UNIVERSITY FOR DEVELOPMENT STUDIES

EFFECTS OF PROCESSED FALSE YAM TUBER ON NUTRIENT METABOLISM AND PHYSIOLOGY OF BROILER CHICKENS

AHADZE KING YAW

THESIS SUBMITTED TO THE DEPARTMENT OF ANIMAL SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN ANIMAL NUTRITION

MARCH, 2020

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BY

AHADZE KING YAW (B.Sc. Agriculture, Dip Education, MSc. Animal Nutrition)

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DECLARATION

Student

I hereby declare that this thesis is the result of my own work and that no part of it has been presented for another degree in this University or elsewhere.

Candidate's Signature…….Date……..

Name: AHADZE KING YAW

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

Principal Supervisor's Signature…….Date……

Prof. Herbert K. Dei (PhD)

(UDS, Ghana)

ABSTRACT

Six separate experiments were carried out to assess the effects of anti-nutritive elements in the false yam tuber on physiology and metabolism of broiler chickens. In experiment 1, the nutrient composition of the untreated sun-dried false yam tuber was determined. It comprised on dry matter basis 5.34% crude protein, 4.35% ether extract, 2.50% Ash and 3140.81Kcal metabolizable energy/kg. Also, the mineral composition of the untreated sun-dried false yam tuber revealed the presence of potassium, magnesium, calcium, phosphorus, sodium, iron, zinc and copper. In experiment 2, phytochemical screening of the untreated sun-dried false yam tuber showed, on dry matter basis, the presence of alkaloid (1.53%), phytate (2.97%), oxalate (2.64%) and mucilage (11.25%). In Experiment 3, the effects of processing on the nutrient and anti-nutritive factors of the false yam tuber were determined. Processed products used were Blanched false yam (BFYM), 0.1M Sodium hydroxide treated false yam (NaTFYM), 0.1M Sodium hydroxide treated blanched false yam (NaTBFYM) and Fermented false yam (FFYM). The macro-mineral elements which were consistently and significantly higher ($P < 0.05$) after processing when compared to the unprocessed ones are phosphorus, calcium, potassium and the trace elements iron, zinc, copper and manganese. Generally, processing caused reduction in phytate (27.95%), alkaloids (37.25%), saponins (68.18%), terpenoids (69.57%), oxalates (84.47%) and mucilage (100%) in the fermented false yam. Experiment 4 investigated effects of prolonged fermentation on microbial content of fermented false yam product and its safety. The following microorganisms were identified in the fermented false yam product: *Enterobacter coloacae, Agglumerans* and *Bacillus spp* all of them amounting to a value of 2.48×10^7 CFU/ml. In addition the yeast and mould cell counts averaged 2.5×10^5 CFU/ml. Experiment 5 involved toxicity study using Sprague Dawley rats for a period of 90 days to determine potential toxicity of the residual anti-nutritional factors in the processed false yam products. Rats fed on the processed false yam products inclusion rates of 5, 10 and 20g/kg did not show any visible clinical signs, deaths, or toxic effect on the animals' behaviour. Haematological values measured showed a significant ($P < 0.05$) reduction in HGBT, HCT and RBC levels of rats fed on 20g/kg. Biochemical parameters such as ALT and AST measured for the treatment rats showed significant difference ($P<0.05$) from the control rats. Organs such as kidneys, spleen, lungs and liver of rats fed on the 10g/kg and the 20g/kg of the processed false yam products were

significantly (P < 0.05) reduced compared with the control. Experiment 6 investigated the effect of residual anti-nutritive factors in the processed false yam products on growth, blood metabolites and carcass characteristics of broiler chickens. A total of 350 Cobb broilers at four weeks of age were fed seven experimental diets for four weeks using 2x3 factorial designs. The experimental diets comprised fermented false yam (FFYM) and 0.1MSodium hydroxide treated blanched false yam (NaBFYM) which replaced portions of the maize on weight by weight basis at inclusion levels of 0%, 5%, 10% and 15%. The control diet (0%) contained no processed false yam products. The highest daily feed intake was reported in birds fed on the fermented false yam treatment. Daily live-weight gain of birds on the treatment diets decreased as the inclusion rate increased showing significant difference (P<0.05) at 15% when compared with the control. Haematological parameters measured for birds on the processed false yam such as WBC, RBC, HB, MCHC, RDW-CV, PDW, MPV and PCT were significantly different (P<0.05) from those of the control birds. It can be concluded that fermented and 0.1M sodium hydroxide treated blanched false yam tuber can replace maize at 10% inclusion in diets with minimal residual anti-nutritive effect on digestibility and growth of the broiler chicken.

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DEDICATION

I dedicate this thesis to my parents the late Mr. Lawrence K. Ahadze and Mrs Comfort Benewoe for their indefatigable support and trust they had in me by investing their resources and time in my education.

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LIST OF ABBREVIATIONS

AST: Aspartate Aminotransferance

ALP: Alkaline Phosphatase

ALT: Alanine Aminotransferase

LDH- Lactate Dehydrogenase

SG: Specific Gravity

GGT- Gamma glutamyl transferase

MCHC: Mean Corpuscular Haemoglobin Concentration

WBC: White Blood Cells

RBC: Red Blood Cells

HCT: Haematocrit

HGB: Haemoglobin Concentration

RDW-SD: Red Cell Distribution width (Standard Deviation)

RDW-CV: Red Cell Distribution Width

MPV: Mean Platelet Volume

PDW: Platelet Distribution Width

PLCR: Platelet Larger Cell Ratio

ALB: Albumin

MCV: Mean Corpuscular Volume

MCH: Mean Corpuscular Haemoglobin

PLT: Platelets

LYM%: Lymphocyte Percent

LYM#: Lymphocyte Absolute Value

CK: Creatinine Kinase

CHAPTER 1

1.0 Introduction

The major components of poultry feedstuff are made up of carbohydrates and proteins from plants and animal sources (Tewe and Egbunike, 1992). There is increasing demand for carbohydrate and protein foods from both plant and animal sources as human population increases and livestock industry expands. The high demand for these commodities in both human and animal nutrition has led to corresponding increase in the cost of these items. Carbohydrate, a major source of energy in poultry feed, constitutes 50% of poultry feed can be obtained from starchy staples such as root crops (cassava), cereals (maize) and other stem tubers (yam and potatoes) (Tewe and Egbunike, 1992). There is competition between human beings and livestock, especially non-ruminants for maize as a source of carbohydrate. The high cost of maize and its eractic supply has led to the search for locally available, cheap alternative sources of carbohydrate (Tewe and Egbunike, 1992).

False yam is one of such close substitutes for maize. According to Fay (1987), the false yam plant is in the family of *Icacineacae*. The plant is found in the wild in less rainfall areas of Ghana and other West African countries including Central Africa. It grows vegetatively and produces big tubers in the soil. According to Dei *et al*. (2011a), the false yam (*Icacina oliviformis*) tuber could be a new feed resource alternative to maize, because it has the potential in terms of its nutritive value to reduce the demand for maize as a major ingredient for poultry.

Fay (1991) reported that the false yam tuber contains high carbohydrate with potential of being used as carbohydrate source in poultry diets. The false yam tuber has an objectionable odour and taste, high fibre content and anti-nutritive elements such as oxalates, alkaloids, gums and resins, phytates and triterpenoids which limit the nutritive value of the yam in monogastric diets (Iwe, 2003). Phytate is known to cause a decrease in Ca^{2+} and Na^{+} absorption in the gut. Cheeke and Shull (1985) have reported that saponins in false yam reduce feed intake, decrease digestion, and also

interacts with calcium, phosphorus, lipids and cholesterol absorption in the intestines of monogastric animals. Mucilage forms thick viscous consistency with feed, reducing digestion and absorption. Oxalate decreases mineral absorption. Gum resins in soaked false yam tuber meal affects digestion of fats and protein in layer chickens (Mohammed and Dei, 2013).

According to Fagbemi *et al*. (2005), false yam can only be included in the diet of poultry and pigs if it is subjected to fermentation, drying in the sun, soaking, boiling and drying in the oven. The processing methods suggested are likely to reduce the anti-nutritive factors in the false yam, reduce the undesirable taste and odour, and improve its palatability.

The presence of anti-nutritive elements in feed may be poisonous to human beings or livestock and may cause reduction in feed intake, digestion, absorption of essential nutreints for growth, and reduction in blood parameters or death of animals (Okaka, 2005), hence the need to remove them from such feedstuffs.

Earlier attempts to ameliorate adverse effects of raw false yam tubers and seeds in poultry included boiling for 2 hours and fed at 120g/kg (Dei *et al*., 2011a), soaking for 9 days and fed at 90g/kg (Dei *et al*., 2011b), use of salt-petre solution, fed at 120g/kg (Dei *et al*., 2013b), and aerobic fermentation (9 to 15 days) and fed at 150g/kg (Dei *et al*., 2012c). These studies concentrated on the nutritive value of the processed products and growth performance of broiler chickens. Thus, there is a gap in the literature with regard to nutrient metabolism and physiological effects of residual anti-nutritive factors on physiological parameters such as blood profiles and organs. Besides, other alternative means of removing these anti-nutritive factors in feed have to be looked into, such as the use of akaline material such as sodium hydroxide to treat the false yam before blanching in water and also using completely submerged fermentation technique and other processing combination methods.

Furthermore, studies on the mineral composition in the tuber was inadequately dealt with in previous studies when processing techniques such as boiling, soaking, and salt petre treatments were

carried out on the false yam tuber. Aside this, the residual amounts of minerals in the processed products in the previous studies were not taken into consideration when experimental diets for the studies were formulated. These could have had potential effect on metabolism and physiology of some blood metabolites and organs of the animals concerned.

Another gap in the literature has to do with lack of intensive screening of the bioactive chemicals in the false yam tuber and seeds and their quantification as well as their effects on metabolism and physiology of broiler chickens although it was mentioned theoretically in the literature.

Toxicity study about the false yam tuber is another area which was casually mentioned in the literature review but worth considering which has not been extensively looked into how these metabolites would affect some specific tissues and organs of animals.

Again, the presence of microbial organisms in the fermented products has also not been critically looked into in the literature and how these microorganisms could affect nutrient metabolism, health and safety of the animals. It is for these reasons that the study has become necessary to look at the effect of residual anti-nutritive elements in the false yam tuber, to explore other processing methods, determine the microbial content of the fermented product and toxicity of the processed product on metabolism and physiology of broiler chickens.

1.1 Research Questions

1. Do false yam tuber and seed contain adequate amount of nutritive elements to replace maize in broiler chicken diet?
2. Do false yam tuber and seed contain anti-nutritive elements such as saponins, alkaloid, terpenoids, phytate, and oxalate?
3. Can the secondary metabolites i.e anti-nutritive elements be reduced through simple processing methods?

4. Can the residual anti-nutritive elements in the processed false yam tuber products interfere with haematology, serum biochemistry and nutrient metabolism in the broiler chicken?

1.2 Main Objective

The main objective of the study was to determine the effect of anti-nutritive elements in false yam tuber on physiology and metabolism of broiler chickens.

1.3 Specific Objectives

The specific objectives were to:

1. Determine the proximate composition (CP, EE, CF, Ash, NFE), phytochemical constituents and the mineral composition of the false yam tuber and the seed.
2. Determine simple but effective method of processing false yam tuber to be included in chicken broiler diets.
3. Carry out toxicity test on the processed product using Sprague Dawley rats.
4. Find out how the processed false yam products may affect growth, blood serum biochemistry, haematological parameters and carcass characteristics of broiler chickens.

CHAPTER 2

2.0 Literature Review

2.0.1 Poultry feed supply, shortage and high cost in developing countries

Poultry feed industries in Africa depend largely on the importation of maize and other feed ingredients. The importation puts pressure on the various countries' foreign exchange earnings (Ravindran and Blair, 1991). Fish and maize are the major components of feed ingredients in the diet of poultry. The quantities of these feed ingredients produced locally are inadequate, and expensive due to competition between human beings and livestock for these raw materials. There is, therefore, the need to import the maize and fish to support the feed industries and the poultry farmers (Reddy and Qudraytullah, 2004).

Maize, not only is it used in human and animal diet, but also in the industries for the manufacture of alcoholic beverages such as beer and whiskey (Potter and Hotchkiss, 1995). The increased use of maize to produce ethanol, leading to shortages in the supply of the commodity and its escalating prices in the world market has been a major concern for the poultry industries (Tewe and Egbunike, 1992).

2.1 The use of non-conventional feed stuffs for poultry

Non-conventional feeds are feed ingredients that have not been used traditionally in large quantities in the diets of animals and neither in commercial quantities in rations for livestock (Devendra, 1992). The high demand for cereal products, especially maize which is a principal source of energy in poultry diets, has called for intensified research work to investigate the potential use of other feed resources for poultry feed (Teguia *et al*., 2004). According to Nworgu (2004), an alternative solution is to explore other feed ingredients which are not traditionally used in feed rations for animals but have the potential to replace partially, completely or supplement the existing diets of

livestock. Feed ingredients or new feed resource, which can be used in human nutrition and are easy to come by and are not expensive will be the most economic and practical solution to the problem (Oluyemi and Roberts, 1988). According to Kekeocha (1984), protein and energy components of the diet are normally substituted for and these should be carefully considered since there are differences in the nutrient compositions of the various substitutes.

2.1.1 Crop residues and agro-industrial by-products

These are waste products from field crops after harvesting or processing which are not directly used in human nutrition but can be used to feed livestock. They include; cocoyam peels, maize cobs, cassava peels, cocoa pod husks, and others which are widely used as animal feed (Onyeonagu and Njoku, 2010). Some of these by-products and waste can be included in diets relatively small proportions (Wilkinson, 1988).

Wastes from animal and crop products after the primary produce have been turned into secondary products is called agro industrial by- products. The by-products can be used in livestock diets but not in human nutrition (Farrell, 1997). According to Agreheore (1998), some of the by-products can be used directly in livestock diets others may need further processing in order to release their nutrients before incorporating into animal diets. The nutrients in the agricultural by-products are classified as protein, or energy and combined protein and energy sources. Feeds with greater proportion of carbohydrate are classified as energy source but low in protein. On the other hand, feed with higher proportion of protein is considered protein rich source and low in carbohydrate. Carbohydrate source of feed is molasses (41% CP, 75% DM, 12.7 GE MJ/Kg DM) which is a waste product after processing sugar (Aregheore, 1998).

Dried yeast sludge, the end product of molasses fermentation to alcohol, has been safely incorporated as ingredient to a basal cereal free ration in poultry diets at 20% inclusion level with better growth responses (Garewal, 1969). According to Aregheore (1998), protein sources in agro-

industrial by- products are obtained from seeds of field crops which contain considerable amount of oil. After processing the seeds and extracting the oil what is left is called the cake. Most of these oil seed cakes may contain appreciable amount of proteins. Some of these cakes include soya bean cake (45%), copra cake (18.8% CP) and palm kernel cake (18% CP), which are by-products from their respective oil seeds. Both protein and energy sources which are relatively higher in one by-product include maize, wheat bran, rice, brewers spent grains and pito mash (Aregheore, 1998).

Spent grains (example "pito" mash) contain high B vitamins (Buamah, 1992) and oil palm slurry is rich in ether extract 4-7.3% on dry matter basis (Okai, 1998), CP 96 g/kg, ME 17.76MJ/Kg (Devendra, 1977). This is comparable to maize with 90 g/kg CP and 14.37 MJ/kg. The use of cassava powder and oil palm slurry mixture has been found to be suitable feed ingredients for monogastric animals (Hutagulung *et al*., 1977; Atuahene *et al*., 1987). Sheanut cake is a useful energy and protein source in the diets of monogastrics. Sheanut cake components can be digested by chickens (Card and Nesheim, 1975). Studies have shown that 7.5% and 10% inclusion rates of rice bran in rations for laying hens had no effect on their laying performance (Ersin Sameli *et al*., 2006; Nobakht, 2007).

The major challenge associated with the use of agro industrial by- products from agricultural waste has to do with the high levels of non-starch polysaccharides which are not easily digested especially in monogastric animals. The non- starch polysaccharide cell walls are made up of cellulose, hemicellulose, lignin, and pectin fractions in the by-products. Cassava peels and their tuber remnants may contain low protein, high levels of phytate, hydrogen cyanide (HCN) and polyphenols (tannins) (Akpan and Ikenebomeh, 1995). Other anti-nutritive factors are alkaloid, saponins, triterpenoid, mimosine, cyanide, and other enzyme inhibitors.

The constraints of these ant-nutritive factors are that they can impair the availability of nutrients, depress feed intake, lower digestibility and growth of animals that utilize them (Hathcock and Radder, 1994; Shahidi, 1997).

2.1.2 The false yam plant

The *Icacina oliviformis* plant grows with erect shooting system and leafy in nature. It grows in less rainfall areas in the savanna regions. The tubers produced by the plant are very big, fleshy and fibrous in nature. The false yam plant belongs to the family *Icacinaceae*. Other species of false yam include; *Icacina mannin*, cross River state of Nigeria (Burkhill, 1985), *Icacina trichantha*, in Western Nigeria (Umoren *et al*., 2008). False yam plant is called by different names in different places. The Gambians call it Manankaso, Ghanaians, Tarkwara; Senegalese, Kouraban or Bankanas; West Africans, Basouna; Sudanese, Pane (Kay, 1987). In Ghana, the Ashantes call it 'Abor ntupe', Ewes, 'Anyigba Fe dzi, Hausas and Dagombas call it Tarkwara (Fay, 1991).

False yam grows in the wild mostly in sandy soils in the savanna regions of Sudan, Gambia, Ghana and Senegal (Fay, 1987). The plant flowers and produces bright-red fruits containing single seed and large underground tuber.

2.2 Uses of false yam

2.2.1 Fruit of the false yam

The false yam bears green fruit which turns reddish when ripe. The flesh of the fruit is eaten as snack by children (Dalziel, 1984). NAS (2008) has reported that the fruit may contain vitamin C. False yam seeds are rich in carbohydrate and contain 8% protein (NAS, 2008). The seeds when soaked in water are ground to make flour. The flour can be used for varieties of food (Styslinger, 2011).

2.2.2 False yam seed, tuber and leaves as animal feed

The false yam seeds, leaves, and tubers can be processed to feed animals (Fay, 1987). The tuber contains 74.5% carbohydrate which is the main energy source in poultry ration. Studies by Dei *et al*. (2011b) have reported the use of the false yam tuber and the seeds in poultry diet by soaking and boiling to reduce the anti-nutritive factors. According to Ansah and Aboagye (2011), the false yam leaves can be used to feed rabbits.

2.2.3 Medicinal properties of the false yam plant

The leaf twigs of the false yam in decortions are used for internal haemorrhages, for cough and all chest infections (Burkhill, 1995). The tubers are believed to cure malaria and constipation (Asuzu and Abubakar, 1995). Burkhill (1995) has reported that species of false yam called *Icacina tricahntha* are used as aphrodisiac and can be used to treat soft tumours and mumps (Rufus, 2010). Methanol extract of *Icacina trichantus* has been used significantly to reduce high blood glucose concentration in the mice (Ezeigbo, 2010).

2.3 Nutrient composition of false yam tuber

Table 2.1 and Table 2.2 show the comparison between the nutrient and amino acid composition of false yam seed and the tuber compared to cocoyam, sweet potato and cassava and other root and tuber crops. The protein content of the tuber is low ranging from 4.4-10.4% and fat (0.7-1.74%). Data from Table 2.1 showed that the tuber contains soluble carbohydrate of 51.3 to 74.8%. A higher crude protein value of 16.45% has been reported by Osei *et al*. (2013a) which is more than those reported by NRI (1987) and Dei *et al*. (2011b). The crude fibre content varies from 3.1% (Osei *et al*., 2013a) to 4.3% (Dei *et al*., 2011a). The ash value of the tuber is 1.7% (Osei *et al*., 2013a) and 2.8% (NRI, 1987; Dei *et al*., 2011a).

The variations in the values of the proximate analyses may be due to differences in analytical methods and techniques used and the geographical location, the variety and maturity state of the plant (Okai *et al*., 1995).

The false yam seed has crude protein ranging from 7.4 - 14% and carbohydrate from 65.5 - 80.7% (Table 3). The seed has low fat content. Kay (1973) has reported a fat content of 0.1% and 0.99% by Dei *et al*., (2012a). The ash content reported by Kay (1973) was 0.5% and 0.63% by Dei *et al*. (2012d).

A report by Scott *et al*. (1947) showed that maize, cereal grain for feeding monogastrics, has high energy. The maize grain contains 87% DM, 2.1% ash, 10.2% CP, 4.8% fat, 79.5% NFE, and ME of 16.4 MJ/kg DM (Larbier and Lecltercq, 1994). The nutrient content of the false yam and the seeds are comparable to that of other root tubers like sweet potato, water yam; however, the false yam tuber and seed have higher gross energy than wild cocoyam and cassava.

False yam tuber and seed have similar ash content but higher fibre contents which are comparable to those of wild cocoyam. The raw seed meal of the false yam has higher essential amino acids such as arginine, methionine, phenylalanine, isoleucine, valine, methionine, threonine which are relatively lower in the tuber (Table 2.2). The low amino acids in the false yam tuber and processed seed meal as compared with root and tubers such as cassava calls for dietary supplementation with high protein feeds (Swaminathan and Koclar, 1989).

Table 2.1: Chemical compositions of false yam tuber and seed samples compared with other root and tubers (% dry matter)

False yam tuber sample	Dry matter	Crude protein	Ether extract	Total carbohydrates	Crude fibre	Ash	Nitrogen free extract	Gross energy (kcal/kg	Sources
False	**Yam**	**Tuber**	**Samples**						
Fresh tuber	41.0	4.4	1.6	84.5	-	-	-	-	Fay, (1991)
Dried tuber	88.3	10.3	0.7	74.5	-	2.8	-	-	NRI, (1987)
Sun-dried tuber	86.46	5.41	1.6	53.1(starch)	-	2.2	-	4,067	Dei *et al.*, (2011a)
Sun-dried tuber	87.5	16.45	1.14	-	3.1	1.7	-	3,565	Osei *et al.*, (2013a)
Soaked (9days tuber)	85.5	5.9	1.5	-	15.7 (NDF)	2.1	74.8	4,067	Dei *et al.*, (2010)
Boiled (2hours) tuber	85.7	6.46	0.98	-	4.3 (NDF)	2.76	-	4,139	Dei *et al.*, (2011a)
Boiled (2hours) tuber	86	8.84	1.74	-	3.7	2.33	-	3,677	Osei *et al.*, (2013a)
				False yam seed samples					
Raw seed	81.7	0.5	80.7	-	-	-	-	-	Fay, (1991)
Raw seed	87.0	-	72	0.1	0.5	-	-	-	Kay, (1973)
Raw seed	87.0	0.1	72	-	-	-	-	-	NAS, (2008)
Soaked (9 days) seed	85.9	7.4	0.4	65.5 (starch)	1.7	0.5	-	3,660	Dei *et al.*, (2012c)
Soaked (15days) seed	86.1	8.4	0.56	-	9.9 (NDF)	6.3	80.6	4,282	Dei *et al*, (2012d)

Table 2.1 continues

Other root tubers	Dry matter	Crude protein	Ether extract	Total carbohydrate	Crude fibre	Ash	Nitrogen free extract	Gross energy (kcal/kg)	Sources
Fresh cassava root	37.0	1.0	0.3	-	4.4	-	-	1,141	Woolfe (1992)
Fresh cassava root	32.0	2.2	0.1	-	5.1	2.8	89.0	-	Jalaludin (1997)
Sun-dried cassava root	-	2.9	1.4	-	5.0	2.3	88.4	-	Ravindran *et al*., (1992)
Boiled cassava root	28.3	0.38	0.04	27.4	0.5	0.46	-	1,112	Favier (1977)
Fresh sweet potato	30.0	1.5	0.3	-	3.9	-		-	Woolfe (1992)
Sweet potato	-	4.4	0.6	-	6.9(NDF)	3.10	-	4,091	Noblet *et al*., (1990)
Raw wild cocoyam	92.69	7.21	4.69	-	1.48	5.13	81.58	1,246	Onu and Madubuike (2006)
Cooked wild cocoyam	90.70	7.15	4.22	-	1.49	5.12	82.02	1,246	Onu and Madubuike, (2006)
Fresh water yam	24.0	2.4	0.1	-	1.2	-	-	1,104	Woolfe (1992)
Raw water yam	93.21	10.27	1.15	-	2.31	2.93	76.57	3,756.5	Ezeoch and Ojimelukwe (2012)
Boiled water yam	95.26	8.11	0.15	-	1.52	2.48	83.02	3,639.4	Ezeocha and Ojimelukwe (2012)

Table 2.2: Comparative amino acid compositions (% dry matter) of false yam tuber and seed samples with other root and tubers

Amino acid	Sun-dried tuber	Soaked tuber	Raw seed	Soaked seed	Sun-dried cassava root	Fresh sweet potato	Cocoyam	Sun-dried white yam
Arginine	0.792	0.08	0.94	0.51	0.29	0.34	-	0.660
Glycine	0.094	0.08	0.45	0.32	0.01	-	-	0.525
Histidine	0.115	0.16	0.21	0.16	0.07	0.16	-	0.520
Isoleucine	0.079	0.08	0.62	0.43	0.03	-	0.219	-
Leucine	0.124	0.13	0.75	0.50	0.31	0.57	-	0.750
Lysine	0.192	0.24	0.29	0.19	0.07	0.41	0.241	0.710
Phenylalanine	0.042	0.07	0.44	0.31	0.03	0.54	0.316	0.530
Methionine	0.003	0.01	0.05	0.04	0.03	0.11	0.84	0.548
Threonine	0.077	0.07	0.36	0.24	0.03	0.34	0.257	0.443
Tryptophan	0.021	0.05	0.08	0.01	0.29	-	0.88	-
Valine	0.102	0.13	0.46	0.34	0.04	0.52	0.382	0.333
Tyrosine	-	-	0.28	0.18	0.01	0.18	0.226	0.410
Cystine	-	-	-	-	-	0.16	0.163	0.527
Sources	Dei *et al*. (2011a)	Dei *et al*. (2012a)	Dei *et al*. (2012c)	Dei et al. (2011c)	Gil and Buitrago (2002)	Gohl (1982)	FAO (1970)	Ogunlade *et al*. (2011)

2.3.1 Anti-nutritive factors in raw and processed false yam tuber

Anti-nutrients are substances or chemicals in feed and water which affect the absorption of nutrients in the gut of animals causing depression in growth and in extreme cases food poisoning, Hegarty (1978). The presence of some of these anti-nutrients can be reduced through processing methods and making the false yam a valuable feed resource in the livestock industry.

Studies by several authors have shown that cooking, soaking, fermentation, chemical treatment may reduce or inactivate the anti-nutritional factors. The use of one or combination of methods of processing may prove more efficient than only one method.

2.3.2 Terpenes, (gum resins)

A report by Vanhaelen *et al*. (1986) has shown that the false yam and the seed contain a chemical compound called gum resin, also known as terpenes. Studies by Dei *et al*. (2011a) have reported a value of 3.75% terpenes higher than 0.9% - 2.8% reported by NRI, (1987).

2.3.3 Hydrocyanic acid

False yam varieties such as *Icacina trichantha* and *Icacina senegalensis* have anti-nutritive factors such as phytic acid, oxalic acid, alkaloids, hydrocyanic acid (Umoh, 2013; Umoren *et al*., 2008). Studies by Dalziel (1984) have shown that raw false yam has 0.53mg/100g hydrocyanic acid while blanched oven dried products of the false yam recorded the least value of 0.23 mg/100g. Steeped false yam tubers which have been dried in the sun and oven contain 0.26 mg/100g hydrocyanic acid compared with steeped oven dried (0.25mg/100g). However, higher values of 3.2 to 60 mg HCN/kg have been reported in fresh wild yam tubers (Bhandari and Kawabata, 2004). Bradbury and Holloway (1988) in their studies, reported 19.4mg/kg HCN for roasted garri and 2.5 mg/kg HCN for cassava flour plus water. Hydrogen cyanide levels in some false yam varieties, if present at all, are below the safety level of 0.5 to 3.5 mg/kg body weight which is considered lethal dose for human poisoning by cyanide (Bradbury, 1991). The estimation of hydrogen cyanide in fresh cassava ranges from 15 to 400 mg HCN/kg fresh weight. The presence of hydrogen cyanide, in the form of cyanogenic glucoside in feed when consumed by animals, affects some physiological functions, for example hydrgogen cyanide interferes with the activities of cytochrome oxidase an enzyme associated with the respiratory system which can cause death in the animal.

2.3.4 Oxalic acid

Raw false yam has 256.9 mg/100g total oxalate (Chinma and Igyor, 2007). Raw sample (98.25mg/100g soluble oxalate), blanched oven dried (48.40mg/100g), and blanched sun-dried (92.40 mg/100g soluble oxalate) (Chinma and Igyor, 2007). According to Chinma and Igyor (2007), oxalate in leafy vegetables ranges from (24.65 to 46.22 mg/100g), which is lower than that found in false yam tuber. Bhandari and Kawabata (2004) have reported oxalate levels of 0.06 to 0.197mg/100 g in wild false yam tubers. Onwuka (2005) concluded in their findings that the lethal dose level of oxalate in human beings is 2 to 5 g.

Calcium oxalate level in some varieties of cocoyam is about 780 mg/100g (Bradbury and Holloway, 1988).

2.3.5 Tannin

The tannin content of raw false yam is 21.65 mg/100 g (Chinma and Igyor, 2007). The amount of tannin in blanched sun dried false yam tuber (21.24mg/100g) is higher than steeped sun dried flour (5.13mg/100g). According to Chinma and Igyor (2007), tannin values reported in blanched and steeped false yam tuber are higher when compared to leafy vegetables (0.32 to 0.83 mg/100g) grown in the tropics. In addition, Okwu and Ndu (2006) have shown that the tannin levels in aerial yam 0.08 mg/100g, bitter yam 0.09 mg/100g and water yam 0.04 mg/100g are much lower than those reported for processed false yam products (5.3 to 21.24 mg/100g).

2.3.6 Phytate

Phytate value of false yam ranges between 1.67 and 3.85 mg/100g (Igbedioh *et al.*, 1994). Studies have shown that raw false yam contains phytate level of 3.85 mg/100g, blanched sun-dried of 2.27 mg/100g, and blanched oven dried a least value of 1.671 mg/100g (Igbedioh *et al.*, 1994). In other studies the amount of phytate in pigean pea (0.2%), bambara beans (0.29%), wild yam tubers (0.18

to 0.36%) groundnut (0.29%), cassava (0.4%) and maize (0.16%) are much lower than those reported for processed false yam products (Adeyeye *et al.*, 2000).

2.3.7 Alkaloid

A report by Gernah *et al*. (2007) showed that alkaloid in African locust bean fruit pulp (17.60 mg/100g) is higher than that in the raw false yam (3.12 mg/100g). Again, they found out that alkaloid levels in blanched oven dried (2.17 mg/100g) and blanched sun dried false yam (1.37 mg/100g) were lower than what has been reported in the raw false yam. Furthermore, the alkaloid in some yam varieties such as yellow yam (0.68 mg/100g), water yam (0.74 mg/100g), white yam (0.38 mg/100g), aerial yam (0.88 mg/100g), were also lower than those found in raw false yam. However, Okwu and Ndu (2006) reported that the alkaloid content of bitter yam is comparable to that in blanched sun dried false yam (1.37mg/100g). Gernah *et al*. (2007) concluded that the alkaloid contents in steeped sun-dried (2.05mg/100g) and steeped oven dried (1.950mg/100g) were lower than alkaloid in the raw fresh tuber of the false yam. The alkaloid content of blanched sun-dried false yam is (1.37 mg/100g) which compares favourably with that of bitter yam (1.68 mg/100g) (Okwu and Ndu, 2006). Also, the alkaloid in potato, potato glycol in doses of 20 mg/100g causes intestinal upsets (Okwu and Ndu, 2006).

2.4 Anti-nutritional factors and their effect on monogastric animals.

Anti-nutritional factors such as saponins, alkaloid, phytates, oxalates, triterpenes, tannins, hydrocyanic acid etc. have physiological effects on monogastric animals such as; reduction in feed intake, growth, digestibility, and reduction in mineral absorption.

2.4.1 Reduction in feed intake

Studies have shown that saponins in alfalfa taste bitter and foams in water. The presence of this anti-nutrient reduces feed consumption in monogastric animals (pigs and chicks) (Cheeke and Shull,

1985). Shequier *et al*. (1989) have also reported similar observations when processed leaves of *Sesbania sesban* having saponin content of 0.71% were included in a maize basal diet fed to broiler chickens.The toxic compound, canavanine, in jack bean has been found to reduce feed intake at 300g/kg in the diet of non-ruminants (Tschiersch, 1962). Mucuna bean meal inclsusion in poultry ration caused a decrease in feed consumption of layer birds and this was attributed to the presence of bioactive factors such as trypsin and tannins in the mucuna seeds (Carew *et al*., 2003). Saponin content of 9g /kg has been reported to cause a decrease in feed consumption in broiler chicks (Jenkins and Atwal, 1994) and the reason assigned was the bitter taste.

2.4.2 Reduction in growth performance

Hegarty (1978), in their studies, observed that when 5-10% *Lucaena leucocephala* leaf meal was included in the ration for rabbits, pigs and poultry, the results showed a considerable decrease in growth of all the animals. According to Hegarty (1978), the presence of mimosine in the leaf of *Lucaena leucocephala* combined with the pyridoxyl destroying the activities of B_6-containing enzymes transaminase. Also, mimosine immobilizes metals such as zinc needed by transaminase enzymes and hence inhibiting the activities of amino acids.

Tannins values of 5-20 g/kg in faba beans and sorghum grains incoporated into the ration of broiler chikens led to poor growth performance in the birds and sometimes causing deaths (Igi *et al*., 2004). Lectin (50%), trypsin (40%) and other anti-nutritive factors (10%) in raw soya beans caused a reduction in the body weights of the rats placed on unprocessed soya beans (Igi *et al*., 2004). Olumu (1995) has reported that goitrogenic substances present in soya and groundnut caused enlargement of the thyroid gland.

Thyroid gland secretes thyroxine, which is an important hormone responsible for important chemical reactions in the animal body. Inadequate production of this hormone may cause a reduction in growth of animals. Cocoa pod husk meal when supplied in greater quantities to animals

especially, the monogastrics, the concentration of theobroma in the cocoa pods caused a decrease in the weights of these animals (Owusu-Domfe, 1972; Clarke and Clarke, 1979; Penkam, 1984; Atuahene *et al*. (1998). Processed cassava based diets are known to cause reduction in body weights of rats and pigs (Tewe *et al*., 1976; Tewe and Maner, 1981; Tewe, 1983). They attributed the reduced growth rate to HCN. The residual HCN in the processed cassava tuber inhibited the thyroid gland to take up iodine thereby caused a decrease in the quantity of thyroxine needed by the animal for body development (Tewe, 1991). According to MacDonalds *et al*. (2002), terpenes impair availability of nutrients hence reduce growth rate.

2.4.3 Reduction in digestibility

Cheeke (2005) defined digestibility as the breakdown of complex food nutrients into simple and absorbable form. Jenkins and Atwal (1994) have reported the effect of saponins on the digestion and absorption of dietary lipids, cholesterol and bile acids. Pusztai *et al*. (1981) have observed that lectin present in legumes impair the absorption of amino acids and carbohydrates. According to Thompson (1993), the presence of the lectins found in the legumes interferes with the lining of the small intestines causing it to be bound to the epithelial cells. This affects absorption of digested nutrients (Lajolo and Genoverse, 2002).

Phytic acid in mucuna leaf meal combines with protein to form phytate protein complex (Singh and Krikorian, 1982). According to Reddy *et al*. (1982), the phytate protein complex formed affects digestion of protein in the gastrointestinal tract of animals due to the inhibitory effects on pepsin, trypsin, and chemotrypsin, thereby affecting the bioavailability of amino acids (Singh and Krikorian, 1982).

Non-starch polysaccharides (NSP) affect availability of energy and other nutrients in the gut. They form viscous gel and hence interfere with enzyme activities and nutrient absorption (Choct, 1999). According to Huisman and Vander Poel (1989), tannin in faba beans forms cross link with protein

and glycoprotein thereby disrupting the activities of digestive enzymes. Studies by Shimoyamada *et al*. (1998) have shown that saponin in legume seeds form saponin-protein complexes thereby reducing protein digestibility. Mohammed and Dei (2013) have reported a decrease in protein and fat digestion when soaked false yam was fed to layer chickens. They attributed the decrease in weight to the activities of gum resins present in the false yam.

2.4.4 Reduction in mineral absorption

Studies by Khare (2000) have shown that most food materials store phosphorus in the form of phytate in their seeds and brans. Phytate interferes with the absorption of nutrients and other mineral elements especially cations like Fe^{+2}, Zn^{+2}, Mg^{+2}, Ca^{+2} in the small intestine forming phytate complex. The enzyme phytase is absent in animals hence affecting the digestion of phytate when diets with high levels of phytate are present in the gastrointestinal tract. Also, phytate complex formed in the small intestine intereferes with the absorption of lipids and proteins (Leiner, 1989). The stepwise reaction of phytic acid in the small intestines of animals has been proposed by Woyengo and Nyachoti (2011). According to them, the phytic acid in a neutral pH environment is incompletely broken down. The partially dissociated phytic acid reacts with metallic cations such as Fe^{+2}, Zn^{+2}, Mg^{+2}, Ca^{+2} to form insoluble complexes (Lyon, 1984). The level of percentage precipitation is highest in zinc (99.5%), followed by calcium (83%), iron (75%) and the least in magnesium, (62%) (Prattley *et al*., 1982), Contrary to this, Davies and Olphin (1979) observed variations in the percentage precipitation of cations in the gastrointestinal tract to be 98, 91, and 80% for zinc, copper and manganese respectively. Erdman (1979) concluded that monovalent mineral elements interact loosely with phytic acid compared with divalent mineral cations. Scheuermann *et al*. (1988) also concluded that monovalent mineral elements are not affected by the presence of phytic acids.

Olumu (1995) reported 0.38% of phytic acid in pigeon pea while Phytic acid content of 624 mg/100g has been reported in cassava root (Marfo *et al*., 1990). Chemical interactions between phytic acid and calcium results in the formation of calcium oxalates which is insoluble preventing calcium absorption and metabolism in the animal's body.

Lonnerdal *et al*. (1989) in their studies examined the caecal contents of rats and realized that Ca^{2+} ion concentration has moved from 0.45 to 17.5% as a result of calcium in the feed supplied to these animals. They attributed the increase in Ca^{2+} ions to the presence of phytic acid which inhibited digestion and absorption. Again, in another study, Zn^{2+} absorption decreased by 84% in the small intestines of rats. A cereal meal containing 0.13% concentration of phytic acid fed to rats caused a 33% reduction in the absorption of Fe^{3+} in the gastrointestinal tract of rats (Kim *et al*., 1993). Furthermore, Rubio *et al*. (1994) in their studies concluded that a diet containing 0.3% phytic acid could reduce absorption of Zn from 42% to only 18% in rats.

