GROWTH RESPONSE AND HAEMATOLOGICAL CHARACTERISTICS OF BROILER CHICKENS FED DIETS CONTAINING SHEA BUTTER *(VITELLARIA PARADOXA* C.F GAERTN) SEED CAKE AS STARTER DIET

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JULY, 2014

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DEPARTMENT OF BIOLOGICAL SCIENCES,
FACULTY OF SCIENCE
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NIGERIA

JULY, 2014

DECLARATION

I declare that the work in this thesis entitled **'Growth response and haematological characteristics of broiler chickens fed diets containing shea butter *(Vitellaria paradoxa* C.F Gaertn) seed cake as starter diet'** has been carried out by me in the Department of Biological Sciences under the supervision of Prof. S.J. Oniye and Dr. T.S. Olugbemi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Ademola Julius OROGUN	……..	……
Name of Student	Signature	Date

CERTIFICATION

This thesis titled "GROWTH RESPONSE AND HAEMATOLOGICAL CHARACTERISTICS OF BROILER CHICKENS FED DIETS CONTAINING SHEA BUTTER *(VITELLARIA PARADOXA* C.F GAERTN) SEED CAKE AS STARTER DIET" by Ademola Julius OROGUN meets the regulations governing the award of the degree of Master of science degree in Biology of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to hundreds of thousands lonely and deserted people out there. May you find a place of shelter and succour.

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All glory and honour I give to my creator, keeper and ever present help in times of need, your glory oh Lord you share with no man.

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I acknowledge my colleagues and staff of the Department of Biological Sciences, may the Lord repay you all for your labour of love.

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ABSTRACT

There is high cost of feeding poultry in developing country like Nigeria. To this end, effectiveness of Shea Butter Cake (SBC) [an agro-industrial by-product obtained from the processing of the nuts of the shea tree (*Vitellaria paradoxa*, Gaertn) for fat] as feed meal was investigated in 225 ROSS broilers assigned to five feed diet in 3 replicates with 15 birds per replicate for four weeks. Samples of the shea butter cake, and diets were analysed for their nutrient and gross energy contents using AOAC (2006) methods. Proximate analysis of Shea butter cake revealed 92.57% DM containing 12.70% CP, 5.96% CF, 0.84% oil, 3.77% ash, 76.72%NFE. It also contained necessary minerals such as Ca (1.85mg/100g), K (0.99mg/100g), Mg (0.38mg/100g), Na (1.05mg/100g), Fe (1.06mg/100g), P (0.40mg/100g), Zn (5.676mg/100g), and Cu (0.55 mg/100g). Antinutrients determined in SBC were phytate (4.60 mg/100g), Hydrogen cyanide (2.90 mg/100g), saponins (1.15 mg/100g) and tannins (0.11mg/100g). Essential amino acid encountered included arginine (1.89g/Kg), histidine (0.50g/Kg), isoleucine (1.01g/Kg), leucine (1.34g/Kg), lysine (1.28g/Kg), methionine (0.38g/Kg), phenylalanine (1.75g/Kg), valine (1.25g/Kg) and threonine (0.95g/Kg); while the non-essential amino acids were glutamic acid (3.56g/Kg), aspartic acid (1.96g/Kg) cystine (0.35g/Kg), proline (1.30 g/Kg), tyrosine (1.10g/Kg), serine (1.25g/Kg), alanine (0.75g/Kg) and glycine (1.25 g/Kg). The control diet (diet1) contained 0% SBC while diets 2, 3, 4 and 5 contained shea butter inclusion at 10, 20, 30 and 40% respectively; all diets were iso-nitrogenous and iso-caloric. Measured parameters were live body weight, body weight gains, and feed intake. Feed conversion ratio and feed cost per kilogram diet were calculated. Haematological parameters obtained after the 28-day trial showed that Packed cell volume, Haemoglobin, White blood cell and Mean corpuscular

haemoglobin concentration had the following range 21.63 -25.53%, 7.21- 8.51g/dl, 13.61-14.26x10^3µl and 26.60 – 28.38% respectively. The highest live body weight (LBW) of 968.33± 4.41g and Body weight gain (BWG) of 538.33±6.00g g were obtained with 10% SBC which did not differ significantly ($P<0.05$) from values obtained with 0% SBC, while poorest LBW and BWG values of 900.00±2.89g g and 521.67± 5.83g respectively were obtained with 40% SBC diet. Feed consumption for chicks under 0(control), 10 and 20% dietary treatments were similar but they were significantly different ($P<0.05$) to those on diets 4 and 5; chicks on diet 1 (0% SBC) consumed highest (969.55g) and chick on diet 5 (40%) consumed the least (919.47g). Feed conversion ratio (FCR) however increased with increase in SBC inclusion levels from 1.050 in diet 1(control) to 1.080 in diet 5.The health of the birds were not affected in majority of the dietary treatments as all indices obtained were within the normal values. A reduction in live body weight, feed consumption, body weight gain was observed when inclusion level of SBC in the diet exceeded 10%. Feed cost per Kg of diets and feed cost per Kg weight gained decreased from ₦94.00-₦84.00 and ₦98.70-₦90.72 respectively as the inclusion level of SBC in the meal increased from 0 to 10%. It was concluded that the inclusion level of 10% SBC meal did not impose any detrimental effect in the birds and production cost was reduced. Additional processing methods for shea butter are recommended which may further reduce the antinutrients and improve the availability of nutrients for animal growth and development. The use of SBC in feeding of other animals is recommended.

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ABBREVIATIONS

₦ = Naira

A.B.U= Ahmadu Bello University

A.O.A.C= Association of Official Analytical Chemists

ANFs= Antinutritional factors

BWG= Body weight gain

CF= Crude fiber

CP= Crude protein

DM= Dry matter

DNA= Deoxyribonucleic acid

EE= Ether extract

FCR= Feed conversion ratio

FI= Feed intake

g = Gram

Hb= Haemoglobin

HCT= Haematocrit

HPLC= High Performance Liquid Chromatography

LBW= Live body weight

MCHC= Mean corpuscular haemoglobin concentration

Ml= Millilitre

MT= Metric tonnes

NFE= Nitrogen Free Extract

PCV= Packed Cell Volume

RNA= Ribonucleic acid

SBC= Shea Butter Cake

SE= Standard Error

W/V= Weight per Volume

WBC= White Blood Cell

μl= Microlitre

CHAPTER 1

1.0 INTRODUCTION

1.1 Background of the Study

The domestic chicken (*Gallus gallus domestica)* is an important domestic animal worldwide, especially for its nutritious flesh that is used as a source of food (Unang, 2003), food security and revenue for rural households (Dieye *et al.,* 2004). In the developing world, smallholder poultry rearing is a common practice in most rural and some urban areas, where the demand for the birds is expanding as a result of population growth, increased urbanization, and rising incomes (David, 2004; Schneilder and Polnick, 2010).

The West African poultry sector, particularly in Nigeria faces high production costs and technical constraints (Schneilder and Polnick, 2010). Feed accounts for 70 - 80% of the total cost of broiler production in Nigeria (Ademola and Farinu, 2006) with the high cost of the conventional poultry feed stuffs stemming directly from their competition with human staple (Esonu *et al*., 2001). Of the total cost of feed, about 95% is used to meet energy and protein requirements, about 3-4% for major minerals, trace mineral and vitamin requirements, and 1 to 2% for various feed additives (FAO, 2011). Recent advances in modern poultry production have further increased the competition for conventional feedstuffs between livestock industry, agricultural product-dependent industries (confectioneries and distillers) and humans (Bolu and Adakeja, 2008).

In Nigeria, the poultry feed industry is heavily dependent on grains such as maize, millet and sorghum; and oilseed resources such as groundnut cake, soybean cake, cotton seed cake and palm kernel meal (RMRDC, 2003). It has therefore become imperative to

explore other alternatives for the feed industry in order to reduce the current stress on human food supply.

One of the key agro-forestry species in Africa, particularly Nigeria is the shea butter tree, *Vitellaria paradoxa* syn. *Butyrospermum paradoxum* (Sapotaceae). The shea tree produces fruits which is cherished and eaten by humans and animals; the nut of this fruit is processed to give shea butter, while the residue or by product is the shea nut cake (Dei *et al.,* 2008). The shea nut cake is an end product; available in large quantity and generally disposed off via incineration, because it is considered as "useless". Approximately 500,000MT of this cake are produced annually in the savanna region of West Africa (Okai and Bonsi, 1989).

1.2 Statement of Research Problem

By the year, 2020, the world population is estimated to be 8 billion with most of the population growth coming from developing countries (Singh and Makkar, 2009). This expected surge in population will definitely impact on the animal industry as more animal protein will be demanded. Much of the increase in global demand for poultry products are in developing countries. While global consumption of poultry products, especially poultry meat in Nigeria, has consistently increased over the years, and this trend is expected to continue (FAO, 2011). The need for increased animal protein consumption of the rural and urban Nigeria populace in the face of rising population and inflation has resulted in the increase in cost of conventional animal protein sources (Agbogidi and Okonta, 2011).

Feed accounts for 75-80% of the total cost of poultry production in Nigeria and this is largely due to the high cost of conventional feed stuffs stemming directly

from their high demands as staple food by humans (Esonu *et al*., 2001). Rapid growth in the poultry industry is having a profound effect on the demand for feed and raw materials (FAO, 2011). In 2003, Africa imported some 182,000 tonnes of chickens from the European Union (CTA, 2004). Feeding broilers during the early weeks of life represents a challenge to nutritionists and flock managers (Plumstead, *et al.,* 2007).

1.3 Justification

In most developing countries, the major sources of protein in commercial poultry production are fishmeal and oil seed cakes which are usually scarce, expensive and used extensively by other livestock and humans (Gadzirayi *et al.,* 2012). The replacement of expensive and imported conventional feed ingredients particularly those of protein origin (fish, soybean and groundnut cake) with cheap, locally available and non-human consumable substitutes in feed represents a suitable strategy for reducing the total feed cost of poultry production in Nigeria (Tuleun and Igba, 2008). Therefore, any major reduction or decrease in the cost of feed input will extensively reduce the overall cost of production and increase the profit margin of the farm (Owen *et al.,* 2012), thereby increasing their wealth profile and standard of living.

Early age nutrition of broilers is fundamental to growth (weight) and health status, and consequential to overall performance of the birds. The improvement in the nutritional status of the young broiler is dependent on a number of mechanisms, all of which rely on the bird metabolising adequate amounts of energy and other nutrients. Furthermore, the attainment of a good 7-14day weight gain, together with good uniformity, is increasingly important (Firman and Boling, 1998; Plumstead *et al.,* 2007). The early weeks represents an ever-increasing proportion of the broiler production cycle; a good start leads to a uniform flock of chicks with a good 7-14 days weight, which is correlated to the slaughter

weight of the birds (Agbede *et al.*, 2012). These days are not only crucial and critical, but determine the final weight gain and profitability of the venture.

The shea butter cake, which is a by-product that is locally available and non-human consumable, may assist in fundamentally reducing the cost of feed in poultry production, particularly in the first few weeks of broiler production.

1.4 Aim and Objectives

The aim of this study is to evaluate the growth response and haematological characteristics of broiler chickens fed diets containing shea butter cake (*V. paradoxa)* as starter diet.

The specific objectives were to:

a. determine the proximate composition, anti-nutritional factors, minerals and amino acids profile of dried *V. paradoxa* cake
b. determine the growth response of broilers fed starter diets containing different inclusion levels of shea butter cake
c. determine the haematological characteristics of broiler chicks fed shea butter cake as starter diets
d. determine the cost implication of feeding broiler chicks with SBC starter diet.

1.5 Hypotheses

a. The proximate composition, anti-nutritional factors, mineral and amino acids content of dried shea butter seed cake are low
b. There is no significant difference in growth response of broiler chicks fed starter diet containing different inclusion levels of shea butter seed cake and chick fed starter diet devoid of SBC

c. There are no significant differences in the haematology of broiler chickens fed diets containing different inclusion levels of shea butter cake as starter diet in comparison to chicken fed starter diet devoid of SBC.
d. The cost implication of incorporating Shea butter cake in starter diet is not lower than starter diet devoid of SBC.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Overview of Poultry Production

Broilers dominate the world poultry consumption contributing about 70 percent to the world poultry market, turkeys account for about 8 percent while other poultry provides the balance of 22 percent (Hacina *et al*., 1998). According to FAO (2011), the production of 230 million tons of all meat was composed of about 65.2 million tons of poultry meat, 56.3 million tons of beef and 89.5 million tons of pork. The world meat economy has been characterized by the rapid growth of the poultry sector. Its share in total meat output went from 13 percent to 28 percent in the last three decades. In recent years, poultry has contributed effectively to the buoyancy of the meat trade (Iheukwumere and Herbert, 2003). Poultry farming is a sub-sector of livestock, which has been developed from backyard chicks rearing to commercial farming during the past five decades (Altaf-Ur-Rahman *et al.,* 2009).

Estimates for the combined annual production of the world's poultry industries put the total at about 95 million metric tons of meat and approximately 63 million metric tons of eggs (Horizon, 2010). FAO (2010) reported that 281.5 million metric tonnes of all meats are been produced worldwide and that poultry meat accounted for about 92.3 million tons of this total, poultry's output compared with 103.6 million tons for pork and 64.7 million tonnes of beef. Total broiler production over the period 2009-2019 will grow by 1.8% annually to reach 79.36 million metric tonnes, while the international trade in broiler meat grows by 1.3% per year to reach 8.29 million metric tons (FAO, 2011). Poultry production is an increasingly important agricultural industry

in the world, poultry meat and egg account for about 10% of the total amount of all meat, eggs and milk produced in the world each year (Onu and Madubuike, 2012).

In Africa the total poultry population of the continent was estimated as 113.3 million chickens, 15 million ducks and 7 million turkeys in 1998 (Guaye, 2000). Production scale being 1,781,701 metric tonnes (MT) of hen eggs, 2,269,000 MT of chicken meat, 32,824MT of turkey meat and 52,989MT of duck meat (Guaye, 2000). The rapid expansion of the poultry industry is very evident in countries like Brazil, Morocco, Nigeria and Saudi Arabia (Daghir, 2009).

The industry has undergone remarkable growth over the years in Nigeria. According to Cardi *et al.* (2005), the Nigerian poultry industry expanded with the domestic meat production increasing from 172,000 MT in 1999 to 211,000 MT in 2005. Poultry enterprise is prominent among livestock production and contributes substantial amount of meat and eggs for human consumption in Nigeria (Onu *et al.*, 2011). However, the expansion is not keeping pace with domestic consumption requirements and is being hampered by fluctuations in the supply of good quality feed (Onu *et al.*, 2011).

One of the most important roles of animal production is to provide high quality protein for human consumption (Araujo, 2004). The high demand for chicken meat, low capital input required, early market age, rapid return over invested capital and the small space required for poultry production have increased awareness that chicken farming is a profitable venture. Poultry production can play an important role in poverty alleviation and in the supply of quality protein to rural people (Araujo, 2004). Poultry farming has become an important money spinning industry in Nigeria (Adeyemo *et al.*, 2010). However, as beneficial and interesting poultry seems, this sub-sector is bedevilled by

high off-farm input prices particularly feed prices, this has made a greater number of poultry farmers to produce below capacity (Onu and Madubuike, 2012).

2.1.1 Contribution of poultry to the economy

To alleviate low protein intake Nworgu *et al.* (2000) suggested that broiler production is the fastest and easiest route since they are prolific, possess a high feed conversion ratio and are accepted by all, irrespective of religion. Poultry production is unique in that it offers the highest turn over rate and the quickest returns to investment outlay in the livestock enterprises (Osman, 2007). Poultry production, especially broiler production offers the greatest promise of increasing the quality and quantity of animal protein intake in Nigeria because of the high economic value, short generative interval and fast growth rate that can result in the production of meat within 8 weeks (Onu and Otuma, 2008).

