

**AMINO ACID REFERENCE VALUES FOR SELECTED FEEDSTUFFS USED
IN THE NIGERIAN POULTRY INDUSTRY**

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CERTIFICATION

This is to certify that this work "Amino Acid Reference Values for Selected Feedstuffs used in the Nigerian Poultry Industry" was carried out by OKATA, UGOCHUKWU EDWIN (20104885628) in partial fulfillment for the award of the degree of Master of Science (M.Sc.) in Animal Management in the Department of Animal Science and Technology of the Federal University of Technology, Owerri.


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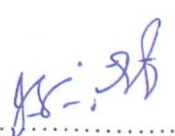

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DEDICATION

This work is dedicated in loving memory of my late father Mr Edmond Okata who gave me the courage, to my beloved mother Mrs. M.U. Okata who is still around and strong to guide and advise me and also to my siblings.

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ABSTRACT

The main objective of this study was to establish reference protein composition values for selected local feedstuffs such as dry brewers spent grain (DBSG), cassava meal (CAM), palm kernel cake (PKC) and groundnut cake (GNC) for the Nigerian feed industry by determining amino acids profiles and amino acids compositional quality scores. The selected local feedstuffs were each sourced from five locations from northern and southern parts of Nigeria such that there were five samples for each raw material and 20 samples in all. The samples were analysed at Evonik Industry Laboratory, Germany. Results were generated on dry matter (DM), crude protein (CP) standardized to a dry matter content of 88%, CP as is, total amino acids (Taa) without NH_3 , and NH_3 content. Total amino acids Essential amino acids, non-essential amino acids, amino acid scores such as qualitative, quantitative and chemical scores were calculated and values subjected to descriptive statistics such as mean, standard deviation and coefficient of variation across samples values from different locations. This study showed that all the sampled local feedstuffs were high in dry matter content, with values ranging from $89.304 \pm 0.857\%$ recorded for CAM to $93.006 \pm 0.456\%$ recorded for PKC. However, the coefficient of DM variation was less than 1.00%. CP content of the DBSG, CAM, PKC and GNC sampled (as is), ranged from $0.988 \pm 0.230\%$ for CAM to $44.430 \pm 6.600\%$ for GNC, while the CV between the different samples ranged from 4.518% recorded for PKC to 23.279% recorded for CAM. The Taa ranged from $0.837 \pm 0.182\%$ recorded for CAM to $40.025 \pm 5.831\%$ recorded for GNC, with high CVs of 21.744% for CAM and 14.568% recorded for GNC values, indicating wide variations in Taa of samples from different locations. Methionine content of the feedstuff ranged from the $0.014 \pm 0.005\%$ recorded for CAM to the $0.441 \pm 0.039\%$ recorded for DBSG, with CVs of 35.714% and 11.004 for CAM and GNC respectively. Lysin values ranged from $0.045 \pm 0.006\%$ recorded for CAM to $1.439 \pm 0.162\%$ recorded for GNC, with CVs across five samples of each material being generally higher than 10% except for PKC. Again, threonine concentrations

ranged from the $0.039 \pm 0.008\%$ recorded for CAM to the $1.150 \pm 0.146\%$ recorded for GNC, while coefficient of variations between individual sample of DBSG and PKC were less than 10% CV, but wide between samples of CAM and GNC. Isoleucine values ranged from $0.035 \pm 0.011\%$ recorded for CAM to $1.499 \pm 0.225\%$ recorded for GNC, however, the CV between CAM and GNC were greater than 10%, while DBSG and PKC recorded less than 10% CVs. Mean values of leucine ranged from $0.068 \pm 0.032\%$ recorded for CAM to $2.771 \pm 0.394\%$ recorded for GNC, while the phenylalanine content ranged from 0.047 ± 0.017 recorded for CAM to $2.262 \pm 0.354\%$ recorded for GNC. Qualitative amino acid scores showed that GNC was the richest protein source and was rich in all the essential amino acids with exception of methionine. DBSG was rich in four essential amino acids (leucine, valine, phenylalanine and arginine), while PKC was rich only in arginine and moderate in isoleucine, leucine, valine and phenylalanine. CAM was poor in all essential and non essential amino acids contents. The quantitative scores of the amino acid profiles of the sampled feedstuffs showed that on mean Taa bases, PKC contained 47.92% essential amino acids while GNC contained 43.24%, DBSG (42.21%) and CAM (41.19%). The percentage mean total neutral amino acids in the feedstuffs ranged from 67.58% recorded in PKC to 90.68% recorded in DBSG indicating that these are the most abundant amino acids in the feedstuffs. It was concluded that Nigerian GNC based diets should be supplemented with synthetic methionine, while DBSG based diets should be supplemented with lysine, methionine and to some extent threonine for them to drive optimal performance in monogastric animals. Nigerian PKC cannot be regarded as a protein source since it requires fortification with synthetic sources of almost all the essential amino acids. CAM based diets should be supplemented with all the synthetic amino acids, especially the limiting amino acids. Practical feeding trials with monogastric livestock should be carried out to validate these results, since the study was limited to laboratory analysis.

Key words: Amino acid profiles, protein scores, dry matter, feedstuffs reference values

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Poultry is any of the domesticated and commercialized types of birds used for production of eggs and meat for human food and for other purposes (Jurgens, 2002; NRC, 1994). Modern poultry production in any part of the world is based on the manipulation of genetic and environmental factors that affect intensively farmed poultry. This includes feeding well-balanced and hygienically produced diets to highly productive lines of birds (Omenka and Anyasor, 2010). A well balanced diet is that which contains all components such as carbohydrate, proteins, fats, vitamins and minerals in their right proportions (Hamdy, 2008). The main factors influencing the nutritive value of a diet are however the ingredients employed and their chemical compositions (McDonald *et al.*, 2002).

Inadequate nutrition is a major cause of low live-weight gains, infertility and low yields in poultry in Nigeria and many producers have expressed challenges in feeding their animals optimally for commercial purposes. Feed insufficiency has been shown to be due to stiff competition with the need for human food in many developing countries, particularly for the fast growing and prolific monogastric species of poultry and pigs (Tewe, 2001; Odunsi, 2003). Constraints to feeding and feed formulation using conventional feedstuffs include; high cost of feedstuffs driven chiefly by the competition between humans, food industry and farm animals for these feedstuffs (Okoli *et al.*, 2009), anti-nutritional factors/growth inhibitors in locally available feedstuffs (Emenalom *et al.*, 2004; Okoli *et al.*, 2012), higher rates of indigestibility, losses of quality under poor storage conditions (Hossain *et al.*, 2011) and variations in chemical composition due to soil differences, climatic changes and differences in processing methods (Okoli *et al.*, 2003; Omede, 2010).

Earlier efforts at overcoming these constraints included identification of cheaper and locally available feed materials, development of more efficient feeding practices and use of better converting animal (Udedibie, 2003; Esonu, 2009; Kategile, 1982). However, expanding the utilization of available feeds, including non- conventional feed resources, represents possibly, the most important goal for sustaining intensive poultry production industry in Nigeria. Thus, a wide range of alternative feedstuffs have been demonstrated to be available for feeding in commercial poultry production in Nigeria (Udedibie, 2003; Esonu, 2009; Emenalom, 2002; Uchegbu, 2005; Anyanwu, 2002; Etuk, 2008; Okoli *et al.*, 2003). The current challenge however is to formulate diets that contain these feedstuffs in the appropriate proportion without compromising the nutritional requirements of the bird and recommended official standards (Uchegbu *et al.*, 2009). Many of the feedstuffs also contain considerable amounts of harmful endogenous nutritional substances which must be eliminated from the feedstuff before it could be effectively utilized for poultry feeding.

Furthermore, in the semi-commercial system commonly practiced in the country, only part of the feedstuff requirement is purchased from commercial millers, therefore, there is always the possibility of on-farm mixing or dilution of purchased feeds with locally available alternative feedstuffs (Mmeremikwu, 2001). Again, in low-input family poultry system, locally available, alternative feedstuffs are used to supplement the scavenging feed base (Ravindran and Blair, 1991; Sonaiya, 1990).

Feed ingredients are generally classified as carbohydrate (cereal grains like maize, sorghum, millet and tubers like cassava etc), animal proteins (fishmeal, meat meal and blood meal etc), plant/vegetable proteins (groundnut cake, soybean meal etc), fiber (spent grain, wheat offal, palm kernel cake, rice offal etc), micro-minerals (iron, iodine, copper,

cobalt, manganese, selenium, molybdenum etc), macro-minerals (calcium, phosphorus, magnesium, sodium, potassium, chlorine and sulphur etc) and vitamin premixes (Esonu, 2006). Except for water, these nutrients have to be provided by the ingredients that make up the diet (Leeson and Summers, 2000).

Animal feeds are compounded by means of least cost formulation (Esonu, 2006) that requires the composition of the diet to be correct in terms of energy, protein, minerals, vitamins and fiber (Thomas, 1998). In poultry rations, the limiting nutrients are usually energy and some of the essential amino acids, such as methionine and lysine (Leeson and Summers, 2000). In formulating poultry rations therefore, the nutrients to be considered are protein, energy, fat, vitamins and water. Specifically, for each species (chicken, turkey, duck etc) of poultry, NRC (1994) suggested requirements for 14 amino acids, 12 minerals, 13 vitamins, and one fatty acid.

Protein is required for the synthesis of body tissues (particularly muscle), physiological molecules (such as enzymes and hormones), feathers and for egg production, and also provides small amount of energy. Protein content of a feedstuff is generally measured as crude protein, which is simply nitrogen contained in the feedstuff x 6.25. (Leeson and Summers, 2000). The component building blocks of proteins are amino acids. The ones essential for the bird include methionine, lysine, tryptophan, threonine, phenylalanine, leucine and isoleucine (Esonu *et al.*, 2006). Methionine would usually be the first-limiting amino acid in grain and soybean meal diets (Jurgens, 2002; NRC, 1994).

Groundnut, (*Arachis hypogaea*) is the major source of plant protein in Nigeria. The country produces about 4.5% of the overall world volume, making it relatively available (FAO, 1994). The cake produced after oil extraction (GNC) may contain anti-nutritional

factors (mycotoxins) produced by various fungal organisms that thrive on the nut. These negative factors can be reduced during processing. The methods of oil extraction include, press or expeller, solvent extrusion and prepress solvent extraction. These methods contribute to variations of its biochemical compositions.

Palm kernel cake (PKC), obtained from palm fruit (*Elaeis guineensis*), is an agro-industrial by-product that is produced locally in Nigeria and within the West Africa sub-region in sizable quantities (Boateng *et al.*, 2008). It is therefore readily available and cheap, particularly in Southern Nigeria (Onwudike, 1986 and Olumu, 1995). It is aflatoxin free, palatable, and has considerable potential as carbohydrate, fiber and protein source (Sundu *et al.*, 2006). Industry adapted processing methods include solvent or hydraulic press which account for variations in its biochemical compositions.

Energy sources are largely made up of intact plant carbohydrates that are directly utilizable by man. Energy can also be made up from the unconventional sources, such as sorghum, cassava and other agro-industrial by-products, if these are properly processed to meet the criteria for their efficient utilization by the different livestock species (Tewe and Bokanga, 2001). The energy needs of different animals are calculated by determining the metabolizable energy of feedstuffs used in formulating the diet (Pagot, 1992). Metabolizable energy means basically the part of the feed which the animal is able to utilize (Adefope and Wright, 2005).

Cassava, a high energy crop, is available throughout the year in Nigeria with production capacity of 14 million tones (Ayodeji, 2005). It is the 3rd most important food source in the tropics after rice and maize (Polso and Spencer, 1991). According to Calpe (1991), the use of cassava in animal feed accounts for only 2% of cassava utilization in Africa.

The limiting factor of cassava utilization in animal production however is its hydrogen cyanide content, which can be reduced by processing methods to produce cassava meal (Udedibie *et al.*, 2008; Okoli *et al.*, 2012).

Fats are also needed in animal diets and are concentrated forms of energy per unit of weight. They contain two quarter times as much energy as either carbohydrates or proteins. They are sources of essential fatty acids that aid in metabolic processes and also the building block of lipid (Esonu, 2006). Dietary protein and energy content must be specified to maintain the proper ratio of protein to energy so that birds can consume an adequate amount of protein. The protein requirement or amino acid requirements can be defined accurately only in relation to the energy density. Also, the degree of fat deposition in meat producing birds can be affected by this relationship (Kellems and Church, 1998).

Fibers are mostly bulky materials in feedstuff which have high lignin content of minimal value to humans and when consumed by selected species of animal provide a means for converting relatively low-quality raw material to high-quality products for use as human food. It has been noted that several of the available by-products and wastes in Nigeria are mostly fibrous materials which have limited value for non ruminant animals (Tewe, 2001). Enzymatic supplementations and palletization of feeds allow increased usage of fibrous residues in non-ruminant feeding (Tewe, 2001).

Brewer's spent grain is a by-product of beer brewing consisting of the residue of malt and grain which remains in the mash-kettle after the mashing and lauterin process (Beldman *et al.*, 2004). It consist primary of grain husks, pericarp and fragments of endosperm. By mass, it consists of about half carbohydrate, with the rest being mostly proteins and lignin

(Pirkko, 2008). It is a bulky material in animal feeding and it is readily available and cheap but the limiting factor is high lignin content, which makes its digestion difficult (Deltoro *et al.*, 1982). Its nutrient content vary from plant to plant and also depending upon the type of substrate used (barley, maize, sorghum, etc), proportion fermented and fermentative process used. Some industries dry and sell it as dried brewer's grain, while others have it available as wet brewer's grain (Wadhea *et al.*, 1995).

Vitamins are organic chemicals containing carbon, which help to control body processes and are required in small amounts for normal health and growth (Esonu, 2006). Fat soluble vitamins like vitamin A, D, E, and K require fats for proper absorption and many vitamins require some protein to be properly digested and utilized. The water soluble vitamins are B1, B2, B6, B12 and C (Budvari, 1996; Esonu, 2006). Minerals on the other hand are inorganic elements that come from the soil and water, and are absorbed by plants or eaten by animals. Among the twenty elements that function in animal nutrition, carbon, hydrogen, oxygen and nitrogen are regarded as the non-mineral elements. The other sixteen are referred to as the mineral elements. Micro-minerals are required in trace amount, while macro-minerals are required in relatively large amounts (Esonu, 2006).

1.2 Problem Statement

There are wide variations in the biochemical compositions of local feedstuffs from Nigeria (Adepoju *et al.*, 2010). The reasons for these variations have been traced to lack of standardization of processing methods (Okoli *et al.*, 2012), inadequate laboratories and analytical equipment as well as poorly trained laboratory staff (Chonde and Doulla, 2003). Furthermore, variations in plant varieties, soil types and soil nutrients at the varied locations on which the plants grow have been shown to influence the nutrient composition of feedstuffs derived from them (Okoli *et al.*, 2001 and 2003).

Lack of a functional feed quality scheme in Nigeria has lead to poor regulation of standards in the animal feeds production resulting in many sharp practices in the industry (Omede, 2004 and 2010). For example, cases of commercial feed ingredients dilution with less expensive materials have been observed in the industry, although these are rarely reported. For example, the moisture content of commercial feeds may be deliberately increased to reduce cost of production (Omede, 2004) or ammonia and urea may be used to improve the crude protein analysis value of low quality fish meal (Okoli *et al.*, 2009).

Current published information on the nutrient compositions of Nigerian feedstuffs remain at best fragmented (Aduku, 1993), since they were derived from non-standardized experiments which were not targeted at generating reference values for the country. The need for such important production information has raised the critical questions; what should be regarded as reliable nutrient composition values of basic feedstuffs produced in the country and specifically, what should be regarded as the reference protein composition values of Nigerian feedstuff?

1.3 Study Objective

The main objective of this study is to establish the reference protein composition values of selected local feedstuffs including dried brewers spent grain (DBSG), palm kernel cake (PKC), processed cassava meal of flour (CAM) and ground nut cake (GNC).

Specifically the study will

1. Determine the dry matter and moisture content of the selected feedstuffs
2. Determine the amino acid profiles of the selected feedstuffs
3. Calculate the amino acids compositional quality scores of the selected feedstuffs
4. Establish reference protein composition data of the selected raw materials

1.4 Justification

Routine analysis of feedstuffs for information on their nutrient composition is a good management practice. This is because nutrient compositions of many locally available feedstuffs have correlation with processing methods (Adepoju *et al.*, 2010). The desired nutrient information on such locally available feedstuffs includes amino acid, mineral, proximate and toxicological status. Specific information on the amino acid compositions of local feedstuffs is critical to their optimized utilization in the formulation of compound feeds. This is even more important in the present globalized economy since accurate knowledge of nutrient compositions of local feedstuffs creates room for reductions in environmental impact of animal production, especially with regards to carbon footprint, global warming potential, eutrophication and acidification (Redshaw *et al.*, 2010).

The detrimental effects of the variations in the biochemical compositions of feedstuffs may be borne more by small holder farmers who are usually unable to formulate their own animal diets and may not have access to standard laboratories for feedstuff analysis. This is critical since feed accounts for more than 60% of the cost of intensive poultry production in Nigeria (Okoli *et al.*, 2007).

It is therefore not surprising that small holder poultry farmers in Nigeria highlighted high cost and poor quality feed as a critical constraint of production (Okoli *et al.*, 2004). It is therefore imperative to develop reference values for important feedstuffs employed in feed formulation in the country since such reliable data could form the bases for calculation of the nutrient compositions of available on farm and commercial poultry diets.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nigeria Poultry Industry

Nigerians own many types of farm animals including poultry. Poultry ranks highest in number among the farm animals in the farm; mostly 80 – 90 % owned by small scale farmers (Idi, 2000). Poultry production is increasing very rapidly and the consumption of its products is at its peak compared to other kinds of meat beside beef (Bukar, 2003). The population of poultry in the country was estimated by FDLPCS (1992) to be 104.3 million comprising 72.4 million chickens, 15.2 million pigeons, 11.8 million ducks, 4.7 million guinea fowl and 0.2 million turkeys Turkey and chicken however make up the species of commercial poultry production (FAO, 2006). Poultry are therefore animal resources available to even the poorest families (Aini, 1990). It has proved to be a particularly versatile group of domestic animal species that are adapted to a wide range of climatic conditions.

The poultry industry is an important and integral part of agriculture sector in Nigeria that deals with domesticated and commercialized types of birds and is vital for the supply of day old chicks, meat, egg and manure (Jurgens, 2002; NRC, 1994) as well as source of additional income for small farmers and major source of income for commercial poultry/livestock owners. Poultry production has long been recognized as one of the quickest means for rapid increase in protein supply in the shortest run. Of recent, there has been recorded improvement in the poultry production sub-sector in Nigeria, with its share of the Gross Domestic Production (GDP) increasing in absolute terms. Poultry eggs and meat contribution to the livestock share of the GDP increased from 26 % in 1995 to 27 % in 1999 (CBN, 1999; Adeoleji *et al.*, 2014). There has also been relative ease of

compounding efficient feeds using easily available local feedstuffs (Afolobi and Ojo, 2009; Adedeji *et al.*, 2014).

Productivity of most local breeds however remain below their genetic potential, which is due usually to various factors like inadequate management, poor quality feedstuffs and feeding, low reproductive management, prevalence of diseases and lack of various support services such as artificial insemination (Okoli *et al.*, 2005; Khanum *et al.*, 2007; Okoli *et al.*, 2009). There are two distinct poultry production systems in Nigeria, namely commercial and rural poultry production systems as found in most developing countries of Africa and Asia (Adene and Oguntade, 2006). Each of these two systems is associated with characteristics of stock, scale, husbandry and productivity that define it as a distinct production system. However, the industry is essentially a “two way” production system in which the traditional or rural production system exists side by side with the commercial system (Chima, 2011).

Within these groups can still be found three sub-groups namely, intensive, extensive and semi-intensive systems. Thus, poultry production systems in Nigeria are influenced by endemic factors such as type of birds, housing, socio-economic background of practitioners, disease incidence, financial sources and feeding as well as products disposal methods and channels (Adedeji *et al.*, 2013). The peculiar challenges associated with industrial intensive poultry production, make rural or family poultry production in Nigeria popular (Alabi and Aruna, 2005; Chima *et al.*, 2012).

2.1.1 Commercial poultry production system

The Commercial poultry production system as the name implies is industrial in its prototype and therefore based on large, dense and uniform stocks of modern poultry

hybrids. Commercial system is the dominant production system in developed countries, and this sector has also recently expanded in many developing countries including Nigeria. Large vertically integrated production units and use of high-producing modern strains of birds characterize commercial systems. In these systems, feed is the most important variable cost component, accounting for 65 to 70 percent of production costs (Adene and Oguntade, 2006). High productivity and efficiency depend on feeding nutritionally balanced feeds that are formulated to meet the birds' nutritional requirements. It is capital and labour intensive; as well as inputs and technology demanding (Leeson and Summers, 2005). Commercial poultry production in any part of the world, particularly Africa is therefore based on the manipulation of genetic and environmental factors that affect the intensively farmed poultry.

However, quality feed remains the foundation on which commercial poultry productive efficiency is built among other determinants. There is no other factor that is directly or indirectly related to proper nutrition and high performance of poultry that is more critical than feed quality evaluation, control and ration consistency (Richardson, 1994). According to Jones (2005), under commercial production, the overall mission of feed formulation and manufacturing is to provide farmers with efficiently manufactured feeds that are correctly delivered to their facilities and consistently contain the available materials required by animals for body maintenance, growth and reproduction having considered the nutrient and physical characteristics of the feed raw materials.

Commercial intensive poultry farming in Nigeria, also provides optimum conditions for the concentration of pathogens and transmission, due to the crowding of thousands of birds in enclosed warm, and dusty environments conducive for disease transmission (Chima *et al.*, 2012). Furthermore, selection of birds for faster growth rate and higher

meat yield leaves the bird's immune system less able to cope with infections; while the higher degree of genetic uniformity in the population makes disease spread easier (Delany, 2003).

2.1.2 Rural/traditional poultry production system

The rural poultry production system is by convention a subsistence system which comprises stocks of non-standard breeds or mixed strains, types and ages. It is generally of small scale, associated with household or grass root tenure and involves little or no veterinary inputs. The rural poultry sector is therefore in its original sense, household or individual holding and occupation which has however been extended to non-village settings in peri-urban localities, mainly by the middle class dwellers (Adene and Oguntade, 2006). The common features to all these intermediate grades are in their subsistence scale generally, with minimal or no inputs and labour overheads.

The system is, however mostly distributed in rural areas of Nigeria where the rural poor practice it. The indigenous chickens are in general hardly, adapted to rural environment, where they survive on little or no input and adjust to fluctuations in feed availability. They make up about 98% of the total poultry numbers kept in Africa (Gueye, 2003). Specifically, they constitute 80% of the poultry type raised in rural areas in Nigeria (RIM, 1992). The majority of Nigerians because of the pigmentation, taste, leanness and suitability for special dishes (Horst, 1989) prefers their products. The indigenous poultry species represent valuable resources for livestock development because their extensive genetic diversity could provide better breed improvements adapted to commercial poultry production in tropical countries (Sonaiya, 1990). More recent works reveal that the different ecotypes could be grouped into two major categories on the basis of body weight as heavy ecotype and light ecotype (Momoh *et al.*, 2007).

These two extremes of traditional and commercial production systems has in-between them the semi-commercial system (Daghir, 1995), which is characterized by small to medium-sized flocks (50 to 500 birds) of local, crossbred or “improved” genotype stock, and the purchase of at least part of their feed from commercial compounders. According to (Glatz, 2009), several feeding strategies may be used in two of these systems: include on-farm mixing of complete rations, using purchased and locally available feed ingredients; dilution of purchased commercial feeds with local ingredients and blending of a purchased concentrate mixture with local ingredients or whole grains. However, available information shows that the scale of these distinct prototypes operation can range from stocks of a few units or a few dozens of a variety of poultry birds (species) in the rural poultry production system/household poultry to tens or hundreds of thousands of chickens in the grades of commercial poultry (Adene and Oguntade, 2006).

It was predicted during the 1990s that most increases in poultry production during the next two decades will occur in developing countries, where rapid economic growth, urbanization and higher household incomes, will increase the demand for animal proteins (Ravindran and Blair, 1991). Several other factors such as genetic progress in poultry strains for meat and egg production, better understanding of the fundamentals of nutrition and disease control, have contributed to the consistent growth in world poultry production. Thus, it has been an innovator and applicator of advancing technology and knowledge to keep meat and egg prices relatively constant for decades. This includes feeding well-balanced and hygienically produced diets to highly productive lines of birds (Omenka and Anyasor, 2010).

2.2 Nigeria Feedstuffs (Raw Materials) Industry

Nigeria is blessed with wide range of feed resources such as oilseeds, grains and agro-industrial by-products which could be used in the formulation of good quality livestock feeds. Feedstuffs/raw materials are any components of a diet that serve some useful purposes in the overall formulation of livestock/poultry ration. Most raw materials provide one or more sources of nutrient to the animal. Besides, some feed raw materials are purposely included to provide just bulk or what could be described as physical density, while some are included to reduced oxidation of the easily oxidized nutrients and some others simply to emulsify fats, provide color or rather as acceptability requirements (Adejoro, 2004). These locally available feed resources in Nigeria have the potential to support a flourishing livestock in particular poultry industry, but their under-utilization by the farmers has led to feeding challenges in the livestock industry. This could be traced to inadequate information based on location and localization of feed resources, processing, storage and quality enhancement. It has also been associated with long time dependence by major players in the livestock industry on conventional and imported feed resources, while cheap local feed resources suffer neglect and low patronage. This is critical since high cost of meat, egg and their products in the country is due to the high cost of feed inputs. (Reddy and Rao, 1996) reported that feed accounts for 65 - 70% of the total cost in the intensive system of animal production, while Pond *et al.* (1995) stated a similar figure of 50 - 80%.

