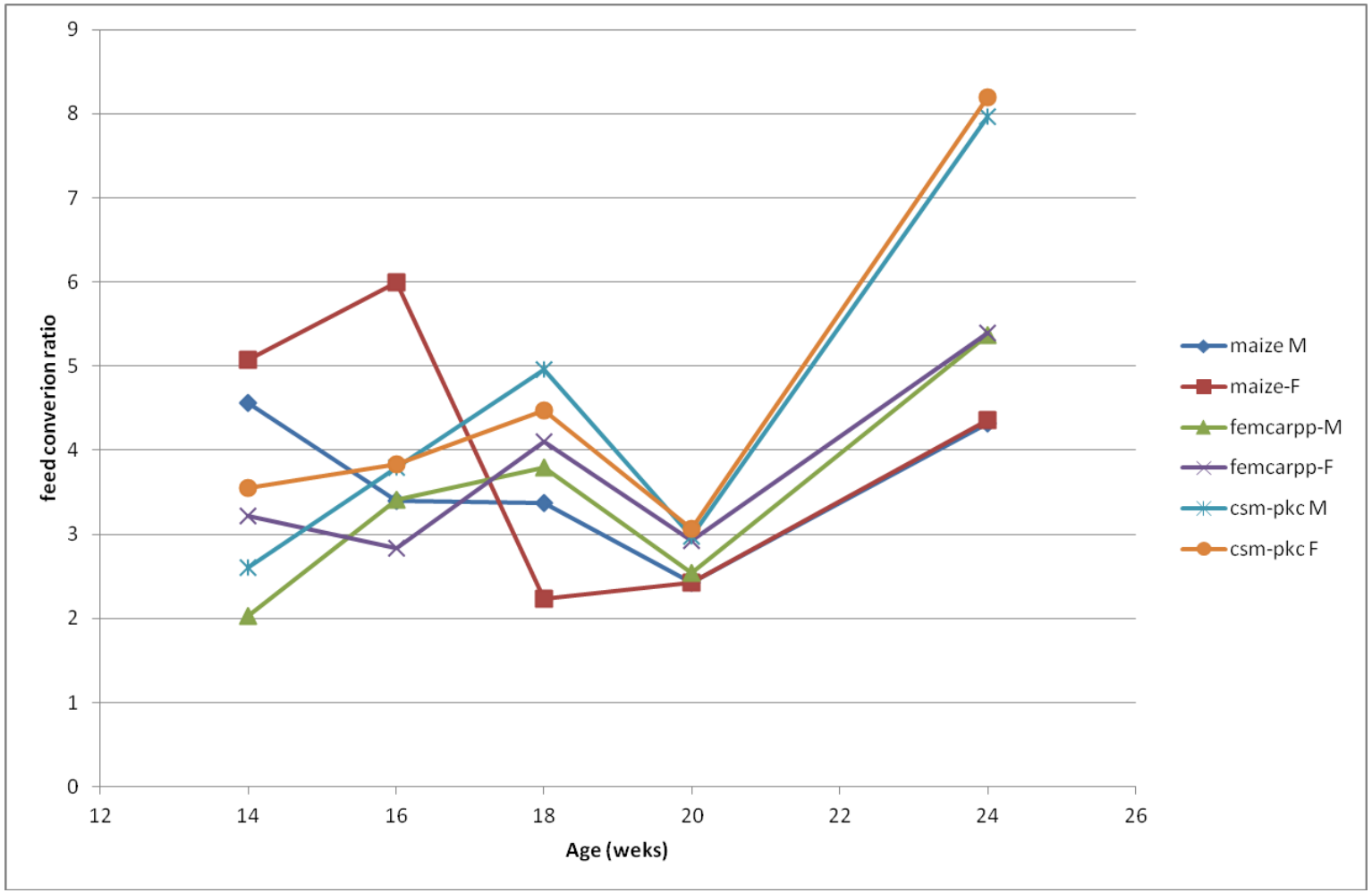
feed converion ratio
Age (weks)
0
1
2
3
4
5
6
7
8
9
12
14
16
18
20
22
24
26
maize M
maize-F
femcarpp-M
femcarpp-F
csm-pkc M
csm-pkc F

Figure 4.12: Graph of feed conversion ratio of grower pigs fed experimental ration against age

The pigs fed CSM-PKC mix was significantly ($p < 0.05$) less tender than those fed FEMCARPP and maize based diets. These suggest that CSM-PKC mix impacted negatively on meat quality and consumers may discriminate against meats from such pigs. However, no significant differences were found for hedonic (degree of likeness) among treatment groups.