2.4.5 Effect of anti-nutritive factors on haematology and serum biochemistry

Activities of enzymes in body fluids are necessary indicators whch help to detect whether diseases are imminent or a particular organ in the body is malfuntioned. Nabil *et al*. (2011) in their studies observed that *Jatropha curcus* seeds when fed to broilers increased the level of blood serum enzymes such as alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. They attributed the effect to anti-nutritive element in the *Jatropha curcus* which might have caused damage to the liver cells (hepatocytes) causing the leakage of these enzymes into the blood stream.

According to Aniagu *et al*. (2004), the level of ALT in the cystosol hepatocyte of the liver is a better indicator than ALP and AST. The level of this enzyme therefore can be used to assess the extent of damage to the liver by anti-nutritive factors. The presence of bilirubin in the blood serum is standard indicator of liver diseases. An increase in the concentration of bilirubin in the blood serum is an

indication of hepatocellular damage or the obstruction of the hepatic biliary tract of the liver (Samia *et al*., 1992). The levels of creatinine and urea concentration in the blood serum are important indicators of the proper functioning of the kidney (Ojo *et al*., 2013). The liver breaks down creatine to creatinine which is a means by which ammonia is removed from the body through blood vessels in the glomerular capsule of the kidneys. When there are defects in the kidneys, the filtering rate of the creatinine is affected leading to a rise in the ammonia concentration in the blood. Again, defects in the kidney may lead to an increase in urea in the blood (Sumiati *et al*., 2007; Ojo *et al*., 2013). *Jathropha curcas* inclusion in the diet of broilers caused a rise in cholesterol and reduction in the concentration of the protein (hypoproteinemia) (Eisenbarth *et al*., 2007). The hypoproteinemia observed might have been caused by phorbol esters, an anti-nutritive element present in the *Jatropha curcas*, which could have caused damage to hepatocytes and death of cells where proteins are manufactured. Again, phorbol ester activities disrupt the functions of protein kinase C (PKC) and activities of other enzymes associated with protein synthesis for example, DNA polyamines and other acivities like cell gene expression, processes and cell differentiation (Goel *et al*., 2007; Nabil *et al*., 2011). According to them, this could have caused hypoproteinemia in the serum of the chicken fed with graded levels of *Jatropha curcas*. Reddy *et al*. (1982) also attributed the decreases in total protein to inhibition of protein utilization in the broilers. Increase in serum cholesterol in broilers fed graded levels of *Jatropha curcas* could have been caused by decreased in activities of lipase enzymes produced by the pancrease as a result of inhibition activity of the phorbol esters on insulin production (Liu and Lin, 1997; Goodman and Gilman, 1985; Ojo *et al*., 2012). Omage *et al*. (2002) have reported similar observation in the rise of cholesterol level in the blood of chickens blocking arterial veins leading to the heart and eventually affecting the heart of the chicken which can lead to death of the birds.

Amaefule (2002) has reported the presence of haemaglutinin in pigeon pea which affects blood formation and consequently reducing pack cell volume. Akinwutimi *et al*. (2004) have reported a decrease in haemoglobin and red blood cell counts compared with an increase in white blood cell counts when more than 50% tiger nuts meal was fed to broiler chickens. According to Tewe (2006), high cyanide levels in the diet of growing pigs induced haematological changes in these animals.

2.4.6 Toxicity

High levels of hydrocyanic acid in cassava can cause death in animals because it inactivates cellular respiration thereby causing death of poultry (Salkowski and Penny, 1994). *Napoleona imperialis* seed meal contains oxalate an anti-nutritive element which at higher level of inclusion in diets of animals affects the absorption of calcium ions and also causes irritation and wearing of the gastrointestinal tract which can cause mortality in animals (Kumar and D'Mello, 1991). Raw sorghum containing tannins above 30-70g/kg concentration in diets for poultry can cause death (Salunke and Chavan, 1990; Farrel *et al*., 1999; Iji *et al*., 2004).

2.5 Processing methods on anti-nutritional factors

Anti-nutritional factors are chemical materials in feed and water which inhibit dietary nutrients digestion and absorption in the gastrointestinal tract of animals. The nutritional value of the false yam can be improved by the use of processing methods such as soaking, boiling, heat and chemical treatment etc. which can eliminate or inactivate bioactive factors.The use of combination of methods of processing may prove more efficient than only one method.

2.5.1 Heat treatment

Heat treatment, as a processing method includes; cooking or boiling, the use of moist heating and dry heating. Cooking or boiling causes volatile compounds in feed to evaporate and also reduces

chymotrypsin and trypsin inhibitors and other soluble materials if the solution in which they are cooked or boiled is poured away (Amaefule and Obioha, 2001).

The use of heat treatment inactivates anti-nutritional factors for example, in tropical legumes such as cowpea, soya beans, pigeon pea, and lima beans.

Processing leguminous feed by heating improves protein quality and physiological activities against trypsin and haemaglutinin in these feed by disrupting and breaking the peptide bonds in the protein. This exposes the unfold structure of the protein to improve digestive enzymes intereactions (Sathe *et al*., 1984). According to D'Mello (1982), the extent to which anti-nutrients are removed from a feeding material are associated with temperature, size of feed particle and duration of heating and which type of heating methods adopted whether moist heating or dry heating. They concluded that moist heating is better than dry heating in reducing anti-nutrients in feed materials. When *Phaseolus vulgaris* are cooked in water and the solution is discarded, 30-40% of the anti-nutritive factor polyphenol is removed (Bressani and Elias, 1980). A study by Udedibie and Nwaiwu (1988) boiled jack beans at different durations of 30, 60, 90 and 120 minutes. Phytochemical examination of the raw and cooked beans showed that 60 minutes cooking of the beans significantly reduced the heat sensitive bioactive chemicals such as alkaloid, terpenoids, hydrogen cyanide and saponins (Amaefule and Obioha, 2001).

Raw and heat treated pigeon pea seed meal diets were fed to broiler chickens. The growth performance of birds on the raw diet was poorer compared to birds fed on the heat treated beans (Amaefule and Obioha, 2001).

In another study, Kaankuka *et al*. (2000) boiled soya beans for 30 minutes or lesser time 20 minutes and later fed to growing pigs. The growth of pigs on the 30 minutes boiled beans diet was better than pigs fed 20 minutes boiled soya meal diet. According to Ogundipe (1980), water vapour treated

soya beans in the diet of broiler chickens resulted in higher weights when compared with cooking, boiling, and roasting.

Boiling reduces cyanogenic glucoside in sweet potato, yellow yam and cocoyam. It also removes phytic acid in cocoyam (Omoruyi *et al*., 2007). Trypsin inhibitor in kidney beans, cowpea and pea was considerably reduced when the beans were subjected to cooking, roasting, autoclaving, microwave cooking and boiling (Khattab and Arntfield, 2009). Trypsin inhibitor activity in soya beans was reduced by boiling soaked soya beans for different time periods compared with the raw seeds (Rasha *et al*., 2011). Subjecting soaked soya beans to different times of boiling and comparing the levels of trypsin inhibitors to that of the raw seeds, they concluded that soaking and boiling were better methods of processing than a single method.

In addition, they realized that the most effective methods of inactivation of trypsin inhibitor activities are boiling (90 min) and autoclaving (for 10 min). Boiling white kidney beans reduced the phytic acid concentration by 21% in the red and 24% in the white beans (Rehman and Salariya, 2005). According to Iyayi and Taiwo (2003), dry heating treatment can reduce the non-protein factor, L-Dopa (L-3, 4-dihydroxyphenylalanine) in mucuna bean. Siddhuraju *et al.* (1998) have also reported of similar reductions of L-Dopa in mucuna after roasting the seeds.

2.5.2 Soaking

Soaking is a processing method used to reduce soluble anti-nutritional substances in feed or foodstuff, by discarding soaking solution. Vidal-Valverde *et al*. (1992) have suggested that chemical constituents in soaked feed materials may interact in the soaked solution resulting in a decrease of the chemical constituents in the feed. Feed ingredients containing saponins when soaked in water the anti-nutritive element (saponin) which is bitter is reduced. The treated feed ingredients became more acceptable to animals when used in diets (Joshi *et al*., 1989).

Baltra *et al*. (1986) observed 58-66% reduction of anti-nutritive element called trypsin in lentil seeds when the seeds were left in water for one day. Bioactive substances in jack beans such as canavanine and canaline which are water solube are completely reduced when the seeds are soked in water (Leiner, 1980). Dei *et al*. (2011a) reported a false yam inclusion level of 5-10% in layer diet after soaking for 14 days to reduce the antinutritive factors in the false yam tuber and seeds. Soaking cassava and changing of the soaking medium over a period of days, reduced cyanogenic glucoside in cassava (Cooke and Maduagwu, 1985). Prolonged soaking of cassava tubers' for six days in water reduced hydrocyanic acid by 97% (Bourdoux *et al*., 1983). Elhady and Habiba (2003) have reported the presence of phytic acid in beans. After soaking the beans in water for 24 hours the concentration of the phytic acid was reduced by 36%. Trypsin inhibitor activity was reduced from 9-18% by hydration (Shimelias and Rakshit, 2007). Erdman and Pneros Schneirer (1994) reported a reduction in polyphenol and oxalate that inhibit the absorption of calcium and iron by soaking. Aqueous extraction methods have been employed in the removal of chlorogenic acid in sunflower seeds (Dominguez *et al*., 1993).

Moneam (1990) has reported the use of soaking and cooking to enhance the removal of anti-nutritive factors inhibiting protein and starch enzymes activities in food substances. These methods removed cyanide concentration (20%) from fresh cassava root tubers.

The concentration of phytic acid in beans was reduced by 57 - 58% after soaking in water for 12 hours before cooking (Nergiz and Gokgoz, 2007). Okai *et al*. (1995) have reported the presence of tannin an anti-nutritive factor in shea kernels. However, after processing kernels by soaking and boiling in water, the tannin concentration was reduced by 40-70%.

Soaking of dehullled and whole bean of mucuna in water for 24 hours reduced non protein factor L-Dopa (L-3, 4-dihydroxyphyenylalanine) by 30% while in the whole bean by only 6% (Nyirenda *et al*., 2002).

2.5.3 Fermentation

According to Frazier and Westhof (1988), fermentation is a process of using bacteria, mould and yeast to metabolize raw material, forming new products with higher value than the initial material. One of the several functions of fermentation according to Steinkraus (1995) is the enrichment of the diet and removal of toxic components. Lactic acid, an organic acid, was used to breakdown saponins and tannins in feed materials, thereby detoxifying these compounds (Yosioka *et al*., 1996). Fagbemi *et al*. (2005) in their studies reported that trypsin and phytic acids in oil seeds were reduced drastically after subjecting the seeds to fermentation processes. Tannin in shea nut meal was reduced by wet incubation of sheanut meal (Annongu *et al*., 1996).

2.5.4 Chemical treatment

Ologhobo *et al*. (1993) in their study used four different solvents (ether, ethanol, alkali and acid) to extract the anti-nutritive factors in faba beans. They concluded that the alkali was a better solvent extractor of the anti-nutritive elements than that of the ether, alcohol and acid. In a similar study by D'Mello and Walker (1991), potassium bicarbonate was used to detoxify jackbeans. Canavanine, the anti-nutritive factor solubilized in the alkali.

Fernandez *et al*. (1993) used (0.07%) sodium bicarbonate and 0.1% citric acid solutions to extract trypsin inhibitors in faba beans by soaking in their respective solutions. The sodium bicarbonate solution extracted more of the trypsin inhibitors than the ctric acid solution. According to them, the trypsin inhibitor is more stable in citric acid solution. Again they realized that the use of urea in detoxifying these native proteins resulted in the interaction between the hydrogen bonds in the trypsin inhibitor with the urea leading to the breaking up of the peptide bonds in the anti-nutritive elements rendering them less reactive and thereby exposing their structures to more reactive enzymes (Rawn-David, 1983).

Udedibie and Nkwocha (1990) used (3%) aqueous solution to soak jack beans for one week. They reported the release of strong ammonia gas odour. The treated beans were washed with fresh water, boiled for 60 minutes, dried at a temperature of 80ºC and milled. The milled jack bean meal was used in a feeding trial on broiler chicks. They concluded that 25% of the jack bean meal in a chicken diet was tolerable for the broiler chickens. In another experiment, 2.5% of urea was mixed with crushed jack beans and stored for one week in a sack. After one week the mixture was toasted for 20-30 minutes in a hot pan until the product was crispy. The toasted jack bean meal (10 - 20%) were used to prepare rations for finisher broiler chickens and fed for 35 days. Udedibie *et al*. (1994) concluded that 20% urea treated jack bean meal was tolerable for the broiler chickens.

2.6 Processed false yam tuber and their nutritional value for broiler chickens

Studies by Dei *et al*. (2011a) have shown that when false yam tuber is chopped into pieces, sun-dried for 5 days and milled, it can be included in maize based diet for chickens at 3% on weight by weight basis. The studies showed that the partial substitution did not affect growth performance of chickens. In another experiment, they evaluated the effect of soaked false yam tuber meal on growth performance of broilers. In that experiment, chopped false yam tuber was soaked in water for 9 days, sun-dried on concrete floor, and milled into gritty powder. The milled powder replaced maize at inclusion levels of 0, 30, 60, and 90, g/kg in a diet for broiler growers fed from 21 to 56 days of age. The proximate nutrients in the sun –dried false yam tuber meal, compared with that of maize appeared to be similar except those of the crude protein in sun dried false yam (5.9%), maize (10.2%) and neutral detergent fibre in sundried false yam (15.7%) and maize (2%). The nitrogen free extract in the tuber was high (74%) indicating a high concentration of soluble carbohydrate such as starch. The high gross energy content of 17MJ/kg DM recorded for the sun-dried false yam tuber was attributed to the high content of carbohydrate (Dei, 2011a). Feed intake of the control birds was comparable to birds on the processed false yam tuber indicating that the processing

method of soaking reduced the bitter content in the feed material thereby increasing feed intake. In the same way, feed utilization in the control birds was similar to birds fed on processed false yam tuber meal, indicating similar growth rate.

In another experiment Dei *et al*. (2011a) the soaking duration was extended from 9 days to 15 days and the product replaced portions of the maize on weight by weight basis in maize-fish meal diet at 120 and 150g/kg.

The proximate nutrients in the sun-dried false yam tuber meal were similar to what was reported earlier in terms of the energy and the soluble carbohydrate. However, certain important amino acids found in maize, were higher than in the tuber. They are; phenylalanine, glycine, leucine, arginine, isoleucine, valine, methionine and threonine. A low crude protein content of the tuber demands that other ingredients high in protein are included in order to balance for the low protein. Studies by Dei *et al*. (2011a) showed that sun-dried false yam tuber meal when included at 12 and 15% affected the development of broiler chickens due to decreased feed consumption. The higher inclusion level of the false yam tuber meal was based on the assumption that prolonged soaking would further improve its nutritional value. The reduction in growth was attributed to residual bioactive substances still present in the sun-dried false yam tuber meal. According to MacDonald *et al*. (2002), terpenes reduced availability of nutrients and reduced growth performance of animals.

Studies were carried out by using salt petre (0.1 molar Concentration) to soak false yam tuber for 12 days during which the solution was discarded every three days (Dei *et al*., 2013a). At the end of the 12 days, the samples were rinsed in fresh water and dried in the sun before grinding into powder. The basis of using the saltpetre (potassium nitrate) was that it interacts with anti-nutrients such as terpenes and reduced its presence in feed materials (Pommer, 2003).

The processed product replaced portions of the maize on weight by weight basis for maize fishmeal diet at 80, 100 and 120 g/kg. The studies showed that saltpetre treated fasle yam product diet improved the performance of the birds and up to 120 g/kg diet was tolerable (Dei *et al*., 2013a).

In another experiment, false yam tuber was soaked in salt solution at a concentration of 0.1%, dried, milled and used as replacement for maize-fish meal based grower diet at 80,100, and 120 g/kg. They observed that salt treated false yam meal diet affected development of the broiler chickens (Dei *et al*., 2013b).

2.6.1 Boiled false yam tuber

Dei *et al*. (2011a) fed boiled false yam tuber meal diet to broiler chickens to evaluate the growth response of the birds. The chopped tuber was boiled in water for 2 hours at a ratio of one part of fresh sliced false yam tuber to one part of water. The water was poured away and the samples were dried in the sun for some days and then milled into a gritty meal. Portions of the maize were replaced by the boiled false yam meal on weight by weight basis (0, 30, 60, and 90g /kg) in a broiler grower diet. Broiler chickens were put on the diets from 3 to 8 weeks. The residual bioactive substance (gum resins) found in the sun-dried false yam tuber meal was 37.5 g resin/kg DM. Boiling has been effective in reducing the resins by 39% (37.5 g resins/kg DM to 22.88 g resins kg DM). The growth response of broiler chickens to boiled sun-dried false yam tuber meal is an indication that up to 90g/kg could be tolerated by the birds. In a similar experiment, boiled false yam tuber meal was used at higher dietary levels replacing maize (w/w) at 0, 120 and 150 g /kg in a grower broiler chicken diet and fed from 21-56 day of age. There was a clear indication that including boiled false yam tuber meal at 120 and 150 g /kg in the diets of broilers had adverse effect on growth (Dei *et al*., 2011a). The experiment was repeated by using boiled false yam tuber meal at 50 and 100g /kg in broiler grower diets (Osei *et al*., 2013a). They concluded that feeding the boiled

tuber meal at 100 g /kg had adverse effect on broiler performance. This reinforces the fact that the boiled tuber cannot be fed beyond 90 g /kg.

Dei *et al*. (2013c) in their studies used soaked or boiled yam tuber meal to examine the growth response of the broiler chickens. The control diet was without false yam tuber meal and the treatments diets having treated false yam tuber meal replacing portions of maize at the levels of 80, 100, or 120 g/kg were tested. They concluded that combined methods of soaking and boiling false yam tuber improved the feeding value and the birds were able to tolerate levels up to 120g /kg.

2.6.2 Fermentation

According to Antai and Nkwelang (1999), fermentation of *Icacina manni* paste for six days reduced the concentration of anit-nutrients present in the paste. The concentration of terpenes and other unidentified bioactive elements in the false yam tuber including the seeds were reduced by fermentation. In another study, Teog (2010) evaluated the extent to which fermentation can improve on the use of raw false yam tuber (*Icacina oliviformis*) meal on broilers. Fermentation was done by native microorganisms, where water was added to the raw tuber flour to form thick paste in a plastic container for three days after which it was sun-dried for 7 days. The fermented product replaced maize (w/w) at 30, 60, or 90, g/kg in maize-based grower diets. The nutritive value of fermented product for poultry was determined based on growth performance of broilers. The results indicated that feed consumption decreased when the fermented false yam meal progressively increased in the ration. This is an indication that the bitter compound in the tuber was not reduced to a level that makes the feed palatable to the birds. The reduction in feed intake resulted in growth depression and consequent poor utilization of the diets containing fermented false yam product. They suggested that the growth depression observed in the broiler chickens could have come from the bioactive compound (gum resins) in the product, an indication that natural fermentation for 3 days was

inadequate to have caused sufficient reductions in the anti-nutrients to a level that is acceptable to the birds.

2.6.3 Processed seed meal and their nutritional value for broiler chickens

Dei *et al*. (2011b), placed the seeds of false yam plant in water for 3 days while the soaked water was discarded every 24 hours. The seeds were then rinsed in fresh water and dried in the sun for 7 days and ground into gritty meal to improve its nutritional value. The treated seed meal substituted for maize on weight by weight basis at the levels of 0, 50, 75, and 100g/kg to prepare a broiler grower chicken diet and fed from 3 to 8 weeks of age. The study showed that up to 100g/kg replacement of maize was acceptable for the broiler chickens. Soaking of the seeds might have reduced the concentration of the gum resins and other unidentified anti-nutritive factors, making it more palatable for the birds.

In another experiment by Dei *et al*. (2012c), the seeds were placed in water for the durations of 9, 12, or 15, days and the soaked water was discarded every 3 days. The seeds were rinsed with fresh water and dried in the sun for 7 days and ground into gritty meals. Each of the processed product replaced maize (w/w) at 100 g/kg in a grower diet. They concluded that soaking duration has no appreciable effect on nutrient composition of the seed meal. They also stated that soaking the seeds for 12 days improved their nutritive value for broilers.

Dei *et al*. (2011b) in their studies, examined how boiling affects the nutrients in the false yam seeds. Crushed seeds of the false yam were boiled in water for 30 minutes, rinsed in fresh water and dried in the sun for 7 days and ground into gritty meal. The treated seed meal substituted for maize by weight on weight basis at inclusion levels of 0, 50, 75, and 100 g/kg in a grower broiler diets and fed from 3 to 8 weeks of age. The results showed that boiling the seed for 30 minutes and including it in the diet at 50 g/kg or more had no adverse effect on feed intake, but adversely affected broiler chick growth performance. This suggests that boiling had no effect on the bioactive compounds in

the seed. The bioactive chemicals are possible contributory factors which could have affected feed digestibility hence poor digestion and utilization of feed. It appears that boiling duration may have been too short to influence the major anti-nutritive factors in the seeds.

Dei *et al*. (2013d) examined how boiling false yam seeds under different durations affect the nutrients in the seeds on broiler chickens. The seeds of the false yam plant were boiled in water for the durations of 1, 2, or 3, hours, dried in the sun for 5 days, and milled into coarse texture. Each of the processed products was substituted for maize at 100g/kg (w/w) in broiler finisher diets.

The results indicated decreased feed consumption, feed conversion efficiency and weight gain for broiler chickens fed boiled false yam seed meal rations. The growth performance of all the chickens fed on the treated false yam seed diet was similar. This suggests that boiling may not be the most appropriate method for detoxifying the seeds for feeding broilers.

Mensah *et al*. (2013) carried out a study by feeding seeds of false yam soaked (12 days) and boiled (2 hours) for broiler chickens. The processed product replaced portions of the maize at inclusion levels of 80, 100 and 120 g/kg in maize-fish meal based grower diets. The result showed that feeding soaked boiled false yam seed meal at the levels tested resulted in lower growth performance. They observed that seeds that were boiled were quite hard therefore not easily digested since particles of the boiled seed meal were seen in the droppings. This is a further indication that boiling as a method is not appropriate for detoxifying the seeds for birds.

2.6.4 Processed tuber and seed meals and their nutritional value for layer chickens

Dei *et al*. (2012 d) studied how soaked false yam seed meal affects the development of pullets (2-5 months old) and subsequent egg production. The feeding of soaked false yam meal up to 100 g/kg in growing pullet diets showed no adverse effect on their growth. In addition, they concluded that soaked false yam seed which has been milled can be added to the diets of layer chickens up to 100 g/kg without affecting their egg laying performance (Mohammed and Dei, 2012). Soaking the tuber

prior to boiling was evaluated on growth and subsequent egg production of pullets and it was found that it had adverse effects on growth performance and subsequent egg production when included at 50 g/kg or more in the diets of pullets. Mohammed and Dei (2013) evaluated the effect of soaked false yam tuber meal on apparent nutrient digestibility of layers (19-35 weeks of age). In this study, chopped tubers were soaked in water for a duration of 12 days and the soaked water was discarded every 72 hours.The tubers were washed with fresh water, dried in the sun for 7 days and ground into coarse flour. The milled flour was used to replace portions of the maize on weight by weight basis at the inclusion levels of 0, 50, 75 and 100 g/kg layer diet and fed from 19 to 35 weeks of age. The results showed that addition of soaked false yam meal to the diets had adverse effect on crude protein and crude fat digestibility. This is an indication that the residual concentration of the resins (terpenes) after soaking might be high enough to have metabolic effect even though resins level was not determined. This could have influenced the digestibility of protein and fat in the diets. According to Macdonald *et al*. (2002), terpenes anti-nutrient affects digestion and absorption of essential nutrients in the gastrointestinal tracts of animals. Feed consumption in all the treatments were similar but egg production trend declined as the soaked false yam tuber meal increased in the diets. The increased graded levels affected protein and fat digestibility of hens. Residual concentrations of ANFs might have affected major nutrients (protein and fat) which are components of egg, hence laying performance was reduced.

2.7 Production and nutritional value of other roots and tuber crops

Cassava, sweet potato and cocoyam are good sources of energy in the diets of animals.They produce cheap food energy. They produce more dietary energy per hectare than cereals. Cassava production in the world in the year 2007 is estimated to be 288 million tons. Africa alone accounts for 52%, Ghana, 9,650 million tons, (Table 2.3). Based on the yield per hectare, sweet potato can produce 15

-20 tonnes depending on planting density. The nutrients in the root and tubers and their by-products are shown in Table 2.4.

The extent of the practical use of the root and tuber crops in non-ruminant (pigs and poultry) feeding programmes is limited by the following; They are succulent and 2/3 of their weight is water, and they have low dry matter content (25 - 32% DM). The bulky nature of the produce makes post-harvest handling difficult, increase transport costs and makes processing and preservation difficult.

The major nutrient component of root and tuber is starch. The protein content is low ranging from 2.7 - 7.9%. The by-products of root and tuber for example vines, leaves and peels contain high levels of crude fibre (12.1 - 16%). Some contain toxic or anti-nutritive factors. For example, cassava contains cyanogenic glucosides which have unpalatable taste and toxic. Cocoyam contains alkaloids which are toxic and can cause swellings in the mouth, throat and irritation in the gut of animals. Root and tuber crops are easily contaminated by microbial organisms due to high moisture content. Also, the dried products are dusty when milled into powder (Tewe and Egbunike, 1997).

Table 2.3: World cassava production (x10^3 tonnes)

	Year			
	2006	**2007**	**2008**	**2009**
Nigeria	45721	34410	42770	4500
Dem. Rep of Congo	14989	15004	15020	15036
Ghana	9638	9650	9700	10000
Angola	8810	800	8900	9000
Mozambique	6765	5039	8400	9200
Tanzania, United	6158	6600	6700	6500
Rep. of Uganda	4926	4456	4942	4500
Malawi	2832	3239	3700	4000
Madagascar	2359	2400	2405	2000
Other Africa	15251	15354	15923	16233
Africa	117449	104952	118461	121469
Latin America	36311	36429	37024	36606
Asia	70465	75882	77631	83715
World	224483	217536	233391	242069

Source: FAO, (2009).

Table 2.4: Composition of roots, tubers, their by-products and cereals (% dry matter)

	Dry matter	Crude Protein	Crude fibre	Fat	Ash	Starch	Gross Energy (kcal/kg)
Roots and tubers							
Cassava Root	31.9	2.70	3.10	0.80	3.70	76.5	3909.40
Cassava root (ensiled)	34.50	2.00	2.90	0.50	3.4	74.10	3010.18
Sweet potato tuber	30.10	2.50	1.53	0.60	1.0	72.40	4061.00
Cocoyam tuber	24.90	7.90	1.90	0.70	5.20	77.9	3474.60
Root and tuber by-products							
Cassava peel (dry)	94.90	8.20	12.50	3.10	5.40	-	2460.00
Cassava leaves (dry)	92.20	29.00	14.90	6.70	11.6	-	2532.37
Sweet potato vines (dry)	86.60	23.10	16.00	5.30	4.02	-	2269.58
Cocoyam leaves	8.20	20.60	12.10	11.70	12.10	-	2314.60
Cereals							
Maize	87.0	10.0	1.3	4.0	2.0	71.8	4096.5
Guinea corn	88.4	9.9	2.9	2.4	2.1	74.6	3940.9

Source: Olajide *et al*. (2011)

2.7.1 Uses of cassava roots, in diets for poultry and pigs

Tewe and Egbenuke (1997) in their studies concluded that cassava peel and the flour can be added to poultry diet at inclusion level of 10%. In addition, 20% cassava peel and 40% cassava flour has been successfully incorporated into chicken layer diet. According to Olugbemi *et al*. (2010), cassava flour fortified with *Moringa oleifera* leaf meal at 5 and 10% level could replace portions of maize at of 55.6% and 83.33%, in chicken diets. The inclusion levels did not affect productivity and haematology of the chicken.

A report by Balogun and Bawa (1977) has shown that fresh harvested cassava tubers when chopped into pieces or parboiled in water and dried, can be fed to pigs. According to the report (Table 2.6), 57% of the cassava peel could replace maize partially and this was cost effective. Furthermore, 60% and 100% substitution in the diet of growing and finishing pigs with dietary composition of brewers dried grain, fish meal and groundnut cake did not affect their growth.

Cassava tuber rmeal diet, with a protein level of 20% was fed to growing and finishing pigs *ad-libitum* throughout the duration under consideration. They concluded that the growth and carcass characteristics of the pigs improved (Ospina *et al*., 1995).

Table 2.5: Root and tuber crop substitution for maize

Root and tuber crops	Substitution for maize	Comment	Source
Cassava	40% (pigs) 20-30% (poultry) 40% (cattle) 40% (goat and sheep)	20% most economical for layers, high level acceptable for broiler than layers. Higher milk and fat yields and live weight gains for dairy cattle	Gomez *et al*. (1984) Devendra (1977)
Sweet potato	25% (poultry) 50% (pig)	Supplement 0.2-0.5% of lysine. Horses, mules and hogs, for lactating dairy cows (satisfactory)	Yeh *et al*.(1985)
Dalo	20-25% replacement of maize in broiler diets	At levels of 20-40% of cocoyam improved growth rate in pigs	Anigbogu (1997) and Abdulrashid and Agwunobi (2009); Esonu (2000)

2.7.2 Importance of sweet potato in non-ruminants diet

According to An, (2004), sweet potato belong to the family of convulvaceace which is widely grown in the warm climatic zones of Africa. In monetary terms, it costs higher to produce cereals than sweet potato (Huarg, 1982). Because of its readily digestible carbohydrate, sweet potato can be included in the diet of monogastric animals (Oboh, 1987).

The protein casein found in milk is similar to the protein content (30-40%) in sweet potato called patatin (Liedl *et al*., 1987). Up to 50% potato chips could be substituted for maize-soya bean diets without a significant depression in growth (Table 2.6). However, the limitations to the use of the product in poultry nutrition are due to reducing sugars present and the dustiness associated with the processed product (Akinmutin and Osuagwu, 2008).

2.7.3 Use of cocoyam in diets of poultry and pigs

According to Obioha (1972), cocoyam is less costly to produce, and readily available when compared with other tuber crops and cereals. The energy content of cocoyam is high, less costly to cultivate (Hahn, 1984) and less vulnerable to diseases, insects and pests invasion. Cocoyam starch particles are smaller in sizes and this makes them to be easily digested when compared to other tuber crops (Lyonga *et al*., 1986; Ezedinma, 1987).

2.7.4 Limiting factors in cocoyam, as food for man and animals.

The bioactive chemicals present in cocoyam are oxalates, phytates, tannins and saponins (Agwunobi *et al*., 2002). Oxalates serve as a defense mechanism and storage reserve for calcium (Smith *et al*., 1982). Abdulrashid and Agwunobi (2009) reported 25% replacement of maize using cocoyam meal in broiler diet. Studies by Esonu (2000) have shown that for cocoyam species (*Canadium hortulanum*) up to 20% level of inclusion has been successfully incorporated into chicken diets. According to Uchegbu *et al*. (2010), 15% of sun-dried cocoyam has been found to be acceptable to be included in chicken diet. In a related studies, 20% inclusion level of dried cocoyam tuber has been found tolerable for broiler chickens (Onu *et al*., 2001); Onu *et al*., 2009).

Onu *et al*. (2009) and Huisman (1995) have observed that anti-nutrients in cocoyam such as tannins and trypsin inhibitors, affect protein and energy utilization in broiler chickens.

According to Esonu (2000), cooking and drying corms, leaves, and petiole of cocoyam plant before feeding to pigs ensure reduction or removal of oxalic acid present in the plant. Up to 20-40 % of the dry matter of the treated leaves, petiole and the corms could be tolerated by growing pigs. Ohaemenyi (1993) in a study concluded that cocoyam variety *Xanthosoma sagittifolium* corms need to be boiled for some duration in order to reduce the anti-nutritive factors in them before being included in the diets of monogastric animals.

Table 2.6: Symptoms and mode of elimination of toxic components of Roots, Tubers and their by-products.

Roots, Tubers and by-products	Toxic/Anti-nutritional factor	Typical Levels (mg/kg DM)	Primary associated symptoms	Mode of elimination
Fresh cassava root	Hydrocyanic acid	233-1150	Vomiting, dizziness, weakness, diarrhea, death	Fermentation, boiling, grating, cooking, ensiling, sun-drying
Fresh cassava peel	Hydrocyanic acid	1300-2250	Vomiting, dizziness, weakness, diarrhea, death	Fermentation, boiling, Grating, sun-drying, cooking,ensiling
Dried cassava roots	Hydrocyanic acid	14-65	Vomiting, dizziness, weakness, diarrhea, death	Fermentation, boiling, Grating, sun-drying, cooking,ensiling
Dried cassava waste	Hydrocyanic acid	57.2		Fermentation, boiling, Grating, sun-drying, cooking,ensiling
Fresh cassava leaves	Hydrocyanic acid	2650-7200		Fermentation, boiling, Grating, sun-drying, cooking,ensiling
Cocoyam tuber	Irritating/acridity substance Oxalic acid Saponin	- 45.3 (g/100 gDM) 0.53-7.9 (g/100 gDM)	Irritation/burning sensation Precipitation of calcium, interference with mineral absorption Bitter taste	Cooking, sun-drying, roasting fermentation
Sweet potato	Trypsin inhibitor	-	Impairment of proteolytic digestion	Cooking, sun-drying

Source: Tewe *et al*. (1976)

2.7.5 Inferences from Literature Review on False Yam

False yam, an alternative new feed resource contains high carbohydrate which is comparable to maize used in livestock production. Bioactive chemicals which are present in food crops include; saponins, mucilage, phytate, oxalate lower the crop's nutritive value for livestock production. It has been established that most of these anti-nutritive factors interfere with physiological and metabolic activities of livestock when they are consumed. A research into such chemicals which are anti-feedants and some being toxic using rats and broiler chickens is necessary to enhance the use of the crop in feeding livestock.

The search for effective and efficient methods of processing such as soaking,, fermentation, chemical treatment, heat treatment, or a combination of the methods to eliminate or decrease these bioactive chemicals, may encourage the use of the false yam tuber as an alternate source of feed in livestock production.

The conventional energy source of feed ingredient, maize, is being competed for by humans and livestock in their diets. The false yam plant is the new energy feed resource presently under investigation. It grows in the wild in most of the savanna regions in Ghana, which is tolerant to adverse weather conditions and diseases.

CHAPTER 3

3.0 General Materials and Methods

3.1 Source of false yam and its preparation for the studies

False yam tubers, growing in the wild of environs of Nyankpala in the Northen Region, were used in the studies. The harvested tubers were peeled and sliced into thin sections about 1 cm thick and put into six groups of known weights of 1000 g. Each group was subjected to a specific processing method. Processing methods used were:

1. Sun drying the false yam tuber in the fresh state
2. 0.1M sodium hydroxide treatment
3. 0.1M sodium hydroxide treatment plus blanching
4. Blanching of false yam
5. Fermentation of the false yam for 14 days.
6. Raw false yam tuber

3.2 Sun-drying of false yam

The sliced raw false yam tubers (1000 g) were dried in the sun for 1 week making sure the dried weight was constant. The dried tubers were packaged in transparent polythene bag and labelled as **SDFYM**.

3.3 0.1M sodium hydroxide treatment

The second group of the raw sliced tubers was soaked in 0.1M sodium hydroxide for three days. The soaked solution was drained at the end of the 3rd day. The pieces of the soaked false yam tubers were washed in clean water and separated into two groups. One group was then dried in the sun for seven days packaged and labelled **NaTFYM**. The other half was blanched three times in hot water at about 90^{o}C for 30 minutes and later sun-dried for seven days, packaged and labelled **NaTBFYM**.

3.4 Blanched false yam

The third group of the raw sliced false yam tubers was blanched in hot water at a temperature of 90^{o}C for half an hour and left in the water for one day. After 24 hours, the water was poured away and the sliced tubers were blanched for the second time for 30 minutes and left in the water for another 24 hours. The process was repeated for the third time. At the end of the 72 hours, the sliced pieces of the blanched tubers were sun dried for 7 days packaged and labelled **BFYM.**

3.5 Fermented false yam for 14 days

The fourth group of raw sliced false yam tubers was placed in water until the 14th day when they were removed. The soaked solution was replaced every 24 hour interval with fresh water. At the end of the 14th day, the fermented pieces of the false yam tubers were sun-dried for 7 days, packaged and labelled as **FFYM** for further chemical analysis.

The fresh and dried samples were separately milled by using a blender. The milled false yam tuber samples were put into transparent polythene containers labelled, and kept at 4^{o}C in a fridge. This was done in order to reduce error before their proximate, mineral and anti-nutrient screenings.

3.6 Qualitative Analysis of Phytochemicals

The screening of the phytochemical constituents in the false yam tuber and seeds were done by using the powder of the milled samples and extracts in alcohol using the protocols by Harborne (1998); Trease and Evans (1989) and Sofowara (1993).

3.7 Preparation of reagents

Maeyer's reagent: This is a mixture of potassium iodide and mercuric chloride. About 5 g Potassium iodide was weighed and added to 20 ml of distilled water. Similarly, 0.355g Mercuric chloride was

also weighed and placed in separate 60 ml distilled water. The two solutions were mixed together and 100 ml of distilled water was added to make the mark.

Dragendorff's reagent: This is a mixture of two solutions A and B in a ratio of 1:1. Solution A is made up of 1.7g of basic bismuth nitrate plus 20g of tartaric acid. These two substances were placed in 80ml distilled water stirred until they dissolved. In solution B, about 16 g of potassium iodide was weighed and dissolved in 40 ml of distilled water. The two solutions (A and B) were then mixed in a 1:1 ratio.

3.7.1 Test for alkaloids

Methanol extracts were collected from 0.5-0.6g for processed false yam tuber. Eight millilitres of 1% HCl was added to the methanolic extract warmed and filtered. Dragendorff's and Mayer reagents were added separately to 2ml of the filtrate. The formation of white precipitate or turbidity or no precipitate is an indication of the presence or absence of alkaloid in the feed material being tested.

3.7.2 Test for steroids

Acetic anhydride (2ml) was added to 0.5 ml of the methanolic extract of the false yam tuber. This was followed by the addition of 2 ml of concentrated sulphuric acid. The mixture was agitated for thorough mixing. The formation of a greenish colour is an indication of steroid or when the colour changed from violet to blue.