More recently, the growth of the animal feed industry in Nigeria has allowed considerable use of industrial by-products, agricultural by-products and wastes, some of which although containing potentially toxic components, can be safely included in compounded feeds in relatively low proportions. Various agro-industrial by-products (AIBPs) and other non-conventional feedstuffs such as brewers spent grains, cocoa pod

husk, dried coffee pulp and palm kernel meal among others (Okai, 1995; Tewe, 2004 Uchegbu *et al.*, 2005) have been evaluated in Nigeria as potential feed ingredients for the non-ruminant farm animals. Feedstuffs and commercial marketed feeds are blended from different feed raw materials, hence the endogenous quality of the individual raw materials and their ratios of blending reflect on the final feed product made available to the animal (Omede, 2010). Sundu *et al.* (2005a) reported that this may be due to the fact that each ingredient has its own characteristics, either physically or chemically. However, in developing countries like Nigeria, monitoring feed quality has been restricted to proximate evaluation which only reveals the biochemical quality of feeds and feedstuffs. This does not give the appropriate quality picture of the feedstuff. However, when properly analyzed, including setting of standard on processing of these feedstuffs, results obtained may provide answers to many questions on field variation in animal performance (Omede, 2008; Okoli *et al.*, 2012). For example, with adequate rate of feedstuffs and feeds supply, animals performance in many farms in Nigeria have been observed to decline, and this may be attributed to feed raw materials quality and a wide variability in nutrient composition (Limcango-lopez, 1987; Omede, 2004; Jones, 2005; Okoli *et al.*, 2007a,b,c; Uchegbu *et al.*, 2008; Uchegbu *et al.*, 2009a).

2.3 Classifications of Livestock Feedstuffs

2.3.1 Classification according to industrial uses

Feedstuff could be classified according to their industrial usage into conventional and non-conventional feedstuffs. They can also be classified broadly according to their nutritional values into roughages and concentrates. The roughages constitute mainly industrial by-products and agricultural wastes, while concentrates constitute energy and protein sources, which may be of plant or animal origin.

A. Conventional feedstuffs

Traditionally these feedstuffs have been used in animal feeding and/or are used in commercially produced rations for animals. Conventional poultry feedstuffs usually include many cereals like maize, sorghum, wheat, oat, barley; and a few cereal byproducts such as wheat-bran, animal and vegetable protein sources like fish meal, meat meal, soybean meal and groundnut cake. The whole ration is fortified with adequate minerals and vitamins either in chemically pure or through ingredients known to be rich in these nutrients. With the cost of feed soaring high and the availability of conventional ingredients becoming scarce, intensive and continuous efforts are being made to determine the nutritive value of agro industrial by-products to replace more costly ingredients in poultry rations (Wilson, 1987). According to Ghadge *et al.* (2009) and Okoli *et al.* (2012), the common feedstuffs utilized in compounding poultry feeds in Nigeria include maize, sorghum, soybean meal, groundnut cake, fish meal and limestone. The proximate values of these feedstuffs are shown in table 2.1.

Generally, constraints to feeding and feed formulation in Nigeria using conventional feedstuffs include; high cost of feedstuffs driven chiefly by the competition between humans, food industry and farm animals for these feedstuffs (Okoli *et al.*, 2009), anti-nutritional factors/growth inhibitors in locally available feedstuffs (Emenalom *et al.*, 2004; Okoli *et al.*, 2012), higher rate of indigestibility due to simple stomach, losses of quality in storage condition (Hossain *et al.*, 2011) and variations in chemical composition due to soil difference, climatic changes and differences in processing methods (Okoli *et al.*, 2003; Omede, 2010).

i. Maize: About 70 - 80 % of the variable costs of poultry production are feed related (Louw *et al.*, 2011). Maize grains and soybeans are major ingredients of poultry diets and

Table 2.1: Composition of some conventional feedstuffs in poultry industry

<i>Feedstuffs</i>	<i>% Dry matter</i>	<i>% CP</i>	<i>% CF</i>	<i>% T A</i>	<i>M E (Kcal)</i>
maize	89.70	10.80	1.30	3.70	409.70
sorghum	96.80	13.00	4.01	2.60	394.10
SBM	91.40	38.00	2.70	9.10	
GNC	97.00	34.20	6.90	5.00	260.00
Fish-meal	96.20	49.15	3.46	18.65	220.00
Limestone	-	-	-	-	-

SBM = Soybean meal; GNC = Groundnut cake; CP = Crude Protein; CF = Crude Fiber;

TA = Total Ash; ME = Metabolizable Energy

Source: (Okoli *et al.*, 2012; Ghadge *et al.*, 2009).

are costly. Maize grains which usually form the bulk of poultry diets have many other uses in Nigeria. It is highly digestible and contains very little fibre, it contains the highest oil and energy content of all cereals but it is low in protein, fibre and minerals (Babiker *et al.*, 2009), especially lysine, and sulphur-containing amino acids (methionine and cyteine). The yellow varieties are a good source of vitamin A and xanthophyll. The latter is responsible for the yellow skin in certain breeds of fowl. Averagely its' metabolizable energy and crude protein values are 3350 kcal/kg and 7.5% respectively (Batal and Dale, 2011).

ii. Sorghum: It is majorly for energy supply. The feeding value of sorghum is similar to that of maize with ME (kcal/kg) 3288, CP % 11 compared with maize ME 3350 kcal/kg and CP 8.5% (NRC, 1994). The nutrient profile of sorghum is complementary to protein sources in typically formulated maize based poultry rations anywhere in the world, but it has higher protein content, quite palatable and maybe used in place of maize. Sorghum meal is a good source of some amino acids and its digestibility compares favorably with corn (Nyanno *et al.*, 2007), especially when considering newer sorghum varieties that are now produced in the USA. The fat content of grain sorghum and thus the energy value for poultry is slightly lower when compared to maize (Kriegshauser *et al.*, 2006), but this difference is easily balanced in rations with other sources of energy such as animal by-product meals or oils. Compared to corn, grain sorghum contains reduced quantities of yellow xanthophylls required for egg yolk pigmentation and skin coloration for broilers (Cramer *et al.*, 2003). In some cases where the customer prefers lighter meat products, sorghum may be used to reduce carcass pigmentation for marketing advantages.

iii. Groundnut cake: It is quite palatable and is widely used as a source of protein in poultry rations. It contains about 46% protein and metabolizable energy (ME of

3.27Kcal/Kg). Thus, most groundnut cake consumed internally in Sub-Saharan Africa during the 1970s and the 1980s may have gone to poultry because of rapid poultry development during the period. The main constraint to its utilization is its ease of contamination with toxic fungi usually due to bad storage. The most dangerous of these substances is aflatoxin Okoli, 2005).

iv. Soybean cake: Soybean meal (SBM) is the primary protein source in poultry diets worldwide. In general, most databases on ingredient composition provide information for two types of SBM: regular SBM (R-SBM) with 44 to 45% CP and high-protein SBM (HP-SBM) with more than 47% CP (NRC, 1994; INRA, 2002; FEDNA, 2003). Many of these tables report similar or slightly different digestibility coefficients of the amino acids for these two types of SBM and none of them takes into account the origin and processing conditions applied to the soybeans during the production of the SBM. However, recent data (Fickler, 2005; de Coca-Sinova *et al.*, 2008; Valencia *et al.*, 2008) indicate that the amino acids profile and quality of commercial batches of SBM are variable and that the digestibility of the nutrients might not be as uniform and predictable as is generally accepted. In fact, van Kempen *et al.* (2006) reported that the chemical composition of SBM varies widely according to the variety and the origin of the beans, an observation that might have important implications in practical feed formulation.

v. Fish meal: Fish-meal is one of the best poultry feedstuffs as a source of animal protein. Its composition varies widely depending upon whether it is made from whole bony fish or fish cannery scraps. It is generally manufactured from wild-caught, small marine fish that contain a high percentage of bones and oil, and usually deemed not suitable for direct human consumption. The fish caught for fishmeal purposes solely are

termed "industrial". Most tropical fish-meals contain 45 to 55% protein. The presence of fish scales reduces its feeding value.

vi. Oyster-shell/Limestone: Limestone is a source of calcium as well as oyster shell that contains more than 38% calcium. Calcium source remains the main concern of farms since related to egg quality is egg shell quality. After the egg shell is formed, it cannot be remade, and egg shell defects will result in product down grading due to the presence of cracks, deformities and irregularities, with consequent significant economic losses (Ito *et al.*, 2006). Kussakawa *et al.* (1998) mentioned that, in order to be marketed, the egg shell of eggs must be strong enough to withstand the vagaries of lay, collection, grading and transportation until they reach the final consumer. However, according to Leeson and Summers (2005), approximately 7 to 8% of the eggs produced present some kind of egg shell damage caused by different reasons that directly affects egg marketing. Moreover, eggshell integrity is essential to the preservation of internal egg quality. Calcium is an essential mineral for egg shell quality, and it is the main component of the egg shell, which consists of 95% calcium carbonate (Miles, 1993). Adequate calcium supplementation to layer diets is therefore critical, as its deficiency may cause reductions in egg size and production, poor egg quality, with consequent high percentage of broken eggs and increased layer mortality (Dell'Isola and Baião 2001; Geraldo *et al.*, 2006).

B. Non-conventional feedstuffs

These are feedstuffs that have not been traditionally used in animal feeding and/or are not normally used in commercially produced rations for animals (Umesh *et al.*, 2014). A common feature about feeds is that the traditional feeds of tropical origin tend to be mainly of annual crops, animals and industrial origin. In this sense, the non-conventional

feedstuffs term could really be more appropriately referred to as “new feeds” (Amata, 2014) and this term is increasingly being used.

Poultry and livestock production may continue to be ulcerative if costly conventional feedstuffs are not replaced with cheaper and non-conventional available feedstuffs (Dauda *et al.*, 2009). However, there are limitations to their use, since most non-conventional feedstuffs are compromised by their low protein, anti-nutritional factors and high fiber (the non-starch polysaccharides) content coupled with high levels of anti-nutrients (Iyayi *et al.*, 1997).

A large number of agro-industrial by-products, some common tropical browse plants, leaf meals and animal wastes (Amata, 2014) which have been identified, processed and used for feeding of farm animals are designated as a group of unconventional or non-conventional feeds; however, in some countries such as India and Pakistan, what may now be classified as unconventional may in fact be conventional/traditional owing to the fact that it may have been in use as livestock feed over a long time. An example is wheat straw which is very widely used in these two countries. In addition, the availability of unconventional feedstuffs, especially of plant origin, is dependent to a large extent on the type of crops being cultivated and the prevailing degree of application of the crop technology (Younas and Yaqoob, 2005). Many non-conventional feedstuffs are available to livestock producers in West Africa and in particular Nigeria, but due to traditional practices or poor understanding of their value/limitations, lack of adequate nutritional information, other uses, or presence of some deleterious constituents (Yakubu, 1992) like alkaloids, toxic amino acids, phenolic compounds, tannins, trypsin inhibitors, carcinogens, glucosinolates etc usage can be and often is limited (Devendra, 1983).

In the near future, the variety and quantity of by-products and edible wastes are expected to increase, and disposal options for many of these wastes, such as landfills, will become more limited and costly. The utilization of non-conventional feed ingredients is gaining ground daily in Nigeria, for instance, Adeyemo and Oyejola (2004) reported that poultry droppings could be used to replace blood meal in guinea fowl diet up to 40% dietary level without any adverse effect on performance. Thus, the role of livestock in recycling and "adding value" to many of these by-products and wastes will become increasingly important as a viable waste management option which is being advanced as a method for preventing environmental decay and increasing food supplies (Joglekar *et al.*, 1983; SWFN/CAN/BA/NRC, 1983; Duru and Uma, 2003).

Animal nutritionist are continually making efforts to source cheaper and readily available unconventional feed materials that will replace or substitute the conventional feedstuff, in order to significantly reduce the cost of animal production (Okah, 2004). However, cost is not the only factor to be considered, since variations in processing methods, availability and feed form (pellet, meal, wet or dry) can also impact the value of these feeds (Gomez, 1982).

Examples of non-conventional feedstuffs include discarded biscuits, bakery waste, rice bran, blood meal, corn cob, maize bran, cassava peel, cassava chips and copra cake. Others are oil palm slurry, groundnut skins; brewer's spent grains, molasses, sugar beet pulp, citrus pulp, yeast and wheat bran among many others that are location specific. Feed costs and animal competition with humans for feed items suggests strongly that alternative energy sources such as residues of crop harvest should be used partially or wholly to replace maize in livestock diet to reduce cost of meat production and to make available the major crops for human consumption (Ngou and Mafeni, 1983). Data in table

2.2 highlights some of the non-conventional feedstuffs used for feeding poultry and their nutrient composition.

2.3.2 Classification of feedstuff according to nutritional composition values

Monogastric livestock feeds provide the basic nutrients required for animal (poultry) production, including energy, proteins/amino acids (macro-nutrients), and minerals, vitamins and other micro-nutrients. Feeds may be broadly classified as concentrates and roughages, depending on their composition as shown in figure I.

a. Roughages are feeds with low density of nutrients, usually having crude fibre content above 18% on DM basis. These include agro/industrial by-products most fresh and dried forages and fodders. Definitions of these feeds and their nutrient contents vary in literature (FAO, 1983). By-products from sugar industry molasses is an important by-product of the sugar industry. Its production of molasses is dependent on the production of cane or beet sugar. Molasses has been used for many years as a cheap source of energy in the ration of farm animals. Molasses contain about 3.25% crude protein. It has been included in animal feeds at the levels of 5 - 10%. These levels are mostly used as (i) a carrier for urea impregnation of poor quality roughages (ii) a binder for commercial pelleted feeds for the convenient and economic feeding of livestock and as (iii) a sweetener for increasing the voluntary intake of compounded feeds.

b. Concentrates are feeds that contain high density of nutrients, usually low in crude fibre content (less than 18% on dry matter (DM) basis and high in total digestible nutrients (FAO, 1983). Concentrates may be fed in raw or milled forms as individual feeds (sometimes referred to as straights), or may be blended or formulated into balanced rations for particular production purposes (compound feeds) and are manufactured by

Table 2.2: Composition of some non-conventional feedstuffs

<i>Feedstuffs</i>	<i>CP %</i>	<i>EE %</i>	<i>CF %</i>	<i>Cal ME (Kcal/kg DM)</i>
C F chaff	1.4	1.1	10.2	3436
C P meal	2.2	1.1	4.3	2460
Cowpea T	17.0	2.6	20.3	1005
Melon pulp	8.6	43	31.1	1148
C M sieving	0.8	1.5	9.0	1787
Soybean T	16.6	4.0	25.4	2096
Plantain pulp	4.1	0.6	0.1	1004
Yam peel M	6.4	5.0	7.3	136
F by-product	44.3	29.1	0.0	5055 □
Neem leaves	17.5	4.2	12.3	752
Dry rumen D	11.53	8.79	15.30	-----

Source: (Sonaiya 1990; Okata, 2007)

* The energy value for the fish products is gross energy. CP = Crude Protein, ME = Metabolizable Energy

EE = Ether Extract, CF = Crude Fiber, CF chaff = Cassava Fermented Chaff, CP meal = Cassava Peel Meal, Cowpea T = cowpea testa, C M sieving = Cassava Meal Sieving, Soybean T = Soybean Testa, Yam peel M = Yam Peel Meal, F by-product = Fish by-product and Dry rumen D = Dry Rumen Digesta.

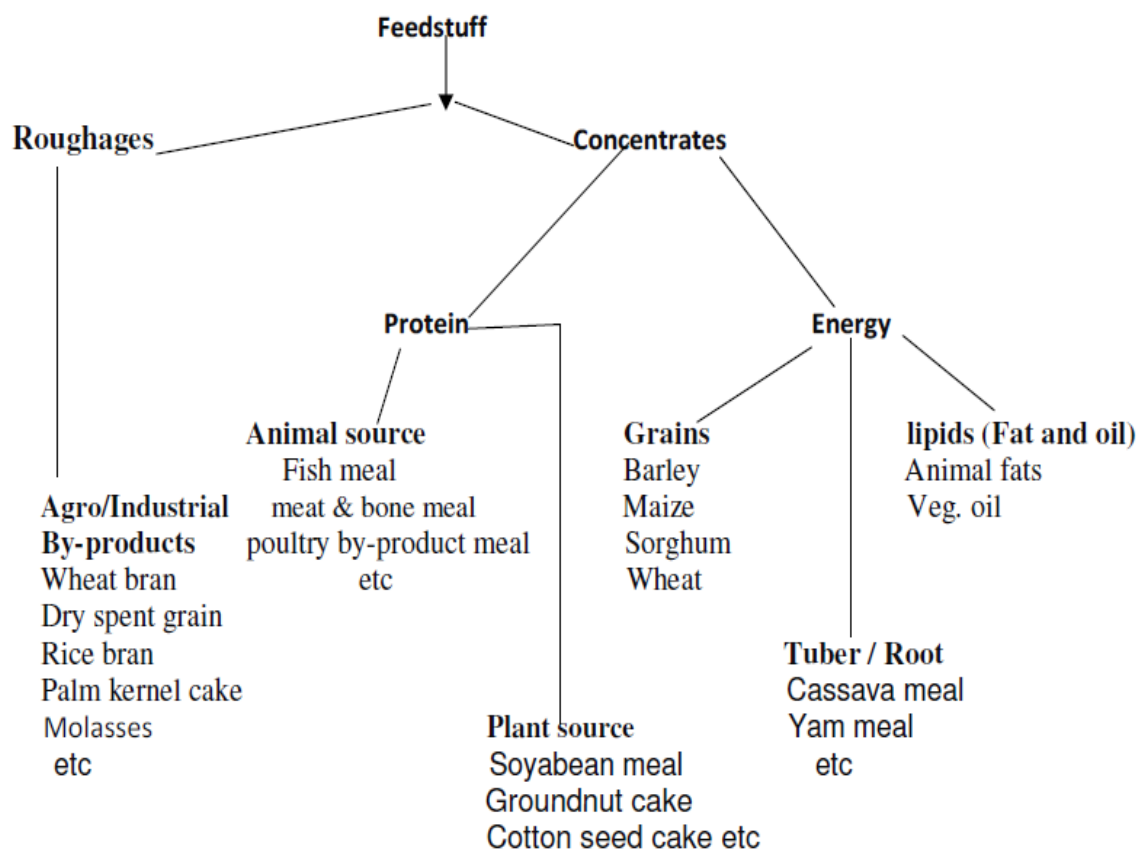


Fig. 1: Classification of poultry feedstuff according to nutritional value

Source: (Leeson, 2005)

feed compounders as meal type, pellets or crumbles (Wondra, 1995). The main factors influencing the nutritive value of a diet are the ingredients employed and their chemical composition (McDonald, 2002).

i. Proteins are complex organic compounds of high molecular weight. As with carbohydrates and fats, proteins contain carbon, hydrogen and oxygen, but in addition they all contain nitrogen and generally sulphur (McDonald, 2002). Feedstuffs with over 20% crude proteins are referred to as protein concentrates. Proteins (also known as polypeptides) are made of amino acids arranged in a linear chain and folded into a globular form. Amino acids are produced when enzymes, acids or alkalis hydrolyze proteins. Although over 200 amino acids have been isolated from biological materials, only 20 of these are commonly found as components of proteins. Dietary requirements for protein are actually requirements for the amino acids contained in the dietary protein (NRC, 1994).

Crude protein contains both true protein and other nitrogenous products (non-protein nitrogen), but only the true protein portion is able to be digested by animals. However, contrarily to monogastrics, ruminants are able to convert non-protein nitrogen to true protein through the activities of rumen bacteria. Also, in the case of ruminants, all the essential amino acids can be synthesized by the rumen microorganisms, which theoretically make this class of animals independent of a dietary source once the rumen microorganisms have become established (Coffey, 2008). However, maximum rates of growth or milk production cannot be achieved in the absence of supply of dietary amino acids in a suitable form (McDonald, 2002). Non-ruminant animals cannot synthesize the essential amino acids fast enough to meet the animals needs, therefore those essential amino acids must be provided in the ration (Merchen and Titgemeyer, 1992).

The main functions of proteins are in cellular metabolic activities which make them to be continuously needed to replace dying body cells and to supply materials for building body tissues such as ligaments, hair, hooves, skin, organs, and muscle are partially formed by protein. Thus, proteins have an important role as basic structural unit, and are also needed for metabolism, hormone, antibody and DNA production. When proteins are fed in excess, they are converted to energy and fat. Protein concentrates are of two types based on their origins that is plant and animal origin.

Animal proteins are considered good-quality proteins since they contain a good balance of essential amino acids. Animal by-product originate from the slaughter houses for large animals and poultry and fish processing factories. It includes such products as: meat and bone, offals, blood, bone, intestines, poultry heads and feet, fat, feathers, horns, hooves, animal hair, stomach, rumen content and the carcasses of animals disqualified by the Veterinary Services. Dairy by-products not utilized for food production (whey, casein, butter milk) as well as tannery by-products are included in this group. Animal and poultry manure, which are currently used as feed ingredients, can also be included in this group (Kazimerz, 2003).

Plant proteins are thought to be poor-quality proteins because they lack some amino acids. Monogastric animals need balanced ration with the right balance of essential amino acids. If grains are combined in the correct combination they will provide a balanced ration. Soybean meal is most commonly used. High protein feeds therefore include oilseed meals, animal protein feeds, and grain protein feeds.

ii) Carbohydrates are chemical compounds essentially of carbon, hydrogen and oxygen, made up of sugars, starches, cellulose and lignin, which are referred to as energy

concentrates. The main functions of this nutrient are as energy that powers the muscular movement, as a source for body heat and as building block for other nutrients. Its dietary excess is stored as fat. Simple carbohydrates (sugar and starches) are referred to as nitrogen free extract (NFE) and are mostly present in cereal grains such as maize, wheat, sorghum, barley and oats. Total concentrates fed to livestock are comprised of feed grains fed and by- product feeds fed. Crude fiber (cellulose mainly) is another components of carbohydrates.

Tubers/Roots are plants yielding starchy roots, tubers, rhizomes, corms and stems. They are used mainly for human food as such or in processed form for animal feed, manufacturing of starch, alcohol and fermented beverages including beer. A tuber is an enlarged storage tips of a rhizome or an underground stem with leaves reduced to scales or scars subtending the auxiliary buds with edible modified stems, while root crops are plants with edible modified roots (Kawakami, 1978). Certain root crops, notably bitter cassava, contain toxic substances, as a result, certain processes must be undertaken to make the product safe for animal feed. Apart from their high water content (70 – 80%), these crops contain mainly carbohydrates (largely starches that account for 16 - 24% of their total weight) with very little protein and fat (0 - 2% each) (OSU, 2010).

iii. Fats and oil are lipids which are chemically defined as neutral chemical compounds essentially of carbon, hydrogen and oxygen, but contain more carbon and hydrogen atoms than carbohydrates for this reason fats have 2.25 times as much energy value than carbohydrates (McDonald *et al.*, 2002). They are insoluble in water but soluble in alcohol, ether and chloroform. Lipids are easily stored in the body, with their main functions being source of fuel (stored at higher conc./g than carbohydrates), source of heat, insulation, body protection (cushioning), carrier of fat-soluble vitamins (A, D, E,

K). In addition, added fat minimizes dust in a mixed or pelleted feed and has immune functions through essential fatty acids (Leeson and Summers, 2001) and are important constituents of the structure of cells. At room temperature fats are solids, oils are liquid, and oil is usually derived from plant, while fat is derived from animals. Sources of oil include soybean oil, corn oil and fish oil, while product fats include lard or tallow from livestock rendering. Lipids essentially provide cheap energy source, reduce dust in feed manufacturing and increase feed palatability.

2.4 Factors Influencing the Use of Unconventional Feedstuffs in Livestock Feed Formulation

2.4.1 Nutrient composition and nutrient availability

Unconventional feedstuffs can be highly variable in quality and nutrient composition due to often; there are no quality control processes in place. More so, there can be considerable variations in the soil type on which the crops grew, for example, type of fertilizer application (Enujeke, 2013) as well as plant-to-plant variations in processing methods that will lead to inconsistency in by-products sourced from different companies (Iyayi and Davies, 2005).

Nutrient content analysis is therefore a good management practice, in particular for unusual feedstuffs that may be prone to large variation. Feedstuff composition tables (NRC, 1982; NRC, 1996; NRC, 1998) can be used but the composition tables are based only on averages and information on hand at the time of their publication. In addition, nutrient compositions of some feedstuffs have changed over time due to changes in the raw ingredients and (or) changes in processing methods. The nutrient information needed also depends on the type and class of livestock being fed.

2.4.2 Potential health hazards free

Many unconventional feedstuffs may contain toxic substances such as mycotoxins from molds, high sulfur levels and high nitrates (Amata, 2014), disease organisms and/or anti-nutritional factors (Iyayi *et al.*, 2006; Omede, 2008). If toxic substances are present, the unconventional feedstuff should not be considered unless the deleterious factor(s) like high amounts of anti-nutritional components can be eliminated or neutralized inexpensively. Many commercial feed analysis laboratories can screen for mycotoxins such as aflatoxins and for potentially harmful bacteria, and can analyze for sulfur content and heavy metals.

2.4.3 Gross composition and quality

A visual gross appraisal of the potential alternative feedstuff should be done prior to delivery for identification purposes and to ensure consistency of composition. The Association of American Feed Control Officials (AAFCO, 2004) official publication gives detailed descriptions and nomenclature of many feedstuffs. Proper identification of the feedstuff is important as there is much information already published on many potential unconventional feedstuffs. This information could be useful in initial assessments of whether or not one may use an alternative feedstuff.

2.4.4 Effect on end-products

The alternative feedstuff when included in the diet should not harm the end product, for instance, affecting the taste and quality of the meat or compromise food safety. For example, a feedstuff high in unsaturated fat (i.e., peanut kernels) when fed at a high level in the diet to pigs can result in carcasses with soft, oily fat (Amata, 2014).

2.4.5 Special handling, processing and storage requirements

Many alternative feedstuffs may require special transport, handling, storage, dehydration (due to high moisture content) and detoxification processes, mixing and feeding compared to traditional feedstuffs (Amata, 2014). There is an urgent need for processing techniques that are economic and practicable. These additional requirements may inhibit the use of the alternative feedstuff due to the cost, or the lack of special equipment to store and process them.

2.5 Dry brewers' spent grain (DBSG)

Beer is the fifth most consumed beverage in the world apart from tea, carbonates, milk and coffee with estimated annual world production exceeding 1.34 billion hectolitres in 2002 (Fillaudeau *et al.*, 2006). In the manufacture of beer, various residues and by-products are generated. The most common ones are spent grains, spent hops and surplus yeast, which are generated from the main raw materials (Mussatto, 2009). Brewers spent grains (BSG) are the solid remains from the wort preparation process and consist of a complex mixture of maize grit, sorghum spent grains and malted sorghum spent grain, pericarp and fragments of endosperm (Forssell *et al.*, 2008). In Nigeria, due to the banning of the importation of malted barley into the country in 1988 by the federal government, Nigerian brewers use local sorghum and maize (Uchegbu, 1995).