Onu and Otuma (2008) further emphasized that the most efficient and economic means of providing high quality animal protein in developing countries is through the expansion and sustenance of the poultry industry. Daghir (2009) reported that poultry meat production has shown much higher growth than any other type of meat production during the past decade with the rate of increase in chicken meat averaged 5.7% per year since 1990. Ilo *et al.* (1996) reported that the expansion of the nation's commercial poultry holds a great promise to bridging the animal protein gap in the country within the shortest possible time. Poultry industry played important role in alleviating animal protein deficiency in the last two decades throughout the world via increased availability of poultry eggs and meat (Pervez and Abdul, 2011).

2.1.2 Effect of food security on the emerging population

The world population is growing geometrically and it is projected to be 8 billion by 2020; it is however, pathetic to note that the developing world which is under pressure with the challenge of food security, is contributing most to this increase (Singh and Makkar, 2009). Globally, the relationship between food availability particularly protein in low income countries and rising population is increasingly perilous and for some, it has attained a crisis proportion (Omuikhoje, 2008). Food insecurity and lack of access to health care play a major role and explain persistence of malnutrition which is often related to protein-energy and micronutrients deficiencies (West *et al.,* 2006). Adeola and Olukosi (2009) reported that as human population continues to grow, with the greatest growth expected in countries that are already suffering from chronic hunger and malnutrition, there will be need to ensure food safety for all, especially the more susceptible sector of human population. In developing countries, more than 250 million children are affected by malnutrition; and 178 million of them under the age of five years present growth retardation (Shetty, 2010).

FAO (2011) predicted that global meat production and consumption will rise from 233 MT in year 2000 to 300 MT by 2020, and milk from 568 to 700 million tonnes over the same period. These predictions show a massive increase in animal protein demand, needed to satisfy the growth in the human population, and the increasing affluence of the emerging economies (Afolayan *et al.,* 2002). Many developing countries like Nigeria, (with over 150 million people) are yet to achieve self-sufficiency in food production (Ajaja *et al.,* 2003). In Nigeria, the high cost of animal protein has made the protein intake to fall below 10g compared to the recommended daily intake of 35g (FAO, 2007). The might of the developed nations of the western

world can be attributed to their ability to feed themselves, export to other countries and embark on food aids to needy countries facing food crisis (Onu and Otuma, 2008).

2.2 Challenges Faced in Broiler Production

2.2.1 Poor quality of ingredients used in the manufacture of feed

Many of the raw materials are not properly processed and handled while some others are adulterated. Ademola and Farinu (2006) stated that frequently moisture content, aflatoxin level and other microbial contaminant of raw material are never evaluated prior to purchase and use in the manufacture of poultry feeds. Consumption of such feeds by birds often results into serious health problems, depressed productive (in case of broilers) and reproductive performance (in case of layers). In extreme cases of contaminated feeds high mortality often occurs.

2.2.2 Supply of poor quality chicks

This is a major source of sub-optimal production and reproductive performance of flocks in the country. The problem is often exacerbated for the fact that poor quality chicks are not always realised early enough until considerable level of cost has been incurred in raising the chicks (Owen *et al.,* 2012). Araujo *et al.* (2004) observed that there are no strict and compulsory quality control measures in the hatchery or in the market in most developing countries.

2.2.3 High feed cost

General knowledge exists that feed constitutes the highest and most expensive input in any livestock farm, especially poultry (Owen *et al.,* 2012). Ani *et al.* (2012) reported that the major factor militating against intensive animal production in Nigeria

is the high cost of feed and feed ingredients; especially the conventional energy and protein feed ingredients like maize, soybean cake and groundnut cake. They further explained that feed account for 70% of total cost of producing poultry intensively. The production of low quality feed lead to many problems in broiler production resulting in poor performance and decreased benefits (Pervez and Abdul, 2011). The price of feed is the single most expensive item on the list of expenses of a farmer because of their financial constraints (Afolabi *et al.,* 2011). The conventional protein feedstuffs for poultry such as soyabean, groundnut cake and fish meal are scarce and expensive because they are competed for by humans as food and other industrial uses (Kwari *et al*., 2011a).

There may be some divergence from the use of cereals for feed but the need for protein feeds cannot be avoided (Afolabi *et al.,* 2011). The animal feed industry uses 77% of the soybean meal produced primarily as an amino acid and protein source in diets (Kerley and Allee, 2003). The most important and expensive feedstuffs are the energy sources, usually maize which accounts for the largest proportion of about 50-55% of the poultry diet (Afolayan *et al.,* 2002). Kwari *et al.* (2011b) were of the opinion that the use of maize as sole energy source in poultry diet formulations is becoming unrealistic on account of the decline in the land cultivable to maize as a result of climatic change, the use of maize grain as a staple food for humans and several industrial uses. The escalating rise in the cost of maize is brought about by its declining production conditions and stiff competition for its use by man and other livestock species (Hamzat and Babatunde, 2003). Energy source constitutes 45-60% of finished feeds for monogastric animals and birds eat to satisfy their energy requirement (Tewe and Egbunike, 1992). Agbede *et al.* (2012) was of the opinion that the high inclusion

rate of maize translates into high cost of feed because of seasonality of maize production and competition for it by man and livestock. The small-scale and semi-commercial broiler farmer is faced with the problem of buying small quantities of feed at an inflated price.

The rapid growth of broilers demands that they be supplied with high quality diets to sufficiently cater for their nutrients requirements (Adeyemo *et al*., 2010). The protein required by broiler depends primarily on the amount needed for maintenance of health, tissue integrity and for productive purposes (Adeyemo *et al.,* 2010). The growth in demand for livestock products suggests that there will be a consequent rise in demand for animal feed, not only of cereals but of other feeds and particularly proteins (Afolayan *et al.,* 2002). The ever-increasing cost of poultry feeds with the attendant increase in the cost of chicken and eggs shows that there is need to explore the use of alternative feed ingredients that are cheaper and locally available (Ani *et al.*, 2012).

2.2.4 Inadequate access to and high cost of veterinary services

As poultry production can be considered an important development tool for promoting food security at household level, there have for a many years been various initiatives to improve production and to provide veterinary services such as vaccination to protect chickens from Newcastle disease and other diseases (Permin and Pedersen, 2009). However, weak relationships and poor collaboration between public veterinary services and the private poultry sector, result in ineffective disease control, which affects productivity and profitability. Despite its rapid growth, importance and contribution to national economies and food security, the commercial poultry sector faces major challenges that require innovative thinking among and engagement between, all stakeholders within countries (Lindberg, 2012). Presently the issue of

provision of veterinary services to the livestock community, and the division of tasks between government and private veterinarians is debated (Lindberg, 2012). However, this discussion is not very relevant for poor smallholder poultry owners, as their production system is unable to bear the full costs of the veterinary services required. For the foreseeable future, it is envisaged that veterinary services to the poor must be subsidised, be it by outside donor funding or by national government veterinarians (FAO, 2011). Another constraint to the delivery of veterinary services is the lack of properly trained and qualified personnel, and the lack of coverage in vast and sparsely populated areas. In order to overcome this situation, paraveterinary personnel, such as veterinary assistants or vaccinators, who have undergone shorter training than vets may need to be employed to make up for the deficits in available veterinarians (Saleque, 2000) or the existing infrastructure could be optimised by the establishment of joint public health facilities, e.g., diagnostic laboratories, serving both the human and animal sectors (Larsson, 2006).

The provision of disease control services has to date dealt with the classical concept of veterinarians fighting disease outbreaks or preventing major diseases by vaccinations. It should be stressed, however, that this approach is not easily adopted by a poor target audience, and it should be further stressed that an important part of disease prevention is based on good management practices that should be an integral part of smallholder poultry development programmes. Further, the discipline of ethnoveterinary medicine based on locally available medicinal plants has as yet only been sporadically researched (Sonayia, 2000). Disease control delivery programmes for smallholder poultry have to date been restricted to vaccination campaigns against Newcastle disease the most important and devastating disease in poultry production, while control

programmes for other poultry diseases remain unattended to. Over the past decade a substantial amount of research has been invested in the development and application of a thermostable vaccine for Newcastle disease (Oakeley, 2009).

2.2.5 Housing, credit facilities and farm equipment availability

The basic requirements of a poultry house are that it provide enough space, protection from weather and predators (dogs, possums, foxes, etc.) and allow for movement of air (Owen *et al.,* 2012). Space requirements depend on the type of chicken such as for egg production, exhibition or meat production (Agbogidi and Okonta, 2011). The mortality rates of chicks brooded in kerosene- and grid-based systems are 7 and 10 per cent, respectively (Owen *et al.,* 2012). Most chick deaths are caused by carbon dioxide or carbon monoxide gas emissions or by outbreaks of fire. The traditional method of leaving chicks to be brooded by their mothers for their first few days of life results in mortality rates of 60 to 70 percent (Aengwanich and Simaraks, 2004).

Despite the acknowledged importance of poultry production, Akanji (2007) opined that it is characterized by low production level due to limited finance for the procurement of basic poultry equipment and materials. This is in line with Hamzat and Babatunde (2003) who opined that small-scale poultry farmers do not have adequate capital and resources to expand their scale of operations. The result of this is that many of the small-scale poultry farmers are not encouraged to increase their productivity. Several farmers are faced with uneasy access to loan and credit procurement (Agbogidi and Okonta, 2011). Adeola and Olukosi (2009) also confirmed that credit facilities or loans from financial institutions are not accessible to the poultry farmers in the rural areas. Market price fluctuation and non availability of land and space are also constraints to increase of poultry production. Adeola and Olukosi (2009) opined that

lack of technical knowledge is a major constraint that militates against poultry production and that mortality was the major constraint to village chicken productivity.

2.2.6 Temperature and heat stress management

Broiler chicken is a homeotherm that can live comfortably only in a relatively narrow zone of thermo-neutrality (Shutze *et al.*, 1996). The changes in environmental temperature below and above the thermal comfort zone have a negative effect on bird performance. Both low and high temperatures act in a negative way (Graczyk, 2003). Low environmental temperature increases feed intake and decreases body weight gain and feed efficiency, thus negatively influencing the performance of broiler chickens. As Cahaner and Leenstra (1992) proved, broiler chickens adapted more easily to lower than to higher temperature. The optimal temperature range for efficient production for broiler chickens over 4 weeks of age is 18 - 21˚C (Aengwanich and Simaraks, 2004). Shutze *et al.* (1996) reported that short-term cold conditioning of chickens at an early age could induce an improvement either in thermo tolerance during cold challenge or in performance of chickens exposed to an optimal environmental temperature.

The increase in energy requirement as a result of exposure to low environmental temperature implies necessary changes in the cardiovascular system to accommodate the energy needs. Thus, increases in haematocrit, haemoglobin concentration, blood volume, liver and heart muscle weight have been observed in broiler chickens and turkeys exposed to low environmental temperature (Yahav, 2002). Yahav *et al.* (1997) reported linear relationship between haematocrit and heart weight under constant temperature. The observed relationship indicates an adaptation of heart mass to the changes in work load associated with the changes in blood flow resistance. Vecerek *et al.* (2002) documented that abdominal fat content was affected by environmental

temperature. In cooler environment, more energy was dissipated as heat and thus fat accumulation was decreased. At three weeks of age, body weight and feed conversion were better for the chicks brooded at warmer temperatures. A point that should be considered is the difference in air temperature and floor temperature.

2.2.7 Concerns about environmental pollution

One of the major challenges of commercial poultry is waste management. Large quantity of poultry waste is inevitably generated on continuous basis in commercial production to the extent of the intensity of operation (Dozier, 2001). The waste, if not managed properly, can be inimical to human health and the water quality. For example, bacteria in poultry wastes can contaminate drinking water and may cause potentially serious illnesses (Graczyk *et al.*, 2003). High level of nitrates (a form of nitrogen that develop naturally in poultry wastes) in drinking water may particularly harm young livestock. Nutrients in poultry waste that enter streams also may lower oxygen levels and kill wildlife.

Odours from commercial poultry operations are repugnant, especially when the waste is not properly managed (Cheng *et al*., 1999). This has been documented to be a major source of conflict within communities where the enterprise is located within or near residential areas (Cheng *et al*., 1999).

2.3 Shea Tree and its Products

One of the key agro forestry species in Africa is the shea butter tree, *Vitellaria paradoxa* syn. *Butyrospermum paradoxum* (Lovett, 2005). It belongs to the family Sapotaceae, order Ebenales which contains 600 species distributed in 50 genera (Leroy, 1982; Guignard, 1986). Shea tree populations are wild and diverse in phenotype and genotype (Fontaine *et al.,* 2004), with two subspecies taxonomically defined viz *paradoxa*

and *nilotica* (Lovett, 2005). Common names include: ‘karite’ (French), ‘ori-oyo’ or ‘ori’ (Yoruba) and ‘kadanya’ (Hausa). It occupies a 500 - 700 km wide and 5000 km long stretch of African savannah from Senegal to Ethiopia (Goreja, 2004). It is localized between the latitudes 9° and 14°N in West Africa, 7° and 12° N in central Africa and 2°N and 8°N in East Africa (Goreja, 2004). This zone corresponds to the wetter central and southern parts of dry countries in Africa (Fig 1) such as Burkina Faso, Mali and Niger and the drier northern parts of wetter countries such as Benin, Cameroon, Côte d'Ivoire, Ghana, Nigeria, Togo and Uganda (Elias and Carney, 2007; Sanou and Lamien, 2011).

Africa processed Shea butter exports increased from 1200MT in 1998 to 3200MT in 2000 (Garba *et al*., 2011). Nigeria is the leading producer of Shea nuts and it is estimated that 355,000MT was produced in 1999 which amounted to 58% of the African production and 414,000MT in 2005 which amounted to 60.5% of the African production.

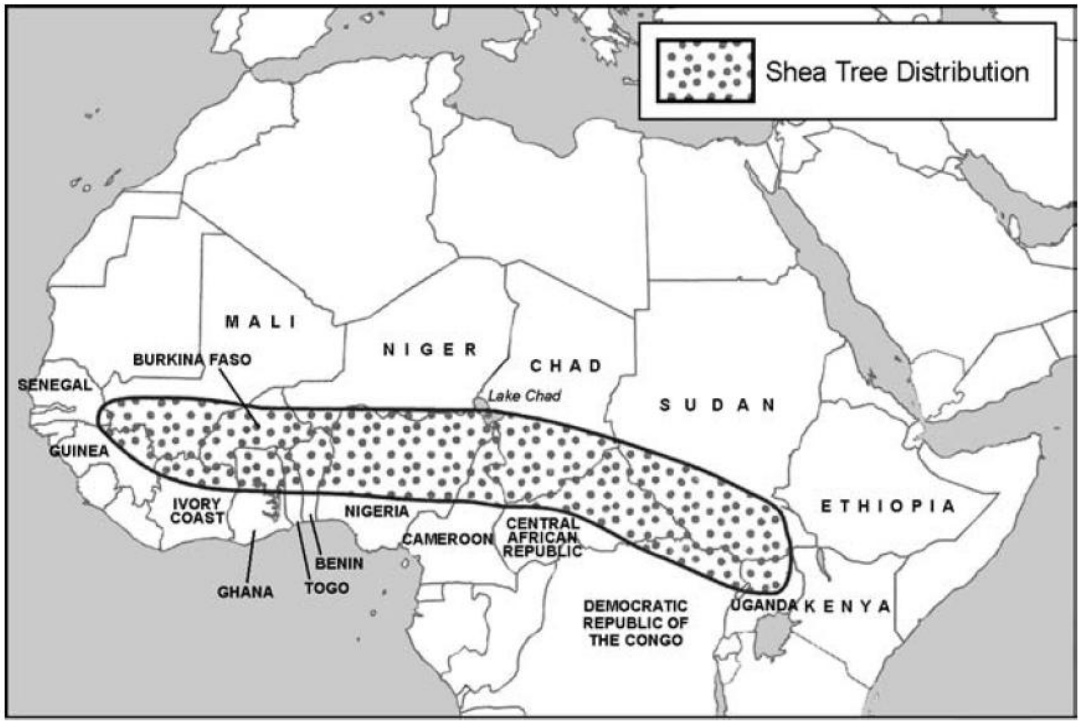


Figure 1: Distribution of shea trees in Africa (Elias and Carney, 2007).