3.7.3 Test for terpenoids

Methanolic extract of the false yam (2ml) was added to 2 ml of $CHCl_3$ in a test tube. Three millitre of concentrated sulphuric acid was added to the side of the test tube for it to form a layer with the mixture. Reddish brown colour formation is an indication of the presence of terpenoids.

3.8 Proximate composition

The AOAC (2000) procedure was used to determine the proximate compositions; crude protein, ether extract, nitrogen free extract, crude fibre and ash of the false yam tuber and the seeds.

The Nitrogen free extract was determined by subtracting the sum of ether extract, ash, crude protein and crude fibre from 100 as depicted by the formular NFE % = 100 - [(EE% + %ASH + %CP + CF)]. The metabolisable energy was estimated by the equation: ME (Kcal/kgDm) = 37 % x % Protein + 81.8 x % Fat + 35.5 x % NFE (Pauzenga, 1985).

3.9 Determination of the Anti-nutritional factors

3.9.1 Phytate determination

The method of Asieber (1987) was used to determine phytate. Four grams of the processed false yam tuber meal was placed in 100 ml of 2% HCl and the solution was left for 3 hours duration. The solution was then filtered using a filter paper. The filtrate (25ml) was placed in a conical flask. Ammonium thiocyanate (3M) solution was used as an indicator by adding 5 drops. Distilled water measuring 53.5ml was added to the mixture to obtain the required acidic pH. A standard solution of iron (III) chloride was prepared by using 0.000195g of iron per ml in distilled water. The standard iron (III) solution was titrated against the filtrate with ammonium thiocyanate (3M) solution until a brown yellowish colouration lasted for 5 minutes. Phytic acid in the false yam was estimated by the formula:

% Phytic Acid = 8.24 x t x 100 x wt of sample/1000

Where t = titre value

3.9.2 Oxalate Determination

The protocol prescribed by Asieber (1987) was followed to determine the level of oxalate in milled false yam tuber and it seeds.

False yam tuber meal (about 1g) was weighed into 75 ml of 1.5 N H_2SO_4 in 250 ml beaker. The mixture was stirred intermittently using a glass rod for 1 hour before finally filtering with a filter paper. 0.1$KMNO_4$ solution was placed in a buirette and titrated against 25 ml of the hot exract of the filtrate until a faint pink colour was obtained.

3.9.3 Saponin Determination

The method described by Birk *et al.* (1963) as modified by El-Difrawi and Hudson (1979) was followed to determine the saponin content in the false yam tuber and the seeds. Processed false yam meal weighing 20g was extracted in 100 ml of 20% aqueous ethanol. The mixture was stirred intermittently using glass rod for 12 hours. At the end of the 12 hours, the mixture was filtered using filter paper. Another 200 ml of 20% aqueous ethanol was used to re-extract the residue. The two extracts were bulked together and the volume reduced to 40 ml using vacuum extractor. Forty millilitres of the ethanol extractor was placed in a separating funnel. Diethyl ether (20 ml) was then added and agitated. Two separate layers were observed in the separating funnel, i. e. the ether and aqueous layers. The aqueous layer was drained into a beaker and the ether layer discarded. NaOH solution was added to the aqueous solution until the required pH of 4.5 was obtained. N-butanol (60 ml) was then added and 10 ml of NaCl aqueous was used to wash the butanol extract combination two times. The resultant solution was then dried in a fume cupboard. Crude saponin obtained was weighed and the following formula was used to obtain the saponin content:

Saponin (%) = weight of residue x 100/sample weight.

3.9.4 Alkaloids

The protocol prescribed by Obadoni and Ochuko (2001) was used to determine the alkaloid content in the false yam and the seeds. A mixture of acetic acid in ethanol (about 200ml) was placed in 250m1 beaker. Five grams of processed false yam meal was added, stirred and the solution left for a

duration of 4 hours. At the end of the 4 hours the solution was filtered. The filtrate was reduced to one quarter of the original volume. Few drops of concentrated NH_4OH were added to the filtrate. The white precipitate observed is the presence of alkaloid. The precipitate was then dried and weighed.

3.9.5 Polyuronides (pectins, mucilages, gums and resins)

The methods of Obadoni and Ochuko (2001) were used in determining the gums and resins contents. Aqueous extract of the processed false yam meal (about 2ml) was added dropwise in a test tube where 10ml of alcohol or acetone have already been placed. A thick precipitate formed was separated off by filtration or centrifigution and washed with alcohol. The residue was then dried and weighed as the resins present in the false yam tuber.

3.9.6 Determination of terpenoids

Terpenoids determination was followed by the protocol described by Fegurson (1956). Fifty grams of sample was dissolved in 300 ml of 70% ethanol. Filtrate was concentrated at 42^{o}C using vacuum extractor to reduce the volume of the mixture. The concentrated residue was dissolved in 0.5ml acetic acid and washed with chloroform (50ml). The residue was dried and weighed as total terpenoids.

3.10 Mineral Analysis of Processed False Yam

One gram of the false yam tuber meal was ashed in a muffle furnace at a temperature of 550^{o}C for 4 hours. The ashed samples were rinsed into centrifuge tubes and placed on a mechanical reciprocating shaker for 5minutes for proper mixing.

The ashed solutions were centrifuged for some few minutes at speed of 300 rpm. The centrifuged ash solution was again diluted with 100 ml distilled water. The topmost part of the solution was

decanted into a pre- washed clean glass container in order to determine the mineral elements such as Mg, Ca, P, Na and K using standard analytical grade solutions of the various minerals.

3.10.1 Determination of calcium

The clear supernatant solution of the sample obtained earlier (about 5ml) was placed in a conical flask. Potassium hydroxide solution (10%) measuring 10ml was added. The following indicators were then added KCN (2%) 5 drops, TEA (30%) 1 ml, and EBT (1 drop). The mixture was swirled for thorough mixing. EDTA 0.02N was titrated against the mixture in the conical flask. A change of colour from red to blue signified the end point of titration.

Calcium in mg = Titre value of EDTA x 0.40

$$\% \text{ Calcium} = \frac{mg\ Calcium}{Sample\ wt} \times 100$$

3.10.2 Determination of magnesium by Colorimetric method

Five millilitres of the ashed solution was placed in a concial flask. A buffer solution of a mixture of 5 ml NH_4Cl and NH_4OH was then added. Indicators such as TEA (30%) 1 ml, KCN (10%) 3 drops, and EBT (few drops) were added to the solutions in the conical flask. The resultant solution was shakened to ensure thorough mixing and was then titrated with 0.02N EDTA. A change of colour from red to blue is an indication of the end point.

Magnesium in mg = Titre value of EDTA x 0.24

$$\% \text{ Mg} = \frac{mg\ Magnesium}{Sample\ wt} x\ 100$$

3.10.3 Determination of Phosphorus (P)

Phosphorus was determined using colorimetric method. Vanadomolybdate reagent is a mixture of two solutions ammonium molybdate and ammonium vanadate. About 22.5g of the ammonium molybdate was weighed and dissolved in 400 ml distilled water. Similarly, 8.25 g of the ammonium

vanadate was also dissolved in 300 ml of boiled distilled water. The two solutions were then mixed together and allowed to cool to room temperature. Nitric acid measuring 250 ml was then added to the solution followed by 1000 ml of distilled water in order to dilute the mixture. 0.2195g of (Potassium hydrogen phosphate) was dissolved in 1000 ml distilled water to prepare a standard solution. This solution contains 50 µg P/ml. In order to prepare standard curves, standard solutions measuring 1, 2, 3, 4, 5, and 10 ml were placed in 50 ml volumetric flasks and vanadomolybdate reagent (10 ml) was added to each flasks. Distilled water was added until the 50 ml mark was reached. The phosphorus content in the respective flasks became 1, 2, 3, 4, 5 and 10 µg P/ml. Jensey 6051 colorimeter at wavelength of 430nm was used to measure the absorbance of the concentrations of phosphorus in the various flasks. The calibration curves were obtained by plotting absorbance against concentrations.

Five (5) ml of the processed false yam tuber ash digest was placed in a 50 ml conical flask. Vanadomolybdate reagent was then added followed by distilled water until the 50ml mark was reached. The mixture was allowed to stand for a while for the colour to develop. When a stable yellow colour was developed, colorimeter readings of the absorbance of the phosphorus were read at 430 nm. The absorbance read was used to determine the P content from the standard curve.

The % P in the digest was determined by the formula: P content (g) in 100 g sample (% P) = $\frac{C\ x\ df\ x\ 100}{1\ 000\ 000} = \frac{C\ x\ 1000\ x\ 100}{1\ 000\ 000} = \frac{C}{10}$

Where C = concentration of P (µg /ml) as read from the standard curve;

df= dilution factor, which is 100 *10 = 1000, as calculated below:

1 g of sample made to 100 ml (100 times);

5 ml of sample made to 50 ml (10 times)

1 000 000 = factor for converting µg to g

3.10.4 Method for determination of potassium (K) and sodium (Na)

Photometric method was used to determine the potassium and sodium in 2 ml each of the processed false yam ash digest. A combined solution of 1000ppm was prepared from NaCl and KCl which have been dried at 105 ^{o}C in an oven for duration of 4 hours. NaCl weighing 2.54g and KCl (1.98g) each was dissolved in distilled water (200 ml). The two solutions were mixed together to form 1000 ml. Standard curve of 200, 400, 600 and 800 ppm was developed for K. Also, standard curve of 20, 40, 60 and 80 ppm was developed for sodium. The flame photometer (Model PEP7, Jenway, United Kingdom) with butane gas was used to read the absorbance. Also, the digested ash solutions made from HNO_3 and $HClO_4$ were read on the flame photometer. Based on the standard curves developed, the concentrations for Na and K were determined using the absorbance observed from the sample.

1 g of the processed false yam tuber may contain (µg) = C x df

K content (g) in 100 g plant sample, (% K) = $\frac{C \times df \times 100}{1000\,000} = \frac{C \times 100 \times 100}{1000\,000}$

$= \frac{C}{100}$

df = dilution factor, which is 100 x1 = 100,

- 1.0 g of sample made up to 100 ml (100 times)
- 1000 000 = factor for converting µg to g

Where C = concentration of K (µg / ml) as read from the standard curve

3.10.5 Method for determination of iron (Zn), manganese (Mn), iron (Fe) and copper (Cu)

Sample solution of the processed false yam ashed tuber digest was used to determine Mn, Fe, Zn, and Cu using Atomic absorption spectrophotometer. The setups of the equipment in terms of the acetylyn pressure 50-60 psi, and a voltage of 208-240 V were set to recommended values.

Also, the correct wavelengths of the elements concerned Mn 279.5nm, Fe 248.3nm, Zn 213nm, Cu 324.8 nm, their files and hollow cathode lamps were selected. Standard solutions of the elements to be analysed were used to develop calibration curves. Processed false yam tuber ash digest was used to analyse for each of the elements. For the calibration curves developed, X represents the concentration of the element in the processed false yam ash digest and Y the absorbance. In order to determine the concentration of a particular element substitute, its absorbance reading into calibrated equation and this gives the concentration of the element in mg/L.To determine the mass concentration of a particular element in 100 ml of the digest multiply by 0.1L this gives a total mass of the element in solution. The % amount of the element in the sample of solution is then determined by

$$\text{Conc. (Cu, Fe, Mn, Zn) (mg/kg)} = \frac{\text{Conc x V}}{\text{Sample weight (g)}}$$

Conc = concentration measured from Atomic Absorption Spectrophotometer

Where V= volume of sample of false yam ashed digest solution used =100 ml, Sample weight = 1.00g

CHAPTER 4

EXPERIMENT 1: NUTRIENT COMPOSITION OF FALSE YAM TUBER AND SEEDS

4.0 Introduction

Previous studies by Dei *et al*. (2011a) on the false yam tuber did not compare the nutrient composition and the metabolisable energy in the tuber and seed. The false yam tuber and seed were harvested from the same location which is presumed to have experienced the same environmental conditions such as temperature, rainfall and soil characteristics. However, it has been reported by Okai (1998) that differences in the nutrient content of food crops come about as a result of age, geographical location, maturity and the variety of the crop. The interaction of these climatic factors could create microclimate in this location which has potential effect on the amount and types of the nutrient concentrations in the false yam plant. Furthermore, the mineral constituents of the false yam tuber and seed have not been compared in earlier studies Dei *et al*. (2010a) although, they were passively mentioned. It was for these reasons that the current study was carried out to examine the nutrient compositions of the false yam tuber and seed meals.

4.1 Objectives

1. To determine the proximate compositions of processed false yam tuber and seed meals
2. To calculate the metabolisable energy content of false yam tuber and seed meals

4.1.1 Hypothesis

The proximate nutrients in processed false yam tuber and the seed will not differ when standard methods are used.

4.1.2 Materials and methods

4.2 For the methods refer to general materials and methods section 3.0

4.2.1 Treatments and experimental design

Three treatments, the raw or unprocessed false yam tuber, sun-dried false yam tuber and seeds arranged in complete randomized design were used. Each of the treatment samples was replicated three times for the proximate and mineral analysis.

4.2.1.1 Proximate Analysis

The AOAC (2000) methods of proximate analysis of feed were used to determine the crude protein, moisture, crude fibre, ash, ether extract, dry matter and nitrogen free extract contents of false yam tuber meal which has been kept in the fridge.

4.2.2 Determination of Moisture Content

An empty small beaker was weighed as (W1) using an electronic weighing scale. Two grams of the processed false yam tuber meal/seeds were weighed into this beaker and the weight recorded as W2. This was then placed in an oven and dried to constant weight at a temperature of 105^{o}C for 24 hours. The beaker was removed the next day, allowed to cool in a desiccator for some minutes and weighed as (W3).

$$\% \text{ Moisture content} = \frac{\text{(weight loss due to drying)} \times 100}{\text{(weight of sample)}}$$

$$\% \text{ Moisture Content} = \frac{(w2-w3) \times 100}{(w2-w1)}$$

4.2.3 Determination of Crude Fat

Five grams (5g) of false yam tuber meal/seeds were weighed into a filter paper (w1). The filter paper was wrapped with a thread and placed in a thimble of Soxhlet apparatus. A solvent n-hexanae

was used in the Soxhlet apparatus to extract the fat from the false yam tuber meal for 5 hours. At the end of the 5 hours, the oil- solvent mixture was poured into a pre-weighed beaker (w2) and placed in an oven set at about 60ºC for 30 minutes. The evaporated oil- solvent mixture was placed in a desiccator and allowed to cool. This was then weighed as W3.

$$\% \text{ Crude Fat Content} = \frac{(\text{weight of fat extracted}) \times 100}{(\text{weight of sample})}$$

$$\% \text{ Crude Fat Content} = \frac{(w_2 - w_3) \times 100}{(w_1)}$$

4.3.3 Determination of Crude Protein

Micro-kejeldahl apparatus was used to determine the crude protein in the false yam tuber meal/seeds. Four processes are involved in the determination of crude protein. The first stage involves digestion of the false yam tuber meal using Micro-kejeldahl apparatus. The second stage is distilling the digested product using 25 ml of NaOH solution into receiving flask containing 25 ml of 2% boric acid. The third stage is titration of the distillate using standard 0.01 HCl. Determination of ammonia content and finally the determination of the crude protein content of the false yam tuber meal/seeds. About 2 g of the false yam tuber meal/seeds was weighed and transferred into micro-kejeldahl flask. 10% H_2SO_4 measuring 10 ml was added to the sample plus one tablet of catalyst. The content of the flask and its sample turned black and was heated in a fume cupboard for a duration of 3 hours to ensure complete digestion. The next step was that the digested sample which now appeared colourless was diluted with distilled water to reach the 100ml mark. Ten millilitres of this was pipetted into distillation flask, and 25 ml of 40% NaOH was added to it. The entire mixture was cloudy due to excess NaOH making the solution alkaline.Twenty five millilitres of 2% boric acid was measured into a conical flask. 2 drops of mixed indicators were added. The boric acid and drops of the mixed indicators in the receiving flask turned red. The digested sample in the

distillation flask was allowed to distill into the receiving flask. The condenser end of the distillation apparatus was ensured to be completely immersed in the boric acid solution. As the distillation continued the pure colour of the boric acid in the receiver flask began to turn green indicating the presence of ammoania. This process continued until 50 ml mark was reached. The condenser end was washed with distilled water and the receiver flask removed. The content of the receiver flask was titrated with 0.01 HCl. The green colour in the receiver flask turned pink which was an indication of the end point of the titration. The titre value recorded was then used to determine the nitrogen content of the sample by a formula: Lastly the crude protein content of the false yam tuber/seeds was determined by multiplying by 6.25.

$$\% \text{ Nitrogen content} = \frac{(\text{Titre value} \times M \times 0.0014 \times Df \times Cf \times 100)}{(\text{weight of sample})}$$

Where;

Cf = correction factor = 10

Df = Dilution factor = 50

M = Molarity of HCl = 0.01M

% Crude protein = % Nitrogen x 6.25

6.25 is a constant for food sample.

4.3.4 Crude fibre determination

False yam tuber meal (2g) was weighed into 500cm^3 beaker which contained 200ml of 1.25% of H_2SO_4 solution. One gram of asbestos was added to it and the solution boiled for 30 minutes. The content of the beaker was poured into a Buchner funnel which has been lined with muslin cloth for filtering. The filtrate was boiled in 200 ml of NaOH and filtered. The second filtrate was washed twice with some amount of alcohol followed by petroleum ether. The washed residue was placed in a pre-weighed beaker (w2). The residue was placed in an oven at 105^{o}C for 24 hours to dry to a

constant weight. The residue was removed and allowed to cool in a desiccator. The weight of the residue plus the beaker was determined as (W3). The crude fibre was determined as;

$$\% \text{ crude fiber} = \frac{W_1 - W_2 \times 100}{W_3}$$

Where;

W_1 = original weight of sample

W_2 = weight of residue before oven drying

W_3 = weight of residue after oven drying

4.3.5 Carbohydrate Content Determination

The soluble carbohydrate is the NFE, the nitrogen free extract. The NFE is determined by difference. The sum of NFE is equal to the sum of the percentage Crude protein, Ash, Moisture, Crude Fat and Crude Fibre contents, subtracted from 100.

% Carbohydrate = NFE = 100 - (% moisture + % Ash + % crude fibre + % crude protein+ % crude fat).

4.3.6 Mineral content determination

Standard analytical procedures were used to determine the mineral content of the false yam tuber meal and the seed. Two grams (2g) of the digested ash samples were used to prepare 100ml standard solutions from which minerals such as iron, potassium, phosphorus, zinc, sodium, calcium and magnsesium were determined in three independent replicates by the protocols prescribed by AOAC, (2000).

4.3.7 Energy content

The metabolizable energy was calculated using the equation of Pauzenga (1985).

ME (Kcal/kg DM) = 37 X % Protein + 81.8 x % Fat + 35.5 x NFE

4.3.8 Statistical analysis

Data were analyzed using Graph pad Prism (Graphpad Prisim version 7, www.graphpad.com)

Three independent replicate samples for each treatment were used for every parameter under consideration. The results are presented in tables as means ± standard error of mean (SEM).

The differences in the mean values were subjected to One-way ANOVA and Dunnett's multiple comparisons test was used to separate the means using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla, California, USA (www.graphpad.com). Significant differences were declared at a probability of $P<0.05$

4.4 Results

The proximate nutrient composition of the false yam tuber and the seed are presented in (Table 4.1). The crude protein of the false yam tuber was 5.34% which was lower than that recorded in the seed (11.42%). The crude fibre in the sun-dried tuber recorded a higher value (7.29%) than in the seed (1.19%). Nitrogen free extract in the seed was 74.34% and sun-dried tuber 72.52%. The metabolisable energy value for the seed (3392.90kcal/kg/DM) was higher than the sun-dried tuber (3127.87.12kcal/kgDM).

Table 4.1: Proximate composition and caloric values of raw, sun-dried false yam tuber and seeds

PARAMETERS	SDFYM	SEM	RFYM	SEM	FYMS	SEM	P-value
Dry matter (%)	92 ^{a}	0.058	81^{b}	0.173	93.5^{c}	0.00	0.0001
Crude protein (%)	5.34^{a}	0.015	4.70 ^{b}	0.0173	11.42 ^{c}	0.012	0.0001
Crude fibre (%)	7.29 ^{a}	0.00	3.57 ^{b}	0.012	1.19 ^{c}	0.017	0.0001
Ash (%)	2.5 ^{a}	0.017	1.52 ^{b}	0.012	2.5^{a}	0.006	0.0001
Ether extract (%)	4.35 ^{a}	0.017	1.75^{b}	0.016	4.05^{c}	0.006	0.0001
Nitrogen free extract (%)	72.52^{a}	14.55	69.46^{a}	12.37	74.34^{a}	1.351	0.1063
Metabolisable energy (kcal/kg DM)	3127.87^{a}	0.936	2782.88^{b}	2.014	3392.90^{c}	1.258	0.0001

RFYM: Raw false yam; SDFYM: Sun dried false yam; FYMS: false yam seed. Means in the same row with different superscripts are considered significant ($p < 0.05$). SEM: Standard error of mean, P: probability

The mineral compositions of the sun-dried and seed of the false yam presented in Table 4.2 show the presence of phosphorus, magnesium, calcium, sodium, potassium, iron, manganese, copper and zinc.

Table 4.2: Mineral constituents of raw, sun-dried false yam tuber and seeds

MINERAL/ELEMENT	SDFYM	RFYM	FYMS
Calcium (Mg/g)	0.42 [a]	0.21[b]	0.19 [c]
Phosphorus (%)	0.10 [a]	0.13[b]	0.16 [c]
Iron (Mg/g)	10.90 [a]	7.70 [b]	6.55 [c]
Copper (Mg/g)	3.53[a]	2.30[b]	1.80 [c]
Magnesium (%)	0.42 [a]	0.26 [b]	0.34 [c]
Manganese (%)	0.75 [a]	1.03 [b]	0.85[a]
Potassium (%)	0.88 [a]	0.89 [a]	0.09 [b]
Sodium (%)	0.10 [a]	0.08 [a]	0.12 [a]
Zinc (Mg/g)	0.75 [a]	1.80 [b]	2.70 [c]

RFYM: Raw false yam; SDFYM: Sun dried false yam; FYMS: false yam seed. Means in the same row with different superscripts are significantly different at a probability of ($p < 0.05$).

The amount of phosphorus, sodium, and potassium for the false yam seed were higher compared to the dried tuber values (Table 4.2).

4.5 Discussion

4.5.1 Proximate composition of nutrients in the false yam tuber and seeds

The high crude protein content in the seeds and moderate amount in the sun dried tuber may be due to the presence of alkaloid compounds identified in the tuber 1.53g/100g and the seeds 1.00g/100g Tables 5.1 and 5.2, when they were screened for anti-nutritive factors. In addition, high pungent ammonia gas produced in the fresh and chipped tubers and milled seeds the next day might also be a contributory factor to the crude protein. This pungent smell of ammonia gas was confirmed by using red litmus paper which changed to blue proving the presence of ammonia. The ammonia gas could have come from ammonia compounds present in both the seed and the false yam tuber. This could have contributed to the high crude protein in the false yam seed, moderate in the tuber, which is fairly comparable to what was recorded by NRI (1987) as 10.3% CP and Osei *et al*. (2013a) as 16.4% CP but similar to that reported by Dei *et al*. (2011a) as 5.41% CP. The crude protein in the seed (11.42%) is comparable to the crude protein in fresh water yam tuber (10.27%) as reported by Ezeocha and Ojimelukwe (2012). However, crude proteins in both the false yam seed and the tuber are higher than that reported in cassava root (2.7%), and sweet potato tuber (2.5%) but fairly comparable to cocoyam tuber (7.9%), (Olajide, 2011). The high to moderate crude proteins in both the false yam seed and tuber is a good indication of the possible substitution for maize as a carbohydrate source of energy in the diets of monogastrics. However, the high crude fibre content of the tuber makes the false yam seeds a better choice in monogastric nutrition. The crude fibre content in the sun-dried tuber (7.29%) was significantly ($P< 0.05$) higher than the crude fibre in the false yam seed (1.19%). Thus, the crude fibre in the present study is quite higher than what has been reported by Osei *et al*. (2013a) in dried false yam tuber (3.1%). The total carbohydrate content of the false yam seed (74.34%) was significantly ($P < 0.05$) higher than in the the dried tuber (72.52%). This corresponds fairly to what has been reported by NRI (1987) in dried false yam tuber

(74.4%) but lower than that reported by Dei *et al*. (2011a) as 53.1% (in the form of starch) in sun – dried false yam tuber. The total carbohydrate content in the dried false yam tuber and the seed ranged from 69.46%-74.4% which is comparable to the carbohydrate reported in cassava (74.10%) sweet potato tuber (72.40%) cocoyam tuber (77.9%) maize (71.8%) and guinea corn (74.6%), (Harvey, 1980). The false yam seed and the tuber are good sources of soluble carbohydrate which is the main energy source in the diets of livestocks.

The dry matter contents of the sun-dried tuber and the seed 92% and 93.5% respectively are higher than the dry matter in sweet potato (30.10%), cocoyam tuber (24.9%) and cassava root (31.9%) reported by (Olajide *et al*., 2011). The low moisture content implies that the shelf - life of the sun-dried false yam tuber and the seed can be prolonged.

Metabolizable energy content ranged from 2782.9 - 3392.9 kcal/kg DM in the sun-dried tuber and the seed of the false yam (Table 4.1). The energy values are comparable to those in cassava (2881.1kcal /kg DM), sweet potato (2711.8 kcal /kg DM) and cocoyam tuber (3115.0 kcal /kg DM) but lower than that of maize (4096.5 kcal /kg DM) (Harvey, 1980).

4.5.2 Mineral composition of the false yam tuber and the seeds

All the minerals analyzed for in the false yam tuber and the seeds, i. e. copper, manganese, sodium, potassium, phosphorus, magnesium, calcium, zinc, and iron had higher values than those contained in cassava, potato and some cereal crops. The iron contents of the sun-dried, and false yam seeds ranged from 6.55 mg/g to 7.70 mg/g and are higher than those reported for cassava (0.27mg/g), raw potato (0.78mg/g), yellow corn (2.71mg/g) and sorghum (4.4mg/g) (Gil and Buitrago, 2002). In addition, potassium (880 – 1090 mg/g), calcium (0.21 - 0.42 mg/g), magnesium (0.26 - 0.42%), phosphorus (0.10 - 0.13%), and sodium (0.08 – 0.12 mg/g) in the false yam tuber and the seed were higher than their respective values in cassava 271 mg/g, 16 mg/g, 21 mg/g, 27 mg/g, and potato (421 mg/g), (12 mg/g), (23 mg/g), (57 mg/g). These corroborate similar findings by Gil and

Buitrago (2002). The trace minerals were zinc (1.8 - 3.75 mg/g), copper (1.8 - 3.53 mg/g) and manganese (750-1030 mg/g) in the raw, sun-dried false yam tuber and the seeds (Table 4.1) and were also higher than their respective values reported in cassava (0.34 mg/g, 0.1 mg/g, and 0.384 mg/g), and sweet potato, (0.29 mg/g, 0.108 mg/g, and 0.153 mg/g).

Comparing the nutrient components in the false yam tuber and seed meals with that of maize also suggests that, the ether extract, crude fibre, crude protein, mineral elements and metabolisable energy are failrly comparable to that of the maize. Thus, the false yam tuber can be considered as a new feed resource from the nutritional point of view.

4.6 Conclusion and Recommendations

The false yam tuber and the seed are valuable source of energy, micro and macro nutrients. Therefore, there is the need to conduct further study to determine the phytochemical constituents in the false yam tuber and the seed and their levels of concentration.

CHAPTER 5

5.0 EXPERIMENT 2: PHYTOCHEMICAL SCREENING OF THE FALSE YAM TUBER AND THE SEEDS

5.1 Introduction

Plant parts such as the (leaves, stem, roots, flowers, fruits, seeds and bark) may contain chemicals which are primary or secondary synthetic products. Some of the primary synthesized products of plants are carbohydrates, sugars and amino acids and proteins. The secondary products are the anti-nutritients which include alkaloids, terpenoids, tannins, phenols and saponins (Igile, 1996). The distribution of these chemicals in terms of quantity and quality may differ from parts of the various species of plants (Baquar, 1989). Some of the anti-nutrients may be toxic to animals while others may have medicinal properties. According to Baquar (1989), the concentration of these chemicals in parts of plants may vary form plants of various species depending on the age, climatic and ecological factors. Studies by Fransworth and Moris (1976) suggested that many of these anti-nutrients serve as defence mechanism for the plants from being attacked by insects or infected with diseases by micro-organisms such as fungi, bacteria and viruses and also being eaten by herbivores (Fransworth and Moris, 1976).

It was established from Experiment 1 that sun-dried false yam tuber and seeds contain appreciable amount of primary nutrients. Thus, the use of the false yam as a new feed resource has the potential to serve as substitute for the cereals such as maize which constitute major energy source in poultry nutrition (Dei *et al.*, 2011a). Preliminary studies have shown that only 3% inclusion level of the untreated tuber that is sun-dried tuber can be fed to broiler chickens with no adverse effect on their growth performance (Dei *et al.*, 2012c). Again, only 5% inclusion level of the seed can be used by broiler chickens (Dei *et al.*, 2012c).This is because the untreated tuber and seed meals contain antinutritional factors that depress growth of broiler chickens (Dei *et al*. 2011a).

Therefore, the main problem of limited use of both the tuber and seed meals in diets for chickens is the presence of ANFs. However, there is limited information on the types and levels of the ANFs present in the tuber and seed meals.

Thus, there is the need to screen both tuber and seeds for phytochemicals present as well as quantify those present. This study therefore sought to characterize the ANFs in the false yam tuber and seeds and also quantify the anti-nutritive elements to be identified.

5.1.1 Objectives

1. To screen the anti-nutritive elements in the false yam tuber and seed meals.
2. To determine the concentration levels of these anti-nutritive elements.

5.1.2 Hypothesis

Anti- nutritive elements and their concentration levels in the false yam tuber and the seed will not differ when determined in extracts or milled specimens using standard procedures.

5.2 Materials and methods

5.2.1 Collection of the plant

Refer to *Section 3.1*.

5.2.1.1 Treatments and experimental design

Three treatments; the raw false yam tuber, sun-dried false yam tuber and seeds arranged in complete randomize design were used for phase 1 and 2 of the study. Three independent replicate samples for each treatment were used for every parameter under consideration.

5.2.2 Phase 1: Phytochemical screening of the false yam tuber and seeds

Anti-nutritive factors screened were; Saponins, polyuronides (mucilage, pectins, or resins), phenolic compounds, Tannins, steroids, terpenoids, flavonoids, anthracenocides, alkaloids and Cyanogenic glucosides

Phytochemical screening of the false yam and plant seeds for anti-nutrients were carried out using the milled and extracts in alcohol following the procedure described by Sofowara (1993), Trease and Evans (1989) and Harbone (1973).

5.2.3 Phase 2: Quantitative determination of phytochemical constituents

Refer to *Section 3.9.1*.

5.2.3.1 Statistical analysis for phase 2

Data were analyzed using Graph pad Prism (Graphpad Prisim version 7, www.graphpad.com)

Three independent replicate samples for each treatment were used for every parameter under consideration.

The differences in the mean values were subjected to One-way ANOVA for the treatments. Dunnett's multiple comparisons test was used to separate the means using GraphPad Prism version

7.00 for Windows, GraphPad Software, La Jolla, California, USA (www.graphpad.com). Significant differences were declared at a probability of $P<0.05$

5.3 Results

Alcoholic extract of the false yam tuber and the seed were carried out on saponins, steroids, polyuronides (mucilage, pectins and resins), alkaloid, cynogenic glucosides, flavonoids, terpenoids and reducing sugar. The results of the phytochemical screening as presented in Table 5.1, showed that saponins, alkaloids, polyuronides, steroids, terpenoids were present in the false yam tuber and the seeds. These findings corroborate similar findings by Umoh (2013) in the false yam tuber. However, in the present study, cynogenic glucosides, tannins and phenolic compounds were absent in both the false yam tuber and the seed meals but present in samples reported by Umoh (2013). The differences could have been due to the type of varieties and the area where the false yam plants were harvested.

Table 5.1: Phytochemical screening of the constituents of false yam tuber and the seed

Phytochemicals	RFYM	SDFYM	FYMS
Reducing sugar	+	+	+
Polyuronides (mucilage, pectin, resin)	+	+	+
Saponins	+	+	+
Phenolic compounds	–	–	–
Tannins	–	–	–
Steroids	+	+	+
Terpenoids	+	+	+
Flavanoids	–	–	–
Anthracenocides	–	–	–
Alkaloids	+	+	+
Cyanogenic glucoside	–	–	–

RFYM: Raw false yam; SDFYM: Sun dried false yam; FYMS: false yam seed.

Phytochemical compound when present is represented by (+); Phytochemical compound when absent is shown as (-).

Also, phytochemical screening using the milled samples obtained from the sun-dried tuber and seeds confirmed the presence of triterpenoids, saponins, polyuronides (mucilage), alkaloids, phytates and oxalates. Phytochemical constituents present in the raw and sundried tuber and seed are presented in Table 5.2.

Table 5.2: Levels of Anti-nutrients in raw, sun-dried false yam tuber and seeds

ANTI-NUTRIENTS	RFYM	SDFYM	FYMS
Terpenoid (g/100g)	0.05 ^{b}	0.23 ^{a}	0.20 ^{c}
Saponin (g/100g)	0.51 ^{a}	0.88 ^{a}	0.58 ^{a}
Polyuronides (mucilage, pectin and resins) (g/100g)	12 ^{a}	11.25 ^{a}	7.5 ^{b}
Alkaloid (g/100g)	0.77 ^{b}	1.53 ^{a}	1.00 ^{c}
Phytate (g/100g)	1.98 ^{b}	2.97 ^{a}	3.23 ^{c}
Oxalate (g/100g)	0.54 ^{b}	2.64 ^{a}	0.72 ^{c}

RFYM: Raw false yam; SDFYM: Sun dried false yam; FYMS: false yam seed. Means in the same row with different supercripts are significantly different ($p < 0.05$).

The concentration of phytate was higher ($P < 0.05$) in the seed (3.23%), followed by sun-dried tuber (2.97%) and the fresh tuber least (1.98%). The terpenoid, saponins and oxalate contents recorded for the sun-dried tuber had higher values 0.23%, 0.88%, and 2.64%, than the fresh tuber 0.05%, 0.51%, and 0.54% respectively. Mucilage content of the fresh false yam was the highest (12%) as against 11.25% for the dried false yam tuber and 7.50% for the seed.

The alkaloid content of the sun-dried false yam was significantly higher ($P < 0.05$) compared with the seed, and the raw false yam (Table 5.2).

5.4 Discussion

Anti-nutritional factors or secondary metabolites in feed when consumed by animals in large quantities affect their growth performance and hence may decrease productivity of these animals (Kumar, 1992).

These anti-nutrients found in the tuber and seed meals are potentially harmful to animals. Studies by Prathibha *et al*. (1995) have shown that anti-nutritional factors in diets affect digestion and immobilize essential nutrients in the gastrointestinal tract of animals.The phytate content of the false yam seed (3.23g/100g) was higher than raw false yam tuber (1.98g/100g) and the sun-dried tuber meal (2.97g/100g). Again, the phytate levels in the false yam are higher than in yams found in the wild; 0.18% to 0.36% (Bhandahari and Kawabata, 2004). Furthermore, the phytate levels in maize (0.16%), cassava (0.4%), and cultivated yams (0.47%) are also lower than the reported value for false yam (Adeyeye *et al*.., 2005). Phytate values reported in the present study corroborate similar result obtained by Umoh (2013) who reported phytate value of 3.85mg/100g in sun-dried false yam tuber.

Phosphorus in plants is stored as phytic acid, also known as hexaphosphate of myo-inositol and constitutes about 80% of total phosphorus in plants. Cocoyam and cassava tubers are reported to contain phytic acid levels of 1.75 and 62.4 g/100g, respectively (Umoh, 2013). The presence of phosphate ions in phytic acid may interact with cations such as K^{+}, Fe^{2+}, Mg^{2}, Mn^{2+}, and Zn^{2+} Ca^{2+} in the gastrointestinal tract of animals forming complex compounds of phytate which are not easily digested and absorbed due to lack of phytase enzymes in the small intestines. In addition, deficiencies of essential nutrients and minerals in the small intestines of monogastric animals are attributed to the presence of the complex phytate.

The least amount of oxalate content was recorded in the raw false yam tuber 0.54g/100g, followed by false yam seed 0.72g/100g and the highest amount of 2.64g/100g was recorded in the sun-dried

false yam tuber (Table 5.2). Salts of oxalate are formed from the interaction between oxalic acid and mineral elements such as sodium, potassium, calcium and magnesium. The chemical combination results in the formation of oxalate salts. The observed values of oxalate (24.65 to 46.22 mg/100g) in leafy vegetables as reported by (Chima and Igyor, 2007) are lower when compared with oxalate values present in false yam tuber and seed meals. Again, oxalate values of 0.06 to 0.197mg/100g found in some wild yams by (Bhandahari and Kawabata, 2004) are lower than the present values observed for the false yam tuber and seeds. Onwuka (2005) has reported 2 to 5g of oxalate as the maximum level for humans beyond which it could cause death. *Colocasia* and *Xanthosoma* varieties of cocoyam have been reported to contain 780 mg/100g of oxalate (Bradbury and Holloway, 1988). The presence of oxalic acid in cultivars of taro tubers and the leaves could cause irritation and acridity in the small intestines and swelling of the mouth and throats of animals when eaten raw (Bradbury and Holloway, 1988). Oxalic acid interacts with mineral elements such as potassium, calcium, magnesium and sodium to form insoluble precipitate in the urinary tracts causing the formation of kidney stones. The salts of the oxlate precipitated have sharp needle like structures which can penetrate soft skin.

The alkaloid values reported by Okwu and Ndu, (2006) for white yam (0.38 mg/100g), aerial yam (0.88 mg/100g) and water yam (0.74 mg/100g) are lower than the present values of the false yam, tuber and the seeds**.**

However, the alkaloid values of 1.68 mg/100g reported by Okwu and Okwu (2004) for the bitter yam can be compared with that of the false yam tuber. Alkaloids in compounds are toxic to humans and can cause death. Some alkaloids are however, useful in treating kidney diseases (Konkwara, 1979). Alkaloids in false yam tuber and seed are used to treat pains and muscle spasms in humans and also used as bactericidal material to kill microorganisms (Okwu and Okwu, 2004). Alkaloids in potato can cause gastrointestinal and nervous problems.

Saponin content (Table 5.2) of the sun-dried tuber of the false yam (0.88 g/100g) was higher than that of the fresh tuber (0.51 g/100g) and the seed (0.58 g/100g). Saponin when disolved in water foams and taste bitter. When saponin is intravenously given to animals the blood cell erythrocytes are completely destroyed.