These cereals could be used individually or as a mixture in an appropriate proportion. Mostly, Nigerian brewers (Adewusi and Ilori, 1994) use a mixture consisting of 77% sorghum and 23% maize. For cell immobilization itself, the grain husks are used once and they are composed mainly of a rigid matrix of 30% cellulose, 34.9% hemicellulose and 17.7% lignin (Palmarola-adrados *et al.*, 2005). Continuous beer fermentation is based on a high-density yeast population and mashing process; which is one of the initial

operations in brewery in order to solubilize the malt, unmalted cereals and other starchy products, after the cereals have been wet-mashed to extract the malted sugars for the various brewery products from cereal grains to ensure adequate extraction of the wort (water with extracted matter) (Fillaudeau *et al.*, 2006). Following different separation strategies, the amount of brewers' spent grain (BSG) generated could be about 85% of the total by-products (Reinold, 1997; Mussatto *et al.*, 2006; Tang *et al.*, 2009). However, according to Townsley (1979), spent grain accounts on average, for 31% of the original malt weight, representing approximately 20 kg per 100L of beer produced (Reinold, 1997), which accounts for 30 to 60% of the biochemical oxygen demand (BOD) and suspended solids generated by a typical brewery. The schematic flow chat of BSG production is shown in figure II.

Thus, BSG is a readily available, high volume low cost by-product of brewing and is a potentially valuable resource for industrial exploitation (Robertson *et al.*, 2010). Because of its potential as a usable waste (Aliyu and Bala, 2011), the fact that it is used in Nigeria as animal feedstuff, therefore is a recent trend among animal nutritionist to use it as a non-conventional feedstuffs in order to cut down the cost of feed (Ojeniyi *et al.*, 2010). However, this use is limited, especially in the feeding of monogastric animals mostly due to high crude fibre and moisture content of about 80%, which increases its bulkiness. It therefore needs to be dried before incorporating into poultry rations (Couch, 1978). Sun-drying is the most common method used and this method requires large space and large polythene sheets or concrete floors for drying.

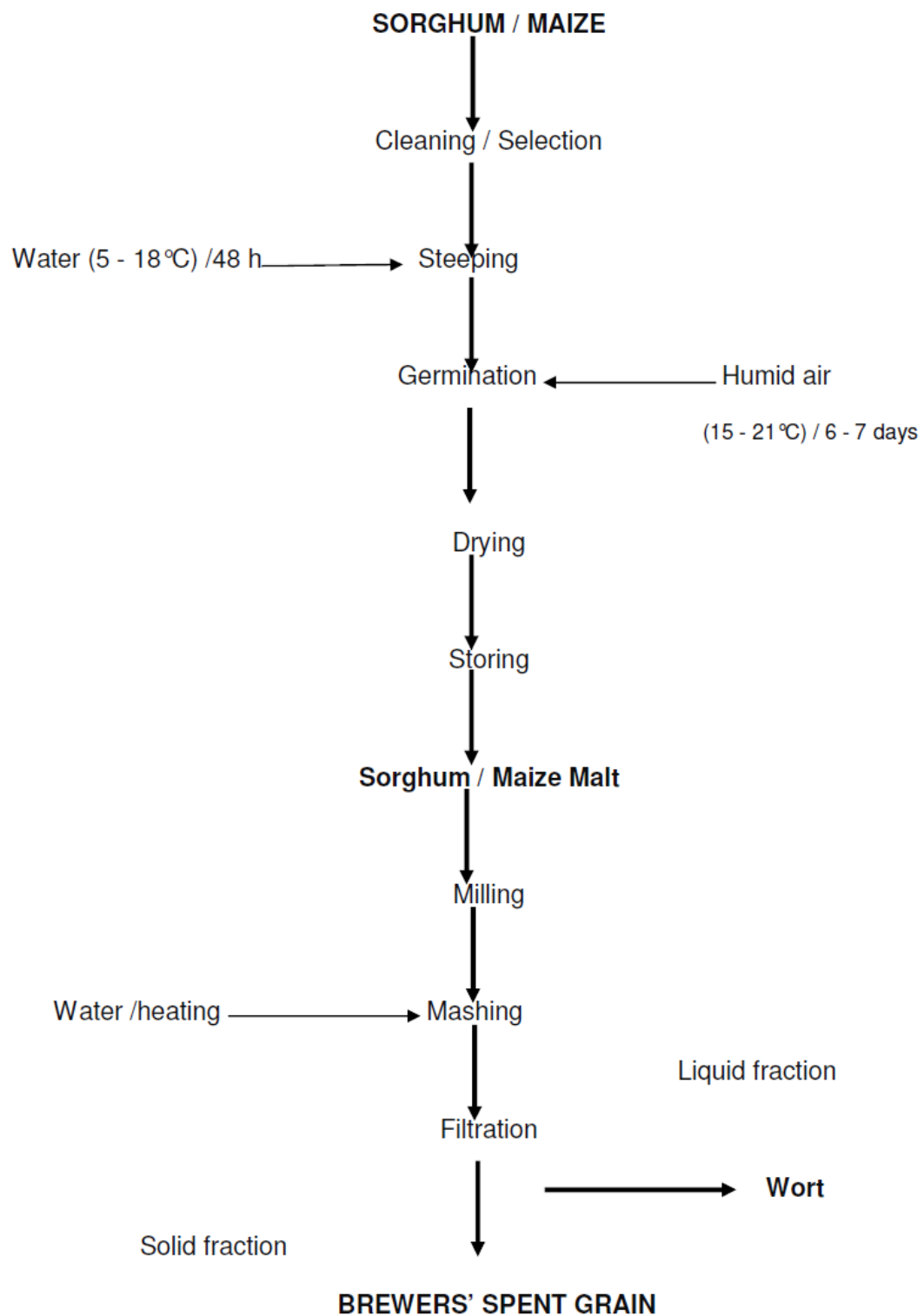


Fig. II: Schematic flowchart of the process to obtain BSG from natural Sorghum / Maize
(Source: Blezinger, 2003; Mussatto *et al.*, 2006).

2.5.1 Nutritive quality of BSG for livestock feeding

The concentration of nutrients in the feedstuff, the efficiency with which absorbed nutrients are used, the proportion of the nutrients digested and the amount eaten are commonly used to assess nutritive value of feedstuffs (Topps, 1989). Several attempts have been made to utilize BSG in animal feeds, production of value-added compounds such as xylitol, lactic acid, among other microorganisms cultivation, or simply as raw material for extraction of compounds such as sugars, proteins, acids and antioxidants (Aliyu and Bala, 2011).

Its nutrient contents vary from one brewing plant to another depending on the type of grain used (sorghum, wheat, corn etc), harvest time, soil / fertilizer type (Enujeke, 2013), extent of fermentation and type of fermentative process (Robertson *et al.*, 2010). Brewers' yeast has been successfully used as a replacer for soya in diets of growing and fattening pigs and sows (Yaakugh *et al.*, 1994; Wlcek *et al.*, 2004), while dry brewers spent grain has been successfully incorporated in broiler finisher ration at the ratio of 1:1 for growth performance of broilers (Ironkwe and Bamgbose, 2012).

Brewer's dried grain is an excellent source of quality by-pass protein. It contains essential amino acids which has made it to become widely used as animal feed (Salama *et al.*, 1995; Wang *et al.*, 2001). It also contains digestible fibre and is rich in essential amino acids (0.9% lysine, 0.4% methionine, 0.4% tryptophane, 1.2% phenylalanine, 1.1% threonine and 1.6% valine). It is therefore higher in protein and amino acids than corn (Almquist, 1972) and has high mineral and B-vitamin content. Specifically, brewer's dried grain has been reported to have better available protein, energy and ash composition than maize and wheat offal (Aletor, 1986; Babatunde, 1989). For example, birds fed 100% BDG replacement of maize have better growth performance than birds on control

diets. Longe and Adetola (1983) and Faniyi (2002) have all recommended the inclusion of about 50% and above of brewer's spent grain in broiler finisher diet. Although BSG can be fed to poultry, its high fibre content and reduced protein digestibility however tend to decrease its nutritional value and metabolizable energy compared to the original grain (Onifade and Babatunde, 1998). As a result, brewers' spent grains are not well suited to the feeding of poultry with high energy requirements such as young broilers. They are better tolerated by older broilers and laying hens.

2.5.2 Protein and amino acids content

Essential and non-essential amino acids of dry brewers spent grain amino profiles are shown in table 2.3. Thus, brewery spent grains have been utilized as feed for animals for many years (Szponar *et al.*, 2003); the presence of fairly amount of nutrient substances among all such as sugars and amino acids aid in its utilization as feed for livestock (Bisaria *et al.*, 1997). In bioconversion of organic wastes, the protein constituent of the complex substrate is usually broken down into amino acids by the proteolytic activity of the degraders (Singh *et al.*, 1988; Barimalaa *et al.*, 1994).

DBSG has been shown to contain 17 amino acids on analysis. These include varied concentrations essential amino acids such as lysine, methionine, histidine, phenylalanine, valine, isoleucine, leucine, tryptophan and threonine as well as non-essential amino acids such as serine, glycine, alanine, cysteine, proline, aspartic acid, glutamic acid and arginine. Of these, the most important in poultry nutrition are cysteine, lysine and methionine (Portsmouth, 1978; Ranjhan *et al.*, 1974). Other amino acids necessary in poultry feeding also detected in the fermented BSG are histidine, arginine, asparagine, glycine, valine, isoleucine leucine, and phenylalanine. Their concentrations are fairly higher than the values recommended for poultry nutrition (Essien and Udotong, 2008),

while that of Methionine is lower than the recommended values for poultry. The percent dry matter values among essential and non-essential amino acids (lysine, histidine, valine, methionine, isoleucine, leucine, phenylalanine and arginine, aspartic acid, glutamic acid, proline, glycine, alanine, cystine) respectively were found to increase as reported by (Onyimba *et al.*, 2014).

2.5.3 Other benefits of BSG

The ingestion of BSG or its derived products provides some health benefits, since dietary fiber has been generally reported to affect some non-infectious diseases (Prentice *et al.*, 1978). Also, incorporation of BSG into monogastric diets is beneficial for intestinal digestion, alleviating both constipation and diarrhea. Such effects were attributed to the content of glutamine-rich protein, and to the high content of non-cellulosic polysaccharides and smaller amounts of β -glucans (Tang *et al.*, 2009). More so, brewery by-products contain increased or high crude protein concentrations: brewer's grains 28 - 30%, brewer's yeast 50 %, brewer's spent grain with hot sludge 50-60%, press liquor 40% and protein sludge from press liquor 50 - 60%. These are palatable and readily consumed when in good condition and are quite rich in protein (27 - 33% DM) (Robertson *et al.*, 2010), which makes them valuable sources of protein. The protein value can be affected by the heat applied during the brewing process, which can be beneficial to ruminants but tend to be detrimental to monogastric animals. However, it is usually fed to poultry in dried form, as it is easier to store and more stable than the wet form (Onifade and Babatund, 1998).

Table 2.3: Amino acids profile of dry brewers spent grain

(a) Essential amino acids % of dry brewers spent grain

<i>Amino acid</i>	<i>* Poultry requirement</i>	<i>Dry brewers spent grain (%)</i>
Valine	0.8 - 1.0	1.0
Methionine	0.7 - 0.8	0.2
Isoleucine	0.8 - 0.8	0.8
Leucine	1.4 - 1.6	2.7
Phenylalanine	0.7 - 0.8	1.5
Histidine	0.4 - 1.4	0.4
Lysine	1.1 - 1.2	1.5
Threonine		2.0

(b) Non-essential amino acids % of Dry brewers spent grain

Arginine	1.2 - 1.4	1.6
Serine		1.6
Glutamic acid		2.8
Proline		1.0
Glycine	1.0 - 1.1	1.1
Alanine		2.0
Cystine	0.3 - 0.4	0.3
Aspartic acid	0.8 - 0.9	3.5

(Onyimba *et al.*, 2014; Portsmouth, 1978)

a. Culturing for microorganisms

The common characteristic of the brewery by-products such as BSG, which includes for high moisture content of about 80%, protein and polysaccharide contents make it particularly susceptible to microbial growth and degradation through bacterial and fungal actions (Asurmendi *et al.*, 2013). The presence of resident microflora initiates these processes within the shortest time, in an attempt to utilize it as sole carbon source (Robertson *et al.*, 2010).

BSG been used to cultivate *Bifidobacterium adolescentis* 94BIM, *Lactobacillus* sp. (Novik *et al.*, 2007), actinobacteria, especially *Streptomyces* (Szponar *et al.*, 2003), *Pleurotus ostreatus* (Gregori *et al.*, 2008), *Penicillium janczewskii* (Terrasan *et al.*, 2010), and *Penicillium brasilianum* (Panagiotou *et al.*, 2006) among others. Thus, BSG is recommended as a suitable medium for isolation and maintenance of unknown strains and is therefore highly suitable for screening and production of new biologically active substances and fast spores production (Szponar *et al.*, 2003).

b. Enzyme synthesis

BSG is rich in hemicellulose (30 to 35%) (Russ *et al.*, 2005), its components constitute 1, 4- β linked xylose backbone with a heterogeneous substituents such as Larabinose, O-acetyl, ferulic acid, p-coumaric acid and 4-methylglucuronic acid (Panagiotou *et al.*, 2006). Thus, BSG has been effectively used as a carbon source for feruloyl esterase and xylanolytic enzyme production by *Talaromyces stipitatus* as well as *Humicola grisea* var. *thermoidea* and *Penicillium janczewskii*, respectively (Mandalari *et al.*, 2008; Terrasan *et al.*, 2010). *Streptomyces avermitilis* CECT 3339 also produces feruloyl esterase and (1 \rightarrow 4)- β -D-xylan xilanohydrolase, while growing on BSG (Bartolomè *et al.*, 2003). Thus, microorganisms growth on BSG produce a number of enzymes that aid in its utilization

such as endoxylanases, β -xylosidases, α - arabinofuranosidases and esterases (Mandalari *et al.*, 2008). However, the substrate composition as well as the strain of organism used determines the enzyme type and activity. The presence of digestible and non-digestible organic residues makes BSG, a potential substrates on which amylolytic organisms could be cultured for the production of β -amylase and amyloglucosidase (Adeniran *et al.*, 2008). Other enzymes of interest include xylanases, feruloyl esterases and α -L-arabinofuranosidases.

c. Lactic acid production

Lactic acid (2-hydroxy propanoic acid) has been commonly applied in foods, the chemical industries, fermentations, and pharmaceuticals (Ali *et al.*, 2009). Recently, however, there has been an increasing interest in lactic acid production because it can be used as a precursor of poly-lactic acid (PLA) production. However, the realization of this potential is dependent on whether lactic acid could be produced at a low cost form competitive on a global scale (Bai *et al.*, 2008).

The major challenge in commercial production of latic acid is use of expensive carbon sources such as glucose, sucrose or starch. The use of such raw materials is not economical because lactic acid is a relatively cheap product. Thus, exploitation of cheap raw material sources would be beneficial. Agro-industrial residues like BSG are attractive alternatives to substitute these costly raw materials (Mussatto *et al.*, 2007a, 2008b).

2.6 Cassava Meal (CM)

Cassava (*Manihot esculenta* Crantz), is one of the non-conventional energy sources in livestock production mostly in tropics (Tada *et al.*, 2004). It outstrips maize in terms of starch content per unit mass (Teles *et al.*, 1995). Cassava is mainly cultivated because of

its starchy storage roots and it is considered one of the most important staple foods in the tropical countries (Lebot, 2009; Ecocrop, 2011). Cassava, also known as cassava, manihoc, tapioca, Brazilian arrowroot or yuca originated from tropical America and was first introduced to the Congo basin, Africa by the Portuguese around 1558 (Akoroda and Ikpi, 1992). Its tuber is the most productive crop in terms of energy yield per unit land area (Coursey and Haynes, 1970; Oke, 1978; Okezie and Kosilowsld, 1982; Ravindran, and Blair, 1991). It represents the fourth largest source of energy following rice and maize in the tropics, besides being a very important animal feed and industrial raw material (Cardoso and Souza, 2002).

African cassava production surpassed 145 million tonne in 2011; approximately 57% of the global crops that year of 256 million tone (FAOStat, 2013). Nigeria alone contributed 36% of all African production, which is approximately 52 million tonne (FAOStat, 2013). In contrast to Latin America (about 14% of global production, mainly from Brazil) and Southeast Asia (about 32% of global production from Thailand and Indonesia), where the majority of cassava is exported for industrial purposes or animal feed, about 70 to 80% of cassava produced in Nigeria is utilized for human consumption (Dada *et al.*, 2010).

Inspite of these important agricultural and nutritional role played by cassava, its food value is greatly compromised by the presence of endogenous cyanogenic glycosides, especially linamarin and lautrastralin, which under several prevailing tropical conditions are readily hydrolysed to liberate hydrogen cyanide (Esonu, 2006; Udedibie *et al.*, 2008).

Several processing methods such as ensiling and drying have been tried and found to be effective in reducing the cyanogenic glycoside content of cassava products (Phuc *et al.*,

2000; Enyenihi *et al.*, 2009; Udedibie *et al.*, 2009). Udedibie *et al.* (2008) and Enyenihi *et al.* (2009) showed that sun drying of cassava tubers could not completely eliminate hydrogen cyanide from it, however a mixture of cassava tuber, BSG and palm oil was able to reduce HCN to about 25 ppm level in cassava products (Udedibie *et al.*, 2012). Aladi *et al.* (2013) also subjected mixtures of cassava and palm kernel cake to solid state fermentation using *Aspergillus niger* as fungal inoculants and reported excellent products with enhanced crude protein and amino acid values. Okoli *et al.* (2013) recently studied the physiochemical and hydrogen cyanide content of three processed cassava products used for feeding poultry in Nigeria and concluded that they are excellent energy sources for monogastric animal feeding.

2.6.1 Cassava processing for livestock feeding

The general schematic flow chart of the process to obtain cassava meal for livestock feed is shown in figure III. Washing is usually the first step, followed by peeling. The roots are then sliced, either by hand or mechanically. Cassava chips may have different sizes and shapes (rectangular, cube, thick slice) depending on the slicing and drying methods. Drying may be natural or artificial. Sun-drying is done on concrete floors or on trays. Sun-drying is a very labour intensive operation, requiring about 35 – 40 laborers/ha of drying floor. Chips dried on trays are better-looking and more uniformly dried than those dried on concrete floors. Artificial drying is done using static or moving bed dryers, or rotary dryers. Cassava chips can be sold directly, ground into cassava meal, or pelletized (Tewe and Egbunike, 1988). During pelletizing, chips are heated, moistened and then forced into continuous die presses. Pelletizing may result in a product that is 25 – 40% denser, more uniform, more durable, less dusty and easier to handle (Hahn *et al.*, 1992).

Peeling operations require time. Alternative methods to produce chips and pellets without peeling have been developed. One such method consists in grating and chopping unpeeled tubers, mixing them with cassava foliage in a 4:1 ratio and passing the mixture through a pelletizer (Tewe, 2004; Ogbuokiri *et al.*, 2014). In humid places where sun-drying is not easy, cassava roots can be ensiled alone (clean cassava roots + 0.5% salt) or in mixture with rice straw or cassava leaves (Premkumar *et al.*, 2001; Ngoan *et al.*, 2002; Kavana *et al.*, 2005). Cassava peels represent 5 –15% of the cassava tuber weight; which is obtained after water-cleaning and peeling. Solid fibrous residue remaining after starch extracted from root make up to 17% of the tuber. Quality and appearance varies with age, time after harvest, and industrial equipment used in processing the tubers.

2.6.2 Nutritive value of cassava meal for livestock feeding

It is widely known that cassava root products are rich in carbohydrates but low in protein, amino acids and all other nutrients and thus are used mainly as sources of energy. For example, proximate values of processed cassava products from Nigeria were reported as crude protein (2.56 – 2.90%); Nitrogen free extract (78.50 – 84.23%) and metabolizable energy (2842.23 – 3142.71 MJ/kg) among others (Okoli *et al.*, 2012). In using cassava root products as cereal substitutes therefore, approximately 15 to 20% extra protein source is needed (Khajarn *et al.*, 1982). Cassava therefore has potential to serve as a primary feed ingredient in livestock feeding programs due to its nutrient composition, and this could help reduce the competition between livestock, particularly poultry and man for maize.

The nutrient constituent of cassava by-product can be affected by factors such as variety, rootstock, growing conditions, maturity, climate and processing/ handling. Cassava Leaf Protein Concentrate (CLPC) was evaluated as equi-protein replacement of fish meal

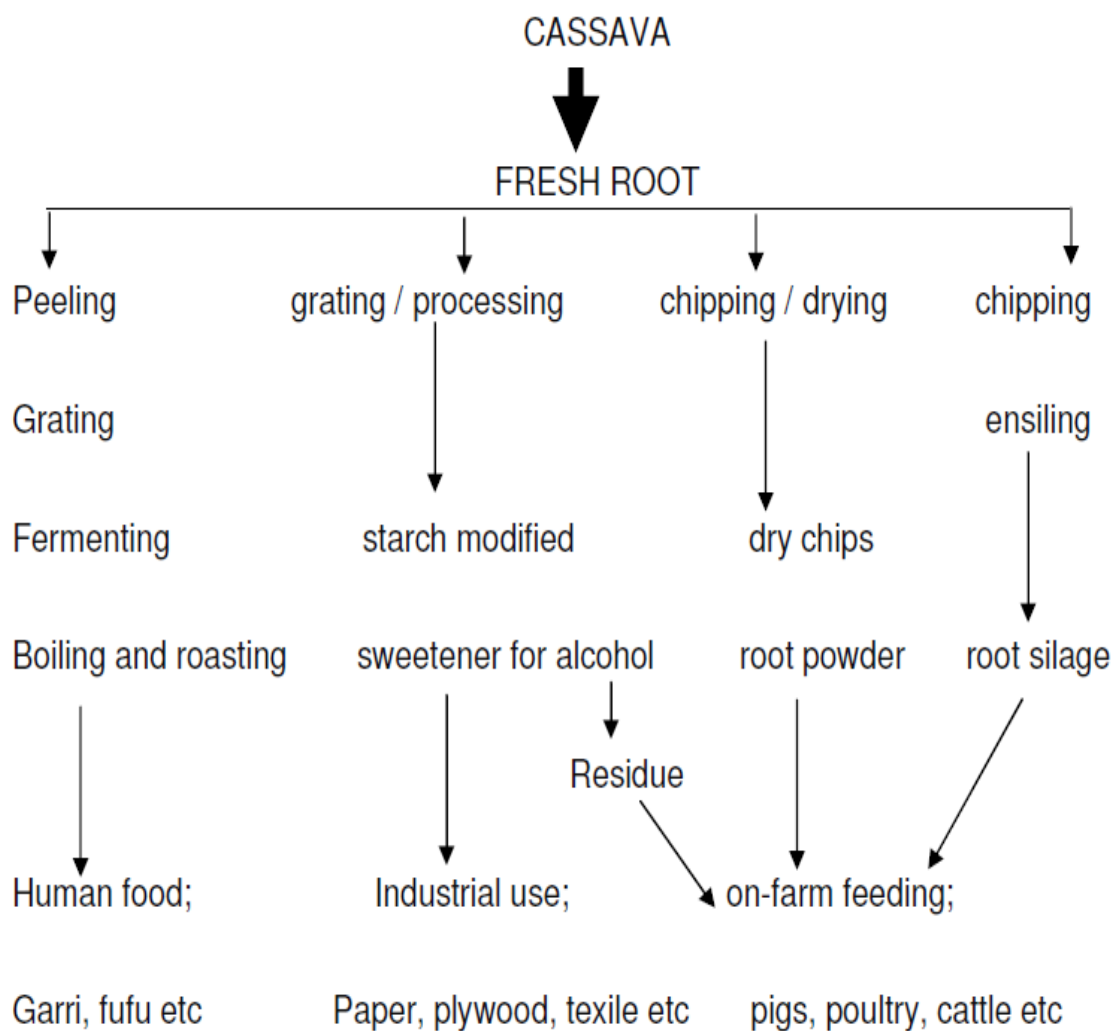


Fig. III: Schematic flowchart of the process to obtain CM for livestock feed

(Ukachukwu, 2005; Howeler, 2003; Boscolo *et al.*, 2002a; 2002b; Nwokoro *et al.*, 2005; Scapinello *et al.*, 2005)

protein in layer diets by (Fasuyi, 2006) with value of crude protein 29.0% (Madalla, 2008) which is better when compared with the tuber. Feeding trials with ruminants resulting in little reduction in performance when 100% cassava replaced maize became a window opportunity for non-ruminant feeding trial particularly poultry (Gohl, 1991). Thus, cassava has been shown to outstrip maize in terms of starch content per unit mass (Teles *et al.*, 1995) and starch content of about 60 – 70%. However, the level of protein is very low; being approximately 2.5% of dry matter (Ogunjobi and Ogunwolu, 2010; Mario, 1999).

The proteins of cassava tubers are rich in arginine but poor in methionine, lysine, tryptophan, phenylalanine and tyrosine, hence the need for complimentary argumentation with legumes. Maner (1972) reported that a fresh cassava based diet had an identical nutritional value to a corn-based diet fed gestating pigs. The cassava-fed sows, also maintained on pasture, had an increased still-birth rate and slightly inferior weight gains post-lactation. Studies have also been carried out on the reproductive performance of rabbits fed cassava-based diets over three breeding periods. Results demonstrated that the performance of pregnant and lactating does did not differ significantly from those receiving non-cassava diets, in terms of litter size and birth and weaning weight of offspring (Omole and Onwudike, 1982). Essential and non-essential amino acids of cassava meal amino profiles are shown in tables 2.4a and b.