Shea tree can grow up to 12.2 meters or more depending on the protection given to it. The tree bears fruits at maturity and starts flowering by early November. The fruit, which is green in colour has a fleshy edible pulp that contains carbohydrate (41.3 g/100 g), protein (0.7 - 1.3 g), ascorbic acid (196.1 mg/100 g), iron (1.93 g/100g) and calcium (36.4 mg/100 g) (FAO, 2007). On the global scale, the species has made remarkable contributions in the food and cosmetic industries through shea butter (Boffa *et al*., 1996). Locally, *V. paradoxa* is valuable in traditional medicine, provision of fuel wood and in the production of soap, candle and pomade (Awoleye, 1995). Its dietary importance at the local level is derived from the fat extract which is used for cooking as well as the fruit pulp which is consumed by humans and livestock (ICRAF, 2000). The protein-rich caterpillars (*Cirina butyrospermi*) associated with the species are considered a delicacy among Yoruba and Nupe (Ande, 2004), as well as the Tiv ethnic groups in Nigeria (Ugese *et al*., 2005).

2.3.1 Distribution of shea butter tree

Vitellaria paradoxa (C.F. Gaertn) is a small to medium-sized deciduous tree that occurs in a wide swathe of territory above 1° of latitude in tropical Africa (Hall *et al.*, 1996). It is indigenous to the Guinea savannah belt of West Africa, found across 19 African savannah countries, covering an estimated 1 million km^2 between Senegal and north-western Uganda; with rainfall within the 600mm to 1500mm rainfall isohyets from Senegal in the west to Uganda in the east (Salle *et al.*, 1991; Okullo *et al.*, 2004).

According to Okullo *et al.*, (2004), the shea tree occurs mainly on open dry land, covering the Guinea and Sudan savannah areas as well as the lower Sahel regions of the Nigeria. Nigeria with 425,000 MT and Burkina Faso with 85, 000 MT is the leading producers, Ghana; and finally Mali in the near vicinity (FAO, 2005).

Table 2.1: Trends in Shea Nut Production (MT)

Year	Ghana	Nigeria	Cote d'ivore	Togo	Benin	Mail	Burkina Faso	total
1965	36,000	54,000	3,000	1,000	7,000	74,000	74,000	212
1970	44,000	67,000	3,000	3,000	8,000	82,000	82,000	251
1975	48,000	88,000	4,000	5,000	7,000	78,000	78,000	276
1980	44,000	110,000	9,000	9,000	9,000	85,000	85,000	346
1985	55,000	100,000	60,000	20,000	26,000	85,000	85,000	506
1990	52,000	289,000	20,000	6,000	7,000	85,000	85,000	548
1995	52,000	384,000	36,000	8,000	15,000	85,000	85,000	656
2000	65,000	369,000	36,000	8,000	15,000	85,000	85,000	648
2002	65,000	370,000	36,000	8,000	15,000	85,000	85,000	649
2004	60,000	425,000	36,000	8,000	15,000	85,000	85,000	645

Source: FAO (2005)

2.3.2 Shea butter tree

The shea nut tree according to Lovett and Haq (2000) is a multi-purpose tree and highly valued for the oil obtained from the nut. *V. paradoxa* is a foremost economic tree in the *Sapotaceae* family (Maranz *et al.,* 2004).

Horizon, (2010) gave description of *V. paradoxa* as a small to medium-sized tree 7-25m high; much branched, dense, spreading, round to hemispherical crown. Bole is short (3-4 m but exceptionally 8 m), diameter ranging from 0.3-1m, but most frequently 0.6m. Slash pale pink, secreting white latex. Bark conspicuously thick, corky, horizontally and longitudinally deeply fissured, with a life span of about 200 years but needs between 8 and 15 years until the first harvest (Aremu and Nwannewuibe, 2011).

The tree leaves are long, dense clustered and spirally arranged while petioles are 5-15cm long, Juvenile leaves are rust-red and pubescent, later coriaceous, glabrous and dark green, shining, 12-25cm long and 4-7cm wide, leaf margin are wavy and bent. The flowers develop in the axils of scale leaves, at the extremities of dormant twigs, from buds formed 2 years previously. Inflorescence are dense fascicle 5-7.5cm in diameter, at the end of a flowering twig, each usually containing 30-40 flowers, though 80-100 have been recorded. Individual flowers white or creamy-white, about 1.5 cm in diameter and subtended by scarious, brown, ovate or lanceolate bracteoles, which are abscised before flower opening.

The trees fruit at the end of the dry season and are harvested during rainy season between the months of May and August (Okullo *et al.,* 2004). The fruit takes 4-6 months to develop and each tree produces 15-20kg of fruits (Karin, 2004). Fruits grows in bunches, ovoid in shape like an avocado with deep green or brown colour; measuring

about 5-8cm long and 3-4cm wide, elliptic, a yellow-green or yellow berry with thick butter-like, mucous pericarp; generally containing only 1 oval or round red-brown seed (the shea nut), surrounded by a fragile shining shell with a large, round, rough hilum on a broad base. When unripe the shell has an ivory white colour, when it is ripe it has a dark brown colour (Aremu and Nwannewuibe, 2011). The sweet pulp is edible and inside the fruit is a nut surrounded by a thin shell containing a hard kernel and a whitish almond-like nut which form 50% of its weight, called shea butter (Aremu and Nwannewuibe, 2011). The fresh nut size and shape of particular trees are distinctive (Boffa *et al.*, 2004) containing 41% water, 18% residue, 21% oil and 20% husk (USAID, 2007).

The shea tree does not only provides shea fruit, kernels and butter but it is also a source of fuel (charcoal), shade, medicine, traditional apiculture for placing hives and traditional cultural ceremonies (Maranz *et al.*, 2004). Other parts of the tree such as sap, leaves and roots have industrial potential applications (USAID, 2007).

2.3.3 Shea butter oil and its uses

After shelling, the shea kernels are processed into shea oil or butter which is tallow like substances that solidifies at room temperature to form a yellow, cream or grey colour (Adikini, 2002). The shea oil is used as food sauce, cosmetic, soap making and traditional medicine (it is a very beneficial remedy against aches and pains and sores and wounds for which it is applied as an unguent) and is also traded in the international markets as a valuable raw material in the cosmetic, chocolate and pharmaceutical industries (USAID, 2007). It has been reported by many authors including Tallantire and Goode (1995), and FAO (2007) that shea oil comprises fatty acids and vitamins which are essential in the human diet.



Plate I: Shea butter seeds

As a result, Africans have mostly depended on it as their substitute for the valuable dairy butter and natural source of vitamins (FAO 2007).

2.3.4 Shea butter processing/ extraction

The United State Agency for International Development USAID (2007) reported that technologies that have been used for extracting vegetable oil including shea butter included traditional boiling, mechanical pressing and solvent extraction. However, for decades, shea butter has been processed by labour intensive indigenous traditional boiling method which has affected the quality of shea butter oil (Masters and Puga, 1994). Improved methods such as mechanical pressing (both manual and hydraulic) are being adopted in many African countries (FAO, 2007).

2.3.4.1 Traditional boiling extraction

The nuts are mainly collected in the early hours by women and children (Asante, 1987). Matured fruits may also be plucked and buried or kept in air-tight containers for it to ripe after which the fruits are depulped and the nuts boiled and/or sun dried to prevent germination (Esien, 2003). The nuts are roasted at a temperature close to 120^{o}C, roasting is stopped when a deep brown colour is obtained and nut can easily be broken. The roasted nuts are pounded using mortar and pestle to obtain a brown-black paste, the paste is later grinded into paste on a flat stone surface. The paste is beaten using warm water to keep the paste at high temperature, upon through beating the mass is washed once or twice before boiling. To obtain the butter the creamy mass is boiled in a cooking pot. When the women extract shea butter, a maximum of six to twelve kg of kernels is processed at a time. This is because some processing steps need to be done on the same day with limited time intervals.

2.3.4.2 Mechanical pressing extraction

This processing technique mainly involves the crushing of the shea kernel into coarse particle sizes (FAO 2007). The paste is soaked in little hot water before pressing to release the shea butter. The method was developed to improve the efficiency of production of shea butter since traditional boiling method was found to be labour intensive and wasteful (Masters and Puga, 1994).

2.3.4.3 Solvent extraction

Solvent system is mainly used in laboratory experiment although it is sometimes used for commercial extraction in developed countries. According to FAO (2007), this method is not usually used in domestic and commercial shea butter extraction due to the high costs involved, environmental problems and lack of technical skills in developing countries.

2.4 Chemical and Nutritional Composition of Shea Butter Fruit and Shea Butter

Shea butter is a slightly yellowish or ivory-coloured vegetable fat extracted from the kernels of the fruit of *V. paradoxa* (Honfo *et al.*, 2011).

2.4.1 Physico-chemical composition of shea oil

Colour, refractive index and viscosity play an important role in determining the quality of any oil because they give physical specification for description of the oil. While the colour of oil comes from natural colouring matters from α- and β-carotene, xanthophyll and chlorophyll, the refractive index is ratio of light in vacuum to speed of light in the oil under examination and the viscosity is material friction acting within the oil. These are parameters for characterization of oils and assessment of the level of

purity. Shea butter have been reported to have a pale yellow, cream or grey colour solid at room temperature and refractive index of 1.467 (Adikini, 2002). However, a report by Stryer (1988) indicated that variation in the physical parameters of vegetable oils might be associated with changes in the un-saturated fatty acids due to oxidation, polymerization and isomerisation.

2.4.2 Chemical parameters

Wiesman *et al.* (2003) reported Shea butter to contain high levels of UV-B absorbing triterpene esters, including cinnamic acid, tocopherols (vitamin A), and phytosterols (campesterol, stigmasterol, beta-sitosterol and alpha-spinosterol). They also showed that Shea butter does contain a high percentage of unsaponifiables, such as triterpenes (cinnamic acid esters, alpha- and beta-amyrin, parkeol, buytospermol, and lupeol and hydrocarbons such as karitene).

Acid value is a measure of acid hydrolysis that has occurred in the oil or fat, peroxide value is used to quantify the primary oxidative products in the oil due to substantial amount of unsaturated fatty acids (Pearson, 1991). According to Wiesman *et al.* (2003) draft standard for shea butter, the shea butter acid value is less than 6.0 mg/KOH/kg, peroxide value is less than 10 mEq/kg, saponification value is between 170-190 mgKOH/g and iodine value, $40I_2$/100g. Although Adikini (2002) reported values within the range of USAID (2007) for shea butter samples from Uganda, higher values were reported by Kapseu *et al.* (2007) and Tano-Debrah and Yoshiyki (1994) for West African shea butter samples. These variations in chemical parameters were attributed to processing, fruit harvesting and kernel storage methods (USAID, 2007).

According to Maranz and Weisman (2004), tocopherols are also chemical parameters that are important in nutrition and cosmetic properties of oils because they act as good anti-oxidants. Their presence also causes oxidative stability of the oil. Although shea butter has been reported to have over 800mg/100g of α-tocopherol in West African shea butter, the shea butter from Uganda has 29mg/100g- the lowest in Africa (Maranz and Weisman, 2004). This variation in the α-tocopherol values was associated with environmental conditions. It should be noted, however, that low level of tocopherols can result in a serious decrease in the protective power of any oil against auto-oxidation (Choe and Min, 2006).

2.4.3 Proximate composition of shea fruit pulp

Fruits are generally poor sources of proteins and oil; they contain reasonable amounts of carbohydrates, fibre, minerals and vitamins C (Pearson, 1991). According to CRIG (2002) the shea fruit is a seasonal source of calories in the sub Saharan Africa with edible pulp containing 41.2g of carbohydrates, 0.7-1.3% proteins and 196mg/100g vitamin C (CRIG, 2002). A report by Cheng *et al.* (1999) indicated that while carbohydrates are good sources of energy, proteins can catalyze, regulate, protect and provide energy. Vitamin C on the other hand is essential for normal growth and development of the human body.

Minerals can be classified as either macro or micro. The macro minerals consists of calcium (Ca), sodium (Na), potassium (K) and magnesium (Mg) while micro minerals are iron (Fe), zinc (Zn), selenium (Se), copper (Cu) and many others. In Ghana, the shea fruit pulp has been reported to contain Ca (36mg/100g), Mg (26mg/100g) and iron (1-3g/100g) and others in small quantities (FAO, 2007). According to Maranz *et al.* (2004) the most abundant mineral in shea fruit is potassium

with values as high as 130mg/100g in West African countries and 400mg/100g in Uganda. Even other minerals were reported by Maranz *et al.* (2004) to be higher than those reported by CRIG (2002) and FAO (2007). These varying mineral compositions across Africa were found to be due to varying environmental conditions.

2.5 Anti-nutritional Factors in Shea Butter Cake

Anti-nutritional factors (ANFS) may be defined as those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms (e.g. inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed) which exert effects contrary to optimum nutrition (Kumar, 1992). Anti-nutritional factors are present in different food substances in varying amounts, depending on the kind of food, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances (Inuwa *et al.*, 2011).

2.5.1 Oxalate

The name oxalate is attributed to its occurrence in plant oxalis (Wood sorel). The earliest interest in the toxicity of oxalate arose because of instances of severe or fatal human poisoning following the eating of large quantity of the leaves of certain plants such as rhubarb, known to contain relatively large amounts of oxalates (Osagie, 1998).

2.5.2 Phytate

Phytic acid (Inositol hexaphosphoric acid) forms insoluble salts with essential minerals like calcium, iron, magnesium and zinc in food rendering them unavailable for absorption into the blood stream (Bingham, 2006). About half of the phytic acid

phosphorus content taken by man is excreted unchanged. The remaining unavailable or utilization, the knowledge of phytate content can be made use of in calculating the quantity of phosphorus available out of the total phosphorus in a diet (Robert and Yudkin, 1999). Phytate (*myo*-inositol hexakisphosphate) is the common storage form of phosphorus in plant seeds and cereal grains (Reddy *et al.*, 1992). Phytate is considered to be an anti-nutritional factor for humans and animals because of its high chelating ability with cations and complex formation with the basic amino acid group of proteins, thus decreasing the dietary bioavailability of these nutrients (Martinez *et al.*, 1996). Phytate is not metabolized by monogastric animals, which have low levels of phytate-hydrolyzing enzymes in their digestive tracts. These un-metabolized phytates pass through the intestinal tract and are excreted outside and caused environmental problems by eutrophication of surface water resources (Raboy, 2001).

2.5.3 Trypsin Inhibitor

The presence of trypsin inhibitors in the diet leads to the formation of irreversible condition known as enzyme-trypsin inhibitor complex. This causes a drop in intestine trypsin and a decrease in protein digestibility, leading to slower animal growth (Bolhuis, 2003).

2.5.4 Cyanogenic Glycoside

Cyanogenic glycosides (cyanide) being an effective cytochrome oxidase inhibitor interferes with aerobic respiratory system. Hydrocyanic acid does not occur free, but combines with sugar to form a non-toxic compound known as cyanogenic glycoside (Bolhuis, 2003). Respiratory chain at the cytochrome oxidase level is lost

during soaking and cooking so that its content in vegetables and fruits poses no danger of toxicity (Bolhuis, 2003).