Saponins present in the false yam tuber and the seed meals have been found useful in treating some health problems (Sodipo *et al*., 1991). This finding in the present study supports an earlier observation made on the processed products which have been kept for three months without mycotic infection. Ladan *et al*. (2009) has suggested that extracts of the false yam tuber and the seed may be useful in chemotherapy against mycotic infections.

In a study using alfalfa saponin (0.71%) in chick and pig diets, the results showed decreased feed consumption, which translated into slow growth rate in the animals (Cheeke and Shull, 1985).

Similar observation was reported for the leaf meal of *Sesbania sesban* having 0.71% saponin fed to chickens (Shqueir *et al*., 1989). Saponins in gastrointestinal tracts of animals interact with cholesterol forming hypocholesterolemia preventing the absorption of the cholesterol (Johnson *et al*., 1986). Mucilage content (Table 5.2) of the raw and sun-dried false yam tuber are significantly ($P < 0.05$) higher (12 and 11.25% respectively) than the false yam seed (7.5%).

Mucilage is a carbohydrate made up of arabinose, xylose, galactose, galacuronic acids and rhamnose components and when present in water swells up and becomes thick increasing intestinal viscousity. Wang *et al*. (2008a, 2008b) have reported that food materials containing more oil and mucilage may increase the intestinal viscousity thereby affecting the digestion of the protein in the feed due to inaccessibility of the enzymes to interact with the protein to break it down.

5.5 Conclusion and Recommendation

Phytochemical screening and quantification of the false yam tuber and the seed revealed anti-nutritive factors such as oxalates, mucilage, steroids, phytate, triterpenoids, alkaloid and saponins. The presence of these secondary metabolites lends credence to the fact that the false yam tuber and the seeds cannot be fed directly to livestock without undergoing processing. It is recommended that suitable, simple but efficient processing methods should be developed in order to make the product more useful in the livestock industry.

CHAPTER 6

6.0 EXPERIMENT 3: EFFECT OF PROCESSING ON NUTRITIVE VALUE AND ANTI-NUTRITIVE FACTORS IN FALSE YAM TUBER AND THE SEEDS

6.1 Introduction

It was established in Experiments 1 and 2 that the false yam tuber and the seeds are good sources of micro and macro nutrients and energy; however, its utilization is limited by anti- nutritive factors. Experiment 3 was conducted to determine the effect of processing on the nutritive value and anti-nutritive factors in the false yam tuber and seeds.

Plants and their parts including leaves, stem, roots, flowers, fruits and seeds used as food materials in both livestock and human diets, may contain biologically active constituents (Igile, 1996). These bioactive compounds may be toxic or advantageous in biological systems. The bioactive constituents are also called secondary metabolites or anti-nutrients. Anti-nutrients are chemical substances which interfere with digestion and absorption of essential nutrients and minerals in the gastrointestinal tracts of animals. Anti-nutrients are the major determinants as to whether plants parts can be used in human and animal diets. Some of the anti-nutrients occur naturally in plants others might have been acquired through fertilizer and pesticide applications on food crops and other plants **(**(Igile, 1996)**.** Examples of anti-nutrients are alkaloids, coumarins, gossypol, cardiac glucosides, trypsin inhibitors, phytates, oxalates, cyanogenic glucosides, flavonoids, saponins and tannins. According to Kertsen *et al*. (1991) and Sugano *et al*. (1993), some anti-nutrients may be deleterious or important in livestock and human nutrition and health related problems depending on the quantity in the diet, that is the level of lethal dosage which may be harmfiul or beneficial to animals and humans. According to Oakenfull and Sidhu (1989) and Soetan (2008), some anti-nutrients are used in pharmacology in health-related issues. Poverty in Africa, ignorance, inadequate nutritional education has killed thousands of people as a result of the consumption of anti-nutritive

factors in food materials as poison especially cynogenic compounds and others which are less known but poisonous (Igile, 1996).

There is the need for constant surveillance to be kept on plants used as feed stuff in human and animal nutrition because of the continuous use of new plant varieties in animal and human diets.

According to Osagie (1998), plant breeders through their research activities to develop plants which are resistant to diseases and can produce more yields have rather introduced undesirable materials into the plants. Osagie (1990) has reported the safe use of some food materials when consumed individually. However, in combination with some other food substances could be detrimental depending on the quantity consumed. For example the consumption of less protein foods in combination with food containing tannins, the protein will be immobilized by the tannins in gastro-intestinal tracts of animals.

Chemical in plants could cause biochemical and pharmacological reactions when consumed by humans and animals **(**Amadi *et al*., 2006; Soetan, 2008**).** Application of processing methods such as boiling, fermentation, soaking and germination and other methods like genetic manipulations could reduce or eliminate some of these bioactive chemicals in plants (Soetan, 2008). A study by Piorrock *et al*. (1984) has shown that bioactive chemcials in plants and their by-products could be reduced through boiling. Plant foods which contain tannins, phytate and oxalate which are poisonous to humans and animals could be rendered safe through cooking before eaten (Enechi and Odonwodu, 2003).

Sathe *et al.* (1984) evaluated the effect of heat as a processing method on secondary metabolites such as trypsin inhibitors and haemaglutinins in legumes. The heat destroyed the hydrogen bonds in the peptide linkage in such native proteins. The destruction of the protein structure exposes them to better digestive enzyme attack. A study by Carlini and Udedebie (1997) reported that cooking reduces anti-nutritive factors such as alkaloids, terpenoids, cyanogenic glucosides and saponins in

jack beans. A study on the nutrients and the bioactive chemicals in sorghum was carried out by using different processing methods such as cooking, sprouting, and fermentation. According to Ikemefua *et al*. (1991), a combination of processing methods such as fermentation and cooking were effective in reducing anti-nutritive factors to acceptable levels than the sole method of heat processing.

Additionally, they observed that processing enhanced enzyme activitites in such a way that they could interact with complexes to release undesirable substances. According to Kazanas and Fields (1991), fermentation increases lipid content of food as a result of increased activities of more lipolytic enzymes leading to production of more free fatty acids and glycerol and thus enhancing the flavor, scent and taste of the end products.

The use of chemicals in processing food materials has been explored by Ologhobo *et al*., (1993). According to Ologhobo *et al*. (1993), alkali was used in the extraction of anti-nutrients from jack beans which was more effective than by acid, ether or alcohol treatment. The use of 0.07% sodium bicarbonate solution decreased considerably the presence of trypsin inhibitor in faba beans better than 0.1% citric acid solution (Fernandez *et al*., 1993). In another studies, Udedibie and Nkwocha (1990) soaked raw jack bean seeds in urea solution (3%) for one week in a room. Fresh water was then used to wash the beans at the end of 7th day. The beans were then dried at 80 ^{o}C in the oven. The urea treated jack beans were milled and used in broiler chickens feeding trial. They concluded that inclusion level of 25% was tolerated by the broiler chicken. Cyanoglucosides in sweet potato, cocoyam, and yellow yam reduced drastically to lower levels after subjecting these food crops to roasting, cooking and boiling as processing methods (Omoruyi *et al*., 2007).

According to Vidal-Valverde *et al*. (1992), soaking is used to remove soluble anti-nutritional elements in feed materials, when the soaking solution is discarded or poured away. Metabolic

reactions in the feed materials and the soaking medium can also affect some of the chemical constituents in the feed.

Joshi *et al*. (1989) have reported that saponins have bitter taste and foamy properties in feed materials. According to them, the bitterness in feed materials can be minimized through repeated washing with water. The resultant product becomes palatable due to the reduction in bitterness in the feed. Experiment 3 therefore sought to determine suitable but simple processing techniques to detoxify the anti-nutrients observed in experiment 2 and at the same time maintain fair balance of nutrients in the false yam tuber. At this stage the false yam seeds were dropped because of the difficulties obtaining the seeds at that time of carrying out the experiments.

6.1.1 Objectives

1. To determine how various processing techniques could be used to lower the quantity of bioactive constituents in the false yam tuber:
   a. Fermentation of the false yam tuber for 14 days
   b. 0.1M sodium hydroxide treatment of the false yam tuber
   c. blanching of false yam tuber in hot water
   d. Sun-drying of the false yam tuber
   e. Sodium hydroxide treated blanched false yam tuber
2. To determine the effects of those processing methods on the proximate nutrients in the false yam tuber.

6.1.2 Hypothesis

Anti-nutritional levels and nutrient composition of the false yam tuber will not differ by using simple processing techniques.

6.2 Materials and methods

6.2.1 Processing methods

Refer to section 3.1

6.2.1.1 Treatments and experimental design

Six treatments of the processed false yam tuber products; sun-dried false yam tuber, blanched false yam tuber, sodium hydroxide (0.1M) treated false yam tuber, sodium hydroxide (0.1M) treated blanched false yam tuber, and fermented false yam tuber arranged in complete randomize design were used. Three independent replicate samples for each treatment were used for every parameter under consideration

6.2.2 Determination of anti-nutritive factors

Refer to section 3.9

Treatments of the false yam tuber included;

1. Fermentation of the false yam tuber for 14 days (FFYM)
2. 0.1M sodium hydroxide treatment of the false yam tuber (NaTFYM
3. blanching of false yam tuber in hot water (BFYM)
4. Sun-drying of the false yam tuber (SDFYM)
5. Sodium hydroxide treated blanched false yam tuber (NaTBFYM)
6. Raw false yam tuber (RFYM)

6.3 Data analysis

The differences in the mean values were subjected to One-way ANOVA for the treatments and Dunnett's multiple comparisons test was used to separate the means using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla, California, USA (www.graphpad.com). Significant differences were declared at a probability of $P<0.05$

6.4 Results

The results of the proximate nutrient composition of the false yam are presented in Table 6.1. The crude protein content of the various processed false yam tuber products did not show any significant ($P<0.05$) change in value with respect to the processing methods used. The highest percentage loss in value was obtained in the blanched false yam (6.34%) compared with the sun-dried false yam tuber. The lowest moisture content recorded was in the sodium treated false yam (3.83%), followed by the sodium treated blanched false yam (4.5%), fermented (5%), blanched false yam (7.5%) and the highest in the sun-dried false yam (7.9%). The crude fibre in the 0.1M treated false yam yielded the highest value of (9.20%) and the least in the blanched false yam (4.71%). The nitrogen free extract, which represents the soluble carbohydrate, was significantly high ($P<0.05$) in the sodium hydroxide treated blanched false yam (78.51%) and the least recorded was in the raw false yam tuber (69.48%).

Among the processing methods, blanched false yam recorded the highest metabolizable energy value (3270.81Kcal/kgDm) compared to the other processing techniques.

Table 6.1: Proximate composition and caloric values of raw and processed false yam tuber

PARA-METERS	RFYM	SEM	SDFYM	SEM	BFYM	SEM	NaTFYM	SEM	NaTBFYM	SEM	FFYM	SEM
DM (%)	81[b]	0.577	92. [a]	0.577	92.5 [a]	0.577	96.17 [b]	0.882	95.5 [c]	0.577	95 [d]	0.577
CP (%)	4.70[a]	0.017	5.34 [a]	0.015	5.00 [a]	0.580	6.10[a]	0.58	6.10[a]	0.58	6.30[a]	0.58
CF (%)	3.57 [a]	0.577	7.29 [b]	0.020	4.71 [c]	0.012	9.20 [d]	0.058	5.75[e]	0.012	8.53[f]	0.015
Ash (%)	1.50[a]	0.058	2.50 [b]	0.012	1.50 [c]	0.058	3.50 [d]	0.058	2.00 [e]	0.058	1.50[f]	0.058
EE (%)	1.75[a]	0.185	4.35 [b]	0	4.32[c]	0	5.2 [d]	0	3.14 [e]	0.00	3.16 [d]	0.00
(%)	69.48[a]	0.83	66.36[a]	1.455	76.97[b]	0.857	72.17[c]	1.14	78.51[d]	0.58	75.51[e]	0.866
Kcal/m)	2783.59[a]	2.014	3140.81[b]	0.9358	3270.81[c]	0.882	3213.10 [d]	0.583	3269.66 [e]	0.768	3172.19 [f]	0.572

RFYM: Raw false yam; SDFYM: Sun dried false yam; BFYM: Blanched (3x) false yam; NaTFYM: Sodium hydroxide (0.1M) treated false yam, NaTBFYM: Sodium hydroxide (0.1M) treated blanched false yam; FFYM: Fermented false yam (14 days). Means in the same row with different supercripts are considered significant ($p < 0.05$). SEM: standard error of mean.

The results of the residual minerals found in the processed products of the false yam tuber are shown in Table 6.2. The mineral elements identified which were consistently high after processing when compared to the unprocessed ones are phosphorus (0.13%) in sodium hydroxide treated fasle yam and sodium hydroxide treated blanched false yam, calcium (0.53%) in sodium hydroxide treated fasle yam, potassium (0.81%) in blanched false yam and the trace elements (mg/100g), iron (7.40) in blanched false yam, zinc (3.03) in fermented false yam, copper (3.45) in sodium hydroxide treated fasle yam, manganese (0.85) in sodium hydroxide treated blanched false yam, respectively.

Table 6.2: Residual mineral element in raw and processed false yam tuber

MINERAL /ELEMENT	RFYM	SDFYM	BFYM	NaTFYM	NaTBFYM	FFYM
Mg (%)	0.26^{b}	.42 ^{a}	0.27^{b}	0.31^{b}	0.27^{b}	0.28^{b}
Ca (Mg/g)	0.21^{b}	0.42 ^{a}	0.30^{b}	0.53^{c}	0.34^{d}	0.41^{ad}
Mn (%)	1.03^{b}	0.75 ^{a}	0.63^{a}	0.83^{ba}	0.85^{a}	0.70^{a}
Na (%)	0.08^{a}	0.10^{a}	0.09^{a}	0.09^{a}	0.07^{ab}	0.05^{b}
K (%)	0.89^{a}	0.88^{a}	0.81^{b}	0.28^{c}	0.45^{d}	0.12^{e}
P (%)	0.13^{b}	0.10^{a}	0.20^{c}	0.13^{d}	0.13^{d}	0.01^{e}
Fe (Mg/g)	7.70 ^{b}	10.90^{a}	7.40^{b}	7.28^{b}	7.15^{b}	5.55^{c}
Zn (Mg/g)	1.80^{b}	3.75^{a}	3.15^{c}	2.90^{d}	2.80^{d}	3.03^{d}
Cu (Mg/g)	2.30^{b}	3.53^{a}	2.35^{b}	3.45^{a}	1.83^{b}	2.98^{a}

RFYM: Raw false yam; SDFYM: Sun dried false yam; BFYM: Blanched (3x) false yam; NaTFYM: Sodium hydroxide (0.1M) treated false yam; NaTBFYM: Sodium hydroxide (0.1M) treated blanched false yam; FFYM: Fermented false yam (14 days). Means in the same row with different supercripts showed significant ($p < 0.05$).

The residual anti-nutritional factors in the processed products of the false yam tuber are shown in Table 6.3. The residual anti-nutritional factors terpenoids, saponins, mucilage, alkaloids, phytate and oxalate were high in the sun-dried tuber and were considerably ($P<0.05$) decreased by the processing techniques used. Fermented false yam for 14 days had the highest reductive percentage of 27.95, 37.25, 68.18, 68.80, 84.47, and 100% in phytate, alkaloids, saponins, terpenoids, oxalate, and mucilage, respectively. However, the highest terpenoid reduction (81.19%) was recorded in the blanched false yam.

Table 6.3: Residual amouints of anti-nutritional substances in processed false yam tuber

ANTI-NUTRIENTS (%)	RFYM	SDFYM	BFYM	Loss (%)	NaTFYM	Loss (%)	NaTBFYM	Loss (%)	FFYM	Loss (%)
Terpenoid	0.05 [b]	0.23 [a]	0.04 [b]	82.61	0.07c	69.57	0.06 [c]	73.91	0.07 [c]	69.57
Saponins	0.51 [a]	0.88 [a]	0.40 [b]	54.55	0.45 [b]	48.86	0.40 [b]	54.55	0.28 [c]	68.18
Polyuronides (mucilage, pectin and resins)	12 [a]	11.25 [a]	0 [b]	100	7.5 [c]	33.33	0 [b]	100	0 [d]	100
Alkaloids	0.77 [b]	1.53 [a]	1.22 [a]	20.26	1.75 [a]	14.38	1.92a [b]	0.25	0.96 [c]	37.25
Phytate	1.98 [b]	2.97 [a]	2.80 [c]	5.72	2.47 [c]	16.84	2.31 [c]	22.22	2.14 [c]	27.95
Oxalate	0.54 [b]	2.64 [a]	0.18 [c]	93.18	0.48 [c]	81.82	0.63 [d]	76.14	0.41 [e]	84.47

RFYM: Raw false yam; SDFYM: Sun dried false yam; BFYM: Blanched (3x) false yam; NaTFYM: Sodium hydroxide (0.1M) treated false yam; NaTBFYM: Sodium hydroxide (0.1M) treated blanched false yam; FFYM: Fermented false yam (14 days). Percentage losse were computed by using the SDFYM as standard against other processing methods. Means in the same row with different superscripts were considered significantly different ($p < 0.05$).

6.5 Discussion

The different processing methods showed significant ($P<0.05$) variations in the proximate compositions except the crude fibre in 0.1M sodium hydroxide treated false yam (9.57%), fermented false yam (8.95%), and ash in blanched false yam (1.62%), fermented false yam (1.58%). Lower crude fibre in blanched false yam and the sodium hydroxide treated blanched false yam when compared to sun-dried false yam could be due to thermal processing in 90^{o}C water which affected the solubility of dietary fibre significantly ($P< 0.05$) which is made up of mucilage dissolving in the hot water leaving small quantity of the insoluble dietary fibre comprising of cellulose and lignin. This confirms similar observation by Madhusud- han (2009). The ash contents in blanched false yam (1.62%), and fermented false yam (1.58%) were low when compared to the sun-dried false yam (2.72%) due to leaching of more soluble minerals into the hot or normal water used during the processing. The most affected minerals for the blanched false yam and fermented false yam were magnesium, calcium, potassium and iron.

Blanching of the false yam tuber as a processing method decreased the crude protein content of the tuber. The seemingly lower crude proteins in blanched false yam (5%) when compared with sun-dried false yam (5.34%) may be due to effect of heat which could have denatured part of the protein. Another contributing factor could be attributed to nutrients leached into the hot water used in the blanching or chemical changes such as oxidation and reduction might have occurred in the medium. This corroborates the findings of FAO (1999) that some minerals when present in food undergo chemical changes such as oxidation reduction reactions affecting nutrients present in the food.

The high metabolisable energy value recorded with blanched false yam (3270.81 Kcal/kg) and sodium hydroxide treated blanched false yam (3269.66 Kcal/kg) when compared with false yam dried in the sun (3140.81 Kcal/kg) could be due to the application of heat which raptured more cells containing starch granules and thereby increasing the soluble starch content of the material.

In addition, thermal degradation of insoluble dietary fibre into components of monosaccharides could have led to increase in the metabolisable energy. This agrees with the findings of Kutos *et al*., (2003).

Fermentation of the false yam as a method of processing appeared to have affected the proximate nutrients, minerals and the anti-nutritional factors in the false yam as depicted in Tables 6.1, and 6.2. According to Iyayi and Losel (1999), fermentation is made up of aerobic and anaerobic processes. In both processes, food materials are immersed in water for one week (submerged fermentation).

The highest crude protein (6.30%) recorded in the fermented false yam tuber could have been caused by enzymes produced by micro-organisms in the fermented medium which might have caused the break down of carbohydrates and lignocelluloses into protein hence causing the slight increase in the protein level in the fermented false yam product.This supports similar observation reported by Hwei-Ming *et al.* (1994) and Balagopalan (1996).

Mineral elements in the processed false yam are shown in Table 6.2. Raw false yam when chipped into thin pieces appeared creamy brown initially but as soon as it was ground it turned into black dough suggesting the presence of mineral element(s) which has been oxidized. Another clear observation was the pungent smell of urine when the chipped products were heaped together for 24 hours. The gas evolved was tested with red litmus paper which turned into blue colour confirming the presence of ammonia gas. The ammonia gas could have come from alkaloid compounds in the false yam. Phytochemical analysis indicated the presence of alkaloid compounds (Tables 5.1 and 5.2). Nitrogen atoms are part of constituents in the structure of alkaloid compounds. Depending on the number of nitrogen atoms present in the alkaloid structure, the alkaloid compounds could be described as 1^{o}, 2^{o} or 3^{o} amines which contribute to the basicity of the alkaloid (Sarker and Nahar, 2007).

The mineral analysis (Table 4.2) has confirmed the presence of high amount of iron in the raw and sundried false yam tuber (7.70-10.90 mg/100g) and this could have been the reason why the fresh dough from the raw yam turned black immediately after grinding due to the oxidization of the iron elements. Other important micro elements present (mg/100g) are zinc, copper and manganese. The highest loss of mineral elements occurred with processing methods such as; fermented false yam, sodium hydroxide treated blanched false yam and the blanched false yam. These reductions were recorded in minerals such as potassium (86.36% loss), phosphorus (90% loss), sodium (50%) and iron (49.08%) for the fermented false yam. However, phosphorus and manganese in sodium hydroxide treated false yam and sodium hydroxide treated blanched false yam appreciated in values and these may be due to some chemical reactions with the sodium hydroxide used in the extracting medium.

Phytochemical constituents (Tables 5.1 and 5.2) observed included phytates, oxalates, sterols, tri-terpenes, saponins, alkaloids and mucilage. However, hydrogen cyanide and tannins were absent. The highest concentrations of saponins and mucilage were in the sun-dried false yam. These anti-nutritional factors were reduced significantly ($P< 0.05$) by the processing techniques adopted. The saponins, terpenoids, mucilage, alkaloids contents (g/100g) of sun-dried false yam were 0.88, 0.23, 11.25, 1.53, respectively; phytate and oxalate (mg/100g) 2.97 and 2.64 respectively. These values were considerably reduced by other processing methods.

However, fermented false yam, and blanched false yam appeared to have the least of the residual anti-nutritive factors and hence the best among the processing methods adopted. The percentage reductive values of the anti-nutrients in fermented false yam were saponin 68.18% loss, tri-terpenes 68.80% loss, mucilage 100% loss, alkaloids 37.25% loss, phytate 27.95% loss and oxalate 84.47% loss.

Blanched false yam percentage reductive values recorded are saponin (54.55% loss), tri-terpenes 81.19% loss, mucilage 100% loss, alkaloids 20.26% loss, phytate 5.72% loss and oxalate 93.18% loss. The alkaloid contents of sodium hydroxide treated false yam and sodium hydroxide treated blanched false yam after processing increased above the alkaloid content in the sun-dried false yam. The reason may be that the alkaloids in the false yam tuber which are sources of ammonia could have reacted with the sodium hydroxide to precipitate more of the alkaloid compounds. Mucilage, another anti-nutritive factor identified in the false yam, was consistently high in the raw false yam (12%) when compared to the processed ones like sun-dried false yam (11.25%), blanched false yam (0.00%), sodium hydroxide treated false yam 7.50%, sodium hydroxide treated blanched false yam (0.00%) and fermented false yam (0.00%). The high percentage reductions of mucilage could be explained by the fact that its constituent units which are made up of arabinose, xylose, rhamnose, galacuronic acids and galactose are water or alkaline soluble. However, in the gastrointestinal tracts of animals, they form thick gelatinous consistency. The viscous mixture affects digestion of protein and absorption of essential nutrients in feed materials thereby causing growth impairements in chicken (Alzueta, *et al*., 2003). According to Wang *et al*. (2008a, 2008b), food materials with high oil and mucilage compositions affect enzyme accessibility to the substrate protein thereby affecting the breakdown of protein by these enzymes.

Another factor may be non- specific interactions between proteins and polysaccharides in the food slowing down the interactions between these nutrients and enzymes (Degen *et al*., 2007). Another processing method which contributed to the reduction of the concentration levels of bioactive factors in the false yam was the use of 0.1M sodium hydroxide solution and also blanching it in hot water apart from the fermented false yam tuber as shown in (Table 6.3). This supports similar findings by Ologhobo *et al*. (1993) who observed greater extractability of anti-nutritional factors in a base soluble fraction like potassium bicarbonate to detoxify jack beans.

The percentage reduction of phytate content of the processed false yam using all the processing methods adopted was not appreciable. This implies that considerable amount of phytate was still present in the processed products.

6.6 Conclusion and Recommendation

The processing techniques adopted had insignificant effect on the proximate and caloric components of the processed products. However, the levels of anti-nutritive factors in the processed products were considerably reduced, with the fermentation for 14 days being the best among the processing methods adopted. Because of the prolonged fermentation for 14 days of the fermented product, it is recommended that the level of possible microbial contamination should be investigated by experiment if the product is to be used in the diets for monogastrics.

CHAPTER 7

7.0 EXPERIMENT 4: EFFECT OF PROLONGED FERMENTATION ON MICROBIAL CONTENT OF FERMENTED FALSE YAM (14 days).

Fermentation is the sum total of chemical reactions which leads to the breakdown of complex nutrients by yeast or bacteria or a combination of the two microorganisms to produce organic acids, alcohol, and carbon dioxide. Alternatively, it is the biological conversion of complex carbohydrates in food by yeast or bacteria or a combination of the two to produce organic acids, alcohol, and carbon dioxide (Stein Kraus, 1998).

Fermentation is a processing method of food materials in water which has been in use for quite a long time to process food materials for humans and animals. According to Iyayi and Losel (1999), fermentation is an aerobic and anaerobic processes involving soaking of food in water for periods extending from 1 to 8 days (submerged fermentation). Soaking on the other hand is a processing method to remove soluble anti-nutritional factors in food or feed stuff by discarding the soaking solution Iyayi and Losel (1999). Fermentation of food materials may take 3 to 8 days to accomplish (FAO, 1998). In both fermentation and soaking there is the possibility of metabolic reactions which will affect the constituent compounds of the material in question (Vidal-Valverede *et al.*, 1992). Acetic acid, alcoholic and putrefactive fermentations are caused by micro-organisms such as moulds, bacteria and yeast. Each of these micro-organisms produces specific enzymes responsible for the breakdown of complex nutreints in food substances (Hassan *et al*., 2005). Fermentation can be either natural or controlled. In natural fermentation, food materials are placed in water and left for four days to one week in a room to ferment. In the natural state of fermentation there are no deliberate additions of microorganisms or heating of the medium. Controlled fermentation on the other hand, involves the deliberate addition of lactic acid bacteria into the medium where the food has been placed to initiate and complete the fermentation process.The end product has desirable

scent and taste and contains vitamins and minersls. The sour taste comes from the lactic acid produced at the end which helps to preserve the product (Achi, 1992). Products from controlled fermentation includes; vinegar, pickles, olives, dairy, krants etc.

Fermentation can also be grouped into liquid substrate and solid substrate fermentations. In the liquid substrate fermentation microbes are grown in a liquid medium which contains almost all the nutrients needed for growth. These microorganisms produce microbe derived enzymes. This type of liquid fermentation is used in most industries to produce probiotics, dairy products, tannin of leather, curing of tea, coffee, bakery products such as acetic acid, butyric acid, lactic acid, etc. On the other hand, solid substrate fermentation involves the use of fungi which are cultured on the surface of substrates to produce hypha which can penetrate deep into the substrates. This is an added advantage over single cell micro-organism which grows on the surface of substrate and uses the nutrients for growth and ultimately for fermentation. Okoli *et al*. (2006); and Cegielska-Radziejewska *et al*. (2013) in their studies reported that some yeast and mould need oxygen inorder to grow. They require water activity of 0.85. However, the yeast requires much higher moisture than the mould. Temperature requirements for these microorganisms ranged from 10 to 35 ^{o}C and a pH of 2 to 9, thus acidic and alkaline mediums. According to them the degree of damages done by microorganisms are associated with the substrate type, microorganisms and the extent of invasion. Mould and yeast can cause 5 to 100% loss in the nutrients in any food material causing the entire food material to decompose (Okoli *et al*., 2006; Cegielska-Radziejewska *et al*., 2013). The mould and yeast may affect both processed and unprocessed food crops and even those in storage. Some of the food crops they attack include cereals, root crops, fruits, pulses and vegetables. The detectability of these microorganisms on food materials depends on visible manifestations they leave on the affected food materials. Some of the signs and symptoms they leave on spoiled food materials include colour, white cottony mycelia, spots of rot, slime and scabs. Yeast and mould infection of

food crops may cause substantial loss to producers, consumers and processors. Mould may produce toxic materials called mycotoxin which is not easily destroyed by heat although the organisms may die during cooking. Fungi presence on food materials affects quality of nutrients since they feed on them producing abnormal scent and taste to the feed (Cegielska-Radziejewska *et al.*, 2013). According to Pitt and Hocking (2009), mould species which are toxic are in the family of *Fusarium*, *Penicillium Alternaria*, and *Aspergillus*. The common mycotoxin found in feed and food include; *Deoxynivalenol, Zeralenone*, *T2 toxin*, *Aflatoxins, Patulin, Fumonisins* and *Ochratoxin A*. When feed affected by fungi is consumed at a lower concentration by poultry for a longer period of time, the mycotoxin in the feed accumulates in the birds and thus affecting their physiological functions. Feed utilization is impaired, translating into poor growth and ultimately affecting their productivity. On the other hand, higher consumption of fungi infected feed may cause quick damage to specific organs such as liver, and the immune system and sometimes leading to death (Mabbett, 2004; Cegielska Radziejewska, 2013). The dough from cassava tubers have rough textures when initially processed, however, with time the cellulose in the dough are broken down by microbial cellulase such as *Candida tropicalis, Rhizopus spp, Zygosacchromyces*, *Geotrichum candidum*, *Bacillus spp*, mainly *subtilis* to produce smooth textures of the dough (Amoa-Awua and Jackobsen, 1995). In addition, souring of cassava dough occurs through the production of lactic acid bacteria, *Leucono mesenteroides, Lactobacter brevis*, and *Lactobacterium plantarum.* Furthermore, the cyanogenic glucoside in the cassava dough produces hydrogen cyanide a toxin which affects the physiology of animals. The enzyme linamarase in the dough and those ones produced by mould, yeast and *Lactobacillu*s help in the breakdown of the cyanogenic glucoside to detoxify the dough.

During the fermentation of the cassava dough microorganisms (*Candida spp, Lactobacillus,* yeast *and* mould) contribute to the production of volatile compounds such as ethylacetate, 3-methyl-1-butanol acetoin, isoamylalcohol, 1-propanol, acetoin and amyl which impart organoleptic properties

such as scent, flavour and good taste to the fermented product (Oyewole and Odunfa, 1990; Jespersen *et al*., 1994).

This study was a follow up to Experiment 3 where one of the processing methods used was fermentation of the false yam tuber for 14 days which proved to be the best among the processed products. The present study was to examine the effect of prolonged fermentation on microbial content of the fermented product.

7.1 Objectives

1. To identify the type of microbes present in the fermented product.
2. To estimate the load of microbes in the fermented products.

7.2 Material and methods

7.2.1 Study area

Refer to *Section 3.1*

7.2.1.1 Treatments and experimental design

Fermented false yam tuber samples arranged in complete randomize design were used. Three independent replicate samples of the treatment were used for every parameter under consideration.

7.2.2 Preparation of fermented false yam tuber

Refer to *Section 3.5*

7.2.3 Enumeration of microbial organisms

In this study, a viable count was the main method used. Plate Count Agar (PCA) (Oxoid CM0325, Oxoid Ltd, England), Malt Extract Agar (MEA) (Oxoid CM59, Oxoid Ltd, England) and 1% Bacteriological Peptone (BP) (Sigma P0556, Sigma-Aldrich, USA) were prepared following the methods prescribed by the manufacturers.

Nine millilitres (9.0 ml) of the BP was distributed into capped test tubes. Autoclave was used to process the PCA, MEA and the BP at 121^{o}C for 15 minutes. In order to keep these materials in the molten state, they were put into water bath at a temperature of 50^{o}C.

The molten media were poured into sterile petri dishes and allowed to solidify. The agar plates were turned upside-down (inverted) and incubated at 37^{o}C for 18-24 hours to check for sterility.

7.2.4 Serial Dilutions

Four sterilized test tubes were used in the dilution process. In each of the sterilized test tube was placed BP measuring 9.0 ml and labelled with dilution factors as follows: 10^{-1}, 10^{-2}, 10^{3}, and 10^{-4}. Ten-fold serial dilution was made from the original concentration (1/1 Dilution or 100) of the sample (one part of the milled sample was mixed with 9 parts of sterile BP for a total of ten parts,

yielding 10-fold dilutions or 1/10 dilution ratio). Using a micropipette, 1/1 Dilution was mixed and 1.0 ml transferred into 10^{-1} dilution and the pipette tip discarded. The content of 10^{-1} dilution was also mixed and 1.0 ml transferred into the 10^{-2} dilution and the pipette tip discarded.

The procedure was continued up to the 10^{-4} dilution as described by Yousef and Carlstrom (2003) and Alexandar (2014).

7.2.5 Spread plate method

One hundred microlitres (100 µl) or 0.1 ml of 10^{-1} dilution was transferred into the solidified PCA and MEA. Using a glass spreader, the inoculum was spread onto the agar surface evenly. This procedure was continued up to the 10^{-4}dilution and a duplicate made for each dilution. The inoculated plates were inverted, and cultured for 24 hours at a temperature of 37ºC. The MEA plates were also cultured for fungi for 48 to 72 hours at a temperature of 27ºC. Growth pattern on agar surface was examined after the period of incubation.

7.2.6 Culturing, isolation, and identification of microorganisms

A loop full of suspended sample in BP was cultured on each of the following: Xylose Lysine Deoxycholate (XLD) agar (Oxoid CM0469, Oxoid Ltd, England) plate for gram-negative bacteria, Blood agar (FlukaBiochemika 70133, Flukachemika, GmbH, Switzeland) plate for gram-positive bacteria, and Sabouraud dextrose agar (Oxoid CM0041, Oxoid Ltd, England) for fungi. Gram-staining was performed on all isolates to determine their Gram reactions.

For the Gram-negative rods/enterobacterioceae identification was done using standard identification test kits (API 20E, BioMerieux, France) (Alexander and Strete, 2001). Catalase test was performed on Gram-positive cocci and rod cells using hydrogen peroxide, 3% H_2O_2 (10 volume solution) (Alexander and Strete, 2001; Cheesbrough, 2006), while macroscopic and microscopic examination was used to confirm the presence of fungi in the sample.

7.2.7 Counting of colonies

Colony counter (Stuart SC6, Bibby Sterilin Ltd, UK) was used to count all visible colonies. Plates with fewer colonies than 20 colonies were considered to have insufficient numbers to be reliable [too few to count (TFTC)] and plates with more than 300 colonies were too crowded to count accurately [too numerous to count (TNTC)]. Thus, count colony forming unit per millilitre or gram (CFU/ml or CFU/g) was calculated using the relations outlined by (Alexander and Strete, 2001).

$$\text{Dilution factor} = \frac{\text{Sample volume}}{\text{Sample volume} + \text{Diluent volume}}$$

$$\text{Count (CFU/ml or CFU/g} = \frac{\text{Average No. of colonies from duplicate plates}}{\text{Dilution factor} \times \text{Volume plated}}$$

7.3 Results

The microbiological results are presented in Table 7.1. The predominant microorganisms identified in the fermented false yam were gram negative rods *Enterobacter coloacae* and *Enterobacter agglumerans*, and gram positive rod *Bacillus spp.* In addition, yeast, and moulds were also identified.

Table 7.1: Isolation and biochemical characterization of organisms from 14- day fermented false yam tuber

Sample code	Colony morpho-logy	Gram stain reaction	Xylose lysine	Deoxy-Cholate	Blood agar	Subou-rand dextrose	Cata-layse Test	Organisms identified
FY_1	Rods	+	+	+	_	_	+	*Enterobacterco loacae Enterobacter*
FY_2	Rods	+	+	+	_	_	+	*agglumerans*
FY_3	Positive rods	+	_	_	+	_	+	*Bacillus spp*
FY_4	Positive oval Shape	+	_	_	_	+	_	*Moulds*
FY_5	Positive oval shape	+	_	_	_	+	_	*Yeast Cells*

FY_1- FY_5: sample codes for fermented false yam for 14 days, +: Positive, -: Negative

The coliform forming unit per mililitre for both negative and positive bacteria was $2.48X10^7$cfu/ml and that of the mould and the yeast was $2.50\ X10^5$cfu/ml (Table 7.2).

Table 7.2: Colonies counted per millitre of the fermented false yam tuber samples

Test code	Test conducted	Results (cfu/ml)
FY-01/14	APC/37°C/24hrs/PCA	$2.48\ X\ 10^7$
	Yeast/Moulds/25°C/5days/MEA	$2.50\ X\ 10^5$

7.4 Discussion

The bacteria counts were very high in the fermented false yam (Table 7.2). The value obtained for the *Enterobacter cloacae, Agglumerans and Bacillus spp* was 2.4 x 10^7 CFU/g. The value obtained was greater than the food value of < 10^4 Cfu/ml reported by Ossai Ochonogor, (2012). This is an indication that the fermented false yam has been contaminated during the 14 days of fermentation

These findings corroborate similar studies by Olukoya *et al*. (1991); Mensah *et al.* (2002) and Yahoah Manu *et al*. (2010). The incidence of bacteria contaminations may be due to un-hygienic nature of the environment where the fermented false yam was dried. The area was close to a poultry house and so human movement might have encouraged multiple contaminations. Also, transfer from hands of processors and flies can cause contamination. This supports the findings of Yassin and Almonqatea (2010). According to Pelczar *et al*. (2005), bacteria which live in the gastrointestinal tracts of animals and humans are the Coliform bacteria and the most prominent ones are the faecal coliforms.

Yeast and mould cell counts were 2.5 x 10^5 CFU. The value obtained was within the recommended value of 1, to 3 x 10^5 $CFUg^{-1}$. The safety of a product and for that matter the hygienic quality depends on the number of harmful microorganisms inhabiting this food. The counts of the fungi propagules should not exceed the values of 1 to 3 x $10^5 CFUg^{-1}$. The concentration of mycotoxin present in the food helps to categorize the food as wholesome or unwholesome (Dalcero *et al*., 1998). Krnjaya *et al*. (2008) in their studies observed that total count of fungi propagules in a feed determines the hygienic quality and safety of that feed. It also helps to estimate the level of mycotoxin concentrations in the feed. Fungal contamination of the false yam tuber, that is, the raw materials might have occurred during pre-harvest and post- harvest periods of production, processing, transportation and storage. Del Pilar Monge *et al*. (2012) have reported similar observation that mould and mycotoxin presence vary in feed materials. According to them, the

conditions prevailing in the area, humidity, the season of the year and the geographical location influence the growth and mycotoxin production by the fungi.

The abnormal scent of the original false yam at the end of the fermentation duration produced very nice alcoholic aroma which is an indication of the activities of yeast. This corroborates similar findings of Amoa-Awua *et al*. (1996) who suggested in their studies that the nice alcoholic scent produced during cassava fermentation by yeast and lactic acid bacteria were mainly products of lower molecular weight of alcohol and esters.

