NUTRITIONAL EVALUATION AND IMPROVEMENT OF SHEA NUT *(Vitellaria paradoxa,* Gaertn.) MEAL FOR POULTRY

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ABSTRACT

Shea nut *(Vitellaria paradoxa,* Gaertner or *Butyrospermum parldi,* Kotschy) meal, a solid residue from the shea fat industry, is available in large quantities in West Africa. Six collected samples (2 non-industrial and 4 industrial or expeller meals) had a range of nutrient compositions [g/kg dry matter (DM) basis] of crude protein (CP) (103.9 - 143.6) with low concentrations of lysine (30.0 - 41.7 g/kg CP) and methionine plus cystine (33.4 - 39.7 g/kg CP); ether extract (101.4 - 394.8), ash (46.9 - 75.6) and total non-starch polysaccharides (NSP) (305.9 - 396.6) as well as gross energy (22.8 - 27.4 MJ/kg DM). A major part of the variability was due to the large differences in the amounts of residual fat. A broiler bioassay indicated that shea fat had a low metabolisable energy (22.0 MJ/kg DM) compared to soybean oil (39.8 MJ/kg DM). Also, two broiler bioassays showed that the shea nut meals had low energy availability as indicated by their low TMEn values (12.6 - 15.5 MJ/kg DM). Another broiler assay showed that the mean nutrient utilisation efficiencies of the meals were relatively low for dry matter (0.645), crude protein (0.585) and lipid (0.665). The major anti-nutritive factors identified were saponins (4.0 - 6.3 g/kg DM) and tannins (35.1 - 201.3 g/kg DM). There was no evidence of theobromine being present in any of the six samples. There was a strong negative correlation ($P<0.05$) between dry matter metabolisability and total NSP content of the meal. A broiler growth experiment showed that there was a quadratic decline ($P<0.001$) in feed intakes, growth rates and feed conversion efficiencies when shea nut meal was included at increasing dietary levels in nutritionally complete diets. The untreated shea nut meal can be fed at 20 g/kg in a broiler diet. However, the treatments of the shea nut meal by hot water extraction, natural fermentation with added polyethylene glycol, and fermentation using either *Aspergillus niger* or a mix of *Bacillus subtilis* and *Bacillus licheniformis* gave improvements ($P<0.001$) in the growth

rates of broiler chickens when incorporated at 90 g/kg in the diets. A fungal fermentation using *A. niger* and *Ceriporiopsis subvermispora* was further investigated. The treated meals gave increased ($P<0.001$) broiler growth rates compared to the untreated meal when incorporated at 90 g/kg. Combinations of both fungal organisms or with polyethylene glycol gave no further improvement ($P>0.05$) in growth performance. Another bioassay that involved shea nut meal fermentation using *A. niger* and *C. subvermispora* confirmed their potential in improving its nutritional value to broiler chickens. The experiment also showed that the fungal fermentation process should, in preference, be completed in a closed container in order to avoid contamination by other organisms. The *A. niger* fermentation process resulted in substantial reductions in the concentrations of saponin (86.7%), total proanthocyanidins (34.5%) and hydrolysable tannins (52.9%). Another broiler growth experiment that examined the effect of defatted shea nut meal fermentation using *A. niger* showed there was no significant difference in growth performance between 'as-received' and defatted meals. *A. niger* fermentation of shea nut meal improved ($P<0.001$) the growth rates of broiler chickens when the fermented meals were included at 100 g/kg in the diets in comparison to the unfermented meal, but the growth rates obtained were lower ($P<0.001$) than for the birds fed the control diet. It was evident from this study that the untreated shea nut meal has a low nutritive value that can be improved through fermentation, particularly using *A. niger*. The nutritional improvement of shea nut meal achieved in this study still falls far short of what is expected for it to become valuable for the poultry feed industry. These fermentation methods using *A. niger* or *C. subvermispora* require further improvements to provide satisfactory feed products.

DECLARATION

This is to confirm that the work herein reported has been carried out solely by the author. However, information obtained from the work of others was duly acknowledged in the references. Also, no part of this work has been submitted for any other degree.

Herbert K. Dei

DEDICATION

This thesis is dedicated to the memory of my mother (Akpodoe Gogovi) and grandfather (Wode Gogovi).

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PUBLICATIONS FROM THESIS

A. JOURNAL ARTICLES

1. Dei, H.K., Rose, S.P. and Mackenzie, A.M. 2006. Apparent metabolisable energy and digestibility of shea *(Vitellaria paradoxa)* fat, cocoa *(Theobroma cacao)* fat and soybean oil in broiler chicks. *British Poultry Science* 47 (5): 607-612.

2. Dei, H.K., Rose, S.P. and Mackenzie, A.M. 2007. Shea nut *(Vitellaria paradoxa) meal as a* feed ingredient for poultry. *World's Poultry Science Journal* 63 (4): 611624.

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B. CONFERENCE PAPERS

1. Dei, H.K., Rose, S.P. and Mackenzie, A.M. 2006. Apparent metabolisable energy and digestibility of shea fat in broiler chicks. *British Poultry Abstracts* 2 (1): 35-37.

2. Dei, H.K., Rose, S.P. and Mackenzie, A.M. 2006. Variability of nutritive value of shea nut *(Vitellaria paradoxa)* meal for poultry. *World's Poultry Science Journal* 62 (supplement): 340-341.

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5. Dei, H., Rose, P. and Mackenzie, S. 2007. Variability of dietary energy of shea nut *(Vitellaria paradoxa)* meal for poultry. *Proceedings of the Ie European Symposium on Poultry Nutrition (pp. 15-18), 26-30 August 2007,* Strasbourg, France.

LIST OF ABBREVIATIONS

AME	Apparent metabolisable energy
ANOVA	Analysis of variance
BSA	Bovine serum albumin
CP	Crude protein
DM	Dry matter
EE	Ether extract
NFE	Nitrogen-free extract
NDF	Neutral detergent fibre
NSP	Non-starch polysaccharides
MJ/kg	Megajoule per kilogram
P	Probability
FFA	Free fatty acid
g/kg	Gram per kilogram
g/b/d	Gram per bird per day
°C	Degrees Celsius
%	Percentage
R	Correlation coefficient
R^2	Coefficient of determination
RDF	Residual degrees of freedom
SED	Standard error difference
SD	Standard deviation
SNM	Shea nut meal
Syn.	Synonym
TMEn	True metabolisable energy corrected for nitrogen

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CHAPTER ONE

GENERAL INTRODUCTION INTRODUCTION

The shea tree *[Vitellaria paradoxa* C.F. GAERTNER, syn. *Butyrospermum parkii* (G. Don) KOTSCHY] also called `Karite' in French is the most common tree found in the Sahelian parklands over very large areas of Africa (Leakey, 1999). Nut production across the West African sub-region is estimated at 250,000 tonnes annually (Pesquet, 1992), but has been increasing as a result of using the fat extracted (shea butter) as a cocoa butter substitute in chocolate, confectionery and cosmetics (Hall *et al.*, 1996). At the moment, the residue variously referred to as "shea nut meal (SNM)" or "shea nut cake" or "shea butter cake" has no economic value. It is mainly disposed of through incineration that can cause some environmental pollution. Hence, there is a need to find alternative uses for this agro-industrial by-product.

The world's growing population, the increasing demand for poultry products, and the shortage of major feed ingredients may require utilisation of less conventional raw materials including shea nut meal in poultry feeds. A major constraint to increasing poultry productivity in Ghana is the scarcity and fluctuating quantity and quality of the year-round supply of conventional feedstuffs such as maize and fishmeal. In order to meet the projected high demand for poultry products, it is imperative that there is better utilisation of non-conventional feed resources (e.g. agro-industrial by-product) which do not compete for use directly in the human food chain (Buamah, 1992). There is also a need to improve the nutritional quality of agro-industrial by-products like `shea nut meal', which will not only give a continued supply of inexpensive poultry feed ingredient but also can bring economic benefits to rural women who are engaged in shea fat production and ensure food and nutrition security at the household level.

Preliminary studies have shown that shea nut meal contains moderate amounts of crude protein (approximately 16%) and residual fat (approximately 13%) that can provide some nutrients in poultry diets (Morgan and Trinder, 1980; Atuahene *et al.,* 1998). Presently, there is a relative lack of detailed information on nutrient composition and nutrient availability of shea nut meal samples produced in Ghana and how processing methods (i.e. traditional or expeller) affect these variables. There is, therefore the need to evaluate and explain the variability of nutrient supply in the different types of shea nut meal presently available in Ghana. The biological availability of the economically important nutrients provided by shea nut meal is poorly defined. There is a need to determine and explain the availability of the main nutrients and to understand how different processing methods may alter nutrient availability.

Shea fat is an important, but highly variable, component of shea nut meal. Feeding of fat (e.g. soybean oil) is a common method to increase the energy density of poultry diet. It also provides essential fatty acids. It is envisaged that shea fat could exhibit low digestibility and metabolisable energy value when fed to poultry due to the saturated nature of the fat (Badifu, 1989). In that case, the fat content of the shea nut meal could be a contributory factor to its poor utilisation by poultry. The metabolisable energy value of shea fat as a dietary fat for poultry has not been documented. The lack of detailed understanding of the available energy value of this fat has resulted in it being difficult to reliably estimate the energy contribution of the variable amounts of residual fat that occur in different samples of the shea nut meal. An understanding of the metabolisable energy value of variable inclusion levels of fat in poultry feeds is required to enable the efficient use of shea nut meal and possibly shea fat in poultry diets.

The few experiments that have examined growth responses of poultry to shea nut meal diets (Adeogun *et al.,* 1989; Atuahene *et al.,* 1998; Olorede *et al.,* 1999) have found poor and variable growth performances. The poor utilisation of shea nut meal by poultry in preliminary experiments has been attributed variously to the presence of anti-nutritive factors such as saponins, tannins and theobromine (Annongu *et al.,* 1996a; 1996b; Atuahene *et al.,* 1998). However, there is a lack of data that convincingly demonstrates the harmful effects of a particular anti-nutritive factor. Hence, there is a need to understand whether anti-nutritive factors limit the nutrient utilisation from diets containing shea nut meal. Second, this information would underpin experimental strategies to investigate whether different processing methods or further treatment of shea nut meal can reduce the concentration of the biologically important anti-nutritive factors. The adverse effects of these anti-nutritive factors could be counteracted through various nutritional strategies including fermentation, enzyme supplementation, tannin binding agents, or irradiation procedures. Nutritional strategies tried so far have been natural fermentation and hot water treatment with some degrees of success. However, these approaches are limited in scope and need to be examined in more detail.

1.2 OBJECTIVES

The overall objectives of this PhD project were:

- To determine the digestibility and metabolisable energy value of shea fat as a dietary fat for poultry.
- To determine and explain the variation in nutrient composition of different Ghanaian shea nut meal samples.
- To determine and explain the variation in nutrient availability and energy availability of different Ghanaian shea nut meal samples.

- To test the hypothesis that the growth performance of poultry (broiler chickens) fed different dietary levels of shea nut meal can be predicted by the variation in the available nutrient content of the shea nut meal.
- To determine the content of anti-nutritive factors in shea nut meal and to describe and explain their biological activity in growing poultry.
- To test the hypothesis that the content of the biologically important anti-nutritive factors in shea nut meal can be reduced by further treatment of the processed meal and that these procedures can improve the growth performance and health of growing poultry.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Shea trees *[Vitellaria paradoxa*, C.F. Gaertner., syn. *Butyrospermum parkii* (G. Don) Kotschy] belong to the family Sapotaceae. It is also called `Karite' in French. It is the most common tree *(Figure 2.1a, Figure 2.1b)* found in the Sahelian parklands over a very large area of Africa (Leakey, 1999). A mature tree is about 5 m in height whose productivity in terms of quantity of fruit (15-50 kg of fresh fruit per year) and the quality of the kernel (free acidity) varies considerably (Hall *et al.,* 1996). It grows naturally in the wild in the dry Savannah belt of West Africa. It is present in Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Democratic Republic of Congo, Ghana, Guinea, Guinea Bissau, Mali, Niger, Nigeria, Senegal, and Togo (Hall *et al.,* 1996). In Ghana, the shea nut trees occur extensively in the Guinea Savannah and less abundantly in the Sudan Savannah (Adomako, 1985). The trees are found over almost the entire Northern Ghana, over about 77,650 square kilometres covering Western Dagomba, Nanumba, Southern Mamprusi, Eastern and Western Gonja, Lawra, Tumu and Wa. Eastern Gonja has the densest stands. There are also sparse areas of shea cover in other parts of Ghana such as Brong-Ahafo, Ashanti, Volta and the Eastern Region. Across the West African sub-region, the trees are conserved by the local people because of their high social, economic and ecological value (Hall *et al.,* 1996). The time taken by the tree to reach fruit-bearing age (10-25 years) is a large disincentive to commercial cultivation of the tree (Hall *et al.,* 1996). Nut production is about 3-6 kg of dry kernels per tree, which varies considerably between trees and years (Boffa *et al.,* 1996) since the trees are exposed to the vagaries of the environment such as bush fires in the wild. The nuts are collected from the wild, mainly by local women and children either for sale or domestic use.

The edible fat (i.e. shea fat) from the nuts is called `shea butter' and is the main dietary fat of the indigenous people from these regions. It is used internationally in the production of chocolate, sweets, baking fat and cosmetics. At the national or sub-regional level, only a few estimates of nut production are available. Adomako (1985) estimated total shea nut production in northern Ghana at 135, 000 t per annum, and indicated that about 70% of the nuts collected were processed and consumed locally. The amounts of nuts collected and processed have been increasing over the past decade as result of using the shea butter as a cocoa butter substitute (50-100 g/kg) in chocolate (Lipp and Anklam, 1998) and raw material for confectionery (Padley *et al.*, 1994) and in cosmetics (Hall *et al.*, 1996). There have been various interventions by governments (Adomako, 1985) and non-governmental organisations to increase the nut yield of the tree, and nut collection by local women as well as improving methods of fat extraction by women groups.

The edible fruit contains a kernel rich in fat. It is estimated that 10 kg of dried kernels will yield about 5 kg of shea fat (Leakey, 1999). After industrial (i.e. expeller and sometimes solvent) fat extraction, a by-product referred to as shea nut meal or cake remains. Some material is also available as a residue after non-industrial, water-based fat extraction methods. Even though large quantities of the nuts (100,000t per annum) are processed (Okai and Bonsi, 1989), the meal produced has no economic value at present in West Africa and is often incinerated, so contributing to environmental pollution. Meanwhile, the poultry industry in West Africa is facing acute shortages of conventional poultry feed ingredients with a consequent dwindling in productivity and profitability.

Shea nut meal is now receiving increased attention as a potential feed ingredient for poultry due to the increased amounts that are available. There is a need to explore the utilisation of this by-product as it represents a home-produced and potential supply of inexpensive raw material for poultry diets in West African countries. Thus the objectives

of this review were to assess the methods of production, chemical composition, poultry performance and methods of counteracting any anti-nutritive factors associated with using shea by-products in poultry diets.

Figure 2.1a: Shea trees on the range

Figure 2.1b: shea tree bearing fruits (shea nuts)

2.2 METHODS OF PRODUCTION 2.2.1

Non-Industrial

The processing of shea nuts to obtain fat is carried out either by non-industrial processing (i.e. local people using traditional methods) or by large industrial companies. Nonindustrial processing procedures may vary from one locality to another, but essentially involve the use of water for fat extraction *(Figure 2.2a, Figure 2.2b).* The nuts are roasted in a clay oven or metal pan at 150°C for 1-2 hours, depending on the moisture content, a process considered to be complete when the nuts become dark in colour, exude oil and begin to crack (Hall *et al.,* 1996). The roasted nuts are ground in a corn mill or pounded in a mortar to form a paste, which is then worked in cold water by hand so that the fat rises to the surface and can be collected. The residue left is sun-dried and stored.

Fat extraction by non-industrial methods is relatively inefficient and results in large amounts (>200 g/kg) of residual fat in the shea nut meal. The typical efficiency rates of fat extraction range between 15 and 34% of the weight of fat per kg of kernels processed, but can be improved up to 85% through interventions of appropriate technology into some of the processing stages (Hall *et al.,* 1996). Although large amounts of nuts are processed using the non-industrial methods, there are no conscious efforts to use the meal for feeding animals. Thus after the fat emulsion is collected, the residue is usually thrown away. Also, fat extraction is carried out mostly by individual households in small lots scattered over a wide geographical area. Most of the traditional processors only process nuts when the fat is required for sale or home consumption. The non-industrial sector may not presently be a reliable source of the meal for the feed industry. However, formation of co-operative groups in the processing of the nuts, as well as the use of semi-industrial methods for fat extraction (e.g. semi or fully automated), may overcome this problem as well as ensure improved uniformity of the shea nut meal.

Figure2.2a: Kneading of ground shea kernel by hand to extract fat

Figure 2.2b: Collection of fat emulsion

2.2.2 Industrial

The industrial fat extraction method involves the use of hot presses (filter, screw, hydraulic) and possibly followed by the use of chemical solvents to exract the fat. Even though higher efficiency rates can be achieved with solvent extraction of fat, the screw-press method *(Figure 2.3a, Figure 2.3b)* is predominantly the sole method used in the West African industry because of the convenience and economics of the process (Hall *et al.*, 1996). The expeller process involves preheating the nuts by means of steam and fat extraction via a screw-press machine, leaving the residue, which still contains moderate amounts of residual of the fat (100-200 g/kg). About 50,000 tonnes of shea nut meal are produced each year by companies located in the sub-region of West Africa (Okai and Bonsi, 1989), making it a reliable source with more consistent quality.

Figure 2.3a: Shea kernels on conveyor belt

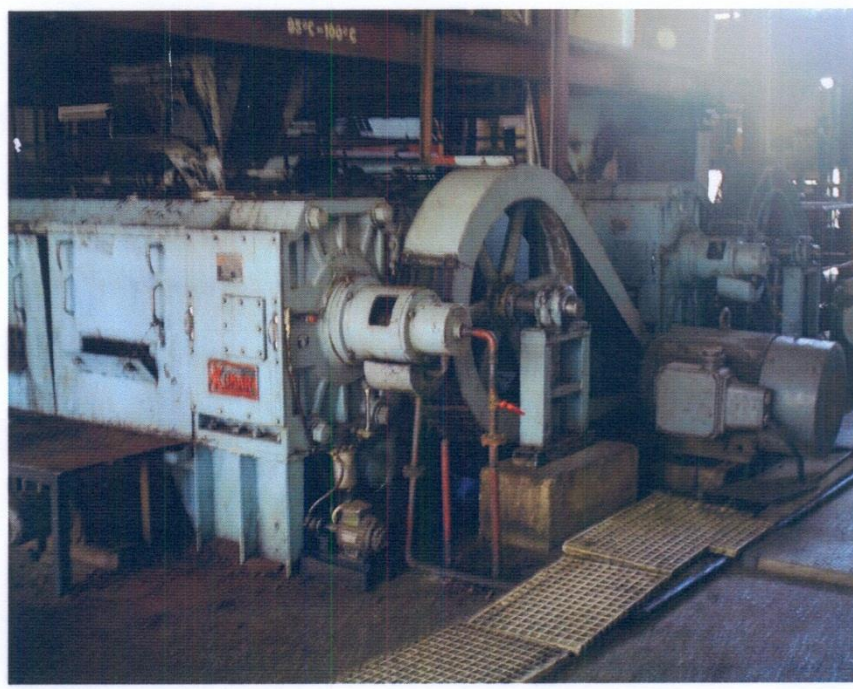


Figure 2.3b: The screw-press machine

23 CHEMICAL COMPOSITION

There are discrepancies in the reported chemical composition of shea nut meal that can be attributed to differences in processing methods, the handling of the nuts prior to processing, or seasonal effects on nut production. Differences and errors in analytical methods and techniques may also be a cause of variation (Okai *et al.,* 1995). Also, wide genetic variations have been observed in the shea tree biotypes of *Vitellaria* in the West African region (Hall *et al.,* 1996), which may vary in their nutrient compositions.

The use of a non-conventional feedstuff in poultry diets can give reasonable performance only if diets are formulated correctly or their anti-nutritive factors removed. In this regard, nutrient levels, bioavailability, and anti-nutritive factors and their effects on bird performance must all be considered in determining the usefulness of shea nut meal as a feed ingredient for poultry.

2.3.1 Macro-Nutrient Content of Shea Nut Meal

Shea nut meal has a macronutrient composition *(Table 2.1)* that is approximately similar to palm kernel meal (INRA, 2004), but has a high and variable content of crude fat. In most cases these macronutrients have been determined using proximate analytical methods that have limitations for defining its feed qualities (Morgan and Trinder, 1980). Most criticism of the proximate system of feed analysis has been focused on the crude fibre, ash and nitrogen-free extract (NFE) fractions. Ideally, protein ought to be represented by an analysis for true protein. In proximate analysis, crude protein content is calculated from the nitrogen content of the food, some of which may be of non-protein nitrogen compounds in the food. Lipids should be analysed by a method that determines truly metabolisable lipids. Some lipids extracted in the ether extract fraction such as waxes have no metabolisable energy value. Carbohydrates ought to be separated from non-nutritive lignin and partitioned into at least two major fractions: (1) fibrous carbohydrate, digestible by

symbiotic microorganisms in the digestive tracts of herbivorous animals; and (2) soluble carbohydrate, digested by enzymes secreted by the digestive system of all animals. Lignin, the major contaminant of concern, is present in both crude fibre and NFE fractions in variable amounts. Van Soest (1963) emphasised the failure of the crude fibre and NFE to give a nutritional separation of fibre, and stressed the need for a clear nutritional definition of fibre, based upon nutritional availability, exclusive of chemical methods and physical properties. Thus the high nitrogen-free extract fractions reported in shea nut meal can be misleading, since a variable proportion of the crude fibre such as cellulose, lignin and hemicelluloses as well as organic acids may be contained in the nitrogen-free extract (McDonald *et al.*, 1995). Also, NFE is the difference between the sum of all the five fractions (moisture, ash, crude protein, ether extract and crude fibre (expressed in g/kg) substracted from 1000; thus all analytical errors in the determination of the other fractions would be reflected in the NFE. NFE includes sugars, fructans, starch, pectins, organic acids, pigments, hemicellulose, cellulose, lignin and acid-insoluble ash. Apart from the cellulosic materials, the presence of latex or gum (e.g. pectins) has also been reported in shea kernels (Kershaw and Hardwick, 1981). Nevertheless, the nutrient compositions *(Table 2.1)* are indications that the meal has some nutritional potential for poultry. *Table 2.1* also shows how variable the nutrient content of shea nut meal can be; probably the residual fat content being the major cause of variability.

Table 2.1: Proximate composition of nutrients (g/kg) in shea nut meal obtained from industrial and non-industrial sources (dry matter basis)

Nutrient	[1]Greenwood (1929)	[1]Busson (1965)	[1]Jacobsberg (1977)	[1]Morgan &Trinder (1980)	Gohl (1981)	Farinu (1986)	Okai *et al.* (1994)	Atuahene *et al.* (1998)	Rhule (1999)	Olorede *et al.* (1999)
Crude protein	121-142	80-250	80-150	127-171	171	202	138	162	189	181
Ether extract	54-220	30-200	30-200	17-104	40	362	175	134	123	158
Crude fibre	53-73	60-120	60-120	108-173	120	85	138	95	75	107
Ash	60-69	50-60	50-60	65-76	76	33	53	42	48	48
Nitrogen-free extract	527-675	480-500	480-500	531-654	593	318	496	567	565	506

[1]Adapted from Hall *et al.* (1996)

2.3.1.1 Fat and fatty acids

The fat contents of different shea nut meal samples are highly variable *(Table 2.1).* This can be attributed mainly to the different efficiencies of fat extraction methods within the industry (Womeni *et al.,* 2002). Gohl (1981) indicated that the fat content of oilseed residue depends on the method used to remove the oil. The average fat content in the dry kernels ranges between 400 and 600 g/kg (Loszner, 1986; Adu-Ampomah *et al.,* 1995); but could vary widely depending on tree biotype. Other causes of variation in the fat content of the meal could be due to the methods of fat extraction, kernel preparation practices prior to fat extraction, agronomic factors, or lipolytic enzymes of the kernel itself or of microorganisms (Kershaw and Hardwick, 1981; Badifu, 1989; Tano-Debrah and Ohta, 1994).

The shea fat is made up mainly of triglycerides, which constitute 860 g/kg (Badifu, 1989) of the non-polar lipid fraction (960 g/kg of the total lipid). The rest of the non-polar lipids are sterols (100 g/kg), diglycerides (30 g/kg) and free fatty acids (10 g/kg). The triglycerides are esters of glycerol and fatty acids. The carbon atoms of the glycerol part are numbered 1-3. The fatty acids in the 1- and 3-positions are most often interchangeable but the fatty acid at 2-position is important for nutritional and functional aspects of fats (Lipp and Anklam, 1998). Twenty-one (21) triglycerides have been identified of which shea stearin (2-oleoyl-distearin) and shea olein (1-stearoyl-diolein) made up 600 g/kg of the fat (Sawadogo and Bezard, 1982). The typical fatty acid profile of shea fat obtained from different geographical locations is summarised in *Table 2.2a.* The data reveal that geographical origin seems to have an influence on the quality of the fat. According to Kershaw and Hardwick (1981) and Masters (1992), the quality of the fat produced may vary with some kernels yielding solid fat and others oil. However, all samples demonstrate the presence of the predominant fatty acids such as stearic, oleic, linoleic, palmitic and linolenic acids; of which stearic and oleic acids are the major fatty acids. According to Sawadogo and Bezard (1982), the fat contains —460 g/kg oleic acid (60% in the 2-

Position, 440 g/kg stearic acid (24% in 2-position), and —60 g/kg linoleic acid (85% in the 2-position).

The characteristic pattern of the fatty acids and occurrence of oleic acid at the 2-position of the glycerol backbone (Lipp and Anldam, 1998) seem to explain the fat's suitability for the formulation of hydrogenated products and cocoa butter equivalents. The melting range (3436°C, Tano-Debrah and Ohta, 1994) is similar to that of cocoa butter (32-35°C) which is near mouth temperature (37°C). The fatty acid structure of shea fat is also similar to that of cocoa fat; but differs greatly from that of soybean oil *(Table 2.2b)* that is regarded as good dietary oil for poultry. Soybean oil is highly digestible (0. 96) in poultry with high AME value (38.5 MJ/kg DM) due to the presence of high amounts of unsaturated fatty acids such as linoleic and linolenic acids (Wiseman and Salvador, 1991; Leeson and Summers, 2001).

Inclusion of fat is a common method to increase the energy density of a poultry diet. Not only is it a source of energy but it also provides essential fatty acids. Fats are fed mainly as glycerides (i.e. triglycerides). In the small intestine, the fats undergo lypolysis and the fatty acids released are emulsified and then packaged into micelles for absorption. The intestinal digestibility of fatty acids is highly variable due to the dietary amount (Sell *et al.,* 2001) and source (Castrovilli *et al.,* 1994; Blanch *et al.,* 1996; Dauml-nicke *et al.,* 1999) of fat added to the diet. When a fat source influences metabolisable energy, it is generally attributed to the nature of its fatty acid composition. Small intestine digestibility is often lower for saturated fatty acids than for unsaturated fatty acids (Renner and Hill, 1961). The 18 carbon unsaturated fatty acids (i.e. oleic, linolenic and linoleic acids) are more digestible in the small intestine than the 18 carbon saturated fatty acid (i.e. stearic acid). Therefore oils which are relatively unsaturated have a higher dietary energy value than the more saturated fats. Similarly, hydrogenation of oils or hydrolysed fat (i.e. free fatty acids)

is associated with a reduction in dietary energy value (Young, 1961; Wiseman, 2002).

Several factors are known to be responsible for the improved digestion of unsaturated fats compared with those that are saturated. These include the greater ability of unsaturated fatty acids to infiltrate the bile salt micelle (Freeman, 1969), the effect of the degree of saturation on pancreatic secretory activity (Ballesta *et al.,* 1990; Deschodt-Lanckman *et al.,* 1971), the morphology of the small intestine may be altered by the degree of saturation (Cera *et al.,* 1988), and a low molecular weight binding protein of the intestinal mucosa possibly binds unsaturated fatty acids in preference to saturated fatty acids (Octner *et al.,* 1972).

Both shea fat and cocoa fat have either C16:0 or C18:0 as the major fatty acids in the *sn*-1/3 position of the glycerol backbone (Lipp and Anklam, 1998). Thus the low digestibility values observed for cocoa fat resulted from decreased lypolysis and possibly also low absorption of triglycerides with high levels of long-chain saturated fatty acids in sn-1/3 position. The position of a fatty acid in the triglyceride molecule is also important; a fatty acid in the *sn-2* position is directly absorbed, whether saturated or unsaturated (Vila and Esteve-Garcia, 1996). If the stearic acid is esterified at the 1-or-3-position, it is released as free fatty acid, and in the presence of calcium and magnesium it is poorly absorbed due to soap formation (Mattson *et al.,* 1979; Brink *et al.,* 1995). The fatty acid structure of shea butter is also similar to that of cocoa butter; but differs greatly from that of lard, soybean oil, maize oil or poultry oil *(Table 2.2b),* which are regarded as good dietary oils for poultry. These oils are highly digestible in poultry with high AME values *(Table 2.2c)* due to the presence of high amounts of unsaturated fatty acids such as linoleic and linolenic acids (Wiseman and Salvador, 1991; Leeson and Summers, 2001).

The fatty acid profile of shea fat in relation to soybean oil *(Table 2.2b)* indicates that, the digestibility and energy value of the fat are likely to be poor as a result of its low unsaturated-to-saturated fatty acids ratio (-1.0) due to the predominance of stearic acid in the fat structure. Stearic acid has been implicated in the poor digestibility and low AME

Wiseman and Salvador, 1991).

Studies involving rats have shown poor digestibility of cocoa fat, which has a similar fatty acid profile to shea fat when compared with maize oil or soybean oil (Hoagland and Snider, 1943; Apgar *et al.*, 1987; Chen *et al.*, 1989). In a preliminary study, Hoagland and Snider (1943) reported the digestibility coefficient of cocoa butter (0.63) to be lower than that of soybean oil (0.99) or maize oil (0.98) when the diet contained 50 g/kg fat. This was confirmed by Apgar *et al.* (1987), who observed the digestibility coefficient of cocoa fat (0.59) to be significantly ($P<0.05$) lower than that of maize oil (0.93) at 50 g/kg level of inclusion in the diet. A similar trend was reported by Chen *et al.* (1989) in rats fed cocoa fat and maize oil. The digestibility coefficient of cocoa fat (0.526) was significantly ($P<0.05$) lower than that of maize oil (0.834), suggesting that the decreased bioavailability of cocoa fat was due to its high content of long-chain saturated fatty acids.