Table 4.5.2 Carcass and organ weights of grower-finisher pigs fed FEMCARPP or CSM-PKC mix as replacements for maize

PARAMETERS	Treatments			SEM
	Maize	FEMCARPP	CSM-PKC mix	
Live weight Kg	41.40	35.53	32.73	5.892
Carcass weight (kg)	27.69	23.43	21.32	4.133
Dressing percentage (% live weight)	66.91	65.80	64.91	1.044
lean cuts (% of Live weight)	47.91	46.82	46.20	0.789
Lean cuts (% carcass weight)	71.50	71.16	71.18	0.981
% ham	19.74	18.25	19.50	0.505
% shoulder	16.44	15.83	15.13	0.500
% loin	11.73	12.75	11.56	0.989
Fat cuts				
% Spare ribs	7.69	8.89	8.98	0.674
% Jowl meat	5.53	5.77	5.40	0.355
% Belly	4.54	4.09	3.58	0.534
Offal				
% Head	8.89	9.53	9.89	0.648
% Trotters	2.59	2.58	2.63	0.148
% Tail	0.45^{a}	0.39^{b}	0.38^{b}	0.016
% Liver	1.87	1.72	1.74	0.127
% Lung	0.79	0.93	0.85	0.097
% Heart	0.43	0.47	0.49	0.047
% Testes	0.72	0.45	0.49	0.303
% Kidney	0.31	0.21	0.19	0.118
% GIT*	18.21	19.24	20.28	3.266

*Calculated value

^{a,b} – mean values with different superscript are significantly different ($p<0.05$)

Table 4.5.3 Meat quality characteristics of grower-finisher pigs fed FEMCARPP or mixture of Cassava meal and Palm Kernel cake as replacements for maize

PARAMETERS	TREATMENTS			SEM
	Maize	FEMCARPP	CSM-PKC mix	
Drip loss (%)	6.43	6.10	7.84	1.147
Water holding capacity (%)	49.93[a]	46.82[b]	43.55[c]	1.300
Cooking loss (%)	41.00[a]	38.73[b]	34.47[c]	1.559
Organoleptic assessment				
Juiciness	6.33	6.16	5.25	0.475
Tenderness	6.50[a]	6.64[a]	5.00[b]	0.387
Connective tissue amount	6.33	6.33	5.42	0.533
Flavour intensity	5.92	6.00	5.67	0.387
Hedonic rating	5.92	6.90	6.33	0.717

[a ,b ,c] – mean values with different superscript are significantly different ($p<0.05$)

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusions

From the results of the study, it can be concluded that blending of cassava root pulp and palm kernel cake improve the physical characteristics of the products for poultry feeding. The three inoculation techniques (direct or batch inoculation with *A. niger* and spontaneous inoculation) were able to enhance the nutritive value of the products for poultry and pigs. The mean crude protein content of fermented samples increased from 9.75 % to 14.16 % in *A. niger* inolcuated samples, 15.37 % in batch inoculated samples (14.67 %) in spontaneously inoculated samples.

Under spontaneous inoculation technique, *Aspergillus oryzae* and *Rhizopus spp* were the principal fungal implicated, while contributions of *A. niger* and *Mucor spp* were minor. *A. niger* was found to be more efficient in degrading fibre in the substrate than the other fungi implicated in the other treatments but the fungi implicated in the spontaneously inoculated samples were effective in increasing the level of crude protein than the pure cultures of *A. niger* inoculation. Total fungal counts varied significantly ($p < 0.001$) with the method of inoculation. *A. niger* inoculated samples had significantly ($p < 0.001$) lower counts than the spontaneously and batch inoculated samples. However, fermentation of the mixture under spontaneous inoculation will be easier to adopt by the farmers under prevailing farm conditions and environment.