2.6.3 The challenges of using cassava meal in monogastric livestock feeding

Common characteristics of cassava meal is high crude fiber, ash levels in peels, inclusion level, and anti-nutritional factor (Asaolu *et al.*, 2012) and deficiencies of specific nutrients such as amino acids (particularly Methionine and tryptophan), fatty acids, minerals, and vitamins (Montagnac *et al.*, 2009a). Similarly, high moisture content, pro to

Table 2.4: Amino acids profile of cassava meal

a) Essential amino acids

<i>Amino acid</i>	<i>Cassava meal (g/100g)</i>
Valine	0.15
Methionine	0.58
Isoleucine	0.33
Leucine	0.43
Phenylalanine	0.25
Histidine	0.13
Lysine	3.00
Arginine	9.02

b) Non-essential amino acid of Cassava meal

Aspartic acid	0.39
Threonine	1.49
Serine	0.30
Glutamine	8.90
Proline	0.27
Glycine	0.29
Alanine	6.11
cystine	0.65

(Omeire, 2012; Eka, 1998)

deterioration in wet fractions and dustiness of dried materials are of practical considerations during storage, handling and utilization (Garcia and Dale, 1999; Apata and Babalola, 2012).

i Effect of inclusion level

A lot of research works have been done to define the optimum level of cassava inclusion in animal diet and to modify the plant's chemical and physical properties that restrict its use (Montilla *et al.*, 1976). Low values of weekly live weights were associated with increased cassava inclusion levels in broiler chicks ration (Tada *et al.*, 2004). This is attributed to very low crude protein, high crude fiber and also anti-nutritional factors which reduced bioavailability of some nutrients (Ojewola *et al.*, 2006; Khempaka *et al.*, 2009). Although earlier studies suggested that lower inclusion rates (8 – 10%) must be maintained, more recent studies with broilers suggest that a substantial proportion as much as 50 – 75% (Olugbeme *et al.*, 2010) of energy ingredients can be replaced with cassava chips or flour with no decrease in production, provided diets are balanced with regard to other nutrients, cyanide levels are less than 141 mg/kg, and diet is not overly dusty (Apata and Babalola, 2012). However, as cassava rations contain cyanide, it has to be treated in order to enhance its utilization in poultry rations (Agunbiade *et al.*, 2001). Addition of palm oil (3%) to cassava meal was shown to improve broiler diet texture and palatability (Kana *et al.*, 2012). Aniebo (2012) examined the performance of broilers placed on composite cassava root meal-based broiler starter diets supplemented with palm oil, methionine or palm oil plus methionine and reported good performance results.

Udedibie *et al.* (2009 and 2012) used dried cassava tuber meal mixed with brewers dried grain and palm oil at the ratio of 6:3:1 to stimulate maize in the diet of laying hens to achieve lower cost of egg production. The optimal inclusion level and utilization of the

processed cassava meal would therefore depend upon the processing method used. Studies (Tewe *et al.*, 1976; Obioha and Anikwe, 1982; Tewe and Kasali, 1986; Okoli *et al.*, 2012; Aladi *et al.*, 2014) indicated that ensiling and fermentation are the most efficient methods, while oven-drying is the least efficient method for cyanide reduction in fresh cassava meal (Okoli *et al.*, 2012).

ii Anti-nutritional factors

Many researchers (Vogt, 1966; Ojewola *et al.*, 2006; Khempaka *et al.*, 2009) researchers have reported depressed performance of cassava-fed chickens. Toxic hydrocyanic acid (HCN) is released from the cyanogenic glycosides during hydrolysis through exposure to the extra cellular enzyme linamarase present in the root peel of cassava (Butler and Kennedy, 1965) to release linamarin and some ethyl linamarin. Linamarin is the major cyanogenic glycosides of cassava with (93%), while ethyl linamarin accounts for 7% (Chauynarong *et al.*, 2009; Casadei, 1988; Butler and Kennedy, 1965). The reaction proceeds in two steps (Nartley, 1978) viz: cyanogenic glycoside is degraded to sugar and cyanohydrin (x-hydroxynitrile); cyanohydrin then dissociates to ketone and hydrocyanic acid. Thus, for linamarin the glycoside is first hydrolyzed by linamarase to produce B-D-glucopyranose and 2- hydroxyisolentynonitrile or acetone-cyanohydrin, after which the latter is degraded to acetone and HCN (Tewe *et al.*, 1980; Mahungu *et al.*, 1987). HCN affects livestock through acid hydrolysis in the intestine and by glucosidases of the liver and other tissues (Padmaja and Panikkar, 1989). The respiratory process in animal tissues is obstructed by HCN through deactivation of the cytochrome-oxidase enzyme system (Pudek and Bragg, 1974).

In the whole plant, linamarin is synthesized from the amino acid, valine, while ethyl linamarin is synthesized from isoleucine. Thus, both cyanogenic glycosides are not

harmful to the plant but are sources of aspartic and glutamic acids. However, both cyanogenic glycosides concentration, resulting from the action of hydrolytic enzymes found in plant, is influenced by the nutritional status, variety and age of the plant (Ravindran, 1988). High HCN levels were particularly found more in bitter varieties than in sweeter varieties due to breakdown products of hydrolysis of cyanogenic glycosides in the presence of linamarase (Tewe and Iyayi, 1989).

Several research methods have been adopted to abate the anti-nutritional factor (HCN) of cassava. These include grating and sun drying (Tewe *et al.*, 1976), ensiling (Obioha and Anikwe, 1982), fermentation (Tewe and Kasali, 1986), boiling (Longe, 1980), freezing (Obioha *et al.*, 1983), oven drying (Tewe and Kasali, 1986; Osei and Twumasi, 1989), sun drying (Osei and Duodu, 1988; Esonu and Udedibie, 1993), parboiling and sun drying (Salami, 2000). It is evident from the studies conducted by Esonu and Udedibie (1993) and Salami (2000) that parboiling prior to sun drying had no advantage over sun drying alone in terms of reduction of cyanide content of cassava meal. HCN levels as well as bitterness in plants, has been shown to decrease with plant maturity (Borin *et al.*, 2005), as well as with fertilization. Significant effects of fertilizer type have been recently demonstrated with organic fertilizer which resulted in lower cyanide content in both leaves and tubers of two cassava varieties compared with inorganic fertilization (Faazah *et al.*, 2013).

2.7 Groundnut Cake (GNC)

Groundnut, or peanut (*Arachis hypogaea*) is a species in the legume family Fabaceae, native to South America, Mexico and Central America (Gibbon and Pain, 1985). Groundnuts have been known to man as an important food crop for many centuries. However, they acquired economic importance only two centuries ago and even as late as

60 years ago in the developed and developing countries (Demba, 1985). It is a major source of edible oil and protein meal and considered highly valuable for human and animal nutrition especially in the developing world.

Groundnut is grown mainly for its oil, protein, plant residue and seed cake. More than half of the world groundnut production is crushed for expulsion of oil, diverted mainly as edible oil (Carley and Fletcher, 1995). Groundnut cake (GNC) is the one of the most commonly used poultry feed ingredient in Nigeria. Smith, (1990) observed that groundnut cake is very palatable and the quality of the protein is good, ranking it close to that of soya bean meal. GNC is however a poor source of essential amino acids such as lysine and methionine. Even though it is highly palatable and has better binding properties for pelleting than soybeans (Lovell, 1989) it may invariably be infested with *Aspergillus* sp., which will produce aflatoxins under favorable conditions.

2.7.1 Nutrient value of GNC in poultry feeding

Groundnut cake, a by-product of oil extraction, is an excellent livestock feed because of its high protein content (Aletor and Ojelabi, 2007; Ezekiel *et al.*, 2011). The cake contains 45 - 60 crude protein, 22 - 30% carbohydrate, 3.8 - 7.5% crude fiber and 4 - 6% minerals (Desai *et al.*, 1999; NRC, 2001; NCSAF, 2000).

Groundnut cake is alternative protein source, since it is deficient in sulphur amino acids such as methionine and cystine followed by lysine (Ranjhan, 1999; Ovie, 2007).

Therefore, adequate supplementation with animal protein is necessary since its crude protein has sub-optimal amounts of cysteine and methionine, although the limiting amino acid is lysine (McDonald *et al.*, 1992). Again, its amino acid quality improves in artificial

Table 2.5: Amino acid profile of GNC

a) Essential amino acids of GNC

<i>Amino acid</i>	<i>Groundnut cake (%)</i>
Valine	2.9705
Methionine	0.3394
Isoleucine	2.0425
Leucine	2.0656
Phenylalanine	1.7052
Histidine	0.5785
Lysine	0.4582
Arginine	3.3551
b) Nonessential amino acid	
Aspartic acid	3.6182
Threonine	1.9403
Serine	1.4528
Glutamine	3.9025
Proline	1.799
Glycine	1.4446
Alanine	1.7279
cystine	0.4921

(Maneemegalai and Reena Prasad, 2011)

diets when reinforced with lysine and methionine. However, when groundnut cake is used in high cereal diets, such supplementations also ensures that the requirement of vitamins B12 and calcium are met, especially for fast growing animals such as poultry and pigs. GNC amino acid profile as reported by Maneemegalai and Reena-Prasad (2011) are shown in tables 2.5 for essential and non-essential amino acids.

2.7.2 The Challenges of using groundnut cake in livestock feeding

i Anti-nutritional factor: Naturally mycotoxins and other fungal metabolites are found in peanut / groundnut cake from Nigeria. Several food / feed contamination by fungi and consequent release of secondary metabolites into the food / feed materials have been well documented (Jimoh and Kolapo, 2008; Ngoko *et al.*, 2008; Sulyok *et al.*, 2010). However, aflatoxins have been predominantly and most extensively studied mycotoxins while very little is known about other fungal metabolites such as (ochratoxins, Versicolorin A, and Versicolorin C) among many others (Banu and Muthumary, 2008; Younis and Malik, 2003).

Chemical and microbiological investigations revealed that the toxic effects produced by groundnut cake had resulted from the presence of quantities of four secondary metabolites of the mould *Aspergillus flavus* in the diet. As these compounds fluorescence either green or blue in ultraviolet light, they were designated;

(1a) aflatoxin B1 and (1b) aflatoxin B2

(2a) aflatoxin G1 and (2b) aflatoxin G2

Furthermore, due to the presence of some vital nutrients in the parent material; groundnut, it is prone to contamination by numerous moulds which secrete toxins (Gachomo *et al.*, 2004; Jimoh and Kolapo, 2008) that are possibly carried over into the

processed snack. Almost all agricultural commodities will support the growth of the aflatoxin-producing fungi *Aspergillus flavus* and *A. parasiticus*. Formation of aflatoxins can occur during the pre- and post-harvest stages of food production as long as a suitable environment for mould growth is provided. Optimal conditions for aflatoxin production are a water activity in excess of 0.85% and a temperature of 27° C.

These secondary metabolites act as anti-nutritional factors either by destroying or inhibiting some vital nutrients in the parent material and by posing threat to animal health. Aflatoxin B1 is metabolized to aflatoxin M1 (AFM1) in the liver which could be lethal by causing acute toxicosis and death in livestock. Ascorbase is an enzyme released by plant cells in response to damage. Processes such as harvesting change ascorbic acid in food to oxalic acid, which is an organic dicarboxylic acid that readily forms insoluble salts with calcium and magnesium (Pathak, 1997). Oxalic acid and its soluble salts are both corrosive and poisonous. This reaction increases rapidly during storage, especially in warm climate.

Aflatoxin could be abated, in agro-residues in poultry feeds through exposure to sunlight (solar radiation). For example, γ -radiation (60Co) and microwave heating were investigated in artificially contaminated feed samples. Photodegradation of aflatoxin by sunlight has also been found to cause significant decrease in both B1 and the total aflatoxins. Moreover, the degrees of aflatoxins were dependent on exposure time (Aziz and Youssef, 2002).

2.8 Palm Kernel Cake (PKC)

The fruit of the Oil Palm (*Elaeis guineensis*) consists of an outer mesocarp and inner hard shelled nut containing the palm kernel. Palm oil is extracted from the mesocarp, while

palm kernel oil comes from the kernel of the nut. Prior to oil extraction, the outer shell should be separated after cracking. PKC is a by-product of the palm oil industry. Two types of oil extraction process are employed, either screw press (expeller) or solvent extraction. The solvent extracted PKC has lower oil content, ranging from 1.2 - 5.0 %, while the expeller pressed PKC has 4.5 - 17.3 % (Tang, 2000). There has been a dramatic increase in global production of PKC with annual growth of 15% over the last two decades (FAO, 2002). Total global production of PKC amounted to about 8.2 million metric ton in 2012 (MPOB, 2012), while an earlier paper estimated yields of 0.04 t/ha/year for PKC (Devendra, 2006). Its consistent availability and cost effectiveness, compared to conventional feedstuffs make it a material of interest to nutritionist. Palm kernel meal is aflatoxin free, palatable and has considerable potential as a carbohydrate and protein source. However, chemical analysis of palm kernel meal showed that its nutrient content ranges widely, depending upon the oil extraction process, the species of the palm nut and the amount of shell content remaining in the meal (O'Mara *et al.*, 1999). Thus, PKC is widely used as a moderate source of protein and energy in different livestock such as dairy cow (Carvalho *et al.*, 2006), pig (Adeschinwa, 2007), rabbit (Orunmuyi *et al.*, 2006), laying hen (Chong *et al.*, 2008) and broiler chickens (Mardhati *et al.*, 2011).

2.8.1 Nutritional value of PKC in livestock feeding

Its low nutritive value, grittiness due to high amount of lignin (13.6%) in PKC, also the contamination with nut shell (Knudsen, 1997) makes this feedstuff fibrous and potential for deterioration in unhygienic conditions. A large amount of PKC is discarded causing environmental challenge. This is a problem for palm kernel cake producing countries, such as Nigeria and will continue to create environmental problems in the future.

Livestock (poultry, pig, rabbit etc) has been found effective medium for recycling this agro by-product into animal protein to combat the future environmental hazard.

Proximate composition of PKC was found as; Crude protein 15.32 ± 0.95 , Crude fibre 14.39 ± 1.18 , Ether extract 1.75 ± 0.08 , Total Ash 4.35 ± 0.50 , NFE 64.19 ± 2.27 and M.E (kcal/kg) 1892 ± 10.53 according to Shakila and Reddy (2014). The use of palm kernel cake in broiler diets has been practiced for several decades, but due to its low level of key essential amino acids (lysine and methionine in particular) , high dietary fibre (particularly in the form of β -mannan) and grittiness have precluded its inclusion in poultry diets.

Contradictory results have been reported on the effect of palm kernel cake on the performance of broilers (Panigrahi and Powell, 1991; Ezieshi and Olomu, 2004; Sundu *et al.*, 2005a). Problems created by the use of palm kernel meal may not be related to the physical properties of palm kernel meal but to its contribution to the overall nutrients in the diet, particularly amino acids and metabolisable energy (Sundu *et al.*, 2004a).

2.8.2 Potential value of PKC to poultry health

Palm kernel cake is aflatoxin free, palatable and has considerable potential as a carbohydrate and protein source. Poultry diets contain a wide variety of carbohydrates, readily digested carbohydrates such as starch and sugars and indigestible oligosaccharides and non starch polysaccharides (NSPs). Numerous studies on the role of indigestible carbohydrates in the digestive tract of chickens over the last two decades have improved our understanding of the role and fate of these carbohydrates. We are now in an era when carbohydrates in poultry diets are not only recognized as a source of energy but also have beneficial effects on chicken health (Sundu *et al.*, 2006). Studies on

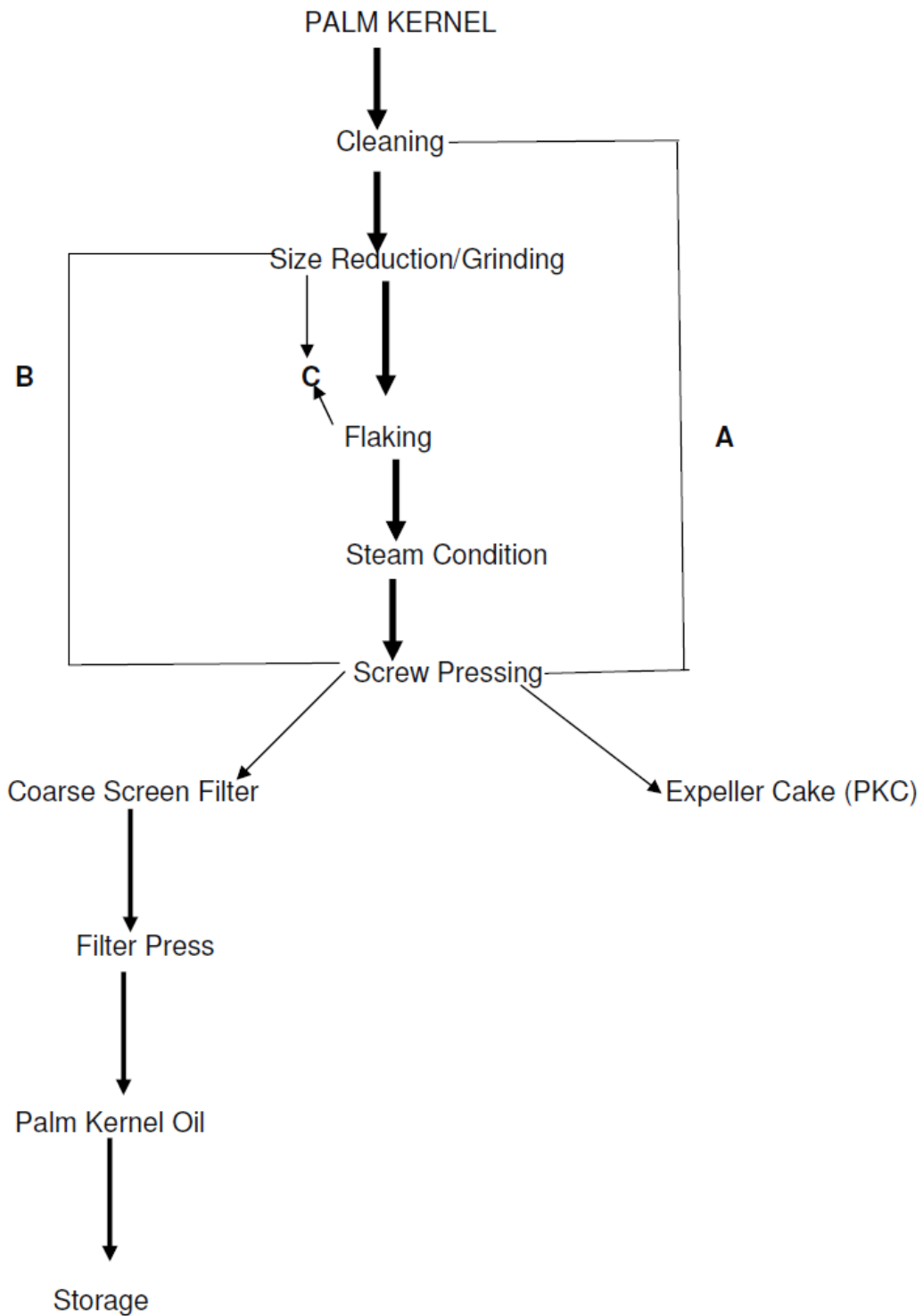


Fig. VI: Flow chart of PKC (MPOB, 1992; Tang, 2000)

- (A) Direct screw pressing without kernel pre-treatment
 (B) Partial kernel pre-treatment followed by screw pressing
 (C) Complete pre-treatment followed by screw pressing

this aspect will probably be more intense in the near future as the use of antibiotics concern about antibiotic resistance and oligosaccharides have been substances of choice to replace antibiotics due to their capacity to block the colonization of pathogen bacteria in the intestine of broilers (Fernandez *et al.*, 2000). Many current findings suggest that palm kernel cake could replace commercial manno-oligosaccharide as a prebiotic to improve chicken health and immunity (Allen *et al.*, 1997; Fernandez *et al.*, 2000 and Fernandez *et al.*, 2002). The beneficial effects of such compounds in the diet are not limited to improved health status of the host and inhibition of the intestinal pathogens (Gibson *et al.*, 2004) but also reduced production of putrefactive compounds (Cumming and Macfarlane, 2002) which could be related to N or amino acid catabolism. The efficacy of mannose based carbohydrates, either as manno-oligosaccharides (MOS) (Lyons, 2002) or mannose (Oyofe *et al.*, 1989) to improve the immune system of animals has been well accepted.

Commercially, the product “Bio MOS” which is extracted from yeast, has been marketed with efficacious effects in 92% of all animal studies (Lyons, 2002). The inclusion of palm kernel meal in the diet improves the immune system of birds, reduces pathogenic bacteria and increases the population of nonpathogenic bacteria such as *Bifidiobacterium sp* (Fernandez *et al.*, 2002) in the intestine, thus, (Allen *et al.*, 1997) found that the addition of 25 g palm kernel meal/kg diet reduced the degree of salmonella colonization in the intestinal tract of broilers consuming feed which was inoculated with *Salmonella kedeugou* or *Salmonella enteritidis*. The birds became clear of infection by three weeks of age while birds fed the unsupplemented diet remained infected. (Zulkifli *et al.*, 2003) conducted research on Newcastle disease antibody titre of broiler chickens fed the palm kernel meal based diets and found that Newcastle disease antibody titre of birds fed palm

kernel meal diets remained constant throughout a week period of heat stress while broilers fed the control diet had a large reduction in titer.

Possible mechanism of this action could be, that dietary Mannan oligosachharides (MOS) from palm kernel meal may attract micro-organisms away from the intestinal binding sites. Mannan oligosachharides have been reported to have receptor sites for the fimbriae of *E. coli* and *Salmonella sp* which results in elimination of these particular bacteria as the digesta flows out (Spring *et al.*, 2000). Accordingly, colonization of the microbes in that organ decreases and thus the birds are less susceptible to these organisms. These two benefits should be considered as strong recommendations for using palm kernel meal in broiler diets, particularly in palm kernel meal producing countries, not only for increasing bird productivity but also to improve chicken health.

2.8.3 Challenging factors of palm kernel cake on monogastric livestock

i Inclusion level

Owing to its high fibre content, non-starch polysaccharides and shell content, the use of PKC in poultry rations is very limited, with wide variation in the optimum inclusion level. (Yeong, 1987), Abu Hassan and Yeong (1999) has suggested inclusion level of up to 20% in broiler ration, while in layers ration its inclusion could be up to 25% without adverse effect on egg production and quality (Yeong, 1987; Radim *et al.*, 2000). Muscovy ducks can be fed PKC at 30% level without any deleterious effects on performance (Mustafa *et al.*, 2001). However, inclusion of PKC at levels greater than 20 % was reported to reduce egg production and egg quality (Yeong *et al.*, 1981), although in another study egg production reduction was only observed at levels exceeding 40% (Onwudike, 1988).

PKC has been reported not suitable in high percentage for monogastric animals such as poultry and fish due to its high indigestible carbohydrates and moderate protein contents (Jørgensen *et al.*, 2010; Iluyemi *et al.*, 2006) and increase in faecal weight attributed to either increased mass of undegraded fiber or increase in water retained by the degraded fibre (Selvendran *et al.*, 1987). Several studies have been carried out enhancing the nutrient content of PKC for poultry for digestion and inclusion level enhancement. Topics include enzyme treatment and solid-state fermentation of the PKC. Enzymic depolymerization of PKC releases digestible sugars that will be fully absorbed and metabolized by poultry. Supplementation with specific enzymes can improve nutrient digestibility and has worked efficiently to break down mannans in PKC (Saenphoom *et al.*, 2010). Broilers can be fed diets containing 30% fermented PKC without any adverse effect on performance (Noraini *et al.*, 2008).

Some common fungi that have been utilized in fermentation of PKC include *Rhizopus stolonifer*, *Trichoderma spp* and *Aspergillus niger* (Suriani *et al.*, 2008; Abdeslahian *et al.*, 2010; Aladi *et al.*, 2014). Thus, fermentation with *Aspergillus niger* was reported to increase the true metabolizable energy of PKC from 5.5 MJ ME/kg to 8.1 MJ ME/kg (Abdul Rahman *et al.*, 2010). Essential and non-essential amino acids of Palm kernel cake are presented in table 2.6 a and b respectively.

2.9 Crude protein (CP)

The basic nutrient that cannot be compromised in the choice of feedstuffs for feed formulation and preparation is protein (Zeitler *et al.*, 1984). It is important to optimise the utilization of proteins provided by energy sources (i.e. cereal grains), as protein sources are more expensive. Proteins from animal sources, such as meat, poultry, fish, eggs etc,

Table 2.6: Amino acid profile of PKC for livestock feeding

a) Essential amino acids of Palm kernel cake

<i>Amino acid</i>	<i>Palm kernel cake (%)</i>	
Valine	0.81	0.77
Methionine	0.25	0.29
Isoleucine	0.52	0.52
Leucine	1.07	0.99
Phenylalanine	0.68	0.65
Histidine	0.30	0.28
Lysine	0.56	0.44
Arginine	2.18	1.96

b) Nonessential amino acid

Aspartic acid	1.10	1.26
Threonine	0.40	0.47
Serine	0.55	0.67
Glutamine	3.33	2.86
Proline	0.62	0.54
Glycine	0.69	0.72
Alanine	0.66	0.63
cystine	0.2	0.20

(Boateng *et al.*, 2013; Yeong, 1981; Shakila and Reddy, 2014)

provide all nine indispensable amino acids in adequate amounts and for this reason are considered “complete proteins”. Proteins from plants, legumes, grains, nuts, seeds, and vegetables tend to be deficient in one or more of the indispensable amino acids and are called ‘incomplete proteins’ (Nwachukwu, 2007). However, cost effective, maximum utilization of grain protein will reduce ration costs unlike tuber feedstuffs such as cassava meal which is principally a source of carbohydrate (Ogunjobi and Ogunwolu, 2010). For this fact, cassava meal based rations must be supplemented with protein, amino acids, fat, minerals, and vitamins at higher levels than are needed in cereal-based diets.

Protein synthesis, affects virtually every aspect of metabolism in living cell. Amino acids are the key regulators of various pathological and physiological processes, including immune responses, as well as being used for the synthesis of all of the other N-containing compounds in the body, including hormones, neurotransmitters, nucleotides (RNA and DNA), histamine, polyamines such as spermine and spermidine etc. These observations are mentioned to highlight the impact that amino acids have on overall body metabolism and the likely importance that optimizing amino acids nutrition has on health, fertility and production performance of livestock (Esonu, 2006).

Unfortunately, there is a wide-spread belief that whenever crude protein concentrations are lowered, performance is negatively affected. Burnham (2005) speculates that this belief stems from researchers (Neto *et al.*, 2002; Bregendahl *et al.*, 2002) who lowered crude protein concentrations beyond practical formulation and then did not supplement back with sufficient amounts of limiting amino acids other than methionine (Met) and lysine (Lys). On a practical basis, however, bird performance can be hindered by excessively lowering crude protein in diets due to a number of factors other than the reduction of crude protein itself. According to Waldroup (2000), these factors can

include: reduced potassium levels, altered ionic balance, lack of nonessential amino acids, imbalances among certain amino acids (e.g. branched chain amino acids), and/or potential toxic concentrations of certain amino acids.