The metabolisable energy value of shea fat as a dietary fat for poultry has not been documented. Shea fat is an important, but highly variable, component of shea nut meal. The lack of detailed understanding of the available energy value of this fat has resulted in it being difficult to reliably estimate the energy contribution of the variable amounts of residual fat that occur in different samples of the shea nut meal. An understanding of the metabolisable energy value of variable inclusion levels of fat in poultry feeds is required to enable the efficient use of shea nut meal and possibly shea fat in poultry diets. Also, it is possible that some shea fat may be used in poultry diets in the future.

Table 2.2a: Fatty acid profiles of shea fat[a] (g/kg total fatty acids)

Palmitic ($C_{16:0}$)	Stearic ($C_{18:0}$)	Oleic ($C_{18:1}$)	Linoleic ($C_{18:2}$)	Linolenic ($C_{18:3}$)	Arachidic ($C_{20:0}$)	References
57	410	490	43	-	-	Banerji *et al.* (1984)
40	580	330	30	-	20	Hogenbrink (1984)
66	468	514	84	21	5	Jacobsberg (1977), Ivory Coast
42	225	680	49	1	-	Jacobsberg (1977), Tchad
38	441	438	67	16	-	Jacobsberg (1977), Benin
40	432	439	66	3	16	Kanematsu *et al.* (1978)
80	370	500	50	-	-	Meara (1979)
32	435	459	58	13	-	Baldrick *et al.* (2001)
36	444	424	59	17	-	Tano-Debrah and Ohta (1994), Ghana
40	460	410	70	10	-	Badifu (1989), Nigeria
33	443	456	55	-	13	Sawadogo and Bezard (1982), Burkina Fa
46[b]	428[b]	467[b]	57[b]	12[b]	14[b]	

[1]Values may not total 1000 g due to trace amounts of other fatty acids not reported or rounding of figures.

[b]The mean values of the fatty acids

Table 2.2b: Comparison of fatty acid composition of shea fat with selected dietary fats/oils (g/kg total fatty acids)[1]

Fatty acid	Shea fat[2]	Cocoa fat[3]	Tallow [4]	Lard[7]	Palm oil[4]	Soybean oil[4]	Sunflower oil[5]	Corn oil[6]	Poultry oil[6]
Lauric acid (C12:0)	-	-	2	-	3	1	-	-	-
Myristic acid (C14:0)	-	-	23	16	14	2	-	-	6
Palmitic acid (C16:0)	46	253	249	224	488	161	60	112	232
Palmitoleic acid (C16:1)	-	-	39	21	1	6	-	-	71
Stearic acid (C18:0)	428	376	206	177	55	61	64	21	64
Oleic acid (C18:1)	467	327	405	461	364	251	284	269	430
Linoleic acid (C18:2)	57	28	66	80	73	452	581	579	179
Linolenic acid (C18:3)	12	2	10	21	2	66	1	8	6
Arachidic acid (C20:0)	14	12	-	-	-	-	6	5	2

[1]Values may not total 1000 g due to trace amounts of other fatty acids not reported or rounding of figures
[2]Table 2.2a
[3]Lipp and Anklam (1998)
[4]Wiseman and Salvador (1991)
[5]Ortiz *et al.* (1998)
[6]Waldroup *et al.* (1995)
[7]Huyghebaert *et al.* (1988)

Table 2.2c: Digestibility and metabolisable energy values of triglycerides in broiler chickens fed selected dietary fats/oils

source	Digestibility (%)		Metabolisable energy (MJ/kg)	
	3-4 weeks	>4 weeks	1-3 weeks	7.5 weeks
Soybean oil	96[+]	96[+]	38.5*	38.5*
Corn (maize) oil	84[+]	95[+]	-	41.3[#]
Lard	92[+]	93[+]	30.8***	-
Beef tallow	70[+]	76[+]	30.9*	32.9*
Menhaden oil	88[+]	97[+]	35.9[#]	37.6[#]
Palm oil	74**	-	27.7*	32.3*
Sunflower oil	85****	88****	-	40.4[#]

[+]Leeson and Summers (2001)
*Wiseman and Salvador (1991)
** Zumbado *et al.* (1999)
***Huyghebaert *et al.* (1988)
****Ortiz *et al.* (1998)
[#]NRC (1994)

2.3.1.2 Protein and amino acids

There are variations in the crude protein contents of shea nut meals *(Table 2.1).* Even though these differences in protein contents have not been examined in detail, it is highly probable mat the Shea nut meal processmg memous *mat are* llSeC1 III Luc iuuuzuy *way uc* responsible. The removal of the residual fat by solvent extraction methods may increase the crude protein content proportionately in the final meal.

The crude protein content of shea nut meal is similar to that of expeller palm kernels (INRA, 2004) or cereal by-products such as maize gluten feed, wheatfeed and rice bran (NRC, 1994). Thus, it could replace these agro-industrial by-products in poultry diets and contribute a moderate amount of dietary protein. So far, there has been only one published article showing the amino acid profile of shea nut meal *(Table 2.3).* Tryptophan, an essential amino acid, for poultry has not been reported. The concentrations of the usual limiting amino acids for poultry, such as lysine and methionine, are relatively low *(Table 2.3).* Its amino acid profile, with the exception of the low amount of arginine, is similar to that of palm kernel meal and wheatfeed *(Table 2.3).* The implication of the low concentrations of lysine and methionine in shea nut meal would be the need for synthetic amino acid supplementation of diets. Annongu *et al.* (1996a) demonstrated the importance of amino acid supplementation of shea nut meal based diets for broilers and recommended the use of synthetic lysine and methionine in poultry diets that contained high proportions of shea nut meal.

Processing conditions, the presence of anti-nutritive factors, physical and chemical structure of the nutrient, and dietary fibre are factors that can influence protein digestibility (Sauer and Ozimek, 1985; Sibbald, 1987; Leeson and Summers, 2001). The digestibility of the protein in shea nut meal for poultry has not been reported nor is there information on the variability between different samples.

Table 23: Comparative amino acid composition (g/kg protein basis) of shea nut meal with selected oilseed meals, maize and wheatfeed

Amino Acid'	Shea nut meal (147.0 g/kg CP)[2]	Soybean meal (475.0 g/kg CP)	Palm kernel meal	Maize (85.0 g/kg CP)	Wheatfeed 160.0 g/kg CP)
Arginine	69.9	73.3	135.0	44.7	71.9
Histidine	28.7	26.9	23.0	27.1	23.1
Isoleucine	41.2	44.6	32.0	34.1	36.3
Leucine	65.4	78.7	60.0	117.6	66.9
Lysine	39.0	62.3	36.0	30.6	43.1
Methionine	14.7	14.1	20.0	21.2	13.1
Cystine		15.2	15.0	21.2	20.0
Phenylalanine	30.9	49.3	39.0	44.7	40.0
Threonine	28.7	39.4	35.0	34.1	30.6
Tryptophan		15.6	10.0	7.1	12.5
Valine	51.5	46.7	57.0	47.0	44.4
Serine	26.5	52.2		43.5	46.9
Glycine	39.0	43.2	48.0	38.8	39.4

'Data are adapted from Elkin (2002). [2]La7ani *et al.* (1984)

2.3.1.3 Polysaccharides

The poor utilisation of shea nut meal by monogastric animals could be in part due to the poor availability of its polysaccharides. In poultry nutrition, polysaccharides are broadly categorised into starch (i.e. digestible portion) and non-starch polysaccharides (NSP) (i.e. undigested portion). Thus polysaccharides comprising largely starch form a major portion of poultry diet with contents ranging from 400 to 700 g/kg (Carre, 2002). The main function is to supply energy for maintenance and production. The NSP *(Figure 2.4)* include cellulose, lignin (a polyphenol often associated with carbohydrates), hemicellulose, pentosans, gums, mucilages, pectic substances and galactomannans Lignin, cellulose and hemicellulose (non-cellulosic) constitute insoluble NSP (often referred to as dietary fibre), while gums and mucilages represent various types of soluble NSP. The insoluble NSP forms the bulk of the total fibre in poultry diets (Carre, 1990). One of the important attributes of insoluble NSP is the ability to absorb large amounts of water and maintain normal motility of the gut (Stephen and Cummings, 1979). However, from nutritional point of view, soluble NSP have negative effects on nutrient digestion and absorption in poultry (Antoniou *et al.,* 1981; Choct and Annison, 1990; Bedford *et al.,* 1991; Annison, 1991) whenever appreciable levels are contained in the diets. The adverse effects, such as altered intestinal transit time, increased endogenous losses of nutrients, and a change of nutrient digestion and absorption pattern (i.e. enzymatic digestion against microbial fermentation) of soluble NSP, are mediated through their ability to increase the viscosity of the digesta, to modify the physiology of the gastro-intestinal tract and to change the ecosystem of the gut. Endogenous losses include secretions, enzymes, mucous and sloughed cells. Change in microbial activity will lead to potential toxin production. It has also been observed that heat processing increases the solubility of NSP, especially in the case of f3-glucans (Vukic Vranjes *et al.,* 1994), which may compromise chick productivity. Shea nut meal contains high levels of polysaccharides *(Table 2.1).* However, it has been observed that 40% of the polysaccharides in shea nut meal are indigestible (Busson, 1965).

Also, Morgan and Trinder (1980) reported high acid detergent fibre [i.e. detergent analytical system (van Soest, 1963)] and low *in vitro* organic matter digestibility of shea nut meal. These reports indicate that the polysaccharides in shea nut meal may comprise largely of NSP. Apart from the cellulosic materials, the presence of latex or gum has also been reported in shea kernels (Kershaw and Hardwick, 1981).

The polysaccharide content of shea nut meal is poorly described and there is a need to provide more detailed chemical analysis. For instance, *Table 2.4* shows a high proportion of nitrogen-free extract in shea nut meal relative to other oilseed meals and wheatfeed. In particular, there is a need to determine whether shea nut meal contains any available starch and in what amount. There is a relatively high fibre content, but there is a need to quantify the amount of insoluble and soluble NSPs. Soluble NSP can cause highly viscous conditions within the small intestines of poultry and there is a need to evaluate whether the soluble NSP content of the shea nut meal could give the high viscous conditions

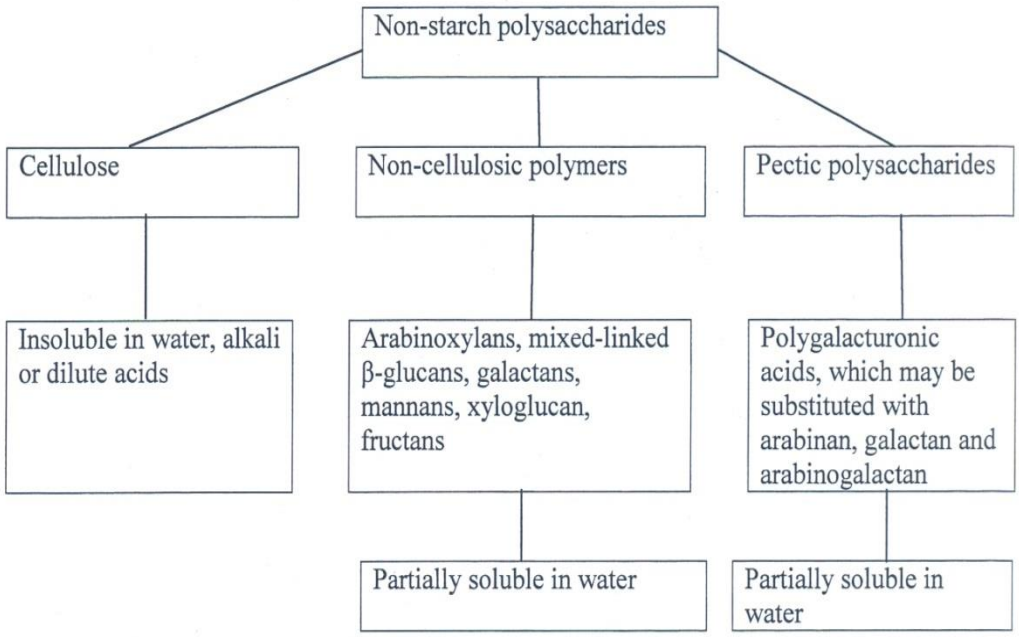


Figure 2.4: Classification of non-starch polysaccharides (Choct, 2002)

Table 2.4: Carbohydrate contents of selected common plant feedstuffs and shea nut meal' (g/kg dry matter)

Feedstuff	NFE	CF	NDF	ADF
Shea nut meal[2]	567	95	101	92
Soybean meal	124	58	125	91
Palm kernel meal	51	167	693	470
Wheatfeed	333	74	365	111

NFE-Nitrogen-free extract, CF-crude fibre, NDF-neutral detergent fibre, ADF-acid detergent fibre 'Unless indicated otherwise, data are adapted from McDonald *et al.* (2002)
[2]Atuahene *et al.* (1998)

2.3.1.4 Gross energy and estimate of metabolisable energy

The gross energy content of one sample of shea nut meal has been reported to be 19.0 MJ/kg DM (Okai *et al.,* 1995). However, the biological availability of this energy in poultry is probably low and variable (Atuahene *et al.,* 1998; Olorede *et al.,* 1999). Atuahene *et al.* (1998) estimated the apparent metabolisable energy corrected for nitrogen to be 7.1 MJ/kg DM. This finding shows that the available energy concentration of shea nut meal may be low relative to its proximate nutrient composition. There is a need for additional evidence that determines the metabolisable energy of shea nut meal and also that describes the variation in metabolisable energy between different shea nut meal samples.

2.3.2 Micro-Nutrients

Shea nut meal has ash contents in the range of 33-76 g/kg DM *(Table 2.1),* but relatively low mineral contents *(Table 2.5).* This indicates the presence of large amounts of sand or soil contamination. The shea kernels are usually dried on the bare floor. The levels of macro-elements as well as trace elements in the meal are relatively lower than those in palm kernel *meal (Table 2.5).* However the levels of trace elements such as copper and zinc reported by Atuahene *et al.* (1998) were excessively high, probably due to contamination. Presently, the literature contains no information on the vitamin composition of shea nut meal.

Table 2.5: Comparison of mineral composition of shea nut meal with selected feedstuffs

Feedstuff	Ash	Ca	P	Mg	Mn	Fe	Zn	Cu
	g/kg DM				mg/kg DM			
Shea nut meal[1]	52.8	2.1	2.0	1.9	31.3	460.7	24.6	4.4
Soybean meal[2]	62.0	3.5	6.8	3.0	32.0	-	61.0	25.0
Palm kernel meal[3]	45.2	3.1	6.2	3.2	144.6	589.4	35.3	23.2
Maize[2]	13.0	0.3	2.7	1.1	6.0	-	16.0	2.5
Wheatfeed	50.0	1.4	9.7	1.8	13.4	5.7	11.4	2.0

[1]Okai *et al.* (1994)
[2]Data adapted from McDonald *et al.* (1995)
[3]Data calculated from INRA (2004)
[4]Data calculated from NRC (1994)

2.3.3 Anti-Nutritive Factors

the availability of nutrients, depress feed intake, and reduce the growth in animals that consume them (Hathcock and Rader, 1994; Shahidi, 1997). However, the presence of a toxic factor *per se* does not preclude the utilisation of the material as a feedstuff. Various processing methods are available to neutralise or detoxify the deleterious components of by-products and waste materials. Besides, provided the level of consumption is low, even the most potent anti-nutritive compounds can be tolerated without any adverse consequences (Yannai, 1980; Ferket and Middleton, 1999).

There is disagreement on the main anti-nutritive factors present in shea nut meal. Some authors found saponins (Gohl, 1981), tannins (Okai, 1990), or both saponins and tannins (Annongu *et al.,* 1996a), while others recorded saponins and theobromine (Atuahene *et al,* 1998) or solely theobromine (Rhule, 1999). The variation in the concentrations of these anti-nutritive factors between shea nut meal samples could be partly related to the great intra-specific genetic variation among the shea tree biotypes (Hall *et al.,* 1996). A more plausible reason for the variation might be the different analytical methods employed by these authors as well as the sophistication of the methods in detecting some of the anti-nutrients. The dietary fibre in shea nut meal has also been recognised as an anti-nutritive factor (Morgan and Trinder, 1980).

2.33.1 Tannins

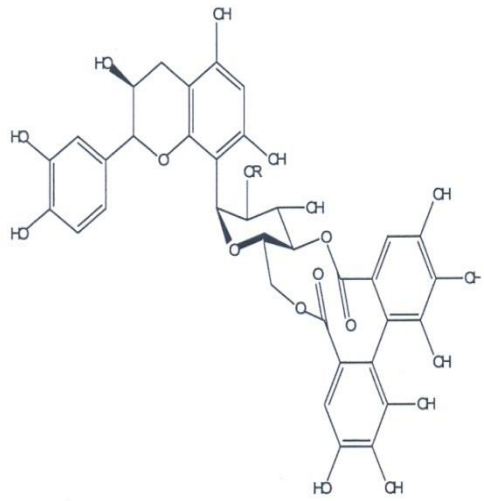
Tannins are a group of plant polyphenols composed of two classes, the "condensed" tannins *(Figure 2.5a)* and the "hydrolysable" *(Figure 2.5b)* (De Bruyne *et al.,* 1999). Inadequate analytical methods have often been used for the assay of tannins in shea nut meal. Okai *et al.* (1995) used the Dennis-Folin 'total-phenol' reagent (AOAC, 1984) and

obtained a concentration of 109.8 g/kg DM, while Annongu *et al.* (1996a) used ytterbium acetate reagent (Reed *et al.,* 1985) and reported a total phenol concentration of 156.4 g/kg DM and an approximate proanthocyanidins (BUT A550) content of 5.3 g/kg DM in the material. Despite differences in analytical methods, environmental factors as well as material preparation for analysis may influence the concentration of tannins in feedstuff samples. Hagerman (1988) observed that tannin extractability may change markedly within a single plant species between seasons; while Butler and Rogler (1992) noted that moist conditions favour the complexing of tannins with proteins so that the tannin might no longer be extracted and assayed, but could still be bioactive active in poultry diets (Mahmood *et al.,* 2006). Generally, the concentrations of tannins in shea nut meal are high relative to other feedstuffs such as sorghum (13.0 g/kg DM, Muindi *et al.,* 1981) and peas (3.5 g/kg DM, Griffiths, 1981). The biological significance of tannins in animal nutrition is related to their characteristic ability to form complexes, both with metal ions and with macro-molecules such as proteins and polysaccharides (De Bruyne *et al.,* 1999). Many studies have shown that dietary tannins result in reduced weight gains and poor feed efficiencies in chicks (Armstrong *et al.,* 1974; Ahmed *et al.,* 1991). Smulikowska *et al.* (2001) found that the tannin content of peas had a pronounced negative effect on protein digestibility ($r = -0.93$; $P<0.05$) as well as metabolisable energy ($r = -0.99$; $P<0.001$) in chicken. Also, tannins occurring in sorghum grains and faba beans depress growth and cause toxicity to chicks (Rostagno *et al.,* 1973; Trevirio *et al.,* 1992; Iji *et al.,* 2004). Nelson *et al.* (1975) observed that when hybrid sorghums of varying tannin content were fed to chicks, there was a significant ($P<0.05$) negative correlation between tannin content and metabolisable energy ($r = -0.62$), dry matter digestion ($r = -0.63$) and bioavailability of amino acids ($r = -0.82$). Tannins have been shown to inhibit *in vivo* activities of trypsin and a-amylase, but increased lipase activity (Longstaff and McNab, 1991), which may affect digestion of proteins and starch. In poultry, small quantities of tannins in the diet cause adverse effects. Levels from 5 to 20 g/kg can cause depression in

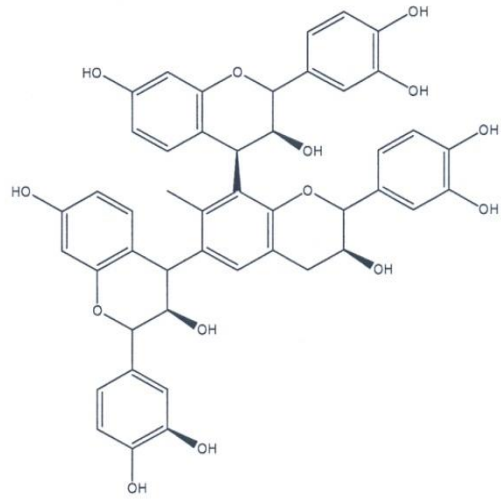
growth and egg production, while levels from 30 to 70 g/kg can cause death (Salunke and Chavan, 1990; Farrel *et al.,* 1999; Iji *et al.,* 2004).

The consequences of astringency and binding of proteins by tannins in diets that include shea nut meal have been reported in studies involving rats (Okai *et al.,* 1995) and poultry (Annongu *et a/.,* 1996b). According to Okai *et al.* (1995), reduction in tannin content of shea nut meal (40-70%) processed either by soaking or boiling in water improved feed intake and protein digestibility in weanling rats. Annongu *et a/.* (1996b) found significant improvements in feed intake, feed efficiency and growth rate as well as haematological variables such as haematocrit, erythrocyte number, leucocyte count and haemoglobin concentration of broilers when the tannin concentration in shea nut meal was reduced by 57% through fermentation (i.e. wet incubation of the meal).

There is a further need to describe and quantify the tannin content of shea nut meal and to describe the variability between samples.



Complexed tannin



Condensed tannin

Figure 2.5a: Proanthocyanidins or 'condensed tannins'

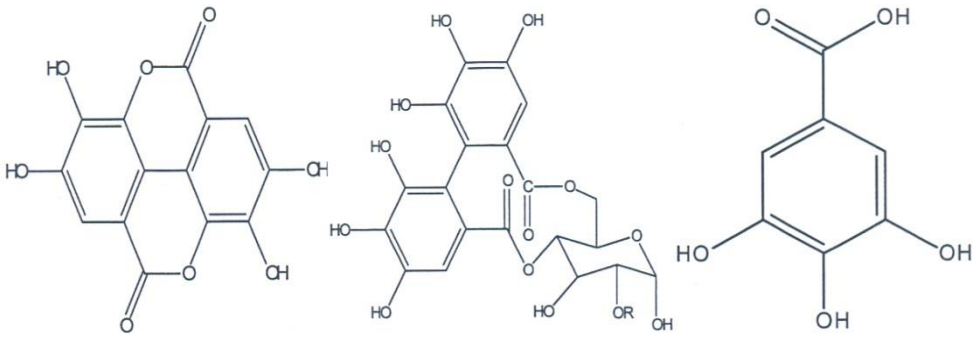


Figure 2.5b: Hydrolysable tannins

2.3.3.2 Saponins

Saponins are a large group of glycosides with a diverse range of properties (Haralampidis *et al.*, 2002), including steroid saponins and triterpenoid saponins. The triterpenoid saponins *(Figure 2.6)* occur in the Sapotaceae family (Sparg *et al.*, 2004) that includes the shea nut *Vitellaria*. Annongu *et al.* (1996b) found Nigerian shea nut meal samples to be relatively higher in saponins (30.0 g/kg DM) than has been reported for Ghanaian samples (3.0 g/kg DM) (Atuahene *et al.*, 1998), High saponin concentrations in feed have been found to significantly affect growth, feed intake and reproduction in poultry (Sim *et al.*, 1984; Terapunduwat and Tasaki, 1986; Potter *et al.*, 1993; Jenkins and Atwal, 1994). High dietary saponin content (9 g/kg) decreased growth rates and feed intake of chicks (Jenkins and Atwal, 1994). These negative effects have been ascribed to several properties of saponins, such as reduced feed intake caused by the bitter taste (Cheeke, 1971) or astringent and irritating taste (Oleszek *et al.*, 1994), reduction in protein digestibility (Shimoyamada *et al.*, 1998) probably by the formation of saponin-protein complexes (Potter *et al.*, 1993), and damage to the intestinal membrane (interact with lipid bilayer) thereby increasing its permeability (Johnson *et al.*, 1986) that facilitates uptake of substances that are normally not absorbed (e.g. toxins, microbes, allergens) and inhibition of active mucosal nutrient transport (Francis *et al.*, 2002). Saponins have also been shown to have haemolytic activity towards red blood cells (Khalil and Eladawy, 1994), as well as complexing minerals such as iron, zinc and calcium (Milgate and Roberts, 1995) thereby rendering them unavailable to the animal The saponin content of shea nut meal reported by Annongu *et al.* (1996b) falls within the range occurring in some common animal feeds such as legumes (Price *et al.*, 1987) that have also been associated with anti-nutritive properties. However, there have been claims that saponins may have beneficial effects in poultry production, but these appear to have been based on rather slender evidence (McNab and Boorman, 2002). There have been small but significant improvements in weight gains (Johnston *et al.*, 1981) and feed efficiency (Johnston *et al.*, 1982) of chickens

fed very low concentration (900µg/kg). Other beneficial effect of low saponin concentatration (250 mg/kg) is reduction in mortality due to ascites in broiler chickens (Anthony *et al.*, 1994).

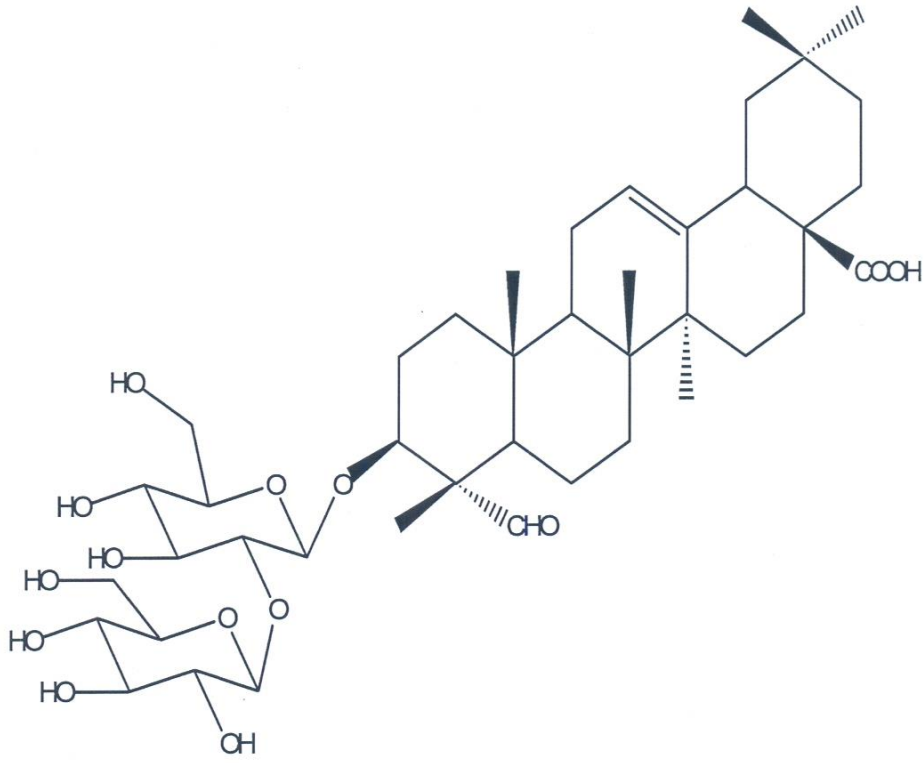


Figure 2.6: Triterpenoid saponins

2.3.3.3 Theobromine

Theobromine (3, 7 dimethylxanthine) is a purine-derived alkaloid that can be toxic to some animals (Strachan and Bennett, 1994). Both Atuahene *et al.* (1998) and Rhule (1999) recorded theobromine concentration of 4.5 g/kg in shea nut meal. This is far lower than the levels (17-25 g/kg) found in cocoa meals (Clarke and Clarke, 1979; Peckham, 1984). Monogastric animals such as mice, pigs and hens fed high levels of cocoa meal either die or have poor growth performance (Owusu-Domfeh, 1972; Clarke and Clarke, 1979; Peckham, 1984). Atuahene *et al.* (1998) attributed the poor performance of broilers fed shea nut meal diets to the diuretic effect of theobromine (Trease and Evans, 1972); however the concentration of theobromine in the diet was not stated.

2.4 Growth Performance of Animals fed Shea Nut Meal

The nutritive value of shea nut meal for all classes of livestock has been so poor that some authors consider it to be unsuitable for feeding livestock (Irvine, 1934; Bourlet, 1950; Jacobsberg, 1977; Louppe, 1994), while others thought there is room for improvement (Okai *et al.*, 1995; Rhule, 1999; Annongu *et al.*, 1996abc; Olorede *et al.*, 1999) provided the anti-nutritive factors present are removed. Although the meal has high carbohydrate content (Morgan and Trinder, 1980), it was reported that 40% of the carbohydrate was indigestible (Busson, 1965) due to its high acid detergent fibre value and low *in vitro* digestibility (Morgan and Trinder, 1980). The meal was also found to cause severe gastric disturbances in livestock (Dalziel, 1937; VITEC, 1986) and toxic effects, which resulted in injury to the digestive mucosa and red blood cell haemolysis, depression in feed intake and low weight gains (Osei-Amaning, 1993; Atuahene *et al*, 1998; Annongu *et al.*, 1996a, 1996b; Olorede *et al.*, 1999), which could lead to death in severe cases (VITEC, 1986). There is disagreement as to the exact source of this toxicity, some attributing it to the presence of saponins (Cheeke, 1976; Morgan and Trinder, 1980; Gohl, 1981; Annongu *et al.*, 1996a) and others to tannins (Busson, 1965; VITEC, 1986; Okai, 1989) or both saponins and tannins (Annongu *et al.*, 1996b).

There is also evidence implicating theobromine for the toxic effects (Atuahene *et al,* 1998; Rhule, 1999). This controversy over the main toxic factor in shea nut meal calls for accurate characterisation of the anti-nutritive factors for their effects to be better determined as this study seeks to investigate.

Despite the gloomy picture of the nutritive value of the shea nut meal, there remains the need for this potential agro-based industrial by-product to be used in the formulation of diets for livestock and poultry, particularly in developing countries of West Africa due to scarcity of conventional feedstuffs. Suggestions to promote the use of shea nut meal include guidelines on dietary inclusion levels for all classes of livestock and poultry, detoxification of the meal, and other practical approaches.

2.4.1 Ruminants

Preliminary studies with ruminants revealed that 250 to 300 g/kg shea nut meal could be tolerated (Morgan and Trinder, 1980). Owing to its poor palatability and digestibility, they advised that the use of shea nut meal should be limited to that of filler, but suggested it could be added to the concentrated rations of ruminants. VITEC (1986) restricted the use of the meal to 50-100 g/kg in ruminant rations, but suggested that it should not to be fed to calves or lambs.

In Ghana, shea nut meal based diet preference test performed on sheep revealed that they preferred the treatment diet with 250 g/kg shea nut meal and 750 g/kg wheat bran to diets that contained higher amounts of shea nut meal in the mixture relative to wheat bran (Essien, 2003). As a follow up study, the growth response by sheep to shea nut meal -based diets fed at 0, 150, 300 g/kg was investigated using West African Dwarf Sheep (Karbo *et al.*, 2004).

