PERFORMANCE OF BROILER CHICKENS FED WATER AND UREA TREATED NEEM (*Azadirachta indica*) KERNEL CAKE AS PROTEIN SUPPLEMENTS

By

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DECLARATION

I, Owusu Frimpong Isaac, hereby certify that the work herein submitted as a thesis for the Master of Science (Animal Nutrition) degree has neither whole nor in part been presented nor is being concurrently submitted for any other degree elsewhere. However, works of other researchers and authors which served as a source of information were duly acknowledged by references of the authors.

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DEDICATION

To my dear mother Felicia Manu, beloved wife, Dina Konadu Frimpong and lovely daughters, Jemima, Felicia, Miriam and Angela

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I am grateful to the Almighty God for His enablement to complete this project. My sincerest gratitude also goes to my supervisor, Professor Mrs C. C. Atuahene for her guidance, assistance, constructive criticism and technical advice without which this project will not have been successful. May the good God richly bless her.

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ABSTRACT

In a study to evaluate the performance of broiler birds fed graded levels of water and urea-treated neem kernel cake (NKC), three hundred (300) day-old commercial broiler chickens (Cobb, 500) were randomly assigned to five dietary treatments for 56 days. Each treatment was replicated thrice in a Completely Randomized Design (CRD). The data obtained was subjected to analysis of variance (ANOVA) and where the analysis indicated significant treatment effect, the means were compared using Duncan's Multiple Range Test (1995). Water and feed were given *ad-libitum*. Major parameters measured included: feed intake, water intake, body weight gain, feed conversion ratio carcass characteristics and biochemical and haematological qualities of the blood. The diets were formulated to be isonitrogenous to replace soya bean meal at 0% NKC, 5% WNKC, 10% WNKC, 5% WUNKC and 10% WUNKC for diets 1, 2, 3, 4, and 5 respectively. The results showed that Average daily feed intake (ADFI), average body weight gain (ABWG), average daily water intake (ADWI), feed conversion ratio (FCR) and feed cost/kg live weight were significantly ($p<0.05$) different across dietary treatments, while the water/feed intake ratio did not show any significant ($p>0.05$) difference. Broilers fed the 0% NKC (control) diet had ADFI, ABWG, ADWI and feed cost/kg live weight values which were significantly ($P<0.05$) better than the birds on the 5% and 10% NKC diets. Results on carcass parameters were also significantly ($p<0.05$) different among the dietary treatments. Liver, gizzard, heart and intestinal weights of birds on the test diets were significantly ($p<0.05$) heavier than those on the control diet. With the exception of the red blood cell (RBC), globulin and total protein, all the haematological and biochemical parameters measured were not significantly ($p>0.05$) different across the dietary treatments. Economics of production revealed that a profit/bird on the control diet was GH¢5.20 as against GH¢3.39, GH¢1.14, GH¢2.76 and

GH¢0.99 for birds on 5% WNKC, 10% WNKC, 5% WUNKC and 10% WUNKC. Values registered for birds fed the 5% inclusion levels of the NKC diets on average final body weight and water intake were significantly ($p<0.05$) higher than those which were on the 10% inclusion levels. It is therefore suggested that replacing soya bean meal with NKC at the inclusion levels used in this study may have a deleterious effect on growth performance of the birds and will also, not be cost effective

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Full words
ADG	**Average daily gain**
ADFI	**Average daily feed intake**
A/G	**Albumin/globulin**
AFBW	**Average final body weight**
AFK	**Autoclaved full-fat neem kernel**
AOAC	**Association of Official Analytical Chemists**
CNSC	**Charcoal-treated neem seed cake**
CP	**Crude protein**
DM	**Dry matter**
ENSC	**Expeller neem seed cake**
ENSCD	**Expeller neem seed cake diet**
FAO	**Food and Agriculture Organisation**
FBM	**Fermented bambara groundnut meal**
FCR	**Feed conversion ratio**
fl	**Fermtoliter**
gl/dl	**Gram/decilitre**
Hb	**haemoglobin**
HCl	**Hydrochloric acid**
HDL	**High density lipoprotein**
HNSC	**Hydraulic press neem seed cake**
HNSMD	**Hydraulic press neem seed meal diet**

KNUST	**Kwame Nkrumah University of Science and Technology**
LDL	**Low density lipoprotein**
LSD	**Least significant difference**
MCH	**Mean corpuscular haemoglobin**
MCHC	**Mean corpuscular haemoglobin concentration**
MCV	**Mean corpuscular volume**
ME	**Metabolizable energy**
mg/dl	**Milligram/deciliter**
MNSC	**Methanol-processed neem seed cake**
NaOH	**Sodium hydroxide**
NCFR	**Non-conventional feed resources**
ND	**Newcastle disease**
NFE	**Nitrogen free extract**
NKC	**Neem kernel cake**
NKM	**Neem kernel meal**
NLM	**Neem leaf meal**
NRC	**National Research Council**
PCV	**Packed Cell Volume**
RBC	**Red blood cell**
RBM	**Raw bambara groundnut meal**
RMNS	**Raw milled neem seed**
RNSMD	**Raw neem seed meal diet**
RNSM	**Raw neem seed meal**
ROBM	**Roasted bambara groundnut meal**
SBM	**Soya bean meal**