2.9.1 Amino acids

The quality of a protein could be determined by its potential to cover the physiological requirements in terms of amino acids for maintenance and performance (growth, reproduction, production of milk and eggs). Dietary supplementation with amino acids beyond their requirements for growth deposition might thus be useful depending on environmental conditions particularly with the evolution to reduce use of medication in livestock production (Kidd, 2004). Based on growth or nitrogen balance, amino acids were traditionally classified as nutritionally essential or nonessential for mammals, birds and fish. It was assumed that all the “nutritionally non-essential amino acids (NEAA)” were synthesized sufficiently in the body to meet the needs for maximal growth and optimal health. In poultry, 22 amino acids are needed to form body protein, and amino acid is divided into two. Nine of the amino acids must be provided in the ration; these are termed essential or indispensable amino acids. The body can make the other amino acids needed to synthesize specific structures from other amino acids which are non-essential amino acids, (Esonu, 2006).

i) Essential amino acid

They are not synthesized in enough quantities by the animal itself (Garthwaite *et al.*, 1998), to meet metabolic needs of the body. Essential amino acids must be supplied to prevent the conversion of essential amino acids into non-essential ones (Wathelet, 2000). They are also known as indispensable amino acids which and include methionine, lysine,

threonine, isoleucine, leucine, valine, histidine, phenylalanine and arginine (Esonu, 2006).

Lysine is often one of the limiting amino acids in broiler diets Han and Baker (1994). Amino acids are critical for muscle development (Tesseraud *et al.*, 1996) and Lys content in breast muscle is relatively higher than other amino acids Vazquez and Pesti (1997). Lysine represents approximately 7% of the protein in breast meat (pectoralis major and minor muscles). Dietary Lys inadequacy has been shown to reduce breast meat yield compared with other muscles (Tesseraud *et al.*, 1996). Therefore, defining dietary AA needs for optimum growth and meat yield is of utmost importance. Vazquez and Pesti (1997) reviewed 16 data sets throughout the world and estimated the dietary Lys requirement as 1.21% for BW gain and 1.32% (total) for feed efficiency for the young chick from 0 to 21 d of age. These estimates based on growth were much higher than the 1.10% dietary Lys requirement recommended by (NRC, 1994). Lysine enables the synthesis of carnitine, which converts fatty acids into energy, dietary lysine needs vary with the response criterion and breast meat has a higher estimate than growth responses (Holsheimer and Ruesink, 1993; Kerr *et al.*, 1999; Sterling *et al.*, 2006) and plays an important role in the production of hormones, antibodies and enzymes. Having a deficiency in lysine can lead to niacin deficiency and cause a health condition called pellagra. A significant decrease in fertility was noted with increasing levels of isoleucine and lysine and also suggestion by (Ekmay *et al.*, 2013) that adequate dietary lysine and isoleucine should be provided for maximum hatching egg production but an excess may affect fertility (Ekmay *et al.*, 2013).

Methionine is usually the first limiting amino acid in most of the practical diets for broiler chicken (Vieira *et al.*, 2004; Han and Baker, 1994). This amino acid aids in the

production of sulphur, which is necessary for normal metabolism, a precursor of cystine (Wallis, 1999) and it is also essential for the synthesis of hemoglobin and glutathione that fights against free radicals which makes it play a major role in antioxidant systems of the cell through various systems (Métayer *et al.*, 2008). Methionine sulfoxide reductase A and B (MSR-A and MSR-B) is an enzymatic system which turns back into methionine. Methionine are considered as the one main target of free radicals in protein and by the action of MSR A or B system reverse to methionine, it can be considered as a free radical scavenging system (Stadman *et al.*, 2005).

Valine Corzo *et al.* (2007) confirmed experimentally that valine was the 4th limiting amino acid in corn-soybean meal diets for 21 – 42 days broilers. Valine is necessary for muscle metabolism by preventing the breakdown of muscle, because it supplies the muscles with an extra glucose responsible for the energy production during physical activity, repair of tissues (Niewold, 2008) and can be useful in the treatment of liver and gallbladder disorders.

Leucine is one of three essential amino acids that increase muscle mass (Etzel, 2004), (Cisneros *et al.*, 1996) showed that high leucine diet for broilers resulted in an improvement in the muscle color. On the other hand, excessive leucine content in the diets resulted in retarded growth rate and increased feed conversion (FC) for 3-week-old, male broiler (Edmonds and Baker, 1987; Smith and Austic, 1978; Farran and Thomas, 1990; D'Mello and Lewis, 1970) and it helps muscle recover after exercise. It also regulates blood sugar through stimulation of insulin release. Insulin, an anabolic hormone, is needed to enable key nutrients, such as glucose, amino acids and creatine, to enter muscle cells. Insulin stimulates protein synthesis, inhibits protein breakdown, and supplies the body with energy. These functions make it invaluable when the body is

stressed. Leucine is used clinically to help the body heal, and it also affects brain function and can be used in place of glucose in 'fasting' states. It also inhibits inflammation, enhances specific immunity (Buyse *et al.*, 2008).

Threonine; this amino acid is needed to synthesize other amino acids that aid in production of collagen. It is also important for antibody production and intestinal physiology. The gastrointestinal tract, while representing only 4 - 6 % of body mass, accounts for 25-50% of whole body protein turnover. The extraction of essential dietary amino acids by the gut might represent 20-70% (Nichols and Bertolo, 2008). The primary fates of these extracted amino acids are synthesis of intestinal proteins which are mainly secreted into the lumen as mucus which protect the gut from pathogens and antinutritional factors. Mucins are glycosylated proteins secreted along the intestinal epithelium and involved in the diffusion and absorption of the nutrients along the digestive tract. Mucins are particularly rich in threonine, proline and serine with Thr representing as much as 28-40% of the total amino acids of mucins. Thr is thus important for gut function and environmental factors impacting the gut integrity might thus impact the need for Thr. Intestinal inflammation (ileitis in pigs) by increasing the mobilisation of endogenous protein appears to reinforce the demand for Thr. Moreover, it has been demonstrated using different environmental conditions such as clean and dirty litter that Cobb birds in the dirty conditions responded better to the Thr supplementation than birds in the clean environment (Kidd and Corzo, 2006). However, a mild coccidial challenge which might slightly affect the intestinal mucosa did not require further threonine supplementation (Kidd *et al.*, 2003). Threonine needs of broilers decline with age (NRC, 1994; Samadi and liebert, 2006) suggesting that threonine may become more important in older birds, possibly because of higher maintenance requirement (Kidd and Kerr, 1996). Amino acid need of birds is affected by sex, male and female responded differently to

dietary amino acid concentrations (Penz *et al.*, 1997; Dozier *et al.*, 2001). However, report of (Holsheimer *et al.*, 1994; Thomas *et al.*, 1995) showed that threonine estimates from male and female requirement were similar.

Phenylalanine is a precursor to catecholamines that regulate the central, peripheral nervous system and cell metabolism (Kidd, 2004; Li *et al.*, 2007; Niewold, 2008) and there are three forms of phenylalanine: D-phenylalanine, L-phenylalanine and DL-phenylalanine.

Arginine aids in gene expression; DNA and protein synthesis; antioxidants; plays an important role in cell division, dietary arginine is required for maximum neonatal growth and embryonic survival (Wu, 2010) and also it aids in energy metabolism (muscle, nerve), healing of wounds by quickens repair time of damaged tissue (particularly bone) (Stechmiller *et al.*, 2005; Witte and Barbul, 2003), removing ammonia from the body, immune function, and the release of hormones (Tapiero *et al.*, 2002; Stechmiller *et al.*, 2005; Witte and Barbul, 2003). It is precursor for the synthesis of nitric oxide (NO) Andrew and Mayer (1999). The nitrate-nitrite-nitric oxide pathway that is monitored through saliva testing can generate Non-L-arginine derived NO.

Histidine controls allergic reaction; vasodilator; gastric acid and central acetylcholine secretion (Li *et al.*, 2007; Niewold, 2008). It aids in regulating and utilizing essential trace elements like iron, copper, molybdenum, zinc, and manganese in poultry. This amino acid is also essential in forming numerous metal-bearing enzymes and compounds, such as the antioxidant super oxide dismutase.

ii) Non-essential amino acid

Non-essential simply indicates that the animal is able to synthesize these amino acids or convert them from one into another. To undertake such amino acid inter conversions the animal requires sources of carbohydrates and suitable nitrogen compounds. They are cysteine, glycine, glutamic acid, aspartic acid, serine, alanine and proline.

Glutamic acid and Proline aid in citrulline free radical scavenger and also for arginine synthesis, dietary glutamine is necessary for intestinal mucosal integrity and some of the traditionally classified NEAA (e.g. glutamine and glutamate) play important roles in regulating gene expression, cell signaling, antioxidative responses, and immunity. Additionally, glutamate, glutamine, and aspartate are major metabolic fuels for the small intestine and they, along with glycine, regulate neurological function (Wu, 2010).

2.9.2 Classification based on chemical principles

Amino acids can also be classified base on chemical principles (pH) into three main categories, the neutral, acidic and basic amino acids. This classification is based on the different types of constituents (R) which are present on the carbon atom in addition to the amino group. Classifying amino acids helps animal nutritionist break them down into smaller byte-size so that one can better understand their functions in livestock ration and orders of limiting amino acids, understanding the effects of limiting amino acids in the diet will allow the nutritionists to minimize or overcome the influence of excess essential amino acids (Kidd *et al.*, 2000; Baker *et al.*, 2002) and help to reduce costs and supplement adequately in poultry nutrition, also it aids in amino acid partition (Ogbuewu *et al.*, 2014) know important ones for reproduction, maintenance and muscle deposit (Wu *et al.*, 2010).

i) Acidic amino acids

Acidic amino acids possess a second carboxyl group in the substituent R position; its R groups with a net negative charge at pH 7.0 are aspartate and glutamate. These amino acids are the parent compounds of asparagine and glutamine.

ii) Basic amino acids

The basic amino acids have an additional basic group and its R groups have a net positive charge at pH 7.0. They are arginine, histidine and lysine.

iii) Neutral amino acids

The neutral amino acids are alanine, asparagine, cystine, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine and tryptophan.

iv) Aliphatic amino acids

Aliphatic R groups are nonpolar and hydrophobic. Hydrophobicity increases with increasing number of C atoms in the hydrocarbon chain. Although these amino acids prefer to remain inside protein molecules, alanine and glycine are ambivalent, meaning that they can be inside or outside the protein molecule. Glycine has such a small side chain that it does not have much effect on the hydrophobic interactions. These include Leucine, Isoleucine, Glycine, Valine, and Alanine (Takei *et al.*, 2006).

2.9.3 Amino acid profile of feedstuffs

The quality of protein required in ration dependent on the animal species, age, genotype and sex as well as on the performance level. It follows therefore that there are two important factors with respect to protein quality, which are the ratio of essential amino acids in the protein and their availability. It is essential to determine the amino acid

profile of feedstuffs used in livestock rations formulated for monogastric animals, as a balanced amino acid profile in the final ration will have a proteins paring effect and provide a more balanced profile of absorbable essential amino acids which allows meeting amino acid requirements with less dietary protein since amino acids are the building blocks for protein synthesis Sibbald (1987). Thus, there is growing recognition that besides amino acid role as building blocks of proteins and polypeptides, some amino acids regulate key metabolic pathways that are necessary for maintenance, growth, reproduction and immunity (Wu, 2009) and they are called functional amino acids, which as follows arginine, cystine, glutamine, luecine, lysine and proline. Once digested and absorbed, amino acids are used as the building blocks of structural proteins. Body proteins are constantly being synthesized and degraded, therefore adequate amino acid supply is critical to support growth or egg production. Amino acid profile determines the quality of proteins in a given sample.

2.10 Amino Acid Requirements for Poultry Production

Amino acids are building blocks for proteins in all animals. The requirements of amino acids in animals are well defined in various sets of recommendations such as those of NRC (National Research Council), USA, etc some researchers have indicated that the (NRC, 1994) lysine requirement for chicks is too low (Vazquez and Pesti, 1997; Kidd *et al.*, 1998; Kerr *et al.*, Si *et al.*, 2001; Labadan *et al.*, 2001). Other studies have reported that Met + Cys levels should be above (NRC, 1994) recommendations (Lumpkins *et al.*, 2007; Si *et al.*, 2004). Although most amino acid requirements established by the (NRC, 1994) considered safe estimates for broiler chicks. Requirements vary depending on the species and age of animals (Ayu, 2007). Amino acids should be supplied either in the form of protein or crystalline amino acids in feed to meet requirements.

The primary limiting amino acid in cereal grains for growing pigs is lysine, followed by the sulphur containing amino acids and threonine (Shimanda and Cline, 1974). Sulfur-containing amino acids, cysteine (Cys) and methionine (Met) are essential for the entire biological kingdom because of their prominent tasks in primary and secondary metabolism (Ravanel *et al.*, 1998; Leustek *et al.* 2000; Saito, 2000, 2004; Droux 2004). It is therefore important to determine the contents of lysine and methionine in feed sources used for ration formulation. Understanding the effects of limiting amino acids in the diet will allow the nutritionists to minimize or overcome the influence of excess essential amino acids (Kidd *et al.*, 2000; Baker *et al.*, 2002) and help to reduce costs. By comparing requirements and the actual amino acids present in feed, the order of ‘limiting amino acids’ can be estimated. The orders of limiting amino acids in broiler and pig feeds are summarized in Table 2.7.

Threonine (Thr) is the third limiting essential amino acid behind methionine (Met) and lysine (Lys) in poultry diet and has profound effects on broiler performance and lysine utilization (Kidd, 2000) and increasing dietary lysine without an increase in Threonine (Thr) may limited breast meat yield (Dozier *et al.*, 2008). Crystalline amino acids should be added to feed in the order of limiting amino acids when the protein content of the feed is reduced, which is the reason why DL-Methionine and L-Lysine HCl were introduced to feed.

Table 2.7: Order of limiting amino acids

<i>Animal type</i>	<i>1st</i>	<i>2nd</i>	<i>3rd</i>
poultry	methionine	lysine	threonine
pig	lysine	threonine	tryptophan

Broiler responses of economic interest, such as body weight (BW) gain, feed conversion ratio (FCR), and breast meat yield (BMV), can be optimized by increasing amino acid (AA) concentrations, improving the AA balance, or both. The AA requirements to maximize a response are lowest for BW gain, increased for FCR, and highest for BMV. A maximum performance response is reached when the response plateaus, whereas an optimal response is the one providing the highest return per input Vieira and Angel (2012). The amino acid requirements for broiler at different feeding stage and broiler breeder feeds, are summarized in table 2.8 a and b.

High concentrations of dietary amino acids are needed to support the rapid growth of meat-type chickens. Body weight of commercial meat-type chickens will increase 50 – 55 fold by 6weeks after hatching NRC (1994) which may be as a result of tissues substantial protein content. Thus, adequate amino acid nutrition is vital to the successful feeding program for this type of chicken.

Methionine+cystein; it has been great argument concerning amino acid requirements for broilers centers on the sulfur amino acids. Many has attempted especially to ascertain the relative proportion needed of these two amino acids with variable results. Many have attributed a share of the argument in estimated requirements to factor such as the sparing effect of choline (Quillen *et al.*, 1961; pesti *et al.*, 1979; or sulfate Ross and Harns, 1970) or negative effects of copper sulfate (Baker and Robbins, 1979). However, another factor that may contribute to the disagreement in result was the comparison of results using crystalline amino acids diets with results using diets based on practical ingredients, thus, this different may relate in part to the incomplete digestion of the portion in the intact ingredient. The cysteine status of the basal diet is a major factor that contributes to the

Table 2.8 a: Broiler (%) amino acid requirements at different age/ration

<i>Amino acid</i>	<i>Starter (0 – 6wks)</i>	<i>Finisher (6 – 8wks)</i>
Arginine	1.25	1.05
Histidine	0.35	0.59
Isoleucine	0.80	0.68
Leucine	1.20	1.01
Lysine	1.10	1.00
Methionine	0.50	0.35
Methionine+cystine	0.90	0.66
Phenylalanine	0.72	0.61
Threonine	0.80	0.69
Valine	0.90	0.76

b. Broiler breeder amino acid requirement (90% DM) Units per hen per day mg/g

<i>Arginine</i>	<i>1,110</i>
Histidine	205
Isoleucine	550
Leucine	1,250
Lysine	765
Methionine	450
Methionine+cystine	700
Phenylalanine	610
Threonine	720
Valine	750

Source: (NRC, 1994)

apparent disagreement in results, especially when diets with intact ingredients are used. Generally a basal diet, considered deficient in sulfur in sulfur amino acid, is supplemented with graded levels of methionine and response determined. Reports for methionine requirement in poultry, for the period of 3 weeks (Waldroup *et al.*, 1979; Tillman and pesti, 1985) are above the NRC (1994) recommendation while (Robbin and Baker, 1980a; Thomas *et al.*, 1985) are at or near that recommendation.

Greater diversity exists among estimates for total sulfur amino acid (TSAA) requirement, as would be expected from the factors indicated above. Feeding crystalline amino acid diets suggests a markedly lower TSAA value (Robbins and Baker 1980a). Though basing TSAA requirements on data using crystalline amino acid was perhaps not justifiable for practical diets, it does not point out that the TSAA requirement could be less if a proper balance between available methionine and cysteine existed.

Arginine; there was significantly change in its recommendation for arginine requirement of broilers. Thus, has eliminated from consideration all studies in which potential lysine – arginine antagonisms are unlikely to occur with practical ingredients NRC (1994).

Lysine; 1.1% requirement of lysine in 3 weeks old broiler in stead of 1.2% as recommended by NRC (1994) which agreed with lysine requirement of broiler reported by (Burton and Waldroup, 1979) but beyond 1.1% lysine the responses were declined and it was found that less than 0.275% lysine was required for maintenance thus, the total requirement of lysine is 414.27mg/day which about 6.84% of total lysine requirement would be utilized for maintenance with 41.43g of average daily feed intake (ADFI), the dietary level of lysine needed would be 1.0% of the diet, this reduction was supported by

previous reports (Edward *et al.*, 1956; Boomgaart and Baker, 1973a,b; Woodham and Deans, 1975; Burton and Waldroup, 1979) while 3 – 6 weeks of age limited research.

Threonine; considerable research has been conducted on the threonine requirement for broilers, majority of the studies supported the value of 0.8% for 0 – 3 weeks of age (Uzu, 1986; Robbin, 1987; Thomas *et al.*, 1987; Smith and Waldroup, 1988b; Austic and Rangel-lugo, 1989). A lot of studies with intact protein diets have been carried out to allow estimation of the requirements for leucine, isoleucine and valine in 21day old broilers (D' Mello', 1974; Woodham and Dean, 1975; Thomas *et al.*, 1988).

The average digestible requirements per breeder per day for cysteine, total sulphur amino acids (TSAA), phenylalanine, isoleucine, valine and crude protein were 477, 901, 689, 830, 799 mg per day and 20.0g per day, respectively (Ekmay *et al.*, 2013) which were above NRC (1994) recommendation for broiler breeder but in (Ekmay *et al.*, 2013) reported values of 1,026 mg per day arginine, 424 mg per day methionine, 916 mg per day lysine, 613 mg per day threonine all were below NRC recommendation.

Table 2.9 Pullets allowed to self-select diets based on protein or energy content seem to voluntarily consume much less protein in early life and more protein as the approach maturity (Summers and Leeson, 1978) than do pullet on more conventional production. However, low-protein or low-lysine starter diets invariably depress the growth rate of layers (Douglas and Harms, 1982; Kwakkel *et al.*, 1991). Low-protein diets have a transitory effect on muscle fiber size rather than any long-term effect on numbers of such fibers (Timson *et al.*, 1983) but there were little indication that excessively high levels of protein have any benefit on growth and development. Data of Keshavarz (1984) and Leeson and Summers (1989) suggested that in Leghorn pullet intake to 140days od age

Table 2.9 a: Layers % amino acid requirement at different age (week)

<i>Amino acid</i>	<i>Chicks 0 – 6</i>	<i>Starter 6 - 12</i>	<i>Grower 12 - 18</i>	<i>Layer 18 above</i>
Arginine	0.94	0.78	0.62	0.72
Histidine	0.25	0.21	0.16	0.18
Isoleucine	0.57	0.47	0.37	0.42
Leucine	1.00	0.80	0.65	0.75
Lysine	0.80	0.56	0.42	0.49
Methionine	0.28	0.23	0.19	0.21
Methionine	0.59	0.49	0.39	0.44
+cystine				
Phenylalanine	0.51	0.42	0.34	0.38
Threonine	0.64	0.53	0.35	0.44
Valine	0.59	0.49	0.38	0.43

was short fall of 1kg, an intake of 1kg of balanced protein during the same period seems to result in maximum growth. Broiler breeder, the total amino acid requirement of an individual breeder hen includes three components: a requirement for maintenance, a requirement for tissue protein accretion and a requirement for egg production. A portion (typically 10 – 15%) of the dietary amino acid ingested is not digested. These are excreted which elevates the nitrogen in the faeces. High nitrogen in the litter results in foot pad lesions and excess ammonia, which can irritate eyes and the respiratory tract. Amino acid inclusion levels of the pullet starter, developer, pre-breeder and breeder feeds have been investigated in a number of laboratories. The effects of absolute levels of protein or amino acids on performance are confounded by differences in feed intake between studies. Attempts to determine and model the relationship between amino acid intake and egg production have been made for example by (Fisher, 1998; Bowmaker and Gous, 1991). Modern breeds are more responsive to dietary amino acid density and have the potential to deposit a lot more breast muscle and less fat. While broilers are fed to optimize growth and meat yield, the propensity for rapid growth and development of a large breast must be controlled in parent stock (www.hubbardbreeders.com).

The requirements of amino acids in animals are well defined in various sets of recommendations such as those of NRC (National Research Council), USA, etc. Requirements vary depending on the species and age of animals Johnson and Fisher (1959). Amino acids should be supplied either in the form of protein or crystalline amino acids in feed to meet requirements, for the laying hen, the protein and amino acid requirement were determined by (Leveille *et al.*, 1958, 1960).

The amino acids requirement for turkeys and turkey breeder are summarized in Table 2.10 and are based on either actual experiments, modeling or are calculated as a ratio

Table 2.10: Turkey and its breeder % amino acid requirement

<i>Amino acid %</i>	<i>0-</i>	<i>4-</i>	<i>8-</i>	<i>12-</i>	<i>16-</i>	<i>20-</i>	<i>Breeder</i>	<i>Breeder</i>
	<i>4wk</i>	<i>8wk</i>	<i>12wk</i>	<i>16wk</i>	<i>20wk</i>	<i>24wk</i>	<i>holding</i>	<i>laying</i>
Arginine	1.6	1.4	1.1	0.9	0.75	0.6	0.5	0.6
Histidine	0.58	0.5	0.4	0.3	0.25	0.2	0.2	0.3
Isoleucine	1.1	1.00	0.8	0.6	0.5	0.45	0.4	0.5
Leucine	1.9	1.75	1.5	1.25	1	0.8	0.5	0.5
Lysine	1.6	1.5	1.3	1	0.8	0.65	0.5	0.6
Methionine	0.55	0.45	0.4	0.35	0.25	0.25	0.2	0.2
Methionine + cysteine	1.05	0.95	0.8	0.65	0.55	0.45	0.4	0.4
Threonine	1.0	0.95	0.8	0.75	0.6	0.5	0.4	0.45
Valine	1.2	1.2	0.9	0.8	0.7	0.6	0.5	0.58
phenylalanine	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.55

Source: (NRC, 1994; Warnick and Anderson, 1973; Hurwitz *et al.*, 1983a; Tuttle and Balloun, 1974; Behrends and Waibel, 1980)

with lysine at the ages in question has been experimented (NRC, 1994). Little experiments have been conducted to determine the amino acid requirements of growing turkeys with the exception of lysine and sulfur amino acids (Hurwitz *et al.*, 1983a) employed body analyses and feed intake together with calculated maintenance needs to estimate requirements.

Isoleucine requirement listed for turkey poult 1% of the diet is based largely on the research of Warnick and Anderson (1973) and it corresponds with the value of 1.03% obtained from modeling by (Hurwitz *et al.*, 1983a). Similarly, (Hurwitz *et al.*, 1983a) base the leucine requirement 1.9% of the diet on the value of 1.86% reported by Warnick and Anderson (1973) and 1.96% from modeling. The frequent limitation of lysine and sulfur amino acids under practical conditions has been well investigated, starting with poult requirement of 1.6% lysine in the diet. This value represents a mean value of 1.55% obtained by (Balloun and Phillip, 1957b), 1.6% (Kummerow *et al.*, 1971), 1.68% (Warnick and Anderson, 1973), 1.5% (Tuttle and Balloun, 1974) and 1.55% (D' Mello' and Emmens, 1975) while the value of 1.42% obtained by modeling (Hurwitz *et al.*, 1973) was noticeably lower than those measured by bioassay. Thus, the sulfur amino acid requirement value of 1.1% for starting poult was derived from the study of 1.04% by (Warnick and Anderson, 1973), 1.05% by Murillo and Jensen (1976b), 1.10% by Potter and Shelton (1979) and 1.1% by Behrends and Waibel (1980), as well as modeling value of 1.05% by (Hurwitz *et al.*, 1983a).

The absolute amino acid requirements change due to genetic or environmental factors, the ratios among them are only slightly affected. Thus, once the ideal AA profile has been determined, the requirement for a single AA (i.e., lysine) can be determined experimentally for a given field situation and the requirements for the other entire AA

calculated from the ideal ratios. The solution to obtaining reliable AA requirements is therefore not to determine the AA requirements, but rather to determine the ideal AA profile for laying hens. Because the indigestible portion varies considerably among feed ingredients, it is highly recommended that diets are formulated on a digestible amino acid basis (Sklan and Noy, 2003). For instance, soybean meal, meat and cottonseed meal contain about the same amounts of total methionine but their methionine digestibility varies widely thus, dietary amino acid ingested is not digested so formulating on a digestible amino acid basis is paramount D' Mello (2003); Friedman and Gumbann (1989). Diets formulated on a total amino acid basis must contain large safety margins to account for the differences in digestible amino acid content of different feed ingredients. By formulating diets on a digestible amino acid basis, safety margins can be reduced and feed ingredients can be more accurately valued based on their content of bioavailable amino acids. The protein and amino acid contents in the diet and their ratio to energy content are important not only for parent performance and hatchability but also for chick quality.