It was found that the inclusion of 150 g/kg shea nut meal in the diet had no significant effect on dry matter intake, daily weight gain or feed conversion efficiency, while feed cost per animal was reduced. High level of inclusion in sheep diets was not attainable because of anti-nutritive factors present in the meal, which resulted in low digestibility (Anto, 2004).

2.4.2 Monogastrics 2.4.2.1 Rats

In an experiment using weanling rats, Okai *et al.* (1994) had shown that the optimum level of shea nut meal in the diet to be 50 g kg $^{1.}$ The adverse effect of feeding high level of the shea nut meal was linked to the presence of tannins and other unidentified factors. When the shea nut meal was analysed for anti-nutritive factors it was found to contain 109.8 g/kg tannins, but did not contain saponins and aflatoxin Bl. In order to detoxify tannins, Okai *et al.* (1995) subjected the shea nut meal to hydro-thermal treatment either by soaking or boiling in water. The tannin content was drastically reduced by soaking or boiling. Despite the reduction in the tannin content particularly through soaking of the meal, the improvement in performances of the rats fed the processed shea nut meal diets did not match that of their counterparts fed the standard control diet. The generally poor growth performance observed with the processed material suggests adverse effects of the residual tannins, or there may be some other unidentified anti-nutritive factors present in the material.

2.4.2.2 Pigs

Morgan and Trinder (1980) and Gohl (1981) had reported that shea nut meal was unpalatable and could be toxic to pigs. These effects were attributed to tannins and saponins in the shea nut meal. In a trial with growing gilts to determine the levels (50, 100, 150 g/kg) of inclusion at which these effects would occur or performance of the pigs after

shea nut meal had been withdrawn from diets, Okai and Bonsi (1989) found that inclusion of shea nut meal depressed growth rate and feed efficiency but not feed intake. However, the post withdrawal growth and reproductive performances were not affected; probably as a result of compensatory growth after a period of poor growth induced by the anti-nutritive factors. In the studies with pigs, Okai (1990) and Rhule (1995) recommended 50 and 100 g/kg shea nut meal in the diets for grower and finisher pigs, respectively. Rhule (1995) partly attributed the negative effect on performance to the presence of theobromine in the diet. Thus shea nut meal was treated hydro-thermally at varying time regimes in order to reduce the content of theobromine (Rhule, 1999). It was found that boiling the meal for 90 minutes reduced considerably the level of theobromine from 4 g/kg in the untreated shea nut meal diet to 2 g/kg in the treated diet that contained 200 g/kg shea nut meal. Subsequent feeding of the treated shea nut meal to either grower or finisher pigs was tolerated up to 200 g/kg in their diets.

2.4.2.3 Poultry

In the preliminary studies carried out so far, the response of poultry has not been encouraging. Even relatively low dietary inclusion levels of shea nut meal in poultry diets *(Figure 2.7)* can decrease feed intakes, growth rates and feed conversion efficiencies (Atuahene *et al.*, 1998). This was attributed variously to anti-nutritive factors present in the meal. However, the following dietary recommendations have been found to have no significant effects on performance of the birds. Feeding of 25 g/kg (Atuahene *et al,* 1998; Dei *et al.,* 2006), or 50 g/kg (Adeogun, 1986; Osei-Amaning, 1993) in broiler chicken diet; 100 g/kg in pullet chick diet (Olorede and Longe, 1999); and 50 g/kg in exotic guinea fowl diet (Dei *et al.,* 2001). The apparent lack of agreement on dietary inclusion level of shea nut meal in broiler chicken diet appears to be due to the processing method (traditional or industrial) of the shea nut meal.

Nevertheless attempts are still being made to improve the utilisation of shea nut meal for poultry with particular reference to broiler chickens. These include protein or amino acid supplementation and detoxification strategies. The poor feeding value of shea nut meal can be linked to poor nutrient availability particularly protein. Thus Olorede *et al.* (1999) tested the hypothesis that the negative effects of shea nut meal on a bird's live weight might be due to the poor utilisation of the nutrients of shea nut meal, particularly protein. Shea nut meal was therefore incorporated up to 200 g/kg in broiler diets that contained 200 g/kg fishmeal as a major source of essential amino acids, particularly methionine, and this was also compared with similar diet with groundnut cake. They found out that incorporating shea nut meal in broiler diet up to 100 g/kg had no significant effects on growth performance (14-42 days of age) using the fishmeal but observed adverse effects with the groundnut cake. The reason being that the fish meal supplied more essential amino acids particularly methionine than groundnut cake in the diet. It is interesting to note that in the gastro-intestinal tract tannins present in the diet can be hydrolysed to gallic acid and partially excreted in the form of 4-0-methyl-gallic acid, utilising the methionine and choline in the feed as a source of methyl groups for the *0*-methylation (Potter and Fuller, 1968); thereby reducing ill-effects of tannins. For instance, addition of 1.5-3.0 g/kg methionine to diets containing high tannin sorghum as the only cereal component has resulted in growth rates similar to those achieved by feeding maize or low tannin sorghum based diets (Armstrong *et al.*, 1974a; Elkin *et al.*, 1978; Luis and Sullivan, 1982). The use of the fishmeal in the study has been shown to be cost effective.

In another study, Annongu *et al.* (1996b) reported significant improvement in weight gain of broilers when fermented shea nut meal was fed at 100 g/kg dietary inclusion level that was supplemented with *lyle* (essential amino acids) and molasses. The results of these studies point to the fact that adequate animal protein supplementation or amino acid

supplementation of shea nut meal based diets can overcome some its nutritional shortcomings.

Annongu *et al.* (1996c), in another study, found that supplementation of shea nut meal diet with polyethylene glycol prevented the deleterious effects of saponins in broilers. However, polyethylene glycol is noted for binding tannins (Silanikove *et al.,* 2001; Landau *et al.,* 2003).

It is evident that, there needs to be some further improvement of the nutritional value of shea nut meal before it can become more useful for the poultry feed industry. Thus there is need to examine these proposed processes of detoxifying or improving shea nut meal for poultry.

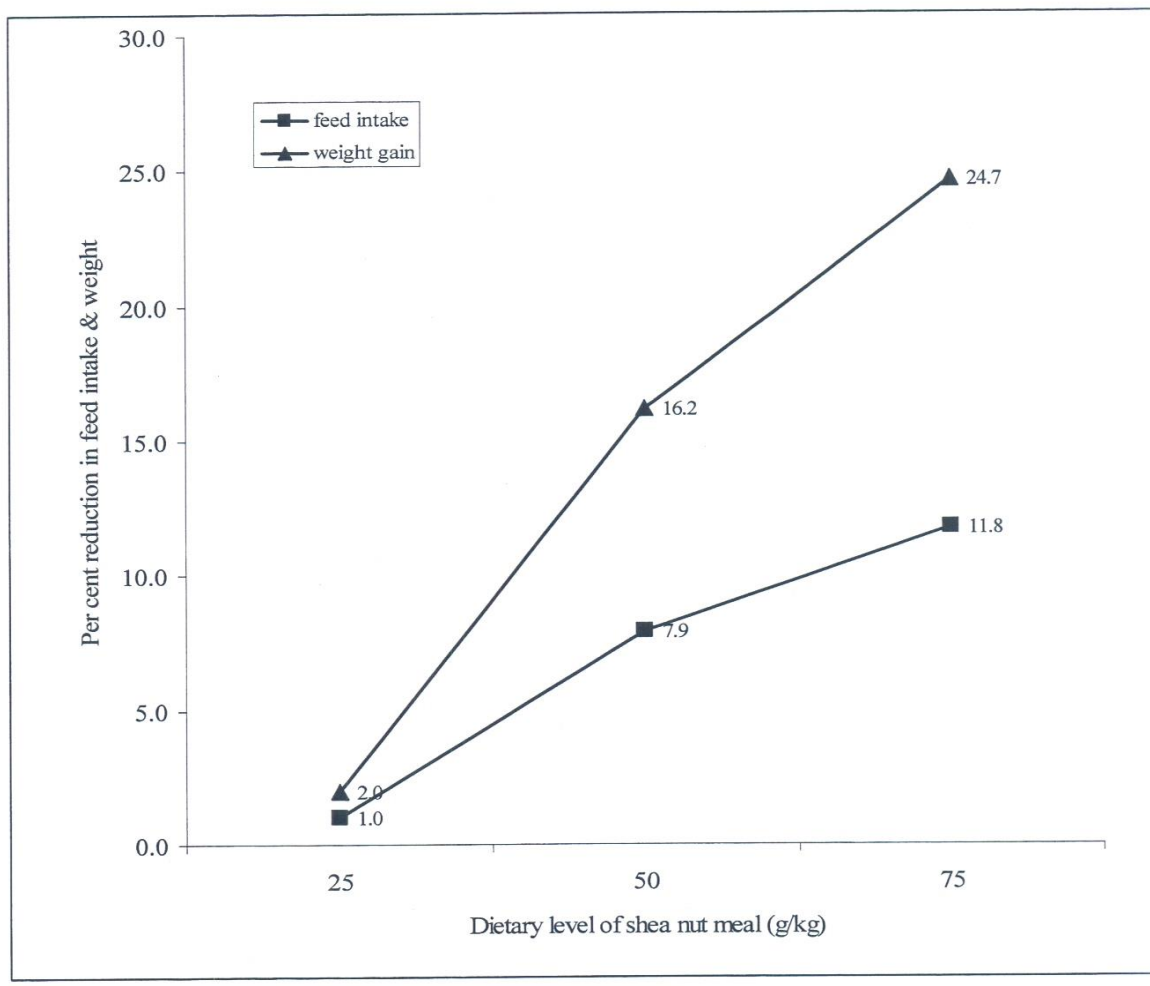


Figure 2.7: Percentage reduction in growth variables of broiler chickens (14-56 d) fed varying dietary levels of shea nut meal (Data adapted from Atuahene *et al.*, 1998)

2.5 Methods for Improving the Nutritive Value 2.5.1

Fermentation

The amounts of tannins and saponins in some foods can be reduced by fermentation, although the mechanism by which these components are eliminated is not fully understood (Reddy and Pierson, 1994). However, the fermentation process can produce organic acids (Delaude, 1974) that break down saponins or tannins; or create conditions for the growth of native microbes (Yosioka *et al.,* 1966) that detoxify these components (Reddy and Pierson, 1994). The microbial degradation *(Bacillus, Corynebacterium, Klebsiella, Aspergillus, Penicillium, Fusarium and Candida spp)* of condensed tannins is found to be less than hydrolysable tannins in both aerobic and anaerobic environments (Bhat *et al.,* 1998). Reichert *et al.* (1980) suggest that, during incubation, tannins may react to form higher oligomeric polymers not readily soluble in water, therefore, are less likely to interfere with digestive enzymes or other proteins. Annongu *et al.* (1996a) fed shea nut meal that had undergone an 8-day fermentation, which reduced the saponin content by 50%, total phenols by 57% and proanthocyanidins by 34%. The fermented shea nut meal gave about a 30% improvement in broiler chicken growth performance compared to the unfermented meal sample when fed at 100 g/kg of the diet. This initial information suggests that fermentation is a useful strategy that requires further study and development.

2.5.2 Water treatment

Tannins are water extractable (Price *et al.,* 1978a) and theobromine is soluble in hot water (Adegbola, 1977). Okai *et al.* (1995) reported that treatment of the meal by soaking in water (1 g shea nut meal in 20 ml water) overnight or boiling (1 g shea nut meal in 20 ml water) for an hour reduced the tannin content in the meal, where soaking removed 40% and boiling removed 70% of the tannins These methods improved feed intake and protein digestibility of shea nut meal in weanling rats, but failed to improve their growth to the

standard of the control diet. It is possible that, in the presence of moisture, the tannins are polymerised to larger molecules that are insoluble and lose their ability to precipitate proteins. However, such complexed-tannins have been shown to markedly depress nutrient digestibility of salseed *(Shorea robusta) meal* by poultry (Mahmood *et al.,* 2006). Okai *et al.* (1995), also, found these processing methods to be associated with a considerable loss of dry matter (45% loss with soaking and 53% loss with boiling). Kumar and Singh (1984) noted 20-70% losses in dry matter when alkalis were used to remove tannins in other feedstuffs. In studies involving pigs, Rhule (1999) compared shea nut meal soaked in cold water overnight with a hydrothermal treatment of shea nut meal. He found that cold water treatment had no effect, but the hydrothermally treated meal gave improved growth rates and feed efficiency in pigs. Even though, the water treatments of shea nut meal have not been evaluated in poultry, considerable loss of dry matter during the process may be a disincentive for this strategy.

2.5.3 Enzymes

The positive dietary effects of enzyme treatments on anti-nutritive factors such saponins and NSP in feeds for poultry have been recognised in the feed industry. Wubben *et al.* (1996) reported that the enzyme, avenacosidase, is capable of detoxifying the saponins in oats. This enzyme was produced by an isolate of the fungus, *Septoria avenae,* which detoxifies the saponins by enzymatic hydrolysis of the sugar chain attached at carbon 3. Another enzyme, soybean saponin hydrolyse, has been isolated from *Neocosmospora vasinfecta* var. *vasinfecta* PF1225 (Watanabe *et al.,* 2004), which also successfully degraded the saponins. It is, therefore, probable that appropriate enzyme supplementation could be useful in ameliorating any adverse effects of saponins in shea nut meal.

Also, the possible adverse effects of soluble NSP could be offset through the addition of exogenous enzymes, such as (3-glucanases and xylanases, which improve bird performance

by increasing nutrient digestibility or by augmenting feed intake. Birds do not produce enzymes that are capable of digesting xylans and P-glucans in feedstuffs (Silversides and Bedford, 1999). Marsman *et al.* (1997) and Zanella *et al.* (1999) demonstrated that addition of commercial feed enzymes to a maize/soybean broiler diet significantly improved weight gain and feed conversion efficiency. It has been reported that enzymes are also effective in diets rich in saturated fats (Nissenin *et al.,* 1993). Shea nut meal contains high levels of soluble NSP and saturated residual fat, so enzyme treatment of the meal would appear to be an appropriate solution.

2.5.4 Irradiation treatment

It has also been observed that irradiation can be used to depolymerise NSP and saponins. Siddhuraju *et al.* (2002) have shown that irradiation of *Sesbania* seeds was able to degrade glycosidic linkages in oligosaccharides with subsequent increase in the levels of free sugars, as has been reported with mung beans (Machaiah *et al.,* 1999). They also observed that soaking *Sesbania* seeds followed by irradiation (6 kGy) reduced the levels of saponin. The authors suggested that the possible mechanism for the reduction was the delinking of the carbohydrate moiety from the aglycone of steroids/triterpenoids bound through glycosidic linkages under the radiation process. Campbell *et al.* (1986, 1987) noted the effectiveness of irradiation (10 kGy) in the depolymerisation of NSP such as P-glucans in oats and barley by improving growth in chicks. This occurred as a result of an increase in p-glucan solubility and reduction in digesta viscosity. It seems the treatment of shea nut meal through irradiation may ameliorate the effects of any saponins and NSP present in the material.

2.5.5 Tannin binding agents

A promising way of overcoming the problem of tannins in feeds has been the use of tannin-binding agents. These are artificial polymers such as water-soluble polyvinyl pyrrolidone (PVP), water-insoluble polyvinyl polypyrrolidone (PVPP) and water-soluble polyethylene glycol (PEG). They contain a large number of oxygen atoms capable of forming hydrogen bonds with the phenolic groups in tannins, and to precipitate them from solutions (Jones, 1965). The addition of tannin-binding agents has been shown to ameliorate deleterious effects of condensed tannins by displacing condensed tannins from tannin-protein complexes (Jones and Magnan, 1977). The positive effect of tannin-binding agents, particularly polyethylene glycol (PEG) has been demonstrated in chicks and rats fed high tannin sorghum, field beans and barley (Ford and Hewitt, 1979) as well as in ruminant species (Silanikove *et al.,* 2001; Landau *et al.,* 2003). Also, the effectiveness of PEG has been demonstrated in chicks fed shea nut meal (Annongu *et al.* 1996c). However, the efficacy of PEG was attributed by these workers to the prevention of deleterious effects of saponins. The authors observed a drastic fall in blood or tissue cholesterol level of the chicks, an effect noted for saponins This study suggests the possibility of PEG reducing the adverse effects of both tannins and saponins in shea nut meal for poultry and therefore requires further study.

2.6 Conclusions from the Literature Review

- A large quantity of shea nut meal will in the future be available for use in the poultry feed industry in West Africa. There is room for increase in current production levels due to increasing global demand for the shea fat as well as interventions by regional governments and non-governmental organisations to boost shea nut production.

- Shea nut meal has a nutrient composition similar to that of expeller palm kernel meal, or some cereal by-products such as wheatfeed with exception of its often high crude fat content; but it contains anti-nutritive factors that reduce feed intake and growth performance of birds. Also, the wide variation that is observed in nutrient composition or nutritive value of shea nut meal samples in the industry will give problems in feed formulation.
- There is inadequate information on the nutrient composition of shea nut meal and the biological availability of these nutrients. In particular, there is a lack of data on amino acid composition of different shea nut meal samples and the biological availability of these amino acids. The meal contains variable amounts of residual fat yet there is no information on the contribution of this fat to dietary energy supply. Two types (expeller and non-industrial) of shea nut meal are currently produced in Ghana and there is also a possibility of variability between different expeller shea nut meal samples. The major factors that cause the variation in their nutrient compositions need to be understood.
- The high concentrations of tannins and to some extent saponins are a major nutritional limitation for shea nut meal. The relatively high content of non-starch polysaccharides is another possible anti-nutritive factor. The adverse effects of these anti-nutritive factors could be counteracted through various nutritional srategies including fermentation, enzyme supplementation, tannin binding agents, or irradiation procedures. Nutritional strategies tried so far have been natural fermentation and hot water treatment with some degrees of success. However, these approaches are limited in scope and need to be examined in more detail.

CHAPTER THREE

EXPERIMENTALS

3.1 NUTRITIONAL VALUE OF SHEA NUT MEAL FOR POULTRY

The previous section identified that there was a lack of basic information on the nutrient composition of shea nut meal. Secondly, there are a number of different sources and methods of production of shea nut meal. There is a need to describe and explain the variation in nutrient contents between different samples.

The hike in prices and occasional scarcity of conventional feed ingredients such as maize and fishmeal for poultry in Ghana has led to the search for suitable non-conventional feedstuffs such as agro-industrial by-products (e.g. oilseed meals including shea nut meal and cereal brans), cassava *(Manihot esculenta),* and some leguminous crops like faba beans *(Vicia faba)* and velvet beans *(Mucuna* spp). The use of less expensive non-conventional feedstuffs can potentially reduce feed costs and give reasonable performance only if diets are formulated correctly or their anti-nutritive factors removed. In this regard, nutrient levels, bioavailability, and anti-nutritive factors and their effects on bird performance must all be considered in determining the usefulness of any non-conventional feed ingredient for poultry.

3.1.1 Chemical Composition of Expeller and Non-industrial Shea Nut Meals

Initially, the aim was to evaluate only an expeller and a non-industrial shea nut meal. However, it was deemed necessary to add a few shea nut meal samples that reflected differences in factories and duration of storage. Also, the removal of the fat in the material was to simulate the industrial process that involved the use of solvent, Hence evaluation of eight samples in this study.

The first objective was to determine the chemical compositions of four expeller and two non-industrial shea nut meal samples collected in two different seasons (2004 and 2005) as well as two defatted samples of shea nut meal collected in 2004 (1 expeller and 1 non-industrial).

The second objective was to determine an in *vitro* viscosity of soluble non-starch polysaccharides of the shea nut meal samples.

3.1.1.1 Materials and Methods

Procurement of shea nut meal samples

Six shea nut meal samples were obtained from Ghana for the study. Four of the shea nut meal samples were produced by the same industrial process (expeller fat extraction), but by different factories [Shebu-Loders Croklaan Ltd, Savelugu (3 samples) and Juaben Oilmills, Juaben (1 sample)]. The industrial expeller shea nut meal process involved steaming (wet heating) of the kernels. The fat was extracted mechanically using a screw-press. Two additional shea nut meal samples were obtained from a local, non-industrial (water-based fat extraction) processor (Christian Mothers Association, Tamale). This material was produced by a traditional method of roasting the kernels, grinding in a mill prior to water-based fat extraction using the hand to knead, scooping off fat emulsion and the collection of the residue for sun-drying. Three of the six shea nut meal samples collected were produced during the 2004 growing season and the other three in 2005. All the samples obtained were stored at ambient temperatures at source (approximately 25°C) and, after transport, in cold storage at 4°C (UK).

Two additional shea nut meal samples were prepared by removing the residual fat from two of the collected shea nut meals (1 expeller meal and 1 non-industrial meal from the

2004 season). The fat in the shea nut meal was removed through continuous extraction with petroleum ether (b. p. 40-60°C) using a Soxtec system (Foss Ltd, UK).

Chemical analyses

The shea nut meal samples were ground in a laboratory mill fitted with limn-mesh size screen.

My matter

Dry matter content of samples was determined by drying the samples in an oven at 100°C overnight using a standard method (Method 934.01 of AOAC, 2000).

Crude protein and amino acids

The nitrogen (N) content of samples was determined by the combustion method (Method 968.06 of AOAC, 2000) using Leco (FP-528 N; Leco Corp., St. Joseph, MI) with EDTA as a standard. The crude protein content of samples was calculated as N x 6.25.

The amino acids in the samples were determined at the Animal Nutrition Division of Degussa Ltd, Germany using an HPLC technique (Biochrom 20; Amersham Pharmacia Biotech, USA). AOAC (2000) methods were used that involved oxidation of the protein with performic acid followed by acid hydrolysis. Tryptophan was determined following alkaline hydrolysis in an autoclave.

Crude fat and free fatty acids

Crude fat content of samples was determined by the ether extraction method (Method 920.39 of AOAC, 2000) using a Soxtec system (Foss UK Ltd) after the samples had been digested by hydrochloric acid (4M) using the wet digestion method.

The free fatty acid content of the fat extracted from the samples was determined by the titration method (Method 940.28 of AOAC, 2000) and expressed as percent oleic acid.

Ash, calcium and phosphorus

The ash content of samples was determined by combustion in a muffle furnace for 24 h at 500°C by AOAC method (Method 942.05 of AOAC, 2000).

Calcium content of samples was determined by AOAC method (Method 968.08 of AOAC, 2000) using an atomic absorption spectrophotometer (Smith-Hieftje 1000; Thermo Electron Corp., Hampstead).

Phosphorus content of samples was determined by AOAC method (Method 965.17 of AOAC, 2000) using a spectrophotometer (DU 640; Beckman, USA).

Non-starch polysaccharides

Total, soluble and non-soluble NSP contents were determined according to AOAC (1998) methods (985.29, 991.42, 993.19, 991.43) and AACC Methods (32.07, 32.21, 32.05) using a Megazyme assay kit (Megazyme International Ireland Ltd). Duplicate analyses were performed on all samples. The NSP profile was not determined because the soluble NSP fraction showed no significant effect on *in vitro* viscosity of the shea nut meal.

Neutral detergent fibre

The neutral detergent fibre content was determined by the method of van Soest (1963) using a neutral detergent solution.

Phenolic compounds

- ***Total soluble polyphenols***

Total soluble polyphenols were determined at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland. The content of total phenolics was determined according to the method described by Naczk and Shahidi (1989) using catechin as a standard. A methanol solution of the extract (0.5 nil), Folin-Ciocalteau's solution (0.5 ml), sodium saturated solution (1 ml) and water (8 ml) were added and mixed well. The absorbance was measured at 725 nm.

- ***Total extractable tannins***

Total extractable tannins of the samples were carried out at the Wildlife Habitat/Nutrition Laboratory of the Department of Natural Resource Sciences, Washington State University, Pullman, USA according to the procedure of Martin and Martin (1982) using the Bovine Serum Albumen (BSA) binding assay. The results obtained as tannin binding capacity in

mg BSA precipitate per g sample were expressed on g/kg dry matter basis. This method would extract both hydrolysable and condensed tannins

- ***Bound plus soluble condensed tannins (proanthocyanidins)***

Bound plus soluble proanthocyanidins were determined at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland. The bound plus soluble proanthocyanidins were assayed colorimetrically by the method of Price *et al.* (1978b) using catechin as a standard. A methanolic solution of the extract (1 ml) and vanillin reagent (5 ml) were added and mixed well. The absorbance of each sample was measured at wavelength of 500 nm.

- ***Soluble condensed tannins (proanthocyanidins)***

Soluble proanthocyanidins were determined at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland. The soluble proanthocyanidins were determined by the acid-butanol reagent method of Porter *et aL* (1986) as cyanidins using an HPLC (Beckman DU 7500, USA) and detected at a 550 nm wavelength.

- ***Hydrolysable tannins***

Hydrolysable tannins were determined at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland. Hydrolysable tannins were determined as gallic acid after enzymatic hydrolysis using tannase according to the procedure of Karamad *et al.* (2006), after which HPLC (Shimadzu Corp., Kyoto, Japan) was used for the detection at 280 nm wavelength. Gallic acids were analysed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan).

Phytate

Phytate was determined according to AOAC method (Method 986.11 of AOAC, 1990) and the method described by Rutkowska (1981) at the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland.

Saponins

The saponin in the samples was determined at the Institute of Agricultural Research and Training, Ibadan, Nigeria by the method of Wall *et al.* (1952). The sample was extracted with ethanol in a soxhlet apparatus, and the extract was defatted with benzene in a continuous liquid-liquid extractor. The addition of butanol formed a butanol-saponin extract that was quantified using rat red blood cells.

Theobromine

The shea nut meal samples analysed were either devoid of theobromine as determined by one laboratory (SCIANTEC, Dalton, UK), or barely detectable (0.4 mg/kg DM) as determined by another laboratory (RSSL, Reading, UK). The theobromine content of the sample was determined at the Reading Scientific Services Ltd, The Lord Zuckerman Research Centre, Reading, UK according to an in-house method (TM 150) especially developed for analysing both theobromine and caffeine in chocolates, cocoa products and instant cocoa powder drinks. It was an HPLC method with a reverse-phase C18 column and DAD detection. The peaks of theobromine from samples were checked both by retention time and the spectral matched against the standard, and the content was calculated according to the response factor of the standard. The detection limit of the method was measured to be 1 mg/kg. Therefore, any data less than 1 mg/kg normally was reported as <1 mg/kg or not detectable (RSSL, Reading, UK).

Gross energy

The gross energy content of samples was determined by adiabatic bomb calorimetry (Model 1261; Parr Instrument Co., Moline, IL) with analar sucrose as a standard.

In-vitro viscosity of soluble NSP in shea nut meal

Feed samples

Both expeller and non-industrial shea nut meals, cereal grains (barley, maize, rye and wheat obtained from CERC, Harper Adams University College, UK and soybean meal (Target Feed, UK) were used for this laboratory experiment. The *in-vitro* viscosity of maize served a negative control, because soluble NSP in maize is negligible. On the other hand, wheat and soybean meal, barley and rye grains may contain appreciable contents of soluble NSP that can become viscous due to the presence of pentosans, 13-glucans and xylans, respectively.

Water extraction methods

Two different water extraction methods were used for the *in vitro* viscosity study. According to the Grosjean *et al.* (1999a) method, a water extract of each sample was prepared from 2.5g of feed sample that was mixed with 10 mL de-ionised water, stirred (15 min), centrifuged at 500 rpm (10 min), the supernatant decanted into a tube and incubated in a water-bath at 100°C (10 min), cooled, centrifuged at 500 rpm (5min) and the supernatant pipetted into a tube and stored at 4°C prior to analysis. According to the procedure described by Pirgozliev *et al.* (2001), the water extract of each sample was prepared from 2.0g of the feed sample and mixed with .8 ml distilled water, vortexed, incubated in a water-bath at 40°C (30 min), centrifuged at 1000 rpm (2min) and the supernatant pipetted into a tube and allowed to stand for 15 min at room temperature prior to analysis.

Two- stage method

In addition, an *in vitro* method that used a partial digestion of the feed material was used. The shea nut meal, maize, wheat and barley samples were subjected to a two-stage *in vitro* digestion assay as described by Bedford and Classen (1993). The commercial enzymes: pepsin and pancreatin (Sigma-Aldrich Chemie GmbH, Steinhein, Germany) were used. Replicates (4 each) of each sample (0.1 g) were incubated with 3 ml of 0.1N HC1, which contained 2,000 U pepsin/ml for 45 min at 40°C with occasional vortexing. After 45 min, 1 ml of 1 MNaHCO3 solution that contained 2 mg pancreatin/ml was added and incubated at 40°C for 2 h with occasional vortexing. After each incubation period, the contents were centrifuged at 3,000 rpm for 20 min, and the supernatant pipetted into tubes and stored at 4°C prior to analysis. Relative viscosities of supernatants after centrifugation were measured using a Brookfield Digital Viscometer (Model DV-II+, Brookfield Viscometers Ltd, Essex, UK) set at 30 rpm. 0.5 ml of each sample was used for viscosity measurements. The viscosities were measured at room temperature (24 °C). Duplicate readings were taken in each case.

Statistical analysis

Type of shea nut meal [expeller (n=4) and non-industrial (n=2)] was considered as a factor and analysis of variance of data was carried out using GENSTAT (8^{th} version). The correlation coefficients between the soluble NSP and determined viscosity of the samples and the proportions of the amino acids within the protein supply were calculated.

3.1.1.2 Results

Nutrient composition

The chemical compositions of six shea nut meal samples (4 expeller and 2 non-industrial) are shown in *Tables 3.1a and 3.1b.* The dry matter contents of the samples ranged between 907.6and 946.2 g/kg. The mean ether extract (EE) of the expeller samples (125.4 g/kg 59

DM) was lower ($P<0.01$) than that of the non-industrial samples (379.0 g/kg DM).

The free fatty acid content of the residual fat in the samples was lower ($P<0.05$) in the expeller samples (108.3 g/kg EE) than the non-industrial samples (364.0 g/kg EE). The mean crude protein level of the non-industrial samples (110.8 g/kg DM) was lower ($P<0.05$) than that of the expeller samples (134.5 g/kg DM). There were significant differences in the levels of the mean lysine ($P<0.05$), methionine plus cystine ($P<0.01$) and tryptophan ($P<0.05$), which were 40.1 versus 33.1 g/kg CP, 39.1 versus 34.3 g/kg CP, and 14.1 versus 13.2 g/kg CP in the expeller and non-industrial samples respectively. However, there was a strong and consistent relationship between the crude protein level and lysine ($r^2 = 0.883$), methionine+cystine ($r^2 = 0.973$) and tryptophan ($r^2 = 0.832$) in the samples. All the samples contained relatively high amounts of total NSP. The mean total NSP in the expeller samples (383.7 g/kg DM) was higher ($P<0.05$) than that of the non-industrial samples (311.4 g/kg DM), but both had similar contents of soluble NSP *(Table 3.1b)*. The only starch detected in the meal was present as resistant starch.