7.5 Conclusion and Recommendation

To conclude, the fermented false yam tuber product was contaminated with *Enterobacter cloacae, Agglumerans and Bacillus spp*. The fermented product can only be used provided the necessary precautionary measures of production, processing, handling, and transportation are considered to avoid excessive microbial contamination.

CHAPTER 8

8.0 EXPERIMENT 5: DIETARY TOXICITY STUDIES OF PROCESSED FALSE YAM USING SPRAGUE DAWLEY RATS

8.1 Introduction

Experiment 5 was a follow up to Experiment 3 and it sought to determine the potential toxicity of the residual concentration of anti-nutritional substances in the false yam products by the use of sub-chronic toxicity studies in Sprague Dawley rats for a period of 3 months. The study aimed at using animals such as Sprague Dawley rats to determine the safety of the processed false yam products.

Toxicology is the study of chemical constituents in plants especially, bioactive factors, drugs and their toxic effect on the brain, the physical condition, the physiology and the well being of animals and humans. These bioactive factors can also cause general weakness in the body and death. Toxicological studies help to make decisions as to whether a new drug should be adopted for clinical use or not (Aneela *et al*., 2011). Also, per the guidelines of OECD 401, 423, and 425 the use of drug clinically without clinical trial and toxicity studies is not permitted. The use of new drugs or bioactive factors in plants on animals and humans require toxicological trials. Again, in order to determine the lethal dosage of the new found drug or feed material, trials are carried out on animals such as pigs, rats, dogs to ascertain the use of the feed material or drug**.** Three types of approach are adopted in toxicological studies. They are acute, subacute, and chronic. In acute studies, a drug or new natural plant product is given in large quantity in order to find out the immediate response of the animals. That is, the acute toxicity helps to determine the LD^{50} of bioactive factors in plants as well as drugs. In subacute, the test material in question is given to the study animal repeatedly at certain dose range or at sublethal quantity for 15 to 20 days. The biological and chemical reactions of the tissues of the animals are then monitored closely. With regards to chronic studies according to Lipnick *et al*. (1995), the study material is given at different doses for 3 months up to a year inorder

to examine the muatagenic or carcinogenic properties of the drug. The animals concerned are then monitored for any abnormal developments.

The toxicity and quality control of false yam incorporation in diet for animals is of much importance, the reason being that there is inadequate scientific study on the safety of the crop. Few studies if any at all has explored such opportunities for the false yam. It is therefore necessary to examine the safety of the false yam tuber through series of toxicity tests so as to provide useful information regarding its use in animal feed.

8.1.1 Objectives

1. To determine the LD^{50} of the raw, sun-dried, and 0.1M NaOH false yam.
2. To determine the cumulative effect of residual anti-nutritive factors in the processed false yam products on biochemical parameters and tissues of the Sprague Dwaley rats.

8.2. Materials and method

8.2.1 Study area

Experimental trial was carried out at the Animal house in Mampong - Akwapim Research into Plant Medicine.

Phase 1 of the study: LD^{50} of the raw, sun-dried, and 0.1M NaOH false yam products.

8.2.1.1 Treatments and experimental design

Three treatments; fermented false yam tuber, sodium hydroxide (0.1M) treated blanched false yam tuber and sun-dried false yam tuber and three levels of the treatments 5%, 10% and 20% in completely randomized design were used. Thus, 3 X 3 factorial in complete randomize design.

8.2.2 Preparation of the false yam tuber

Sample of processed yam products from the earlier experiment were ground into powder form. 10ml of saline water was used to dissolve 5 g of the milled sample of the false yam tuber.

8.2.3 Experimental animals

Young female Sparague Dawley rats 8-12 weeks old and of an average weight of 200 to 280g were selected based on the information provided by OECD (2001) that LD^{50} test on female rats are slightly more sensitive than male rats.The animals selected were individually marked for easy identification before the start of trial. Three times three factorial, in complete randomize design was used to distribute the animals. The selected animals were placed individually in a metabolic cage to acclimatize them to the conditions prevailing in the animal house (50 to 60% relative humidity and a room temperature of 23^{o} C, light days of 12 hours and nights of 12 hours) for 5 days.

The test material or substance was given at one dose level of 5 g/kg body weight to the selected animals. If mortality occurs at this dose level, then a further testing at the next dose level was

carried out on the animals. If test substance-related mortality is produced further, testing at the next lower level may need to be carried out.

8.2.4 Test procedure

Before carrying out the dosing, animals were deprived of food for 24 hours. The average fasted weight of each animal was used to calculate the dosage levels based on the guidelines 423 provided by OECD (2001). Five grams (5g) of the tested substance was given to each animal by using recommended syringe attached to tubes. Three hours after dosing, the animals were supplied with feed in the various metabolic cages. After the administration of the test material, the animals were monitored every 4 hours for 24 hours duration. At the end of the 24 hours, the animals were observed periodically.

8.2.5 Parameters

The wellness parameters were measured by observing the number of animals which showed behavioural changes in salivation, sleep, changes in fur, diarrhoea, tremors, and deaths and recording them as at when they happen. These observations were carried out continuously for 30 minutes. The animals which were used as control were compared with the material tested animals for any observable changes.

8.2.6 Phase 2 of the study: Cumulative effect of residual anti-nutritive factors of the processed false yam products on performance of Sprague Dawley rats.

8.2.7 Plant material

The prepared false yam products of fermented, 0.1M sodium hydroxide treated false yam and sodium hydroxide blanched false yam, were used.

PROCEDURE

8.2.8 Animals and Housing

Seventy-two 4-week old Sprague Dawley rats of mixed sex from Mampong - Akwapim Research into Plant Medicine were used. Sprague Dawley rats of mixed sex weighing from 160 to 200 g of 4 weeks old were selected. Three times three factorial in complete randomize design was used to distribute the animals. Each animal was assigned to a metabolic cage for 5 days in order for the animals to acclimatise to the conditions prevailing in the animal house such as humidity 50 to 60%, temperature 22 ^{o}C to 23 ^{o}C, a light period of 12 hours and a day period of 12 hours. Normal feed and water were supplied *ad-libitum*. At the end of the 5 days acclimatization, the animals were deprived of feed overnight by withdrawing feed.

8.2.9 Diet Formulation and Feeding

After fasting, seventy-two Sprague Dawley rats were selected taken into consideration the initial body weights and wellness conditions of the animals and assigned to four dietary treatments produced in-house consisting of FFYM, NaBFYM, CONT and SDFYM. Following the results of acute toxicity studies, unprocessed false yam and sodium hydroxide treated false yam did not exhibit any adverse effects at dose level of 5g/kg Sprague dawley rats. Based on these results it was established that 5g/kg should be minimum, 10g/kg as medium and 20g/kg as maximum doses to be included in the treatment diets of the rats.

All formulated diets were iso-caloric and iso-nitrogenous. The compositions of the diets are shown in table 8.1. Each animal per metabolic cage were fed *ad-libitum*. Feed and water were replaced as and when it was necessary.

Table 8.1: Composition of Experimental Diets for Sprague Dawley Rats

INGREDIENT	CONTROL 0%	FFYM 5%	FFYM 10%	FFYM 15%	NABTFYM 5%	NABTFYM 10%	NABTFYM 15%	SDFYM 5%	SDFYM 10%	SDFYM 15%
MAIZE	60	55	50	40	55	50	40	55	50	40
FISH MEAL	10.95	10.95	12.14	10.95	10.95	9.57	9.36	10.95	12.14	9.36
SOYA BEAN MEAL	17.05	17.12	16.10	16.10	16.10	17.48	17.69	16.10	16.10	17.69
WHEAT BRAN	10.00	9.93	9.76	10.95	10.95	10.95	10.95	10.95	9.76	10.95
FFYM	0.00	5.00	10.00	20.00	0.00	0.00	0.00	0.00	10.00	0.00
NABTFYM	0	0	0	0	5	10	20	0	0	0
SDFYM	0	0	0	0	0	0	0	5	10	20
OYSTER SHELLS	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
VITAMIN PREMIX	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
SODIUM CHLORIDE	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
TOTAL	100.00	100.00	100.00	100.0	100.0	100.00	100.	100.0	100.00	100.0
CALCULATED NUTRIENTS										
DRY MATTER	90.89	91.5	92.5	94.4	92.04	93.02	94.4	92.04	92.5	94.4
CRUDE FIBRE	0.82	1.19	1.61	2.03	1.11	1.40	1.68	1.11	1.61	1.68
ETHER EXTRACT	0.40	0.42	0.42	0.42	0.39	0.39	0.38	0.39	0.42	0.38
CRUDE PROTEIN	20.13	20.55	20.47	20.39	19.93	19.73	19.52	19.93	20.47	19.52
METABOLISA-BLE ENERGY	12.29	12.35	12.41	12.46	12.35	12.41	12.47	12.35	12.41	12.47

Vitamin premix supplied (per kg diet): vitamin E = 20 I.U; vitamin D3 = 12,000 I.U.; vitamin A = 10,000 IU ; cobalamin = 0.05 mg; Pyridoxine = 4.0 mg; Thiamain = 2.0 mg; Riboflavin = 3.0 mg; vitamin K = 2.5 mg; vitamin B12 = 0.01 mg; panthotenic acid = 5 mg; nicotinic acid = 20 mg; folic acid = 0.5 mg; choline = 0.2 g; iodine = 0.0014 g; iron = 20 mg; zinc = 0.03 g; copper = 0.006 g; cobalt = 0.25 g; manganese = 0.006 g, anti-oxidant = 0.25 mg, selenium = 0.24 mg; biotin = 0.08 mg.
FFYM: FERMENTED FALSE YAM (14days), NABTFYM: SODIUM HYDROXIDE (0.1M) TREATED BLANCHED FALSE YAM. SDFYM: SUN-DRIED FALSE YAM. CONTROL DIET.

8.2.10 Experimental Design

The average weight of the rats and the sex were used to distribute the rats randomly into 4 main groups. Three times three factorial in complete randomize design was used to distribute the animals.

Three males and 3 females making a total of 6 animals per treatment in each group.The animals were assigned to the four dietary treatments of Control (normal diet with no false yam product), fermented false yam, (0.1M) sodium hydroxide treated blanched false yam, and unprocessed false yam (sun-dried false yam).

8.2.11 General observations

One hour after the animals were dose treated observations such as gross toxicities, behavioural changes and deaths were recorded for each animal and thereafter once a day for 3 months duration. The symptoms recorded for each animal were based on what has been described by Path or Tox System 4.2.2 (Xybion Medical Systems Corporation, USA). Average body weights of the animals were taken weekly as well as feed intake considering amount given and the left over the next day.

8.2.12 Urinalysis, haematology and serum biochemistry

The last week before the end of the experimental trial, samples of blood were taken at the tail end of Sprague Dawley rats into test tubes in which heparin and EDTA were placed to prevent coagulation of the blood collected.The samples collected were then analysed for haematological ansd serum biochemical parameters. Also, urine samples were collected and analyzed by the use of urine chemical analyzer (Clinitek-500, USA) and Multistix 10 SG (Bayer, USA). Urine parameters measured included glucose, pH, protein, bilirubin, ketone body, specific gravity, and volume.

The equipment Sysmex KX-2IN Haematology Analyzer (Sysmex Corporation Kobe, Japan) was used to measure blood parameters such as mean corpuscular volume (MCV), red blood cells (RBC), haematocrit (HCT, haemoglobin (HGB), White blood cells (WBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemaglobin concentration (MCHC), platelets (PLT), lymphocyte absolute value (LYM#), lymphocyte percent (LYM%), red cells distribution width (PDW) and platelet larger cell ratio (PLCR%), and mean platelet volume (MPV).

Blood serum biochemistry was carried out using autoanalyzer (Toshiba 200FR NEO, Toshiba Co., Japan) by centrifuging at 300 r.p.m for 10 minutes. Blood serum parameters measured included; glutamyl transpeptidase (GGT), total protein (TP), alkaline phosphatase (ALP), glutamyl transpeptidase (GGT) albumin (ALB), blood urea nitrogen (BUN), creatinine (CREA), creatinine kinase (CK), alanine aminotransferase (ALT), albumin/globulin ratio (A/G), aspartate aminotransferase (AST), total bilirubin (TBIL), potassium (K), sodium (Na), inorganic phosphorus, chloride (Cl) and calcium (Ca).

8.2.13 Necropsy

At the end of the experimental trial the animals were anathesised using isoflurane. The arterial vein at the neck region was then cut. The kidney, heart, lung, liver and spleen were then removed and weighed.

8.2.14 Histopathological examination

The organs from the experimental rats' i.e. the heart, kidney and liver, were placed in 10% neutral buffered formalin. Embedded organs in paraffin were sectioned at 4 to 6 mm and stained with eosin (Sigma HT110-1-32) and hematoxylin (Sigma MHS-16). Tissues of the various organs sectioned were subsequently mounted in a medium for light microscopy.

8.2.15 Statistical Analysis

Data obtained from phase 2, feeding processed products of the false yam to the Sprague Dawley rats were compared individually with the control diet group using their mean values. The differences in the mean values were subjected to Two-way ANOVA and Dunnett's multiple comparisons test were used to separate means by GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla, California, USA (www.graphpad.com). Significant differences were declared at a probability of $P<0.05$.

8.3 Results

The results in phase 1 revealed that raw false yam tuber, sun-dried and (0.1M) sodium hydroxide treated false yam were not found to be toxic, at a dosal level of 5g/kg of the animal's body weight. The body weights of the experimented animals remained unchanged. Also, health parameters such as behavioural pattern, eyes, skin fur, salivation, sleep, and mucous membrane used for toxicity evaluation were found to be normal in both the control and treated animals. There were no signs of diarrhoea, lethargy, or coma in any of the animals.

Based on the guidelines 423 of oral acute toxicity studies OECD (2001) the LD^{50} value of sun-dried false yam and 0.1M sodium hydroxide treated false yam exceeded 5g/kg of the Sprague dawley body weight.

The results in phase 2 showed that rats fed on the processed false yam at the selected inclusion rate of 5g/kg, 10g/kg, 20g/kg did not show any visible clinical signs, deaths or toxic effect on the animal's behaviour throughout the 90- day feeding trial.

8.3.1 Food and water consumption and Sprague dawley rat weight changes

Feed consumption and body weight changes are presented in Table 8.2.

Table 8.2: Growth performance of Sprague Dawley rats fed on unprocessed and processed false yam

Parameters	Level (%)	False yam treatments				ANOVA		
		FFYM	**NABTFYM**	**SDFYM**	**Mean**	**Factor**	**SED**	**P-value**
Final weight (g) rat	0				216.72[a]	T	7.858	< 0.0001
	5	186.83[b]	178.57[bc]	165.33[c]		L	7.858	< 0.0001
	10	159.75[b]	163.00 [b]	157.03[c]				
	20	149.28[b]	152.31[b]	144.34[c]		T X L	8.025	< 0.0001
	Mean	**165.29**	**164.63**	**155.57**				
Feed consumed (g)/rat/day	0				16.09[a]	T	0.3138	< 0.0001
	5	15.39[a]	15.34[a]	13.49[b]		L	0.3138	<0.0001
	10	15.45[a]	14.90[a]	13.02[b]				
	20	15.84[a]	14.98[a]	12.08[b]		T X L	0.3762	<0.0001
	Mean	**15.56**	**15.07**	**12.86**				
Weight gain (g)/rat/day	0				3.07[a]	T	0.07295	< 0.0001
	5	2.80[b]	2.54[c]	1.64[d]		L	0.07295	< 0.0001
	10	2.50[b]	2.36[b]	1.57[c]				
	20	1.91[b]	1.94[b]	1.45[c]		T X L	0.08739	< 0.0001
	Mean	**2.40**	**2.28**	**1.55**				
Feed/gain	0				5.24[a]	T	0.3170	< 0.0001
	5	5.50[a]	6.04[a]	8.23[b]		L	0.3170	< 0.0001
	10	6.18[b]	6.31[b]	8.29[c]				
	20	8.29[b]	7.72[b]	8.38[b]		T X L	0.3410	< 0.0001
	Mean	**6.66**	**6.69**	**8.30**				
Water consumed (ml)/rat/day	0				29.66[a]	T	1.017	< 0.0001
	5	26.86[b]	22.21[c]	22.39[d]		L	1.017	< 0.0001
	10	25.51[b]	24.49[c]	22.75[d]				
	20	23.72[b]	24.61[c]	19.56d		T X L	0.9331	< 0.0001
	Mean	**25.36**	**23.77**	**21.57**				
Urine passed (ml)/rat/day	0				3.72[a]	T	0.6346	< 0.0001
	5	3.75[a]	2.53[b]	2.57[c]		L	0.6346	< 0.0001
	10	3.77[b]	1.94[c]	2.06[d]				
	20	2.40[b]	4.46[a]	2.09[c]		T X L	0.6022	< 0.0001
	Mean	**3.31**	**2.98**	**2.24**				

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam. SDFYM: sun-dried false yam. Control diet. Means in the same row compared with control showing different supercripts were declared significant ($p < 0.05$). 'P' represents the probability, SED is the standard error of difference, r.d.f.; is the residual degree of freedom, T-treatment, L- level, Polynomial contrast for Treatment X Level interaction.

Average daily feed intake per rat on the false yam products ranged from 12.08 to 15.39 g at 5g /kg diet, 13 to 15.45 g/day at 10 g/kg diet, 12.08 to 15.84 g/day at 20 g/kg diet, compared with rats on the control diet (16.09g/day). Sprage Dawley rats on the 5 to 20 g/kg sun-dried false yam diets consumed less feed (13.49g, 13.02g and 12.0g respectively) which was statistically significant ($P<0.05$) compared with their counterparts (16.09g) fed normal diet.

There were significant differences ($P<0.05$) in the final average weight gain (Table 8.2) of rats fed the normal diet when compared with those on the processed false yam products at 5g/kg, 10g/kg and 20g/kg. The body weights of rats on the treated false yam products decreased. At inclusion or dose rate of 20g/kg, rats fed on the sun-dried false yam diet obtained the lowest body weight gain (144.34g) on the 11th week of feeding compared with those on the control diet (216.72g). Rats fed on the normal diet recorded daily weight gain of 3.07 g/day comparable to the rats fed on fermented false yam (2.80g/day). The rats fed on the sun-dried false yam product recorded the lowest gain of 1.45g/day.

Feed conversion efficiency was poor for all rats fed on the processed false yam products. The lowest feed conversion was recorded for rats fed the 20 g/kg sun-dried false yam (8.38). The feed conversion efficiency for rats on the processed false yam products ranged from 5.50 to 8.38. However, their counterparts on the normal diet recorded the highest feed/gain ratio of 5.24 (Table 8.2).

It was observed that the amount of water consumed per rat per day was twice the quantity of feed intake by a rat for the day. The average amount of water intake by a rat fed on a normal diet in a day was 29.66ml higher than rats placed on the processed false yam products (19.56 to 26.86ml). The highest amount of water was consumed by rats fed normal diet and the lowest were those fed on sun-dried false yam diet (19.56ml/rat/day) (Table 8.2).

8.3.2 Urinalysis

The differences in volume of urine passed by rats fed on the 5g/kg diet on fermented false yam, sodium hydroxide treated blanched false yam, sun-dried false yam and those on the 10g/kg diet of sodium hydroxide treated blanched false yam and sun-dried false yam is shown on (Table 8.2).

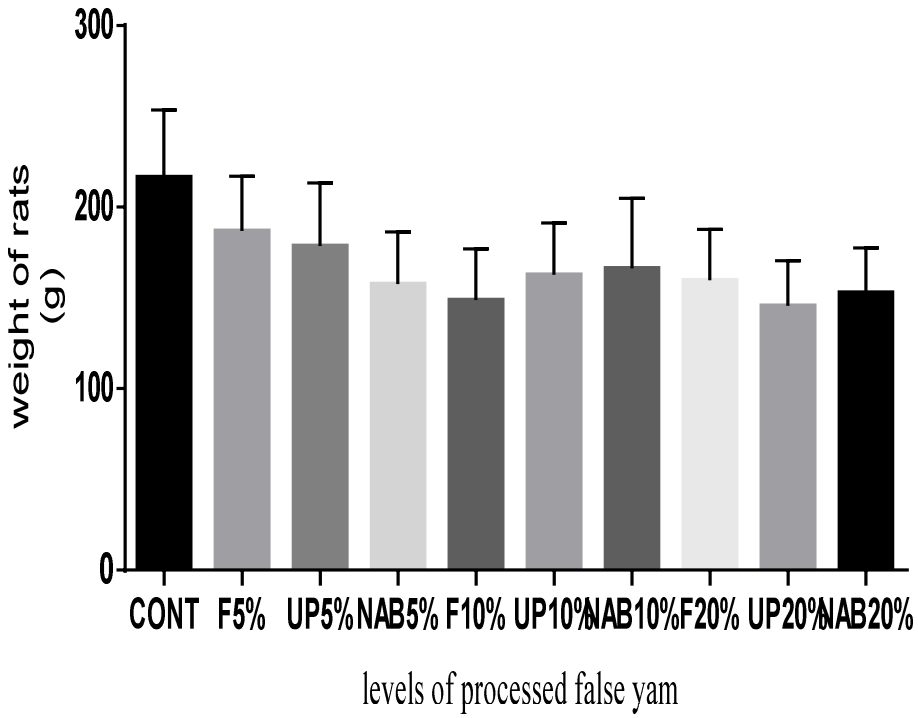
The daily amount of urine passed per rat on the processed false yam products ranged from 1.94 to 4.46ml. The highest amount of urine (4.46ml) was passed by rats fed on the sodium hydroxide blanched fasle yam product at 20 g/kg higher than rats fed on normal diet (3.7ml).The protein content in the urine showed significant difference ($P<0.05$) for rats fed on the 5 to 20 g/kg diet of fermented false yam (0.44 to 0.55 g/l) and those fed on 20 g/kg diet of the sodium hydroxide treated blanched false yam (0.46 g/l) compared with the control group (1.27 g/l) (Table 8.3).

Table 8.3: Effect of varying levels of processed false yam on urinary parameters of Sprague Dawley rats

Parameters	Level (%)	False yam treatments				ANOVA		
		FFYM	NABFYM	SDFYM	Mean	Factor	SED	P-value
KETONE (mg/dl)	0				0.50	T	0.2031	0.3462
	5	0.50	0.82	0.50		L	0.2031	0.2760
	10	0.50	0.59	0.50				0.0968
	20	0.52	0.51	0.52		T X L	0.05194	
	Mean	**0.51**	**0.64**	**0.51**				
PH	0				6.84			
	5	6.33	6.33	6.62		T	0.2903	0.0477
	10	6.33	6.33	6.62		L	0.2903	0.2591
	20	7.50	6.44	6.75		T X L	0.3494	0.1850
	Mean	**6.72**	**6.37**	**6.66**				
PROTEIN (g%)	0				1.27a			
	5	0.44b	0.79a	0.91a		T	0.2698	0.0284
	10	0.49b	0.48a	0.77a		L	0.2698	0.0192
	20	0.55b	0.46c	0.79a				0.0285
	Mean	**0.49**	**0.58**	**0.82**		T X L	0.2816	
BILIRUBIN (mg%)	0				8.67			
	5	8.60	8.60	8.65		T	0.4249	0.2995
	10	8.62	8.57	8.50		L	0.4249	0.9603
	20	8.58	8.63	8.65		T X L	0.09417	0.5201
	Mean	**8.60**	**8.60**	**8.60**				

Means in the same row compared with the control with different supercripts were considered significant at probability level of ($p < 0.05$). ,'P' is probability SED represents standard error of difference, T-treatment, L- level, Polynomial contrast for Treatment X Level interaction.

Results on weight gain of rats are presented in Figs 8.1. Sprague Dawley rats placed on the treatment diets throughtout the entire duration of the experimental trial consistently showed a reduction in body weights. Rats fed 5 g/kg dosage of sodium hydroxide blanched false yam diet, 10g/kg fermented false yam diet and 20 g/kg sun-dried false yam diet recorded the lowest growth rates as shown in fig 8.1. The growth of the rats on the 20g/kg slowed down completely between the 6^{th} and the 8^{th} weeks of feeding. The rats ate small quantity of the feed hence the retardation in growth.



CONT = Control diet
F = Fermented false yam tuber
UP = unprocessed false yam tuber
NAB = Sodium hydroxide (0.1M) treated false yam tuber

Fig 8.1 Mean live weight of sprague Dawley rats fed with diets containing 0% to 20% processed false yam

8.3.3 Haematology and blood chemistry

The results of blood and serum biochemistry are presented inTables 8.4 and 8.5. There were significant ($p<0.05$) reductions in the blood parameters such as HGB, HCT, RBC level of rats fed on the 20g/kg of the treatment diets. Other haematological values, WBC, MCV, MCHC, PLT, LYM (%), LYM #, RDW-SD did not change significantly when compared with their couinterparts fed on the control diet.

Table 8.4: Effect of different levels of processed false yam tuber meal on haematological parameters of Sprague Dawley rats

Parameters	Level (%)	False yam treatments				ANOVA		
		FFYM	NABFYM	SDFYM	Mean	Factor	SED	P-value
	0				14	T	2.559	0.5443
WBC	5	14.47	11.83	13.67		L	2.559	0.7041
(x 10^3/µL)	10	12.77	12.58	12.42				
	20	14.75	12.71	11.82		T X L	1.493	0.2913
	Mean	**14**	**12.37**	**12.64**				
	0				8.57[a]	T	0.1968	0.1017
RBC	5	8.53	8.11	8.48		L	0.1968	0.1819
(x 10^6/µL)	10	8.16	8.28	8.14				
	20	8.28[a]	6.76[b]	8.27[a]		T X L	0.2175	0.0100
	Mean	**8.32**	**7.72**	**8.30**				
	0				14.27 [a]	T	0.4659	0.4302
	5	14.38	13.92	14.27		L	0.4659	0.6377
HGB	10	14.08	14.18	13.69				
(g%)	20	13.73[a]	11.38[b]	14.17[a]		T X L	0.4786	0.0174
	Mean	**14.06**	**13.16**	**14.04**				
	0				44.50 [a]	T	1.332	0.2850
	5	43.29	42.18	44.38		L	1.332	0.1491
HCT	10	40.82	43.42	41.79				
(%)	20	41.91[a]	35.01[b]	43.02[a]		T X L	1.756	0.0142
	Mean	**42.01**	**40.20**	**43.06**				
	0				51.67	T	0.7037	0.1287
	5	50.68	51.91	52.32		L	0.7037	0.6177
MCV	10	52.20	52.40	51.52				
(µm^3)	20	50.64	43.35	52.04		T X L	0.7557	0.0542
	Mean	**51.17**	**49.22**	**51.96**				
	0				16.64	T	0.4075	0.3028
	5	16.84	17.17	17.36		L	0.4075	0.4447
MCH	10	17.32	17.10	16.92				
(pg)	20	16.61	16.54	17.13		T X L	0.440	0.9926
	Mean	**16.92**	**16.94**	**17.14**				
	0				32.25 [a]	T	0.9828	0.3563
	5	33.79	33.07	33.77		L	0.9828	0.5819
MCHC	10	33.19	32.73	33.46				
(%)	20	32.64[a]	26.72[b]	32.98[a]		T X L	0.9454	0.0242
	Mean	**33.21**	**30.84**	**33.40**				
	0				903.67	T	62.81	0.9179
	5	1001.8	1000.9	1036.6		L	62.81	0.1801
PLT	10	938.22	943.06	938.44				
(x 10^3µL)	20	1049.60	955.65	1019.30		T X L	63.73	0.3754
	Mean	**996.54**	**966.54**	**998.11**				
	0				72.61	T	3.206	0.2867
	5	69.91	70.92	66.51		L	3.206	0.4792
LYM	10	69.70	68.82	69.05				
(%)	20	68.98	68.04	66.26		T X L	3.253	0.2238
	Mean	**69.53**	**69.26**	**67.27**				
	0				10.24	T	1.06	0.1712
	5	9.68	8.30	8.29		L	1.06	0.5656
LYM x 10^3	10	9.08	8.78	8.67				
	20	10.34	8.46	7.92		T X L	1.075	0.1287
	Mean	**9.7**	**8.51**	**8.29**				

Table 8.4: Continued

Parameters	Level (%)	False yam treatments			Mean	ANOVA		
		FFYM	NABFYM	SDFYM		Factor	SED	P-value
RDW-SD (%)	0				22.41	T	2.736	0.9900
	5	23.15	22.32	22.47		L	2.736	0.9757
	10	22.82	23.63	23.10		T XL	2.776	0.8647
	20	23.78	21.12	22.44				
	Mean	**23.25**	**22.36**	**22.67**				
MPV (%)	0				6.82	T	0.1798	0.3143
	5	6.57	6.71	6.51		L	0.1798	0.8353
	10	6.64	6.70	6.73		T X L	0.1825	0.4098
	20	6.73	6.55	6.52				
	Mean	**6.65**	**6.65**	**6.59**				
LECO (%)	0				14.75	T	0.2376	0.6368
	5	15.00	14.80	14.75		L	0.2376	0.8292
	10	15.00	15.00	14.83		T X L	0.2220	0.2812
	20	15.00	15.00	14.88				
	Mean	**15**	**14.93**	**14.83**				
SG (%)	0	1.015	1.019	1.018	1.025	T	0.002996	0.7177
	5	1.023	1.021	1.023		L	0.002996	0.4921
	10	1.017	1.021	1.019		T X L	0.003072	0.6324
	20	**1.018**	**1.020**	**1.020**				
	Mean							

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam. SDFYM: sun-dried false yam. Control diet. Means in the same row compared with the control with different superscripts showed significant difference ($p < 0.05$). 'P'is probability, SED represents standard error of difference, T-treatment, L- level, Polynomial contrast for Treatment X Level interaction.

Differences in biochemical parameters such as ALT and AST measured for treatments rats were significantly different ($p<0.05$) compared with values obtained from the control rats (Table 8.5).

Table 8.5: Effect of varying levels of processed false yam on blood chemistry of Sprague Dawley rats

COMPONENT	Level (%)	False yam treatment			Mean	Factor	SED	P-value
		FFYM	NABFYM	SDFYM				
	0				547.56	T	64.21	0.3116
CK-MB	5	641.22	551.53	529.66		L	64.21	0.9841
(µmol/L)	10	546.94	526.56	538.56				
	20	526.72	567.22	498.53		T X L	65.14	0.7612
	Mean	**571.63**	**548.44**	**522.25**				
	0				28.44	T	1.04 7	0.3341
Albumin	5	29.16	30.29	29.82		L	1.047	0.1578
(g/l)	10	28.99	30.76	30.05				
	20	29.52	30.65	30.19		T X L	1.062	0.7891
	Mean	**29.22**	**30.57**	**30.02**				
	0				0.27 [a]	T	0.08312	0.0509
Direct bilirubin	5	0.26	0.47	0.28		L	0.08312	0.0211
(µmol/L)	10	0.21 [a]	0.21 [a]	0.46[b]				
	20	0.26	0.18	0.35		T X L	0.08433	0.1152
	Mean	**0.24**	**0.29**	**0.36**				
	0				0.97	T	0.1304	0.1527
Total bilirubin	5	0.88	0.68	0.91		L	0.1304	0.9311
(µmol/L)	10	0.86	0.90	0.83				
	20	0.77	1.03	0.99		T X L	0.1518	0.3853
	Mean	**0.84**	**0.87**	**0.91**				
	0				93.80	T	15.72	0.9650
Creatinine	5	90.50	94.28	87.02		L	15.72	0.9431
(µ/l)	10	86.17	94.28	85.52				
	20	86.06	84.85	91.67		T X L	15.92	0.9035
	Mean	**87.58**	**91.14**	**88.07**				
Urea	0				17.76a	T	1.366	0.048
(mmol/l)	5	18.18 [a]	16.95 [a]	20.71 [b]		L	1.366	0.2429
	10	19.77	19.28	19.77				
	20	19.89	20.01	18.74		T X L	1.492	0.3786
	Mean	**19.28**	**18.75**	**19.74**				
Total protein	0					T	2.588	0.5465
(g/l)	5	64.22	65.94	67.56	64.33	L	2.588	0.1713
	10	64.73	65.97	69.46				
	20	64.82	68.39	65.21		T X L	2.589	0.5048
	Mean	**64.59**	**66.77**	**67.41**				
	0				482.22 [a]	T	44.78	0.1149
ALP (µ/l)	5	473.11	570.71	479.72		L	44.78	0.0287
	10	535.50 [a]	511.88 [a]	409.17 [b]				
	20	522.94 [a]	475.06 [a]	417.41 [b]		T X L	45.43	0.0802
	Mean	**510.52**	**519.22**	**435.43**				
	0				186.92 [a]	T	12.02	<0.0002
AST	5	144.94 [b]	134.34[c]	140.71[d]		L	12.02	<0.0001
(U/L)	10	150.73 [b]	122.38 [c]	134.13 [d]				
	20	151.26 [b]	134.02 [c]	114.04 [d]		T X L	11.33	<0.0001
	Mean	**148.98**	**130.25**	**129.63**				
	0				52.72 [a]	T	5.624	0.2520
	5	55.72	46.41	46.22		L	5.624	0.0005
ALT	10	44.83 [a]	38.81 [b]	36.06 [b]				
(U/L)	20	42.50 [a]	38.06 [b]	39.24 [b]		T X L	4.113	0.0017
	Mean	**47.68**	**41.09**	**40.51**				

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam. SDFYM: sun-dried false yam. Control diet. Means in the same row compared with the control showing different supercripts were considered significantly different ($p < 0.05$).

'P' represents probability SED is the standard error of difference T is treatment, L- level, Polynomial contrast for Treatment X Level interaction.

High ALP (570.71 and 511.88µ/l) values were recorded by rats on dose level of 5 and 10g/kg of sodium hydroxide blanched false yam product and the lowest (409.17 and 417.41 µ/l) for rats fed on the sun-dried false yam at 10 and 20g/kg diet.

Renal profile measured includes creatin kinase myocardial band and urea. For the creatin kinase myocardial band there was no dose level treatment effect on rats fed on treatment and control diets.

Organs of the rats on the treatment diets showed significant ($P<0.05$) reductions in organ weights (Table 8.6). There were considerable reductions in weight of heart, lung, and kidney weights of the rats fed 20g /kg rate of the sun-dried and sodium hydroxide blanched diets.

Table 8.6: Effect of different levels of processed false yam on organ weights of Sprague Dawley rats

ORGAN	Level (%)	False yam treatments			Mean	ANOVA		
		FFYM	NABTFYM	SDFYM		Factor	SED	P-value
	0				0.77 [a]	T	0.09387	
HEART (g)	5	0.61	0.67	0.73		L	0.09387	0.3492
	10	0.60[b]	0.66 [a]	0.65 [a]				0.0516
	20	0.62	0.57	0.57				
	Mean	**0.61**	**0.63**	**0.65**		T X L	0.06301	0.0524
	0				1.65 [a]	T	0.1215	
LUNG	5	1.55	1.44	1.45		L	0.1215	0.3598
(g)	10	1.48	1.44	1.42				0.5513
	20	1.35 [a]	1.15 [b]	1.14 [b]		T X L	0.1285	0.0019
	Mean	**1.46**	**1.34**	**1.34**				
	0				7.89a	T	0.6749	
LIVER (g)	5	6.16	6.17	6.87		L	0.6749	0.0684
	10	5.50 [b]	6.26 [b]	5.95 [b]				0.0055
	20	6.39 [a]	6.03 [a]	6.25 [a]		T X L	0.7010	0.0480
	Mean	**6.02**	**6.15**	**6.36**				
	0				0.48a	T	0.08789	
SPLEEN (g)	5	0.40	0.59	0.40		L	0.08789	0.1733
	10	0.42 [b]	0.56 [c]	0.36 [d]				0.04009
	20	0.40 [a]	0.38 [ab]	0.39 [b]		T X L	0.03222	0.0103
	Mean	**0.41**	**0.51**	**0.38**				
	0				1.56a	T	0.1577	
KIDNEY (g)	5	1.34	1.17	1.25		L	0.1577	0.1316
	10	1.11 [b]	1.23 [b]	1.12 [b]				0.0066
	20	1.15 [b]	0.98 [b]	1.06 [b]		T X L	0.1493	0.0033
	Mean	**1.20**	**1.13**	**1.14**				

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam. SDFYM: sun-dried false yam. Control diet. Means in the same row compared with the control showing different superscripts were declared significant ($p < 0.05$)., 'P' is the probability, SED represents standard error of difference, T-treatment, L- level, Polynomial contrast for Treatment X Level interaction.

8.3.4 Gross necropsy and Histopathology findings

Tissues of the organs examined did not show any diet related effects when compared with rats on the control diet. Organs such as kidneys, spleen and liver of rats fed on the 10g/kg and the 20g/kg of the treatment diets were highly reduced ($p < 0.05$) compared with the rats on the normal diet. No diet related histopathology findings were made. Tissue examination of the heart, kidney, liver, lung, and spleen are shown in Figures 8.2, 8.3, 8.4, 8.5 and 8.6, respectively.

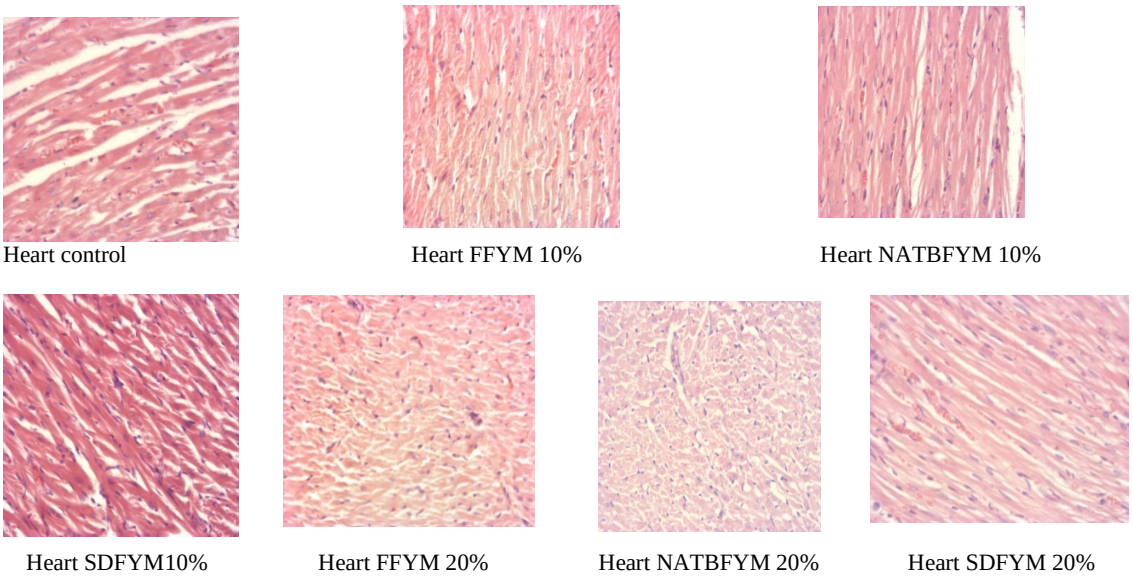
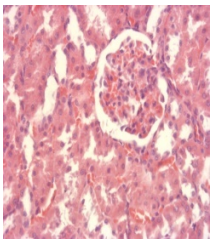
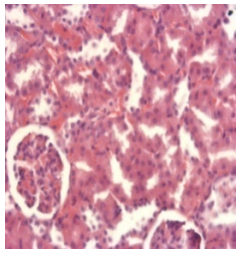
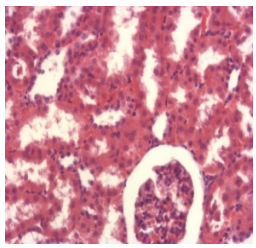
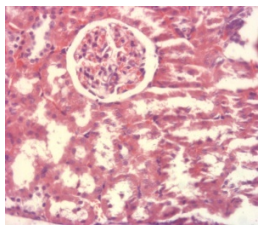


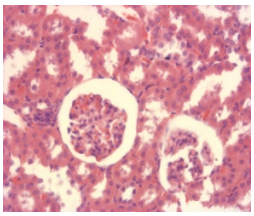
Fig 8.2 Tissues from the heart of Sprague Dawley rats fed on varying levels of processed false yam. The nuclei are clearly visible no bleeding and cross striation of the muscles are conspicuous.