Broiler chicks fed sundried spontaneously inoculated mixture of cassava root pulp and palm kernel cake had lower live weight, feed intake (98.97 g/day), higher feed conversion ratio (2.89) than their counterparts fed maize based diets (111.3 g/day and 2.38 g/day respectively). The cost per kg weight gain of chicks fed the control diet (N 454.23/kg) was higher than those fed diets containing FEMCARPP (N 446.30/kg). These were lower than those of CSM-PKC diets (539.33/kg). The carcass characteristics of the chicks fed the treatment diets did not vary significantly but those fed maize based diets had significantly higher ($p < 0.05$) drip loss (2.24), higher cooking loss (29.86 %) with lower water holding capacity (41.97 %) when compared to those fed FEMCARPP based diets (2.04 %, 21.61 % and 48.03 % respectively). No significant differences were found for most of the haematological parameters. All the parameters were within the normal range for birds raised in similar environments

The live weight of the broiler finishers fed diets containing wet FERMCARPP (2005.2 g) was lower than the control diet (2272.2 g), sun dried FEMCARPP (2177.8 g) and CSM-PKC mix (2146.3 g), though, they consumed less feed than other treatment groups. Their feed conversion ratio did not vary significantly ($P > 0.05$) from those fed diets containing maize, sundried FEMCARPP or CSM-PKC mix. No differences were found in the carcass parameters studied except for full gizzard weight. The meat from chicks fed diets containing wet FEMCARPP was low ($p < 0.05$) on tenderness score (5.00 out 8) while those fed CSM-PKC mix had the highest tenderness score (6.00 out 8) among all treatment groups.

The live weight of weaner pigs fed 100 % maize replacement level (9.42 kg) were significantly ($p < 0.05$) higher than those on maize based diet, whereas those on diets

containing CSM-PKC mix were poor ($p<0.05$) than the control. The feed conversion ratio of pigs on control diet (3.09) was better ($p>0.05$) to those fed FEMCARPP based diets (2.68 and 2.32). These were superior to those fed diets containing CSM-PKC. No significant differences were found for all the haematological parameters evaluated in this study.

Final live weight of grower pigs fed FEMCARPP was similar to those fed the control diet ($p > 0.05$) whereas both were superior to those fed the CSM-PKC based diet. The results showed that sex did not significantly affect the growth performance of the animals. The FCR was similar among pigs fed diets containing maize and FEMCARPP and both were superior to those fed diets containing CSM-PKC. No differences were found for all carcass traits measured except proportion of tail. The water holding capacity of pigs fed maize based rations (49.93) were significantly higher ($p < 0.05$) than those fed FEMCARPP (46.82) which in turn was higher than those fed CSM-PKC mix (43.55). Cooking loss also followed the same trend. The sensory evaluation of meat among pigs CSM-PKC mix was significantly ($p<0.05$) less tender than those fed FEMCARPP and maize based diets.

5.2 Recommendations

It is therefore recommended that:

1. Solid state fermentation is an effective tool for improving physical and chemical properties of the mixture of cassava root pulp and palm kernel cake as feed material in poultry and pig diets. The direct or batch inoculation with *A.niger,* and spontaneous inoculation methods are effective methods of inoculation for the substrate.

2. Spontaneous inoculation technique should be employed by farmers for ease of production at the farm level. The fermented product can be fed fresh though additional labour or technology might be needed for storage.

3. Further research is needed to determine the optimal supplement of other products when birds are placed on the fermented product.

4. The feeding trial should be extended to laying hens, turkeys and rabbits.to establish their productivities and optimum returns or benefits.

5. The technology should be extended to farmers that are often faced with feed crisis resulting from shortage of maize and other grains.

6. Government and her agencies should develop policies and programmes which will encourage farmers to use of the products in place of maize in broilers and swine feed production.

CONTRIBUTION TO KNOWLEDGE

1. This study has revealed that solid state fermentation improves the physicochemical characteristics of a mixture of cassava root pulp and palm kernel cake for poultry feeding within 6 days of fermentation. Spontaneous inoculation or direct/batch inoculation of the mixture with *Aspergillus niger* yield end products of similar characteristics. The technology increased the crude protein content, improved effective fibre and the aggregate structure of the product.