Table 3.1a: Variation in chemical composition of expeller and non-industrial shea nut meal samples[1] (g/kg DM)

Components	Industrial (expeller) shea nut meal					Non-industrial shea nut meal		
	2004s batch 1	2004s batch 2	2005s	2005j	**Mean±sd**	2004c	2005c	**Mean±sd**
Dry Matter (g/kg)	932.9	924.6	925.7	941.2	**931.1±7.67**	946.2	907.6	**926.9±27.29**
Crude Protein (CP)	143.6	133.3	132.4	128.8	**134.5±6.35**	117.7	103.9	**110.8±9.76**
Amino acids (g/kg CP)								
Methionine	22.5	22.1	22.6	22.1	**22.3±0.25**	20.5	21.4	**21.0±0.06**
Cystine	17.2	16.3	17.0	16.3	**16.7±0.46**	12.9	13.8	**13.4±0.06**
Met+Cys	39.7	38.5	39.6	38.4	**39.1±0.07**	33.4	35.1	**34.3±0.12**
Lysine	41.7	41.4	39.6	37.7	**40.1±0.18**	30.0	36.2	**33.1±0.44**
Threonine	41.2	41.1	42.6	40.8	**41.4±0.08**	38.3	41.3	**39.8±0.22**
Tryptophan	14.0	14.0	14.6	13.9	**14.1±0.03**	13.1	13.2	**13.2±0.01**
Arginine	89.1	85.4	90.3	87.4	**88.1±0.21**	71.7	75.6	**73.7±0.28**
Isoleucine	46.5	46.6	46.9	45.9	**46.5±0.04**	43.1	45.6	**44.4±0.18**
Leucine	78.6	78.1	80.3	77.5	**78.6±0.12**	75.0	78.3	**76.7±0.23**
Valine	57.2	57.4	57.8	56.5	**57.2±0.05**	53.8	57.1	**55.5±0.23**
Histidine	28.5	28.2	28.6	27.6	**28.2±0.04**	24.3	26.4	**25.4±0.15**
Phenylalanine	37.6	37.8	38.6	36.9	**37.7±0.07**	36.5	38.9	**37.7±0.17**
Glycine	50.0	49.5	51.2	49.5	**50.1±0.08**	48.7	53.7	**51.2±0.35**
Serine	44.7	44.0	46.6	44.4	**44.9±0.12**	40.5	43.9	**42.2±0.24**
Proline	52.8	52.2	53.3	50.6	**52.2±0.12**	47.7	51.2	**49.5±0.25**
Alanine	57.9	57.4	59.3	57.4	**58.0±0.09**	55.1	57.8	**56.5±0.20**
Asparagine	116.8	114.5	119.0	115.8	**116.5±0.19**	100.6	106.1	**103.3±0.39**
Glutamine	169.4	164.2	172.0	166.8	**168.1±0.33**	155.4	158.7	**157.1±0.23**
Ether Extracts (EE)	101.4	128.3	120.2	151.6	**125.4±20.86**	363.8	394.8	**379.0±21.92**
Free fatty acids	16.2	15.8	10.1	10.0	**13.0±3.44**	178.6	93.6	**136.1±60.10**
FFA (g/kg EE)	160.0	123.0	84.0	66.0	**108.3±41.9**	491.0	237.0	**364.0±179.61**
Total NSP	376.0	381.7	396.6	380.5	**383.7±5.20**	305.9	316.9	**311.4±37.30**
Insoluble NSP	340.4	345.4	353.9	340.2	**345.0±10.40**	274.5	281.8	**278.2±24.10**
Soluble NSP	35.6	36.3	42.7	40.3	**38.7±4.00**	31.4	35.1	**33.3±6.20**
Ash	52.3	50.6	54.2	46.9	**51.0±3.10**	75.6	51.6	**63.6±16.97**
Calcium	3.1	2.6	1.8	1.6	**2.3±0.70**	3.8	3.0	**3.4±0.57**
Phosphorus	2.6	2.3	2.5	2.4	**2.5±0.13**	2.6	2.4	**2.5±0.14**
GE (MJ/kg)	22.8	24.0	23.8	24.3	**23.7±0.65**	26.0	27.4	**26.7±0.99**

[1]Sample code indicates the year of manufacture and the location of manufacture: s-Shebu-Loders Croklaan Ltd (1-batch one, 2- batch two), j-Juaben Oilmills Ltd, c-Christian Mothers Association. NSP-non-starch polysaccharide, EE- ether extract, GE-gross energy, FFA-free fatty acids, sd-standard deviation

Table 3.1b: Variation in the means of the chemical composition of expeller and non-industrial shea nut meal samples (g/kg DM)

	Industrial (expeller) shea nut meal	Non-industrial shea nut meal	Standard error difference	Probability
Dry Matter (g/kg)	931.1	926.9	13.15	0.765
Crude Protein (CP)	134.5	110.8	6.37	0.020
Amino acids (g/kg CP)				
Methionine	22.3	21.0	0.339	0.015
Cystine	16.7	13.4	0.447	0.002
Met+Cys	39.1	34.3	0.737	0.003
Lysine	40.1	33.1	2.351	0.041
Threonine	41.4	39.8	1.098	0.213
Tryptophan	14.1	13.2	0.242	0.016
Arginine	88.1	73.7	1.99	0.002
Isoleucine	46.5	44.4	0.828	0.062
Leucine	78.6	76.7	1.355	0.219
Valine	57.2	55.5	1.090	0.179
Histidine	28.2	25.4	0.726	0.017
Phenylalanine	37.7	37.7	0.903	0.979
Ether Extracts (EE)	125.4	379.0	36.35	0.003
FFA (g/kg EE)	108.3	364.0	92.34	0.041
Total NSP	383.7	311.4	7.505	<0.001
Insoluble NSP	345.0	278.2	5.307	<0.001
Soluble NSP	38.7	33.3	2.765	0.119
Ash	51.0	63.6	7.708	0.177
Calcium	2.3	3.4	0.579	0.124
Phosphorus	2.5	2.5	0.115	0.685
Gross energy (MJ/kg)	23.7	26.7	0.648	0.010

NSP-non-starch polysaccharide, EE- ether extract, FFA-free fatty acids

The chemical analyses of the phenolic compounds in shea nut meal samples estimated different chemical or biochemical properties of phenols. The concentrations of total soluble phenolics (TSP), total extractable tannins (TET) using the Bovine Serum Albumin binding assay, bound plus soluble proanthocyanidins by the vanillin assay (PasV), soluble proanthocyanidins by the butanol assay (PasB) and hydrolysable tannins (HT) in shea nut meal samples are shown in *Table 3.2.* The analytical data as a whole indicated that shea nut meal had a wide range of phenolic components. The data showed that the processing method of shea nut meal had an effect on the extraction of these phenolic components. The mean concentrations of these various components were significantly higher ($P<0.05$) in the industrial meal than in the non-industrial meal *(Table 3.2).*

Despite wide variations in the values determined between different analytical methods *(Table 3.2),* there were statistically significant correlations between the TSP and TET ($r = 0.715$, $P<0.05$); PasV ($r = 0.918$, $P<0.01$); PasB ($r = 0.920$, $P<0.01$); or HT ($r = 0.832$, $P<0.05$). Similarly, there were significant correlations between TET and PasV ($r = 0.670$, $P<0.05$); or PasB ($r = 0.782$, $P<0.05$). However, no significant correlation was observed between TET and HT ($r = 0.314$). There were, also, significant correlations between PasV and PasB ($r = 0.942$, $P = 0.001$); or HT ($r = 0.762$, $P<0.05$) as well as between PasB and HT ($r = 0.718$; $P<0.05$).

The saponin contents of the shea nut meal samples were similar *(Table 3.2).* Also, the phytate contents were similar, but quite low.

The results of the *in vitro* viscosity studies are shown in *Tables 3.3a & 3.3b.* The different types of shea nut meal had similar *in vitro* viscosities. Both peptic and pancreatic

viscosities were not correlated (r = 0.272, r = 0.196, respectively) with the soluble NSP contents of shea nut meal samples.

Comparison of water extraction *in vitro* viscosity of shea nut meal with selected feed ingredients *(Table 3.3a)* showed that its viscosity was similar to that of wheat or soybean, and lower than that of barley or rye by 30-42% and 85-86%, respectively. However, its *in vitro* viscosity was higher than that of maize by 18-26%.

Table 3.2: The anti-nutritive factor composition of six shea nut meal samples[1] (dry matter basis)

Components	Industrial (expeller) shea nut meal					Non-industrial shea nut meal		
	2004s batch 1	2004s batch 2	2005s	2005j	**Mean ±sd**	2004c	2005c	**Mean ±sd**
Phenolic components[2]								
Total soluble phenolics (g catechin/kg)	122.6	113.5	102.9	106.8	**111.5 ±8.66**	26.2	45.5	**35.9 ±13.64**
Total extractable tannins (g BSA ppt/kg)	147.6	119.2	177.7	201.3	**161.0 ±35.73**	35.1	66.1	**51.0 ±21.92**
Bound+soluble proanthocyanidins (g catechin/kg)	258.1	242.7	279.2	201.4	**245.4 ±32.86**	31.9	67.9	**49.9 ±25.42**
Soluble proanthocyanidins (g cyanidin/kg)	17.6	20.0	20.3	18.5	**19.1 ±1.29**	3.9	7.9	**5.9 ±2.84**
Hydrolysable tannins (g gallic acid/kg)	4.7	5.0	3.4	2.9	**4.0 ±1.03**	1.1	1.8	**1.4 ±0.52**
Saponin (g/kg)	4.5	5.8	6.3	4.0	**5.2 ±1.08**	5.8	6.2	**6.0 ±0.28**
Phytate (g/kg)	0.7	0.4	0.8	0.7	**0.7 ±0.17**	0.6	0.7	**0.7 ±0.08**
Theobromine (g/kg)	ld	ld	ld	ld	-	ld	ld	-

[1]Sample code indicates the year of manufacture and the location of manufacture: s-Shebu-Loders Croklaan Ltd (1-batch one, 2- batch two), j-Juaben Oilmills Ltd, c-Christian Mothers Association.
[2]The chemical analyses estimated different chemical or biochemical properties of phenols
Ld-low detection (<1mg/kg)

Table 3.3a: *In vitro* viscosities of water extraction of soluble NSP in shea nut meal compared with selected feed ingredients

	Method 1 (Pirgozliev *et al.*, 2001)	Method 2 (Grosjean *et al.*, 1999a)
Feed ingredient	Viscosity (cP)	Viscosity (cP)
Maize	1.01	1.06
Wheat	1.36	1.41
Soybean meal	1.39	1.36
Barley	1.94	2.24
Rye	8.97	9.20
Industrial shea nut meal[a]	1.39	1.32
Non-industrial shea nut meal[b]	1.32	1.26
Standard error difference	*0.170*	*0.057*
Probability	*<0.001*	*<0.001*

[a]Mean of 4 samples
[b] Mean of 2 samples

Table 3.3b: *In vitro* viscosities of peptic and pancreatin phases of soluble NSP in shea nut meal compared with selected feed ingredients

	Peptic phase	Pancreatin phase
Feed ingredient	Viscosity (cP)	Viscosity (cP)
Maize (negative control)	1.04	1.26
Wheat (positive control: pentosans)	1.17	1.40
Barley (positive control: β-glucans)	1.78	1.79
Shea nut meal[a]	1.04	1.47
Standard error difference	*0.013*	*0.075*
Probability	*<0.001*	*<0.001*

[a]Mean of 6 samples

3.1.1.3 Discussion

Nutrient composition

Considerable variation in the residual fat content of shea nut meal has been noted in previous studies due to variable efficiencies of fat extraction (Womeni *et al.,* 2002). The higher residual fat in the non-industrial samples was expected since the traditional method of fat extraction was known to be inefficient, and so these samples were higher in their gross energy contents *(Table 3.1).* Also, relatively small deficiencies in operational efficiency of the expeller processes could have influenced the level of residual fat in the four expeller meals. The variation in the FFA content of the fat could either be due to a seasonal effect on kernels, different harvesting and nut preparation methods (Hall *et al.,* 1996) or poor storage conditions at source that might cause oxidation.

The crude protein contents recorded in this study varied only within a narrow range for the expeller meals, but varied substantially between the industrial and non-industrial meals. The non-industrial sample was noticeably darker than the industrial sample that indicates that over-heating could be a problem in this method of production. The traditional method involved higher heat treatment than the expeller method. High processing temperatures of oilseeds has deleterious effects on proteins and amino acids due to formation of Maillard reaction products (Hurell, 1990). The determined amino acid concentrations were similar to the values reported (Lazani *et al.,* 1984). However, the present amino acid profile of shea nut meal included the tryptophan content for the first time. Tryptophan is an essential amino acid for poultry. In general, the concentrations of lysine and methionine in all samples were at low levels that were similar to most cereal protein sources. The low concentrations of lysine and methionine in shea nut meal must be considered when formulating diets for poultry. Annongu *et al.* (1996a) demonstrated the importance of amino acid supplementation of shea nut meal based diets for broilers and recommended the

use of synthetic lysine and methionine in poultry diets that contained high proportions of shea nut meal.

All other nutrient components of the shea nut meal had changed proportionately when the residual fat was reduced *(Table 3.4)*. Most of the nutrients contained in the meal had increased proportionately with fat removal from the meal. This was an indication that the residual fat content partly accounted for the major variation observed in the chemical composition of the meal.

Table 3.4: Changes in chemical composition of expeller and non-industrial shea nut meal samples[1] (g/kg DM) after removal of residual fat using petroleum ether

Components	Industrial (expeller) shea nut meal	Non-industrial shea nut meal
	2004s batch 1	2004c
Dry Matter	935.5	936.4
Crude Protein	170.7	203.2
Amino acids (g/kg crude protein)		
Methionine	20.0	nd[2]
Cystine	19.4	nd
Met+Cys	39.4	nd
Lysine	34.4	nd
Threonine	30.1	nd
Tryptophan	nd	nd
Arginine	63.9	nd
Isoleucine	38.8	nd
Leucine	58.9	nd
Valine	47.6	nd
Histidine	26.3	nd
Phenylalanine	32.6	nd
Glycine	37.6	nd
Serine	33.2	nd
Proline	39.5	nd
Alanine	42.0	nd
Asparagine	76.4	nd
Glutamine	118.4	nd
Ether Extracts	3.8	7.5
Total non-starch polysaccharides	416.8	477.0
Insoluble non-starch polysaccharides	377.4	428.0
Soluble non-starch polysaccharides	39.4	48.9
Ash	64.5	128.9
Calcium	3.6	4.9
Phosphorus	2.9	3.8
Gross energy (MJ/kg)	18.9	18.7
Total soluble phenolics (g catechin/kg)	159.3	nd
Total extractable tannins (g BSA precipitate/kg)	295.5	nd
Bound+soluble proanthocyanidins (g catechin/kg)	311.1	nd
Soluble proanthocyanidins (g cyanidin/kg)	21.7	nd
Hydrolysable tannins(g gallic acid/kg)	5.8	nd
Saponin (g/kg)	0.2	0.2
Phytate (g/kg)	0.9	nd
Theobromine (g/kg)	ld	nd

[1]Sample code indicates the year of manufacture and the location of manufacture: s-Shebu-Loders Croklaan Ltd (1-batch one), c-Christian Mothers Association. Ld- low detection

[2]nd- not determined: The amino acid profile and tannin concentrations were not determined for the defatted non-industrial because it was not a sample of major interest as well as due to cost of analysis

Anti-nutritive factors

Tannins

The anti-nutritive factors identified in shea nut meal samples are shown in *Table 3.2*. Of these, phenolic compounds were present in the greatest amounts. The amounts of some of the phenolic components, such as total phenols and soluble proanthocyanidins, recorded in this study were similar to those reported by Okai *et al.* (1994) and Annongu *et al.* (1996a). The quantification of tannins is important for predicting their effects. However, there are problems with the colorimetric analysis that are due to the variable structures of tannin polymers, extraction of non-tannin polyphenolics associated with tannins and the absence of satisfactory standards (Rickard, 1986; Hagerman and Butler, 1989; Butler and Rogler, 1992). Therefore, total phenolics may include non-tannin polyphenolic compounds or tannin precursors. These low molecular weight polyphenol components associated with tannins are more readily absorbed from chick diets than tannins, and may account for the major anti-nutritive effects (Butler and Rogler, 1992). Although hydrolysable and condensed tannins are largely different in structure, they often produce rather similar anti-nutritive effects such as diminished weight gains and lower efficiency of utilisation of nutrients, particularly protein (Butler and Rogler, 1992).

The chemical interactions between tannins and proteins are generally similar for proanthocyanidins and hydrolysable tannins (Hagerman and Kiucher, 1986). Thus protein precipitation assays have been found more useful for evaluating the anti-nutritive effects of tannins than their quantification by colorimetric methods (Martin and Martin, 1982; Rickard, 1986). In this study, the total extractable tannins obtained by the Bovine Serum Albumin binding assay indicated that the amounts of tannins extracted were high enough to cause nutritional problems in birds. The industrially produced expeller samples contained considerably higher levels of tannins than the non-industrial meal *(Table 3.2)*.

This might be due to the mode of processing of the non-industrial meal, which involved the use of water to extract the fat from the kneaded kernels. Moist conditions favour the irreversible complexing of tannins with proteins and possibly other organic compounds so that the tannin could no longer be extracted and assayed (Butler and Rogler, 1992). Also, the non-industrial processing involved relatively high temperatures (--150°C) during the roasting stage of the kernels. Hagerman (1988) observed that tannin extractability from plant tissues might be reduced by drying samples at temperatures above 50°C. The variations in tannin concentrations among the samples, particularly the nonindustrial meals, could be attributed to seasonal effects on shea nut production in the savanna climate of West Africa. According to Hagerman (1988), tannin extractability may change drastically within a single plant species with season and changes in chemical composition. Removal of the residual fat in the expeller sample resulted in a proportional increase in the levels of the phenolic components, but substantially decreased the saponin content *(Table 3.4)*.

Several studies in ruminant species have shown that tannins form strong complexes with protein, and this effect causes negative effects on appetite and nutrient utilisation (Robins *et al.*, 1987; Silanikove *et al.,* 2001). Tannins may bind to cell walls or soluble cell contents (Kumar and Vaithiyanathan, 1990; Reed, 1995) and so reduce the digestion of protein, which in turn may cause decreases in food intake. Tannins are also known to have an astringent taste which reduces palatability (Ahmed *et al.*, 1991). The physical basis for astringency (Jansman, 1993) may be that tannins bind and perhaps precipitate salivary mucoproteins (Mateus *et al.,* 2004). The interactions involved in this effect are thought to include hydrophobic effects and hydrogen bonding (Oh *et al.*, 1980; Haslam, 1989). This would reduce the lubricating property of saliva, give the mouth a feeling of dryness and affect the ability to swallow the food (Mole, 1989). A second more direct way that tannins affect feed palatability may be that tannins directly bind to taste receptors (Mole, 1989).

taste buds in their oral cavity (Anon., 2000). Unlike proanthocyanidins, hydrolysable tannins are subject to enzymatic hydrolysis in the digestive tract (Fahey and Jung, 1989; Mole, 1989). The resulting products include gallic acid, which is readily absorbed and decreases feed intakes (Jung and Fahey, 1983) and growth rates in chicks (Butler and Rogler, 1992).

Similar negative effects of tannins have been reported in studies with chickens using shea nut meal based diets (Adeogun, 1989; Annongu *et al.,* 1996a, 1996b; Atuahene *et al.,* 1998; Olorede *et al.,* 1999) and other monogastric animals such as rats (Okai *et al.,* 1994; 1995) and pigs (Okai and Bonsi, 1989). Other studies involving high-tannin feedstuffs have given poor weight gains in chicks (Armstrong *et al.,* 1974; Ahmed *et al.,* 1991; Smulikowska *et al.,* 2001).

Absorbed tannins may also have systemic effects on the internal body organs such as necroses of the liver and kidneys of chicks (Karim *et al.,* 1978) as well as an involvement in the development of chick leg abnormalities (Elkin *et al.,* 1978). There have been conflicting reports on the effects of shea tannins in poultry. Annongu *et al.* (1996a, 1996c) observed liver congestion, focal necroses and partial alterations in hepatic architecture as well as chick leg abnormalities in broiler chickens fed shea nut meal based diets. Contrary to their fmdings, Atuahene *et al.* (1998) found no changes in the histological characteristics of the liver or leg structure. Also, Olorede *et al.* (1999) found no changes in the internal organs of broiler chickens that could be attributed to tannins in shea nut meal based diets.

Saponins

Saponin concentrations recorded in this study *(Table 3.2)* were within the range (3.0-30 g/kg DM) reported by Annongu *et al.* (1996a) and Atuahene *et al.* (1998). A number of

factors such as environmental and agronomic factors have been shown to affect the saponin content of plants (Yoshiki *et al.,* 1998). One mechanism that might account for growth-depressing effects of saponins is the lowering of feed intake, because of unpalatability, bitterness (Cheeke, 1971) or astringency (Oleszek *et al.,* 1992). Thus depression in feed intakes of birds fed shea nut meal based diets has been attributed partly to its content of saponin (Annongu *et al.,* 1996a; Atuahene *et al.,* 1998).

Removal of the residual fat in the two different shea nut meal samples reduced the saponin contents of the samples considerably *(Table 3.4).* The unsaponifiable material in unrefined shea fat (e.g. sterols) is high relative to other vegetable oils (Padley *et al.,* 1994). Therefore, processing methods that substantially improve fat extraction efficiency (for example solvent extraction processes) from the shea kernels may enhance the quality of this by-product for feeding poultry.

Theobromine

Theobromine is a toxic alkaloid. The shea nut meal samples analysed were either devoid or contained <1.0 mg/kg DM of theobromine, whereas 4.5 g/kg DM is reported in the literature (Atuahene *et al.,* 1998; Rhule, 1999). The relatively high theobromine level reported in the literature might be due to the method of analysis. In the present study, an advanced method (i.e. HPLC) was used; whereas other researchers used an old analytical method devised by Pearson (1970). The theobromine content recorded in this study appeared to be too low to be of any nutritional concern. Nevertheless, the theobromine reported in earlier studies has been implicated in growth depression of broilers (Atuahene *et al.,* 1998) and pigs (Rhule, 1999).

Phytate

Phytate includes mainly inositolhexaphosphate, but also penta and tetraphosphates are present. They bind calcium, magnesium, iron and other metals into unavailable complex salts, therefore, they are considered as negative factors in nutrition. They are set free from their bonds with proteins and phospholipids under processing conditions (Moscicki *et al.*, 2003). Phytate concentrations in the shea nut meal samples were relatively low *(Table 3.2)*, therefore may not pose any nutritional problems in birds.

Non-starch polysaccharides

Chemical analysis of the NSP in shea nut meal indicated there were high levels of insoluble NSP as well as substantial amounts of soluble NSP *(Table 3.1)*. However, the determination of *in vitro* viscosity of shea nut meal gave relatively low values, which were comparable to that of the wheat and soybean.

Generally, the utilisation of NSP in poultry has been found to be far lower than in other monogastric animals (Jorgensen *et al.*, 1996). The physicochemical properties of NSP are known to be responsible for anti-nutritive activity in broiler chicken (Smits and Annison, 1996). Insoluble NSP is considered to be practically indigestible by poultry (Carre, 1993), thus the relatively high insoluble NSP was an indication that the non-fat component of the meal would not be a significant source of dietary energy. Morgan and Trinder (1980) used an *in vitro* study and found a low digestible organic matter and total digestible nutrients in a shea nut meal sample that they attributed to the fibre content of the meal.

Also, the shea nut meal contained moderate amounts of soluble NSP comparable to that of barley (Englyst, 1989). Although soluble NSP has the potential of being digested in birds (Cane, 1993), the birds do not possess endogenous enzymes capable of cleaving and digesting the 13 linked NSP (Williams *et al.*, 1997). Even though the exact mechanism of action of soluble NSP is not known (Smits and Annison, 1996), it is thought to involve

increased viscosity of digesta. The formation of a viscous solution in the intestine impedes the mixing of the gut contents with digestive enzymes or contact between nutrients and absorption sites on the intestinal mucosa (White *et al.*, 1983; Annison, 1993; Bedford and Morgan, 1996; Smits *et al.*, 1997) with consequent inhibition of starch, lipid and protein digestibility in the birds (Choct and Annison, 1992).

In this study, there seemed to be a low viscosity associated with soluble NSP of shea nut meal that was substantially lower than that of barley or rye *(Tables 3. 3a & 3. 3b).* The water extract viscosity recorded for barley was within the range (1.3 - 7.0) reported by Grosjean *et al.* (1999b). Heat treatment could influence soluble NSP properties (Smits and Annison, 1996) by reducing *in vitro* viscosity (Campbell *et al.*, 1983). The presence of pentosans has been reported in shea kernels (Kershaw and Hardwick, 1981), while mixed-linked f3-glucans are found in barley (McNab and Smithard, 1992). Therefore, the similarity between *in vitro* viscosities of wheat and shea nut meal suggests the soluble NSP in shea nut meal may not be of any major nutritional concerns for poultry.

3.1.1.4 Conclusion

The chemical evaluation of the six shea nut meal samples has shown high and widely variable fat contents. Also the fat contained variable amounts of free fatty acids. The samples contained moderate contents of crude protein that were low in lysine and methionine plus cystine. The samples had high levels of total NSP, but *in vitro* viscosity of the soluble NSP fraction was relatively low. The gross energy contents of the samples were high. The samples were high in tannins and saponin. The removal of the residual fat in the meal considerably reduced the content of saponin, but increased proportionately the concentrations of other chemical constituents.

The nutrient compositions of the samples have shown that the meal could provide some nutrients and energy in poultry diets. However, its amino acid profile would require amino acid supplementation with synthetic lysine and methionine plus cystine. Also, the wide variations observed in nutrient compositions of shea nut meal samples could cause feed formulation problems.

The concentrations of tannins and saponins determined in the shea nut meal samples were quite high, and these high levels could have adverse effects on growth performance of birds. Therefore, further treatments of the meal to counteract, reduce or eliminate these anti-nutritive factors would be imperative.

3.1.2 Value of Shea Fat for Poultry

The chemical compositions of shea nut meals evaluated in the previous section **(section 3.1.1)** have shown there to be variable fat contents. Therefore, the content of fat in the meal may contribute to a large proportion of its overall dietary available energy. Although dietary fat of good quality contributes substantially to the available metabolisable energy for poultry (Wiseman *et al.*, 1986), the nature of shea fat may limit its supply of dietary energy. Therefore, there is a need to investigate the utilisation of this fat in poultry diets.

Shea fat has a relatively high economic value to the regions; therefore it is unlikely to be intentionally produced as an animal feed. However, some downgraded or unwanted batches may become available occasionally to the animal feed industry. Shea fat is also an important component of shea nut meal, the residue obtained after fat extraction.

Shea fat is mostly comprised of saturated fats due to the presence of large amounts of stearic acid but, unlike cocoa fat, it does not contain any significant amount of palmitic acid. Even though there are detailed data on the fatty acid composition of shea fat (Lipp and Anldam, 1998), there is a dearth of information on its digestibility and metabolisable energy in farm animals. The specific objectives of this experiment were to compare the lipid digestibility and metabolisable energy of shea fat with soybean oil and cocoa fat when fed to broiler chickens at three different inclusion levels (30, 60, 90 g/kg).

3.1.2.1 Materials and Methods

Experimental samples

The shea fat for the study was supplied by Aarhus Ltd, Denmark, cocoa *(Theobroma cacao)* fat was provided by Cadbury Ltd, UK and soybean oil was supplied by Target Feeds Ltd, UK. A basal diet was prepared that was based on maize, dehulled soybean

meal, wheatfeed and fish meal, but no supplemental fat or oil. Three dietary inclusion levels (30, 60, 90 g/kg) of shea fat, cocoa fat and soybean oil were added to the basal diet that was calculated to contain adequate levels of required nutrients for young broiler chickens *(Table 3.5)*. The determined crude fat (g/kg DM) contents of diets with added lipid at 30, 60 and 90 g/kg including basal diet were: Basal diet (26.9), Soybean oil diets (50.1, 78.8, 102.5), Cocoa fat diets (51.1, 77.1, 108.6); and Shea fat diets (50.0, 76.9, 97.2).

Table 3.5: Calculated composition (g/kg) of the basal feed

Ingredients	Amount
Maize	400
Dehulled soybean meal	300
Fishmeal	30
Wheatfeed	220
Lysine (HCl)	3
Methionine	4
Limestone	4
Dicalcium phosphate	13
Vitamin and trace element premix*	22
Salt	4
Calculated composition (g/kg)	
Crude protein	239.4
Crude fibre	36.9
Calcium	11.4
Phosphorus	7.6
Sodium	2.0
Lysine	15.2
Methionine	7.6
Methionine + Cystine	11.6
ME (MJ/kg)**	11.5

*Vitamin-trace mineral premix for broilers (Ian Hollows Feed Supplement, UK) added per kg of diet: vitamin A, 16,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 mg; thiamine, 3 mg; riboflavin, 10 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.015 mg; nicotinamide, 60 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; biotin, 0.125 mg; choline chloride, 200 mg; iron, 20 mg; cobalt, 1 mg; manganese, 100 mg; copper, 10 mg; zinc, 80 mg; iodine, 1 mg; selenium; 0.2 mg, and molybdenum, 0.5 mg.

**ME calculated using soybean oil ME (36.5 MJ/kg). Calculated dietary ME values of added soybean oil were 11.5 MJ/kg (0 g/kg), 12.2 MJ/kg (30 g/kg), 12.9 MJ/kg (60 g/kg) and 13.6 MJ/kg (90 g/kg).

Broiler assay

Male day-old broiler chicks (Ross 308, UK) were reared in a litter-floored pen and fed a proprietary broiler starter feed for 13 days. One hundred and sixty (160) birds were then randomly selected and two birds placed into one of 80 metabolism cages (0.36 m x 0.36 m x 0.42 m) with wire floors and that were kept in an environmentally controlled room. The experiment was a factorial design (3 lipids x 3 dietary levels) with one additional control (no added lipid). Each cage of birds was fed one of the ten experimental diets for the following eight days. Weight gains and feed intakes were recorded for the whole period and feed intakes were recorded and all egesta and excreta (droppings) were collected for the final four days. The droppings were collected daily and stored at 4°C until the total sample could be dried in a forced-draught oven at 88°C to constant weight. The droppings were sealed in polythene sheets and stored in a chiller to avoid water loss prior to drying. After drying, the droppings samples were ground in a laboratory mill fitted with lmm-mesh size screen and then stored in a sealed container at 4°C prior to chemical analysis.

Chemical analyses

The lipid in the basal diet was obtained by continuous extraction of the prepared basal diet with petroleum ether (b. p. 40-60°C) using a Soxtec system (920.39; AOAC, 2000). The sample of lipid from the basal diet plus the three test lipid samples were analysed for their fatty acid profiles (963.22; AOAC, 2000) using the one-step extraction and esterification procedure of Sukhija and Palmquist (1988). Fatty acid analysis was completed using a gas-liquid chromatograph (Perkin Elmer 8500; Perkin Elmer Life and Analytical Sciences Ltd, USA) fitted with an automatic sampler (Perkin Elmer AS 8300), integrator, and flame ionization detector. Fatty acids were identified by their relative retention times by comparison with retention times of known pure fatty acid standards (Sigma-Aldrich, UK). Free fatty acid, moisture content and peroxide value were determined using AOAC (2000) methods (940.28, 926.12, 965.33).