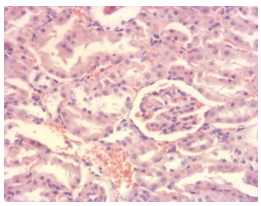

Kidney control


kidney FFYM 10%


kidney FFYM 20%


kidney NATBFYM 10%


Kidney NATBFYM 20%


kidney SDFYM 10%

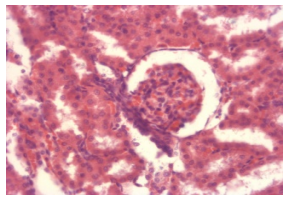
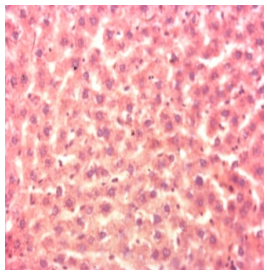
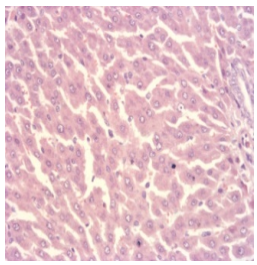
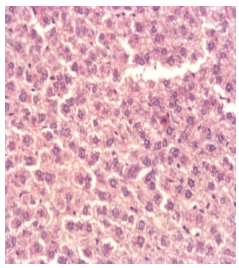
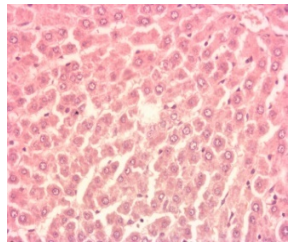

kidney SDFYM 20%

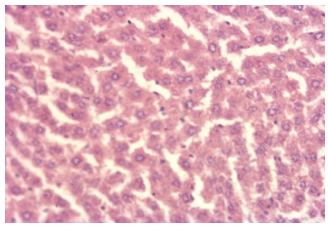
Fig 8.3 Tissues of the kidney from Sprague Dawley rats fed with diets of varying levels of processed false yam. Glomerular cyst and tubular structures in the glomerular capsules are prominently seen.

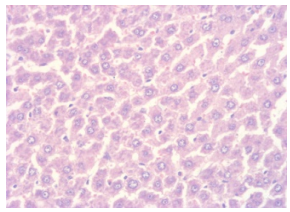

Control liver


Liver FFYM 10%


liver SDFYM 20%


liver FFYM 20%


Liver SDFYM 10%


Liver NATBFYM 10%

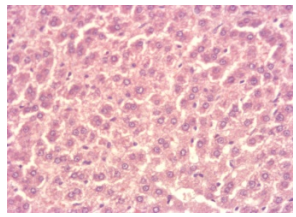
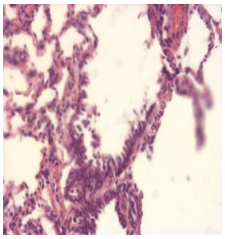
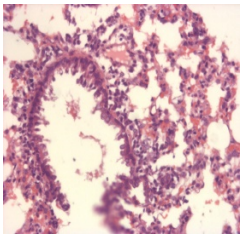
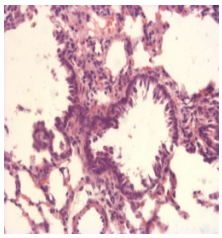
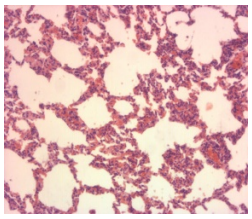

Liver NATBFYM 20%

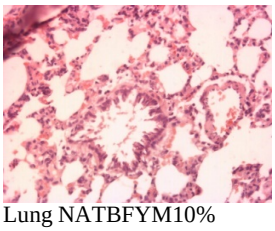
Fig 8.4 Liver tissues from Sprague Dawley rats fed with diets having varying levels of processed false yam. The cells were normal and did not show any degeneration, necrosis and oedema.

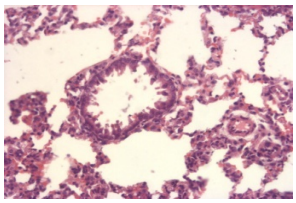

Lung control


lung FFYM 10%


lung FFYM 20%


Lung NATBFYM 20%


Lung NATBFYM10%


Lung SDFYM10%

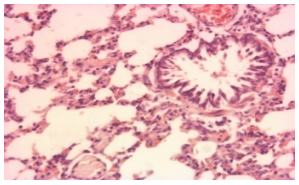
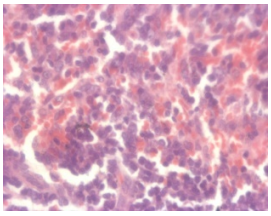
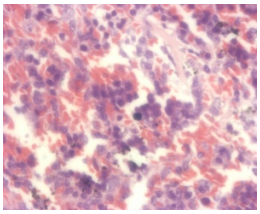
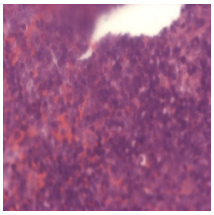
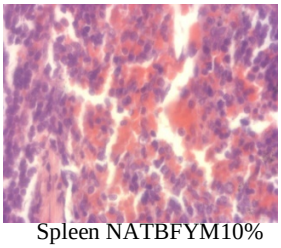

LungSDFYM20%

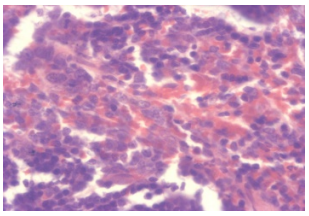
Fig 8.5 Lung tissues from Sprague Dawley rats fed on different levels of processed false yam diet.The lung has normal alveolar sacs with no lesions.

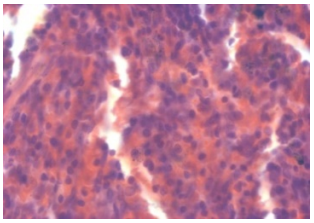

Control Spleen


FFYM10% Spleen


FFYM 20% Spleen


Spleen NATBFYM10%


Spleen NATBFYM20%


Spleen SDFYM10%

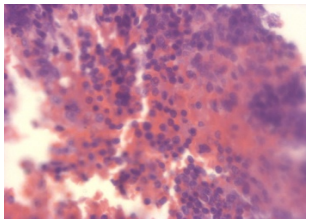

Spleen SDFYM 20%

Fig 8.6 Spleen tissues from Sprague Dawley rats fed on different levels of processed false yam diet. Spleen has normal red and white pulps with central arteriole.

8.4 Discussion

Any new food resource must be subjected to scientific guidelines in an attempt to safe-guard the food for human and livestock consumption. Even though some scientific investigations have been carried out on false yam involving its usefulness in poultry diets, little or no thorough and intensive toxicological work has been done to assess its safety. According to OECD (2001), the guidelines for investigating toxicity in food materials emphasize on body weight changes in the annimals. An accepted limit of 10% decrease in body weight or growth has been fixed under chronic exposure for safety characterization of test material. In the present study, water and feed consumed by the experimental rats and control groups showed significant differences $P < 0.05$. However, a decrease in body weight gains was observed among the treatment groups; 23.73% for rats fed on fermented false yam, 24% for rats on sodium hydroxide blanched false yam, and 28.22% for rats fed on sun-dried false yam compared with the control rats. False yam inclusion in the diets affected the body weight gain of the rats placed on the treatment diets. The residual concentrations of the anti-nutritive factors such as saponin, alkaloid, triterpenoids, phytate, and oxalate in the residual products of the processed and unprocessed false yam may still be a leading contributory factor affecting the growth development of rats placed on the treatment diets. The unprocessed false yam product from previous studies contains relatively higher amounts of the anti-nutritive factors. Although rats fed on it did not show any sign of toxicity, feed consumption decreased at higher inclusion rate of 20 g/kg diet (Table 8.2). The daily decrease in the body weights of rats fed on treatment diets, ranged 1.55-2.40 compared with the control rats 3.07 g/day/rat respectively. This was considered normal with Sprague Dawley rats of similar age and breed in some previous studies reported by Taconic Technical Library, (2003). The apparent decrease in body weights of animals could have been caused by decrease in food and water consumed.

Pathological examination showed no observed colour changes and lesions in the internal organs. The relative organ weights of the control rats' liver, kidney and spleen were higher when compared with rats at dose levels of 10 to 20 g/kg for treatment diets. The low organ weights of the treatments rats could be attributed to commulative effects of residual anti-nutritive elements still remaining in the processed products of the false yam tuber diet. The concentration levels of sponins, alkaloids, terpenoids, mucilage, phytate and oxalates in the processed false yam tuber could have interfered with enzymatic reactions in the gastrointestinal tract, leading to poor digestion and also mineral elements interferance with the anti- nutrients resulting in poor absorption. These observations are similar to what has been reported by Prathiba *et al*. (1995).

Haematological parameters such as HGB, RBC and HCT of rats on the treatment diets were reduced compared to rats placed on the normal diet. The reductions in these parameters could have come from reductions in the spleen and the liver which have direct relationships with RBC, HCT, and HGB formation. The reductions in the haematological factors mentioned could also have triggered a reduction in growth factors consequently resulting in a decrease in body weights of treatment rats. These observations agree to the findings of Nancy (2004) that an increase in HGB, RBC and HCT leads to increase in growth factors and also cause dehydration in animals. The other haematological parameters were normal compared with rats on the control diet.

Serum biochemical parameters such as ALP, ALT and AST for the treatment rats were different from rats placed on the normal diet. The liver, bone and kidney are places of manufacture of ALP (Lakmichi *et al*., 2011). The levels of AST and ALP values in the blood samples collected may be an indication of whether the liver is damaged /injured or not. High levels are indications of possible damage. This corroborates the findings of Pieme *et al*. (2006) and Shashi (2007) that the concentration levels of ALP, ALT and AST are determinant factors whether a disease is imminent

or not. However, no correlatable histopathological changes were observed as shown in figs 8.2, 8.3, 8.4, 8.5 and 8.6.

Renal profile measurements include CK-MB and urea. For CK-MB there was no dose level treatment effect on rats fed on treatment and control diets.

Subchronic test information gathered on specific organ of an animal that has been continuously exposed to test material for longer period of time is useful for toxicity analysis. Pathological examination of the organs of treatment or dose rats did not show any observable alterations in the organs which can be attributed to the test material. However, the liver, spleen and kidney of the treatment groups were significantly ($p<0.05$) decreased when compared with rats on the control diet. The observed changes were found to be related to rats on treatments from 10 to 20 g/kg/diets and were considered to be of toxicological significance.

8.5 Conclusion and recommendation

Sub-acute toxicity study of the unprocessed and processed false yam products did not cause any observable effect on the clinical observations. The blood investigations showed that false yam if consumed by Sprague Dawley rats at a concentration level of 10 to 20 g/kg in the sun-dried and sodium hydroxide blanched false yam diets, may cause a decrease in HGB, HCT and RBC consequently resulting in a decrease in growth rate of the rats. Biochemical parameters such as ALT, and AST were highly affected. The residual concentrations of saponins (0.28 – 0.88%), alkaloids (0.77 – 1.92%) terpenoids (0.05 – 0.23%) mucilage (0 – 12%), oxalate (0.18 – 2.64) and phytates (1.98 – 2.97%) in the processed false yam tuber products could have a cumulative effect on the physiological functions of the rat. Reduced body weights were observed in the animals at a higher treatement dose of (10 to 20 g/kg) which represents an important side effect of false yam and can be determined by further toxicity and pharmacological investigations. Experimental trial using the processed false yam products in feeding should be probed further using broiler chickens.

CHAPTER 9

EXPERIMENT 6: EFFECT OF PROCESSED FALSE YAM PRODUCTS ON GROWTH, BLOOD METABOLITES AND CARCASS CHARACTERISTICS OF BROILER CHICKENS

9.0 Introduction

Experiments 1, 4 and 5 have shown great potentials for the processed false yam products with regard to nutrients, quality and safety. After processing the raw false yam, relatively low levels of anti-nutritional factors were retained in the processed products as reported in the previous experiments. Fermented and sodium hydroxide (0.1M) treated blanched false yam appeared to have responded better when used on Sprague Dawley rats which compared favourably with rats placed on normal diet.

The sub-acute testing of the false yam for toxicity showed no toxicological effect of the residual anti-nutritive factors on Sprague Dawley rats. However, body weights, haematological and serum biochemistry values were reduced. It was therefore necessary to investigate further residues of anti-nutrients in the processed false yam products and their effect on digestion, blood composition, growth and carcass quality of broiler chickens since these are targets for production.

Competition between human-beings and livestock for the conventional feed stuffs needs necessary attention because of the continuous increase in human population which brings with it higher demand for animal protein.

According to Drunna *et al*. (2000), maize constitutes a greater percentage (50%) of carbohydrate source of energy in ration formulation for poultry. In any intensive poultry production feed forms 70% of the total cost (Oluyemi, 1984; Oguntowora, 1984).

Fluctuations in the prices of maize, root and tuber crops and unavailability during certain times of the season calls for an alternate search for feed energy source which is cheaper, less expensive and

readily available. False yam is one of such non-conventional sources of energy. The false yam plant is drought tolerant and forms large tubers underground which weigh over 5kg (NRI, 1987) growing in the wild in the savanna regions especially, in the northern parts of Ghana. It has high nitrogen free extract and mineral elements comparable to that of maize as shown in Experiments 1, 2 and 3, and not easily attacked by insects and diseases when in storage, an observation made on the previous processed false yam products. The anti-nutritive factors found in false yam in Experiments 1 and 2 include saponin, phytates, oxalates, mucilage and alkaloids. The anti-nutritive factors may serve as a defensive mechanism against insects and other pest (Saito *et al*. 1980); Aletor, 1991).

Anti-nutritional factors in false yam tuber can limit maximum utilization by animals in terms of reduction in feed intake, digestibility, mineral absorption and growth. Therefore there is the need to evaluate processed false yam tuber meal containing residues of anti-nutrients.

The experiment aimed at evaluating the effect of the residual anti-nutritive factors in the processed false yam products on growth, carcass characteristics and blood components of broiler chickens.

9.1 Objective

To evaluate the effects of residual anti-nutritive factors in processed false yam products on metabolism and physiology of broiler chickens

9.1.1 Hypothesis

Live weight gain, haematological parameters, serum biochemistry and carcass characteristics of broiler chickens on processed false yam based diets will not differ from control birds on whole maize based diets.

9.2 Materials and methods

9.2.1 Study area

The experimental trial was carried out in the Poultry Unit located at Nyankpala campus of UDS situated geographically in the Guinea Savana Zone at altitude 183m above sea level on longitude 0° 58' 42'' W and latitude 9° 25' 41 '' N (SARI, 2001). The vegetation of the area is generally described as hot-dry Savanna zone. Average temperatures range from 15°C to 42°C. Rainfall experienced in this area is uni-modal with average annual rainfall of 1043 mm, which occurs around April and lasts till November. The dry season begins from November and ends somewhere in March (SARI, 2001). The experiment started on 20th February, 2017 and ended on May, 2017 (8 weeks).

9.2.2 Source and processing of false yam products

This is the same as the description provided in the general materials and methods in Chapter 3.

9.2.3 Experimental birds and design

Five hundred day-old Cobb broiler chicks were brooded under incandescent bulbs of 60-120 watts of energy. The birds were provided with normal starter diets for 4 weeks. During this period the necessary medications and vaccinations were observed. At the end of the 4 weeks and at the begininig of the finisher phase, broiler chickens with an average weight of 900 g were individually distributed randomly into 35 cages with 10 birds per cage. The broiler chickens were assigned to 7 experimental diets (Table 9.1) with 5 replications using 2 x 3, factorial in complete randomized design. Seven experimental diets were formulated comprising fermented false yam (FFYM), and Sodium hydroxide (0.1M) treated blanched false yam (NaBFYM) replacing portions of the maize on weight by weight basis at inclusion levels of 5%, 10% and 15% each. The diet of the control birds contained no (0%) processed false yam products. The experimental diets were formulated to contain the same energy and crude protein contents.

9.2.4 Management of experimental birds

Ten birds per replicate were placed in the same pen enclosed with wire mesh with pen size of 1.8 m x 0.9 m = (1.6m ^{2}/bird) and kept in a conventional poultry house.

The broiler chickens were supplied with experimental feed and water *ad-libitum* under the same conditions in the poultry house. Vaccinations and medications were given to the broiler chickens when there was the need. Feeding trial lasted for 4 weeks. Daily feed intake, body weight gain and mortality were recorded.

Table 9.1: Composition of Experimental Diets

INGREDIENT	CONTROL	FFYM			NABTFYM		
	0%	5%	10%	15%	5%	10%	15%
MAIZE	60	55	50	45	55	50	45
FISH MEAL	10.95	10.95	12.14	10.95	10.95	9.57	9.36
SOYA BEAN MEAL	17.05	17.12	16.10	16.10	16.10	17.48	17.69
WHEAT BRAN	10.00	9.93	9.76	10.95	10.95	10.95	10.95
FFYM	0.00	5.00	10.00	15.00	0.00	0.00	0.00
NABTFYM	0	0	0	0	5	10	15
OYSTER SHELLS	1.30	1.30	1.30	1.30	1.30	1.30	1.30
VITAMIN PREMIX	0.50	0.50	0.50	0.50	0.50	0.50	0.50
SODIUM CHLORIDE	0.20	0.20	0.20	0.20	0.20	0.20	0.20
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
CALCULATED NUTRIENTS							
DRY MATTER	90.89	91.5	92.5	94.4	92.04	93.02	94.4
CRUDE FIBRE	0.82	1.19	1.61	2.03	1.11	1.40	1.68
ETHER EXTRACT	0.40	0.42	0.42	0.42	0.39	0.39	0.38
CRUDE PROTEIN	20.13	20.55	20.47	20.39	19.93	19.73	19.52
METABOLISABLE ENERGY	12.29	12.35	12.41	12.46	12.35	12.41	12.47

Vitamin premix provided (per kg diet): vitamin E = 20 I.U); vitamin K = 2.5 mg vitamin.; vitamin A = 10,000 IU.; vitamin D3 = 12,000 I.U.; Pyridoxine = 4.0 mg; cobalamin = 0.05 mg; Riboflavin = 3.0 mg;Thiamain = 2.0 mg; vitamin B12 = 0.01 mg; choline = 0.2 g; nicotinic acid = 20 mg; folic acid = 0.5 mg; panthotenic acid = 5 mg; manganese = 0.006 g; iron = 20 mg; iodine = 0.0014 g; zinc = 0.03 g; cobalt = 0.25 g; copper = 0.006 g, selenium = 0.24 mg, biotin = 0.08 mg; anti-oxidant = 0.25 mg.

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam.

CONTROL: control diet

9.2.5 Data collection on broiler chicken performance

9.2.5.1 Feed intake

The feed consumed by the broiler chickens were done by estimating the difference in the amount of feed supplied to the broiler chickens per replicate during the week and the left over in the week. The feeds were weighed using electronic scale (JADEVER JPS-1050, Toshiba Co., Japan).

The amount of feed consumed by each bird was then calculated by dviding the amount of feed consumed in the week by the total number of birds in each replicate.

9.2.5.2 Live weight gain

The broiler chickens were weighed weekly in order to obtain the weight gain of the birds during the week. The difference in the final weight gain and the initial live weight was then determined. This was followed with calculation of the daily weight gain by each bird by dividing by the number of days the experiment lasted (28 days).

9.2.5.3 Blood sample collection and processing

Two broiler chickens (female and male) were selected at random from each pen at the end of the 8 weeks feeding trial for blood collection.The blood was taken at about 7.am before feeding. The selected birds were restrained and 2 ml of blood was drawn from their wing veins with syringe and needle. Blood samples collected were placed in test tubes that have been labelled containing Ethylene Diamine Tetra Acetic Acid (EDTA) and mixed thoroughly by shaking inorder to prevent coagulation of the blood.

9.2.5.4 Haematology

Haematology analyzer Sysmex KX-2IN (Sysmex Corporation, Kobe, Japan) was used to process the blood samples collected from the broiler chickens into Haemoglobin concentration (HGB), Red Blood Cells (RBC), White Blood Cells (WBC), Mean Corpuscular Haemoglobin (MCH), Mean

corpuscular volume (MCV), Haematocrit (HCT) Concentration (MCHC), Platelets (PLT), Lymphocyte percent (LYM%), Lymphocyte absolute value (LYM#), Red Cell Distribution Width (RDW), Platelet Distribution Width (PDW), Platelet Larger Cell Ratio (PLCR) and Mean Platelet Volume (MPV).

9.2.5.5 Serum biochemistry

An autoanalyzer (Toshiba 200FR NEO, Toshiba Co., Japan) at 3000 r.p.m was used to process the blood serum for 10 minutes into Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Creatinine (CREA), Blood Urea Nitrogen (BUN), Creatinine Kinase (CK), Total Bilirubin (TBIL), Total Protein (TP), and Albumin (ALB) contents of the blood.

9.2.5.6 Carcass characteristics

At the end of the 8 weeks of the feeding trial the broiler chickens were starved for a period of 24 hours. Two broiler chickens, female and male, were selected from each replicate based on the average mean weight. The birds were then slaughtered by cutting off the jugular vein and allowed to bleed to death. The dead birds were immersed into hot water at a temperature of 70 to 80^{o}C. The defeathered birds were then eviscerated. Organs, such as lungs, liver, emptied intestines, spleen, hearts, kidneys and other parts such as feet, heads etc were weighed.

9.2.5.7 Digestibility trial

According to FAO (2013), digestibility is the percentage of the whole or any single nutrient in the feed not found in the urine or faeces but absorbed from the gastro-intestinal tract for utilization by animals.

The samples of the experimental diets were used in a digestion trial to determine the digestibility of the feed samples containing the nutrients, ether extract, crude protein, ash and dry matter.

9.2.5.8 Experimental design

A total of 35 Cobb broiler chickens of age 8 weeks and an average weight of 1900 g were used for the digestibility trial. Each of the broiler chicken was randomly assigned to a pen (0.4 x 0.3m = 0.12m^2) and a 2 x 3 factorial in complete randomized design was used to distribute the birds to one of the 7 experimental diets and replicated 5 times.

9.2.5.9 Data collection

The experimental diets were weighed daily before supplying them to each bird. The faeces from each replicate were collected by laying plastic sheets on the floor of the wire mesh of each pen. The faeces from each replicate was then weighed and stored under refrigeration at 4^{o}C. At the end of the digestibility trial the faeces samples from each replicate were pulled together to form one sample. The faecal samples were then dried in the oven for 24 hours between 60 and 70^{o}C. The dried faeces were milled and kept in labelled transparent polythene bags for proximate analysis.

9.2.5.10 Proximate analytical procedures

Faecal and experimental diet samples taken were analysed for ether, crude protein, ash and dry matter by the method prescribed by AOAC (2000). All analyses were carried out in triplicates.

9.2.5.11 Coefficients of Digestibility

The apparent digestibility coefficients of dry matter and nutrients were calculated using the formula:

$$\text{Apparent digestibility} = \frac{\text{Nutrient consumed} - \text{Nutrient excreted in faeces}}{\text{Nutrient consumed}}$$

9.2.6 Data analysis

Experimental dietary treatments effects on all parameters collected were subjected to one -way ANOVA for the levels together with the control and factorial analysis for the two factors and their levels. Dunnett's multiple comparisons test was used to compare and separate means using

GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). Treatment means were considered statistically significant at $P<0.05$.

9.3 Results

The growth performance of broiler birds on the varying levels of fermented and sodium hydroxide (0.1M) treated blanched false yam as a substitute for maize are shown in Table 9.2.

Table 9.2: Growth performance and cost of broiler finishers on varying levels of processed false yam meals (4-8 weeks of age)

PARAMETERS	Level (%)	False yam treatments		Mean			
		FFYM	NABTFYM		Factor	SED	P- value
	0			2027.75 [a]			
Average Final Live Weight gain (g) /bird	5	2000.23a	1949.92a		T	138.7	0.8494
	10	1891.11a	1729.31a		L	138.	0.0981
	15	1728.95 [b]	1726.16 [b]		TXL	127.9	0.0315
	Mean	**1873.43**	**1801.94**				
	0			157.50			
Average Daily Feed intake/Bird (g)	5	155.13	146.17		T	5.976	0.1449
	10	155.50	152.84		L	5.976	0.8267
	15	158.70	157.37		TXL	6.234	0.9861
	Mean	**156.44**	**152.13**				
	0			66.09 [a]			
Average Daily weight gain/Bird (g)	5	65.47 [a]	63.64 [a]		T	2.573	0.6163
	10	57.99 [b]	47.07 [c]		L	2.573	0.0001
	15	49.88 [b]	48.74 [b]		TXL	2.093	0.0001
	Mean	**57.78**	**53.15**				
	0			2.44 [a]			
Feed conversion (feed/gain)	5	2.41 [a]	2.32 [a]		T	0.1452	0.6789
	10	2.72 [a]	3.25 [b]		L	0.1452	0.0001
	15	3.22 [b]	3.31 [b]		TXL	0.1577	0.0007
	Mean	**2.78**	**2.96**				
	0			1.91 [a]			
Average cost of feed GH¢/kg diet	5	1.85 [a]	1.85 [a]		T	8.332	0.9984
	10	1.81 [b]	1.79 [c]		L	8.332	0.0161
	15	1.71 [b]	1.75 [b c]		TXL	8.332	0.0160
	Mean	**1.79**	**1.80**				
	0			0.30[a]			
Average cost of Daily Feed in take **(**GH¢/bird)	5	0.29 [a]	0.27 [b]		T	0.0112	0.0306
	10	0.28 [a]	0.27 [a]		L	0.0112	0.0578
	15	0.27 [a]	0.28 [a]		TXL	0.0115	0.1291
	Mean	**0.28**	**0.27**				
Cost of feed/kg weight gain	0			4.66 [a]			
	5	4.46 [a]	4.29 [a]		T	0.2752	0.4029
	10	4.92 [a]	5.82 [b]		L	0.2752	0.0005
	15	5.51 [a]	5.79 [b]		TXL	0.2915	0.0217
	Mean	**5.03**	**5.30**				
				1.91[a]			
Feed cost savings (%)	0				T	0.4860	<0.0703
	5	3.14 [b]	3.14 [b]		L	0.4860	<0.0007
	10	5.23 [b]	6.28 [c]		TXL	0.5880	<0.0001
	15	10.47 [b]	8.38 [c]				
	Mean	**6.28**	**5.93**				

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam. CONTROL: control diet. Means in the same row with different superscripts compared with the control were significantly different ($p < 0.05$). 'P'is the probability; Standard error of difference (SED), T-treatment, L- level, T X L; Polynomial contrast for Treatment X Level interaction.

Feed consumed by broiler chickens daily on the treatment diets ranging between 146.17 and 158.7 g/bird compared favourably with those on the control diet (157.50 g/bird).

The average body weight gain by the broiler chickens decreased linearly when the level of the processed false yam in the diet increased. The average daily weight gain by the control birds recorded the highest value of 66.09 g/bird/day and the lowest 47.05 g/bird/day in 10% sodium hydroxide treated blanched false yam. The daily weight gain by the broiler chickens fed on the fermented false yam at 10% and 15% levels, showed significant difference ($P<0.05$) recording daily weight gains of 57.99g and 49.88g respectively when compared with the control birds (66.09g/bird/day).

Feed conversion ratio values increased significantly ($P<0.05$) when the levels of processed false yam in the diet increased ranging from 2.32 to 3.31 with the 5% sodium hydroxide treated blanched false yam recording the lowest value of 2.32 compared with the control birds (2.44).

The number of birds that died in the control and treatment groups was 3 and 4 respectively.

The final live weight gain of the birds on the treatment diets decreased from 2002.23 g of 5% fermented false yam to 1726.16 g in the 15% sodium hydroxide treated blanched false yam with the control birds recording the highest value of 2027.25 g than the other treatment groups.

The growth pattern of the broiler chickens are presented in a form of bar charts in Fig. 9.1. The growth of broiler chickens on 5-10% processed false yam inclusion in the diet was highly comparable to those on the normal diet. However, at a higher inclusion rate of the processed false yam (15%) in the treatment diets, growth of the birds decreased considerably.

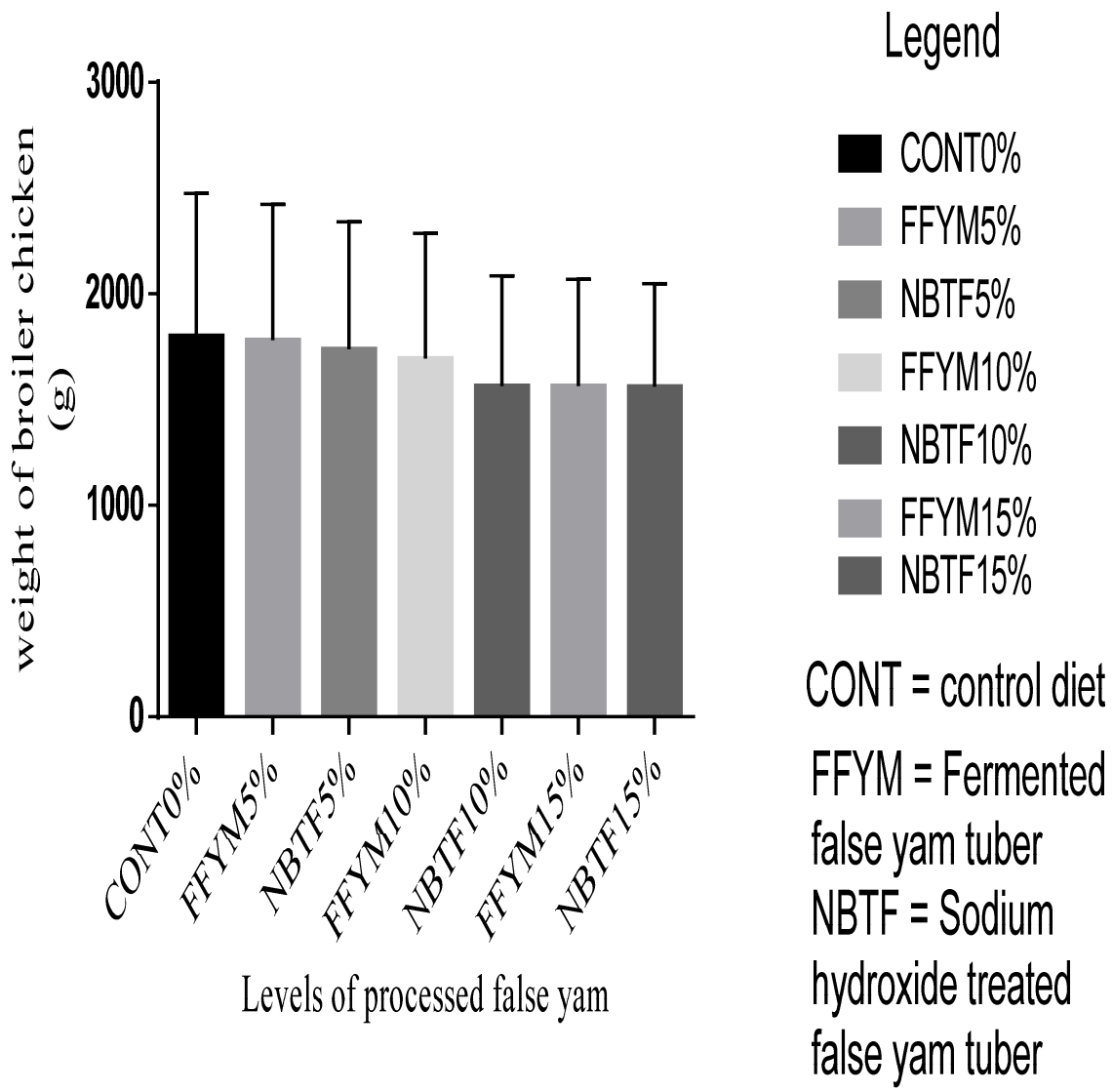


Fig 9.1: Mean live weight of broiler chickens fed with diets containing 0% to 15% processed false yam

The feed cost/kg weight gain of birds on the 10% sodium hydroxide treated blanched false yam diet (Table 9.2) was significantly (P< 0.05) higher with a value of GH¢ 5.82 while the lowest cost of GH¢ 4.29 was recorded for birds fed 5% sodium hydroxide treated blanched false yam diet.

The feed saving cost analysis showed that the 15% fermented false yam diet had the highest (p< 0.05) cost savings of GH¢ 10.47 compared to GH¢3.14, 5.23 6.28 and 8.38 in the 5-15% false yam treated diets, respectively.

The carcass yield analysis indicated higher (P<0.05) values for the birds on the control diet with lower values of organs in the birds fed on processed false yam products. Organs weight such as lungs, kidney and liver of birds fed on the treatment diets were significantly lower (P<0.05) compared to those on the normal diet. However, the weights of crops of the control birds were lower than their counterparts on the treatment diets (Table 9.4).

Table 9.3: Carcass characteristics of broiler finishers fed processed false yam meals (4-8 weeks of age)

PARAMETERS	Level (%)	False yam treatments		Mean			
		FFYM	NABTFYM		Factor	SED	P- value
Live weight (g) / bird	0			2710 [a]			
	5	2582	2518		T	176.5	0.5511
	10	2578	2438		L	176.5	0.3157
	15	2229 [b]	2264 [b]		TXL	175.3	0.0040
	Mean	**2463**	**2407**				
Dressed weight(g)	0			2037 [a]			
	5	1921	1875		T	133.3	0.4668
	10	1881	1741		L	133.3	0.0891
	15	1573 [b]	1566 [b]		TXL	128.8	<0.0001
	Mean	**1792**	**1727**				
Dressing (%)	0			75.11 [a]			
	5	74.53 [a]	74.37 [a]		T	0.8527	0.6612
	10	72.98 [a]	71.58 [b]		L	0.8527	0.0014
	15	70.82 [b]	69.13 [b]		TXL	0.8637	0.0009
	Mean	**72.78**	**71.69**				
Eviscerated weight (g)	0			1730 [a]			
	5	1609 [b]	1545 [c]		T	2.073	<0.0001
	10	1537 [b]	1387 [c]		L	2.073	<0.0001
	15	1210 [b]	1186 [c]		TXL	3.464	<0.0001
	Mean	**1452**	**1373**				
Head (g)	0			13.80 [a]			0.8329
	5	11.20 [a]	11.20 [a]		T	4.630	0.3466
	10	10.80 [a]	11.00 [a]		L	4.630	0.3227
	15	11.20 [a]	10.00 [b]		TXL	3.588	
	Mean	**11.07**	**10.73**				
Feet (g)	0			104.80			
	5	102.40	99.20		T	7.99	0.7826
	10	98.60	100.60		L	7.99	0.7714
	15	90.00	96.60		TXL	8.741	0.4197
	Mean	**97**	**98.8**				

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam. CONTROL: control diet.

Means in the same row with different superscripts compared with the control were significantly different ($p < 0.05$). 'P'is the probability, SED; is the standard error of difference, T-treatment, L-level, Polynomial contrast for Treatment X Level interaction.

Table 9.4: Organ weight of chicken broilers fed False yam Meal

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam. CONTROL: control diet.

PARAMETERS	False yam treatments			ANOVA			
	Levels (%)	FFYM	NABTFYM	Mean	Factor	SED	P-value
Kidney (g)	0			3.60[a]	T	0.1763	0.0001
	5	4.00[b]	3.00[c]		L	0.1763	0.0001
	10	3.80[b]	3.00[c]		T X L	0.1163	0.0001
	15	3.60[b]	2.80[c]				
	Mean	**3.80**	**2.93**				
Lung (g)	0			23.17[a]			
	5	22.27[b]	22.30[b]		T	0.2277	0.0002
	10	21.47[b]	22.43[b]		L	0.2277	0.0008
	15	21.60[a]	20.00[b]		TXL	0.2309	0.0019
	Mean	**21.78**	**21.58**				
Liver (g)	0			58.60			
	5	55.20	61.20		T	**3.767**	0.2954
	10	62.20	59.80		L	3.767	0.7115
	15	60.40	53.40		TXL	4.415	0.3881
	Mean	**59.27**	**58.13**				
Bile (g)	0			5.20[a]			
	5	4.90[a]	4.77[b]		T	0.1466	0.0426
	10	6.13[b]	5.90[b]		L	0.1466	0.0029
	15	6.13[b]	5.40[a]		TXL	0.1262	<0.0001
	Mean	**5.72**	**5.36**				
Proventriculus (g)	0			12.63[a]			
	5	12.30[a]	13.37[b]		T	0.1753	0.0005
	10	13.37[b]	13.42[b]		L	0.1753	<0.0001
	15	12.70[a]	13.07[b]		TXL	0.1186	0.0109
	Mean	**12.79**	**13.29**				
	0			41.80			
Gizzard (g)	5	41.40	40.80		T	3.423	0.9577
	10	45.00	43.00		L	3.423	0.6862
	15	41.00	41.40		TXL	3.362	0.9721
	Mean	**42.33**	**41.73**				
	0			37.80[a]			
Crop (g)	5	40.40[a]	45.40[a]		T	11.24	0.7911
	10	37.50[a]	47.40[a]		L	11.24	0.3751
	15	57.60[b]	64.00[c]		TXL	7.897	0.0179
	Mean	**45.17**	**52.27**				
	0			110.40[a]		14.36	
Intestine (g)	5	120.40[a]	127.60[a]		T	14.36	0.4943
	10	143.80[a]	147.80[a]		L	16.00	0.1117
	15	148.20[a]	169.00[b]		TXL		0.0038
	Mean	**137.47**	**148.1**				

Means in the same row with different superscripts compared with the control were significantly different ($p < 0.05$). 'P'is the probability, SED; is the standard error of difference, T-treatment, L- level, Polynomial contrast for Treatment X Level interaction.