2.5.5 Saponins

Chemically, saponin consists of an aglycone of either steroidal or triterpenoid in nature and one or more glycosides (sugar chains) and occurs in plants that are consumed by humans and animals (EFSA, 2009). It has been reported to reduce feed intake, inhibit growth rate of swine and poultry and show toxicological effects at higher levels in the diet (Livingston *et al.*, 1977). Saponins cause injury to the digestive mucosa and haemolysis in the blood, when fed to non-ruminants at higher levels. It inhibits smooth muscle activities, slows down digesta flow and causes membranous irritations of mouth and the digestive tract (McDonald *et al.,* 1995) as well as hypocholesterolaemia by binding cholesterol, making it unavailable for absorption. They also cause haemolysis of red blood cells and are toxic (Johnson *et al.,* 1986).

In mice and rats, saponin cause local gastro-intestinal toxicity as well as liver and kidney toxicity (EFSA, 2009). It further reduces the activities of digestive enzymes in non-ruminants (Livingston *et al.,* 1977; Longstaff and McNab, 1991). Saponins bind with protein and form complex bonds. Thus, enzymes of protein origin can form bonds with these components and reduce their activities resulting in abdominal disorders. Saponin has the potential as dietary additive at optimum levels in diets favouring high growth rate and better feed efficiency (Yejuman *et al.*, 1998), lower serum cholesterol level (Udea and Shigemizu, 1998) and reduces the emission of ammonia in animals' excreta. According to EFSA (2009) ruminants are more tolerant to saponins than monogastric animals, and can tolerate inclusion levels up to a maximum of 20% of high saponin containing feed ingredient (like *Madhuca mongifolia* seed cake) in the diet.

Among the better-known biological effects of saponins is their capacity to cause lysis of erythrocytes (Khalil and El-Adawy, 1994) and to make the intestinal mucous membrane permeable (Johnson *et al.,* 1986).

2.5.6 Tannins

Tannins are made up of complex phenolic polymers which are classified into two structural groups; hydrolysable and condensed tannins inhibit the activities of some enzymes such as trypsin, chymotrypsin, amylase and lipase. Generally tannin induces negative responses such as astringency, bitterness or unpleasant taste when consumed (Brown, 2008). In poultry small quantities of tannin causes adverse effects. Levels from 0.5 to 2.0 % can cause depression in growth rate and egg production while levels from 3 to 7% can cause death. Hydrolysable tannins are toxic to ruminants when fed at higher levels above 20% (Brown, 2008). Tannin is reported to reduce absorption of minerals (Garrow and James, 1993). Tannins also interfere with dietary iron absorption (Rao and Desothale, 1998). This occurs as a result of formation of complexes with iron which form linkages with proteins during mastication and appears to reduce microbial degradation of protein (Guthrie and Picciano, 1995). The addition of more proteins or amino acids may alleviate the anti-nutritional effects of tannins. Also, moderate levels of tannins (less than 4%) in forage legumes can lead to beneficial response in ruminants resulting in higher growth rate and milk yield (Brown, 2008). This effect may be due to reduction in the digestibility of dietary protein and to a lesser extent that of available carbohydrates and lipids (Brown, 2008). Other adverse nutritional effects of tannins include intestinal damage, interference with iron absorption and the possibility of tannins producing a carcinogenic effect (Griffiths, 2000). Tannins cause decreased feed consumption in animals, bind dietary protein and digestive enzymes to form complexes

that are not readily digestible (Aletor, 2003), decreased palatability and reduced growth rate.

2.6 Nutrition and Feed Management

Nutrition has a main role in the success of poultry industry (Mahmood *et al.,* 2012). The general objective of broiler nutrition is to maximise the economic production performance of broilers. Diets are usually compounded from cereals, such as wheat and maize, as the principal source of energy and protein rich ingredients such as oilseed meals, pulses and animal proteins (Waldroup *et al.,* 2005). Fats or oils are added as additional sources of energy and diets are routinely supplemented with a range of additives, including minerals, vitamins, amino acids, growth promoters, enzymes and medications (Mahmood *et al.,* 2012).

The main components of diets are usually added in ground or crumble form but after the diets have been mixed, they are formed into pellets. Birds are able to discriminate between food sources and when offered a choice between feeds can select a mixture of the major nutrients, such as energy and protein that is broadly appropriate for their individual needs (Mahmood *et al.,* 2012).

2.6.1 Amino Acids

Proteins are made up of amino acids, which perform different functions such as the biosynthesis of tissues and animal products. It is amongst the most expensive nutrient of the feed and broilers have high dietary crude protein requirements (Khan and Zafar, 2005). It is generally accepted that the CP in the diet should represent a balance of essential (EAA) or indispensable and nonessential (NEAA) or dispensable amino acids supporting the needs for maintenance and the tissue accretion (Waldroup *et al.,*

2005). In poultry, 22 amino acids are needed to form body protein, some of which can be synthesized by the bird (non-essential), whereas others can not be made at all or in sufficient quantities to meet metabolic needs (essential). Essential amino acids must be supplied by the diet, and sufficient amount of non-essential amino acids must also be supplied to prevent the conversion of essential amino acids into non-essential amino acids. Additionally, if the amino acids supplied are not in the proper, or ideal, ratio in relation to the needs of the animal, then amino acids in excess of the least limiting amino acid will be de-aminated and likely used as a source of energy rather than towards body protein synthesis. Although all amino acids are equally important and none can be stated as unimportant, yet some being relatively more indispensable than others are known as critical amino acids viz methionine, threonine, lysine and tryptophan (Khan and Zafar, 2005).

2.6.1.1 Methionine

Methionine is universally recognized as the first limited amino acid in broiler chicken's diets based on corn and soybean meal. This serve to improve energy utilization in the body (Brody, 1994) because supplementing methionine resulted in lowering feed conversion ratio while at the same time elevating abdominal fat content. The influence of methionine in broiler diet also serves to improve energy utilisation in the body (Brody, 1994). Methionine may acts as lipotrophic agent through its role as an amino acid in balancing protein or through its role as a methyl. It is showed that methionine as lipotrophic agent is important in diet protein regulation (Adikini, 2002). Reports indicate that the NRC (1994) recommendation of 0.5% and 0.38% methionine for the age of 0 to 3 wk and 3 to 6 wk is too low for obtaining maximum broiler performance (Jahan *et al.*, 2006). Kidd *et al.* (2004) presented data showing improved performance when methionine was added to a corn and soy-bean broiler diet.

Hesabi *et al.* (2009) reported that Supplemental methionine (110 % of NRC recommendation) decreased (P<0.01) proportion of breast and significantly increased thigh to carcass ratio, abdominal fat pad while some research demonstrated supplemental methionine increases breast muscle (Acar *et al.,* 2001). Sufficient intake of dietary methionine is important for the synthesis of proteins (Acar *et al.,* 2001). It may influence the carcass characteristics and visceral organs. Beside the generation of decarboxylated S-adenosylmethionine, methionine is a donor of the methyl groups that participates in the methylation of DNA and proteins, the synthesis of spermidine and spermine and regulation of gene expression (Hesabi *et al.,* 2009).

2.6.1.2 Tryptophan

Tryptophan (Trp) is an essential dietary amino acid for chickens that is necessary for maximum growth and feed efficiency. Trp is equally second or third limiting in typical diets for hens. In diets consisting primarily of corn, it is the first limiting amino acid because of the low Trp content and availability in corn (Bayala, 2005). Ohtani *et al.* (1989) and Jensen *et al.* (1990) suggested that supplemental L-tryptophan improves the egg production rate in laying hens. Trp plays an important role in the brain as a precursor of the neurotransmitter serotonin, which has a major effect on the feeding behaviour of animals among its many functions (Mullen and Martin, 1992). The Trp content in the diet is important for optimal growth performance, and reliable requirement estimates are imperative when formulating diets based on the ideal protein concept (Bayala, 2005). Trp has also been linked with niacin biosynthesis in chickens (Corzo *et al.,* 2005). In general, cereal feedstuffs such as corn, wheat and barley are relatively low in tryptophan (Ryozo and Makoto, 2007).

2.6.1.3 Threonine

Threonine (Thr) is also a major component of plasma γ-globulin in animals. Thr often appears as the third limiting amino acids, although Kidd (2000) argued that there is no clear special need of Thr for immunity in poultry. They also reported that Thr intake also influences components of the immune system while on the contrary Baylan (2006) reported that L-threonine is added to the diet of pigs and poultry in order to exactly match the dietary amino acid balance with the unique nutritional requirements of the animal. Thr is considered to be the third limiting amino acid for broiler chicks fed low protein corn-soybean meal diets (Kidd *et al.*, 2004). Adequate digestible threonine levels are needed to support optimum growth (Kidd *et al.*, 2004) because it serves as important component of body protein and plays an important role as precursor of lysine and serine (Ojano and Waldroup, 2002). Threonine is needed for optimal immune response and gastrointestinal mucine production. It has been reported to improve the liveability of heat stressed broilers (Kidd, 2000). Increased dietary threonine concentration is known to improve nitrogen retention in broiler chicks; therefore changing level of threonine concentration is an important tool to improve nitrogen utilization (Dozier, 2001).

2.6.1.4 Lysine

Lysine is one of the limiting amino acids in broiler diets. The proportion of lysine to all other EAA is of great concern for optimal performance of broilers (Corzo *et al.*, 2002). Increasing dietary lysine levels has been reported to increase carcass protein retention (Sibbald and Wolynetz, 1986). Lysine requirements of broilers are higher in low protein diets for maximum weight gain and feed efficiency. The NRC (1989) recommendation indicated optimum dietary lysine to protein ratio of 0.052, 0.050 and

0.047 during starter, grower and finisher phases, respectively. Nagalakshmi *et al.* (2007) showed dietary lysine to crude protein ratios of 0.066 and 0.077 supported optimum growth rate and feed conversion and they showed the ratio for optimum breast meat yield was lower than for optimum growth rate and feed conversion ratio. Bedford and Summers (1985) showed the increasing lysine to protein ratio in the diet will increase carcass protein and decrease carcass fat. Higher and lower optimum dietary lysine to crude protein ratios have been reported by some workers such as Han *et al.* (1992); Kerr *et al.* (1999); Rezaei *et al.* (2004). Gholam-Reza and Abdolhossein (2013) indicated that optimum dietary lysine to crude protein ratio during the starter phase was 0.062 and 0.69 based on linear broken line and quadratic broken line, receptivity. These values are higher than 0/049 estimated by the NRC (1994) for starter phase, a ratio that is higher than the Han *et al.* (1992) and Rezaie *et al.* (2004) for broiler chickens between three and six weeks old.

2.6.1.5 Non-essential amino acids

Waldroup *et al.* (2005) reported that addition of Non Essential Amino Acid (NEAA) mixture to provide N equivalent to that from EAA was of no apparent benefit when added to diets with 20% CP but a significant improvement when added to diets with 18%. They concluded that the reduced growth associated with the three low CP diets was not due to insufficient non-essential amino nitrogen.

2.6.2 Trace elements

It is widely established that a deficient as well as excessive intake of trace elements could cause an oxidative stress in poultry (Surai, 2002), but the negative consequences of oxidative stress can be reduced by the adequate supplementation of antioxidants into the diet. Cu, Zn, Fe, Mn and Se are considered as antioxidant nutrients.

Fe is a part of catalase, Cu, Zn and Mn are parts of superoxide dismutase and Se is a part of glutathione peroxidase. They all have interdependent roles in the antioxidant protection of organism by the termination of Lacto peroxidase (LPO) reactions (Steiner and Scheidegger, 2007). On the contrary, the same elements as the transitional metal ions have the ability to generate highly reactive hydroxyl radicals through their interactions with reactive oxygen species (Aruoma *et al.*, 1991).

Trace elements play important roles in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living cells of plants, animals and humans (Alhassan *et al.,* 2011). Nollet *et al.* (2008) found no significant differences in the body weight between the groups of broilers fed the diets supplemented with 100, 67, 50, 33 and 17% of recommended dose of the organic form of minerals (Bioplex Cu, Bioplex Fe, Bioplex Zn, Bioplex Mn). The addition of trace elements (Cu, Fe, Zn, Mn and Se) in the organic form into the diet could be reduced to 33% of regular levels with comparison to the supplementation of their inorganic salts, without compromising the body weight of broilers (Nollet *et al.,* 2007). According to Chinajariyawong, (2011), Na and K are very important in water balance and normal functioning of the nerves and muscles and absorption of glucose and glycogen while Ca plays a very important function in bones, nervous system, stimulates some hormonal secretions, and activates some enzymes and blood coagulation. Magnesium on the other hand can assist enzymes involved in the synthesis and breakdown of carbohydrates, fats, proteins and synthesis of DNA and RNA while Fe is a constituent of haemoglobin, myoglobin and a number of many enzymes (Yahav *et al.,* 1997).

2.6.2.1 Effect of trace elements and vitamins on feed intake and utilization

The balance between calcium (Ca) and Phosphorus (P) is also important and the normal contents of starter diets are about 10 g calcium and 4.5 g available phosphorus / kg that is approximate ratio of 2:1 (Durmaz and Gopkinar, 2006). Deficiencies or imbalances of these nutrients can have severe effects on the bone quality and welfare of broilers. The main consequence is rickets, either of the calcium deficiency or phosphorus deficiency type. This occurs when the diet content of either nutrient is too low, or the diet content of one is too high and induces a deficiency of the other (Durmaz and Gopkinar, 2006). Tibial dyschondroplasia (TD) is another consequence of an imbalance when the calcium:phosphorus ratio falls below the optimum (Edwards and Veltmann, 1983).

Phosphorus is the third most expensive component of poultry diets after energy and amino acids (Woyengo and Nyachoti, 2011). However, approximately two-thirds of P in vegetable feedstuffs is poorly digested by poultry because it is bound to phytic acid (PA), which is poorly hydrolyzed by poultry, as they do not produce sufficient amounts of phytase enzyme that hydrolyses PA (Bedford, 2000). Phytic acid is a constituent of plant seeds in which it serves as the major storage form of P (Maenz, 2001). Phytic acid occurs as phytate in spherical inclusions called globoids, which are located within protein bodies (Lin *et al.,* 2005) in cereal grains, grain legumes and oilseeds that constitute the majority of ingredients in poultry feeds. In soyabean, however, most of the phytate (85 to 90%) in protein bodies does not occur as spherical inclusions; it interacts directly with the protein (Prattley and Stanely 1982). Phytic acid is not uniformly distributed in plant seeds, but localized within certain seed components. In wheat (Joyce *et al.,* 2005) and barley (Ockenden *et al.,* 2004) reported that phytate is

concentrated in the aleurone cells, whereas in corn (Lin *et al*., 2005) and oilseeds like soyabean (Prattley and Stanely 1982) and canola (Yiu *et al.,* 1982) it is concentrated in the embryonic cells. Its concentration in various feed ingredients that are commonly used in poultry feeds also varies depending on several factors, including type and variety of ingredient, and year of harvesting (Steiner and Scheidegger, 2007). Rutherfurd *et al.* (2004) and Cowieson *et al.* (2006) found digestibility of PA bound P in broilers to be as low as 10%. The unabsorbed P is excreted via faeces, which can lead to environmental pollution (Toth *et al.,* 2006).