The dry matter and lipid contents of the diets and droppings samples were determined using standard methods (934.01, 920.39; AOAC, 2000).

Calculations

The equation used to calculate the apparent digestibilities of the test fats in the diets was as follows:

$$D F = b / b + (a \times D B)$$

F = test fat

D = apparent digestibility coefficient

B = basal diet

a = proportion of the basal diet

b = proportion of test fat in the basal diet

The AME values of added fats were derived from the difference between the AME value for the basal diet and for that obtained for the basal diet containing a known proportion of added fats. The combination of the added fats was considered to be completely additive. The equation used was as follows:

$$AME F = (AME T — a AME B) / b$$

Where:

F = test fat

T = test diet

B = basal diet

a = proportion of the basal diet in test diets

b = proportion of added fat in the basal diet

Statistical analysis

The experimental data were compared using a randomized block analysis of variance. Tier level of the cages was considered a blocking factor. Any experimental unit (a total of six) that had data that were greater than three standard deviations from the treatment mean was omitted from the statistical analysis for all variables. Linear and non-linear regression techniques with grouping factors (fat or oil) were used to examine the effect of level of dietary fat or oil addition on the lipid digestibility or determined AME respectively of the added lipids.

3.1.2.2 Results

All three lipids *(Table 3.6)* had low free fatty acids and peroxide values. Thus these fats met the suggested quality specifications for feed fats (Palmquist, 2002). The gross energy contents were typical of fats and oils. Shea fat contained predominantly stearic acid, while cocoa fat comprised mainly palmitic and stearic acids. The fatty acid profiles of the fats including soybean oil in this study were typical of those reported in other studies (Banerji *et al.* 1984; Lipp and Anklam, 1998; Wiseman and Salvador, 1991). The lipid in the basal feed was comprised largely of unsaturated C:18, C:20 and C:22 fatty acids *(Table 3.6).* The presence of the long chain fatty acids was probably due to the fishmeal in the basal diet. Eicosapentaenoic acid (EPA, C:20:5n-3) and docosahexaenoic acid (DHA, C:22n-3) are found in fish oil (Hammershoj, 1995).

There were no mortalities in this experiment. The feed intake of birds fed soybean oil compared to those fed shea and cocoa fats was neither affected ($P>0.05$) by the lipid nor level of inclusion in the diets *(Table 3.7).* The gain-to-feed ratio was increased ($P<0.001$) in the birds fed the soybean oil and there was a non-linear effect of level with the greatest gain-to-feed ratio being obtained in the birds fed the 60 g/kg fat/oil additions. No ($P>0.05$) lipid x level of inclusion interaction was observed.

The digestibility coefficients for the dry matter, lipid and gross energy of the shea and cocoa fats were lower than those for soybean oil *(Table 3.8)*. The inclusion of shea fat at 90 g/kg gave lower ($P<0.001$) digestibility of dry matter or lipid than that of cocoa fat. However, dietary energy metabolisabilities of both fats were not significantly different ($P>0.05$) at 90 g/kg dietary level. The 90 g/kg level of lipid inclusion resulted in a decrease ($P<0.01$) in lipid digestibility coefficients but there was also a lipid x level of inclusion interaction ($P<0.01$) that was due to a very low digestibility coefficient of lipid (0.43) in the 90 g/kg shea fat diet. There were no differences ($P>0.05$) in lipid digestibility between the 30 g/kg and 60 g/kg inclusion levels for any of the three test lipids.

There was a significant effect of lipid source on AME ($P<0.001$) of the diets. The determined AME of the soybean oil diets were significantly ($P<0.001$) greater than the shea and cocoa fat diets *(Table 3.9)*. Increasing dietary lipid levels gave increasing ($P<0.05$) determined AME values but there was a lipid x level interaction ($P=0.015$). The increase in AME due to increasing lipid level only occurred up to 60 g/kg for the shea and cocoa fat diets.

Table 3.6: Composition of test lipids and oil extracted from the basal diet

	*Oil in basal diet	Soybean oil	Cocoa fat	Shea fat
Relative proportions of fatty acids (g/kg)				
Myristic acid (**$C_{14:0}$)	5.5	0.0	0.0	0.0
Pentadecanoic acid ($C_{15:0}$)	0.0	0.0	0.0	1.5
Palmitic acid ($C_{16:0}$)	151.7	110.6	259.3	35.6
Palmitoleic acid ($C_{16:1}$)	8.7	0.0	2.9	0.0
Heptadecanoic acid ($C_{17:0}$)	1.2	1.0	1.8	0.0
Stearic acid ($C_{18:0}$)	24.4	36.2	377.4	430.6
Oleic acid ($C_{18:1}$)	253.8	227.1	341.9	449.8
Linoleic acid ($C_{18:2}$)	466.2	546.2	3.1	63.8
Linolenic acid ($C_{18:3}$)	39.3	69.4	1.9	0.0
Arachidic acid ($C_{20:0}$)	4.2	3.3	11.7	15.1
Erucic acid ($C_{20:1}$)	13.0	2.7	0.0	3.6
Eicosapentaenoic acid ($C_{20:5}$)	10.1	4.4	0.0	0.0
Cetoleic acid ($C_{22:1}$)	10.4	0.0	0.0	0.0
Docosahexaenoic acid ($C_{22:6}$)	12.9	0.0	0.0	0.0
Total unsaturated fatty acids	814.4	849.8	349.8	517.2
Total saturated fatty acids	187	151.1	650.2	482.8
Unsaturated/saturated fatty acid ratio	4.36	5.62	0.54	1.07
Moisture (g/kg)	nd	0.0	0.0	0.0
Free fatty acids (g/kg)	nd	13.0	14.0	2.0
Peroxide value (meq/kg)	nd	0.26	0.08	0.13
Gross energy (MJ/kg)	37.54	39.20	39.82	39.93

*Oil extracted from the basal diet
**Carbon chain length followed by number of double bonds
nd = not determined

Table 3.7: Growth performance of broiler chickens (13-21d) fed 3 lipids at 3 levels

Variable	Lipid level (g/kg)	Test lipids			mean	Analysis of variance		
		Soybean oil	Cocoa fat	Shea fat		Factor	SED *r.d.f.=56*	*P*
Mean feed intake (g/bird/day)	0				68.6	Lipids	2.479	0.141
	30	67.0	69.5	67.9	68.2	Level	2.479	0.588
	60	69.8	70.3	71.7	70.6			
	90	64.1	71.9	72.2	69.4			
mean		67.0	70.6	70.6	69.4	Lipids x Level	3.036	0.222
Mean live weight gain (g/bird/day)	0				41.2	Lipids	1.945	0.674
	30	42.2	41.1	41.1	41.5	Level	1.945	0.183
	60	46.9	42.0	43.8	44.2			
	90	41.2	42.4	41.3	41.6			
mean		43.4	41.8	42.1	42.4	Lipids x Level	2.382	0.346
Gain-to-Feed ratio	0				0.61	Lipids	0.0133	<0.001
	30	0.63	0.59	0.60	0.61	Level	0.0133	0.037
	60	0.67	0.60	0.61	0.63			0.433 (L)
	90	0.64	0.59	0.57	0.60			0.020 (Q)
mean		0.65	0.59	0.60	0.61	Lipids x Level	0.0162	0.139

Polynomial contrasts: L-linear effects, Q-quadratic effects, SED-standard error of difference, P-probability, r.d.f.- residual degrees of freedom

Table 3.8: Apparent metabolisability of dietary dry matter, lipid and gross energy in broiler chickens (13-21d) fed 3 lipids at 3 levels

Variable	Lipid level (g/kg)	Test lipids			mean	Analysis of variance		
		Soybean oil	Cocoa fat	Shea fat		Factor	SED *r.d.f.=56*	*P*
Dry matter	0				0.64	Lipids	0.00714	<0.001
	30	0.66	0.64	0.64	0.65	Level	0.00714	0.002
	60	0.67	0.63	0.64	0.65			
	90	0.65	0.63	0.61	0.63	Lipids x Level	0.00874	0.024
mean		0.66	0.63	0.63	0.64			0.144(L)
Lipid	0				0.72	Lipids	0.02895	<0.001
	30	0.85	0.63	0.64	0.71	Level	0.02895	<0.001
	60	0.87	0.59	0.62	0.69			
	90	0.80	0.56	0.43	0.60	Lipids x Level	0.03545	0.003
mean		0.84	0.59	0.57	0.67			0.019(L)
ME:GE	0				0.68	Lipids	0.01082	<0.001
	30	0.71	0.68	0.67	0.69	Level	0.01082	<0.001
	60	0.72	0.67	0.68	0.69			
	90	0.70	0.64	0.63	0.66	Lipids x Level	0.01031	0.019
mean		0.71	0.66	0.66	0.68			0.065(L)

SED-standard error of difference, P-probability, r.d.f.- residual degrees of freedom
Polynomial contrasts for lipid x level interaction: L-linear effects

Table 3.9: Determined AME of experimental diets.

Variable	Lipid level (g/kg)	Test lipids			mean	Analysis of variance		
		Soybean oil	Cocoa fat	Shea fat		Factor	SED *r.d.f.=56*	*P*
AME	0				12.43	Lipids	0.1635	<0.001
(MJ/kg DM)	30	13.28	12.87	12.57	12.91	Level	0.1635	0.001
	60	13.96	13.10	13.08	13.38			
	90	13.94	12.64	12.55	13.04	Lipids x Level	0.2003	0.015
mean		13.73	12.87	12.73	13.11			0.026(L)

SED-standard error of difference, P-probability, r.d.f.- residual degrees of freedom
Polynomial contrasts for lipid x level interaction: L-linear effects

3.1.2.3 Discussion

The experimental data indicated that, in both shea and cocoa fat diets, there was a reduced AME in the 90 g/kg diets. Although it was considered valid to examine this very high dietary level in the experiment, it is not of great practical significance. Practical poultry feeds typically contain a maximum of 50 g/kg of added fats and practical problems of mechanical handling difficulties and pelleted feed quality limit greater amounts being added. Further examination of the data from this experiment could therefore either eliminate, or otherwise deal with the 90 g/kg data, in order that conclusions can be made that are relevant to the commercial feed industry.

Linear regression analysis of the apparent lipid digestibilities of only the two lower inclusion levels (30 and 60 g/kg) of the three diet series indicated that there was neither level effect nor lipid x level interaction. Thus the derived mean digestibility of test lipids in the total lipid supply in the diets indicated lipid digestibility coefficients of 0.95, 0.54 and 0.58 for soybean oil, cocoa fat and shea fat respectively *(Table 3.10). Wiseman et al* (1991) indicated that lipid digestibility decreased when the overall dietary unsaturated-to-saturated fatty acid ratio decreased. In the present experiment, there was also a strong linear relationship (r^2=0.8792) between the total dietary unsaturated-to-saturated fatty acid ratio and lipid digestibility. However, this relationship was not always consistent within each dietary test lipid treatment, particularly in the soy oil treatment. This agrees with the curvilinear model described by Ketels and de Groote (1989). The estimate for soybean oil is similar to the value determined by Mossab *et al.* (2000) for soybean oil in chickens. No digestibility coefficients for cocoa fat appear to have been determined in poultry but the present estimate is similar to the coefficient of fat digestibility reported by Chen *et al.* (1989) from work with rats.

The lower fat digestibility of the shea and cocoa fat was expected. Wiseman and Salvador (1991) demonstrated that saturated fats had a lower digestibility than unsaturated fatty acid in rats, which in the presence of calcium and magnesium is poorly absorbed due to soap formation (Mattson *et al.*, 1979; Brink *et al.*, 1995). Both shea and cocoa fats have either C16:0 or C18:0 as the major fatty acids in the sn-1/3 position of the glycerol backbone (Lipp and Anklam, 1998).

The contribution of the added dietary lipid can be estimated by deduction of the determined AME of that diet from the determined AME of the basal feed (Nitsan *et al.* 1997). The derived estimates for the three lipids used in the present experiment indicated AME concentrations of soybean oil, cocoa fat and shea fat to be 39.8, 26.4 and 22.0 MJ/kg DM respectively *(Table 3.10).*

Prediction of the AME value of fats from the product of their apparent fat digestibility and gross energy concentration has been employed in a number of studies (Wiseman and Lessire, 1987). Wiseman (1990) observed that calculation of AME value of fat from its digestibility and gross energy tended to be lower than a directly determined AME. In the study, calculated AME values obtained by this method were 37.1, 21.5 and 23.1 MJ/kg DM for soybean oil, cocoa fat and shea fat respectively with a standard error of 1.18 MJ/kg. The values for soybean oil and cocoa fat were both lower than the determined values, but the calculated shea fat value was numerically larger. The data therefore indicate that the utilisation of the absorbed lipid hydrolysis products from shea fat may be poorer than expected or the dietary shea fat may have influenced the availability of other nutrients of the diet.

Table 3.10: Apparent lipid digestibility and AME estimates of soybean oil, shea fat and cocoa fat at 2 lipid levels (30, 60 g/kg)

Test lipids	30 g/kg	60 g/kg	Mean
		Digestibility coefficient	
Soybean oil	0.96	0.93	0.95
Cocoa fat	0.55	0.53	0.54
Shea fat	0.58	0.58	0.58
		AME (MJ/kg DM)	
Soybean oil	42.0	37.6	39.8
Cocoa fat	29.5	23.3	26.4
Shea fat	21.2	22.7	22.0

3.1.2.4 Conclusion

This experiment has shown that shea fat contributes to the energy supply to a broiler chicken feed although it had a poor digestibility at high (90 g/kg) dietary levels. The derived AME for shea fat of 22.0 MJ/kg DM gives a valid estimate of its energy concentration at dietary levels of 60 g/kg or less.

3.1.3 Nutrient Metabolisability of Different Shea Nut Meal Samples

There is a relative lack of information on the availability to poultry of the nutrients contained within shea nut meal. There is also a need to evaluate and explain the variability in nutrient availability between the different shea nut meal samples that are presently available in the industry. The previous section **(section 3.1.2.0)** that examined energy availability of shea fat showed that the fat had low metabolisable energy. However, it is not known how shea nut meal samples with varying residual fat would influence dietary nutrient availability.

The objective of this experiment was to determine the metabolisability of nutrients and energy in the six collected shea nut meal samples (4 expeller, 2 non-industrial) and two defatted samples (1 expeller, 1 non-industrial).

3.1.3.1 Materials and Methods

Shea nut meal samples

The samples were as outlined in previous section **(3.1.1.1).**

Broiler assay

Ross 308 male broiler chicks were reared in a solid-floored pen and fed a proprietary crumbled-pellet broiler starter diet (CP=235.0 g/kg, ME=12.5 MJ/kg) for 12 days. The experiment was a factorial design (8 shea nut meal samples x 2 dietary levels) with one additional control (no shea nut meal). The control was duplicated to further enhance the reliability of the control data since metabolisability values would be derived from these data. At 12 days of age, 180 broilers of similar body weight were individually caged (0.3m x 0.3m x 0.36m) and fed one of 17 meal-form experimental diets to 20 d of age. The basal *diet (Table 3.11)* was substituted with shea nut meal at 0, 20 and 40 g/kg. During the 8-day

experiment, the feed offered during the last 4 days was restricted to an amount estimated to be 70% of *ad libitum* feed intake of the control diet based on the measured *ad libitum* feed intake (recorded the previous day) of birds from the same flock kept in similar cages. This was done to avoid any confounding of lower feed intakes due to the presence of dietary shea nut meal. The droppings were collected daily and stored at 4°C until the combined four-day sample was then immediately dried in a forced-draught oven at 60 °C

Table 3.11: Calculated composition (g/kg) of the experimental diets

Ingredients	Basal diet	Shea nut meal diets	
		20 g/kg	40 g/kg
Maize	400	392	384
Dehulled soybean meal	300	294	288
Fishmeal	30	29.4	28.8
Wheatfeed	220	215.6	211.2
Lysine (HC1)	3	2.94	2.88
D,L-Methionine	4	3.96	3.84
Limestone	4	3.96	3.84
Dicalcium phosphate	13	12.74	12.48
Vitamin and trace element premix*	22	21.56	21.12
Salt	4	3.96	3.84
Shea nut meal**		20	40
Calculated composition (g/kg)			
Crude protein	239.4	237.5	235.6
Crude fibre	36.9	36.1	35.4
Calcium	6.6	6.6	6.5
Phosphorus	8.0	7.9	7.8
Sodium	2.0	1.9	1.9
Lysine	15.2	15.0	14.8
Methionine	7.6	7.5	7.4
Methionine + Cystine	11.6	11.5	11.4
ME (MJ/kg)	11.5	11.4	11.3

*Vitamin-trace mineral premix for broilers (Ian Hollows Feed Supplement, UK) added per kg of diet: vitamin A, 16,000 lU; vitamin D_3, 3,000 lU; vitamin E, 25 mg; thiamine, 3 mg; riboflavin, 10 mg; pyridoxine, 3 mg; vitamin B12, 0.015 mg; nicotinamide, 60 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; biotin, 0.125 mg; choline chloride, 200 mg; iron, 20 mg; cobalt, 1 mg; manganese, 100 mg; copper, 10 mg; zinc, 80 mg; iodine, 1 mg; selenium; 0.2 mg, and molybdenum, 0.5 mg.

**Nutrient compositions of all shea nut meal samples assumed to be similar. *Dietary ME was based on an expeller shea nut meal (Atuahene et al., 1998)*

Chemical analyses

The experimental diets were ground and analysed for their contents of dry matter, crude protein, ether extracts (lipids) and gross energy as described previously **(3.2.1.1).**

Calculations

The AME value of the diet was calculated from the gross energy (GE) values of the diet and excreta using the formula:

AMEdiet = [(Feed intake x GEd_{id}) - (Excreta output x $_{GEexcreta)}$]/Feed intake.

An apparent nutrient digestibility coefficient estimate of each sample was derived according to calculation of digestibility of a single feed of a mixed ration (Lloyd *et al.*, 1978).

$S = A + [100 (T-A)]/s$

S is the coefficient of apparent digestibility of the test feed ingredient.

A is the coefficient of digestibility of the basal feed.

T is the coefficient of digestibility of the combination of the basal feed plus test feed ingredient.

s is the proportion of test feed ingredient in the mixed diet (T).

Statistical analyses

Samples of shea nut meal and their rate of inclusion were considered as treatment factors with tier level of cages as a blocking factor. Outliers (20) that were greater than three standard deviations from the treatment means were removed from the data sets for all variables. ANOVA of data and orthogonal contrasts were used to compare the treatment means (GENSTAT, 8th version). The relationship between the determined AME of the samples and their chemical composition was examined by linear regression with groups

(industrial or non-industrial meals) techniques. Also the correlations between nutrient metabolisability and anti-nutritive components of the meal were calculated.

The apparent metabolisable energy content of the samples was not determined due to the very small quantity of the shea nut meal that was used in order not to unduly compromise feed intake of the birds. A strong negative correlation between dietary level (>25 g/kg diet) and feed intake has been demonstrated by Atuahene *et al.* (1998).

3.1.3.2 Results

There were no mortalities in this experiment. Nutrient and gross energy metabolisabilities of shea nut meal samples at two dietary levels are shown in *Table 3.12*. Evaluation of the eight shea nut meal samples showed there was an interaction between the sample and level on lipid metabolisability. There were sample effects on dry matter ($P<0.001$), crude protein ($P<0.01$) and gross energy ($P<0.05$) as well as level effects on dry matter ($P<0.05$), crude protein ($P<0.001$) and gross energy ($P<0.01$). Comparison of nutrient metabolisabilities between expeller meals and non-industrial meals showed significant differences in dry matter (expeller = 0.646, non-industrial = 0.627; SED = 0.0060, $P<0.001$) and gross energy (expeller = 0.667, non-industrial = 0.655; SED = 0.0064, $P<0.01$). Also, the comparison of nutrient metabolisabilities between as-received meals and defatted meals showed only a significant difference ($P<0.05$, SED = 0.0060) in dry matter metabolisability (as-received = 0.640, defatted = 0.633).

A correlation matrix of the mean nutrient metabolisability *(Table 3.12* of the samples and their anti-nutritive factors *(Table 3.2)* is shown in *Table 3.13*. Although there were negative correlations between crude protein metabolisability and some of the phenolic components, saponin and total NSP contents of the meals, they were not significant correlations. However, the correlation ($r = -0.940$) between crude protein metabolisability and total NSP approached significant level ($P = 0.0596$).

There was a non-significant negative correlation between total soluble phenolics and gross energy anti-nutritive factors, but only had a significant ($P<0.05$) correlation with total NSP content *(Table 3.13)* of which the insoluble NSP appeared ($P = 0.0729$) to be the major cause.

The aim of feeding the eight shea nut meal samples to the broiler chicks was to examine the variability in dietary AME rather than growth performance. Although growth data were recorded, the short restricted feeding period and relatively low bird numbers would not give a robust comparison. Therefore only the AME data are considered in this study.

Addition of the eight shea nut meal samples to the balanced diets showed there was an interaction between the source and level on AME. The dietary level of the six original, as-received shea nut meal samples had a significant ($P<0.01$) effect on AME with the 4% level giving a lower AME than the 2% level *(Table 3.14)*. However, this effect was not evident for the two defatted samples. Increasing dietary inclusion levels of these two samples tended to improve the AME of the diets *(Figure 3.1)*. Even though the AME of refined shea fat is approximately 22.0 MJ/kg DM (section 3.1.2), there was no relationship between the level of residual fat in the shea nut meal and AME. However the lack of relationship between total fat level and AME suggests that another quality factor may also have been important. All the shea nut meal samples had residual fats with relatively high FFA contents and, in particular, the non-industrial samples had very high FFA levels *(Table 3.1)*.

Table 3.12: Nutrient and gross energy metabolisability coefficients in shea nut meal samples

Variable			Dry matter		Crude protein		Lipid		ME:GE	
Amount of sample in diet (g/kg)			20	40	20	40	20	40	20	40
	Sample no.[1]	Sample Code[2]								
Industrial (expeller) shea nut meal	1	$2004s_1$	0.656	0.640	0.615	0.580	0.672	0.667	0.673	0.657
	2	$2004s_2$	0.651	0.649	0.594	0.568	0.668	0.653	0.673	0.665
	3	2005s	0.641	0.640	0.582	0.568	0.674	0.657	0.670	0.660
	4	2005j	0.642	0.653	0.593	0.578	0.662	0.618	0.671	0.667
Non-industrial shea nut meal	5	2004c	0.644	0.624	0.589	0.567	0.665	0.636	0.670	0.650
	6	2005c	0.629	0.612	0.602	0.542	0.651	0.638	0.664	0.638
Defatted industrial shea nut meal[2]	7	$2004s_1$	0.638	0.633	0.588	0.585	0.640	0.722	0.662	0.676
Defatted non-industrial shea nut meal[2]	8	2004c	0.632	0.625	0.576	0.520	0.664	0.664	0.660	0.658
Factor			SED (rdf=108)		SED (rdf=108)		SED (rdf=108)		SED (rdf=108)	
Shea nut meal samples (S)			0.0058***		0.0109**		0.0142^{ns}		0.0061*	
Level of inclusion in diet (L)			0.0029*		0.0054***		0.0071^{ns}		0.0030**	
S x L			0.0082^{ns}		0.0154^{ns}		0.0200**		0.0086^{ns}	

[1]Number of replicates per diet: 0% (16), 2% of 1 (9), 4% of 1 (8), 2% of 2 (10), 4% of 2 (8), 2% of 3 (9), 4% of 3 (9), 2% of 4 (9), 4% of 4 (7), 2% of 5 (10), 4% of 5 (10), 2% of 6 (9), 4% of 6 (10), 2% of 7 (8), 4% of 7 (8), 2% of 8 (10), 4% of 8 (9)

[2]Sample code indicates the year of manufacture and the location of manufacture: s-Shebu-Loders Croklaan Ltd (1-batch one, 2- batch two), j-Juaben Oilmills Ltd, c-Christian Mothers Association.

SED-standard error difference, rdf-residual degrees of freedom, ns-not significant (P>0.05), *(P<0.05), **(P<0.01), ***(P<0.001)

The ether extracts contents of defatted samples subjected to acid hydrolysis: Expeller SNM (3.6 g/kg), non-industrial SNM (7.0 g/kg)

Table 3.13: Correlation matrix of nutrient metabolisability and anti-nutritive factors of shea nut meal samples'

	Dry matter	Crude protein	Gross energy	Lipid
Total NSP	-0.9616*	-0.9404	0.5740	0.0409
Insoluble NSP	-0.9271	-0.8587	0.4752	0.2924
Soluble NSP	-0.7880	-0.8619	0.6195	-0.4492
FFA of the fat	0.3858	0.7404	-0.8232	0.8197
Total soluble polyphenols	0.6608	0.9323	-0.8617	0.5600
Bound plus soluble proanthocyanidins	-0.6859	-0.2618	-0.2584	0.8459
Soluble proanthocyanidins	-0.6285	-0.8660	0.7857	0.0454
Hydrolysable tannins	0.3961	0.5255	-0.4621	0.7622
Saponin	-0.7108	-0.6813	0.4018	0.4996

'Degrees of freedom=108
*Correlation coefficient that was significant (P<0.05)

Table 3.14: Apparent metabolisable energy of experimental diets

			AME (MJ/kg DM)		
Amount of sample in diet (g/kg)			0	20	40	Mean
Basal diet			12.16			
	Sample no.	Sample code[1]			
Industrial (expeller) shea nut meal	1	$2004s_1$	12.35	12.02	12.18
	2	$2004s_2$	12.29	12.18	12.23
	3	2005s	12.26	12.18	12.22
	4	2005j	12.26	12.18	12.22
		Mean	12.29	12.14	
Non-industrial shea nut meal	5	2004	12.31	12.01	12.16
	6	2005	12.28	11.90	12.09
		Mean	12.30	11.96	
Defatted industrial shea nut meal	7	$2004s_1$	12.28	12.35	12.31
Defatted non-industrial shea nut meal	8	2004	12.19	12.26	12.22

Factor	SED (r.d.f.=96)	Probability
Shea nut meal samples (S)	0.096	P>0.05
Level of inclusion in diet (L)	0.048	P<0.01
S x L	0.136	P<0.05

[1]Sample code indicates the year of manufacture and the location of manufacture: s-Shebu-Loders Croklaan Ltd (1-batch one, 2- batch two), j-Juaben Oilmills Ltd, c-Christian Mothers Association.

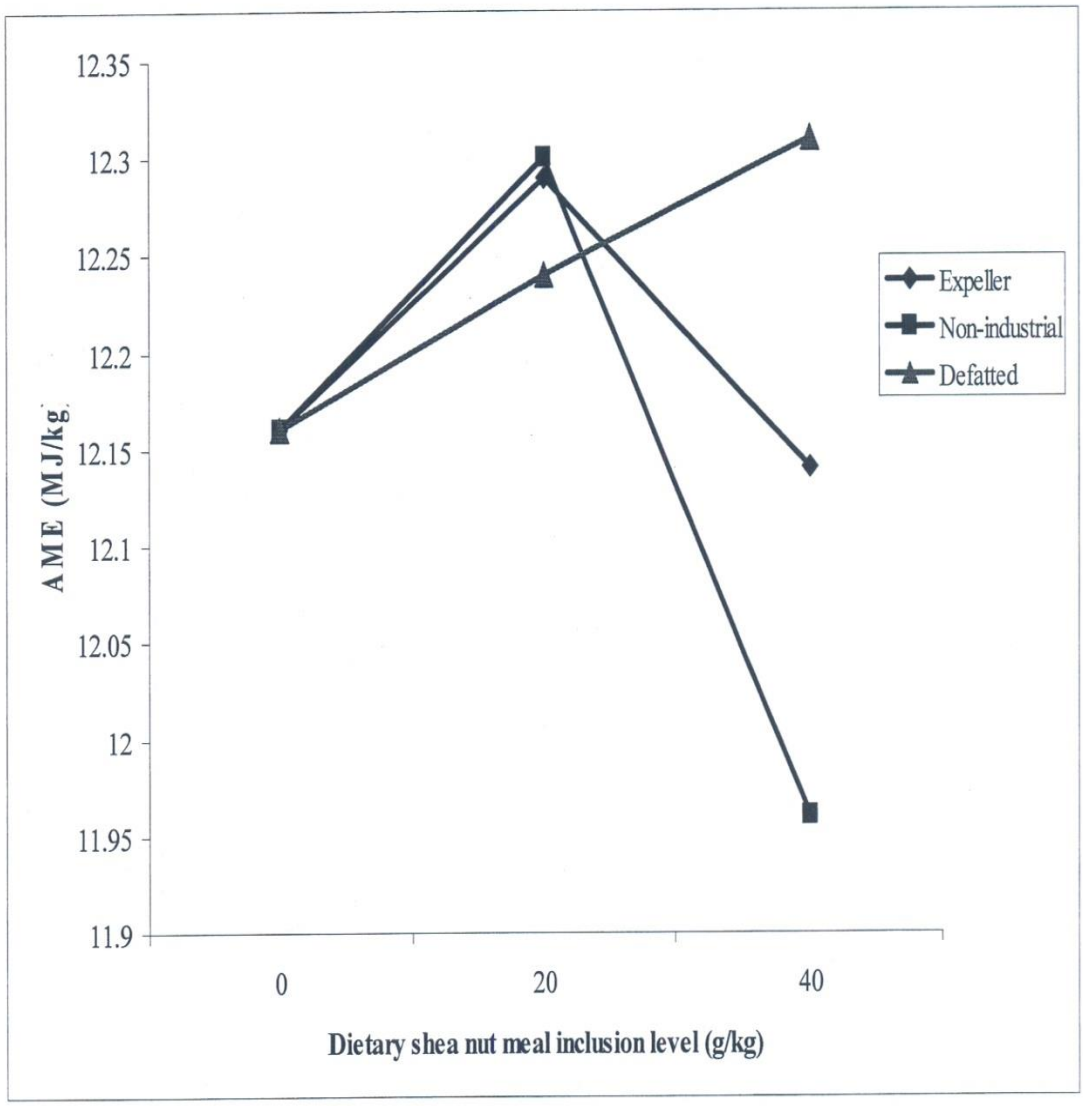


Figure 3.1: Dietary energy of expeller (n=4), **non-industrial** (n=2) **and defatted** (n=2) **shea nut meal samples**

3.1.3.3 Discussion

The nutrient metabolisability of the shea nut meal in general was very poor, particularly in crude protein metabolisability. The low nutrient metabolisability values of the shea nut meals recorded in this experiment could be due to the high amount of NSP. There was a strong negative correlation ($P<0.05$) between dry matter metabolisability and total NSP content of the meal. However, the tannin and saponin contents of the meal could be implicated as well. Poor utilisation of the shea nut meal in poultry diets has been reported (Atuahene *et al.,* 1998; Olorede and Longe, 1999). The reported factors limiting nutrient utilisation of shea nut meal include tannins and saponin (Annongu *et al.,* 1996a), and fibre content (Morgan and Trinder, 1980). The polysaccharides in the samples used in this study were largely non-starch polysaccharides *(Table 3.1),* which are known to have negative effects on nutrient digestion and absorption in poultry (Annison and Choct, 1991). Although the negative impacts of tannin and saponin contents in the meal could not be linked categorically to poor nutrient availability in this experiment, they could be potential anti-nutritive factors due to their effects on feed intakes of birds. The need to counteract the anti-nutritive factors such as tannins and saponins in shea nut meal to improve nutrient utilisation has been suggested by Annongu *et al.* (1996a; 1996c) through the use of fermentation and tannin-binding agents. Even though the data suggest the possibility of NSP as an anti-nutritive factor, the low *in vitro* viscosity of the soluble NSP gives the impression that the use of enzymes to improve its utilisation would not be feasible. Nevertheless, the high insoluble NSP content could be accountable for the ANF effect.