2. *Aspergillus oryze* is the principal fungi implicated in the spontaneously inoculated process, whereas *Aspergillus niger*, *Rhizopus* and *Mucor* species play secondary or supporting roles.

3. The spontaneously inoculated product is effective in replacing maize in broilers and pig diets without deleterious effects on performance, carcass and meat quality of the the broilers and pigs. The product can be incorporated into broilers' diets without further processing, including drying. Feeding the product without further drying reduces feed intake but was cost effective and had no negative effect on carcass and meat quality of broilers.

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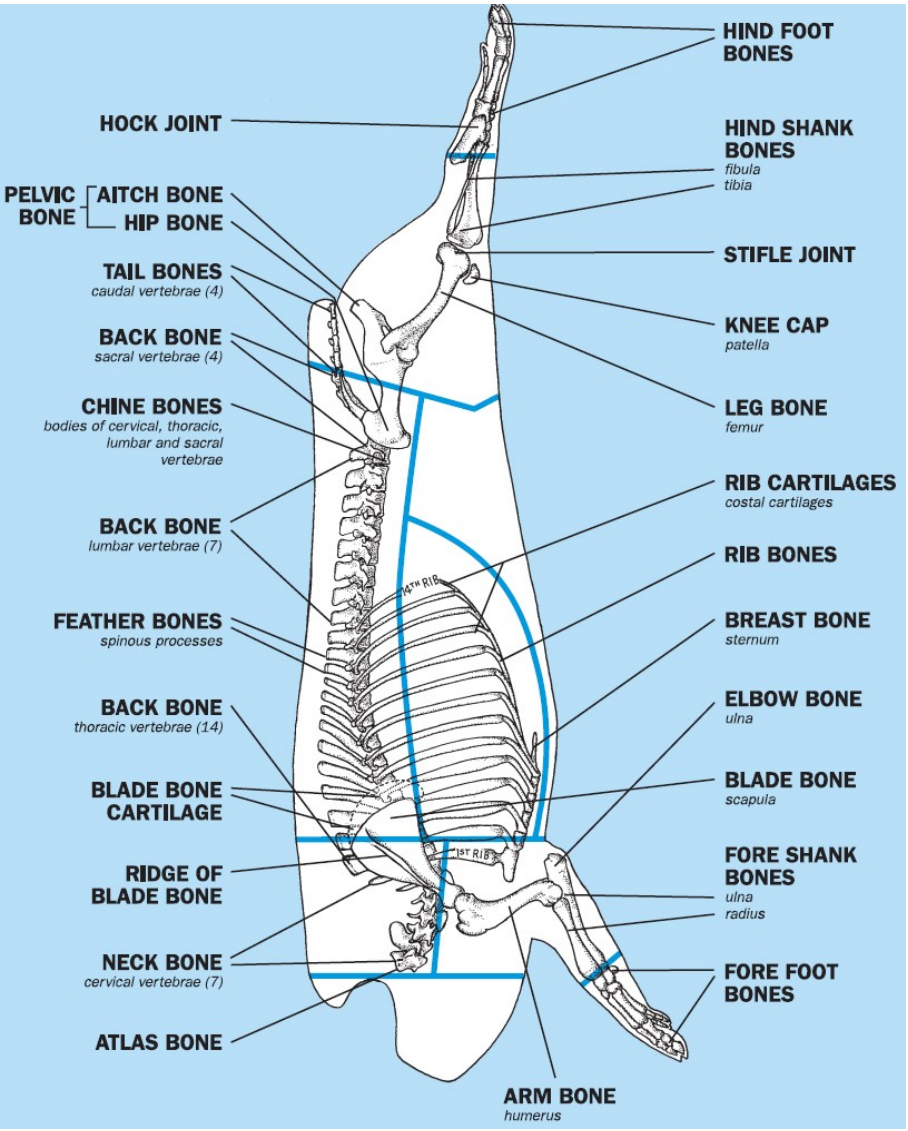
Appendix



Appendix 1: Pig carcass cuts and its bones (FAO, 1991)