Vitamin A, riboflavin and B_{12} are usually low in poultry diets. Inclusion of multivitamin and enzyme in the formulated diet has become an indispensable practice because blending of feed ingredients alone may not ensure all essential vitamins at the right amounts needed for chicken. Some important vitamins like vitamin A, D_3, E, folic acid, pantothenic acid, pyridoxine and riboflavin etc. should be checked carefully in the diet. As the ultimate goal of broiler is to produce more meat within a short period of time, additional multivitamin and enzyme supplement with commercial feed may increase meat production more rapidly. Enzymes and multivitamins prevent deficiency diseases and stimulate growth rate. Beside this, enzyme supplements along with multivitamins reduce mortality, keep birds healthy, increase feed intake, improve digestion and feed conversion rate. Poultry feeds formulated from cereals and their by products contain non-starch polysaccharides (NPS) such as cellulose, xylose, arabinose and galactonic acid which are not easily digested by poultry. Most of the feed ingredients contain some anti-nutritional factors and indigestible part, which hinders feed utilization and bird's performance. These adverse effects may be overcome by supplementation of exogenous enzymes which have been shown to lower viscosity of

intestinal contents and to improve digestibility of starch, protein, fat and apparent metabolisable energy (AME) in broilers fed on diets containing wheat (Bedford, 1995). The dietary administration of enzyme preparations is capable of partly hydrolyzing pentosans and betaglucans and gave significant improvements in performance of broilers fed on different cereal based diets.

2.7 Haematological Characteristics and their Implication in Poultry Production

Haematological profile of animals is an important indicator of physiological or patho-physiological status of the body (Khan and Zafar, 2005). Avian blood differs in cells' characteristics from their mammalian counterparts (Smith *et al.*, 2000). Several factors including physiological (Alodan and Mashaly, 1999) and environmental conditions (Graczyk *et al.,* 2003), diet contents (Kurtoglu *et al.,* 2005), water and feed restriction (Iheukwumere and Herbert, 2003), fasting (Lamosova *et al.*, 2004), age (Seiser *et al*., 2000), administration of drugs, anti-aflatoxin premixes and continuous supplementations of vitamin E (Tras *et al.,* 2000) affect the blood profiles of healthy birds.

Zanu *et al.* (2012) pointed out that haematological analyses are among the methods which may contribute to the detection of some changes in health status and can be a useful aid for diagnosis of diseases in birds. Analysis of normal haematological parameters of chickens is very much essential in diagnosing the various pathological and metabolic disorders and can be used as a tool to assess the health status of an individual (Afolabi *et al.,* 2011). Changes in the haematological parameters are often used to determine various statuses of the body and stresses due to environmental, nutritional and/or pathological factors. Measurement of haematological parameters

provides valuable information in this regards and routinely used in prescribing animal's medicines (Kral and Suchy, 2000).

Esonu *et al.* (2001) had stated that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding. Islam *et al.* (2004) have observed the dependence of blood protein and creatinine on the quality of dietary protein. Talebi *et al.* (2005) reported that with increasing age, the haematological values such as RBC, PCV and Hb contents were significantly increased ($P<0.05$), while erythrocytic indices such as MCV and MCH contents were significantly decreased ($P<0.05$). Avian species are also significantly influenced by poultry diseases including fowl typhoid (Kokosharov and Todorova, 1987), mycoplasmosis (Branton *et al*., 2003), avian coccidiosis (Koinarski *et al*., 2001), infectious bursal disease (Juranova *et al*., 2001), Newcastle disease (Galindo-Muniz *et al*., 2001) and toxoplasmosis (Kaneto *et al*., 1997).

2.8 Use of Shea Butter By-product as Feedstuff

Okai and Bonsi (1989) reported a decrease in feed cost when increasing levels (0, 5, 10 and 15%) of SBC were fed to growing gilts. They found that inclusion of SBC significantly ($P<0.05$) depressed growth rate and feed conversion efficiency but did not affect intake. However, there was a slight decrease (2%) in daily feed intake as the level of SBC increased in the diet. Weight gains of 0.057, 0.048, 0.050 and 0.035 kg/pig/day were recorded for the various levels of SBC (0%, 5%, 10% and 15% respectively) at the end of ten weeks feeding period. The 15% SBC inclusion level recorded the lowest rate of growth and was significantly different ($P<0.05$) from the rest (Okai and Bonsi, 1989). Rhule (1995) stated that, SBC can be fed to grower and finisher pigs at a level of 5% and 10% respectively without any adverse effects on daily weight gain. This was based

on results from pigs fed diets containing 0%, 5%, 10%, 15% and 20% inclusion levels of SBC. The respective average daily gain during the grower phase was 0.05, 0.04, 0.03 and 0.02 kg/day; that of the finisher phase was 0.05, 0.05, 0.05, 0.04 and 0.04 kg/day respectively. The gain of grower pigs on the control diet (0% SBC) was similar to that of 5% SBC diet but differed from levels above 5% SBC level. SBC therefore has been recommended as a potential feed ingredient for pigs at 5-10% inclusion level.

In poultry, Atuahene (1998) stated that SBC could be incorporated in the diet of growing broilers up to 2.5% without adverse effects on performance. The report added that inclusion level above 2.5% (5% and 7.5%) significantly ($P<0.01$) decreased weight gain and that there was a decreased intake as SBC levels increased and consequently a decrease in weight. Olorede and Longe (1999) stated that, pullets could take up to 10% SNC inclusion level in the diet, higher than the 2.5% SBC inclusion level reported by Atuahene (1998). Both authors however agreed that higher inclusion levels of SBC in the diet of poultry birds depress intake and growth rate.

In ruminants, according to Esien (2003), SBC can be incorporated in the diet of sheep up to 50% with wheat or rice bran and will be accepted by the sheep. Salifu (2004) reported that, shea nut cake may be added to the diet of sheep up to 15% without adverse effects on their performance. This was concluded from a study of sheep response to SBC-based diet at 0%, 15% and 30% inclusion levels to form diet 1, 2 and 3 respectively. The results on weight gain were 73.75 g/day, 60.18 g/day and 38.93 g/day for diets, 1, 2 and 3 respectively and there was no significant difference ($P<0.05$) between diets 1 and 2, but diet 3 differed significantly. Issaka (2006) repeated this work at a different location and had similar trends. It was also reported that the dry matter intake increased at 15% inclusion level and decreased at 30% inclusion level with feed conversion efficiencies of 22.36, 17.24 and 10.7 for diets 1, 2 and 3 respectively (0%,

15% and 30% SBC. Shea butter cake can therefore be incorporated into the diet of sheep up to 15% to lower cost and maximize profit (Issaka, 2006). Contrary to this, Annoh (2004) reported no significant difference in feed intake and weight gain among sheep fed three different levels of SBC (0%, 15% and 30% representing diets 1, 2 and 3 respectively) when he added cassava chippings into diet 3, which reduced the concentration of ether extract in the diet. He concluded that SBC can be included in the diet of sheep up to 30% when incorporated with other ingredients that have little or no ether extract such as rice straw and cassava chippings. The potential growth rate of the Djallonke sheep, can be as high as 80 g/day if the diet is adequately supplemented (Berger, 1983). Ngwa and Tawah (1991) reported that supplementation of Djallonke sheep of an average weight of 22 kg with rice straw, groundnut haulms, cotton seed and cowpea vines, recorded average daily gain of 29.24 g, 48.98 g, 52.4 g and 49.19 g/day respectively. An average daily weight gain between 87.0 - 130 g were reported when hybrid sheep developed from Djallonke and Sahalian crosses were fed with cassava peels and pigeon pea waste (Ngwa and Tawah, 1991).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Source of Shea Butter Seed Cake and Day Old Chicks

Shea Butter Cake was obtained from a local shea butter processing factory in Zaki-Biam, Ukum Local Government Area, Benue State, Nigeria. The shea butter cake was air dried for 7 days and then pulverized into powdery form.

Day old Ross 308 broiler chicks were purchased from Zartech® Farm Chicks' depot in Kaduna, Kaduna state. Feed ingredients such as maize, salt, vitamin-mineral premix, groundnut cake, bone meal, and limestone were bought from Samaru and Funtua markets in Kaduna and Katsina states respectively.

3.2 Determination of Proximate Composition of Shea Butter Cake

The analyses were carried out in the Biochemical Laboratory of the Department of Animal Science, Ahmadu Bello University (ABU), Zaria. All the proximate analyses were carried out in duplicate according to AOAC (2006) procedure and the results expressed as percentage of the sample analyzed. The metabolisable energy (M.E) was estimated using Pauzenga (1985) formula:

ME = 35 x CP% + 81.8 x EE% + 35.5 x NFE.

Where:

ME= Metabolisable Energy

CP= Crude Protein

EE= Ether extract

NFE= Nitrogen Free Extract

3.2.1 Determination of moisture content

A clean crucible was dried to a constant weight in an air oven at 110^{o}C, cooled in a dessicator and weighed (W_1). Two grams of finely pulverised sample was weighed into the crucible and then re-weighed (W_2). The crucible and its content were dried in an oven to a constant weight (W_3). The percentage moisture was calculated thus:

% Moisture content = $\{(W_2-W_3)/(W_2-W_1)\}$ x 100%

3.2.2 Determination of ash content

A porcelain crucible was dried in an oven at 100^{o}C for 10 minutes, cooled in a dessicator and weighed (W_1). Two grams of finely pulverised sample was weighed (W_2) into the previously weighed clean crucible which was ignited in the muffle furnace at 550^{o}C for 1 hour and cooled in a dessicator. The crucible and its content were then transferred into the muffle furnace and the temperature was gradually increased until it reached 550^{o}C, the sample was left in the furnace for 8 hours to ensure proper ashing. The crucible containing the ash was allowed to cool to 200^{o}C, the crucible was then removed and cooled in a desiccator until constant weight is obtained (W_3).

% Ash content = $\{(W_2-W_3) / (W_2- W_1)\}$ x 100

3.2.3 Determination of crude lipid content

Four grams of sample was weighed (W_1) into an already weighed clean, dried 500ml round bottom flask containing few anti-bumping granules (W_2) and 300ml of petroleum ether (40-60^{o}C) for the extraction was poured into the flask fitted with soxhlet extraction unit. The round bottom flask and a condenser were connected to the soxhlet

extractor, and cold water circulation was turned on. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 hours. The solvent was recovered and the oil was dried in the oven at 70^{o}c for 1 hour. The round bottom flask and oil were cooled and then weighed (W_3).

% Crude Lipid Content = {(W_2-W_3)/ W_2-W_1} x 100

3.2.4 Determination of crude fibre

Two grams of the defatted sample was weighed (W) into a dry 600cm^3 round bottom flask. 100cm^3 of (0.023M) sulphuric acid was added and the mixture boiled under reflux for 30 minutes. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it is acid free. This was quantitatively transferred into the flask and 100cm^3 of hot (0.312) sodium hydroxide solution was added and the mixture boiled under reflux for 30 minutes and quickly filtered under suction. The insoluble residue was washed with boiling water until it was base free. It was dried to constant weight in the oven set at 100^{o}C. It was then cooled in a dessicator and weighed (W_1). The weighed residue was then be incinerated in a muffle furnace at 550^{o}C for 2 hours, cooled in a dessicator and reweighed (W_2).

The loss in weight on ashing (incineration) = W_1-W_2

Weight of original sample = W

% Crude Fibre = {(W_1-W_2)/W} x 100

3.2.5 Determination of prude protein by Kjeldahl method.

Two gram of the sample was weighed into 100cm^3 Kjeldahl digestion flask and about 1g of catalyst mixture (K_2SO_4 and $CuSO_4$) was added to speed up the reaction. 25ml of concentrated sulphuric acid was added into the flask. The content in the Kjeldahl digestion flask was heated slowly at first in Kjeldahl heating unit frotting subsides and then more vigorously with occasional rotation of the flask to ensure even digestion and avoid over heating of the content. The heating continued until a clear solution was obtained.

After cooling, the solution was transferred into a 100 cm^3 volumetric flask and diluted to mark with distilled water. Ten aliquot of the diluted solution or digest was pipetted into Markham semi macro nitrogen steel and 10cm^3 of 4% sodium hydroxide solution was added. The liberated ammonia was trapped in a 100cm^3 conical flask containing 10cm^3 of 40% boric acid and 2 drops of methyl red indicator. Distillation was allowed to continue until pink colour of the indicator turns green. The content of the conical flask was titrated with 0.1M HCl, with end point indicated by a change from green to pink colour. The volume of the acid used for the distillate as well as the blank was noted.

% Nitrogen = {(0.014 x M x (V_1-V_0))}/ weight of test sample} x 100

Where M= actual molarity of acid,

V_1 = volume of HCl required for 10ml sample solution,

V_0 = volume of HCl required for the blank

Atomic weight of nitrogen = 0.014

Crude Protein = % Nitrogen (N_2) X 6.25

3.2.6 Determination of nitrogen free extract (NFE) or carbohydrate

The total carbohydrate content was determined by difference method. The sum of the percentage moisture, % ash, % crude lipid, % crude protein and % crude fibre was subtracted from 100 (Pauzenga, 1985)

NFE= 100- (crude protein + crude fibre + crude lipid + ash)

3.3 Determination of Anti-nutrient Contents

3.3.1 Determination of hydrogen cyanide (HCN) (Alkaline titration method)

One gram of dried shea butter cake in duplicate was added to 200ml distilled water for 2hr to collect 150cm^3 of distillate. The volume of a 2.5% Volume of 2.5% NaOH was made up to 250cm^3 by adding water to the distillate. Part of the distillate sample (100cm3) was added 8.0cm^3 of 6M NH_4OH solution and 2.0cm^3 of 5% KI, which was then titrated against 0.02M $AgNO_3$ solution using a 10cm^3 micro-burette. The end-point was noted as a permanent turbidity against a black background. Titre values were obtained and cyanogenic glycoside contents calculated using the formula.

Cyanogenic glycoside mg/100g = (TV X 1.08 X EV)/ SM X AL

Where:

TV = Titre value (cm^3)

EV = Extract volume (cm^3)

SM = Sample Mass (g)

AL = Aliquot (cm^3) used.

N/B 1cm^3 of 0.02N $AgNO_3$ = 1.08mg HCN.

3.3.2 Determination of phytate

The phytate content of the sample was determined using the method described by Inuwa *et al.* (2011). This entailed the weighing of 2g of each sample into 250ml conical flask; 100ml of 2% conc. HCl was used to soak the samples in the conical flask for 3 hours and then filtered through a double layer filter paper. Fifty (50ml) of each of the sample filtrate were placed in a 250ml beaker and 107ml of distilled water added to give/improve proper acidity, 10ml of 0.3% ammonium thiocyanate solution was added to each sample solution as indicator and titrated with standard iron chloride solution which contained 0.00195g iron/ml and the end point was signified by brownish-yellow colouration that persisted for 5min. The percentage phytate was calculated.

Calculation

Phytate phosphorus = Iron equivalent x 1.95g of titre

Phytate = (Phytate phosphorus x 3.65g) x 100

3.3.3 Determination of saponins

Saponin extraction was done using acetone and methanol. Crude lipid content of samples was extracted with acetone while methanol was used to extract saponin. Samples (2.0g in duplicate) were folded in a filter paper and put in a thimble and extracted by refluxing in a soxhlet extractor. Extraction was done with acetone in a 250cm^3 capacity round bottomed flask for 3hr after which the apparatus was dismantled and another 150cm^3 capacity flask containing 100cm^3 methanol was fitted to the extractor and extraction sustained for another 3hr. Weight of flask before and after the second extraction was taken to note the change in weight. Methanol was recovered by

distillation after the second extraction and the flasks oven-dried, allowed to cool at room temperature and weighed. Saponin content was calculated from the formula:

Saponin (mg/100g) = ((A-B)/Sw)/100

Where

A = weight of flask and extract

B = weight of empty flask

Sw = sample weight

3.3.4 Determination of tannins

One gram of shea butter cake was dissolved in 10ml distilled water and agitated, left to stand for 30 minutes at room temperature. Each sample was centrifuged and the extract recovered, 2.5ml of the supernatant were transferred into 50ml volumetric flask. Similarly, 2.5ml of standard tannic acid solution was transferred into a separate 50ml flask. A 1.0ml folin -dennis reagent was measured into each flask followed by 2.5ml of saturated Na_2CO_3 solution. The mixture was diluted to 50ml in the flask and incubated for 90minutes at room temperature. The absorbance of each sample was measured at 250nm with the reagent blank at zero. The % tannin was calculated (Jaffe, 2003).

$$T\ (\%) = \frac{(V\text{-}Vo) \times 0.004157 \times 250 \times 100}{W \times 25}$$

where:

V= Final volume

V_0= Initial weight

W= Weight

3.4 Determination of Mineral Contents

The mineral contents (elements) of *V. paradoxa* seed cake: calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were determined using the atomic absorption spectrophotometer (AAS-Buck 205), as described by the methods of AOAC (2006). Phosphorus was determined colorimetrically. All the determinations were done in duplicates. All values was expressed in percentage.