2.11 Nutrient Variations in Livestock Feedstuffs

The nutrient compositions of all feedstuffs vary, and nutritionists must learn to accommodate variation when using them for formulating rations. Feedstuffs with wide variation in nutrient composition are worth less than feeds with narrow variation. The literature on the nutritive value of some feedstuffs contains a wide variation in the published values. Evidence clearly shows that same products from different origins, ecological conditions, with regard to varieties, ages at harvest, storage conditions and processing methods contain widely varying levels of nutrients (Jane, 2011). Considerable differences in chemical composition between different cultivars of sorghum and maize produced at different locations have also been reported (Champbell *et al.*, 1995; Brand *et*

al., 1997). Thus, the actual amino acid profiles of feeds are usually unknown due to wide variation of nutritional composition of feedstuffs. The above sources of variation are considered fixed, i.e., they can be described and replicated for example, Hybrid X may have been bred to produce corn silage with higher than average NDF digestibility, or Distillery Y may dry their distillers grains at very high temperatures causing high concentrations of acid detergent insoluble protein, while another possible fixed source of variation is the analytical laboratory.

Thus, understanding potential sources of variation in feed composition data will help determine which data to use and how to use it. Variation in feedstuffs composition increases risk with its attendant cost (Pearce *et al.*, 1979). If the composition of a feedstuff is highly variable diets formulated using them will either have to be over supplemented to avoid a deficiency (i.e., increased feed costs) or production may decrease because at times the diet does not provide adequate nutrients (Bill, 2007). The nutrient composition of feeds can also be influenced by plant genetics (hybrid, variety, etc). Although great progress has been made in standardizing methods, labs often use slightly different analytical techniques to measure nutrients. If a load of brewers' grains is sampled 15 times and those 15 samples are sent to a lab, you will probably get back 15 different concentrations of protein. The variation could be caused by variation within the load of brewers' grain or it could be caused by random errors at the lab (Belyea *et al.*, 1989). The need to standardize some of the locally available feedstuffs to evaluate their nutrient compositions and regulate the effects of these variations in feedstuffs through referenced values for good genetic potential expression of livestock / poultry industry is therefore imperative.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Materials Sources

The feed raw materials studied were dried brewers spent grain (DBSG), cassava meal (CAM), palm kernel cake (PKC) and groundnut cake (GNC). The samples were selected and collected based on the presences of industries that have some of these products as their by-products and also presences of monogastric animals production industries, especially poultry farms. The four selected locally available feedstuffs were sourced from northern and southern parts of Nigeria. The northern locations were Kano state (Kano) and Kaduna state (Sabongari), while the southern parts were Abia state (Aba and Umuahia), Imo state (Owerri), Enugu state (Enugu), Anambra state (Onitsha) and Rivers State (Port Harcourt).

Table 3.1 shows the distribution of samples collection across the different locations. Each of these raw materials was collected from five locations, such that, there were five samples for each raw material and 20 samples in all. Plates 1 to 4 show pictorial representations of the test samples.

3.2 Samples Collection Procedure

At each samples collection location, three to four bags of the feedstuffs were randomly sampled. Each bag was opened and about 1 kg of the feedstuff collected with a plastic spatula in to a cellophane bag. The materials were then pooled to form the representative sample for that location (Okoli, 2004). Therefore, weight of sample collected at each location was about 5 kg. One sample each of cassava and brewers spent grain was collected fresh and sun dried for 3 – 5 days.

Table 3.1: Sample collection locations

<i>Locations</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Aba	√	√	√	√
Enugu	√		√	
Kano				√
Kadunna				√
Owerri	√	√	√	√
Onitsha	√	√	√	√
Portharcourt	√			
Umuahia		√	√	√

Legend

DBSG = Dried brewers spent grain

CM = Cassava meal

PKC = Palm kernel cake

GNC = Groundnut cake



(a)



(b)

Plate 1: Samples of groundnut cake (GNC)



Plate 2: Samples of dried brewers spent grain (DBSG)



Plate 3: Sample of palm kernel cake (PKC)



Plate 4: Samples of processed cassava CAM

Over 200 grams of each sample was packed, labeled and coded DBSG, CM, PKC and GNC accordingly, with an air tight sealed transparent polyethylene bags and shipped to Evonik Industry laboratory, Germany for amino acid analysis via courier services (EMS). The packages were received at the Evonik Laboratory, Germany in good condition within a week of the shipment. Shipment receipt and acknowledgement are shown in appendix 1 and 2.

3.3 Feedstuff Samples Analyses

3.3.1 Laboratory of Evonik Industries

Analyses procedure employed by Evonik industry laboratories have been detailed in AminoDat[®] 40 (2010). This laboratory procedure complies with the requirements of AOAC (1995) as outlined by Slump and Bos (1985) and Llames and Fontaine (1994).

3.3.2 Laboratory apparatus

The major laboratory equipment and apparatus used included;

a. Amino Acid Analyzer: (Biochrom 30 or 20 plus by Biochrom Ltd., Cambridge, UK).

The model has two-channel A/D converter card on a PC using software for peak integration. The amino grams are simultaneously detected at 570 nm and 440 nm. Columns and appropriate buffer programs also accompany the equipment.

b. Metal container with discharge outlet and lid for performing the oxidation. The external dimensions were 61 x 26 x 52 cm, alternatively a refrigerator may be used.

c. Multiple magnetic stirrers: (Multipoint HP 15, VARIOMG*)

d. Analytical balance; with accuracy of 0.1 mg

e. Thermostatically controlled heating oven: (UT 6060 AR, Thermo Electro LED, Langenselbold, Germany), with acid protected inner walls and timer, adjustable to 110 \pm 1° C.

- f. Automatic pH adjustment with auto sampler: (Metrohm 789 Robotic Sample Processor with autodoser Titrino 719 S, complete with 250 ml plastic titration vessels).
- g. 50 ml laboratory bottles, with thread of DURAN glass (Schott), red PBTP caps with silicone/teflon seal.
- h. Digital pH meter; with accuracy to 3 decimal places.
- i. Ultra centrifugal mill, with ring sieves, < 0.5 and < 0.25 mm, e.g. Retsch ZM 200.

Other laboratory equipment, include 50 ml, 100 ml, 1 l, 2 l and 5 l volumetric flasks, 100 ml and 300 ml Erlenmeyer flasks with glass stoppers, 5 l glass bottles with glass stoppers, 1, 2 and 5 liter glass beakers, 50 and 100 ml PE screw-top bottles, 2 liter thick wall, laminated glass bottles with screw cap enabling purging with nitrogen, variable 50 ml dispenser, variable Eppendorf pipette (up to 5000 μ l), nutsch filter with D4 glass filter, Pasteur pipettes 3 ml one-way, membrane filter (pore size 0.25 μ m, diameter 25 mm) as one-way syringe (5 ml) filter, ultrasonic bath.

3.3.3 Reagents

- a. Chemicals
- b. Reagent solutions. All solutions were prepared with distilled or deionized water.
 - i. *Phenolic formic acid solution, 88%*: 889 g of formic acid was mixed with 111 g water and to this was added about 5 g of phenol. The solution was stored in a dark reagent bottle.
 - ii. *Oxidation reagent (performic acid-phenol)*: 10 ml hydrogen peroxide was mixed with 90 ml of phenolic formic acid solution as above in a 100 ml Erlenmeyer flask. The flask was closed and left to stand for one hour at room temperature in order to induce the formation of performic acid. Then, the solution was placed in an ice bath for 15 min and use immediately.

- iii. *Hydrochloric acid-phenol hydrolysis reagent*, $c = 6 \text{ mol/l}$: 5 g phenol was placed into a 5 l volumetric flask with stopper containing exactly 2.5 l water dissolved while stirring. Exactly 2.5 l of 37% hydrochloric acid was carefully added and mix. The volume was made up with water.
- iv. *Citrate buffer solution*, $c = 0.2 \text{ mol/l Na}$, $\text{pH} = 2.20$ (*sample dilution buffer*): 98 g sodium citrate and 5 g phenol were dissolved in 4 l water in a 5 l glass beaker with stirring. 25 ml thiodiethyleneglycol and 80 ml of 37 % hydrochloric acid was added. The solution was diluted with 800 ml water and the pH adjusted to 2.20 with HCL. This was thereafter transferred to a 5 l volumetric flask and topped up to the mark. Citrate buffer solutions and sodium hydroxide solution were added as eluents for the Amino Acid Analyzer, composition of buffers A – D of the separating program for the Biochrom 30 series analyzers. This is shown in the manufacturer's manual.
- v. *Sodium acetate buffer*, $c = 4 \text{ mol/l}$ for *ninhydrin reagent*: 410 g sodium acetate was weighed. 1472 g potassium acetate and 21.3 g sodium citrate were taken and poured into a 5 l glass beaker. 2 l of water was added and followed by 500 ml acetic acid. The salts were dissolved after cooling the solution was transferred to a 5 l measuring flask and filled to the mark with water.
- vi. *Ninhydrin reagent*: Weigh 1.78 g hydridantin and 20 g ninhydrin into a 300 ml Erlenmeyer flask, 200 ml methanol was added and dissolved by ultrasonication. In laminated 2 l glass bottle 600 ml acetate buffer above, 1200 ml ethyleneglycol and 85 ml hydrochloric acid, 37 %, are mixed and purged with nitrogen for 10 minutes. Then, the methanolic ninhydrin solution is added, mixed and purged with nitrogen for further 10 minutes.
- vii. *Norleucine (NLE) standard*: about 2.0 g norleucine was dissolved – accuracy 0.1 mg – into 500 ml hydrochloric acid and was topped up with water in a 5 l volumetric flask.

The amount of the internal standard norleucine contained in 15.00 ml is calculated (in mg, accurate to 3 decimal places) and marked on the flask.

viii. *Sodium hydroxide solution, $c = 7.5 \text{ mol/l}$* : 1874 g of 32% sodium hydroxide solution was weighed into a 2 l volumetric flask and topped up with water while stirring. It was allowed to cool to room temperature and adjusted exactly to the mark.

ix. *Sodium hydroxide solution, $c = 1 \text{ mol/l}$* : 40 g of sodium hydroxide pellets was weighed into a 1 l measuring flask and was dissolved in 800 ml water, allowed to cool and filled to the mark with water.

x. *Sodium hydroxide solution, $c = 0.4 \text{ mol/l}$* : (regeneration reagent for Amino Acid Analyzer), 16.0 g sodium hydroxide pellets was weighed into 1 l glass beaker and dissolved while stirring in 700 ml water. 0.5 g Titriplex III was added and poured in a 1 l measuring flask while rinsing with water and filled up to the mark.

xi. *Hydrochloric acid, $c = 1 \text{ mol/l}$* : exactly 166 ml hydrochloric acid, 37 %, was poured into a 2 l measuring flask containing about 1 l water. After cooling filled up to the mark.

3.3.4 Calibration

a. Stock solutions: Two mixtures of standard amino acids were weighed in approximately the same composition. The amino acid mixture was dissolved in 100 ml of 0.1 mol/l hydrochloric acid (in the case of the control mixture it is essential to record the total weight!), tight closed and stored frozen. These stock solutions are stable for at least one year in the freezer.

b. Calibration solution: 1.5 ml of the calibration mixture stock solution was poured into a 50 ml glass beaker, then was diluted with citrate buffer solution and dissolved about 2.2 g sodium chloride therein (Fontaine, 2003). The pH was adjusted to 2.20 with sodium hydroxide solution. It was filled up with citrate buffer to about 50 ml volume. The calibration solution was refrigerated until required for analysis for analysis.

c. Control of the calibration: The control solution was prepared by accurately weighing about 1.5 ml of the control mixture stock solution and about 2.5 ml (-15 mg NLE) of the norleucine standard solution was poured into a 50 ml glass beaker, diluted with citrate buffer solution and about 2.2 g sodium chloride was dissolved therein. The pH was adjusted to 2.20 with sodium hydroxide solution. It was then filled up with citrate buffer solution to about to about 50 ml volume. The control solution was refrigerated until required for analysis (Llames and Fontaine, 1994). It must be discharged after one month. Chromatograph the control solution, the amino acid concentrations in the control mixture stock solution was calculated by means of the (RF) response factor values from the completed calibration and determines the difference in percent relative to the expected concentrations for each amino acid. The calibration is considered validated if the deviation is less than 3%.

Note: this procedure is used both for checking the prepared stock solutions and the current norleucine standard solution. The validity of the calibration should be daily monitored in this way.

3.3.5 Sample preparation

a. Oxidation in protein with subsequent hydrolysis: The samples were prepared by meshing into fine particles which can pass through 0.5 mm sieve and each sample was homogenized. Mass of each finely ground sample containing about 10 mg nitrogen was taken (Redshaw *et al.*, 2010; according to Kjeldahl analysis) and poured into a 50 ml laboratory bottle after introducing a magnetic rod the 50 ml laboratory bottle was placed on a magnetic stirrer in a metallic container with discharge outlet and lid for performing the oxidation five milliliter of oxidation reagent performic acid-phenol was added into laboratory bottle that contains the sample and was stirred for 15 mins. The bottle was covered with a loosely applied plastic lid, screw top or foil and left to react for 16 hrs at 0

□ C in a closed container for the oxidation of cystine and methionine (Slump and Bos, 1985).

Thereafter 25 ml of hydrochloric acid-phenol reagent was added and the bottle was placed in the heating oven at 110 °C for 1 hr with a loosely applied screw top to prevent the bottles from cracking. Then the screw top was quickly tightened, and to ensure safety, a hand gloves and eye protector was used. It was allowed to hydrolyzed for further 23 hrs in the heating oven at 110 °C. After completing the hydrolysis the bottles were removed from the heating oven and the bottles with samples were allowed to cool.

b. Internal standard and adjustment to pH 2.20: Fifteen milliliter of norleucine standard solution was pipetted into the bottle using a calibrated dispenser, the hydrolysate was mixed and transferred into a 250 ml plastic beaker (Bech-Andersen and Rudemo, 1990), then about 125 ml citrate buffer was added and also exactly 19 ml sodium hydroxide solution (NaOH C= 7.5mol/L) was added. A portion of the solution was transferred into a 50 mL PE bottle with screw top.

The sample was analyzed using chromatography by passing the same over a membrane filter applied to the column of the Amino Acid Analyser as required (e.g. 30uL).

c. Procedure for sample with very high or low crude protein content: (Llames and Fontaine, 1994): i. In some cases, it is necessary to delay the addition of the nor-leucine standard until the chromatographic measuring solution is being prepared due to some samples with very high or low CP content or wide variations in the amino acid concentrations causing the nitrogen content of the sample weighed to deviate considerably from the required 10 mg because of the limitation on sample size (100 – 800

mg). These result in amino acid peaks in the chromatograms that are either too high or too low, relative to the norleucine peak in the routine procedure.

ii. In some samples containing individual amino acids in extremely high concentration, there may be need for a second measurement following further dilution of the hydrolysate, which would result in too small norleucine peak. With these samples, the oxidized hydrolysate was transferred quantitatively into the 250 ml plastic beaker, combined only with 50 ml citrate buffer and was carefully adjusted to pH 2.20 with NaOH solution.

The final weight of the hydrolysate (TW - yd) was recorded. A portion was frozen in a 100 ml PE screw top bottle or analyzed immediately.

Prior to the measurement, the norleucine solution was weighed into an accurately weighed portion of the hydrolysate (PW - yd) in such a way that the peak areas were similar in size (Mason, 1980). The mixture was then diluted with citrate buffer to the desired analytical concentration. It was passed over a membrane filter and applied to the column of the amino acid analyzer.

Dilution factor: $DF = TW - yd / PW - yd$

TW - yd = total weight of the hydrolysate

PW - yd = weight of the portion used

3.3.6 Process evaluation

Calculation: The calculation was performed by the internal standard method using the peak integration software. The chromatographic raw data stored in the PC are re-integrated if necessary and the calculated amino acid contents were entered into the laboratory information and management system (LIMS) (Redshaw *et al.*, 2010).

a. Calculation of the amino acid response factors (RF values):

= response factor of the respective amino acid

= peak area of the respective amino acid (570nm; Pro, Hypat 440nm)

= peak area of norleucine (channel:570nm)

= weight of norleucine in the stock solution (mg)

= weight of the respective amino acid in the stock solution (mg)

b. Calculation of the amino acid concentration in a sample:

% =

= Peak areas of the respective amino acid

= Peak areas of norleucine

= response factor of the respective amino acid

= weight of norleucine in mg (from 15.00ml standard addition)

= weight of sample in mg

= dilution factor, if required.

(Llames, and Fontaine, 1994).

c. Calculation of the amino acid amounts in the control mixture:

AA (mg) =

= peak area of the respective amino acid

= peak area of norleucine

= response factor of the respective amino acid

= norleucine in mg, calculated from the amount of NLE-solution weighed in

= total weight of the control mixture stock solution (g)

= weight of the proportion of stock solution in the control solution (g)

3.4 Data collection

3.4.1 Overall amino acids values of the feedstuffs

Overall, the follow raw data were generated Dry matter (DM), Crude protein standardized to a dry matter content of 88%, Crude protein as is, Total amino acids without NH₃, NH₃ content, total amino acids

The range of amino acids quantitatively analyzed included essential and non-essential amino acids for poultry. The essential amino acids included Methionine, Lysine, Threonine, Isoleucine, Leucine, Valine, Histidine and Phenylalanine. The Non-essential amino acids analyzed included, Cystine, Methionine+Cystine, Arginine, Glycine, Serine, Proline, Alanine, Aspartic acid and Glutamic acid. Overall the study analyzed for seventeen amino acids, with eight being essential and nine non-essential amino acids.

3. 4.2 Partitioning of amino acids into classes

Using the generated amino acid values, several amino acids quality score were also calculated for the feedstuffs according to the method reported by Ogbuewu, (2012).

1. Total amino acids (TAA) = Σ (Meth + Isoleuc + Phenyl + Lys + Hist + Val + Arg + Thre + leuc + Cys + Glyc + Glut + Ser + Ala + Asp + Prol)

2. Total essential amino acid (TEAA) = Σ (Meth + isoleuc + Phenyl + Lys + Hist + Val + Arg + Thre + leuc)

3. TEAA – (Histidine + Arginine) = Σ (Meth + Isoleuc + Phenyla + Lys + Val + Thre + leuc]

4. % TEAA – (Histidine + Arginine) = $[(\text{TEAA} - (\text{Hist} + \text{Arg}) / \text{TAA}) \times 100]$

5. Total non essential AA (TNEAA) = Σ (Cys + Gly + Glut + Ser + Ala + Asp + Prol)

6. %TNEAA = $[(\text{TNEAA} / \text{TAA}) \times 100]$

7. Total aliphatic amino acids (TAAA) = Σ [Gly + Ala + Val + Leuc + Isoluec]

8. %TAAA = [(TAAA / TAA) X 100]

9. Total neutral amino acid (TNAA) = Σ (Gly + Ala + Val + Leuc + Isoluec + Phenyl + Prol + Ser + Thre + Meth + Cyst + Aspar + Glut]

10. %TNAA = [(TNAA / TAA) X 100]

11. Total basic amino acids (TBAA) TBAA = Σ (Hist + Lys + Arg)

12. %TBAA = [(TBAA / TAA) X 100]

13. Total acidic amino acid (TAcAA) = Σ [Glut + Asp]

14. %TAcAA = [(TAcAA / TAA) X 100]

15. Total sulphur amino acids (TSAA) = Σ (Cys + Meth)

16. % Methionine in TSAA = [(TSAA – Meth / TSAA) X 100]

17. Total polar amino acids (TPAA) = Σ (Asp + Cys + Glut + Lys + Hist + Arg + Thre + Ser)

18. %TPAA = [(TPAA / TAA) X 100].

19. Total non polar amino acid (TNPAA) = Σ (Ala + Gly + Isoleuc + Leuc + Meth + Phenyl + Prol + Val)

20. %TNPAA = [(TNPAA / TAA) X 100]

Legends: AA – Amino acid; Ala – Alanine; Gly – Glycine; Isoleuc – Isoleucine; Meth – Methionine; Phenyl- Phenylalanine; Pro – Proline; Val – Valine; Leu – Leucine; Asp – Aspartate; Tyr – Tyrosine; Lys- Lysine; Hist – Histidine, Arg – Arginine; Thre - Threonine; Ser – Serine; Cys – Cysteine; Glut – Glutamate; Aspar – Asparagine (Source: Ogbuewu *et al.*, 2014).

3.5 Experimental Design and Statistical Analysis

The experimental design was a Completely Randomized Design (CRD) to block the error due to variability of sources of the feedstuffs.

The statistical model is given below

$$X_{ij} = M + t_i + E_{ij}$$

Where,

X_{ij} = individual observation

M = overall mean

t_i = effect of the treatment where $i = 1, 2, 3, \dots, t$

E_{ij} = experimental error

Data generated from amino acid analysis on the below parameters were subjected to analysis of variance and significant differences were established among means, they separated using SPSS statistical software (SPSS, 2011).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Overall Result

4.1.1 Dry matter content of feedstuffs from Nigeria

It has been shown that determining nutrient levels in different feeds on dry matter basis, rather than on as is basis makes a comparison easier because feeds contain different percentages of water. This also allows for comparison between the level of a given nutrient in the dry matter and the level needed in an animal diet (Reiling, 2011). The dry matter (DM) content of DBSG, CAM, PKC and GNC sampled at different locations in Nigeria are shown in table 4.1. Dry matter content of feedstuff ranged from $89.304 \pm 0.857\%$ recorded for CAM to $93.006 \pm 0.456\%$ recorded for PKC, while the coefficient of variation (CV) between the different sample readings was less than 1% indicating that the dry matter values reported in this study could be used as referenced values for the Nigeria feed industry. PKC recorded the highest value of 93.006 ± 0.456 for dry matter content, which agreed with the value reported by Onwudike (1986), Alimo and Hair-Bejo (1995), Sundu *et al.* (2005c) and Sue (2001). The CAM dry matter content obtained in this study was also similar to the report of Abd EL-Baki *et al.* (1993).

4.1.2 Crude protein content of feedstuff standardized to dry matter content of 88%

The crude protein content of feedstuffs standardized to a dry matter content of 88% is shown in table 4.2. It ranged from $0.974 \pm 0.227\%$ recorded for CAM to $42.410 \pm 6.306\%$ recorded for GNC. This GNC value is similar to those reported in India, but slightly lower than that of Ecuador, with the CV readings for these countries being less than 6% (Redshaw *et al.*, 2010). The coefficient of variation between the different samples ranged from 4.544% recorded for PKC to 23.306% recorded for CAM, and

Tables 4.1: Dry matter content (%) of the feedstuff sample in Nigeria

<i>Samples</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	90.77	89.82	92.34	92.21
Sample 2	91.93	89.10	93.38	92.77
Sample 3	91.93	89.22	93.30	92.18
Sample 4	91.81	90.33	92.72	91.72
Sample 5	91.77	88.05	93.29	92.08
Mean± SD	91.642±0.493	89.304±0.857	93.006±0.456	92.192±0.377
CV	0.538	0.960	0.490	0.409

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

Table 4.2: Crude protein (%) content of feedstuff standardized to dry matter content of 88%

<i>Samples</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	25.032	1.127	13.037	45.751
Sample 2	23.730	1.126	13.344	41.500
Sample 3	22.476	1.144	13.139	48.563
Sample 4	24.624	0.643	14.569	44.134
Sample 5	20.809	0.830	13.659	32.102
Mean \pm SD	23.34 \pm 1.719	0.974 \pm 0.227	13.550 \pm 0.617	42.410 \pm 6.306
CV	7.665	23.306	4.554	14.869

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

indicates high variation among the samples. CAM recorded the highest coefficient of variation followed by the 14.869 recorded for GNC. This could be attributed to differences in the variety of crops used in processing the products, fertility of soils the crops were grown on, processing method used by factories to extract the primary products, blending of multiple byproducts together by the manufacturing plant and storage conditions (Sauvant *et al.*, 2004).

The standardized mean referenced values generated from this study will aid the poultry industry in addressing the problem of wide variations in CP values of locally available feedstuffs. For the purpose of this study, CV values of 0 – 10 % are regarded as narrow while those above 10 % are regarded as wide.

4.1.3 Crude protein content as is

The crude protein content of the DBSG, CAM, PKC and GNC sampled (as is), are presented in table 4.3. These ranged from $0.988 \pm 0.230\%$ for CAM to $44.430 \pm 6.600\%$ for GNC, while the CV between the different samples ranged from 4.518% recorded for PKC to 23.279% recorded for CAM. The mean value of CP for DBSG agreed with the value reported by (Santos *et al.*, 2003). Tang *et al.* (2009) reported a CP range of 28 – 30% for brewer's grain, while Robertson *et al.* (2010) reported a range of 27 – 33%. The present range of 20.81 – 25.03% is much lower. Protein values could be affected by heat applied during the brewing process.

The range of CP recorded for CAM in this study (0.64 – 1.14%) is much lower than the 2.5 % reported by Mario (1999) and Ogunjobi and Ogunwolu (2010). Again, it is possible that the processing methods, especially heat application will have affected the protein contents of the samples. Okoli *et al.* (2012) determined the physiochemical and

Table 4.3: Crude protein content (%) of feedstuff sample As is

<i>Samples</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	25.82	1.15	13.68	47.94
Sample 2	24.79	1.14	14.16	43.75
Sample 3	23.48	1.16	13.93	50.87
Sample 4	25.69	0.66	15.35	46.00
Sample 5	21.70	0.83	14.48	33.59
Mean \pm SD	24.296 \pm 1.726	0.988 \pm 0.230	14.320 \pm 0.647	44.430 \pm 6.600
CV	7.104	23.279	4.518	14.855

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

hydrogen cyanide content of similar processed cassava products used for feeding poultry in Nigeria and obtained a CP range of 2.56 – 2.90% in agreement with the report of Ogunjobi and Ogunwole (2010), indicating that the significant variations between the present results and those of earlier published works may be due to the different laboratories in which the analyses were carried out and non-standardization of products processing methods. Adebowale *et al.* (2008) also reported that there were significant changes in chemical composition and pasting properties of cassava grits from different cassava varieties and roasting methods.

Earlier published reports have shown that GNC could yield CP values as high as 45 – 60% (Desai *et al.*, 1999) with average of 54% (NRC, 2001; NCSAF, 2000). The CP results of our sample 5 at 33.59% and sample 2 at 43.75% (Table 4.5) however fell below these reported ranges, indicating the non-standardized nature of processing methods used in Nigeria. Usually products derived from local oil extraction method would contain more oil thus, diluting the CP of the product (Plate 1b), while the industrially processed products will have limited oil content and therefore higher CP (Plate 1a).

Chin (2002) reported a CP range of 15 – 16% PKC produced in Malaysia, while the values from the present results at the range of 13.63 – 15.35% were similar. Ezieshi and Olomu (2007) however, reported a CP range of 14.50 – 19.24 % for PKC samples produced in Nigeria indicating that our present results are low, when compared with Nigerian PKC values in literature. Usually PKC is produced either by solvent extraction (Palm kernel meal) or by mechanical extrusion (Palm kernel cake) and this variation in processing method determines the CP value of the end products (Olomu, 1995).