All the samples had residual fats with relatively high FFA contents and, in particular, the non-industrial samples *(Table 3.1).* This implies that, due to its quality, the residual fat in the shea nut meal may not contribute significantly to its metabolisable energy content as expected *(Figure 3.1).* Thus processing methods that substantially improve fat extraction efficiency (e.g. solvent extraction process) from the shea kernels may enhance the quality

of this by-product for feeding poultry. The reduction in dietary AME with the six as-received shea nut meals *(Table 3.14)* indicated that, the residual fat in the shea nut meal may not contribute significantly to its metabolisable energy content. The defatted shea nut meals had higher metabolisability (AME/GE) (mean of 0.842) compared to the other six shea nut meal samples (mean of 0.526). This gives further evidence that the fat content of shea nut meal may have a deleterious effect on metabolisable energy, although the defatting process also reduced the saponin concentration by 60-66% in the shea nut meal *(Table 3.4).* The unsaponifiable material (e.g. sterols) in unrefined shea fat is high relative to other vegetable oils (Padley *et al.,* 1994).

3.1.3.4 Conclusion

This experiment showed that nutrients in shea nut meal samples in the industry were poorly available in broiler chicken diets. The detrimental effects of tannins, saponins and NSP could account for the poor nutrient availability. It was clear from this experiment that removal of the residual fat did affect dietary energy utilisation of the meal.

Also, the experiment showed that the level of inclusion of the samples in the diets had effects on dietary energy that declined at high dietary levels with the exception of the defatted samples.

It was evident from this experiment that the meal in its present form requires further improvement to become useful for the feed industry.

3.1.4 True Metabolisable Energy and True Amino Acid Availability of shea nut meal

The previous expurimum evaluated nutrients availability of shea nut meal by using a conventional apparent metabolisable energy bioassay. In this, the shea nut meal was included at relatively low inclusion rates. Although this reflects the practical use of the feedstuff, it makes the prediction of nutrient availabilities subject to a large amount of variation.

The bioassay technique used for the measurement of true metabolisable energy (TME) involves the precision feeding of a small amount of the undiluted feed ingredient. Although the feed ingredient is fed at much a higher concentration than would occur in commercial practice, the technique can substantially reduce the variability in the estimation of nutrient metabolisability.

Facilities for the TME bioassay were not available at Harper Adams University College, but some were made available to the project at Scotland Agricultural Centre, Ayr. Only a small number of birds were available for use, so it was decided that the number of shea nut meal samples should be reduced rather than have too low a treatment replication. It was therefore decided to compare only three shea nut meal samples in this part of the project. Two expeller samples and one non-industrial sample were selected for this experiment.

The specific objectives of this experiment were to determine the available energy and amino acids in three samples of shea nut meal (two expeller meals from the same factory but different growing seasons and one non-industrial meal) using a precision-fed broiler assay.

3.1.4.1 Materials and Methods

Shea nut meal samples

The two expeller samples (2004 & 2005) and one non-industrial sample (2004) were procured as described in previous section **(3.1.1.1).**

Broiler assay

Each shea nut meal was fed to one of eight Ross 308 male broilers in a completely randomised design according to an adapted precision feeding technique to determine true metabolisable energy corrected for nitrogen balance (TMEn) (McNab and Blair, 1988). This modified TME bioassay improves the TME method originally devised by Sibbald (1976) by extending the excreta collection time from 24 to 48 h as well as feeding dextrose before and once during the collection period in order to decrease the stress on the birds used for the determination of endogenous losses. The experiment was conducted at the Scottish Agricultural College, Ayr and was approved by the Scottish Agricultural College Animal Ethics Committee. All experimental birds were previously fed the same commercial diet. At 45 d of age, the birds were placed on a raised slatted floor pen with no access to feed, litter or droppings. Water was supplied *ad libitum* throughout the study via a suspended nipple drinker line. After 24 h the birds were given 50 ml of 60% glucose solution. After a further 24 h each bird was fed 30 g of the test samples and placed in individual cages (0.5 m x 0.8 m floor area) designed to collect droppings, at a constant house temperature of 20°C and 23 h of light per day. Birds used for endogenous loss estimation were fed 50 ml glucose solution instead of the experimental diets. The droppings voided by each bird were collected for 48 h, frozen and freeze-dried.

Chemical analyses

The experimental diets and excreta samples were ground and analysed for their contents of dry matter, crude protein, ether extracts (lipids) and gross energy as described previously

(section 3.1.1.1). The amino acid profiles of all samples were determined by SCIANTEC Services Ltd, Dalton, UK. The sample was oxidised with hydrogen peroxide/formic acid/phenol mixture. Excess oxidation reagent was decomposed with sodium metabisulphite. The oxidised sample was hydrolysed with 6M hydrochloric acid for 24 h. The hydrolysate was adjusted to pH 2.2, centrifuged and filtered. The amino acids were separated by ion exchange chromatography using a Biochrom 20 analyser (Biochrom Ltd, Cambridge, UK) and determined by reaction with ninhydrin using photometric detection at 570 nm (440 nm for proline).

Since the method of hydrolysis destroyed tryptophan, data on this amino acid was not reported. Also, data on glycine was not presented because of the glycine yield from acid hydrolysis of uric acid in the excreta (Soares *et al.,* 1971).

Calculations

The TMEn contents of the shea nut meal samples were calculated based on equations of

Sibbald (1976) as follows: TME = [(EI-EO)/FI] + (FEL/FI)

TMEn = TME — (3.44 x ANR/FI) — (3.44 x FNL/FI)

EI is gross energy in feed intake of fed bird

EO is gross energy output in excreta of fed bird

FI is the feed intake of the feedstuffs (30 g)

ANR is apparent nitrogen retention (i.e. difference between nitrogen in feed intake and nitrogen in excreta output of the fed bird)

FEL is gross energy output in excreta of fasted bird (i.e. fasting energy loss from the feed deprived bird)

FNL is fasting nitrogen loss (i.e. nitrogen in excreta of fasted bird)

Nitrogen retained in tissues can be catabolised to yield energy-containing excretory compounds that contribute to fasting energy loss. Therefore, the gross energy excreted was corrected to zero-nitrogen balance using a factor of 34.4 kJ (Hill and Anderson, 1958). The constant , 34.4 kJ (8.22 kcal), was added or subtracted from ME for each gram of nitrogen lost or gained to account for energy required in the excretion of urinary nitrogen.
The TMEn value of each shea nut meal sample was also expressed as a proportion of their gross energy (GE) in order to estimate metabolisability (i.e. assimilation efficiency coefficient).

The true amino acid availability (TAAA) coefficient was calculated for each amino acid according to the equation of McNab (1994) as follows:

TAAA = [(AAC — (AAV — AAVF)]/AAC

AAC is the total amount of amino acid consumed by the fed bird

AAV is the total amount of amino acid voided in excreta by the fed bird

AAVF is the amount of each amino acid voided by the control (fasted) bird during the 48-h

period. This was used to estimate endogenous losses.

Statistical analysis

Shea nut meal samples were considered the treatment factor for the TMEn and TAAA broiler assay. Analysis of variance of data in a completely randomised design and orthogonal contrasts were used to compare the treatment means (GENSTAT, 8th version).

3.1.4.2 Results

The two industrial expeller meals (2004 and 2005) were similar in chemical composition except for free fatty acid (FFA) contents *(Table 3.1).* The non-industrial shea nut meal had a lower ($P<0.001$) TMEn as well as metabolisability than the expeller meals *(Table 3.15).* However, both expeller meals had similar ($P>0.05$) TMEn and metabolisability.

The total amino acid concentrations of crude protein, true amino acid availability (TAAA) and available amino acid concentrations in shea nut meal are listed in *Table 3.16*. The TAAA of all amino acids in the non-industrial shea nut meal were higher ($P<0.001$) than those of the expeller meals. However, both samples had very low TAAA for histidine (0.267 - 0.307), arginine (0.408 - 0.559), methionine (0.470 — 0.571) and lysine (0.436 – 0.609). The mean TAAA of amino acids in the industrial meals was 0.598, whereas that of the non-industrail meal was 0.714.

Table 3.15: True metabolisable energy of three shea nut.meal samples[1]

Component	Industrial (expeller) shea nut meal		Non-industrial (water-based extraction) shea nut meal
	2004s1	2005s	2004c
GE (MJ/kg DM)	22.8	23.8	26.0
TMEn (MJ/kg DM) (Pooled SED=0.532***)	14.38	15.49	12.61
Metabolisability (TMEn/GE) (Pooled SED=0.02206***)	0.635	0.656	0.489

[1]Sample code indicates the year of manufacture and the location of manufacture: s-Shebu-Loders Croklaan Ltd (1-batch one), c-Christian Mothers Association.
***(P<0.001)

Table 3.16: True amino acid availability coefficients (of crude protein) of expeller and non-industrial shea nut meals

Amino acid	True Amino Acid Availability coefficients				Available Content (g/kg CP) of the shea nut meal samples		
	Expeller 2004s$_1$	Expeller 2005s	Non-industrial 2004c	SED (P<0.001)	Expeller 2004s$_1$	Expeller 2005s	Non-industrial 2004c
Essential							
Methionine	0.470	0.479	0.571	0.0010	10.6	10.8	11.7
Cystine	0.664	0.674	0.705	0.0008	11.4	11.5	9.1
Methionine+Cystine	0.448	0.455	0.595	0.0008	17.8	18.0	19.9
Lysine	0.436	0.443	0.609	0.0014	18.2	17.5	18.3
Threonine	0.914	0.928	0.978	0.0012	37.7	39.5	37.5
Arginine	0.408	0.414	0.559	0.0009	36.4	37.4	40.1
Isoleucine	0.668	0.678	0.879	0.0015	31.1	31.8	37.9
Leucine	0.648	0.658	0.778	0.0013	50.9	52.8	58.4
Valine	0.770	0.781	0.896	0.0015	44.0	45.1	48.2
Histidine	0.262	0.267	0.307	0.0006	7.5	7.6	7.5
Phenylalanine	0.723	0.733	0.810	0.0015	27.2	28.3	29.6
Non-essential							
Serine	0.689	0.699	0.907	0.0013	30.8	32.6	36.7
Alanine	0.705	0.716	0.794	0.0011	40.8	42.5	43.7
Asparagine	0.539	0.548	0.669	0.0012	63.0	65.2	67.3
Glutamine	0.551	0.560	0.657	0.0012	93.3	96.3	102.1
Mean of total amino acids	0.593	0.602	0.714		34.7	35.8	37.9

[1]Sample code indicates the year of manufacture and the location of manufacture: s-Shebu-Loders Croklaan Ltd (1-batch one), c-Christian Mothers Association.
SED-standard error difference, P-probability

3.1.4.3 Discussion

Although the non-industrial shea nut meal had a high fat content *(Table 3.1)*, it had a lower ($P<0.001$) TMEn than the expeller meals *(Table 3.15)*. The TMEn of the non-industrial nut meal was 2.3 MJ/kg lower ($P<0.001$) than the mean values of the two industrial expeller meals. The high residual fat content of the non-industrial meal as well as the high concentration of FFA in the fat *(Table 3.1)* might have accounted for its lower energy availability. Shea fat consists mainly of stearic and oleic acids *(Table 3.6)* and has a low unsaturated-to-saturated fatty acid ratio (1.1). Thus it is highly saturated and has relatively low metabolisable energy content as demonstrated in the previous experiment (section 3.1.2). Triglycerides with low unsaturated-to-saturated fatty acid ratios have been shown to have lower metabolisable energy concentrations (Ketels and De Groote, 1989), particularly at high dietary concentrations (Wiseman *et al.*, 1986). Also, there is a negative relationship between the FFA level of a fat and its metabolisable energy (Wiseman and Salvador, 1991, Huyghebaert *et al.*, 1988). In the TME assay, shea nut meal was the sole feed. Therefore, the relatively high fat content of the samples could have markedly reduced the estimate of metabolisable energy; because not only did the meals have a high dietary concentration of saturated fats but also had high FFA concentrations. The high non-starch polysaccharides in the shea nut meals *(Table 3.1)* could be another cause of their relatively low energy availability. Morgan and Trinder (1980) used an *in vitro* study and found a low digestible organic matter and total digestible nutrients in a shea nut meal sample that they attributed to the fibre content of the meal. It is also possible that the high tannin contents of the meals could have had a pronounced negative effect on metabolisable energy (Smulikowska *et al.* 2001).

The precision-fed broiler assay used in this study has been one of the standard methods for estimating amino acid availability in feedstuffs (McNab, 1994). However, the use of broilers instead of adult cockerels is reported to give slightly lower TAAA values (Song *et*

(1965) who suggested that the presence of substantial amount of fat in a feedstuff may interfere with amino acid availability through protein-fat interactions involving carbonyl products reacting with free amino groups.

The low available lysine, methionine, histidine and arginine contents in shea nut meal reduce its suitability for use in poultry feeds. High essential amino acid availability, particularly lysine in feedstuffs is an important factor which determines the protein quality for poultry (Hsu *et al.*, 1977) as well as the economics of using such feedstuffs in least-cost diets (Bryden and Li, 2004). Thus, the use of shea nut meal as a feed ingredient in poultry diets would need further protein supplementation or the use of synthetic amino acids. Studies carried out by Annongu *et a/.* (1996b) and Olorede *et a/.* (1999) demonstrated the importance of amino acid or protein supplementation of shea nut meal based diets for broilers and recommended the use of synthetic lysine and methionine or fishmeal in these diets.

3.1.5 Effects of varying dietary levels of shea nut meal on growth performance of broiler chickens

Previous studies have demonstrated the poor availability of nutrients in shea nut meals for broiler chickens. So far, there is one published report (Atuahene *et al.*, 1998) that has shown the relationship between dietary inclusion levels of shea nut meal and growth performance variables, but with relatively few treatments. Therefore, there is a need for further evaluation of the growth response of broiler chickens fed shea nut meal. This experiment was carried out using a dose-response method to establish a relationship between the dietary level of shea nut meal and growth performance variables. One of the industrially processed shea nut meal samples was used in this experiment, because it was available in a large amount.

The specific objective was to determine the effects of increasing levels (0, 10, 20, 30, 40, 60, 80, 100 g/kg) of expeller shea nut meal on the growth performance of broilers.

3.1.5.1 Materials and Methods

Shea nut meal sample

The expeller shea nut meal (2004 batch 1) was used for this experiment. This was the main sample that had been collected in large amounts for the studies. Also, the aim of the experiment was to establish a relationship between dietary level and feed intake, hence the use of one sample.

Broiler assay

Male day-old broiler chicks (Ross 308, UK) were reared in a litter-floored pen and fed a proprietary broiler feed for 23 days. At 23 d of age, 48 birds of similar weights were randomly selected and each bird placed in a wire-floored cage (0.30m x 0.30m x 0.36m) in an environmentally controlled room. Eight dietary levels (0, 10, 20, 30, 40, 60, 80, 100

g/kg) of expeller shea nut meal were incorporated into a basal diet *(Table 3.17).* The basal diet was formulated to contain adequate levels of required nutrients for young broiler chickens. Each diet was replicated six times and fed for seven days (23-29 d). The logic of substitution of the whole diet rather than replacement of one component of the diet with shea nut meal was to determine the overall effect of using shea nut meal as a dietary ingredient

Table 3.17: Composition of basal diet (g/kg)

Ingredient	Amount
Ground wheat	568
Dehulled soybean meal	150
Full fat soya	150
Sunflower seed meal	44
Soya oil	40
Lysine (HCl)	5
D,L-Methionine	4
Limestone	10
Dicalcium phosphate	5
Salt	4
Vitamins/trace elements/methionine mixture*	20
Calculated nutrients and energy	
ME (MJ/kg)	13.2
Crude protein	211
Lysine	13.8
Methionine + cystine	10.1
Tryptophan	2.5
Calcium	10.2
Phosphorus	5.0
Sodium	1.9
Choline	1.6

*Vitamin-trace mineral premix for broilers (Ian Hollows Feed Supplement, UK) added per kg of diet: vitamin A, 16,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 mg; thiamine, 3 mg; riboflavin, 10 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.015 mg; nicotinamide, 60 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; biotin, 0.125 mg; choline chloride, 200 mg; iron, 20 mg; cobalt, 1 mg; manganese, 100 mg; copper, 10 mg; zinc, 80 mg; iodine, 1 mg; selenium; 0.2 mg, and molybdenum, 0.5 mg.

3.1.5.2 Results

There were no mortalities in this experiment. A strong negative quadratic relationship *(Figure 3.2)* was observed between dietary level of the shea nut meal samples and feed intake (r=0.988). Feed intake was significantly (P<0.001) depressed when the meal was incorporated beyond 30 g/kg diet *(Table 3.18).* There was a general decline in weight gain *(Figure 3.2).* Weight gain declined significantly (P<0.001) when shea nut meal inclusion in the diets was more than 20 g/kg. Even inclusion of the meal at the lowest dietary level (10 g/kg) gave a 9.1% reduction in weight gain relative to those fed the control diet. Feed efficiency, also declined with dietary level with a significant (P<0.001) decline detected beyond 40 g/kg in the diet *(Table 3.18).*

Table 3.18: Effect of dietary level of expeller shea nut meal on feed intake, live weight gain and gain: feed of broilers (23-29 days of age)

Dietary level (g/kg)	Mean total feed intake (g)	Mean total weight gain (g)	Gain:feed
0	775	508.7	0.657
10	781	496.3	0.635
20	749	462.5	0.617
30	698	405.7	0.578
40	624	355.3	0.568
60	511	206.0	0.400
80	410	136.0	0.328
100	344	80.5	0.207
±SED	*31.1*	*23.84*	*0.0310*
Probability	*P<0.001*	*P<0.001*	*P<0.001*

SED-standard error difference

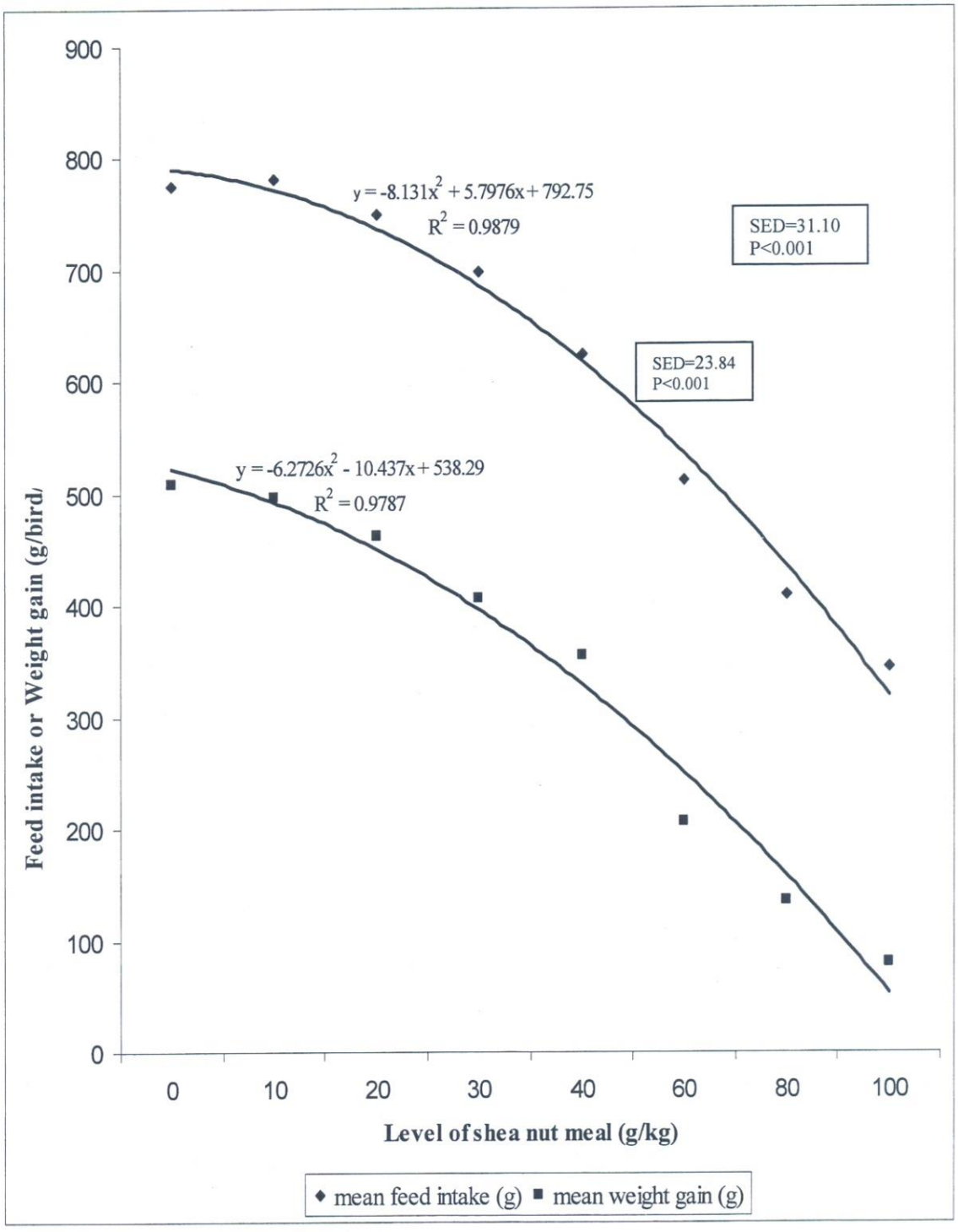


Figure 3.2: Effect of varying concentrations of expeller shea nut meal on feed intake and live weight gain of broilers (23-29 days of age)

3.1.5.3 Discussion

The main limitation of shea nut meal as a feedstuff for animals has been its adverse effect on feed consumption. Thus the decline in feed intake of the birds was expected. Atuahene *et al.* (1998) reported a linear reduction in feed intake (r^2=0.970) of broilers (14-56 d) as opposed to quadratic reduction in feed intake when fed varying levels of shea nut meal in the diets. This could be due to the fewer dietary treatments used in their experiment. Generally, shea nut meal utilisation was very poor even at low dietary levels *(Table 3.18).* Even inclusion of the meal at the lowest dietary level (10 g/kg) gave a 9.1% reduction in weight gain relative to their counterparts fed the control diet *(Figure 3.2),* though the reduction was not significant (P>0.05). Poor feed intake of diets containing shea nut meal has been attributed to anti-nutritive factors such as tannins and saponins (Annongu *et al.,* 1996b; Atuahene *et al.,* 1998) due to their bitterness (Cheeke, 1971) or astringency (Hentgen, 1985). These anti-nutritive factors, particularly tannins were quite high in the samples used in this experiment *(Table 3.2).* This study suggests the need for dramatic improvements in the nutritive value of shea nut meal before it would be useful for the feed industry. The main aspect of the meal to be tackled should be the anti-nutritive factors that limit feed intake with consequent effects on growth.

3.1.5.4 Conclusion

This experiment has shown clearly that high dietary inclusion levels of shea nut meal in broiler diet markedly affect the growth performance of broiler chickens. Thus shea nut meal in its present form has a low economic value for broiler chickens. It was probable that anti-nutritive factors such as tannins and saponin have played a role in its poor utilisation. Therefore, it requires further treatments to counteract the adverse effects of these anti-nutrients. Nevertheless, the untreated shea nut meal could be included in broiler chicken diets up to 20 g/kg without any major effects on bird performance.

3.2 IMPROVEMENT OF SHEA NUT MEAL FOR POULTRY

The previous experiment has shown poor growth performance of broiler chickens. Tannins appear to be a major problem. Various methods of counteracting the tannins in other feedstuffs have been reviewed **(section 2.5).** However, there have been little detailed studies on shea nut meal.

In this study more emphasis would be laid on microbial fermentation of shea nut meal. Other nutritional strategies such as water treatment and the use of polyethylene glycol would be highlighted. Fermentation is an unique process with great potential for recycling some agro-industrial by-products into useful animal feeds in developing countries. The process does not require the use of chemicals and is easy to manage in on-farm conditions or on an industrial scale. Fermentation processes using micro-organisms have been used to improve the nutritive value of some feedstuffs such as soybeans (Chah *et al.,* 1975; Mathivanan *et al.,* 2006), guar meal (Nagra *et al.,* 1998), and koji feed (Yamamoto *et al.,* 2007) for poultry. The desirable characteristics of the fermented products include their acceptability by birds (Nagra *et al.,* 1998) and nutrient availability (Hong *et al.,* 2004). Among the microbes used for fermentation *Aspergilli* featured prominently. *Aspergillus niger is* a fungus that has the capacity to produce enzymes such as hemicellulases, hydrolases, pectinases, lipases and tannases (Pinto *et al.,* 2001; Mathivanan *et al.,* 2006). Thus it has been used extensively in the improvement of agricultural by-products through its action on substrates such as non-starch polysaccharides and proteins (Ong *et al.,* 2007; Aderemi and Nworgu, 2007) or structurally modifying anti-nutritive factors (Hong *et al.,* 2004).

In this section, six treatments that were designed to counteract the tannins were examined. These were (1) Hot water extraction, (2) Natural fermentation, (3) Natural fermentation with tannin binding agent as an additive, (4) Natural fermentation with enzyme as an additive, (5) Fungal fermentation using *Aspergillus niger,* and (6) Bacterial fermentation using a mixture

of *Bacillus subtilis* and *Bacillus licheniformis*. This experiment was designed to give a preliminary screening for an appropriate method to be selected for further studies. The expeller meal used in the previous experiment **(section 3.1.5)** was the only sample evaluated in all of the broiler growth studies.

3.2.1 Comparison of the growth of broiler chickens fed diets containing nutritionally improved shea nut meal samples

The specific objective of this section was to compare the six alternative methods [natural fermentation, natural fermentation with a tannin binding agent (polyethylene glycol) or an enzyme (tannin acyl hydrolase/EC.1.1.20), fungal fermentation *(A. niger)*, bacterial fermentation *(Bacillus subtilis* and *Bacillus licheniformis)* and the use of hot water extraction] of improving the growth performance of broiler chickens fed diets that included three levels of shea nut meal (30, 60, 90 g/kg).

3.2.1.1 Materials and methods

Preparation of samples

The treatments of the six samples for the experiment are shown in *Table 3.19*, and described as follows:

Treatment 1: Hot water extraction

Tannins are soluble in hot water, therefore can be extracted from the material. The shea nut meal was soaked in tap water at the ratio of 1:10 (1 part shea nut meal-to-10 parts of water) and heated in a metal cooking pan. The sample was heated on an electric stove for 90 minutes when the water got to its boiling point. The hot water was drained through a cheese cloth. The sample was dried in a forced-draught oven at 56°C for five days up to about 90% of the dry matter. About 62% of the sample was recovered after the hot water treatment. The dried sample was ground in a mill and stored until mixed with the diets.

Treatment 2: Natural fermentation

This involved fermentation of the shea nut meal by any native micro-organisms (Annongu *et al.*, 1996a). These organisms could either degrade tannins or produce acids that can degrade the tannins. The shea nut meal was soaked in water at the ratio of 1:2 (1 part shea nut meal-to-2 parts of water). The wet sample was gently packed in a plastic container and sealed with a film of polyethylene. It was then incubated for 8 days. The sample was dried and ground as described for the previous treatment.

Treatment 3: Natural fermentation with polyethylene glycol (PEG)

Polyethylene glycol is a family of long chain polymers made up of ethylene glycol subunits. PEG can form complexes with proanthocyanidins in feedstuffs. Twenty grams of PEG 4000 (the number after the name indicates the approximate molecular weight of the

Chain) was dissolved in the water prior to mixing with the sheanut meal at the ratio of 1-to-2, and then fermented, dried and ground as described for the natural fermented sample.

Treatment 4: Natural fermentation with enzyme (tannase)

The addition of tannase to the shea nut meal before natural fermentation was meant to target hydrolysable tannins in the meal, because tannase can degrade hydrolysable tannins in feedstuffs. Fifty miligrams of tannase (EC.1.1.20) was dissolved in the water prior to mixing with the shea nut meal at the ratio of 1-to-2, and then fermented, dried and ground as described for the natural fermented sample.

Treatment 5: Fungal fermentation using A. niger

A. niger is a fungus that has the capacity to produce enzymes, namely hemicellulases, hydrolyses, pectinolytic and lipolytic enzymes (Mathivanan *et al.,* 2006). The *Aspergillus niger* sample used was a laboratory strain isolate obtained from the University of Wolverhampton, UK, cultured by an agar plating technique using Sabouraud dextrose agar (Oxoid Ltd, Basingstoke, UK) and incubated at 24°C for 7 days. *A. niger* spores were harvested by tapping. Spore counts were determined using an haematocytometer according to the Fuchs-Rosenthal technique to be approximately $1.6x10^6$ spores (0.25 g). This sample was fermented, dried and ground as described for the natural fermented sample. Although *A. niger is* an aerobic organism, there would be a production phase under the 'micro-aerobic' conditions that would exist in a closed plastic container (David *et al.,* 2003).

Treatment 6: Bacterial fermentation using a commercial mix of B. subtilis and B. licheniformis

Bacillus subtilis and *Bacillus licheniformis are* closely related and grouped together as the subtilis-group. They produce a broad spectrum of bioactive compounds including volatile

compounds and enzymes such as proteases, lipase and amylases. They can grow under anaerobic conditions (Claus and Berkeley, 1986). One gram of the commercial mix of these *Bacilli* (Provita Eurotech Ltd, N. Ireland) was added to 1 kg of the shea nut meal prior to fermentation. Each gram of the *commercial Bacilli* mix comprised $1.6x10^9$ spores of *B. subtilis* and $1.6x10^9$ spores of *B. Licheniformis*. This sample was also fermented, dried and ground as described for the natural fermented sample. The pH of this sample and other fermented samples was determined using a pH Meter (Model 3510, Jensway, UK). The pH values ranged between 5.9 and 6.1, which suggests some degree of fermentation.