Two grams of ashed shea butter cake powder was weighed into a clean boiling tube. Distilled water (5.0ml) and concentrated Nitric acid (25ml) were added and mixed by shaking gently. The mixture was then refluxed over a water bath at 90^{o}C for 4 hours, cooled and 10ml of concentrated perchloric acid added. The tubes were again refluxed over a water bath at the same temperature for 1 hour and later cooled to room temperature. Concentrated HCl (2ml) was then added to the sample, made up to 100ml with distilled water and filtered. Standard solutions of different minerals earlier prepared in the concentrations of 0.125, 0.25, 0.50, 1.00ppm were used in the calibration with Atomic Absorption Spectrophotometer model AA-63000 using graphite furnace, Shimatzu, Japan.

3.5 Determination of Vitamin C

One millilitre of sample was used to make 1-cm pathlength cuvettes. HPLC was performed with an Isco Model 2350 pump (Isco, Lincoln, NE) with a gradient programmer, an UV-VIS detector, and a Hitachi F-1300 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). The column was a 5-.tm (particle size) Sephasil C18 column, 250 X 4.6 mm from Pharmacia LKB, Uppsala, Sweden. Vitamin C as Dehydroascorbic acid was determined by precolumn derivatization of

dehydroascorbic acid with o-phenylenediamine to its quinoxaline derivative. *E*qual volumes of calibrators and samples were mixed with a 10 mmol/L solution of o-phenylenediamine and incubated at 37^0C 30 mm in the dark. Of this reaction mixture 200 µl was filtered and injected into the HPLC apparatus. The mobile phase consisted of potassium phosphate buffer (80mmol/L) and 200ml/L methanol adjusted to pH 6 and filtered through a 0.2-µm pore-size filter (Millipore, Bedford, MA). The flow rate was 0.75 ml/min. The fluorescence detector was set to 365 nm excitation and 418 nm emission with 15-nm slit bandpass. Under these conditions the fluorescent derivative was eluted after 7 mm.

Derivatization procedure: Ascorbate oxidase (250 U) was dissolved in 2.5 ml of glycerol plus 2.5 mL of a 100 mmol/L monopotassium phosphate solution adjusted to pH 6; divided into portions; and stored at -80^{o}C. Ascorbic acid calibrators were dissolved in water and used immediately, because ascorbic acid degrades spontaneously in aqueous solutions. To 800 µl of ascorbic acid calibrators or samples we added 20µl of ascorbic acid oxidase solution and let this react for 5 mm at room temperature Vitamin C as ascorbic acid was expressed in percentage.

3.6 Determination of Amino Acids

Amino acid composition of shea butter cake was measured on hydrolysates using amino acid analyzer (Sykam-S7130) based on high performance liquid chromatography technique. Sample hydrolysates were prepared. Two hundred milligrams of sample were taken in hydrolysis tube. Then 5ml 6N HCl were added to sample into the tube, tightly closed and incubated at 110°C for 24 hours. After incubation period, the solution was filtered and 200 ml of the filtrate were evaporated to dryness at 140°C for an hour. Each hydrolysate after dryness was diluted with one

milliliter of 0.12N, pH 2.2 citrate buffers, the same as the amino acid standards. Aliquot of 150 µL of sample hydrolysate was injected in a cation separation column at 130°C. Ninhydrine solution and an eluent buffer (The buffer system contained solvent A, pH 3.45 and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 ml/min. The buffer/ninhydrine mixture was heated in the reactor at 130°C for 2 minutes to accelerate chemical reaction of amino acids with ninhydrine. The products of the reaction mixture were detected at wavelengths of 570 nm and 440 nm on a dual channel photometer. The amino acid composition was calculated from the areas of standards obtained from the integrator and expressed as percentages of the total protein.

3.7 Housing

The poultry house was thoroughly disinfected, fumigated with 1 part of potassium permanganate pellets to 3 parts of formalin. Thereafter, the house was sealed for 2 weeks before the arrival of the experimental broiler chicks.

3.8 Feed Formulation

Five iso-nitrogenous and iso-caloric rations were formulated to meet the NRC, (1994) recommendations. The diets were formulated using Win Feed 2.6 (Win Feed Ltd., Milton, Cambridge, UK) by linear programming.

The starter (0-4 weeks) diets were formulated to provide 23% CP. The control ration diet (1) contained no SBC while others (2, 3, 4, and 5) were formulated to 100 gkg^{-1}, 200gkg^{-1}, 300gkg^{-1} and 400gkg^{-1}, of maize and groundnut cake with SBC. The crude protein and the metabolisable energy (ME) was analysed and calculated for all diets.

3.9 Experimental Design

A total of 225 day-old Ross broiler chicks (mixed sex) were randomly allotted to the five (5) treatment diets in triplicate (15 birds per replicate) for four weeks. Each pen

was 1.05×1.30m in measurement and the pens were allotted randomly to different experimental units.

The brooding temperature was 35±2^{o}C during the first week and it was gradually decreased after each week. Continuous illumination was provided by 100 Watts electric bulb lights in all the pens throughout the experimental period to provide warmth to the newly hatched chicks to ensure satisfactory growth.

3.10 Routine Vaccinations

The chicks were prophylactically treated against bacterial infections at the second week using terramycin (chick formula) soluble powder (50g in 50 litres) and against coccidiosis using embazin forte® at 30 g per 50 litres water on the 18th day. Chicks were vaccinated against Newcastle disease using La sota vaccine (7th and 21st day) and Gumboro (14th) using Gumboro vaccine.

3.11 Data Collection

3.11.1 Weight determination

The chicks were weighed individually at the start of the experiment to determine the initial weight, subsequently weekly for four (4) weeks; the average body weight gain was calculated. Seven hundred and fifty grams of feed was offered ad libitum daily to each experimental unit. Similarly, the residual feed in feeders was weighed daily. At the end of each day, the average feed intake was calculated.

Table 3.1: Composition of Formulated Starter Broiler Diet (23%) CP

	Diets (% Inclusion Levels)				
Ingredients	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)
Maize	57.99	44.62	35.53	27.07	17.55
Groundnut	35.26	36.33	34.72	36.18	35.69
Shea Butter cake	-	10.00	20.00	30.00	40.00
Bone Meal	2.75	2.75	2.75	2.75	2.75
Salt	0.30	0.30	0.30	0.30	0.30
Vit. Min Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
DL. Methionine	0.20	0.20	0.20	0.20	0.20
Limestone	3.00	3.00	3.00	3.00	3.00
Calculated analysis					
Crude protein (%)	22.99	22.99	22.99	22.99	22.99
Methionie (%)	0.50	0.48	0.48	0.45	0.45
Calcium (%)	1.22	1.23	1.22	1.24	1.23
Phosphorus (%)	0.74	0.79	0.77	0.83	0.81
Lysine (%)	1.25	1.23	1.21	1.23	1.21
ME Kcal/Kg	2798	2798	2798	2798	2798
Cost per Kg (₦)	94.00	90.00	88.50	85.25	84.00

The daily feed intake was recorded to adjust feed intake for mortality, when it occurred. Mortality records were kept throughout the entire experiment.

3.11.2 Feed conversion ratio (FCR) determination

Feed conversion ratio (FCR) was estimated as:

Feed intake (g)/ Body weight gain (g)

3.11.3 Haematological indices

Two chicks per treatment were randomly selected and 2ml of blood was obtained via the wing veins using sterile gauge 25 needles and syringes at the 4^{th} week of the study after 3 hours of starvation. The blood was transferred into tubes containing EDTA (Ethylene diamine tetra-acetic acid).

The Packed Cell Volume (PCV) was estimated using the standard Micro Haematocrit Centrifugation technique (HCT). This involved thoroughly mixing a freshly collected sample of blood and filling two micro-capillary tubes to approximately two-third of their volume with the blood sample and sealing the empty ends with a Bunsen flame. The tubes were thereafter placed in a micro-haematocrit centrifuge in an opposite direction to be balanced, while the sealed end facing outwards. They were centrifuged for 3 minutes at 1500 revolution per minute (r.p.m). Packed cell volume (PCV) % was read from the micro-haematocrit centrifuge reader. Haemoglobin (g/100ml) is approximately 1/3 of the PCV value (Coles, 1986).

White blood cell is 1/6 of the PCV value obtained.

Mean Corpuscular Haemoglobin concentration (MCHC): This is the concentration of haemoglobin in one red blood cell. It is the amount of haemoglobin

expressed in relation to the volume of red blood cell, and also calculated using the formula by Tort *et al.* (1988).

MCHC= Hb x 100/PCV measured in g/dl

3.11.4 Economics of production

The market cost of the ingredients as at the time of the study was used to calculate the total cost of the feed per 100 kg of the diet, cost of feed per kg diets (₦). Total cost of feed consumed per bird (₦) and the cost of feed/Kg weight gain was calculated thus:

Total cost of feed (₦) = Total feed intake x cost of feed (₦)/Kg

Cost of feed (₦)/Kg weight gain = FCR x cost of feed (₦)/Kg

3.12 Data Analysis

All values obtained for weight, feed intake, body weight gain, feed conversion ratio, PCV, Hb, WBC, MCHC, feed cost/100kg, total cost of feed, feed cost/kg weight gain were subjected to Analysis of Variance (ANOVA) and where significant differences were observed, means were separated using General Linear Model of SAS. The results were considered as significant when p values were less than 0.05.

CHAPTER 4

4.0 RESULTS

4.1 Proximate Composition of Shea Butter

Table 4.1 shows the proximate composition of shea butter cake (SBC). The result showed that 92.57% dry matter (DM) of Shea butter cake contained 12.70% crude protein (CP), 5.96% crude fibre (CF), 0.84% oil, 3.77% ash content, 76.72% nitrogen free extract (NFE). It also showed that the elemental composition of shea butter cake, to be Ca (1.85mg/100g), K (0.99mg/100g), Mg (0.38mg/100g), Na (1.05mg/100g), Fe (1.06mg/100g), P (0.40mg/100g), Zn (5.676mg/100g), and Cu (0.55 mg/100g). *Vitellaria paradoxa* seed cake contained phytate (4.60 mg/100g), Hydrogen cyanide (2.90 mg/100g), saponins (1.15 mg/100g) and tannins (0.11mg/100g) (Table 4.2).

The amino acid composition of the cake showed that it contained both essential and non-essential amino acids. The essential amino acids were Arginine (1.89g/Kg), Histidine (0.50g/Kg), Isoleucine (1.01g/Kg), Leucine (1.34g/Kg), Lysine (1.28g/Kg), Methionine (0.38g/Kg), Phenylalanine (1.75g/Kg), Valine (1.25g/Kg) and Threonine (0.95g/Kg); while the non-essential amino acids were glutamic acid (3.56g/Kg), aspartic acid (1.96g/Kg) Cystine (0.35g/Kg), proline (1.30 g/Kg), tyrosine (1.10g/Kg), serine (1.25g/Kg), alanine (0.75g/Kg) and glycine (1.25 g/Kg) (Table 4.3).

4.2 Weight Performance of Chicks Fed Shea Butter Cake Diet

The initial weight of the chicks at the onset of the experiment (day 1) is shown in Table 4.4.

Table 4.1: Proximate and Elemental Composition of Shea Butter Cake

Nutrient	Values	Units
Dry matter	92.57	% in Dry Matter(DM)
Crude protein	12.70	% in DM
Crude fibre	5.96	% in DM
Oil	0.84	% in DM
Ash content	3.77	% in DM
Nitrogen Free Extract	76.72	% in DM
Calcium	1.85	mg/100g
Potassium	0.99	mg/100g
Magnesium	0.38	mg/100g
Phosphorus	0.40	mg/100g
Sodium	1.05	mg/100g
Iron	1.06	mg/100g
Zinc	5.676	mg/100g
Copper	0.55	mg/100g
Vitamin C	0.09	%

Table 4.2: Anti-Nutritional Constituent of Shea butter cake

Nutrients	(mg/100g of Dry Matter)
Phytates	4.60
Saponin	1.15
Tannin	0.11
Hydrogen cyanide	2.90

Table 4.3: Amino Acid Composition of Shea Butter Cake

Nutrients	(g/Kg)
Methionine	0.38
Lysine	1.28
Threonine	0.95
Arginine	1.89
Isoleucine	1.01
Leucine	1.34
Valine	1.25
Histidine	0.50
Phenylalanine	1.75
Glycine	1.25
Aspartic acid	1.96
Serine	1.25
Glutamic acid	3.56
Alanine	0.75
Tyrosine	1.10
Proline	1.30
Cystine	0.35

Table 4.4: Effect of Experimental Diets on Weight Performance of Birds (g)

	Diets (% Inclusion Level)				
Days of experiment	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)
0	46.37 ± 0.78	46.17 ± 0.29	45.93 ± 0.40	47.17 ± 0.29	46.10 ± 0.17
7	81.18±0.37^{a}	79.63±3.19^{ab}	78.77±2.04^{bc}	77.57±0.71^{c}	77.48±0.85^{c}
14	211.66±6.00^{a}	210.40±5.53^{a}	204.40±3.55^{a}	185.87±1.24^{b}	181.76±1.48^{b}
21	445.0±2.88^{a}	430.0±2.88^{a}	399.33±2.96^{b}	382.67±3.71^{bc}	378.33±3.00^{c}
28	968.33±15.89^{a}	968.33±4.41^{b}	930.00±2.89^{b}	915.00±7.64^{b}	900.00±2.89^{b}

Means with the same superscript in each row are not significantly different (P>0.05)

The initial live body weights of the day old chicks were similar and there was no significant difference ($p>0.05$) in the mean weight across the various treatments at the start of the trial (Table 4.4).

By week 1 of the feeding trials live body weight (LBW) of bird in treatments 1 and 2 were similar but significantly different ($p<0.05$) from treatment 3, 4 and 5. Birds on diets 1 and 2 performed best. During week 2, chickens on dietary treatment 1, 2 and 3 were similar but were significantly different ($p<0.05$) from those on diets 4 and 5. Chickens in treatment 4 and 5 had the least weight during the week (Table 4.4).

Live body weight during week 3 followed similar pattern to that of week 1. However, by week 4, the live body weight (LBW) in the control diet (0% inclusion level) was highest and was significantly different ($p<0.05$) from the other treatments. Birds on 30 and 40% inclusion levels of shea butter cake had the lowest live body weight over the 4 weeks of experiment. Birds on 0% inclusion level (diet 1) and 10% inclusion levels (diet 2) were similar in LBW for three out of the total four weeks of the experiment. The range of final live weight at 4 weeks per bird was between 968.33 ±0.54g and 1088.33 ±11.055g (Table 4.4).