Broiler chickens fed on 5-10% of fermented and sodium hydroxide treated blanched false yam showed a significant increase in the crops and kidneys while a decrease in the lung and kidney weights of those on the 5-10% sodium hydroxide treated blanched false yam fed birds when compared with their counterparts on normal diet (Table 9.4).

The coefficients of the digestibility trial are presented in Table 9.5.

Table 9.5: Effect of processed false yam tuber meals on nutrient digestibility of broiler chickens

NUTRIENT (%)	Level (%)	False yam treatments		Mean	ANOVA		
		FFYM	NABTFYM		Factor	SED	P- value
	0			**76.86**[a]			
CP	5	77.04[a]	80.16[b]	78.60	T	0.8315	0.0123
	10	77.86[b]	79.63[b]	78.75	L	0.8315	0.0422
	15	74.98[b]	76.08[b]	75.53	TXL	0.8356	0.1560
	Mean	**76.63**	**78.62**				
	0			**59.6**[a]			
EE	5	53.91[b]	66.03[c]	59.97	T	0.8356	0.0001
	10	66.24[b]	72.54[c]	69.39	L	0.8356	0.0001
	15	63.92[b]	63.96[c]	63.94	TXL	0.8356	0.0029
	Mean	**61.36**	**67.51**				
	0			**57.55**[a]			
ASH	5	63.94[b]	62.5[b]	63.22	T	1.248	0.0051
	10	65.03[b]	77.98[c]	71.51	L	1.248	0.0001
	15	58.84[a]	69.46[b]	64.15	TXL	0.8466	0.0001
	Mean	**62.60**	**69.98**				
	0			**41.35**[a]			
CF	5	37.68[b]	48.69[c]	43.19	T	0.8342	0.0001
	10	30.71[b]	54.62[c]	58.02	L	0.8342	0.0001
	15	44.21[b]	38.25[c]	41.23	TXL	0.8342	0.0012
	Mean	**37.53**	**47.19**				

FFYM: fermented false yam (14days), NABTFYM: sodium hydroxide (0.1M) treated blanched false yam. CONTROL: control diet.

Means in the same row with different superscripts compared with the control were significantly different ($p < 0.05$). 'P' is the probability SED; is the standard error of difference, T-treatment, L- level, Polynomial contrast for Treatment X Level interaction.

The nutrients in the processed false yam products i. e. (crude protein, crude fibre, dry matter, ether extract) increased ($P< 0.05$) linearly from 5% to 10%. However, at a higher inclusion level of 15%, the proximate nutrients showed a significant ($P <0.05$) decrease.

Haematological parameters such as RBC, HB, WBC, HCT, MCHC, RDW-CV, MPV, PDW and PCT (Table 9.6) measured for broiler chickens fed on the processed false yam products were different ($P<0.05$) from that of the control birds.

Blood parameters such as HCT, RBC, MPV, RDW-SD, HB and RDW-CV in the 5%, 10% and 15% FFYM group showed significant decreases ($P<0.05$); and elevated values in the MCHC (5% NaBTFYM and 15% FFYM), MPV 5% NaBTFYM and WBC in 5%, 10% and 15% FFYM when compared with values obtained from birds in the control group (Table 9.6).

Table 9.6: Effect of processed false yam tuber meal on haematological parameters of broiler chicken (4-8 weeks of age)

PARAMETERS	Level (%)	False yam treatments ANOVA		Mean	Factor	SED	P-value
		FFYM	NABFYM				
WBC count (10^6mm^3)	0			**22.74** [a]	T	0.1643	<0.0001
	5	38.36[b]	17.02[c]		L	0.1643	<0.0001
	10	36.23 [b]	29.66 [c]				
	15	27.26 [b]	19.03 [c]		T X L	0.1758	<0.0001
	Mean	**33.95**	**21.90**				
RBC count (10^6/ml)	0			**2.59** [a]	T	0.02261	<0.0001
	5	2.47 [b]	2.60[a]		L	0.02261	<0.0001
	10	2.37 [b]	2.33 [b]				
	15	2.46 [b]	2.48 [b]		T X L	0.01841	<0.0001
	Mean	**2.43**	**2.47**				
HB (g/dl)	0			**8.07** [a]	T	0.07492	<0.0001
	5	7.63 [b]	7.99 [a]		L	0.07492	<0.0001
	10	7.19 [b]	7.06 [c]				
	15	7.42 [b]	7.75 [c]		T X L	0.02824	<0.0001
	Mean	**7.41**	**7.60**				
HCT (g%)	0			**32.08** [a]	T	0.4602	0.0012
	5	30.18 [b]	30.92 [b]		L	0.4602	<0.0001
	10	29.37 [b]	28.88 [b]				
	15	29.30 [b]	30.04 [b]		T X L	0.2825	<0.0001
	Mean	**29.62**	**29.95**				
MCH (pg)	0			**123.59**	T	0.8800	0.9795
	5	122.61	119.09		L	0.8800	0.5788
	10	123.80	124.09				
	15	120.75	120.88		T X L	0.6890	0.9795
	Mean	**122.38**	**121.32**				
MCHC (g/dl)	0			**25.13** [a]	T	0.1831	0.0003
	5	25.27 [a]	25.94 [b]		L	0.1831	0.0125
	10	24.51 [b]	24.62 [b]				
	15	25.43 [a]	25.79 [b]		T X L	0.2056	0.0003
	Mean	**25.07**	**25.45**				
PLT (x 10^3 µL)	0			**21.13** [a]	T	0.2968	<0.0001
	5	19.74 [b]	11.31 [c]		L	0.2968	<0.0001
	10	17.42 [b]	14.34 [c]				
	15	20.02 [b]	17.50 [c]		T X L	0.1367	<0.0001
	Mean	**19.06**	**14.38**				
RDW-SD (%)	0			**38.85** [a]	T	0.4369	<0.0001
	5	33.04 [b]	37.00 [c]		L	0.4369	0.0213
	10	37.98 [a]	37.70[a b]				
	15	38.72 [a]	36.19 [b]		T X L	0.4019	<0.0001
	Mean	**36.58**	**36.96**				
RDW-CV (%)	0			**9.19** [a]	T	0.1304	<0.0001
	5	7.89 [b]	9.18[a]		L	0.1304	0.0056
	10	8.62 [b]	8.74 [b]				
	15	9.12 [b]	8.71 [b]		T X L	0.1689	0.0227
	Mean	**8.54**	**8.88**				

Table 9.6: Continued

PARAMETERS	Level (%)	False yam treatments		Mean	ANOVA		
		FFYM	NABFYM		Factor	SED	P-value
PDW (%)	0			**10.32** [a]	T	0.1045	
	5	8.97[b]	10.31[a]		L	0.1045	<0.0001
	10	10.67 [b]	8.39 [c]				<0.0001
	15	8.22 [b]	9.02 [c]		T X L	0.0399	<0.0001
	Mean	**9.29**	**9.24**				
MPV (%)	0			**9.59** [a]	T	0.04115	
	5	8.96 [b]	9.73 [c]		L	0.04115	<0.0001
	10	9.13 [b]	9.15 [b]				<0.0001
	15	7.27 [b]	8.72 [c]		T X L	0.01636	<0.0001
	Mean	**8.45**	**9.19**				
PLCR (%)	0			**25.40**[a]	T	0.2161	
	5	19.84 [b]	26.20 [c]		L	0.2161	<0.0001
	10	25.84 [a]	20.98 [b]				<0.0001
	15	17.77 [b]	18.83 [c]		T X L	0.1734	<0.0001
	Mean	**21.15**	**22.00**				
PCT (%)	0			0.019	T	0.0047	
	5	0.018	0.012	0.015	L	0.0047	0.2928
	10	0.017	0.013	0.015			0.4968
	15	0.010	0.016	0.013	T X L	0.0051	0.2866
	Mean	**0.015**	**0.014**				

FFYM: Fermented false yam (14days), NABTFYM: Sodium hydroxide (0.1M) treated blanched false yam. Control diet. Means in the same row compared with control with different superscripts showed significant difference ($p < 0.05$). P' is the probability SED; standard error of difference, T-treatment, L- level, Polynomial contrast for Treatment X Level interaction.

Serum biochemistry indices measured for the treatment groups which were significantly ($P < 0.05$) different from the control birds included; albumin, globulin, ALT, Direct bilirubin, GT, Creatinine, urea, sodium, potassium and chloride (Table 9.6).

Table 9.7: Effect of processed false yam tuber meals on blood chemistry of broiler chicken (4-8 weeks of age)

BLOOD CHEMISTRY	Level (%)	False yam treatments			ANOVA		
		FFYM	NABFYM	Mean	Factor	SED	P-value
ALBUMIN	0			**18.16** [a]	T	0.6148	0.0035
(g%)	5	16.57[b]	18.81[a]		L	0.6148	<0.0001
	10	14.75[b]	15.64[b]				
	15	17.34a[b]	15.86[b]		T X L	0.5823	0.0043
	Mean	**16.22**	**16.77**				
GLOBULIN	0			**20.30** [a]	T	1.774	0.0004
(g/%)	5	23.75[a]	28.50[b]		L	1.774	<0.0001
	10	17.97[b]	22.26[c]				
	15	21.53[a]	24.38[b]		T X L	0.5963	0.0226
	Mean	**20.74**	**21.61**				
PROTEIN	0			**40.69** [a]	T	0.7635	<0.0001
(g%)	5	43.26[b]	46.20[c]		L	0.7635	<0.0001
	10	34.74[b]	37.03[c]				
	15	41.69[a]	43.13[b]		T X L	0.8227	0.0117
	Mean	**39.32**	**39.97**				
	0			**7.94** [a]	T	0.4606	0.0974
ALT	5	7.31[a]	8.34[a]		L	0.4606	0.4044
(IU/L)	10	6.67[a]	7.64[a]		T X L	0.9701	0.0019
	15	9.34[b]	7.79[a]				
	Mean	**7.70**	**9.62**				
	0			**2.60** [a]	T	0.0669	<0.0001
DIRECT	5	1.40[b]	3.34[b]		L	0.0669	<0.0001
BILIRUBIN	10	1.13[b]	1.26[b]				
(µmol/L)	15	1.38[b]	1.44[b]		T X L	0.0929	<0.0001
	Mean	**1.30**	**2.01**				
TOTAL	0			**2.46** [a]	T	0.1875	<0.0001
BILIRUBIN	5	1.64[b]	5.28[c]		L	0.1875	<0.0001
(µmol/L)	10	3.58[b]	2.93[c]		T X L	0.1211	
	15	4.47[b]	4.78[c]				<0.0001
	Mean	**3.23**	**4.33**				
	0			**14.15** [a]	T	0.1392	<0.0001
GT	5	13.25[b]	15.55[c]		L	0.1392	<0.0001
(U/L)	10	14.68[b]	16.96[c]				
	15	14.33[a]	11.54[b]		T X L	0.1883	<0.0001
	Mean	**13.97**	**14.74**				
	0			**25.74** [a]	T	0.5266	<0.0001
CREATININE	5	32.79[b]	46.07[c]		L	0.5266	<0.0001
(mmol/L)	10	39.05[b]	51.23[c]				<0.0001
	15	51.00[b]	39.29[c]		T X L	0.2292	
	Mean	**40.95**	**45.53**				
	0			**1.09** [a]	T	0.0492	<0.0001
UREA	5	0.77[b]	2.22[c]		L	0.0492	<0.0001
(mmol/L)	10	1.95[b]	2.55[c]				
	15	1.36[b]	1.49[c]		T X L	0.0490	<0.0001
	Mean	**1.36**	**2.09**				

Table 9.7: Continued

BLOOD CHEMISTRY	Level (%)	FFYM	NABFYM	Mean	ANOVA Factor	SED	P-value
	0			**153.9**	T	2.835	
SODIUM	5	154.8 [a]	145.4 [b]	[a]	L	2.835	0.0044
(mmol/L)	10	144.1 [b]	136.9 [c]				<0.0001
	15	126.3 [b]	127.1 [b]		T X L	0.8455	<0.0001
	Mean	**141.73**	**136.47**				
POTASSIUM	0			**10.32**	T X L	0.0599	
(mmol/L)	5	7.89 [b]	7.45 [c]	[a]	L	0.0599	<0.0001
	10	7.43 [b]	8.25 [c]				<0.0001
	15	6.75 [b]	6.97 [c]		T X L	0.1144	<0.0001
	Mean	**7.36**	**7.56**				
	0			**103.9**	T	1.083	
CHLORIDE	5	107.60 [b]	102.20 [a]	[a]	L	1.083	<0.0001
(mmol/L)	10	105.50	103.30				0.1303
	15	96.80 [b]	97.38 [b]		TX L	1.048	<0.0001
	Mean	**103.3**	**100.96**				

FFYM: Fermented false yam (14days), NABTFYM: Sodium hydroxide (0.1M) treated blanched false yam. Control diet. Means in the same row compared with control having different superscripts showed significant difference ($p < 0.05$). 'P' is the probability, SED; is the standard error of difference, T-treatment, L- level, Polynomial contrast for Treatment X Level interaction.

9.4 Discussion

Broiler chickens on the false yam treatment diets almost consumed the same amount of feed as the control birds but gained less weight. The increase in consumption of feed by the treatment groups may be due to better processing of the false yam tuber by fermentation and the use of sodium hydroxide (0.1M) treated blanched false yam. It was observed that fermentation removed the offensive odour which was a characteristic of the raw false yam, by producing volatile aroma compounds which are believed to be produced by yeast and lactic acid bacteria. Also, fermentation improved palatability and reduced anti-nutritional factors present in the false yam (Table 6.3) thereby contributing to a higher feed consumption. This corroborates similar findings by Amoa-Awua *et al*. (1996) who fed fermented cassava dough to broiler chickens. The use of sodium hydroxide treatment on false yam might have solubilized the cellulose and hemi-cellulose constituents of the plant cell wall of the false yam tuber ingredient thereby making the plant cell wall accessible to microbial enzymes for quick digestion. These observations are in line with that of Fahey (1993). According to Carlinix and Udedebie (1997), blanching is a heat treatment processing method of food using hot water which affects the breakdown of thermolabile anti-nutritional compounds in feed materials causing their reductions. These anti-nutrients include; terpenoids, saponins, alkaloids, cynogenic glucoside, etc.

Feed conversion ratio values for the broiler chickens fed on the treatment diets increased linearly when the levels of the processed false yam products increased from 5% to 15% in the diets. The implication is that as the level of inclusion increases in the diets, the less the utilization of the diets by the birds. The birds could not extract the required nutrients from the feed because of the effects of residual anti-nutritional substances present in the diets which reduced feed digestibility and utilization.

From previous experiments (Tables 4.3 and 6.3), residual anti-nutritive factors such as saponins, alkaloids, phytate, oxalates were still present in the processed false yam tuber meals.

The amounts of phytate and oxalate contents of the sodium hydroxide treated blanched false yam were 2.31 and 0.63 g/100g respectively; while percentage alkaloid and phytate reductions in the sodium hydroxide treated blanched false yam were 0.25% and 22.22% respectively with oxalate (84.47%) recording the highest loss.

The negatively charged phosphate ion in phytic acid is precipitated by metallic cations such as Zn^{2+}, Fe^{2+}, Ca^{2+}, Mn^{2+}, and Mg^{2+} to phytate. The phytate in the gastrointestinal tract of monogastric animals can form complexes with essential nutrients and other mineral salts causing deficiencies of these substances since there are no phytase enzymes in the intestine to breakdown these complexes.The residual phytate in the processed false yam might have complemented this reaction. The findings corroborate similar work by Prathiba *et al*. (1995) that anti-nutrients can cause deficiency in essential nutrients and also prevent thorough digestion when consumed.

The residual alkaloid content of fermented and sodium hydroxide treated blanched false yam ranged between 0.96 and 1.92 g/100g. Some alkaloids have been reported to have effect on gastrointestinal and nervous disorders leading to muscular spasms (Aletor, 1993). This occurs at a concentration level of more than 20 mg/100g sample. The residual amount of saponins interacts with cholesterol in the intestine to form a complex that is unavailable for absorption and utilization by the monogastric animals. It has been reported that physical and chemical interactions of nutrients such as cholesterol and glucose with saponins reduce their absorption in the gastrointestinal tract of animals (Johnson *et al*., 1986).

Although oxalate appeared to have been the most reduced anti-nutritive factor in the fermented false yam product, residual amount of 0.41 and 0.63 mg/100g in the sodium hydroxide treated blanched false yam could still have interfered in the metabolic activities of the broiler chicken. Oxalates

interact with minerals such as magnesium and calcium forming complexes and making them unavailable for absorption (Oke, 1969).

This goes to confirm some earlier observations made in experiment 6 on muscular weakness and paralysis among broiler chickens around week 7 of production.

Oxalate reacts with calcium ions in the gastrointestinal tract of animals to form calcium oxalate this leads to hypocalcaemia due to immobilization of the calcium ions by the oxalate. The precipitate of the calcium salts in the renal tubles causes urinary disorders such as blockage, calculi and lesions in the kidney (Oke, 1969). The effect of these residual anti-nutritive factors could have caused poor digestion of nutrients in the treatment diets, absorption and utilization.

Digestibility analysis of the nutrients in the treatment diets are presented in Table 9.5. Sodium hydroxide treatment diets appeared to have better digestion of almost all the nutrients; ether extract, ash, crude protein and dry matter. This could have been due to solubilization of the cellulose and hemi-cellulose of the plant cell wall of the ingredient by the hydrolytic action of the NaOH solution, making it more accessible to enzyme activities. This supports similar observation reported by Fahey *et al*. (1993).

The residual anti-nutritional factors analysis in the processed false yam products are presented in Table 6.3. Terpenoid, saponin, alkaloids, phytates, and oxalates in the processed yam indicated lower values compared to the sundried product.

The poorest feed: gain value was observed in the 15% inclusion level of the processed false yam in the diet which could have been caused by higher accumulation of residual bioactive compounds such as phytate, saponin and alkaloid which are the predominant residual toxicants still present in the processed false yam tuber affecting the extraction of the digested nutrients in the treatement diets. The anti-nutritive factors do not act individually but may combine with other nutrients to form

complexes. These complexes lead to lower feed utilization at higher levels of substitutions observed in the experimental trial.

The impact of the residual bioactive compounds could have caused reduction in nutrient utilization and weight gain. The carcass parameters observed showed that live weight, eviscerated and dressed weights were lower with broiler chicken birds on the processed false yam diets than birds on the control diet.

Gross pathological examination on the organs showed no lesions or changes in colour, or any abnormalities. These observations give an indication that the substitution of the maize based diets with the levels of 5 to 15% false yam pose no serious threat to some organ development and functions.

There were some changes in the blood components of the birds fed on the treatment diets when compared with those on the control diet. However, the examination of the tissues of the various organs of the treatment birds did not show any pathological changes which can be attributed to treatment related diets. Moreover, the variations were found to be within limits.

The cost evaluation analysis (Table 9.2) showed that the cost of feed/kg of diet of birds on the control diet was the highest (¢1.91) with the lowest on the broiler chicken fed 15% fermented false yam diet (¢1.71). The lowered cost in the false yam treatment diet was attributed to the fact that the false yam which was used to partially substitute for the maize in the treatment diets was not bought. The 10% sodium hydroxide treated blanched false yam diet feed cost/kg weight gain was highest ($P< 0.05$) with an amount of GH¢ 5.82 while the lowest was recorded for the 5% sodium hydroxide treated blanched false yam (GH¢ 4.29). This was due to lower weight gain recorded by birds fed the 10% sodium hydroxide treated blanched false yam, lower cost of the sodium hydroxide used in processing the false yam and a poor feed conversion ratio value observed for the birds. The feed saving cost analysis showed that the 15% fermented false yam had the highest ($p<0.05$) cost

saving of GH¢ 10.47 as compared to GH¢ 4.66 of the control diet. The increament in the cost saved by using processed false yam to replace portions of the maize was attributable to the low cost of the treated fermented false yam used as alternative energy source when compared with the complete maize-based diet.

9.5 Conclusion

It was concluded that processed false yam by fermentation for 14 days and sodium hydroxide (0.1M) treated blanched, could partially substitute for some portions of maize, thereby enhancing digestibility and metabolism of feed. The processed false yam products could best substitute for maize at 5-10% with minimum growth interference and high cost saving. The final live weight gain by the broiler chickens and their carcass characteristics were not negatively affected by the effect of the processed false yam products.Thus any of the processing methods of false yam could be used in the poultry industry.

CHAPTER 10

10.0 General discussion

The nutrient compositions of the false yam tuber and seed observed were in line with similar reports presented by other researchers (Dei *et al*., 2013; Osei *et al*., 2013a). However, there are slight variations in crude fibre, ether extract, nitrogen free extract and the crude protein. The variations could be due to season of harvesting, geographical location and agronomic conditions as well as analytical methods used. The nutrient compositions of both the tuber and seed particularly in terms of carbohydrate content suggest their use as alternate feed ingredient as substitute for maize. In addition, the study showed much higher ammonia content in both the tuber and the seed culminating in higher crude protein (114g/kg) in the seed and moderate (53g/kg) in the tuber. This is an indication that the true protein contents of the false yam tuber and seed could be lower than that reported in the study. Nonetheless, they appeared to supply adequate amounts of crude protein just as in maize and other cereals and therefore can be a close substitute for maize when comapared with other root and tuber crops in the diets of monogastric animals Dei *et al*. (2011a).

The mineral compositions of both the tuber and seed are comparable to that of maize with higher levels of phosphorus, calcium, potassium, zinc and iron. There is therefore no need to supplement the diet with more of such mineral elements particularly iron which is abundant in both the tuber and seed. The high content of the iron may help reduce anaemic conditions in animals.

The screening and quantification of anti-nutritive compounds in the false yam and seed showed the presence of alkaloid, mucilage, saponins, steroids, terpenoids, phytate and oxalates. This means that using the false yam tuber and seed as a sole diet or in combination with other ingredients could pose serious health problems to monogastric animals. There could be a problem of leg impairment as a result of mobilization of calcium in the diet due to the presence of phytate and oxalate in the false

yam tuber. Phytates and oxalates immobilize positive metallic ions, for example, Mn^2, Fe^{2+}, Mg^{2+}, $Zn^{2+,}$ Ca^{2+} forming complexes of nutrients which are unavailable for absorption and utilization.

Another serious physiological effect of the presence of anti-nutritive factors on monogastric animals may be retarded growth caused by saponins in feed materials. Saponins in the small intestines bind with cholesterol causing hypocholesterolaemia. Saponins, through physicochemical interactions, also interfere with glucose and cholesterol absorption in the small intestines. Mucilage in the tuber and seed, if not removed, could cause digesta to be thick and viscous. The thick consistency caused by this mucilage makes enzyme inaccessible to proteins in the food material and this reduces the protein digestion (Wang *et al*., 2008a, 2008b).

The anti-nutritive factors do not act individually, but act in combination with other nutrients thereby making food less digestible unabsorbable and inefficient utilization. Utilization of the false yam tuber in diets of monogastrics may require some form of processing before inclusion in the diet of monogastric animals.

Processing methods employed on the fresh false yam tuber as means of detoxifying bioactive constituents identified in the false yam tuber included soaking and discarding the water, or through repeated washing (Vidal-Valverde *et al*., 1992). Cooking, boiling and blanching eliminate thermolabile and bioactive constituents including saponins, terpenoids, cynogenic glucoside and alkaloids (Udedebie, 1992). A possible combination of any of these methods can be used to detoxify false yam as observed in this study.

Sodium hydroxide solution (0.1M) used in the processing of the false yam tuber showed promising results (Table 6.3). The texture of the processed product was observed to be smooth. This could have been due to the break down of the components hemicellulose, lignin and cellulose fractions of the cell wall of false yam products by the sodium hydroxide.

According to Fahey *et al*. (1993), the solubilization of the components of the cell wall was as a result of changes caused by the sodium hydroxide interaction and breaking of the bonds of the sub units holding these constituents in place and ultimately causing dissolution and also enhanced microbial enzymes access to the constituents of the plant materials.

Fermentation of false yam tuber for 14 days had the highest reductive percentage of anti-nutritive factors in phytate (28%), alkaloids (37%), saponins (68%), terpenoids (69%), oxalates (84%) and mucilage (100%). This presupposes that even the best processing method identified in the present study still left residual anti-nutritive factors which had negative impact on metabolism and physiology of the broiler chickens. It was observed that fermentation removed the offensive odour which was a characteristic of the fresh false yam tuber, improved the flavour, energy, palatability and fairly balanced mineral element retention in the product after processing.

However, the prolonged fermentation resulted in the product being contaminated with *Enterobacter cloacae*, *Agglumerans, Yeast, Mould and Bacillus spp*. It was noted that careful handling, processing and transportation of the fermented product could eliminate some of these threats.

The processed false yam tuber products with their residual anti-nutritive factors were further investigated for a possible toxicity in Sprague Dawley rats. The results of both acute and sub-acute toxicity studies of the raw and the processed products did not show toxicity at a minimum concentration of 5 g/kg body weight and a maximum of 20 g/kg body weight

Sun-dried, fermented and sodium hydroxide treated blanched false yam when consumed by Sprague Dawley rats at level of 10-20g/kg for a duration of 3months, reduced values of blood components such as HGB, HCT and RBC. Similarly, serum blood chemistry componenets such as AST and ALT were significantly affected.

However, none of these haematology and blood serum alterations in these indices reflected in the histopathological examination carried out on sections of tissues taken from the lungs, liver, spleen,

kidney and the heart. This means that the processed products were safe for consumption by livestock. In addition to this the results presupposes that the processing methods used reduced the anti-nutritive components in the false yam tuber.

Broiler chickens fed on the fermented false yam products showed an increase in feed intake due to better method of processing the false yam tuber by fermentation for 14 days. Fermentation, as a method of processing, decreased the concentration of bioactive constituents in raw false yam such as saponin by 68%, alkaloid by 37%, phytate by 27.95% and oxalate by 84.47%. Fermentation also, improved the flavour, energy, protein and palatability of the product. Similarly, for sodium hydroxide treated and blanched false yam meal, saponin, oxalate and phytate contents were reduced considerably (Table 6.3). Again, the cellulose, hemicellulose of the plant cell wall was solubilized making the carbohydrate more accessible to enzyme activities.

Feed intake by broiler chikens on the treatment diets compared with birds fed the control did not show any significant difference $P < 0.05$ this did not reflect on the final live weight gain by the broiler chickens. Birds fed the treatment diets gained less weight ranging from 1726.16g to 2002.23 g compared to that of their counterparts on the normal diet (2027.75 g). Birds fed on the treatment diets showed poor feed gain ratio ranging from 2.32 to 3.31due to the fact that nutrients in the diets were not efficiently digested, absorbed and utilized. This is a clear indication of the effects of the residual anti-nutritive factors after processing. This suggests that further processing methods are required to render the false yam anti-nutritive free.

Digestibility trials showed that fermented and sodium hydroxide treated blanched false yam recorded higher values ranging from 74.98 to 80.16%, however, these values decreased when the quantity of the false yam tuber in the diet increased to 15%.

The effect of the residual anti-nutritive factors translated into the carcass and organ weights. The live weight, dressed weight and eviscerated weights relatively showed a lower development in the

treatment diets compared with the control birds. Organs such as lungs, liver and kidney were lower in development. However, the crops of birds on the treatment diets were better developed probably due to higher consumption of diets and their low rate of emptying into the proventriculus. The level of blood components measured in animals are used to obtain fair knowledge on how the various organs and systems are working, whether they are meeting their nutritional requirements or not and also to assess the disease condition of that particular animal. When there are changes in the levels of blood componets it explains better how nutritional factors and additives in the diet affect that particular animal. The functions of the enzymes, GGT, GPT and GOT are also, better explained when haematological components are known. These enzymes are used to assess feed, drugs and possibilities of diseases in animals. Progressive accumulations of bioactive compounds in the feed as well as in the drugs in an animal, gives indications of the toxic effect of the feed and the drug in advance before the occurance of the real effect (Alikwe *et al.*, 2010). The high levels of some haematological parameters triggered the defense mechanisms in the birds against diseases and transportation of nutrients. According to Fredrick (2010), if the levels of haematological components measured in livestock are high, pre-suggest that the animals are about falling sick. The mean value of haemoglobin (Hb) recorded in the broiler chickens decreased significantly when 15% of processed false yam was included in the diet. Haemoglobin of broiler chickens fed the processed false yam ranged from 7.09 to 7.91 g/dl as compared to the control birds (8.06 g/dl). These values are comparable to that of White leghorn (8.80 g/dl) and Rhode Island Red (8.70 g/dl). Haemoglobin values of 12.13, 11.63, 10.30 and 10.30 g/dl have been reported for Japanes quails, guinea fowl, geese, and turkey respectively. The activeness of these birds is a contributory factor for higher levels of haemoglobins compared with the domesticated chicken. These observations support similar findings by other researchers (Oyewale, 1991; Kundu *et al.*, 1993).

The present RBC values are in line with those reported by Oyewale and Ogwnegbu (1987). They attributed the differences to age and species. Haematocrit values decreased significantly when the amount of processed false yam tuber in the diet increased. The value was significant at 15% inclusion level for both fermented false yam and sodium hydroxide treated blanched false yam included in the maize based diets (Table 9.4).

The WBCs were significant in the birds fed on processed false yam tuber included in a diet formulated with the maize as the main source of energy. Higher WBCs were observed in broiler chickens fed on 5-10% inclusion levels. High WBCs count is an indication of imminent toxicity of the bioactive components in the feed before the real manifestation of the toxic effects. The high values were an indication that the birds were vulnerable to diseases. This is in agreement with earlier researchers (Alikwe *et al*., 2010; Frederick, 2010).

MCHC increased significantly in broiler chickens fed on processed false yam tuber diet at 15% inclusion level. The narrow variations in the values of MCHC in the present study could be due to differences in residual anti-nutritive factors present in the experimental diets. This corroborates the findings of Sahn *et al.,* (2002). Furthermore, other researchers (Gendi *et al*., 2000) have attributed differences in MCHC to the genetic make-up and age of chickens. Adenkola and Ayo (2009) reported on the effect of time of blood sampling, Olayemi and Ojo (2007) suggested variations to be caused by temperature and environment, while Midilli *et al*. (2004) reported of climate.

The total levels of protein, globulin and albumin increased significantly in the treatment diets (Table 9.5), revealing higher activity of the liver which could have been caused by the residual anti-nutritive factors in the processed false yam. This is in line with what has been reported by others (Kumari *et al*., 2007; Yousef *et al*., 2010). This increased significantly with the treatment groups on the processed false yam than the control birds. The globulin in the serum was obtained by subtracting albumin concentrations from total protein. The globulin values obtained for the

treatment birds fed on the processed false yam produts increased significantly when compared with the values obtained for those fed the control diet as presented in (Table 9.5).The globulin levels in the serum is a manifestation of the health status of the chickens of the treated group and this has a direct relationship with the humoral immune levels in the broiler chickens. This agrees with the findings of Qasem *et al*. (2015).

Nitrogen excretion into the blood comes mainly from urea production in the liver which is filtered by the kidneys. In the current study, serum urea increased significantly in birds fed the processed false yam. Higher rate of urea production in the liver or its excretion in the kidneys are attributed to the effect of residual anti-nutritive compounds which still may persist in the processed false yam tuber. This is consistent with the reports of Srimal (1997) and Amin and Abdou (2012). The lowest uric acid produced by birds fed on the 5% fermented false yam was as a result of lesser amount of anti-nutritive residues left in the processed false yam product. This enhanced the activities of the nephrons in the kidneys to increase the excretion of urinary wastes from the blood of treatment broiler chickens. This is similar to the report by Srimal (1997).

Direct bilirubin values were significantly low for the broiler chickens on processed false yam products ranging from 0.70-3.40 µmol/L with an exceptionally, highest value of 3.40 µmol/L recorded in the 5% sodium hydroxide treated blanched false yam as compared to the control birds (2.58 µmol/L) (Table 9.6). These low values are confirmation of low growth and paralysis observed in the treatment birds. According to Rizvi *et al*. (2008), the low growth and paralysis are associated with ALP enzyme and bilirubin activities which are concerned with growth and bone formation play important roles in the physiology and metabolic activities of animals.

The liver contains the enzymes ALT, ALP, AST, and LDH. Higher levels of concentrations of these enzymes are indications of liver damage. ALT, GT and CRT activities were significantly affected by the partial substitution of the maize based diet with graded levels of the processed false yam

products. The high values of ALT and CRT at a higher inclusion level of 15% are indicative of the possibility of the effect of the residual activities of the anti-nutritive factors identified in the false yam products. This is in support of the work done by Kaplan *et al*. (2003).

Plasma electrolyte elements such as Na, K and Cl^- showed significant reductions when level of the processed false yam tuber inclusion in the normal diet increased (Table 9.5). The reduction in the plasma electrolytes (Na, K and Cl^-) in the present study might have been caused by high level of residual phytate in the diet. This corroborates the findings of Oblerleas *et al*. (1981) that phytate levels in diets affect absorption and utilization of K, Ca, Zn and Na. The residual phytate levels of the fermented and sodium hydroxide treated blanched false yam were high (Table 6.3).

The feed saving cost analysis showed that the 15% fermented false yam had the highest cost saving of 10.47% compared with birds fed on the normal diet. The increased saving cost of the fermented false yam was due to free sourcing of the false yam in the wild and the low cost of the method of processing the fermented false yam product used to partially replace maize in the respective diets as alternative energy source compared to the conventional source of energy from maize used in the control diet. The implication of this is that the fermented false yam could be a better substitute for maize in terms of profit and safety when undertaking broiler chicken production.

10.1 General conclusion and recommendations

10.1.1 Conclusion

- False yam tuber and the seed tested in this study had adequate amounts of nutrients to be used as partial substitute for energy source in mono-gastric nutrition.
- Anti-nutritive factors identified in the false yam tuber and the seed included; saponins, alkaloids, mucilage, triterpenoids, phytate and oxalates.
- Processing of the false yam tuber by fermentation for 14 days and sodium hydroxide (0.1M) treated blanched products reduced the anti-nutritive factors such as saponin, alkaloids, mucilage, triterpenoids, phytate and oxalates to minimal levels.
- The anti-nutritive factors in the unprocessed and processed false yam products had no toxicological effect on Sprague Dawley rats. The minimum lethal dosage LD^{50} for the false yam was above 5 g/kg.
- Feeding up to 10% of the processed false yam products in the diets of broiler chickens had no serious effects on the growth development of the birds.
- Digestibility was enhanced in the processed false yam products of 5-10% inclusion in the diet but could not reflect on the weight gain of the birds.
- Some haematological parameters and blood serum metabolites were affected by processed false yam tuber diet but could not be confirmed by histopathological examinations.
- Fermented false yam had the highest cost saving of 10.47% when compared to other experimental diets used and therefore recommended for partial replacement of maize as an alternative source of energy in monogastric diets.

10.2 Recommendations

- Fermentation of false yam tuber for 14 days and sodium hydroxide treatment blanched should be preferred methods for reducing antinutritive factors in false yam tuber.

- It is recommended that suitable, simple but efficient processing methods (experiment) should be investigated to tackle the residual anti-nutritional factors in the tuber in order to make the product more useful in the livestock industry..
- It is recommended that further investigations should be carried out with the processed false yam seeds using broiler chickens.
- Exogenous Phytase enzyme should be incorporated in the processed false yam tuber diet to enhance phosphorus, calcium and energy digestion and absorption.
- Ileal digestibility trial in broiler chickens should be carried out to determine the digestible products absorbed.
- Poultry farmers can safely use 10% of 14-day fermented false yam tuber meal in broiler chicken diets.