Table 3.19: Experimental treatments using expeller shea nut meal

	Treatments	Source of added material	Amount added
1	Hot water extraction	Tap water	20 kg/kg
2	Natural fermentation	-	-
3	Natural fermentation + PEG	PEG 4000, Sigma-Aldrich, UK	20 g/kg
4	Natural fermentation + Tannase	EC.1.1.20, Sigma-Aldrich, UK	50 mg/kg
5	Fermentation using *A. niger*	Laboratory isolate, University of Wolverhampton, UK	0.25 g/kg ($1.6x10^6$ spores)
6	Fermentation using a mixture of *Bacillus subtilis* and *Bacillus licheniformis*	Provita Eurotech Ltd, N. Ireland	1 g/kg comprising *B. subtilis* ($1.6x10^9$ spores) and *B. licheniformis* ($1.6x10^9$ spores)

Broiler assay

Ross 308 male broiler chicks were reared in a solid-floored pen and fed a crumbled-pellet broiler starter diet (CP=235.0 g/kg, ME=12.5 MJ/kg) for 10 days. Ninety-two birds of similar body weight were housed individually at 10 days of age in cages (0.3m x 0.3m x 0.36m). This experiment was a factorial design (7 shea nut meal samples x 3 dietary levels) with one additional control (no sheanut meal). Each of the six treatments *(Table 3.19)* as well as the unfermented shea nut meal was incorporated at 30, 60 and 90 g/kg in a basal diet *(Table 3.20)*. Each of the experimental diets including the basal diet was replicated four times and fed in a mash form from 10 to 18 days of age. Maize rather than wheat was used in the successive experiments, because maize has been the main energy feed ingredient in West Africa including Ghana.

D,L-Methionine	2.0	1.94	1.88	1.82
Dicalcium phosphate	10.0	9.7	9.4	9.1
Limestone	5.5	5.34	5.17	5.01
Vitamin and trace element premix*	3.0	2.91	2.82	2.73
Salt	3.0	2.91	2.82	2.73
Shea nut meal**	-	30	60	90
Calculated composition (g/kg)				
Crude protein	230.8	228.2	225.6	223.0
Lysine	13.5	13.3	13.1	12.8
Methionine	5.9	5.8	5.7	5.7
Methionine+cystine	9.8	9.6	9.6	9.4
Calcium	7.3	7.2	7.2	7.1
Phosphorus	7.6	7.5	7.3	7.2
Metabolisable Energy (MJ/kg)	12.2	12.3	12.3	12.4

*Vitamin-trace mineral premix for broilers (Ian Hollows Feed Supplement, UK) added per kg of diet: vitamin A, 16,000 IU; vitamin D_3, 3,000 IU; vitamin E, 25 mg; thiamine, 3 mg; riboflavin, 10 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.015 mg; nicotinamide, 60 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; biotin, 0.125 mg; choline chloride, 200 mg; iron, 20 mg; cobalt, 1 mg; manganese, 100 mg; copper, 10 mg; zinc, 80 mg; iodine, 1 mg; selenium; 0.2 mg, and molybdenum, 0.5 mg.

**Nutrient compositions of the shea nut meal samples are assumed to be similar. Dietary formulations are based on an expeller shea nut meal

Statistical analyses

Samples and rate of inclusion were considered as treatment factors with tier level of cages as a blocking factor. ANOVA of data and orthogonal contrasts were used to compare the treatment means (GENSTAT, 9th version). Regression techniques were used to examine the effect of dietary level on growth variables.

3.2.1.2 Results

The pH of the fermentation treatments ranged between 5.9 and 6.1. The untreated shea nut meal decreased growth variables similarly to the results of the previous experiment. There were significant differences between the samples ($P<0.05$) as well as their dietary inclusion levels ($P<0.001$) in all growth variables *(Table 3.21)*. There was no interaction ($P>0.05$) between the samples and dietary level. The growth performances of all the birds fed all the shea nut meal diets were lower ($P<0.05$) than those fed the control diet.

There were no mortalities in this experiment. Although birds fed untreated shea nut meal had similar feed intakes as their counterparts fed the meal treated with hot water, they had lower weight gains ($P<0.01$) and feed conversion efficiencies ($P<0.001$).

There were no significant differences between the untreated meal group and the groups fed either the naturally fermented meal or naturally fermented meal with the enzyme (tannase) in their growth performances. However, the birds fed the PEG treated meal had higher feed intakes ($P<0.05$) and weight gains ($P<0.01$) than those fed the untreated meal. There were no differences *in* feed conversion efficiencies between these treatment groups.

Although birds fed untreated shea nut meal had similar feed intakes as their counterparts fed the meal fermented with bacterial organisms, they had lower weight gains ($P<0.01$) and feed conversion efficiencies ($P<0.01$).

The birds fed the meal fermented with *A. niger* had high ($P<0.05$) performance in all growth variables relative to the group fed the untreated meal. However, they had similar performances to the group fed the naturally fermented meal with PEG. Apart from differences in feed intake ($P<0.05$), birds fed the meal fermented using *A niger* and bacterial organisms had similar weight gains and feed conversion efficiencies.

Generally, there were no significant differences among the nutritionally improved shea nut meal samples in terms of their effects on broiler chick growth performances.

Table 3.21: Growth performance of broiler chickens (10–18 d) fed hot water treated and fermented shea nut meals (SNM) at 3 dietary levels

Variable	Level (g/kg)	Untreated SNM	Hot water treated SNM	Fermented SNM					Mean	Analysis of variance		
				Natural	Natural + Tannase	Natural + PEG	Commercial *Bacilli* mix	*A. niger*		Factor	SED (*rdf*=62)	P
Feed	0								40.1	SNM (S)	3.296	0.015
intake	30	39.6	40.1	42.3	39.7	41.6	39.9	37.8	40.1			
(g/b/d)	60	31.4	34.0	31.8	30.7	38.2	28.8	35.6	32.9	Level (L)	2.289	<0.001
	90	23.3	33.7	29.5	28.1	37.8	26.7	38.7	31.1			<0.001 (L)
Mean		31.5	35.9	34.5	32.8	39.2	31.8	37.4	35.2	S x L	4.037	0.272
Weight	0								26.7	SNM	2.400	0.008
gain	30	23.2	25.1	26.6	24.3	25.7	24.7	24.0	24.8			
(g/b/d)	60	13.4	17.5	15.4	13.4	18.9	13.4	19.8	16.0	Level	1.667	<0.001
	90	6.2	15.5	11.8	10.1	14.2	12.4	18.3	12.6			<0.001 (L)
Mean		14.2	19.4	17.9	15.9	19.6	16.8	19.6	18.6	S x L	2.940	0.443
Gain-to-	0								0.66	SNM	0.0423	0.003
Feed	30	0.59	0.63	0.63	0.61	0.61	0.62	0.64	0.62			
Ratio	60	0.42	0.51	0.46	0.44	0.50	0.49	0.55	0.48	Level	0.0294	<0.001
	90	0.27	0.46	0.40	0.36	0.38	0.47	0.47	0.40			<0.001 (L)
Mean		0.43	0.53	0.50	0.47	0.49	0.53	0.55	0.51	S x L	0.0518	0.600

Polynomial contrasts: L-linear effects, SED-standard error of difference, P-probability, r.d.f.- residual degrees of freedom

3.2.1.3 Discussion

Generally growth performance of the control birds was lower than the breed average. Probably, housing conditions (e.g. temperature) or stress of transfer from deep litter to metabolism cage at an early age was responsible. Nevertheless, growth trends of all birds fed the experimental diets were distinct. The poor utilisation of the untreated shea nut meal was expected from the previous experiment as well as in other studies (Annongu *et al.,* 1996a, Atuahene *et al.,* 1998; Olorede *et al.,* 1999; Dei *et al.,* 2006).

Hot water treatment of the meal gave an improvement in weight gain and feed conversion efficiency over the untreated meal. Similar findings were reported in weanling rats (Okai *et al.,* 1995) and pigs (Rhule, 1999) fed hot water treated meal in the diets. Okai *et* a/. (1995) attributed the improvement in the nutritive value of the meal to a substantial reduction (70%) in the concentration of tannins. However, in their study the improvement in feed intake and protein digestibility of the hot water treated meal was not sufficient to equal those of the birds fed the control diet. They suggested that this might be due to the presence of other anti-nutritive factors. However, the major drawback of this method to eliminate tannins in feedstuffs is the considerable loss of dry matter (Kumar and Singh, 1984; Okai *et al.,* 1995). In the present study, 38% of the meal was lost in the drained water after hot water treatment.

Natural fermentation of the meal tended ($P>0.05$) to enhance its nutritive value for broiler chicks. Annongu *et al.* (1996a) reported a significant ($P<0.05$) improvement in the nutritive value of the fermented meal when it was fed to adult broiler chickens, which they attributed to the reductions in tannins (57%) and saponin (50%). Addition of tannase to the meal before natural fermentation did not change ($P>0.05$) the growth performance of the birds, probably due to the low amount added, or low hydrolytic activity. But addition of PEG had a greater ($P<0.01$) improvement on the weight gains of the birds; since PEG may have displaced some of the condensed tannins from tannin-protein complexes (Jones and Magnan, 1977).

The main lilitation of PEG as an additive for the improvement of the meal would be its high cost.

The use of exogenous micro-organisms for the fermentation of the meal resulted in a substantial improvement in its nutritive value. *A. niger* in particular, increased feed intake by 18.7% compared to the untreated group. Microbial degradation of tannins has been reported (Bhat *et al.,* 1998). However, microbial degradation of condensed tannins is shown to be lower than that of hydrolysable tannins (Bhat *et al.,* 1998). Both types of tannins are high in shea nut meal *(Table 3.2). A. niger* strains have been recognised as having a potential tannase synthesis ability (Pinto *et al.,* 2001), which catalyses the hydrolysis of ester and depside bonds in hydrolysable tannins, as tannic acid, releasing glucose and gallic acid. Other reports have shown that feeding broilers with diets containing soybean cultured with *Aspergilli* significantly improved growth and feed utilisation in broilers by increasing the availability of nutrients (Chah *et al.,* 1975; Mathivanan *et al.,* 2006). Although the bacterial fermentation of the shea nut meal gave a similar improvement in nutritive value, the use of *A. niger* for the fermentation would be a better option than the commercial *Bacilli mix* in terms of cost.

3.2.1.4 Conclusion

It was evident from this experiment that all microbial fermentation methods including natural fermentation with PEG, as well as hot water treatment, improved the nutritive value of shea nut meal for broiler chickens. Feeding of diets containing the nutritionally improved shea nut meals at the high inclusion level (90 g/kg) did not improve growth performance of the birds to the same level as obtained for the control diet. The loss of a substantial amount of dry matter through hot water treatment is a disincentive of this method for improving its nutritive value.

3.2.2 Variability of growth response of broiler chicks fed nutritionally improved shea nut meal samples fermented with fungal organisms *(A. niger* or *C. subvermispora)* and polyethylene glycol

The results of the screening experiment (section 3.2.1.0) have shown the potential of shea nut meal fermentation with fungal organism *(A. niger)* or natural fermentation with PEG as an additive as potential methods for improving the nutritive value of shea nut meal for broiler chickens. This improvement in the nutritive value of the meal was thought to be due to reduction of tannin concentrations. In this section, another fungal organism (white rot fungus) *(Ceriporiopsis subvermispora)* that could not be obtained at the time of the previous experiment due to lengthy procurement procedures, was evaluated together with *A. niger* and PEG. Both fungal organisms are thought to degrade different components of tannins, while PEG is known for its action on condensed tannins. It is not known how shea nut meal fermentation with these fungal organisms individually or in combination with PEG would influence growth performance of broiler chickens. Also, it is not known whether the growth response obtained using the individual fungal organisms and PEG in the previous experiment would be consistent. That is, if any variability in bird growth response in the utilisation of the nutritionally improved shea nut meals.

The specific objective of this experiment was to determine the effects of shea nut meal fermentation with fungal organisms *(Aspergillus niger* and *Ceriporiopsis subvermispora)* and polyethylene glycol (PEG 4000) on the growth performance of broiler chicks when included at 90 g/kg in nutritionally complete diets.

3.2.2.1 Materials and Methods

Preparation of samples

The treatments of the seven samples ror me experiment are shown in *table 3.22*. The fermentation procedures, drying and grinding of the samples were the same as described for the previous fermented samples **(3.2.1.1).** However, changes in the fermentation procedures were described as follows:

Treatment 1: Fungal fermentation using A. niger

This sample was prepared using *A. niger* as described previously (3.2.1.1).

Treatment 2: Fungal fermentation using Ceriporiopsis subvermispora

Ceriporiopsis subvermispora (ATTC number 90466) was obtained from the American Type Culture Collection (ATTC, 10801 University Blvd., Manassas, VA 20110-2209). The white rot fungus or C. *subvermispora* has been shown to be capable of degrading tannins in the leaves of *S. lespedeza* (Gamble *et al.*, 1996). The pure culture that was lyophilized was reconstituted and subcultures were made with the help of ATTC medium (#200 YM or Difco 0712) and incubated at 24°C for 7 days. The cultures were preserved at low temperature (approximately 4°C) till the organisms were used for solid state fermentation. The spores were harvested by scraping. Spore counts were determined using an haematocytometer according to the Fuchs-Rosenthal technique to be approximately $1.6x10^6$ spores, which were equivalent to 0.49 g.

Treatment 3: Natural fermentation with polyethylene glycol (PEG)

This sample was prepared by natural fermentation with PEG 4000 as described previously (3.2.1.1).

Treatment 4: Fermentation with A. niger and C subvermispora

Shea nut meal fermentation using *A. niger* and C. *subvermispora (Table 3.22).*

Treatment 5: Fermentation with A. niger and PEG

Shea nut meal fermentation using *A. niger* with PEG 4000 as an additive *(Table 3.22).* -

Treatment 6: Fermentation with C. subvermispora and PEG

Shea nut meal fermentation using *C. subvermispora* with PEG 4000 as an additive *(Table 3.22).*

Treatment 7: Fermentation with A. niger, C. subvermispora and PEG

Shea nut meal fermentation using the fungal organisms with PEG 4000 as an additive *(Table 3.22).*

Table 3.22: Fermentation treatments of expeller shea nut meal using *A. niger, C subvermispora* and PEG

	Treatments	Amount added (g/kg)
1	*A. niger*	0.25
2	*C. subvermispora*	0.49
3	Natural fermentation + PEG	20.00
4	*A. niger* + *C. subvermispora*	0.25 + 0.49, respectively
5	*A. niger* + PEG	0.25 + 20.00, respectively
6	*C. subvermispora* + PEG	0.49 + 20.00, respectively
7	*A. niger* + *C. subvermispora* + PEG	0.25 + 0.49 + 20.00, respectively

Broiler chick assay

Ross 308 male broiler chicks were reared in a litter-floored pen and fed a proprietary broiler starter feed for 11 days. At 11 days of age, 72 broilers of similar body weight were individually caged (0.3m x 0.3m x 0.36m). The seven fermented and one unfermented shea nut meal samples were each included at 90 g/kg into a basal diet *(Table 3.20).* The basal diet served as an additional control (standard diet). Each of the nine meal-form experimental diets (8 replicates) was fed to the birds from 11 to 19 d of age. The rationale for the high inclusion rate (>90 g/kg) in this experiment was to obtain improvements at a dietary level that would be acceptable to the poultry feed industry. Most feedmills would not be interested in using the product at <90 or 100 g/kg. However, such high inclusion rate might mask subtle improvements at 90 g/kg diet; which might be acceptable for the traditional poultry sector.

Statistical analysis

Fermented samples were considered as treatment factors with two additional controls (basal diet and unfermented shea nut meal) using GENSTAT (10^{th} version). The tier level of cages was considered as a blocking factor. ANOVA of data and orthogonal contrasts were used to compare the treatment means.

3.2.2.2 Results

The pH of the fermented shea nut meals are shown in *Table 3.23.* The reduction in the pH of the fermented samples in this experiment was an indication of better fermentation than in the previous experiment. The growth performance of chicks fed the untreated meal was poor and followed the same trends as in the previous experiments. There were no differences in the growth variables between the individual fungal organisms, or between the fungal organisms and natural fermentation with PEG. Also combinations of both fungi

or each fungus and PEG showed no significant changes in growth performance.

There were no mortalities in this experiment. There were no significant differences between the untreated meal and individual fungal fermentation treatments. Although birds fed the untrated meal and PEG fermentation treatment had similar ($P>0.05$) feed intake, the latter gave better ($P<0.05$) feed conversion efficiency. All combined fermentation treatments gave higher ($P<0.001$) growth performances than that of the untreated meal.

All shea nut meal treatments gave lower ($P<0.001$) growth performance than the standard control diet *(Table 3.24)*.

Table 3.23: pH of fermentation treatments of expeller shea nut meal

	Treatments	pH of fermented materials
1	*A. niger*	5.4
2	*C. subvermispora*	5.4
3	Natural fermentation + PEG	5.6
4	*A. niger* + *C. subvermispora*	5.5
5	*A. niger* + PEG	5.5
6	*C. subvermispora* + PEG	5.5
7	*A. niger* + *C. subvermispora* + PEG	5.5

PEG-polyethylene glycol

Table 3.24: Growth performance of broiler chickens (11 - 19 d) fed shea nut meal fermented with *Aspergillus niger, Ceriporiopsis subvermispora* and polyethylene glycol at 90

Variable ratio	Mean feed intake (g/bird/day)	Mean live weight gain (g/bird/day)	Gain-to-feed
Control	52.2e	36.4[8]	0.70[8]
Unfermented shea nut meal	19.9[e]	4.6[d]	0.22[d]
Fermented shea nut meals			
A. niger	22.7[de]	7.3[ed]	0.29[°]
C. subvermispora	22.4[de]	7.4[ed]	0.30[e]
PEG	25.0[ede]	10.2e	0.38[e]
A. niger + *C. subvermispora*	27.0'[1]	11.4e	0.426`
A. niger + PEG	29.2e	11.5[e]	0.39[°]
C. subvermispora + PEG	26.8e	10.5[e]	0.39[e]
A. niger + *C. subvermispora* + PEG	33.9[b]	17.0[6]	0A8[b]
Standard error difference	*2.967*	*2.611*	*0.056*
Probability	*<0.001*	*<0.001*	*<0.001*

PEG-polyethylene glycol
Means with different superscripts are statistically significant (P<0.001)

3.2.2.3 Discussion

The improvement obtained in growth performance in this experiment was not as great as in the previous experiment (section 3.2.1) when *A. niger* or PEG was used. There was no apparent reason for this, even though the fermentation seemed to be better than in the previous experiment as indicated by pH *(Table 3.23).* Like the previous experiment, the growth response of the standard control birds was less than expected. Nevertheless, the results obtained suggest the potential of the fungal treatments as well as that of PEG to improve the nutritive value of shea nut meal. Also, the data showed the possibility of variability in the procedure in the improvement of the nutritional value of shea nut meal for birds. Environmental factors may inadvertently influence experimental results, thereby masking the potential of the dietary treatments.

The growth data indicate consistently superior ($P<0.001$) feed conversion efficiency for the chicks fed diets containing shea nut meal fermented with both *A. niger* and *C. subvermispora* as well as natural fermentation with PEG, as compared to the unfermented shea nut meal group *(Table 3.24).* This was an indication that some of the anti-nutritive factors, most probably tannins, might have been degraded.

3.2.2.4 Conclusion

It was evident from this study that a solid state fermentation of shea nut meal using *A. niger or C. subvermispora* has the potential of improving its nutritive value for poultry. However, the nutritional improvement achieved in this study fell far short of the level expected of a commercial feed ingredient. Therefore, this novel approach requires further research and development. Although natural fermentation with PEG as an additive has a potential to also improve the nutritional value of shea nut meal, its high cost may preclude it from being commercially viable.

3.2.3 Effects of fungal *(Aspergillus niger* or *Ceriporiopsis subvermispora)* fermentation on the nutritive value of shea nut meal for broiler chicks

Aspergillus niger (Pinto *et al.,* 2001) or *Ceriporiopsis subvermispora* (Gamble *et al.,* 1996) can degrade tannins. Microbial fermentation can also reduce the concentration of saponins in a feedstuff (Reddy and Pierson, 1994). Also, solid state fermentation using fungal cultures such as *Aspergilli* can improve the nutritive value of feedstuffs for broiler chickens (Mathivanan *et al.*, 2006; Yamamoto *et al.*, 2007). The potential uses of these two organisms to improve the nutritional value of shea nut meal by fermentation have been demonstrated in the previous experiment (3.2.2). Therefore there is a need for further research on the solid state fermentation process using these fungal organisms.

If fermentation of shea nut meal is to be viable, a practical system of fermentation would need to be applied at the oil extraction factory or on-farm. It is unlikely that controlled fermentation conditions would be available at these sites. There is a need to examine whether the method of fermentation is an important factor in the nutritional improvement of shea nut meal. The simplest practical fermentation method would be to apply the fungal organisms to the shea nut meal and then leave windrowed. Alternatively, after the application of the fungal organisms, the material could be compacted and sheeted to exclude some, but not all, air and reduce other fungal or bacterial contamination. Two laboratory-based fermentation methods were devised to examine these two fermentation options.

Therefore, this study was undertaken to determine if the fermentation of shea nut meal using *A. niger* or *C. subvermispora,* or a combination of the two organisms could change tannin and saponin contents or change significantly the growth performance of broiler

chickens relative to the unfermented shea nut meal or a basal diet (containing no shea nut meal). A second objective was to compare two methods of fermentation for the same variables of chemical composition, nutrient availability and broiler chicken growth performance for each of the three fungal organism treatments *(A. niger, C. Subvermispora or a combination of both organisms*). Eight dietary treatments were compared.

3.2.3.1 Materials and Methods

Culturing of fungal species

The two fungal organisms *(A. niger, C. Subvermispora)* were cultured as described previously **(sections 3.2.1.1 and 3.2.2.1).**

Preparation of fermented fungi-treated shea nut meal samples

The expeller shea nut meal (2004, batch 1) was divided into seven lots. Six of these shea nut meal samples were mixed with water in the ratio 1:2 and the spores of *A. niger* (0.25g) were added to two samples. The spores of *C. subvermispora* (0.49g) were added to two further shea nut meal samples and a mixture of spores of *A. niger* (0.25g) and *C. subvermispora* (0.49g were added to the two remaining shea nut meal samples. Each of the two fungal treatment samples was allocated to one of two fermentation treatment methods. Method 1 involved fermentation of the wet material in an opened plastic container, whereas in Method 2, the material was firmly compacted and then the plastic container was closed and sealed with adhesive film. All the samples were kept in a room at ambient temperature (24°C) for eight days.

The pH of the fermented samples was determined using a pH Meter (Model 3510, Jensway, UK). The mats of fungal growth on the surface of samples in the opened containers were scraped-off before drying. Each sample was dried at 56°C and ground.

Broiler chick assay

The six fermented and one unfermented shea nut meal samples were each included at 90 g/kg into a basal diet *(Table 3.20)*. Ross 308 male broiler chicks were reared in a litter-floored pen and fed a proprietary broiler starter feed for 10 days. At 10 days of age, 64 broilers of similar body weight were individually caged (0.3m x 0.3m x 0.36m) and fed one of the 8 meal-form experimental diets (8 replicates) to 18 d of age. During the last 4 days of the 8-day experiment, the droppings were collected daily and stored at 4°C until the combined four-day sample was dried in a force-draught oven at 60°C.

Chemical analyses

The ground shea nut meal samples were analysed using standard methods for dry matter, nitrogen, ether extract, ash and gross energy as well as phenolic compounds and saponin as described previously **(section 3.1.1.1.2).** Each sample of bird droppings was also analysed for dry matter, nitrogen and gross energy.

Statistical analysis

A randomised block analysis of variance was used to compare the treatment means using GENSTAT (10^{th} version). The tier level of cages was considered as a blocking factor. Orthogonal contrasts were used to compare the treatment means. The three fungal treatments and two methods of fermentation were considered as treatment factors with two additional controls (basal diet and unfermented shea nut meal diet)

3.2.3.2 Results

The pH of the fermented shea nut meals were 5.3 (opened container) and 5.6 (closed container) indicating that a significant amount of fermentation had taken place in each of the six samples. The proximate nutrient compositions of all fermented fungi-treated and unfermented shea nut meal samples were similar *(Table 3.25)*. However, there were appreciable reductions in hydrolysable tannins (44-79%) as well as saponins (10-87%) in the fermented samples *(Table 3.26)*. The reduction in hydrolysable tannins was greatest when both organisms were present in the meal fermented by method two (closed container). The fungal degradation of proanthocyanidins was less than that of hydrolysable tannins in both fermentation methods *(Table 3.26)*.

The nutrient metabolisabilities of the experimental diets were higher (P<0.05) for the control (maize-soybean meal diet) than any of the shea nut meal containing diets *(Table 3.27a).* However, dietary AME of the unfermented meal was similar to that of the other control. Although there were no significant differences between the fermented and unfermented meals, dietary AME of the fermented meals tended (P=0.052) to be lower. This may have been due to the utilisation of some of more available nutrients by the fungal organisms (Egli and Zinn, 2003).

A correlation matrix of the chick growth and nutrient metabolisability variables of the fermented samples and their contents of anti-nutritive factors is shown in *(Table 3.27b).* There were no significant correlations between the variables. However, the correlation (r = -0.794) between crude protein metabolisability and saponin approached a significant level (P = 0.059).

There were no mortalities in this experiment. There were no significant differences (P>0.05) between the two fermentation methods in variables studied. The birds fed the fermented shea nut meal treatments had higher (P<0.001) feed intakes, weight gains and feed conversion efficiencies compared to the birds fed the unfermented shea nut meal *(Table 3.27a).* Also, all growth variables of birds fed the unfermented meal were far lower (P<0.001) than those of birds fed the basal diet (maize and soybean meal-based). Although the birds fed the shea nut meal fermented with both fungal organisms had lower (P<0.05) feed intakes, there were no significant differences in their weight gains or feed conversion efficiencies compared to birds fed the fermented meals using the individual fungal organisms *(Table 3.27a).*

Table 3.25: Chemical composition of unfermented and fermented fungi-treated samples of shea nut meal (g/kg dry matter basis)

Treatments		Crude protein	Ether extract	Ash	Neutral detergent fibre	Gross energy (MJ/kg)
Unfermented shea nut meal		143.6	101.4	52.3	455.7	22.8
Fermented shea nut meals						
A. niger	Method 1	143.1	117.0	49.6	498.1	23.9
	Method 2	141.9	113.5	50.0	470.3	23.6
C. subvermispora	Method 1	143.0	116.3	55.4	448.6	23.8
	Method 2	141.2	115.3	53.6	467.0	23.6
A. niger + C. subvermispora	Method 1	142.0	111.2	53.6	451.9	23.6
	Method 2	139.3	120.0	49.4	467.4	24.2

Table 3.26: Total soluble phenolics, proanthocyanidins, hydrolysable tannins, free gallic acid and saponins in unfermented (control) and fermented fungi-treated samples of shea nut meal (dry matter basis)

Treatments		Total soluble phenolics (g catechin/kg)	Bound+soluble proanthocyanidins (g catechin/kg)	Soluble proanthocyanidins (g cyanidin/kg)	Hydrolysable tannins (g gallic acid/kg)	Free gallic acid (g gallic acid/kg)	Saponins (g/kg)
Unfermented shea nut meal		116.7	253.5	18.2	3.4	1.4	6.0
Fermented shea nut meals							
A. niger	Method 1	103.2	186.7	16.6	1.5	2.5	3.7
	Method 2	91.1	166.0	13.7	1.6	1.8	0.8
C.	Method 1	102.6	200.2	15.1	1.5	2.7	1.1
subvermispora	Method 2	83.3	166.8	11.5	1.6	1.7	1.7
A. niger + *C.*	Method 1	93.9	191.3	14.1	1.9	2.7	5.4
subvermispora	Method 2	96.7	166.4	12.7	0.7	2.5	0.8

Table 3.27a: Growth performance of broiler chicks (10-18 d), apparent metabolisable energy, dry matter and crude protein metabolisability coefficients of basal diet and diets containing 90 g/kg of unfermented and fermented fungi-treated samples of shea nut meal

Treatments		Feed intake (g/b/d)	Weight gain (g/b/d)	Gain: Feed (g/g)	Dry matter metabolisability coefficient	Crude protein metabolisability coefficient	AME (MJ/kg)
Basal diet (no shea nut meal)		59.0	41.3	0.71	0.80	0.72	13.4
Unfermented shea nut meal		23.0	5.0	0.21	0.75	0.57	12.7
Fermented shea nut meals							
A. niger	Method 1	34.5	14.3	0.39	0.68	0.44	11.1
	Method 2	37.9	15.4	0.37	0.73	0.57	12.3
	Mean	**36.2**	**14.9**	**0.38**	**0.71**	**0.51**	**11.7**
C. subvermispora	Method 1	33.2	12.2	0.36	0.72	0.54	12.1
	Method 2	33.5	13.1	0.39	0.68	0.53	11.4
	Mean	**33.4**	**12.7**	**0.38**	**0.70**	**0.54**	**11.8**
A. niger + *C. subvermispora*	Method 1	32.2	11.1	0.33	0.71	0.49	12.3
	Method 2	27.3	8.8	0.31	0.69	0.55	11.5
	Mean	**29.8**	**10.0**	**0.32**	**0.70**	**0.52**	**11.9**
Probability of treatment differences							
Orthogonal treatment comparisons							
Basal vs. Fermented meals		***	***	***	*	**	*
Unfermented vs. Fermented meals		***	***	***	ns	ns	ns
Fermented meals only							
Methods of fermentation (M)		ns	ns	ns	ns	ns	ns
Fungal organisms (F)		*	ns	ns	ns	ns	ns
M x F interaction		ns	ns	ns	ns	ns	ns
SED for comparing:							
Individual treatment means (n=8)		2.865	2.665	0.056	0.034	0.063	0.678
Fermentation method means (n=24)		1.654	1.663	0.032	0.020	0.036	0.391
Fungal organisms means (n=16)		2.026	2.037	0.039	0.024	0.044	0.479

*SED-significant error difference, n-number of replicates, ns-not significant (P>0.05), *P<0.05, **P<0.01, ***P<0.001*

Table 3.27b: Correlation matrix' of some chick growth and nutrient metabolisability variables, and anti-nutrients in the fermented shea nut meal samples

	Feed intake	Weight gain	CPM	AME	HT	Pas	TPas	TSP
Weight gain	0.968							
CPM	-0.016	-0.122						
AME	0.290	0.044	0.484					
HT	0.689	0.578	-0.314	0.430				
PAs	0.284	0.315	-0.633	-0.045	0.219			
TPAs	0.029	-0.045	-0.480	0.240	0.415	0.709		
TSP	-0.155	-0.120	-0.413	-0.092	-0.221	0.862	0.677	
Saponins	0.010	-0.028	-0.794	0.030	0.583	0.386	0.487	0.131

'Coeicients between -0.81 and +0.81 were not (P>0.05) statistically significant.
CPM-crude protein metabolisability, AME-apparent metabolisable energy, HT-hydrolysable energy, Pas-soluble proanthocyanidins, TPas-bound + soluble proanthocyanidins, TSP-total soluble phenolics

3.2.3.3 Discussion

The amounts of tannins and saponins in some feeds can be reduced by fermentation (i.e. wet incubation of a feedstuff), although the mechanism by which these components are eliminated is not fully understood (Reddy and Pierson, 1994). The fermentation process can create conditions for the growth of microbes (e.g. *Aspergillus)* that break down tannins (Bhat *et al.,* 1998). However, the microbial degradation of condensed tannins (proanthocyanidins) is less than that of hydrolysable tannins in both aerobic and anaerobic environments (Bhat *et al.,* 1998). In this study, the fungal fermentation treatments resulted in higher reductions in hydrolysable tannins than proanthocyanidins *(Table 3.26).* Annongu *et al.* (1996) reported a poor reduction (34%) in proanthocyanidins in naturally fermented shea nut meal, probably due to the action of endogenous micro-organisms. *A. niger* strains are noted for tannase synthesis ability (Pinto *et al.,* 2001; Ramirez-Coronel *et al.,* 2003) that can degrade hydrolysable tannins into glucose and gallic acid. As is evident from the results, gallic acid concentrations increased (21-93%) in the fermented samples *(Table 3.26).* Also, *A. niger* is capable of ligno-cellulolytic enzyme production such as cellulases and xylanases (Villena and Gutierrez-Cornea, 2007), which can degrade tannins associated with cell wall polysaccharides. *C. subvermispora* can degrade condensed tannins (Gamble *et al.,* 1996) as well as hydrolysable tannins (Aikin *et al.,* 1995) through the production of ligno-cellulolytic enzymes that degrade fibre. C. *subvermispora* is among the most selective lignin-degraders in the wood industry.