4.3 Feed Consumption of Chicks Fed Shea Butter Cake Diet

During week 1, feed intake by chicks on diets 1, 2, 3 and 4 were similar but feed intake by chicks on diet 5 was significantly different ($p<0.05$) from the other 4 diets. Chicks on diet 5 consumed the least feed (Table 4.5).

Table 4.5: Weekly Feed Consumption of Broilers at Different Dietary Inclusion Levels of Shea Butter Cake (g)

	Diets (% Inclusion Levels)				
Days of experiment	1(0%)	2(10%)	3(20%)	4(30%)	5(40%)
7	102.82±0.20^{a}	101.92±0.36^{a}	101.67±0.55^{a}	101.25±0.38^{a}	98.15±0.35^{b}
14	183.00±1.38^{a}	183.20±0.79^{a}	179.58±0.82^{b}	179.33±0.65^{b}	177.20±0.81^{b}
21	272.65±1.50^{a}	270.70±0.65^{a}	262.08±1.02^{b}	252.20±0.88^{c}	241.26±0.76^{d}
28	411.08±0.85^{a}	413.67±0.79^{a}	406.75±0.29^{bc}	404.44±0.56^{c}	402.85±1.02^{c}
Final Feed Intake	969.55^{a}	969.48^{a}	950.08^{a}	937.22^{b}	919.47^{c}

Means with the same superscript in each row are not significantly different P>0.05

By week 2, feed intake by chicks on 0% (diet 1) and 10% (diet 2) inclusion levels were similar however there were significant difference ($p<0.05$) between these inclusion levels and those on 20, 30 and 40% inclusion levels of SBC (Table 4.5).

By week 3, feed intake by chicks on 0% (diet 1) and 10% (diet 2) inclusion levels were also similar, however there were significant difference ($p<0.05$) in feed intake between birds on inclusion levels 20, 30 and 40% inclusion levels of SBC. Birds on diets 1 and 2 consumed most while birds on diet 5 fed least (Table 4.5).

By week 4, level of consumption for chicks on 0 and 10 % inclusion level of SBC were similar; similarity also existed between 20, 30 and 40% inclusion levels. However there were significant differences ($p<0.05$) between feed intake in diet 1(0% inclusion level) and 2 (10% inclusion level), and diet 3 (20% inclusion level), 4 (30% inclusion level) and 5(40% inclusion level) (Table 4.5).

Final feed intake (FI) showed that chicks under 0% (control), 10% and 20 % inclusion level of shea butter cake meal were similar, there were significant differences ($p<0.05$) in final feed intake for birds on diets 4 and 5 to those on diets 1 (control diet), 2 and 3 (Table 4.5).

Over the 4 weeks of experiment, chicks on 0 and 10 % inclusion level consumed the highest feed while chicks on diet 5 fed least (Table 4.5).

4.4 Body Weight Gain of Chicks Fed Shea Butter Cake Diet

Mean body weight gain (BWG) for week 1 showed no significant differences ($p>0.05$) within the dietary treatments 1, 2 and 3. Broilers chicks fed dietary treatment 4 and 5 (30% and 40% inclusion levels) were significantly different ($p<0.05$) from the control diet (Diet 1). Chicks fed diet 4 had the least body weight gain while treatments 2 and 3 performed similar to the control diet (Table 4.6).

During week 2, BWG of chicks on 0% and 10% inclusion levels (130.48±5.87g and 130.72±3.25g respectively) were not significantly different ($p>0.05$), on the other hand those on 30 and 40% inclusion (108.31±1.03g and 104.28±0.97g) were significantly different ($p<0.05$) from the control diet (0%). Chicks on diets 1 and 2 had the highest BWG while those on diets 4 and 5 had the least BWG (Table 4.6).

During week 3, dietary treatment 1 (223.34±13.99g) and 2 (219.60±5.69g) gave the best performance in BWG as there were no significant difference ($p>0.05$) between them while those on diets 4(196.79±3.47g) and 5(196.57±2.56g) had the worst performance and were significant different ($p<0.05$) from the control diet (diet 1) (Table 4.6).

By the end of week 4, there was no significant differences ($p>0.05$) across all the five dietary treatments. Numerically, chicks on diet 2 (538.33±6.00g) had the best BWG (Table 4.6). The final BWG of birds on diets 1 and 2 were similar while there was significant differences ($p<0.05$) between chicks on diet 3, 4 and 5 compared to those on diet 1 and 2 (Table 4.6).

Table 4.6: Weekly Body Weight Gain of Broiler Chicks Fed Shea Butter Cake as Starter Diet (g)

	Diets (% Inclusion level)				
Days of experiment	1(0%)	2(10%)	3(20%)	4(30%)	5(40%)
7	34.82±0.72^{a}	33.52±0.16^{a}	32.83±0.19^{ab}	30.40±0.17^{c}	31.34±0.26^{bc}
14	130.48±5.87^{a}	130.72±3.25^{a}	125.63±1.92^{b}	108.31±1.03^{bc}	104.28±0.97^{c}
21	223.34±7.26^{a}	219.60±5.69^{ab}	194.94±3.50^{b}	196.79±3.47^{bc}	196.57±2.56^{bc}
28	523.33±18.56^{a}	538.33±6.00^{a}	532.33±7.88^{b}	530.67±4.70^{b}	521.67±5.83^{c}
Final BWG	921.97^{a}	922.17^{a}	884.07^{b}	867.83^{b}	853.90^{b}

Means with the same superscript in each row are not significantly different P>0.05

4.5 Feed Conversion Ratio (FCR) of Broilers to Different Dietary Levels

The feed conversion ratio (FCR) for the 4 weeks is presented in Table 4.7. There were significant differences ($p<0.05$) in FCR between chicks in dietary treatments 3, 4 and 5 in the first week while those on dietary treatment 1 and 2 were similar. Chicks on diets 1 and 2 performed best while those on diet 5 (40% inclusion level) had the worst FCR (Table 4.7).

During week 2, broiler chicks on dietary treatments 1, 2 and 3 were similar however significant differences ($p<0.05$) existed between them and those on dietary treatments 4 and 5 (Table 4.7). Chicken on diets 1, 2 and 3 had the best FCR while those on diets 4 and 5 had the worst FCR (Table 4.7).

During the third week, there was no significant difference ($p>0.05$) in the FCR of birds fed diets 1 (0% inclusion level), 2 (10% inclusion level) and 5 (40% inclusion level) but there were significant differences ($p<0.05$) between diets 3 and 4 and the control diet (0% inclusion level). Chicken on diet 1 had the best FCR (Table 4.7).

By the end of the 4^{th} week, chicks on 0 and 10% inclusion level of SBC had similar FCR and were the best in feed conversion but were significantly different ($p<0.05$) in FCR to those on 20, 30 and 40% inclusion levels. The final FCR for chicks on dietary treatment 2 and 3 were similar to those on the control diet (diet 1), while there were significant difference ($p<0.05$) between those on diets 4 and 5 compared to the control diet (Table 4.7).

Table 4.7: Feed Conversion Ratio of Broilers at Different Dietary Treatments

	Diets (% Inclusion Level)				
Days of experiment	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)
7	2.96±0.062^{a}	3.04±0.005^{ab}	3.10±0.006^{b}	3.33±0.020^{c}	3.13±0.036^{d}
14	1.41±0.070^{a}	1.40±0.029^{a}	1.43±0.016^{a}	1.66±0.011^{b}	1.70±0.014^{b}
21	1.17±0.040^{a}	1.23±0.035^{ab}	1.35±0.026^{c}	1.28±0.026^{bc}	1.23±0.013^{bc}
28	0.79±0.026^{b}	0.77±0.009^{b}	0.77±0.007^{a}	0.76±0.011^{a}	0.77±0.011^{a}
Final FCR	1.050^{a}	1.053^{a}	1.073^{a}	1.077^{b}	1.080^{b}

Means with the same superscript in each row are not significantly different P>0.05

4.6 Blood Characteristics of Chicks in Response to Dietary Treatments

Packed cell volume (PCV) value (25.17±0.06%) for broilers on diet 2 was similar to (25.53±0.06%) for broilers on diet 1(control diet), while PCV values for broilers on diets 3 and 4 were also similar but they differ significantly ($p<0.05$) to those of the control diet. Broilers on diet 5 also had PCV value which was significantly different ($p<0.05$) from those on the control diet. Packed cell volume values for chicken on diets 1, 2, 3 and 4 was within the acceptable range of 22-35% but values for those on diet 5 was slightly below the range (Table 4.8).

Haemoglobin (Hb) (8.39±0.02g/dl) for broilers on diet 2 was similar to (8.51±0.02g/dl) for broilers on diet 1(control diet), while Hb count for broilers on diets 3 and 4 were also similar they differ significantly ($p<0.05$) to those on the control diet. Broilers on diet 5 also had Hb count which was significantly different ($p<0.05$) from those of the control diet. Haemoglobin counts for chicken in all dietary treatments were within the acceptable range of 7-13g/dl (Table 4.8).

White Blood cell (14.19±0.11 $x10^3\mu l$) for broilers on diet 2 was similar to (14.26±0.11 $x10^3\mu l$) for broilers on diet 1(control diet), broilers on diets 3 and 4 were also similar but significantly different ($p<0.05$) to those of the control diet. WBC count for Broilers on diet 5 was also significantly different ($p<0.05$) from those of the control diet. White Blood cell for chicken in all dietary treatments was within the acceptable range of 13-20 $x10^3\mu l$ (Table 4.8).

Finally, Mean corpuscular haemoglobin concentration (MCHC) (27.53±0.02) for broilers on diet 2 was similar to (27.53±0.02) for broilers on diet 1(control diet), while MCHC for broilers on diets 3 and 4 were also similar but differ significantly ($p<0.05$) to

Table 4.8: Effect of Shea Butter Cake Diets on Some Blood Variables

	Diets (%inclusion level)					
Parameters	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)	Normal Values
Packed Cell Volume (%)	25.53±0.06[a]	25.17±0.06[a]	23.33±0.16[b]	23.13±0.18[b]	21.63±0.03[c]	22-35
Haemoglobin(g/dl)	8.51±0.02[a]	8.39±0.02[a]	7.78±0.05[b]	7.71±0.06[b]	7.21±0.01[c]	7-13
White Blood Cell ($x10^3\mu l$)	14.26±.011[a]	14.19±.011[a]	13.89±.028[b]	13.86±.030[b]	13.61±.006[c]	13-20
Mean Corpuscular Haemoglobin Concentration (%)	27.53[a]	28.38[ab]	27.16[b]	27.93[b]	26.60[c]	26-35

Means with the same superscript in each row are not significantly different P>0.05

those of the control diet. Broilers on diet 5 also had MCHC which was significantly different ($p<0.05$) from those of the control diet. Mean corpuscular haemoglobin count for chicken in all dietary treatments were within the acceptable range of 26- 35% (Table 4.8).

4.7 Economy of Production

The cost implication of feeding broilers with diets containing shea butter cake is presented in Table 4.9; the cost per 100Kg of feed showed a reduction in the cost of feed from diet 2-5, i.e. from ₦ 9000-₦8400, the control diet (0% inclusion) was ₦9,400 for 100Kg while the cost per Kg for diets 1, 2, 3, 4 and 5 was ₦94.00, ₦90.00, ₦88.00, ₦85.25 and ₦84.00 respectively. Feed cost (₦) reduced as the inclusion levels of SBC increased. Feed cost was lower for the SBC diets than the control (0%). The total cost of feed consumed per bird; cost of feed intake per bird (₦) (₦91.13-₦77.73 and cost of feed per kilogram gain (₦) from (₦98.70-₦90.72) showed a reduction as the level of inclusion increased from 0 to 40% (Table 4.9).

Table 4.9: Cost of Feeding (₦) With Different Dietary Treatments

	Diets (%inclusion level)				
	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)
Feed cost/100Kg (₦)	9400.00	9000.00	8800.50	8525.00	8400.00
Feed cost/Kg (₦)	94.00	90.00	88.00	85.25	84.00
Total Feed intake (g/bird)	969.2	969.48	950.08	937.22	919.46
No. of days on feed	28	28	28	28	28
Total cost of feed/Chicks (₦)	91.11	87.25	83.61	79.89	77.23
Feed cost(₦)/Kg weight gain	98.70	94.77	94.42	91.81	90.72

CHAPTER 5

5.0 DISCUSSION

5.1 Proximate Composition of Shea Butter Cake

The 12.7% Crude protein of Shea butter cake (SBC) in this study (Table 4.1) is higher than 9.2% CP reported by Ugese *et al.* (2010) for shea butter cake but in contrast to the untreated shea butter cake meal crude protein of 14.36% (Dei *et al.,* 2008); 14.12% (Rami-Reddy *et al.,* 2008); 16.24% (Atuahene *et al.,* 1998) and 17.31% (Zanu *et al*., 2012) for shea butter cakes. The decrease in crude protein value of shea butter cake is attributed to the leaching of nutrients due to soaking and boiling during the lipid extraction process and also to denaturation of protein by heating. The low crude protein for SBC can also be traced to the long heating period during the processing of the cake, Ojiako *et al.* (2010) reported that longer heating involved in boiling affect the crude protein of seed cakes. The traditional method used in the processing of the shea butter cake involves higher heat treatment than other known methods, and high processing temperatures of oilseeds has deleterious effects on proteins and amino acids due to denaturation associated with the formation of Maillard reaction products (Hurell, 1990).

The oil content of SBC (0.84%) in this study (Table 4.1) is higher than (0.50%) in shea butter cake reported by Zanu *et al*. (2012) but lower compared with 1.20% reported for unfermented shea butter cake (Dei *et al.,* 2008). The considerable variation in residual fat content of shea nut cake may be associated with variable efficiencies of fat extraction methods (Hall *et al*., 1996). The higher residual fat in SBC reported in this study was expected since the traditional method of fat extraction is known to be inefficient, and as such higher fat residue is anticipated in the by-product. Variation in fat content of shea butter cake may also be due to geographical location of the shea

butter trees which is supported by Maranz and Wiesman (2004) who reported variation in lipid content across four climatic zones of several African countries.

The dry matter content (92.75%) in the shea butter cake (Table 4.1) observed in this study was within the range of 85-95% (NRC, 1994; Donlaporn and Worapot, 2011). A number of factors may have contributed to the moisture content of the seeds and they include timing and method of harvest and environmental conditions, such as humidity and rain. Dry matter content below 85% normally leads to spoilage of feed ingredients that is related to mould growth (Hamito, 2010); therefore, the high dry matter content of SBC confere a good shelf-life on it, and this view concur with the findings of Mutayoba *et al.* (2011) that high dry matter content is also beneficial for livestock farmers because it increases the unit value of the feed components.

Shea butter cake in this study contained 76.72% nitrogen free extract (NFE) which is a high pointer of its richness in carbohydrate content and, hence a high caloric provider for broilers (Zanu *et al.,* 2012). High NFE in the SBC is a reflection of its high energy value. It is therefore likely that SBC can be used as substitute for maize which has 82% NFE (Mutayoba *et al.,* 2011).

The crude fibre (CF) of SBC is low (5.96) when compared to 9.5% reported by Atuahene *et al.* (1998), 6.70% (Rami-Reddy *et al*., 2008) and 12.6% (Ugese *et al.,* 2010). Although the CF is low, but falls within the recommended values of 5.5-7% (Nollet, 2008). Fibre is often used as a negative index for measuring the nutritive value of feed ingredients and in the prediction of its total digestible nutrients and net energy (Van Soest *et al*., 1991). The moderate crude fibre content of SBC suggests a reasonable nutritive value for broilers, particularly when included at a low level (Abeke *et al*., 2008).