Overall, GNC recorded the highest value for CP as is, followed by DBSG. The latter result may be due to protein rich malt used in brewing, which in turn affected the grains as earlier reported by Oster *et al.* (1977).

Dietary CP requirement is essentially a misnomer since CP requirement is actually based on amino acid content of the protein. Once protein is digested and absorbed, amino acids are used as building blocks of structural proteins, metabolic proteins, enzymes and precursors of several body components (Primot and Melchior, 2002). Since body proteins are constantly being synthesized and degraded, adequate amino acids supply is critical to support growth and egg production (Applegate, 2008). Again, since it is the feed formulator's task to produce feed recipes with given nutritional values, irrespective of the raw materials to be mixed having different and variable characteristics, there is always the need for such formulations to be guided by reliable CP and amino acids values of available feedstuffs. This is so, since a major objective of feed formulation is to control the nutritional values of the resulting compound feeds. Thus, in environments such as Nigeria, where nutritional content of feedstuffs are particularly variable due to crop variety, fertilization and technological treatments among many others, amino acids estimates of feedstuffs used to formulate compounded feeds should be frequently updated (Wieser and Seilmeier, 1998; Sauvant *et al.*, 2004).

4.1.4 Total amino acid (Taa) content of feedstuffs

The total amino acid content of DBSG, CAM, PKC and GNC sampled at different locations in Nigeria are presented in table 4.4. The Taa content was higher in GNC samples than in other samples because it is the only oil seed cake among the samples studied. The higher values are also reflective of the higher CP values of the GNC samples, indicating that the CP values were derived from actual proteins in the samples.

Table 4.4: Total amino acid content (%) of sample material without ammonia

<i>Samples</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	23.689	0.945	11.578	43.026
Sample 2	22.884	0.819	11.429	39.737
Sample 3	20.733	0.997	11.360	43.145
Sample 4	22.912	0.566	12.725	40.304
Sample 5	18.854	0.716	11.931	29.223
Mean \pm SD	21.814 \pm 1.987	0.809 \pm 0.174	11.805 \pm 0.560	39.087 \pm 5.727
CV	9.109	21.508	4.744	14.652

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

Other Taa values also followed the same pattern for their CP content, indicating that the samples were relatively free of urea, ammonia or any other nitrogen source adulteration (Esonu, 2006). Thus, high protein feedstuffs like DBSG and GNC also had greater amounts of amino acids. Taa to CP ratio is usually constant within a feedstuff category. Thus, a significantly lower amino acid to CP ratio in a sample should suggest presence of a non-protein nitrogen source (AFNOR, 2005).

The total amino acids recorded in this study ranged from the $0.809 \pm 0.174\%$ recorded for CAM to $39.087 \pm 5.727\%$ recorded for GNC. The CV recorded for CAM and GNC were wide, indicating the effects of different processing methods of the companies, fertilization, soil type and variety of the crops (Wieser and Selmeier, 1995; Sauvant *et al.*, 2004). The high Taa content of BDG reported here is probably because of the protein rich malt used in brewing which in turn affected the grains. Because of the wide CV recorded for CAM and GNC, there is the need to increase the replicates of analyses by collecting and analyzing more samples from other locations in the country. Quantification of Taa is usually necessary to appropriately determine the available amino acid contents of feedstuff (Esonu, 2006; AFNOR, 2005). Therefore, for each amino acid being considered, the best nutritional criteria are the quantity that is available to the animal's metabolism. This amount is approximated to the quantity absorbed at the end of the ileum (Wesseling and Liebert, 2002).

4.1.5 Ammonia content of feedstuff sample

The ammonia contents of the sampled feedstuffs are shown in the table 4.5. Mean values ranged from the $0.032 \pm 0.005\%$ recorded for CAM to the $0.94 \pm 0.16\%$ recorded for GNC. All the materials recorded CV values of $> 10\%$ across sampling locations except GNC that recorded 7.079% . The results also showed that feedstuffs that recorded high

Table 4.5 Ammonia Content of feedstuff sample

<i>Samples</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	0.705	0.037	0.201	1.019
Sample 2	0.625	0.036	0.230	0.944
Sample 3	0.559	0.032	0.224	1.126
Sample 4	0.643	0.024	0.234	0.938
Sample 5	0.510	0.029	0.219	0.680
Mean \pm SD	0.608 \pm 0.076	0.032 \pm 0.005	0.226 \pm 0.016	0.941 \pm 0.164
CV	12.500	15.625	7.079	17.428

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

CP such as GNC and DBSG also recorded higher ammonia content indicating that the ammonia were probably derived from denatured proteins. The wide CVs calculated for these feedstuffs also suggest that processing methods influenced ammonia production in these feedstuffs.

Ammonia is a non-protein nitrogen source which may be utilized by enteric microbes for the synthesis of protein, especially in ruminants. The concentration of ammonia in feedstuff is thus used as a bench mark in most analyses since the presence of ammonia defines the intensity and length of fermentation undergone by the feedstuff.

4.1.6 Total amino acid content of the feedstuff sampled

The total amino acids content of the sampled materials ranged from $0.837 \pm 0.182\%$ recorded for CAM to $40.025 \pm 5.831\%$ recorded for GNC (Table 4.6), with high CVs of 21.744% for CAM and 14.568% recorded for GNC values indicating wide variations in Taa of samples from different locations. The differences between the Taa of the sample material without ammonia (Table 4.4) and the overall Taa of the materials (Table 4.6) were however minimal.

4.2 Essential Amino Acid Values of the Test Feedstuff

According to a review by Miller (2004) on protein nutrition requirements of livestock, monogastric animals such as poultry require nine to ten amino acids which their body cannot synthesize, together with sources of amino nitrogen which could be used for synthesis of the remaining amino acids. These amino acids, that cannot be synthesized are the essential amino acids and include, methionine, lysine, threonine, isoleucine, leucine, valine, histidine, phenylalanine and arginine (Esonu, 2006). Two amino acids, cysteine and tyrosine could be synthesized in the body but only from essential amino

Table 4.6 Total amino acids content of the feedstuff sample

<i>Samples</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	24.394	0.982	11.779	44.045
Sample 2	23.509	0.855	11.659	40.681
Sample 3	21.292	1.029	11.548	44.271
Sample 4	23.555	0.590	12.959	41.242
Sample 5	19.364	0.745	12.150	29.903
Mean \pm SD	22.42 \pm 2.06	0.840 \pm 0.18	12.019 \pm 0.57	40.028 \pm 5.88
CV	9.146	21.744	4.782	14.568

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

methionine and phenylalanine respectively. Thus, they are usually referred to as semi-essential amino acids. The amount of arginine derived from protein synthesis may not be adequate due to their break down to produce urea and therefore, a dietary supply may be needed to promote growth in young animals (Miller, 2004). Again, arginine is an essential amino acid for poultry and fish but in mammals it is synthesized as part of urea cycle. Similarly, glycine and serine may not be synthesized in sufficient quantities in rapid growing animals and those under high metabolic demands and are thus, sometimes termed essential.

4.2.1 Methionine content of sampled feedstuffs

Methionine concentrations of the feedstuffs sourced at different locations in Nigeria are shown in table 4.7. Methionine content of the feedstuff ranged from the $0.014 \pm 0.005\%$ recorded for CAM to the $0.441 \pm 0.039\%$ recorded for DBSG. The CVs between the different sample readings were high in CAM and GNC (35.714% and 11.004% respectively), while the values for DBSG and PKC readings were less than 10%. The mean value of methionine content ($0.441 \pm 0.039\%$) in DBSG samples in this study agrees with the value reported by Adeola *et al.* (1994); Lawrence *et al.* (1995) and Yin *et al.* (2002). Thus, the sampled DBSG when compare with methionine content of other bulk fibrous materials such as maize and wheat bran is a quality protein source comparable to oil seeds (Attah-kotoku, 2009). AminoDat (2010) reported higher methionine values of 0.59, 0.25 and 0.48% for DBSG, GNC and PKC respectively although there figures confirm the finding that DBSG contains higher methionine value than GNC.

Because of the major role it plays in humans and animals, methionine is classed as a functional amino acid. It participates and regulates key metabolic pathways to improve

Table 4.7: Methionine content of the test feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	0.483	0.019	0.271	0.431
Sample 2	0.466	0.011	0.267	0.414
Sample 3	0.420	0.019	0.266	0.440
Sample 4	0.451	0.009	0.294	0.462
Sample 5	0.386	0.011	0.278	0.342
Mean \pm SD	0.441 \pm 0.039	0.014 \pm 0.005	0.275 \pm 0.012	0.418 \pm 0.046
CV	8.843	35.714	4.364	11.004

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

health, survival, growth, development and reproduction of organisms (Jankowski *et al.*, 2015). Broilers may not require more than 0.5 and 0.38% methionine in starter and finisher diets respectively. Since it is one of the known sulfur amino acids and its deficiency will usually be first noticed as poor feathering in chicks (MSUES, 2014).

4.1.8 Lysine content of sampled feedstuffs

The lysine content of the sampled materials at different locations in Nigeria is presented in table 4.8. These ranged from $0.045 \pm 0.006\%$ recorded for CAM to $1.439 \pm 0.162\%$ recorded for GNC. The CVs across five samples of each material were generally higher than 10% except for PKC. Lysine level was found to be highest in GNC and lowest in CAM. This is expected since GNC is the only oil seed by-product among the materials sampled. Again, the present DBSG lysine mean value of 0.778% did not agree with earlier reports of Porthsmouth (1978) at 0.20%, however, the published data by AminoDat (2010) gave a higher value of 0.38% for PKC.

Lysine is the most important amino acid for monogastric species, as it is the first limiting amino acid for pigs and the second for poultry after methionine. It ensures optimal feedstuff utilization, better growth rates and improved meat quality (Schutte and De Jong, 1999). Lysine requirements of poultry are specifically influential by dietary, environmental and genetic factors. Because lysine is mainly involved in protein deposition, its requirement increases with the potential protein gain. Therefore, birds and pigs with higher growth rate have higher requirement (Relandeau and Le Bellego, 2004). Based on digestible amino acids ratios, relative lysine in layer diets is estimated to be 84% methionine + cysteine. Again, in practical poultry diet, supplementation of methionine and lysine provides a means for increasing the efficiency of protein utilization and as a result nitrogen excretion is reduced. After these two, threonine and glycine,

Table 4.8: Lysine content of the test feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	0.607	0.054	0.477	1.440
Sample 2	0.826	0.042	0.384	1.415
Sample 3	0.885	0.045	0.406	1.565
Sample 4	0.769	0.038	0.466	1.591
Sample 5	0.802	0.047	0.427	1.183
Mean \pm SD	0.778 \pm 0.104	0.045 \pm 0.006	0.439 \pm 0.039	1.439 \pm 0.162
CV	13.368	13.333	8.888	11.258

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

especially in young chicks may also become limiting by reducing the dietary level of protein (Schutte and De Jong, 1999). Again, because of this major function, lysine is usually chosen as the reference amino acid. This means that the essential amino acid requirement for growth could be expressed as a combination of a lysine level that satisfied the broiler requirement plus adequate ratio to lysine of the other essential amino acids according to the ideal protein concept (Relandeau and Le Bellego, 2004).

4.2.3 Threonine content of sample feedstuffs

The threonine contents of the sampled materials are presented in table 4.9. The mean values for threonine concentrations ranged from the $0.039 \pm 0.008\%$ recorded for CAM to the $1.150 \pm 0.146\%$ recorded for GNC. However, there were limited variations between individual sample of DBSG and PKC, which were less than 10% CV, but wide variations between individual samples of CAM and GNC. Earlier studies by Porthsmouth (1978) reported a threonine value of 2.00% for DBSG, which was much higher than the 0.83% obtained in the present study. However, the 0.99% reference value reported by AminoDat (2010) is similar to the present finding, just as it also agrees with the 1.19 and 0.41% published for GNC and PKC respectively by the same AminoDat (2010). In advanced commercial livestock operations, threonine is usually the second limiting amino acid after lysine for pigs and the third after the sulphur amino acids (methionine and cysteine) and lysine for poultry. It is utilized in protein synthesis and other biological functions such as gut integrity and immunity. As lysine, threonine is indispensable for body protein deposition and growth; its deficiency will affect dietary lysine utilization and growth performance (Ajinomoto, 2008). Its deficiency will also lead to disorder of digestive functions. It is therefore, important to determine the threonine requirement that corresponds to the physiological stage of animals in order to establish balance and efficiency of feed.

Table 4.9: Threonine content of the test feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	0.858	0.044	0.409	1.226
Sample 2	0.869	0.037	0.400	1.136
Sample 3	0.815	0.043	0.396	1.281
Sample 4	0.880	0.026	0.450	1.199
Sample 5	0.730	0.045	0.423	0.906
Mean \pm SD	0.830 \pm 0.061	0.039 \pm 0.008	0.416 \pm 0.022	1.150 \pm 0.146
CV	7.349	20.513	5.288	12.696

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

Again, endogenous losses of threonine to lysine ratio have been reported to be up to 174% in poultry for ileal juice and 132% for excreta. This specified trait of threonine, linked to its metabolic functions highlights the importance of relying on standardized and not true digestibility values since the form takes systematic endogenous losses into account (Ajinomoto, 2008).

4.2.4 Isoleucine content of sampled feedstuffs

The mean values of isoleucine concentration of each sampled feedstuff at different locations in Nigeria are presented in table 4.10. The values ranged from $0.035 \pm 0.011\%$ recorded for CAM to $1.499 \pm 0.225\%$ recorded for GNC, however, the CV between CAM and GNC were greater than 10% indicating wide variations of sample values, while DBSG and PKC recorded less than 10% CVs. The mean value of Isoleucine content of DBSG is within the range reported by Donkoh and Attah-Kotoku (2009) and Portsmouth (1978). The value is however lower than the 1.11% published by AminoDat (2010). Similarly, the present values for GNC (1.49%) and PKC (0.481%) are in agreement with the 1.49 and 0.46% published by AminoDat (2010).

Amino acids such as lysine, threonine and methionine + cystine have been extensively studied. However, requirements of other amino acids such as isoleucine must be determined in order to produce balanced ration for the fast growing modern birds. Isoleucine has been established as one of the limiting amino acids in low protein, corn-soybean diets for laying hens (Harms and Russels, 2000). This is especially true when progressive reduction in protein content of diets leads to a situation where certain amino acids like valine and isoleucine affect the animal performance (Peganova and Eder, 2002).

Table 4.10: Isoleucine content of the test feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	1.033	0.048	0.455	1.656
Sample 2	1.017	0.036	0.481	1.540
Sample 3	0.922	0.044	0.477	1.637
Sample 4	1.048	0.025	0.508	1.554
Sample 5	0.865	0.024	0.486	1.106
Mean \pm SD	0.977 \pm 0.080	0.035 \pm 0.011	0.481 \pm 0.019	1.499 \pm 0.225
CV	8.188	31.429	3.950	15.010

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

Under such estimations isoleucine ratio with branched chain amino acids such as lysine becomes critical to optimal production performance (Adello *et al.*, 2012).

4.2.5 Leucine content of sampled feedstuffs

Leucine contents of the test feedstuff are presented in table 4.11. Mean value in the sampled feedstuff at different locations in Nigeria ranged from $0.068 \pm 0.032\%$ recorded for CAM to $2.771 \pm 0.394\%$ recorded for GNC. However, the CVs between sampled feedstuffs were greater than 10% indicating wide variations between unit samples. This could be as a result of different processing methods, varieties, soil type and fertility of the soil in which they are grown or random errors at the lab (Brand *et al.*, 1997; Belya *et al.*, 1989). The 1.01% leucine content of PKC reported by Shakila and Reddy (2014) was higher than our present result, while the 2.77% reported here was higher than the 2.06% reported by Maneemegalai and Prasad (2011). The 2.70% reported by Porthmouth (1978) was also higher than the 2.27% reported here for DBSG. However, our values agree with the reference data published by AminoDat (2010) for PKC and DBSG, while the GNC value is higher than ours. Leucine is involved in blood sugar regulation through stimulation of insulin; it also inhibits inflammation and enhances specific immunity (Buyse *et al.*, 2008). Cisneros *et al.* (1996) showed that high leucine diets for broilers result in improvement in the muscle color.

4.2.6 Valine content of sampled feedstuffs

Table 4.12 shows the valine contents of the test feedstuff, sampled at different locations in Nigeria. GNC had the greatest mean value of valine content among all the samples, and was within the range reported by Babiker (2012). The mean valine value of DBSG at 1.25% is higher than the 1.05% reported by Porthsmouth (1978) but lower than the 1.51% reference value reported by AminoDat (2010).

Table 4.11: Leucine content of the test feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	3.114	0.090	0.841	3.064
Sample 2	2.357	0.058	0.881	2.814
Sample 3	1.696	0.112	0.861	3.040
Sample 4	2.642	0.041	0.946	2.839
Sample 5	1.572	0.040	0.898	2.096
Mean \pm SD	2.276 \pm 0.647	0.068 \pm 0.032	0.721 \pm 0.219	2.771 \pm 0.394
CV	28.427	47.059	30.374	14.219

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

Table 4.12: Valine content of the test feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	1.292	0.060	0.661	2.048
Sample 2	1.293	0.047	0.702	1.924
Sample 3	1.214	0.056	0.694	2.032
Sample 4	1.313	0.032	0.742	1.924
Sample 5	1.132	0.034	0.702	1.355
Mean \pm SD	1.249 \pm 0.075	0.046 \pm 0.013	0.700 \pm 0.029	1.857 \pm 0.286
CV	6.005	28.260	4.143	15.401

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

However, our mean PKC valine value at 0.72% may be said to be similar to the 0.67% reported by AminoDat (2010). The CV between samples were wide in CAM, which recorded 28.260% and GNC that recorded 15.401% but relatively narrow in DBSG and PKC with readings of 6.00% and 4.14% respectively. Porthsmouth (1978) recommended a valine content of 0.8 – 1.00% in poultry diets.

Because of recent trends that require reductions in dietary crude protein levels in broiler feeds in order to reduce faecal nitrogen excretions, it has been evaluated that depending on the requirement assumed for different amino acids, valine, isoleucine, tryptophan, and arginine should be considered as the next generation of limiting amino acids in broiler feeds. Based on the need to optimize the ratio of valine and isoleucine to that of lysine in broiler diets, Corrent and Bartelt (2011) showed that valine is the 4th limiting amino acid in vegetable broiler diets based on wheat and corn, while the valine:lysine and isoleucine:lysine requirements of broilers should be 80 and 67% respectively in order to optimize performance. However, L-valine supplementation in combination with L-threonine provides the optimal opportunity to formulate technically, economically and environmentally better broiler feeds.

4.2.7 Histidine content of sampled feedstuffs

Table 4.13 highlighted the histidine composition of the feedstuff sampled at different locations in Nigeria. In this study mean and standard deviation values of histidine content ranged from $0.019 \pm 0.005\%$ recorded for CAM to $1.019 \pm 0.146\%$ recorded for GNC. The CVs between the samples were greater than 10% except for PKC with a reading of 4.400%. Histidine content of GNC and CAM were lower than those reported by (Babiker, 2012) and (Lekule, 1988) respectively. The 0.40% histidine content of DBSG reported by Porthsmouth (1978) was similar to the value reported in the present study.

Table 4.13: Histidine content of the feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	0.593	0.023	0.248	1.107
Sample 2	0.521	0.017	0.241	1.028
Sample 3	0.472	0.025	0.241	1.131
Sample 4	0.497	0.012	0.268	1.063
Sample 5	0.414	0.017	0.253	0.767
Mean \pm SD	0.499 \pm 0.066	0.019 \pm 0.005	0.250 \pm 0.011	1.019 \pm 0.146
CV	13.226	26.316	4.400	14.328

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

However, the DBSG value reported by AminoDat (2010) was much higher (0.63%), while GNC values were similar at 1.03% and 1.02% respectively. Histidine is intricately involved in a large number of metabolic processes ranging from the production of red and white blood cells to regulating antibody activity. Dietary histidine levels of 0.4 – 1.5% results in optimal growth of poultry (Portsmouth, 1978). However, histidine toxicity in chickens often manifests as reduced growth, poor feathering and proventricular enlargement, although it is possible that other factors in the diet may augment these effects (Dhawale, 2005).

4.2.8 Phenylalanine content of sampled feedstuffs

Table 4.14 presents the phenylalanine content of the sampled feedstuff at different locations in Nigeria. The mean and standard deviation values ranged from 0.047 ± 0.017 recorded for CAM to $2.262 \pm 0.354\%$ recorded for GNC. However, CVs between the samples reading were 4.498 and 6.342% for PKC and DBSG, and 15.649 and 36.170% for GNC and CAM respectively, indicating wide variation in samples of GNC and CAM but narrow variation in samples of PKC and DBSG. Phenylalanine content of GNC in this study was higher than those reported by (Maneemegalai and Prasad, 2011) but lower than those reported by (Babiker, 2012), while the values for DBSG agrees with value reported by Onyimba *et al.* (2014). CAM phenylalanine composition in this study was lower than the value reported by Lekule (1988). However, the AminoDat (2010) reference values for GNC (2.23%) and PKC (0.53%) were similar to values reported in this study, while the DBSG value of 1.53% was higher than the values from this study.

A phenylalanine requirement range of 0.7 – 0.8% has been reported for poultry (NRC, 1994; Portsmouth, 1978). This indicates that diets based on GNC and DBSG as sources

Table 4.14: Phenylalanine content of the test feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	1.378	0.052	0.549	2.519
Sample 2	1.429	0.064	0.574	2.319
Sample 3	1.334	0.060	0.561	2.507
Sample 4	1.421	0.026	0.615	2.310
Sample 5	1.217	0.031	0.589	1.653
Mean \pm SD	1.356 \pm 0.086	0.047 \pm 0.017	0.578 \pm 0.026	2.262 \pm 0.354
CV	6.342	36.170	4.498	15.649

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

of protein may not require supplemental sources of the amino acid. Tyrosine and cysteine can be synthesized from phenylalanine and methionine respectively and are referred to as conditionally essential because they must be in the diet if phenylalanine or methionine levels are inadequate (Aiello and Mays, 1978). Phenylalanine is essential, plays a key role in the biosynthesis of other amino acids, and is important in the structure and function of many proteins and enzymes (NCBI, 2015).

4.2.9 Arginine content of sampled feedstuff

Arginine contents of sampled feedstuffs at different locations in Nigeria are presented in table 4.15. The mean and standard deviation values of tested materials ranged from $0.036 \pm 0.008\%$ recorded for CAM to $4.889 \pm 0.769\%$ recorded for GNC. The CVs between sample readings were greater than 10% in GNC and CAM, but less than 10% in PKC and DBSG. The mean value of arginine content recorded for GNC in this study is lower than those reported by the FAO (2002), Babiker (2012). Similarly our 1.06% value for DBSG is lower than the 1.6% reported by Portsmouth (1978). The reference AminoDA_t (2010) values of arginine for GNC (5.28%), PKC (1.61%) and BSG (1.60%) were different from our present mean values for these feedstuffs, with the 1.74% value for PKC being higher and others. The arginine requirement for poultry has been reported to range from 1.2 – 1.4% indicating that GNC and PKC based diets may not need extra supplementation of this amino acid (Portsmouth, 1978).

Arginine adequacy in the feed helps to improve egg production, egg weight, modulates lipid metabolism towards reducing total body fat accumulation to improve meat quality and increase antioxidant defense under normal condition. L-arginine has ability to alleviate stress and to normalize growth performance. Dietary supplementation may

reduce ascites under high temperature and improves immune response to common diseases in the farm (Fouad *et al.*, 2012).

Table 4.15: Arginine content sample feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	0.991	0.047	1.823	5.482
Sample 2	1.151	0.038	1.578	5.025
Sample 3	1.157	0.040	1.648	5.365
Sample 4	1.060	0.028	1.911	5.005
Sample 5	0.958	0.028	1.749	3.565
Mean \pm SD	1.063 \pm 0.091	0.036 \pm 0.008	1.742 \pm 0.133	4.889 \pm 0.769
CV	8.561	22.222	7.635	15.729

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

4.3 Non-Essential Amino Acids Contents of Sampled Feedstuffs

The non-essential amino acids are those that could be synthesized by livestock and include cysteine, glycine, glutamic acid, aspartic acid, serine, alanine and proline (FAO, 2002). Tables 4.16 to 4.22 show the values of individual non-essential amino acid in the sampled feedstuffs.

4.3.1 Cysteine content of sampled feedstuffs

Cysteine content of sample feedstuffs are presented in table 4.16. The mean values ranged from $0.014 \pm 0.004\%$ recorded for CAM to $0.520 \pm 0.064\%$ recorded for GNC. The CV between all the samples was higher than 10% indicating wide variation between the samples. The mean value of cysteine for GNC in this study at 0.416% is less than those reported by the FAO (2002). The mean cyseine level for DBSG (0.55%), GNC (0.59%) and PKC (0.16%) published by AminoDat (2010) were generally similar to the values reported here. However our values were higher than the mean obtained in Ecuador but similar to that of Guatemoda (AminoDat, 2010). According to Porthmouth (1978) the poultry requirement for cysteine ranges from 0.3 – 0.4%.

Cysteine and methionine are important sulfur amino acids. Cysteine participates in the synthesis of keratin in feathers. In the case of cysteine deficiency nutrient deposition in the breast muscles may be reduced because keratine synthesis is a priority (Wylie *et al.*, 2001). Methionine and cysteine are essential for body maintenance being part of metabolic process and also cell synthesis and renewal. Numerous studies in orally fed animals show that dietary cysteine can replace part of the methionine requirement in growing pigs, chicks, humans and rats (Kim and Bayley, 1983; Finkelstein *et al.*, 1986; Graber *et al.*, 1971; Di Buono *et al.*, 2001).