The fermentation process can produce organic acids that breakdown saponins (Delaude, 1974). The reduction in saponin concentrations in the fermented shea nut meals could be due to the production of organic acids. Many authors have reported the importance of *A. niger* in the industrial production of citric acid using agro-industrial by-products as substrates (Leangon *et al.,* 1999; Vandenberghe *et al.,* 2000).

Heng *et al.* (2006) reported degradation of saponins in peas at acidic pH. Annongu *et al.* (1996) attributed the reduction in saponin (50%) in naturally fermented shea nut meal to organic acids.

The fermentation of shea nut meal using both fungal organisms in the same culture failed to elicit far greater reductions of tannins or saponins than individual organisms. This was probably due to antagonistic activity between the two fungal organisms. Although the antagonism in other studies (Shrivastava *et al.,* 1996; Sipiczki, 2006) include competition for space and nutrients, and production of enzymes that breakdown cell wall. Thus microbial fermentation of shea nut meal should preferably involve single micro-organisms.

Generally, the effects of the fungal treatments on the tannins in particular were not as great as expected. As such, the residual tannins in the fermented meals could still have adverse effects on the growth of broiler chickens. Fungal fermentation processes can be affected by several factors such as type of substrates (Lu *et al.,* 1995; David *et al.,* 2003), aeration conditions (Meijer *et al.,* 2007) and water activity (Romero *et al.,* 2007) on fungal growth and productivity. Also, as the fungal cells divide and the culture develops, the amount of biomass makes the culture congested. This is a recognised ongoing practical problem of fungal fermentation (Ward *et al.,* 2006). This may limit mass-transfer processes in the culture system of enzymes and metabolites produced or retard growth of the fungal organisms. Thus there is a need to subject fermentation methods of the shea nut meal to further studies. These may include the use of other natural or genetically-engineered fungal organisms and manipulation of the growth conditions of the microbe in order to provide acceptable feed products.

The poor growth performance of birds fed unfermented shea nut meal *(Table 3.27a)* was expected. Many studies involving shea nut meal have shown reduced feed intakes, weight

gains and feed conversion efficiencies in broiler chickens (Annongu *et al* 1996; Atuahene *et al.,* 1998; Olorede *et al.,* 1999), rats (Okai *et al.,* 1995) and pigs (Okai and Bonsi, 1989). These authors have attributed the poor utilisation of shea nut meal in monogastric diets variously to the presence of anti-nutritive factors such as tannins and saponins. The biological significance of tannins and saponins in poultry nutrition is related to their characteristic adverse effects on feed intake (Cheeke, 1971; Armstrong *et al.,* 1974) and nutrient utilisation (Shimoyamada *et al.,* 1998; Smulikowska *et al.,* 2001). In this study, the adverse effects of tannins and saponins probably resulted in low feed intakes as well as low nutrient metabolisability in the unfermented meal *(Table 3.27a).*

The chicks fed diets containing shea nut meal fermented with the fungal organisms had an improved growth performance compared to the unfermented shea nut meal group (Table 4). Annongu *et al.* (1996) also reported significant improvements in feed intakes and growth rates of adult broilers when fed shea nut meal that had undergone natural fermentation. Other reports have shown that chicks fed diets containing guar meal (Nagra *et al.,* 1998), soybeans (Mathivanan *et al.,* 2006) and koji feed (Yamamoto *et al.,* 2007) cultured with *Aspergilli* significantly improved growth rates of broiler chickens. In this study, there were substantial improvements in the feed conversion efficiencies of the fermented meals *(Table 3.27a).* This could be due to protein-sparing effects, as a result of reductions in the concentrations of anti-nutrients (Nelson *et al.,* 1975). All anti-nutrients in the fermented meals tended ($P>0.05$) to be negatively correlated to the crude protein metabolisability *(Table 3.27b),* particularly saponins ($P=0.059$). However, the residual concentrations of these anti-nutrients were still high. Thus growth rates of birds fed the fermented meals were relatively poor compared to that of birds fed control diet *(Table 3.27a).* Within the six fermented shea nut meal treatments, there were no strong correlations between bird growth performance and determined tannin and saponin levels *(Table 3.27b).* This may have been just a reflection of the high variability in animal-based

experiments; but the tannin break-down products may also have had significant effects on bird growth performance. Despite the lack of correlations between the available tannins and growth variables, dietary tannins have been shown to inhibit *in vivo* activities of trypsin and a-amylase (Longstaff and McNab, 1991), which may affect digestion of proteins and starch. Also, total phenolics may include non-tannin polyphenolic compounds or tannin precursors. These low molecular weight polyphenol components associated with tannins are more readily absorbed from chick diets than tannins, and may account for the major anti-nutritive effects (Butler and Rogler, 1992).

Some *Aspergillus* species produce mycotoxins (aflatoxins and ochratoxin A) in foods including fermented products that may cause disease in poultry, humans and other mammals. However, mycotoxin contamination of foods by *Aspergilli is* a very complicated issue. The biological factors allowing the growth of the *Aspergillus* species that produce mycotoxins are dependent on the fungus, the product and the location (Miller, 2002). The only fungi that have been unequivocally demonstrated to produce aflatoxins are *A. flavus, A. parasiticus* and *A. nomius* (Miller, 2002). Also, *A. ochraceus* is likely the most important *Aspergillus* species involved with ochratoxin A contamination. Although *A. niger* has been reported to produce ochratoxin A (Abarca *et al.*, 1994), it is not clear which of the *Aspergillus* species in addition to *A. ochraceus* may be important (Miller, 2002). The lack of any detrimental effects of the fermented shea nut meal using *A. niger* in this study suggested no health risks was associated with its use.

3.2.3.4 Conclusion

It was evident from this study that a solid state fermentation of shea nut meal using *A. niger or C. subvermispora* has the potential of improving its nutritive value for poultry. Also, the use of a closed container to prevent contamination should be preferred. The fermentation process can be scaled up at industrial level with minimal costs.

The nutritional improvement of shea nut meal achieved in this study still falls far short of what is expected for it to become valuable for the feed industry. The fermentation methods presently used require further research to enable a nutritionally valuable feedstuff to be produced.

3.2.4 Comparison of the growth of broiler chickens fed diets containing nutritionally improved shea nut meal and wheatfeed.

The previous experiment (section 3.2.3.0) had shown the potential of a solid state fermentation of shea nut meal using *Aspergillus niger* to improve its the nutritive value for broiler chickens. The inability of the nutritionally improved shea nut meal to compare favourably with the basal feed (i.e.based on maize and soybean meal) suggests that it may have been due to the fact that the shea nut meal had a lower nutrient composition than the basal feed. Shea nut meal has similarities in its nutrient composition to wheatfeed (NRC, 1994). Wheat by-products are used extensively in poultry diets in Western Africa. These by-products are often imported, thus can be scarce and expensive. Therefore, the use of nutritionally improved shea nut meal to replace wheatfeed would provide an alternative and available feed resource.

Therefore, this study was undertaken to determine if the fermentation of shea nut meal, that is an expeller product or a defatted expeller product, using *A. niger* could change the growth performance of broiler chickens relative to the unfermented shea nut meal or wheatfeed in nutritionally balanced diets.

3.2.4.1 Materials and Methods

Culturing of *Aspergillus niger*

The fungal organism *(A. niger)* was cultured as described previously (section 3.2.2.1).

Preparation of fermented fungus-treated shea nut meal samples

The expeller shea nut meal (2004, batch 1) was divided into two lots. The fat in one lot was removed through continuous extraction with petroleum ether (b. p. 40-60 °C) using a Soxtec system (Foss Ltd, UK). Each lot of shea nut meal samples was mixed with water in the ratio 1:2 (1 part shea nut meal: 2 parts water) and the spores of *A. niger* (0.25g) were added.

The samples were fermented in a closed container for eight days as described earlier (3.2.3.1). Each sample was spread on a polythene sheet and dried in a room at 30-40 °C for 6 days and ground.

Broiler chicken husbandry

The two fermented samples and one unfermented shea nut meal sample were each substituted at 100 g/kg for wheatfeed in the diets *(Table 3.28)*. Ross 308 male broiler chicks were reared in a litter-floored pen and fed a proprietary broiler starter feed for 22 days. At 22 days of age, 128 broilers of similar body weight were caged (0.6m x 0.6m x 0.9m) in groups of four and fed one of the 4 meal-form experimental diets (8 replicates) to 36d of age. The experiment was arranged as a randomised complete block design with a pen as the experiment unit. The group body weights were measured at 36 d. Feed and water were provided *ad libitum*. Feed intakes and feed efficiency data were recorded for the grower phase.

Table 3.28: Composition of the experimental diets

Ingredients (g/kg)	Basal diet	Shea nut meal diets
Maize	570.0	570.0
Dehulled soybean meal	220.0	220.0
White fishmeal	50.0	50.0
Wheatfeed	100.0	
Soybean oil	25.0	25.0
Lysine hydrochloride	2.5	2.5
D, L-Methionine	2.5	2.5
Threonine	0.5	0.5
Monocalcium phosphate	8.0	8.0
Limestone	15.0	15.0
Vitamin and trace element premix'	3.0	3.0
Salt	3.5	3.5
Experimental shea nut meal sample[2]		100.0
Calculated composition (g/kg)		
Crude protein	209.3	208.4
Lysine	13.2	13.2
Methionine + Cystine	9.5	9.6
Metabolisable Energy (MJ/kg)	13.0	13.5

Vitamin-trace mineral premix for broilers (Ian Hollows Feed Supplement, UK) added per kg: 800 mg Retinol, 150 mg Cholecalciferol, 1.25 g Tocopherol, 150 mg Thiamin, 500 mg Riboflavin, 150 mg Pyrodoxine, 750 mg Cyanocobalamin, 3 g Nicotinamide, 0.5 g Pantothenic acid 75 mg Folic acid 6.25 g Biotin, 12.5 g Choline chloride, Iron 1 g 50 mg Cobalt, 5 g Manganese, 0.5 g Copper, 4 g Zinc, 50 mg Iodine, 10 mg Selenium, and 25 mg Molybdenum.

[2]*Nutrient compositions of the experimental shea nut meal samples were assumed to be similar. Dietary formulation was based on an expellr shea nut meal*

Chemical analyses

The ground feed samples were analysed using standard methods for dry matter, nitrogen, ether extract, ash, gross energy, tannins, saponins, neutral detergent fibre and amino acids as described previously (section 3.2.1.1).

Statistical analysis

A randomised block analysis of variance was used to compare the treatment means using GENSTAT (10th version). Each cage of 4 pens was considered as a blocking factor.

3.2.4.2 Results

The unfermented and fermented expeller shea nut meal samples had similar proximate nutrient compositions. However, the defatted meal had higher concentrations of crude protein, but similar amino acid profile to the others. Obviously it had a very low crude fat content as well as a lower gross energy content *(Table 3.29).* The composition of the wheatfeed was typical (Sarmiento-Franco *et al.,* 2000). Comparatively, there were similarities in the concentrations of most of the nutrients including amino acids between the wheatfeed and the shea nut meal samples. However, it had lower neutral detergent fibre content than all the shea nut meals. Also, its crude fat content was lower than the expeller shea nut meals, but was high relative to the defatted shea nut meal *(Table 3.29).* Also, all the experimental diets appeared to have similar nutrient composition. The control diet had very low concentrations of tannins *(Table 3.30).*

There were no mortalities in this experiment. The birds fed the fermented shea nut meal treatments had higher ($P<0.001$) feed intakes, weight gains and feed conversion efficiencies than the birds fed the unfermented shea nut meal *(Table 3.31).* However, the growth performance of birds fed all the shea nut meals were lower ($P<0.001$) than those fed the wheatfeed based control diet. Nevertheless, there was no difference in feed intakes

between the control birds and their counterparts fed the diet containing as-received fermented shea nut meal. The birds fed the intact and defatted shea nut meal fermented with *A. niger* had similar (P>0.05) growth performance.

Table 3.29: Chemical composition of wheatfeed, unfermented and fermented fungus-treated samples of shea nut meal (g/kg dry matter basis)

Variable	Wheatfeed	Unfermented shea nut meal	Fermented shea nut meal	Defatted-fermented shea nut meal
Crude protein (CP)	176.0	143.6	165.9	198.2
Amino acid' (g/kg				
Methionine	13.0	16.4	17.4	17.3
Cystine	19.4	15.7	14.2	15.2
Methionine+Cystine	32.4	32.1	31.6	32.5
Lysine	42.1	33.6	31.6	34.1
Threonine	29.2	28.4	30.3	32.0
Arginine	60.9	68.7	63.2	67.2
Isoleucine	35.0	38.8	33.6	35.8
Leucine	59.0	62.7	59.4	62.8
Valine	53.1	49.3	45.2	47.7
Histidine	28.5	23.9	23.2	23.8
Phenylalanine	39.5	31.4	30.3	32.0
Glycine	50.5	37.3	38.1	39.5
Aspartic acid	64.8	83.6	80.7	85.6
Serine	43.4	32.1	34.8	35.2
Glutamic acid	174.9	133.6	128.4	136.5
Alanine	45.4	42.5	41.9	44.4
Tyrosine	29.2	31.4	29.0	30.9
Proline	45.4	39.6	39.4	37.9
Ether extract	37.5	101.4	130.8	4.9
Ash	55.1	52.3	57.7	69.8
Neutral detergent fibre	341.5	455.7	415.8	450.5
Gross energy (MJ/kg)	19.4	22.8	23.2	19.4

'Tryptophan content was not determined

Table 3.30: Chemical composition of experimental diets (g/kg 'as fed' basis)

Variable	Control diet	Unfermented shea nut meal	Fermented shea nut meal	Defatted-fermented shea nut meal
Crude protein	199.9	197.3	198.2	215.1
Amino acid[s]				
Methionine	5.6	4.9	5.2	5.1
Cystine	2.9	2.9	2.9	3.0
Methionine+Cystine	8.1	7.8	8.1	8.1
Lysine	12.0	11.3	11.4	10.7
Threonine	7.6	7.3	7.4	7.0
Arginine	11.5	11.5	11.4	11.4
Isoleucine	6.9	7.9	7.8	7.9
Leucine	14.4	14.9	15.0	14.9
Valine	9.5	9.3	9.5	9.2
Histidine	5.6	5.2	5.2	4.8
Phenylalanine	8.9	8.6	8.6	8.4
Ether extract	63.0	81.3	82.9	59.3
Ash	65.3	66.4	67.4	69.0
Neutral detergent fibre	97.5	128.0	109.7	125.8
Gross energy (MJ/kg)	16.9	17.8	17.6	17.2
Total soluble phenolics	1.86	5.80	3.60	4.87
Bound plus soluble	0.79	5.25	2.60	4.90
Soluble proanthocyanidins	0.00	5.44	2.24	5.27
Hydrolysable tannins	0.00	0.23	0.02	0.04

[1]Tryptophan content was not determined

Table 3.31: Growth performance of broiler chickens (22-36 d) fed diets containing 100 g/kg of wheatfeed, unfermented shea nut meal, and *Aspergillus niger* fermentation of as-received and defatted samples of shea nut meal

Variable	Control	Unfermented shea nut meal	Fermentation of intact shea nut meal	Fermentation of defatted shea nut meal	ANOVA SED (n=8)	ANOVA Probability
Feed intake (g/b/d)	142.0[a]	103.2[c]	136.4[ab]	127.2[b]	3.65	<0.001
Weight gain (g/b/d)	81.5[a]	45.3[c]	66.8[b]	60.3[b]	2.88	<0.001
Gain-to-feed ratio (g/g)	0.57[a]	0.43[c]	0.49[b]	0.47[b]	0.010	<0.001
Final body weight (kg/b)	2.163[a]	1.661[c]	1.958[b]	1.874[b]	0.04002	<0.001

SED-standard error difference
n-number of replicates (experimental unit=4 birds)
[a,b,c]Means within a row lacking a common superscript differ (P<0.001)

3.2.4.3 Discussion

The *A. niger* fermentation treatments of shea nut meal samples *(Table 3.31)* resulted in a substantial increase in feed intakes. The feed intake of fermented intact shea nut meal was similar to that of the control (wheatfeed based), while fermentation of the defatted meal gave 90% of the feed intake of the control. This was a clear indication of appreciable reductions in anti-nutritive factors such as tannins that were described in the previous experiment (3.2.3).

The poor growth performance of birds fed unfermented shea nut meal *(Table 3.31)* was expected as demonstrated in the previous experiment (3.2.3). There were lower feed intakes with a consequent lowering of weight gains and feed conversion efficiencies in the broiler chickens.

Broilers fed diets containing shea nut meal fermented with *A. niger* had an improved growth rate but it was only 74-82% of the control birds. Their feed conversion efficiency was also 82-85% of the control. The reduced growth performance of the birds fed the fermented shea nut meal could still be due to the effects of residual tannins (Annongu *et al.* (1996). It has been reported that small quantities of tannins in the diet (5-20 g/kg) caused depression in growth (Farrel *et al.,* 1999; Iji *et al.,* 2004). The residual concentrations of tannin components in the unfermented shea nut meal were within the reported range of adverse tannin concentrations *(Table 3.30),* while those of the fermented meals were close to the lower limit of the range. Therefore attempts should be made to further reduce the tannins in the fermented material. Also, the absence of a significant difference in growth performance between birds fed the intact and defatted shea nut meals again indicates that the residual fat may be of no additional value. However, removal of the residual fat would give a better consistency in the nutrient availability of the material.

It was evident that the fermentation treatments did not improve nutritive value relative to the control to an acceptable level. Probably, the high replacement level of wheatfeed by the fermented meal should be adjusted. A lot of work needs to be done to upgrade the fermentation methods.

Wheat by-products such as wheatfeed and wheatbran are often imported and used extensively in the West African sub-region in poultry diets. Although the fermented meal in its present form did not match the nutritive value of the wheatfeed used in this experiment, it however, demonstrated its usefulness as a potential feed ingredient. The improvement achieved in this study would be better applicable to the traditional poultry sector. Also, there is the possibility of refining the fermentation process to further enhance the quality of shea nut meal. The shea nut meal at present has no economic value; therefore fermentation with *A. niger* is promising. Further improvement of this fermentation method would make this home-produced material an alternative feed resource for feeding poultry.

3.2.4.4 Conclusion

It was evident from this study that a solid state fermentation of shea nut meal using *A. niger* had improved substantially (40.3%) the growth performance of broiler chickens, even though it did not match that of wheatfeed (79.9%) with reference to the unfermented shea nut meal. Therefore, this method of improving the nutritive value of shea nut meal requires refinement for it to become more useful in poultry rations.

CHAPTER FOUR

GENERAL DISCUSSION

4.1 GENERAL DISCUSSION

4.1.1 Chemical composition and nutrient availability of shea nut meal

The six shea nut meal samples collected for this study were analysed for their chemical components using modem analytical methods. Thus this project has provided essentially more detailed information about the nutrient composition of shea nut meal than earlier reports. Although the data confirmed most of the nutrient concentrations reported by other researchers, there are some chemical components that were poorly defined in those reports (Morgan and Trinder, 1980). This study provided new information such as characterisation of the non-starch polysaccharides, concentration of tryptophan, digestibility of amino acids, FFA content of the residual fat, and the absence of theobromine as an anti-nutritive factor in the meal. Also, there was paucity of information on nutrient availability.

The shea nut meal contained high amounts of NSP *(Table 3.1)* that constituted approximately 56% of total polysaccharides. The other fraction of the polysaccharides was made up of resistant starch. Both polysaccharides fractions are poorly utilised by poultry (Williams *et al.*, 1997). This contributed to the low metabolisable energy availability of the meals *(Table 3.15)*. There was a strong negative correlation ($P<0.05$) between total NSP and dry matter metabolisability *(Table 3.13)*. The insoluble NSP content appeared to be the major cause ($P=0.07$). Morgan and Trinder (1980) attributed poor utilisation of shea nut meal to the fibre content. Although the soluble NSP fraction was relatively high, there was no evidence that it contributed to high viscous conditions.

The single amino acid analysis of shea nut meal that had been previously reported did not include tryptophan, an essential amino acid for poultry. Also, there was no information on amino acid availability in the meal. The determined tryptophan concentration in the meal *(Table 3.1)* was 22-31% more than that of its ideal balance for poultry, but the concentrations of lysine and methionine were only 55-67% and 76-87% respectively of their ideal balance. This study also showed that the availability of the amino acids, particularly lysine and methionine, was poor *(Table 3.16).* In general, the amino acid composition of shea nut meal protein does not complement the imbalanced amino acid composition of cereals. It therefore does not have the potential to replace protein concentrates, such as fishmeal or soybean meal, within a practical broiler chicken feed. The low metabolisable energy and poor amino acid composition result in it being only a replacement for cereal by-products, for example wheatfeed or wheat bran, within poultry feeds.

The nutrient composition data confirmed earlier reports that there existed a wide variation in the fat contents of the shea nut meals due to processing methods. The fatty acid profile of the fat *(Table 3.6)* also corroborated earlier reports, which showed the predominance of stearic and oleic acids. Thus the fat had a very low unsaturated-to-saturated fatty acids ratio (1:1) with a consequent lowering of its metabolisable energy relative to soybean oil *(Table 3.10).* However, none of earlier reports determined the FFA of the fat in the meal. The FFA content of the fat tended to be correlated ($P>0.05$) negatively with gross energy metabolisability *(Table 3.13).* This could be partly responsible for the poor metabolisable energy availability. The presence or absence of the fat in the meal did not change the determined metabolisable energy of the meal at low inclusion levels *(Table 3.14)* and appeared to improve dietary energy availability at high inclusion levels. In order to have meals with consistent composition, it would be preferable for the meal to contain low levels of fat. Thus processing methods such as solvent extraction (Womeni *et al.,* 2002)

and enzyme-assisted extraction (Tano-Debrah *et al.*, 1994) that remove considerable amounts of fat from the kernels may not only be beneficial to the fat industry but also to the feed industry.

A few earlier reports (Atuahene *et al.*, 1998; Rhule, 1999) attributed the poor nutritive value of the meal partly to the theobromine content (4.5 g/kg DM). However, analysis of all the samples in this study using HPLC showed that the meals contained very low, and not significant, concentrations (<1 mg/kg) of theobromine. Therefore, this anti-nutritive factor, might not pose the nutritional challenge to poultry as originally thought. The adverse effects of theobromine reported were similar to those commonly associated with tannins and saponins.

The nutritional shortcomings of untreated shea nut meal are such that it has significant adverse effects on broiler growth performance, even at dietary level beyond 20 g/kg. This very low inclusion rate cannot be acceptable to the feed industry. Hence the need for its improvement to an acceptable level required for feeding poultry. For most feedmills, dietary inclusion rates around 100 g/kg would be acceptable. Nevertheless, any significant improvement in its nutritive value below expectation of the commercial poultry sector may be useful for the traditional poultry sector, whereby birds scavenge for food.

4.1.2 Variability between shea nut meals

The fat content of the shea nut meal was found to be the major cause of variation between different samples collected for the study. Other chemical components, with the exception of tannins, tended to have relatively small variation. Although the concentrations of tannins in expeller meals were far higher than those of the non-industrial meal *(Table 3.2)*, the tannins in the latter may have been poorly extracted due to the non-industrial processing method that involved the use of water for the fat extraction (Butler and Rogler, 1992).

Therefore variation in the different types of shea nut meals evaluated in this study was due principally to the fat content as well as its quality. The metabolisable energy content of the non-industrial meal was lower than that of the expeller meal *(Table 3.15),* because of its high fat content coupled with the high FFA content of fat *(Table 3.1).*

Heat processing of shea nut meal generally affected protein and amino acid concentrations as well as their availability. Despite significant ($P<0.001$) differences in amino acid concentrations and availabilities between the industrial and non-industrial meals, their overall protein crude protein metabolisability *(Table 3.12)* and digestible amino acid profile *(Table 3.16)* were similar. The higher concentrations of amino acids in the expeller meals compensated for their lower availability.

4.1.3 Improvement of nutritive value of shea nut meal

This study demonstrated that there are a number of possibilities of improving the nutritive value of shea nut meal through the use of hot water treatment, natural fermentation with PEG as an additive, and microbial fermentation *(Table 3.21).* However, the fermentation of shea nut meal using *A. niger* was found to be quite promising. The rationale for using high inclusion level of the fermented meal was to provide a product acceptable to feedmills. However, the fermented meal could be used at lower dietary inclusion levels both for the commercial and traditional poultry sectors. The fermented product would be more beneficial at current dietary level recommendation (25 g/kg) of the untreated shea nut meal (Atuahene *et al.,* 1998). The fact that the fermented material gave broiler growth performance close to that of wheatfeed at high dietary level demonstrated its potential as substitute of this cereal by-product that is used extensively in poultry diets in West Africa. It therefore could replace the wheatfeed/bran at low dietary levels. Presently, the shea nut meal has no economic value, thus its use would be cheaper than wheatfeed/bran; since solid state fermentation processes of agricultural by-products are known to be cost effective.

The fermentation procedure is quite simple. On a large-scale, the equipment that will be required include feed mixer, fridge for the storage of the fungus, and plastic containers. The fermented material can preferably be sun-dried on plastic sheet or cemented floor due to the hot climate in Ghana. Also, *A. niger* can easily be cultured to produce large amounts of spores for the fermentation from the initial stock.

factors such as type of substrates (Lu *et al.,* 1995), aeration conditions *(Meijer et al.,* 2007) and water activity (Romero *et al.,* 2007) on fungal growth and productivity. Although low concentrations of tannins and saponins in poultry diets may be beneficial due to their health benefits, the residual levels in the fermented meal were too high. Also, the presence of other anti-nutritive factors such as NSPs needs to be addressed in conjunction with the fermented product in the diet by lowering the inclusion level. There is a need to subject fermentation method of the shea nut meal using *A. niger* to further studies. These may include the use of other potent strains of *A. niger* that are genetically-engineered or manipulation of the growth conditions of the microbe in order to provide acceptable feed products.

The nutritional improvement of shea nut meal achieved in this study still falls short of what is expected for it to become valuable for the commercial feedmills *(Table 4.1)*. From economic viewpoint, it would be preferable to practise mild feed restriction (e.g. 10% restriction) during periods of feed scarcity, which is rampant almost every year in the West African sub-region, rather than feed the nutritionally improved shea nut meal in its present form. Further improvements in the nutritional improvement of shea nut meal are essential for. it to become a useful feed ingredient in the West African poultry industry, the ultimate goal for this home-produced by-product.

Table 4.1: Relative improvement in growth variables of broiler chickens fed diets containing shea nut meal fermented using *A. niger* in relation to unfermented shea nut meal and control (maize-soybean meal based diet)

		Relative improvement (%)	
	Age of birds (d)	Feed intake	Weight gain
Fermented versus Unfermented shea nut meal (100%)			
Experiment 3.2.1	10-18	166	295
Experiment 3.2.2	11-19	114	159
Experiment 3.2.3	10-18	157	298
Experiment 3.2.4	22-36	132	147
Mean		***142***	***225***
Fermented shea nut meal versus control (100%)			
Experiment 3.2.1	10-18	97	69
Experiment 3.2.2	11-19	43	20
Experiment 3.2.3	10-18	61	36
Experiment 3.2.4	22-36	96	82
Mean		***74***	***52***

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

- Shea fat as a dietary fat for poultry has low apparent metabolisable energy content relative to soybean oil.
- Shea nut meal had moderate crude protein contents, but high contents of crude fat and total NSP with wide variation in the crude fat content.
- The dietary metabolisable energy content of shea nut meal was low relative to its proximate nutrient composition.
- The efficiency of nutrient utilisation of shea nut meal was generally low.
- Removal of residual fat in shea nut meal influenced the chemical composition, but did not affect nutrient utilisation efficiency or dietary energy availability.
- Tannins and saponins were identified as the major anti-nutritive factors in shea nut meal.
- The untreated shea nut meal was poorly utilised by broiler chickens.
- Fermentation of shea nut meal using fungal organisms, particularly *A. niger* reduced substantially the contents of tannins and saponin with consequent improvement in growth performance of broiler chickens.
- The improvement in nutritive value of shea nut meal through fermentation procedures was short of expectation; therefore it would not be acceptable to poultry feedmills in its present form; but may be useful in traditional poultry feeding systems.

5.2 RECOMMENDATIONS

- In this study the overall aim was to improve the nutritive value of shea nut meal to an acceptable dietary level for commercial feedmills. Thus high dietary inclusion levels (90-100 g/kg) were used. Even though the improvements in the nutritive value achieved in this study fell short of what were expected for the commercial poultry sector, it was evident that the improved material might be useful when used at low dietary inclusion levels.
- The nutritionally improved shea nut meal can be valuable for inclusion at low dietary levels in the village poultry diets.
- The replacement of wheatfeed/bran with fermented shea nut meal at low dietary levels (<100 g/kg) would be beneficial as an alternative feed resource, since the expected cost of the fermented material would be lower than that of imported wheatfeed/bran.
- There is room for further improvements of the fungal fermentation method using *A. niger*. This could be done through manipulation of the growth conditions of the fungus or use of genetically-engineered fungus that would be more effective in the breakdown of tannins to produce acceptable feed products. Thus, it is strongly recommended that further research be conducted on the current improvement methods.

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