The 3.77% ash content for Shea butter cake obtained in this study is an indication of its low level of mineral elements. Higher ash content has been reported in several raw materials used for poultry and other livestock. For instance, 5.5% ash of shea butter kernel was reported by Ugese e*t al.* (2010), while Rami-Reddy *et al.* (2008) reported 10.25% for shea butter extract, Atuehene *et al.* (1998) reported 4.2% and Dei *et al.* (2008) reported 5.23% ash for unfermented shea nut meal. The low level of ash content may be linked to leaching of the minerals during cooking, this claim is supported by Nagalakshmi (2007) that leaching of soluble minerals in water affect the level of minerals hence the ash content. The low level of minerals in SBC in this study may be connected with low level of ash. Mineral content of SBC in this study such as Ca (1.85%), P(0.40), K, Na and Mg was low in comparison to Ca(3.0), P(2.4) reported for shea butter cake (Dei *et al*., 2008), but in contrast, higher than Ca (0.50%), P (0.27) reported by Rami-Reddy (2008). The low mineral content of SBC may be due to loss of soluble forms of these fractions into the boiling water. Kankengi *et al.* (2003) reported that soaking over-night and cooking for one hour generally reduce the levels of all the major minerals except calcium. Therefore the use of shea butter cake as starter diet in broiler will require fortification with these mineral elements, which can be obtained from addition of vitamin-mineral premixes.

Shea butter cake contains moderate Copper, zinc and manganese which are important trace elements for development of the red blood cells and immune system which are also related with cholesterol and lipid metabolism (Aksu *et al.*, 2010), lending credence to its use possibly as poultry feed meal.

The tannin concentration of SBC (0.11mg/100g) in this study (Table 4.2) is also low compared to 0.16mg/100g and 0.34mg/100g reported by Dei *et al.* (2008) for fermented shea butter nut and unfermented shea butter cake respectively. This low level

of tannin can be attributed to the long heat processing of the SBC during production; USAID (2007) reported that SBC passed through boiling at a temperature of 120^{o}C which invariable reduced the level of tannin in the SBC. The maximum reduction of tannin observed in the boiled kernel agrees with the observation of Kankengi *et al.* (2003) that cooking is an efficient method of reducing the tannin content of feeds. Tannins have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity, which may be part of the reason for the low performance of broilers to higher inclusion level of SBC in this study. Tannins also have the ability to complex with vitamin B (Mutayoba *et al.,* 2011). Tannin level in this study is slightly within Levels from 0.5 to 2.0 % which can cause depression in growth rate and egg production but less than levels from 3 to 7% which is known to cause death in poultry.

Hydrogen Cyanide found in the dry matter of SBC in this study (2.90mg/100g) is relatively high for early day poultry birds, and therefore may require further reduction process if better result is desirable. The cyanide ions available in SBC is high and will therefore have adverse effect in inhibiting enzyme systems; depress growth through interference with certain essential amino acids and utilization of associated nutrients (Soetan and Oyewole, 2009).

Phytate concentration (4.60mg/100g) recorded for SBC in this study (Table 4.1) is within the tolerance level for poultry birds reported by Inuwa *et al.* (2011), phytates in foods are known to bind with essential minerals (such as calcium, iron, magnesium and zinc) in the digestive tract, resulting in mineral deficiencies (Bello *et al.,* 2008). They bind minerals to form insoluble salts, thereby decreasing their bioavailability or absorption (Muhammad *et al.,* 2011). Phytic acid binds trace elements and macro-elements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract thereby making dietary minerals unavailable for absorption and utilisation by the body. It can

also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis (Treviño *et al.,* 1992). Moreover, the phosphorus in phytate has been considered to be largely unavailable to animals because of the limited capacity of monogastric species to hydrolyse phytate in the small intestine.

Saponin constituent of SBC in this study is relatively low and maybe accommodated by the physiology of broilers as starter diet because high saponin causes hypocholesterolaemia by binding cholesterol, making it unavailable for absorption (Fasuyi and Nonyerem, 2007). Other studies reported that high saponin values can affect animal performance and metabolism through erythrocyte haemolysis, reduction of blood and liver cholesterol and reduction in nutrient absorption. Saponins have also been reported to alter cell wall permeability and therefore produce some toxic effect when ingested (Bolhuis, 2003). The effect of saponin on chicks even at low concentration have been reported to reduce growth, feed efficiency and interfere with the absorption of dietary lipids, cholesterol, bile acids vitamins A and E (Johnson *et al.,* 1986; Jensen *et al.,* 1990).

Some amino acid concentration, such as methionine (3.8g/kg), leucine (13.4g/kg), histidine (0.5g/kg) in this study were lower than 5.4g/kg, 16.5g/kg and 5.8g/kg respectively reported by Dei *et al.* (2008) for unfermented shea butter cake. Heating which is known to denature protein and amino acids may be responsible for the low concentration of the amino acid. On the contrary Cystine (3.5g/kg), threonine (9.5g/kg), arginine (18.9g/kg), isoleucine (10.1), valine (12.5g/kg) and phenylalanine (17.5g/kg) were on the high side when compared to 3.2g/kg, 8.1g/kg, 12.7g/kg, 8.7g/kg, 10.3g/kg, 5.8g/kg and 9.5g/kg respectively; but Lysine (12.8g/kg) in this study concur with 12.5g/kg reported by Dei *et al.*(2008). The level of essential and non-essential amino acids in SBC in this study is a pointer to its potential as a substitute to protein source in

poultry feed ingredients. The results of amino acids composition clearly depict *V. paradoxa* seed cake as a potential protein source comparable with other conventional protein sources especially of plant origin (Shutze, 1996), but may require minor fortification with some amino acids particularly the essential amino acid for better growth of broiler chicks.

5.2 Weight Performance of Chicks Fed Shea Butter Cake Diet

The improvement in the performance of broilers fed the control diet (0%) and 10% inclusion level of SBC compared with other dietary treatments may be due to low crude fibre and antinutritional factor and palatability of the diets which enhance consumption and hence increase in weight of the broilers. The declining trend in growth observed in weekly live body weight as the inclusion of shea butter cake increases is similar to the findings of Olorede and Longe (1999); Annongu *et al.* (2006). The higher inclusion of SBC above 10% did not enhance the performance of the birds; similar findings were reported on unfermented shea nut cake by Dei *et al*. (2008). This may be attributed to the presence of higher concentration of anti-nutrient tannin in the SBC as inclusion levels increased. Studies have shown that high dietary tannins result in reduced live body weight and poor feed efficiencies in birds (Treviño *et al.*, 1992; Iji *et al.*, 2004), and this has a pronounced negative effect on protein digestibility (Smulikowska *et al.*, 2001).

5.3 Feed Consumption of Birds Fed Shea Butter Cake Diet

Feed intake was inversely related to the increasing levels of shea butter cake in the diets, and higher inclusion of SBC does not favour the feed intake of the birds. The decreased feed intake of broilers on diets containing 20% SBC and above may be due to poor palatability of the SBC meal and the presence of antinutritional factors as the

inclusion level increased. Esonu *et al.* (2001) and Onu *et al.* (2011) reported that reduction in feed intake is associated with palatability arising from residual antinutritional factors and likely high fiber content. The decline in feed consumption with increasing levels of shea butter cake meal in this study is similar to the result of Atuahene (1998), and Olorede and Longe (1999) during which pullets feed consumption reduced as the inclusion level exceeded 10% shea nut cake in the diet.

5.4 Body Weight Gain of chicks fed Shea Butter Cake Diet

Body weight gain has a similar trend to feed intake. The decreased weight gain at higher SBC inclusion level was partly due to low feed intake, which is related to the poor palatability of shea butter cake diets (Zanu *et al., 2012*).

The poorer growth rate of broiler chicks fed 40% inclusion level of SBC based diet may be attributed to the lower feed intake and poor palatability of the ingredient. Olorede and Longe (1999) and Dei *et al.* (2008) had earlier observed growth depression when SBC was included at higher percent (>10%) in pullet chicks' diet also. The decreasing weight has been related to the fact that weight gain in broiler is directly related to feed intake, the quality of the feed as well as how efficiently the birds utilize the feed (Egbunike *et al.,* 2009).

The reduced weight gain of birds fed 40% inclusion diet compared to birds fed 10 and 20% inclusion diets could also be partly ascribed to the higher crude fibre content of 40% inclusion diet since high crude fiber is known to affect feed intake and hence weight in poultry (Onu, 2010). The lower weight gain of birds fed diet 5 (40%) might also be due to the negative effect of the anti-nutritional factors such as tannin which has been reported to interfere with the biological utilization of protein and to a lesser extent available carbohydrate and lipids (Esonu, 2001). Agbede *et al.* (2012) and Ajaja *et al.* (2003) had similarly observed that low fibre content of diets affect nutrient

digestion and utilization which may also precipitate metabolic dysfunction with attendant weight reduction in monogastric animals. It is not unlikely that the birds were able to satisfy their energy requirement without meeting their requirement for other essential nutrients, particularly amino acids which are needed for tissue synthesis, more so at the higher (>10%) inclusion levels of SBC.

5.5 Feed Conversion Ratio (FCR) of Broiler to Different Dietary Levels

The overall feed conversion ratio (FCR) for birds in 10 and 20% inclusion level (1.05) and (1.07) respectively in this study was relatively low compared to 2.5 and 3.3 reported by Zanu *et al*. (2012) for cockerels fed shea butter diet. Dei *et al.* (2008) had earlier reported lower FCR of 1.79 for broiler fed unfermented shea butter meal.

The high FCR of the birds fed higher inclusion level of shea butter cake may be due to the inability of the young chicks to tolerate anti-nutritional factors found in the diet.

The superior FCR of birds on 0 and 10% SBC inclusion diets to other inclusion level (20%, 30% and 40%) suggests that there was enhanced availability, digestion, absorption and utilization of the nutrients in the cake by broilers. level. Similar observations have been reported by Dei *et al.* (2008) while feeding broilers with shea butter meal.

5.6 Blood Characteristics of Chicks in Response to Dietary Treatments

The selected haematological parameters data (PCV, Hb, WBC and MCHC) obtained in this study though differed significantly among the group but were within normal range for packed cell volume (21-35%), haemoglobin (7-13 g/dl) and MCHC (26-35%), in broilers (Fasuyi and Nonyerem, 2007). This observation suggests that the health of the birds were not compromised throughout the study period. The values obtained for all

the treatment groups indicate nutritional adequacy of all diets, since values did not indicate mal-or under nutrition.

The lower haematological data obtained in birds fed 20%, 30% and 40% inclusion diets may be ascribed to the higher concentration of tannin in SBC diet. Tannin has been reported to have negative effect on feed intake as well as dry matter and protein digestibility and invariably blood parameters (Vecerek *et al.,* 2002). The low availability of minerals in SBC may as well cause reduction in the packed cell volume (Kaya *et al.,* 2006), red blood cell count and haemoglobin concentrations (Hill and Matrone, 2006).

5.7 Economy of Production

The cost/benefit ratio analysis (cost of feed to raise 1kg broiler flesh) showed that cost per kilogram of feed was inversely related to increasing dietary levels of SBC in the diet. The reduction in the cost of the feed (₦/Kg feed) on the shea butter cake - based diets was attributed to the price difference between maize and shea butter cake. Shea butter cake was not purchased *per se* but cost of transportation incurred estimated at ₦ 1.50/kg against ₦ 45.17/kg of maize as at the time of the study.

The reduction in price with increase in the inclusion level could be attributed to the partial replacement of the more expensive maize and soyabean with SBC that has a very low cost (Olorede and Longe, 1999). The use of the SBC diets showed that cost per kg of feed and cost per kg of live weight gain decreased as the SBC content of the diet was increased. So, it may result in significant economic returns which clearly indicate that SBC is more economical for broiler production compared to groundnut cake. Zanu *et al.* (2012) who reported that the feed cost per kg of live weight gain was reduced as the level of SBC in the diet increased from beyond 10% inclusion level.

This is the major thrust of nutritionists, that is, to lower feed cost without compromising the feed quality (Onu, 2010). The reduction in cost per kilogram feed and cost of feed per bird obtained in this study is in line with one of the objectives of this study.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The proximate composition, mineral assay and amino acid profile of shea butter (*V. paradoxa*) seed cake as a potential plant protein feedstuff was investigated. The results of this trial have shown that the anti-nutritional factors, that could impair nutrient utilization by monogastric livestock, are sufficiently deactivated by the processing of the shea butter cake in the factory before inclusion in the diet. The results of the growth performance, feed intake, haematological and feed conversion ratio suggested that the experimental *V. paradoxa* seed cake meal diets up to 10% inclusion levels did not impose any detrimental effect on the health status of chicks. Shea butter cake can be incorporated in broiler starter's diet up to 10% without any adverse effect on the chick's immune status.

The economics of production showed that SBC diets were more profitable as regards the cost of feed per kg weight gain (₦) and thus is cost savings (%). Thus, its use in poultry diet should be maximally exploited as a way of reducing the current breaking pressure on maize as major energy source in poultry diets.

The use of shea butter seed cake which is a by-product in shea butter processing industries, in broiler diets will help to narrow down the price of poultry feed with attendant increase in broiler meat production in sub-sahara Africa, particularly Nigeria.

6.2 Recommendations

i. The inclusion of shea butter cake at level not exceeding 10% is recommended

ii. Additional processing method for shea butter which may reduce the antinutritional factors and improve the availability of nutrients for animal consumption is suggested.

iii. The use of Shea Butter Cake as an energy source rather than a protein source is recommended owing to its high Nitrogen Free Extract

iv. Determination of the fatty acid composition of Shea Butter Cake is recommended, this may give an insight into the likely period of the onset of rancidity and storage characteristics of Shea Butter Cake related diets.

v. Further research in the use of Shea Butter Cake in feeding of other monogastric animals is suggested.

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APPENDICES

4. Average Weight of Day Old Chicks (g)

	Diets (% Inclusion level)				
Replicate	1 (0%)	2(10)	3(20)	4(30)	5(40)
R1	46.6	46.0	46.0	47.0	46.0
R2	47.0	46.5	45.5	47.5	46.3
R3	45.5	46.0	46.3	47.0	46.0

2. Feed Consumption (g/chick/week)

	Diet (% inclusion level)				
Replicate	1 (0)	2(10)	3 (20)	4 (30)	5 (40)
R1	103.20	102.50	102.60	100.50	98.25
R2	102.50	102.00	101.70	101.75	98.70
R3	102.75	101.25	100.70	101.50	97.5
Week 2					
R1	185.20	184.50	180.75	179.25	178.25
R2	180.75	182.90	180.00	178.25	177.75
R3	182.75	182.00	178.00	180.50	175.6
Week 3					
R1	270.00	272.00	261.75	250.55	240.25
R2	272.75	270.00	260.50	252.20	240.80
R3	275.20	270.10	264.00	253.55	242.75
Week 4					
R1	410.50	412.75	407.25	403.78	402.55
R2	410.00	415.25	406.75	405.55	404.55
R3	412.75	413.00	406.25	404.00	401.5

3. Live body weight (LWB) (g/chick/week)

	Diet (% inclusion level)				
Replicate	1 (0)	2(10)	3 (20)	4 (30)	5 (40)
R1	80.50	79.70	79.20	77.40	77.15
R2	81.30	80.15	78.20	78.20	77.4
R3	81.75	79.20	78.90	77.10	77.90
Week 2					
R1	203.33	216.67	208.33	186.67	183.33
R2	223.33	206.20	203.43	184.45	180.40
R3	208.33	208.33	201.43	186.50	181.55
Week 3					
R1	450.00	425.67	398.00	390.00	380.00
R2	445.00	430.00	405.00	380.00	372.50
R3	440.00	435.00	395.00	378.00	382.55
Week 4					
R1	950.00	975.00	925.00	920.00	900.00
R2	955.00	960.00	930.00	900.00	905.00
R3	1000.00	970.00	935.00	925.00	895.00