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APPENDIX

ANOVA table for Growth performance of rat

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-value
Final average weight 5%	Treatment Residual Total	3 283 286	131221 576174 707395	43740 203600	21.484	< 0.0001
Final average weight 10%	Treatment Residual Total	3 273 276	188716 546330 735047	62905 2001.2	31.434	< 0.0001
Final average weight 20%	Treatment Residual Total	3 281 284	232400 598967 831367	77467 2131.6	36.343	< 0.0001
Feed consumed 5%	Treatment Residual Total	3 336 339	313.16 1813.9 2127.1	104.39 5.399	19.336	< 0.0001
Feed consumed 10%	Treatment Residual Total	3 336 339	487.08 1905.1 2392.2	162.36 5.670	28.635	< 0.0001
Feed consumed 20%	Treatment Residual Total	3 120 123	179.6 266.5 446.1	59.87 2.221	26.96	< 0.0001
Daily water consumed 5%	Treatment Residual Total	3 120 123	1224 1619 2843	407.9 13.49	30.23	< 0.0001
Daily water consumed 10%	Treatment Residual Total	3 120 123	800 1925 2725	2667 16.04	16.62	< 0.0001
Daily water consumed 20%	Treatment Residual Total	3 120 123	1599 2028 3626	532.8 16.9	31.53	< 0.0001
Daily amount of urine passed 5%	Treatment Residual Total	3 56 59	21.54 169.1 190.7	7.18 3.02	2.377	< 0.0001
Daily amount of urine passed 10%	Treatment Residual Total	3 56 59	45.87 89.09 135.9	15.29 1.607	9.516	< 0.0001
Daily amount of urine passed 20%	Treatment Residual Total	3 64 67	57.89 197.2 255.1	19.3 3.082	6.261	< 0.0001

Weight gain 5%	Treatment Residual Total	3 120 123	35.74 14.51 50.25	11.91 0.1209	98.54	< 0.0001
Weight gain 10%	Treatment Residual Total	3 120 123	35.83 9.897 45.28	11.79 0.08248	143	
Weight gain 20%	Treatment Residual Total	3 120 123	43.82 14.20 58.03	14.61 0.1184	123.4	< 0.0001
Feed gain 5%	Treatment Residual Total	3 120 123	213.6 186.9 400.5	71.21 1.557	45.73	
Feed gain 10%	Treatment Residual Total	3 120 123	158.4 216.3 374.7	52.80 1.802	29.30	< 0.0001
Feed gain 20%	Treatment Residual Total	3 120 123	227.6 301.2 528.8	75.86 2.510	30.22	< 0.0001

ANOVA table for Haematology of Rats

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-value
WBC 5%	Treatment	3	44.88	14.96	0.7613	0.5290
	Residual	20	393	19.65		
	Total	23	437.9			
WBC10%	Treatment	3	21.18	7.061	0.4030	0.7524
	Residual	20	350.4	17.52		
	Total	23	371.6			
WBC20%	Treatment	3	91.92	30.64	1.272	0.2913
	Residual	67	1614	24.1		
	Total	70	1706			
RBC5%	Treatment	3	2.253	0.7509	2.153	0.1017
	Residual	67	23.36	0.3487		
	Total	70	25.62			
RBC10%	Treatment	3	2.073	0.6909	1.671	0.1819
	Residual	65	26.87	0.4134		
	Total	68	28.94			
RBC20%	Treatment	3	34.74	11.58	4.085	0.0100
	Residual	67	189.9	2.834		
	Total	70	224.6			
HGB5%	Treatment	3	5.601	1.867	0.932	0.4302
	Residual	67	134.2	2.003		
	Total	70	139.8			
HGB10%	Treatment	3	3.331	1.110	0.5686	0.6377
	Residual	65	127.0	1.953		
	Total	68	130.3			
HGB20%	Treatment	3	95.46	31.82	3.623	0.0174
	Residual	67	588.4	8.782		
	Total	70	683.9			
HCT5%	Treatment	3	61.81	20.60	1.290	0.2850
	Residual	67	1070	15.97		
	Total	70	1132			
HCT10%	Treatment	3	144.0	47.99	1.837	0.1491
	Residual	65	1698	26.12		
	Total	68	1842			
HCT20%	Treatment	3	915.5	305.2	3.792	0.0142
	Residual	67	5392	80.47		
	Total	70	6307			
MCV5%	Treatment	3	26.18	8.725	1.958	0.1287
	Residual	67	298.6	4.457		
	Total	70	324.8			
MCV10%	Treatment	3	8.990	2.993	0.5994	0.6177
	Residual	65	324.6	4.993		
	Total	68	333.5			
MCV20%	Treatment	3	866.5	288.8	2.675	0.0542
	Residual	67	7233	108.0		
	Total	70	8100			
MCH5%	Treatment	3	1.551	1.850	1.238	0.3028
	Residual	67	100.1	1.494		
	Total	70	105.7			
MCH10%	Treatment	3	4.442	1.481	0.9027	0.4447
	Residual	65	106.6	1.640		
	Total	68	111.1			
MCH20%	Treatment	3	4.001	1.334	0.03092	0.9926
	Residual	67	2890	43.13		

	Total	70	2894			
MCHC5%	Treatment	3	20.63	9.543	1.098	0.3563
	Residual	67	582.5	8.694		
	Total	70	611.1			
MCHC10%	Treatment	3	14.91	4.968	0.6563	0.5819
	Residual	65	492.1	7.570		
	Total	68	507.0			
MCHC20%	Treatment	3	455.0	151.7	3.345	0.02242
	Residual	67	3038	43.35		
	Total	70	3493			
PLT5%	Treatment	3	17849	5950	0.1676	0.9179
	Residual	67	2.379e+006	35509		
	Total	70	2.397e+006			
PLT10%	Treatment	3	173537	57846	1.679	0.1801
	Residual	65	2.239e+006	34442		
	Total	68	2.412e+006			
PLT20%	Treatment	3	229540	76513	1.052	0.3754
	Residual	67	4.87e+006	72720		
	Total	70	5.102e+006			
LYM X 10^3	Treatment	3	52.19	17.40	1.720	0.1712
	Residual	67	677.4	10.11		
5%	Total	70	729.6			
LYM X 10^3	Treatment	3	27.24	9.081	0.6830	0.5656
	Residual	65	864.2	13.30		
10%	Total	68	891.4			
LYM X 10^3	Treatment	3	81.32	27.11	1.958	0.1287
	Residual	67	927.5	13.84		
20 %	Total	70	1009			
RDSW-SD5%	Treatment	3	7.702	2.567	0.03811	0.9900
	Residual	67	4513	67.36		
	Total	70	4521			
RDSW-SD10%	Treatment	3	13.33	4.443	0.07007	0.9757
	Residual	65	4122	63.41		
	Total	68	4135			
RDSW-SD20%	Treatment	3	61.94	20.65	0.2449	0.8647
	Residual	67	5648	84.30		
	Total	70	5710			
MPV5%	Treatment	3	1.053	0.3511	1.206	0.3143
	Residual	67	19.50	0.2911		
	Total	70	20.56			
MPV10%	Treatment	3	0.2782	0.09272	0.2861	0.8353
	Residual	65	21.06	0.3241		
	Total	68	21.34			
MPV20%	Treatment	3	1.975	0.3583	0.9750	0.4098
	Residual	67	24.62	0.3674		
	Total	70	25.69			

ANOVA table for Organs of rats

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-Value
Heart5%	Treatment	3	0.1513	0.05043	1.140	0.3492
	Residual	18	0.7966	0.04426		
	Total	21	0.9479			
Heart10%	Treatment	3	0.1005	0.03349	3.093	0.0516
	Residual	19	0.2057	0.01083		
	Total	22	0.3062			
Heart20%	Treatment	3	0.1595	0.05315	3.076	0.0524
	Residual	19	0.3283	0.01728		
	Total	22	0.4877			
Lung5%	Treatment	3	0.1513	0.05043	1.140	0.3598
	Residual	18	0.7966	0.04426		
	Total	21	0.9479			
Lung10%	Treatment	3	0.1850	0.06167	0.7219	0.5513
	Residual	19	1.623	0.08543		
	Total	22	1.808			
Lung20%	Treatment	3	0.9807	0.3269	7.256	0.0019
	Residual	19	0.8559	0.04505		
	Total	22	1.837			
Liver5%	Treatment	3	11.552	3.851	2.818	0.0684
	Residual	18	24.599	1.367		
	Total	21	36.150			
Liver10%	Treatment	3	19.452	6.484	5.778	0.0055
	Residual	19	21.319	1.122		
	Total	22	40.771			
Liver20%	Treatment	3	12.757	4.252	3.173	0.0480
	Residual	19	25.465	1.340		
	Total	22	38.221			
Spleen5%	Treatment	3	0.1290	0.04300	1.856	0.1733
	Residual	18	0.4172	0.02318		
	Total	21	0.5462			
Spleen10%	Treatment	3	0.1231	0.04104	3.348	0.0409
	Residual	19	0.2329	0.01226		
	Total	22	0.3560			
Spleen20%	Treatment	3	0.04226	0.01409	4.976	0.0103
	Residual	19	0.05379	0.002831		
	Total	22	0.09604			
Kidney5%	Treatment	3	0.4773	0.1591	2.133	0.1316
	Residual	18	1.342	0.07457		
	Total	21	1.820			
Kidney10%	Treatment	3	0.8026	0.2675	5.549	0.0066
	Residual	19	0.9160	0.04821		
	Total	22	1.719			
Kidney20%	Treatment	3	1.183	0.3942	6.485	0.0033
	Residual	19	1.155	0.06078		
	Total	22	2.337			

ANOVA table for Urinary parameters of rats

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-value
PH5%	Treatment	3	5.689	1.896	2.918	0.0477
	Residual	35	22.75	0.6499		
	Total	38	28.44			
PH10%	Treatment	3	2.702	0.9007	1.380	0.2591
	Residual	52	33.94	0.6526		
	Total	55	36.64			
PH20%	Treatment	3	4.65	1.355	1.665	0.1850
	Residual	56	45.58	0.8139		
	Total	59	49.65			
Protein 5%	Treatment	3	5.679	1.893	3.251	0.0284
	Residual	56	32.61	0.5823		
	Total	59	38.29			
Protein 10%	Treatment	3	6.004	2.001	3.623	0.0192
	Residual	50	27.62	0.5523		
	Total	53	33.62			
Protein 20%	Treatment	3	5.804	1.935	3.563	0.0202
	Residual	52	28.24	0.5430		
	Total	55	34.04			
SG5%	Treatment	3	0.0001422	4.741e+005	0.5227	0.6684
	Residual	56	0.005079	9.070e-005		
	Total	59	0.005221			
SG10%	Treatment	3	0.0001638	5.461e-005	0.8136	0.4921
	Residual	52	0.003490	6.712e-005		
	Total	55	0.003654			
SG20%	Treatment	3	0.0001252	0.0000474	0.5772	0.6324
	Residual	56	0.004049	0.00007231		
	Total	59	0.004175			

ANOVA table for Blood chemistry of rats

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-value
CK-MB 5%	Treatment	3	135087	45029	1.214	0.3116
	Residual	67	2.486e+006	37103		
	Total	70	2.621e+006			
CK-MB 10%	Treatment	3	4817	1606	0.05231	0.9841
	Residual	66	2.026e+006	30695		
	Total	90	2.031e+006			
CK-MB 20%	Treatment	3	45352	15117	0.3892	0.7612
	Residual	67	2.603e+006	38845		
	Total	70	2.648e+006			
Albumin5%	Treatment	3	34.16	11.39	1.154	0.3341
	Residual	67	661.3	9.870		
	Total	70	695.4			
Albumin10%	Treatment	3	55.73	18.58	1.789	0.1578
	Residual	66	685.3	10.38		
	Total	69	741.0			
Albumin20%	Treatment	3	48.92	16.31	1.014	0.3920
	Residual	67	1077	16.08		
	Total	70	1126			
Direct Bilirubin 5%	Treatment	3	0.5087	0.1696	2.727	0.0509
	Residual	67	4.166	0.06217		
	Total	70	4.674			
Direct Bilirubin 10%	Treatment	3	0.7281	0.2427	3.462	0.0211
	Residual	66	4.626	0.07010		
	Total	69	5.354			
Direct Bilirubin 20%	Treatment	3	0.2744	0.09147	2.050	0.1152
	Residual	67	2.989	0.04462		
	Total	70	3.264			
Total bilirubin 5%	Treatment	3	0.8333	0.2778	1.815	0.1527
	Residual	68	10.41	0.1531		
	Total	71	11.24			
Total bilirubin 10%	Treatment	3	0.2065	0.06884	0.3318	0.8024
	Residual	68	14.11	0.2075		
	Total	71	14.32			
Total bilirubin 20%	Treatment	3	0.7037	0.2346	1.029	0.3853
	Residual	68	15.50	0.2279		
	Total	71	16.20			
Creatinine 5%	Treatment	3	604.0	201.3	0.09052	0.9650
	Residual	67	149023	2224		
	Total	70	149627			
Creatinine 10%	Treatment	3	831.7	277.2	0.1281	0.9431
	Residual	66	142828	2164		
	Total	69	143660			
Creatinine 20%	Treatment	3	1000	333.4	0.1890	0.9035
	Residual	67	118188	1764		
	Total	70	1191888			
Urea 5%	Treatment	3	139.9	46.65	2.776	0.0480
	Residual	67	1126	16.80		
	Total	70	1266			
Urea 10%	Treatment	3	80.68	26.89	1.426	0.2429

	Residual	66	1244	18.85		
	Total	69	1325			
Urea 20%	Treatment	3	60.42	20.14	1.045	0.3786
	Residual	67	1292	19.28		
	Total	70	1352			
Total protein 5%	Treatment	3	129.3	43.10	0.7150	0.5465
	Residual	67	4039	60.28		
	Total	70	4168			
Total protein 10%	Treatment	3	293.1	97.70	1.720	0.1713
	Residual	66	3.748	56.79		
	Total	69	4041			
Total protein 20%	Treatment	3	182.2	60.73	0.7881	0.5048
	Residual	67	5163	77.06		
	Total	70	5345			
ALP 5%	Treatment	3	111078	37026	2.052	0.1149
	Residual	67	1.209e+006	18044		
	Total	70	1.320e+006			
ALP 10%	Treatment	3	161030	53677	3.204	0.0287
	Residual	66	1.106e+006	16751		
	Total	69	1.267e+006			
ALP 20%	Treatment	3	9869	32897	2.350	0.0802
	Residual	67	937733	13996		
	Total	70	1.036e+006			
AST 5%	Treatment	3	30443	10148	7.799	0.0002
	Residual	67	87173	1301		
	Total	70	117616			
AST 10%	Treatment	3	41241	13747	12.64	<0.0001
	Residual	66	71803	1088		
	Total	69	113044			
AST 20%	Treatment	3	50515	16838	15.37	<0.0001
	Residual	67	73379	1095		
	Total	70	123894			
ALT 5%	Treatment	3	1191	397.0	1.395	0.2520
	Residual	67	19072	284.7		
	Total	70	20264			
ALT 10%	Treatment	3	2907	969.0	6.762	0.005
	Residual	66	9457	143.3		
	Total	69	12364			
ALT 20%	Treatment	3	2385	794.8	5.613	0.0017
	Residual	67	9488	141.6		
	Total	70	11873			

ANOVA table forProximate composition of raw and processed false yam

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-value
Dry matter	Treatment	5	479.6	95.91	78.48	< 0.0001
	Residual	12	14.67	1.222		
	Total	17	494.2			
Crude protein	Treatment	5	95.26	19.05	19.05	< 0.0001
	Residual	12	12	1		
	Total	17	107.3			
Crude fibre	Treatment	5	72.85	14.57	86.33	< 0.0001
	Residual	12	2.025	0.1688		
	Total	17	74.88			
Ether extract	Treatment	5	22.35	4.471	261.2	< 0.0001
	Residual	12	0.2054	0.01712		
	Total	17	22.56			
Ash	Treatment	5	9.625	1.925	143.7	< 0.0001
	Residual	12	0.1608	0.0134		
	Total	17	9.786			
Nitrogen free extract	Treatment	5	408.30	81.66	4771	< 0.0001
	Residual	12	0.2054	0.01712		
	Total	17	408.5			
Metabolizable energy	Treatment	5	72551	14510	1.172e+006	< 0.0001
	Residual	12	0.1486	0.01238		
	Total	17	72551			

ANOVA table for Residual minerals in processed false yam

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-value
Magnesium	Treatment	5	0.05485	0.01097	109.7	< 0.0001
	Residual	12	0.0012	0.0001		
	Total	17	0.05605			
Calcium	Treatment	5	0.1841	0.03682	301.3	< 0.0001
	Residual	12	0.001467	0.0001222		
	Total	17	0.1856			
Manganese	Treatment	5	0.2958	0.05915	17.83	< 0.0001
	Residual	12	0.0398	0.003317		
	Total	17	0.3356			
Sodium	Treatment	5	0.0048	0.00096	9.600	0.0007
	Residual	12	0.0012	0.0001		
	Total	17	0.006			
Potassium	Treatment	5	1.671	0.3343	3343	< 0.0001
	Residual	12	0.0012	0.0001		
	Total	17	1.672			
Phosphorus	Treatment	5	0.04204	0.008409	68.80	< 0.0001
	Residual	12	0.001467	0.0001222		
	Total	17	0.04351			
Iorn	Treatment	5	46.25	9.251	172.9	< 0.0001
	Residual	12	0.642	0.0535		
	Total	17	46.90			
Zinc	Treatment	5	6.07	1.214	150.2	< 0.0001
	Residual	12	0.097	0.008083		
	Total	17	6.167			

ANOVA table for Anti-nutrient in processed false yam tuber

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P- value
Terpenes	Treatment Residual Total	5 12 17	0.07764 0.001072 0.07871	0.01553 0.00008993	173.8	< 0.0001
Saponin	Treatment Residual Total	5 12 17	0.6357 0.3145 0.9502	0.1271 0.02621	4.850	0.0117
Polyuronides	Treatment Residual Total	5 12 17	507.7 2.28 509.9	101.5 0.19	534.4	< 0.0001
Alkaloids	Treatment Residual Total	5 12 17	3.09 0.2374 3.327	0.618 0.01978	31.24	< 0.0001
Phytate	Treatment Residual Total	5 12 17	2.189 0.0048 2.194	0.4379 0.0004	1095	< 0.0001
Oxalate	Treatment Residual Total	5 12 17	12.35 0.002067 12.35	2.47 0.0001722	14344	< 0.0001

ANOVA tables for Broiler chickenNutrient Digestibility trials

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-value
Crude protein 5%	Treatment	2	20.70	10.35	9.980	0.0123
	Residual	6	6.222	1.037		
	Total	8	26.92			
10%	Treatment	2	11.77	5.885	5.620	0.0422
	Residual	6	6.283	1.047		
	Total	8	18.05			
15%	Treatment	2	5.353	2.6767	2.572	0.1560
	Residual	6	6.242	1.040		
	Total	8	11.60			
Crude fiber 5%	Treatment	2	188.7	94.34	90.38	0.0001
	Residual	6	6.263	1.044		
	Total	8	194.9			
10%	Treatment	2	861	430.5	411.1	<0.0001
	Residual	6	6.283	1.047		
	Total	8	867.3			
15%	Treatment	2	53.31	26.66	25.45	
	Residual	6	6.283	1.047		= 0.0012
	Total	8	59.59			
Ether extract 5%	Treatment	2	220.6	110.3	105.3	0.0001
	Residual	6	6.283	1.047		
	Total	8	226.9			
10%	Treatment	2	251.2	125.6	119.9	0.0001
	Residual	6	6.283	1.047		
	Total	8	257.5			
15%	Treatment	2	37.67	18.84	17.99	0.0029
	Residual	6	6.283	1.047		
	Total	8	43.96			
Ash 5%	Treatment	2	67.33	33.67	14.42	0.0001
	Residual	6	14.01	2.335		
	Total	8	81.34			
10%	Treatment	2	640.9	320.4	298.1	0.0051
	Residual	6	6.450	1.075		
	Total	8	647.3			
15%	Treatment	2	256.2	12.81	119.2	0.0001
	Residual	6	6.450	1.075		
	Total	8	262.7			

ANOVA table for broiler chicken finisher performance

Variate	SOURCE	DF	SSQ	MS	F-RATIO	P-value
Average weight of birds	Treatment	2	62967	31484	0.1637	0.8494
5%	Residual	57	1096000	192272		
	Total	59	11020000			
		2			2.419	0.0981
	Treatment	57	892765	446382		
10%	Residual	59	10520000	184500		
	Total	2	11410000		3.675	0.0315
		57				
	Treatment	59	1202000	600812		
15%	Residual		9320000	163507		
	Total		10520000			
Average feed intake	Treatment	2	1427	713.7	1.998	0.1449
5%	Residual	57	20357	357.1		
	Total	59	21784			
		2				
	Treatment	57	218.5	109.2	0.2803	0.7566
10%	Residual	59	22213	389.7		
	Total	2	22432			
		57				
	Treatment	5	21.75	10.88	0.01396	0.9861
15%	Residual		44412	779.2		
	Total		44434			
Average daily weight	Treatment	2	64.64	32.32	0.4881	0.6163
gain 5%	Residual	57	3774	66.22		
	Total	59	3839			
10%						
	Treatment	2	3643	1822	41.57	0.0001
	Residual	57	2498	43.82		
15%	Total	59	6141			
	Treatment	2	3766	1883	38.59	0.0001
	Residual	57	2781	98.79		
	Total	5	6548			
Feed/gain ratio 5%	Treatment	2	0.1645	0.08226	0.3900	0.6789
	Residual	57	12.02	0.2109		
	Total	59	12.19			
10%	Treatment	2	6.833	3.416	13.74	0.0001
	Residual	57	14.17	0.2486		
	Total	59	21			
15%	Treatment	2	9.093			
	Residual	57	31	4.546	8.359	0.0007
	Total	5	40.09	0.5439		

ANOVA table for broiler chicken finisher performance (Continued)

Cost of feed/kg of diet (GH¢) 5%	Treatment Residual Total	2 24 26	0.000265 2.025 2.026	0.000132 0.0844	0.001567	0.9984
Cost of feed/kg of diet (GH¢) 10%	Treatment Residual Total	2 24 26	2658 7497 10155	1329 1324	4.254	0.0262
Cost of feed/kg of diet (GH¢) 15%	Treatment Residual Total	2 24 26	2660 7497 10157	1330 3124	4.257	0.0262
Cost of daily feed intake/bird(GH¢) 5%	Treatment Residual Total	2 57 59	0.009267 0.071148 0.08044	0.004634 0.001249	3.711	0.0306
Cost of daily feed intake/bird(GH¢) 5%	Treatment Residual Total	2 57 59	0.007859 0.07469 0.08254	0.00393 0.00131	2.999	0.0578
Cost of daily feed intake/bird(GH¢) 10%	Treatment Residual Total	2 57 59	0.0102 0.1369 0.1471	0.005099 0.002402	2.123	0.1291
Cost of feed/kg weight gain(GH¢) 15%	Treatment Residual Total	1.399 43.15 44.55	2 57 59	0.6994 0.7571	0.923	0.4029
Cost of feed/kg weight gain(GH¢) 10%	Treatment Residual Total	14.88 48.44 63.32	2 57 59	7.438 0.8498	8.752	0.0005
Cost of feed/kg weight gain(GH¢) 15%	Treatment Residual Total	14 97.33 111.3	2 57 59	7.002 1.708	4.100	0.0217
Feed cost Savings 5%	Treatment Residual Total	2 6 8	3.024 2.125 5.149	1.512 0.3542	4.268	0.0703
Feed cost Savings 10%	Treatment Residual Total	2 6 8	178.4 31.24 3.121	15.62 0.5201	30.03	0.0007
Feed cost Savings 15%	Treatment Residual Total	2 6 8	34.36 119.5 0.01006 119.5	59.75 0.001677	35641	0.0001

ANOVA table for Carcass of broiler chicken finisher

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
Live weight 5%	Treatment	2	189786	94893	0.6091	0.5511
	Residual	27	4206000	155789		
	Total	29	4396000			
10%	Treatment	2	370027	185013	1.204	0.3157
	Residual	27	4151000	1537723		
	Total	29	4521000			
15%						
	Treatment	2	1438000	719170	6.817	0.0040
	Residual	27	2848000	105499		
	Total	29	4287000			
Dressing weight 5%	Treatment	2	139201	69601	0.7837	0.4668
	Residual	27	2398000	88805		
	Total	29	2537000			
	Treatment	2	439047	219524	2.648	0.0891
10%	Residual	27	2238000	82891		
	Total	29	2677000			
			1459000	729572	13.12	0.0001
15%	Treatment	2	1502000	55619		
	Residual	27	2961000			
	Total	29				
Dressing (%) 5%	Treatment	2	3.055	1.528	0.4202	0.6612
	Residual	27	98.17	3.636		
	Total	29	101.2			
	Treatment	2	63.2	31.6	8.472	0.0014
10%	Residual	27	100.7	3.73		
	Total	29	163.9			
15%	Treatment	2	190.4	95.21	9.210	0.0009
	Residual	27	279.1	10.34		
	Total	29	469.5			
Eviscerated weight 5%	Treatment	2	52796	26398	4096	0.0001
	Residual	6	38.67	6.444		
	Total	8	52835			
				88535	6585	0.0001
	Treatment	2	177070	13.44		
	Residual	6	80.67			
10%	Total	8	177150			
				283126	91005	0.0001
	Treatment	2	566252	3.111		
15%	Residual	6	18.67			
	Total	8	566270			
Head 5%	Treatment	2	39.47	19.73	0.1841	0.8329
	Residual	27	2894	107.2		
	Total	29	2934			
	Treatment	2	141.9	70.93	1.102	0.3466
10%	Residual	27	1738	64.36		
	Total	29	1879			
15%	Treatment	2	245.1	122.5	1.449	0.0327
	Residual	27	1339	148.8		
	Total	29	440	24.46		

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
Heart	Treatment	2	45.07	22.53	4.798	0.0165
5%	Residual	27	126.8	4.696		
	Total	29	171.9			
10%						
	Treatment	2	56.27	28.13	5.972	0.0071
	Residual	27	127.2	4.711		
15%	Total	29	183.5			
	Treatment	2	75.47	37.73	4.779	0.0167
	Residual	27	213.2	7.896		
	Total	29	288.7			
Lung	Treatment	2	5.609	2.805	27.04	0.0002
5%	Residual	9	0.9334	0.1037		
	Total	11	6.542			
	Treatment	2	3.707	1.853	17.37	0.0008
10%	Residual	9	0.96	0.1067		
	Total	11	4.667			
15%	Treatment	2	4.355	2.177	13.55	0.0019
	Residual	9	1.447	0.1607		
	Total	11	5.801			
Liver	Treatment	2	181.1	90.53	1.276	0.2954
5%	Residual	27	1916	70.95		
	Total	29	2097			
	Treatment	2	67.2	33.6	0.344	0.7115
10%	Residual	27	2632	97.47		
	Total	29	2699			
15%	Treatment	2	264.3	132.1	0.9803	0.3881
	Residual	27	3639	134.8		
	Total	29	3903			
Bile	Treatment	2	0.3928	0.1964	4.571	0.0426
	Residual	9	0.3867	0.04296		
	Total	11	0.7795			
	Treatment	2	1.885	0.9424	12.00	0.0029
	Residual	9	0.7067	0.07852		
	Total	11	2.591			
	Treatment	2	1.928	0.9641	30.27	0.0001
	Residual	9	0.2867	0.03185		
	Total	11	2.215			
Proventriculus	Treatment	2	2.387	1.194	19.41	0.0005
5%	Residual	27	0.5533	0.06148		
	Total	29	2.94			
	Treatment	2	1.441	0.7203	36.01	0.0001
10%	Residual	27	0.18	0.02		
	Total	29	1.621			
15%	Treatment	2	0.4384	0.2192	7.788	0.0109
	Residual	27	0.2533	0.02815		
	Total	29	0.6918			

ANOVA table for Carcass of broiler chicken finisher (continued)

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
Kidney	Treatment	2	4.51	2.261	16.16	0.0001
5%	Residual	24	3.358	0.1399		
	Total	26	7.88			
	Treatment	2	3.089	1.545	25.25	0.0001
10%	Residual	24	1.468	0.06117		
	Total	26	4.558			
15%	Treatment	2	3.856	1.928	29.27	0.0001
	Residual	24	1.581	0.06587		
	Total	26	5.437			
Crop	Treatment	2	298.4	149.2	0.2364	0.7911
5%	Residual	27	17040	631.1		
	Total	29	17339			
	Treatment	2	634.2	317.1	1.017	0.3751
10%	Residual	27	8419	311.8		
	Total	29	9053			
15%	Treatment	2	3731	1866	4.684	0.0179
	Residual	27	10754	398.3		
	Total	29	14485			
Intestine	Treatment	2	1492	746.1	0.7233	0.4943
5%	Residual	27	27851	1032		
	Total	29	29343			
	Treatment	2	8434	4217	2.380	0.1117
10%	Residual	27	47838	1772		
	Total	29	56272			
15%	Treatment	2	17651	8826	6.893	0.0038
	Residual	27	34572	1280		
	Total	29	522234			
Feet	Treatment	2	1579	78.93	0.2473	0.7826
5%	Residual	27	8618	319.2		
	Total	29	8775			
	Treatment	2	200.3	100.1	0.2621	0.7714
10%	Residual	27	10314	382		
	Total	29	19515			
15%	Treatment	2	1099	549.7	0.8966	0.4197
	Residual	27	10554	613.1		
	Total	29	17653			

ANOVA table for Carcass of broiler chicken finisher (continued)

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
Spleen	Treatment	2	5.067	2.533	2.111	0.1407
5%	Residual	27	32.4	1.2		
	Total	29	37.47			
			3.467	1.733	0.78	0.4685
	Treatment	2	60	2.222		
10%	Residual	27	63.47			
	Total	29				
15%	Treatment	2	4.267	2.133	1.895	0.1698
	Residual	27	30.4	1.126		
	Total	29	34.67			
Gizzard	Treatment	2	5.067	2.533	0.4325	0.9577
5%	Residual	27	1582	58.58		
	Total	29	1587			
	Treatment	2	52.27	26.13	0.3819	0.6862
10%	Residual	27	1848	68.43		
	Total	29	1900			
15%	Treatment	2	3.2	1.6	0.02831	0.9721
	Residual	27	1526	56.52		
	Total	29	1529			

ANOVA table for Haematological parameters of Broiler Chicken

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
WBC 5%	Treatment	2	2189	1095	9011	0.0001
	Residual	24	2.916	0.1215		
	Total	26	2192			
10%	Treatment	2	819.2	409.6	2946	0.0001
	Residual	24	3.337	0.139		
	Total	26	822.6			
15%	Treatment	2	305.6	152.8	6025	0.0001
	Residual	24	0.6087	0.02536		
	Total	26	306.2			
RBC 5%	Treatment	2	0.0965	0.04825	18.87	0.0001
	Residual	27	0.06902	0.002550		
	Total	29	0.1655			
10%	Treatment	2	0.3949	0.1975	116.6	0.0001
	Residual	27	0.04574	0.001694		
	Total	29	0.4407			
15%	Treatment	2	0.1008	0.05042	27.73	0.0001
	Residual	27	0.04909	0.001818		
	Total	29	0.14991			
HB 5%	Treatment	2	1.099	0.5493	19.57	0001
	Residual	27	0.7578	0.02807		
	Total	29	1.856			
10%	Treatment	2	6.033	3.017	756.3	0.0001
	Residual	27	0.1077	0.003989		
	Total	29	6.141			
15%	Treatment	2	2.113	1.056	51.48	0.0001
	Residual	27	0.07625	0.008473		
	Total	29	0.3695	0.02052		
			2..558			
HCT 5%	Treatment	2	18.41	9.207	8.693	0.0012
	Residual	27	28.6	1.059		
	Total	29	47.01			
10%	Treatment	2	59.41	29.71	74.45	0.0001
	Residual	27	10.77	0.399		
	Total	29	70.19			
15%	Treatment	2	41.46	20.73	41.52	0.0001
	Residual	27	13.48	0.4993		
	Total	29	54.94			

ANOVA table for Haematological parameters of Broiler Chicken (continued)

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
MCH 5%	Treatment	2	0.1607	0.08033	0.02075	0.9795
	Residual	27	104.5	3.872		
	Total	29	104.7			
10%	Treatment	2	2.646	1323	0.5580	0.5788
	Residual	27	64.02	2.371		
	Total	29	66.66			
	Treatment					
15%	Residual	2	7.226	3.613	0.9282	0.4075
	Total	27	105.1	3.893		
		29	112.3			
MCHC 5%	Treatment	2	3.759	1.88	11.22	0.0003
	Residual	27	4.525	0.1676		
	Total	29	8.284			
10%	reatment	2	2.189	1.094	5.178	0.0125
	Residual	27	5.706	0.2113		
	Total	29	7.895			
15%	Treatment	2	2.184	10.92	11.29	0.0003
	Residual	27	2.611	0.0967		
	Total	29	4.795			
PLT 5%	Treatment	2	564.8	282.4	641	0.0001
	Residual	27	11.89	0.4405		
	Total	29	576.7			
10%	Treatment	2	231.2	115.6	1238	0.0001
	Residual	27	2.521	0.09337		
	Total	29	233.7			
15%	Treatment	2	69.2	34.6	126	0.0001
	Residual	27	7.417	0.2747		
	Total	29	76.62			
RDW-SD 5%	Treatment	2	176.2	88.1	81.89	0.0001
	Residual	27	29.05	1.076		
	Total	29	205.2			
10%	Treatment	2	7.193	3.596	4.454	0.0213
	Residual	27	21.8	0.8074		
	Total	29	28.99			
15%	Treatment	2	44.98	22.49	42.31	0.0001
	Residual	27	14.35	0.5315		
	Total	29	59.33			

ANOVA table for Haematological parameters of Broiler Chicken (continued)

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
RDW-CV 5%	Treatment	2	11.18	5.59	65.80	0.0001
	Residual	27	2.294	0.08496		
	Total	29	13.47			
10%	Treatment	2	1.806	0.903	6.334	0.0056
	Residual	27	3.849	0.1426		
	Total	29	5.655			
15%	Treatment	2	1.345	0.6723	4.370	0.0227
	Residual	27	4.154	0.1539		
	Total	29	5.499			
PDW 5%	Treatment	2	12.09	6.044	110.7	0.0227
	Residual	27	1.474	0.05459		
	Total	29	13.56			
10%	Treatment	2	30.07	15.04	1880	0.0227
	Residual	27	0.2159	0.007996		
	Total	29	30.29			
15%	Treatment	2	22.41	11.2	958.5	0.0227
	Residual	27	0.3156	0.01169		
	Total	29	22.72			
MPV 5%	Treatment	2	3.421	1.711	202.1	0.0001
	Residual	27	0.2286	0.008466		
	Total	29	3.65			
10%	Treatment	2	1.364	0.6818	509.4	0.0001
	Residual	27	0.03614	0.001339		
	Total	29	1.4			
15%	Treatment	2	27.51	13.75	336.4	0.0001
	Residual	27	1.104	0.04088		
	Total	29	28.61			
PLCR 5%	Treatment	2	240	120	514.1	0.0001
	Residual	27	6.302	0.2334		
	Total	29	246.3			
10%	Treatment	2	144.4	72.18	479.9	0.0001
	Residual	27	4.061	0.1504		
	Total	29	148.4			
15%	Treatment	2	341.7	170.8	561	0.0001
	Residual	27	8.222	0.3045		
	Total	29	349.9			

ANOVA table for Haematological parameters of Broiler Chicken (continued)

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
PCT 5%	Treatment	2	0.00029	0.00014	1.286	0.2928
	Residual	27	0.00301	0.00011		
	Total	29	0.0033			
10%	Treatment	2	0.00019	9.3E-0.5	0.7179	0.4968
	Residual	27	0.00351	0.00013		
	Total	29	0.0037			
15%	Treatment	2	0.00042	0.00021	1.309	0.2866
	Residual	27	0.00433	0.00016		
	Total	29	0.00475			
MCV 5%	Treatment	2	112	56	2.405	0.1093
	Residual	27	628.6	23.28		
	Total	29	740.7			
10%	Treatment	2	1.261	0.6303	0.0377	0.9631
	Residual	27	451.4	16.72		
	Total	29	422.7			
15%	Treatment	2	51.42	25.71	1.147	0.3327
	Residual	27	605.4	22.42		
	Total	29	656.9			

ANOVA table for Blood Biochemistry of broiler chicken

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
Albumin 5%	Treatment	2	26.56	13.28	7.026	0.0035
	Residual	27	51.03	1.89		
	Total	29	77.59			
				31.28	18.45	0.0001
10%	Treatment	2	62.57	1.695		
	Residual	27	45.77			
	Total	29	108.3	13.59	6.723	0.0043
				2.021		
15%	Treatment	2	27.18			
	Residual	27	54.57			
	Total	29	81.75			
Globulin 5%	Treatment	2	339	169.5	10.77	0.0004
	Residual	27	425	15.74		
	Total	29	764			
10%				46.12	25.94	0.0001
	Treatment	2	92.25	1.778		
	Residual	27	48.01			
15%	Total	29	140.3	43.8	4.378	0.0226
				10.01		
	Treatment	2	87.61			
	Residual	27	270.1			
	Total	29	357.7			
Protein 5%	Treatment	2	152	76.01	26.08	0.0001
	Residual	27	78.69	2.915		
	Total	29	230.7			
10%				90.07	26.61	0.0001
	Treatment	2	180.1	3.384		
	Residual	27	91.37			
15%	Total	29	271.5	15.05	5.269	0.0117
				2.856		
	Treatment	2	30.09			
	Residual	27	77.1			
	Total	29	107.2			
ALT 5%	Treatment	2	5.393	2.696	2.542	0.0974
	Residual	27	28.64	1.061		
	Total	29	34.03			
	Treatment	2	8.813	4.406	0.9364	0.4044
10%	Residual	27	127	4.706		
	Total	29	135.9			
15%						
	Treatment	2	14.81	7.407	7.943	0.0019
	Residual	27	25.18	0.9325		
	Total	29	39.99			
Direct Bilirubin	Treatment	2	19.17	95.85	428.5	0.0001
5%	Residual	27	0.604	0.02237		
	Total	29	19.77			
10%	Treatment	2	13.24	6.622	153.5	0.0001
	Residual	27	1.165	0.04315		
	Total	29	14.41			
15%	Treatment	2	9.459	4.729	130.3	0.0001
	Residual	27	0.98	0.0363		
	Total	29	10.44			

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
Bilirubin 5%	Treatment	2	72.91	36.46	207.5	0.0001
	Residual	27	4.744	0.1757		
	Total	29	77.66			
10%	Treatment	2	6.326	3.163	43.11	0.0001
	Residual	27	1.981	0.07337		
	Total	29	8.307			
15%	Treatment	2	31.73	15.86	356.7	0.0001
	Residual	27	1.201	0.04448		
	Total	29	32.93			
GT 5%	Treatment	2	26.87	13.43	138.7	0.0001
	Residual	27	2.615	0.09685		
	Total	29	29.48			
10%	Treatment	2	44.58	22.29	125.8	0.0001
	Residual	27	4.785	0.1772		
	Total	29	49.37			
	Treatment	2	48.76			
15%	Residual	27	1.15	24.38	572	0.0001
	Total	29	49.91	0.0459		
Creatinine 5%	Treatment	2	2131	1066	768.6	0.0001
	Residual	27	37.43	1.386		
	Total	29	2169			
10%	Treatment	2	3251	1625	6190	0.0001
	Residual	27	7.09	0.2626		
	Total	29	3258			
15%	Treatment	2	3196	1598	2239	0.0001
	Residual	27	19.27	0.7138		
	Total	29	3215			
Urea 5%	Treatment	2	11.61	5.804	480	0.0001
	Residual	27	0.3265	0.01209		
	Total	29	11.93			
				5.379	447.4	0.0001
10%	Treatment	2	10.76	0.01202		
	Residual	27	0.3247			
	Total	29	11.08	0.4093	67.60	0.0001
				0.006055		
15%	Treatment	2	0.8186			
	Residual	27	0.1635			
	Total	29	0.9821			
Sodium 5%	Treatment	2	538.1	269	6.695	0.0001
	Residual	27	1085	40.18		
	Total	29	1623			
10%	Treatment	2	1456	728.1	203.7	0.0001
	Residual	27	96.5	3.574		
	Total	29	1553			
15%	Treatment	2	4935	2468	655.2	0.0001
	Residual	27	101.7	3.767		
	Total	29	5037			

ANOVA table for Blood Biochemistry of broiler chicken (continued)

Variate	Source	DF	SSQ	MS	F-RATIO	P-value
Potassium 5%	Treatment	2	47.85	23.92	1333	0.0001
	Residual	27	0.4847	0.01795		
	Total	29	48.33			
10%	Treatment	2	44.46	22.23	339.9	0.0001
	Residual	27	1.766	0.06541		
	Total	29	46.23			
15%	Treatment	2	80.01	40.01	3536	0.0001
	Residual	27	0.3055	0.01131		
	Total	29	80.32			
Chloride 5%	Treatment	2	155.8	77.92	13.2	0.0001
	Residual	27	158.4	5.865		
	Total	29	314.2			
10%	Treatment	2	24.124	12.07	2.20	0.1303
	Residual	27	148.2	5.488		
	Total	29	172.3			
15%	Treatment	2	314.2	157.1	21.7	0.0001
	Residual	27	195.2	7.229		
	Total	29	509.4