Table 4.16: Cystine content (%) of sample feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	0.484	0.016	0.209	0.552
Sample 2	0.468	0.014	0.173	0.531
Sample 3	0.417	0.021	0.178	0.560
Sample 4	0.434	0.012	0.220	0.551
Sample 5	0.352	0.011	0.194	0.407
Mean \pm SD	0.431 \pm 0.0515	0.014 \pm 0.004	0.195 \pm 0.020	0.520 \pm 0.064
CV	11.949	28.571	10.256	12.308

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

4.3.2 Glycine content of sampled feedstuffs

Glycine content of sampled feedstuffs at different locations in Nigeria are shown in table 4.17. Glycine concentration of the test materials ranged from $0.042 \pm 0.010\%$ recorded for CAM to $2.509\% \pm 0.345\%$ recorded for GNC. The CV between different samples reading was less than 10% in DBSG and PKC but greater in CAM and GNC. The mean value of glycine content in the sampled GNC agreed with those reported by NRC (1994). The mean glycine values of DBSG, PKC and GNC published by AminoDat (2010) at 1.11, 0.62 and 2.59% respectively were generally higher than our values (0.87 and 0.65%) but similar to our GNC value (2.51%). Glycine was first shown to be needed in the diet of growing chickens by Almquist (1994). It affects protein synthesis not only as building block for protein itself, but is required for the formation of DNA, RNA, creatinine and uric acid (Ngo *et al.*, 1977). It is thus considered a semi-essential amino acid in young broilers since chicks can synthesize it, but there is still a considerable amount to be supplied in the diet (Waldroup *et al.*, 2005).

4.3.3 Serine content of sampled feedstuff

The serine compositions of sampled feedstuffs at different locations in Nigeria are shown in table 4.18. Serine content was highest in GNC and could be as a result of it being the major oil seed cake among other samples. The values ranged from $0.041 \pm 0.009\%$ recorded for CAM to $2.010 \pm 0.291\%$ recorded for GNC, while CV between different samples reading was less than 10% for PKC but greater than 10% for other samples. AminoDat (2010) published serine values for DBSG (1.22%), PKC (0.54%) and GNC (2.13%) were similar to the values reported in this study. Chickens require glycine and serine for optimum growth. Serine can be formed by the addition of alpha carbon to glycine via the reversible enzyme, serine hydroxymethyltransferase (Shemin, 1946). The

Table 4.17: Glycine content of sample feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	0.862	0.053	0.613	2.558
Sample 2	0.924	0.051	0.642	2.415
Sample 3	0.890	0.048	0.630	2.895
Sample 4	0.910	0.030	0.694	2.699
Sample 5	0.845	0.030	0.655	1.979
Mean \pm SD	0.886 \pm 0.033	0.042 \pm 0.010	0.647 \pm 0.031	2.509 \pm 0.345
CV	3.725	23.809	4.791	13.750

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

Table 4.18: Serine content of sample feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	1.121	0.047	0.572	2.212
Sample 2	1.062	0.041	0.538	1.982
Sample 3	0.952	0.052	0.530	2.271
Sample 4	1.071	0.030	0.626	2.053
Sample 5	0.817	0.033	0.593	1.533
Mean \pm SD	1.004 \pm 0.122	0.041 \pm 0.009	0.571 \pm 0.040	2.010 \pm 0.291
CV	12.151	21.951	7.005	14.478

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

reports of Coon *et al.* (1974) showed that supplementation of chicks diet with glycine produced increases in serum glycine and serine, while supplementation with serine produced increases in plasma serine levels but was not affected by glycine or serine supplementation indicating that synthetic glycine regulates serine and glycine metabolism in chicks.

4.3.4 Proline compositions of sampled feedstuffs

The proline content of sampled feedstuffs at different locations in Nigeria are presented in table 4.19. Their mean values ranged from $0.048 \pm 0.020\%$ recorded for CAM to $2.157 \pm 0.108\%$ recorded for GNC, while the CV between different sample means were less than 10% for DBSG and PKC, but greater than 10% for CAM and GNC. Proline concentration in this study agreed with the report of Maneemegalai and Prasad (2011) for GNC but less than those reported by Onwudike (1986) for PKC. This could be attributed to processing methods with modern machines to extract primary products, which could affect the by-products nutrient composition. Again the mean values reported by AminoDat (2010) for proline in DBSG (2.30%), PKC (0.47%) and GNC (1.89%) were similar to the mean values reported in this study.

According to Austic (1976), proline satisfies by a narrow margin the criterion for dietary essential of chicks. It has been estimated that the chick synthesized 80 – 90% of the total proline requirement for growth since metabolism of arginine, ornithine and glutamic acid gives rise to proline. Indeed cellular metabolism of glutamine and proline are closely interrelated, since they could be inter-converted with glutamate and ornithine via the mitochondrial pathway involving pyrroline-5-carboxylate (Bertalo and Burrin, 2008).

Table 4.19: Proline content of sample feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	2.095	0.059	0.455	2.102
Sample 2	2.282	0.043	0.467	1.889
Sample 3	2.263	0.076	0.448	2.099
Sample 4	2.093	0.027	0.495	1.870
Sample 5	2.050	0.033	0.479	1.388
Mean \pm SD	2.157 \pm 0.108	0.048 \pm 0.020	0.469 \pm 0.019	1.870 \pm 0.291
CV	5.007	41.667	4.051	15.561

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

4.3.5 Alanine content of sampled feedstuffs

Alanine content of the sampled feedstuffs at different locations in Nigeria are shown in table 4.20. The mean values of sampled materials ranged from the $0.058 \pm 0.018\%$ recorded for CAM to $1.708 \pm 0.226\%$ recorded for GNC. The CV between different samples was less than 10% for PKC but greater than 10% for the other samples. The alanine content of GNC in this study agreed with the value reported by Maneemegalai and Prasad (2011) but is less than the value reported by Babiker (2012). The alanine mean value of PKC in this study at 0.56% was less than the value reported by Onwudike (1986) but higher than the value reported by Othman *et al.* (2013). Similarly, the alanine mean value of DBSG at 1.48% was higher than the value reported by Donkoh and Attokotoku (2009). Again, the reference values reported by AminoDat (2010) for DBSG (1.42%), PKC (0.55%) and GNC (1.74%) were similar to the mean values of 1.48, 0.56 and 1.71% recorded respectively in this study.

Alanine can be manufactured in the body from pyruvate and branched chain amino acids such as valine, leucine and isoleucine. It is the most commonly produced by reductive amination of pyruvate. Since transamination reactions are readily reversible and pyruvate pervasive, alanine can easily be formed and thus has close link to metabolic pathways such as glycolysis, gluconeogenesis and the citric acid cycle. It also arises together with lactate and generates glucose from protein via the alanine cycle (Nelson and Cox, 2005).

4.3.6 Aspartic acid contents of sampled feedstuffs

Aspartic acid composition of sampled feedstuffs DBSG, CAM, PKC and GNC at different locations in Nigeria are presented in table 4.21. The mean values ranged from $0.084 \pm 0.016\%$ recorded for CAM to $5.006 \pm 0.795\%$ recorded for GNC, with CV calculated between different feedstuff samples being less than 10% for DBSG and PKC

Table 4.20: Alanine content (%) of sample feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	2.108	0.075	0.525	1.863
Sample 2	1.513	0.054	0.564	1.715
Sample 3	1.044	0.080	0.549	1.881
Sample 4	1.734	0.040	0.599	1.757
Sample 5	0.988	0.043	0.569	1.324
Mean \pm SD	1.477 \pm 0.472	0.058 \pm 0.018	0.561 \pm 0.027	1.708 \pm 0.226
CV	31.957	31.034	4.813	13.232

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

Table 4.21: Aspartic acid composition of sample feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	1.678	0.099	1.082	5.618
Sample 2	1.708	0.084	1.085	5.144
Sample 3	1.558	0.092	1.074	5.520
Sample 4	1.766	0.057	1.199	5.105
Sample 5	1.449	0.086	1.124	3.643
Mean \pm SD	1.632 \pm 0.127	0.084 \pm 0.016	1.113 \pm 0.052	5.006 \pm 0.795
CV	7.782	19.048	4.672	15.881

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

but greater than 10% for CAM and GNC. GNC as the only oil seed cake among the samples recorded 5.00% aspartic acid, while that of PKC was 1.11% and less than the value reported by Onwudike (1986) and Mohd *et al.* (2013). The aspartic acid value for GNC in this study was higher than those published by Maneemegalai and Prasad (2011), while the DBSG values at 1.63% was also higher than those reported by Donkoh and Attoh-kotoku (2009). Again, AminoDat (2010) reported reference aspartate values of 5.1, 1.06, and 2.03% for GNC, PKC and DBSG respectively, indicating similarity with our values in all samples except for DBSG that recorded a lower mean value. However, according to Porthmouth (1978) the poultry requirement for this amino acid ranges between 0.8 – 0.9%.

Aspartic acid is believed to be a variety of amino acids since asparagines and glutamine are synthesized from aspartic acid as their precursor. It is present in several proteins and functions in the form of neurotransmitter having a tendency to exist within the central nervous system (Aiello and Mays, 1998). With glutamic acid, it plays the important role as general acids in enzyme active centers, as well as maintaining solubility and ionic character of proteins (TBP, 2003).

4.3.7 Glutamic acid content of sample feedstuffs

Glutamic acid composition of sampled feedstuffs DBSG, CAM, PKC and GNC at different locations in Nigeria are presented in table 4.22. The mean values ranged from $0.172 \pm 0.027\%$ recorded for CAM to $8.162 \pm 1.268\%$ recorded for GNC, with CV between DBSG and PKC samples being less than 10%, while CAM and GNC values were higher than 10% indicating wide variation in values of individual samples of CAM and GNC. Onyimba *et al.* (2014) reported a much lower glutamic acid value of 2.8% in DBSG in agreement with Porthmouth (1978). However, the 5.27, 8.45 and

Table 4.22: Glutamic acid content (%) of sample feedstuff

<i>Sample</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Sample 1	4.992	0.159	2.388	9.148
Sample 2	4.998	0.182	2.452	8.446
Sample 3	4.694	0.184	2.401	8.921
Sample 4	4.823	0.133	2.692	8.322
Sample 5	4.277	0.203	2.512	5.976
Mean \pm SD	4.757 \pm 0.297	0.172 \pm 0.027	2.489 \pm 0.124	8.162 \pm 1.268
CV	6.243	15.698	4.982	15.535

DBSG = dry brewers spent grain, CAM = cassava meal, PKC = palm kernel cake, GNC = groundnut cake, S D = standard deviation, CV = coefficient of variation, SEM = standard error of mean.

2.50% reference glutamic acid values reported by AminoDat (2010) are similar to the values reported in the present study indicating that GNC and DBSG are rich in this amino acid. Misner (2015) reported that glutamic acid or its ionic forms, glutamate and glutamine are underrated beneficial amino acids found in most high protein feeds. Although they are regarded as non-essential amino acids, they play important roles during exercise demand. Glutamic acid is biosynthesized from a number of amino acids including ornithine and arginine and is reported to be a common excitatory neurotransmitter in the central nervous system (Misner, 2015).

4.4 Quality Scores of the Amino Acids Contents of the Sampled Feedstuffs

Protein quality evaluations aim to determine the capacity of food protein sources and diets to meet the protein and essential amino-nitrogen requirements in order to satisfy the metabolic needs for amino acids and Nitrogen (FAO, 2013). Over the years many protein scores have been developed for evaluating the quality of animal feed sources. Scores such as essential amino acids indices comprising chemical scores (essential amino acid of the sample / essential amino acid of the whole egg x 100), chemical score to crude protein ratio and total essential amino acid to crude protein content ratio among others have been used to evaluate the quality of non-conventional feedstuffs (Sogbesan and Ugwumba, 2008). However, the nutritive value of proteins depends on their capacity to produce nitrogen and amino acids in adequate amounts to meet the requirements of livestock. In this study three simple scoring techniques, qualitative, quantitative and chemical partitioning of the amino acids profile of the sampled feedstuffs was adopted.

In the qualitative method, the feedstuffs were evaluated on whether they are rich (1.0% and above), moderate (0.5 – 0.9%), low (0.1 – 0.4%) and poor (below 0.1%) in their individual amino acids content. For the quantitative scoring, the method previously

described by Ogbuewu (2012) was used to determine values such as total and percentage total essential and non-essential amino acids among others. For the chemical scoring, amino acid chemical characteristics such as acidity, basicity, neutrality, polarity and sulphur content among other where employed to partition the feedstuffs.

4.4.1 Qualitative scores of amino acids profiles of the sampled feedstuffs

Table 4.23 shows the qualitative scores of the amino acids profiles of the sampled feedstuffs. The results showed that GNC was the richest protein source of all the sampled feedstuffs. It was rich in all the essential amino acids with exception of methionine on it scored a moderate content. This means that Nigerian GNC based diets will need to be supplemented with synthetic methionine. In addition the GNC samples were also rich in all the evaluated non-essential amino acids except cysteine on which it returned a moderate score. It would seem from these results that Nigerian GNC amino acids profile may be limited by its content of sulphur amino acids.

The second richest amino acids source from the present study was DBSG, which was rich in four essential amino acids (leucine, valine, phenylalanine and arginine). The feedstuff was specifically low in its methionine and histidine contents. The implication of these results is that Nigerian DBSG based diets will need to be supplemented with lysine, methionine and to some extent threonine for them to drive optimal performance in monogastric animals (Belandeau and Le Bellego, 2004). Smith (1990) observed that GNC is very palatable and that the quality of its protein is good ranking it close to that of soybean meal, but however poor in lysine and methionine. Ranjhan (1999) and Ovie (2007) also reported that GNC is deficient in sulphur amino acids and lysine although in the present study feedstuff was scored rich in lysine. Again, the Nigerian DBSG was found to be rich in all the non-essential amino acids except for cysteine and glycine that

Table 4.23: Qualitative scores of amino acids profile of the samples feedstuffs

<i>Amino acid</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
(a)Essential				
Methionine	L	P	L	L
Lysine	M	P	L	R
Threonine	M	P	L	R
Isoleucine	M	P	L	R
Leucine	R	P	M	R
Valine	R	P	M	R
Histidine	L	P	L	R
Phenylalanne	R	P	M	R
Arginine	R	P	R	R
(b) Non-essential				
Cysteine	L	P	L	M
Glycine	L	P	M	R
Serine	R	P	M	R
Proline	R	P	L	R
Alanine	R	P	M	R
Aspartic acid	R	P	R	R
Glutamic acid	R	L	R	R

Legend: P = Poor (less than 0.1%); L = Low (0.1 – 0.4%); M = Moderate (0.5 – 0.9%); R = Rich (more than 1.0%).

were low and moderate respectively, indicating that this feedstuff is limited specifically by the sulphur amino acids. The relative rich amino acid profile of the DBSG could be explained by the fact that it is the end-product of fermentation processed and therefore contains dead micro-organism such as yeast which has the capacity to enrich its amino acid profile (Robertson *et al.*, 2012). Thus, even though it is basically a fibrous material it has been shown to have better available protein and amino acids than maize and wheat offal (Aletor, 1998; Babatunde, 1989). However, its high fiber content tends limit its nutritional value and metabolizable energy compared to the original grains (Onifade *et al.*, 1998). As a result DBSG is currently used in feeding older broilers and layers in the Nigerian poultry industry.

According to the present results, PKC was scored rich only in arginine and moderate in isoleucine, leucine, valine and phenylalanine and low in other essential amino acids. From these results, Nigerian PKC cannot be regarded as a protein source for monogastric animals since it requires fortification with synthetic sources almost all the essential amino acids for it to drive optimal performance in monogastric animals like poultry. Its use as a protein source by many small holders pig farmers in southeastern Nigeria (Okoli *et al.*, 2009), needs to be evaluated, especially when combined with cattle blood.

Again, the Nigerian PKC was found to be rich in non-essential aspartic acid and glutamic acid, while being moderate in glycine, serine, proline and alanine. The fact that PKC is specifically low in the sulphur amino acids, lysine and threonine confirms its limitations in poultry production unless subjected mixtures of PKC and cassava root pulp to solid state fermentation using *A. niger* as inoculant and improved the crude protein values of the mixtures from about 10% to 19%. However, the

information on the amino acids profile of the product is not available to determine if the quality scores were also improved.

The CAM was score poor in all the amino acids evaluated except glutamic acid for which it scored low. This poor amino acid profile of CAM is in agreement with the results of earlier studies by Eke (1998) and more recently Omire (2012). These indicate that diets formulated with synthetic amino acids (Ogunjobi and Ogunwole, 2010). However, the proteins of cassava tubers have been shown to be relatively high in aginine, glutamine and alanine (Omeire, 2012).

4.4.2 Quantitative scores of the amino acids profiles of sampled feedstuffs

The quantitative scores of the amino acid profiles of the sampled feedstuffs are shown in table 4.24. On mean total amino acids bases PKC contained 47.92% essential amino acids while GNC contained 43.24%, DBSG (42.21) and CAM (41.19%). Again, the percentage values of non-essential amino acids in the feedstuffs ranged from 50.26 in CAM to 55.09% in DBSG. The values of total essential amino acids ranged from 45 to 49.76% while non-essential amino acids ranged from 50.24 to 54.25% (Omoyemi *et al.*, 2015) similar to the present results. Similarly, our values for percentage essential amino acids without histidine and arginine at 28.50% in GNC to 35.22% in DBSG were lower than the range of 42.83 – 46.45% reported for Nigerian leafy vegetables (Omoyemi *et al.*, 2015). These values indicate generally that the proteins in the feedstuffs are of high quality even though lower or poor in quantity in PKC and CAM.

4.4.3 Chemical scores of the amino acids profiles of the sampled feedstuffs

The chemical scores of the amino acids profiles are shown in table 4.25. Chemical classification of amino acid helps nutritionists to break them down into smaller bytes-

Table 4.24: Quantitative scores of the amino acids compositions of the sampled feedstuffs

<i>Parameters</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Mean total and (%)	8.62 ± 1.194	0.31 ± 0.085	4.02 ± 0.164	12.41 ± 1.731
essential amino acids	(42.21)	(41.19)	(47.92)	(43.24)
Mean total and (%)	12.34 ± 1.041	0.46 ± 0.080	6.05 ± 0.295	21.78 ± 3.230
non-essential amino acids	(55.09)	(54.75)	(50.26)	(54.42)
Mean total and (%)	7.91 ± 0.876	0.29 ± 0.080	3.77 ± 0.152	11.39 ± 1.585
content of essential amino acids without Histidine + Arginine	(35.22)	(34.65)	(31.35)	(28.50)
Histidine + Arginine				
Histidine + Arginine	1.56 ± 0.115	$0.055 \pm$	1.99 ± 0.143	5.91 ± 0.914
content (%)		0.013		

Table 4.25: Chemical scores of the amino acids compositions of the sampled feedstuffs

<i>Parameters</i>	<i>DBSG</i>	<i>CAM</i>	<i>PKC</i>	<i>GNC</i>
Mean total and (%)	8.58 ± 1.280	0.27 ± 0.096	3.37 ± 0.141	10.12 ± 1.459
aliphatic amino acids content of feedstuffs	(38.10)	(31.36)	(28.05)	(25.30)
Mean total and (%)	20.35 ± 2.058	0.74 ± 0.166	9.85 ± 0.457	32.68 ± 4.779
neutral amino acids content of feedstuffs	(90.68)	(87.52)	(67.38)	(81.65)
Mean total and (%)	6.39 ± 0.412	0.26 ± 0.039	3.60 ± 0.175	13.17 ± 2.062
acidic amino acids content of feedstuffs	(28.55)	(31.04)	(29.95)	(32.86)
Mean total and (%)	2.34 ± 0.162	0.10 ± 0.017	2.42 ± 0.180	7.35 ± 1.054
basic amino acids content of feedstuffs	(10.50)	(12.05)	(20.13)	(18.36)
Mean total and (%)	0.87 ± 0.089	0.03 ± 0.008	0.47 ± 0.030	0.94 ± 0.109
sulphur amino acids in feedstuffs	(3.89)	(3.27)	(3.91)	(2.35)
Mean total and (%)	9.57 ± 1.292	0.31 ± 0.107	3.80 ± 0.169	13.04 ± 1.844
non-polar amino acids content of feedstuffs	(42.55)	(36.42)	(32.41)	(32.59)
Mean total and (%)	10.99 ± 0.708	0.45 ± 0.066	7.21 ± 0.397	24.20 ± 3.603
polar amino acids content of feedstuffs	(48.54)	(54.37)	(59.88)	(60.13)
Mean % methionine in total sulphur amino acids	(49.34)	(52.27)	(41.35)	(55.41)

size so that one can better understand their functions in livestock rations. This helps to overcome the problem of adding excess essential amino acids in the diet (Kidd *et al.*, 2001; Baker *et al.*, 2002), thereby reducing cost. The percentage mean total neutral amino acids in the feedstuffs ranged from 67.58% recorded in PKC to 90.68% recorded in DBSG indicating that these are the most abundant amino acids in the feedstuffs. This is agreement with the reports of Adeyeye (2013) from a study of amino acids quality of avian eggs and Omoyemi *et al.* (2015) on the amino acids quality of 10 commonly eaten indigenous leafy vegetables of southwestern Nigeria. The second most abundant amino acids were the acidic amino acids which returned a range of 28.55% (DBSG) to 32.86% (GNC). The least abundant amino acids were the basic amino acids that also ranged from 10.50% in DBSG to 20.13% in PKC. Again, these results are in agreement with the reports of Adeyeye (2013) and Omoyemi *et al.* (2015).

Acidic amino acids possess a second carboxyl group in the substituent R position with a net negative charge at a pH 7.0 and include aspartic acid and glutamic acids. Basic amino acids have an additional basic group and their R groups have a net positive charge at pH 7.0. They include lysine, arginine and histidine. The neutral amino acids on the other hand are asparagine, cysteine, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine and tryptophan (NCBI, 2015).

Sulphur amino acids analyzed for in this study are methionine and cysteine and their values in the total amino acids of the feedstuffs ranged from the 2.35% recorded in GNC to the 3.91% recorded in PKC. However, the percentage methionine in the total sulphur amino acids was highest for GNC (55.41%) and lowest for PKC (41.35%). These values are slightly higher than those obtained by Adeyeye (2015) in leafy vegetables but lower than the values for avian eggs (Omoyemi *et al.*, 2013).

The primary limiting amino acids for poultry are the sulphur amino acids especially methionine which are essential because of their prominent tasks in primary and secondary metabolisms (Saito, 2004; Droux, 2004). Usually a basal diet considered deficient in sulphur amino acid is supplemented with graded levels of methionine and response determined. The percentage aliphatic amino acids in the total amino acids of the feedstuff ranged from the 25.30% recorded in GNC to the 38.10% recorded in DBSG. The aliphatic R groups are non-polar and hydrophobic and increases hydrophobically with increasing number of carbon atoms in their hydrocarbon chain (NCBI, 2015). These include leucine, isoleucine, glycine, valine and alanine (Takei *et al.*, 2006).

Polar amino acids are those with side chains that prefer to reside in an aqueous environment. For this reason they are generally found exposed on the surface of a protein (Betts and Russel, 2003). Six amino acids have such side chains that are polar but not charged. They include serine, threonine, cysteine, asparagine, glutamine and tyrosine. In the present study the percentage of polar amino acids in the total amino acids ranged from the 48.54% recorded in DBSG to the 60.13% in GNC, while for non-polar amino acids, the values were the reverse. This finding indicates that feedstuffs containing high quality proteins would also yield higher percentage values of polar amino acids.

Generally, wide variations of critical concern were observed in all the parameters evaluated in this study. Coefficient of variations in values from different samples were generally more than 5% indicating the need increased sample size in future studies. Usually feedstuffs with wide variations in their nutrient compositions are of lower values than those with narrow variation. Nutrient compositions of all feedstuffs vary because of origins, ecological conditions, different varieties, age at harvest, storage conditions and

processing methods (Jane, 2011). Thus, the actual amino acids profiles of many alternative feedstuffs such as the ones studied here are usually debatable due to wide variations in nutrient compositions (Brand *et al.*, 1997). The need to standardize the nutrient composition data of some of the locally available Nigerian feedstuffs is therefore imperative since nutritionists must learn to accommodate these variations when using such feedstuffs for ration formulation.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study shows that all the sampled alternative local feedstuffs were high in dry matter content. The mean values obtained in this study could be used as reference values for the country since their coefficients of variation were less than 1.00%, with reference standard value of dry matter ranging from 88.5 – 93.5 % for Nigerian feedstuffs. Moreso, wider variations were demonstrated in crude protein contents of the samples as is, than that of crude protein contents standardized to a dry matter content of 88%, indicating the need to adopt the standardized values as the reference values for the country.

GNC was the richest protein source of all the sampled feedstuffs. It was rich in all the essential amino acids, except the sulphur amino acids methionine and cysteine indicating that Nigerian GNC based diets will need to be supplemented with synthetic methionine. DBSG, was rich in the essential amino acids, leucine, valine, phenylalanine and arginine and was specifically low in its methionine and histidine content indicating that Nigerian DBSG based diets will need to be supplemented with lysine, methionine and to some extent threonine for them to drive optimal performance in monogastric animals.

PKC was rich only in arginine and moderate in isoleucine, leucine, valine and phenylalanine and low in other essential amino acids. Therefore, Nigerian PKC cannot be regarded as a protein source for monogastric animals since it requires fortification with synthetic sources of almost all the essential amino acids for it to drive optimal performance in monogastric animals like poultry. CAM scored poor in all the amino acids evaluated except in the non-essential amino acid, glutamic acid for which it scored

low, indicating that CAM based diets should be supplemented with all the synthetic amino acids required at different stages of development, especially the limiting amino acids.

The Nigerian feedstuffs sampled were found to be abundant in neutral amino acids followed by acidic amino acids, while the least abundant amino acids were the basic ones. Sampled feedstuffs collected from different parts of Nigeria showed high variations in the parameters studied.

5.2 Recommendation

From the findings in this study, the following recommendations are made;

- Practical feeding trials with monogastric livestock should be carried out to validate these results, since the study was limited to laboratory analysis.
- Measures proportionate to the risk of using feedstuffs with wide variations in their protein contents should be applied in using these feedstuffs for feed formulation since national standards are not yet available. These may include adopting the lower mean ranges of analytical values and analysis of samples from different locations before use.
- There is need to harmonize industrial extractions processes that yield most of the feedstuffs analyzed in this study in order to develop appropriate regulations that assign quality grades to the feedstuffs. For examples, quality grades could be assigned to products of known industrial processes according to protein and amino acids values derived from the analyses of these products.
- The standardized mean referenced values from this study are recommended for the poultry industry since they will aid in the addressing problem of wide variations in nutrient values of locally available feedstuffs.

- Feedstuff quality assurance schemes in Nigeria should encompass ingredient quality analysis covering all aspects of production and processing methods of such ingredients in order to develop reference quality scores.
- There is the need to integrate regulatory frame works of regulatory agencies such as Standards Organization of Nigeria (SON), National Institute of Animal Science (NIAS), National Agency for Food and Drug Administration and Control (NAFDAC) and Veterinary Council of Nigeria (VCN) so that common enforcement goals rather than varied objectives and organizational control of feed quality are achieved for the country.

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