SEM	**Standard error of mean**
SL	**Significant level**
SNSC	**Solvent extracted neem seed cake**
SNSCD	**Solvent extracted neem seed cake diet**
SPSS	**Statistical Package for Social Sciences**
TCC	**Technology Consulting Center**
TGS	**Triglycerides**
TP	**Total protein**
UNSC	**Untreated neem seed cake**
WBC	**White blood cell**
WNKC	**Water treated neem kernel cake**
WNSC	**Water treated neem seed cake**
WUNKC	**Water and urea treated neem kernel cake**
µl	**Microliter**

LIST OF FIGURES

CHAPTER ONE

1.0 INTRODUCTION

The current trend in the cost of feed as well as irregular supply of feed (Uko and Kamalu, 2008) poses a threat to the future of the livestock and poultry industry (Sonaiya, 1990). FAO (2008) report, indicated that feed prices have jumped to a record high and further increases are on the horizon due to diminishing supply and increasing demand. According to Kellems (2002), the cost of feeding alone represents approximately 75% of the cost of poultry production. It therefore becomes very imperative to intensify efforts in the search for cheaper, abundant and locally available alternatives that have no direct dietary value to man, for sustainable production (Odunsi *et al*., 2002)

Neem kernel cake (NKC) obtained from the neem seed oil industry is a potential alternative source of non-conventional feedstuff (Odunsi *et al*., 2009). The protein content of NKC (CP: 30-40%) is close to that of soya bean meal SBM (CP: 44%), a highly valued protein ingredient used in poultry diet (Reddy *et al*., 1988).

The neem plant (*Azadirachta indica*) belongs to the family Meliaceae. Neem is a fast growing evergreen plant found in most parts of Africa including Kenya, Nigeria and Ghana (Schmutterer, 1988). The tree grows in a wide range of soils and it is a sturdy tree which can be established in poor dry soils without irrigation (Margraf, 1990). In Ghana, the tree is however established in the rainforest and in the savannah belt (Schmutterer, 1988). A full-grown tree can produce a total of 30 – 100kg of fruits depending on rainfall, soil type and ecotype. Fifty (50) kg of fruits yield 30kg of seed giving 6kg oil and 24kg of seed cake (Ramesh, 2000).

Margraf (1990) reported that the neem plant has many uses; for example, the bark, leaves, fruits and sap help to cure various skin diseases, verneral diseases (e.g. syphilis) and tuberculosis. It can also find uses in antiseptics, remedy against rheumatism and antidote to snake or scorpion bite. Neem is also used for various purposes including pest control, manufacture of shampoos, soaps and tooth paste (Saxena, 1984). Uko and Kamalu (2008) reported that scientists are focusing more attention on the tree for various reasons including; using it as pesticide, antimicrobial, the use of parts of the plant as fertilizer, animal feed and as treatment of malaria.

Though rich in crude protein (300 - 400g/kg), Paul *et al.* (1996) and Elangovan *et al.* (2000) reported that, the use of neem kernel cake as feedstuff might impart unpleasant taste or smell to the meat due to the presence of bitter and toxic triterpenoids like nimbidin, nimbin, azadirachtin and salanin. However, report from Katiyar *et al.* (1996) indicated that no significant differences were noticed in the qualitative and quantitative characteristics of the meat of chickens fed a diet containing 20% neem kernel cake and the control diet.

Processing of neem seed cake is therefore required. Such methodology needs to be tested and perfected for maximum dibiterization of neem kernel cake with minimum cost (Gowda and Sastry, 1998).

Several methods have been used by researchers to detoxify and reduce the bitterness of NKC for animal use. The methods include: alkali treatment of neem seed cake (Reddy *et al*., 1988), water washing of neem seeds (Ranga, 2002) and urea soaking of neem seeds (Gowda and Sastry, 1998). Ranga (2002) observed that water washing removed 83.53% of azadirachtin, 53.13% of nimbin and 35.89% of salanin. Water washing, followed by urea treatment (at 1 kg: 10 litres concentration of urea and water) removed 83.27% azadirachtin and 100% nimbin and salanin.

It is observed from the above experiment that water washing followed by urea treatment of neem seeds seem to reduce the levels of the bitter triterpenoids and should give a better results in feeding trials. Also, several research works have been done on the use of water treated and urea-treated neem seed cake separately (Gowda and Sastry, 1998: Ranga, 2002: Uko and Kamalu, 2005: Uko and Kamalu, 2008) but there is not much information about the concurrent use of these products as broiler feed.

This work was therefore carried out to assess the growth performance, carcass, haematological and biochemical qualities as well as the economics of production of broiler birds fed diets containing water treated and urea treated neem kernel cake.

The following specific objectives were looked into:

- Growth performance of broiler chickens (Feed intake, body weight gain, and feed conversion ratio)
- Carcass characteristics of broiler chickens (Defeathered, dressed, liver, heart, shank, empty gizzard and intestinal weight of the birds)
- Haematological indices (Haemoglobin content, white and red blood cell count, packed cell volume, etc. of the blood)
- Biochemical indices (Cholesterol, total protein, albumin, high and low density lipoprotein levels of the blood)
- Economic importance of the experiment (Total feed intake, feed cost/ live weight gain, selling price/ bird, profit/ bird, etc.)

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Botanical description of neem plant

2.1.1 Taxonomic position of neem as indicated by (Ganguli, 2002; Girish and Shankara, 2008)

Kingdom	Plantae
Division	Mangnoliophyta
Order	Sapindales
Suborder	Rutinae
Family	Meliacae
Sub family	Melioidae
Tribe	Melieae
Genus	*Azadirachta*
Species	*indica*

2.1.2 Botany

Neem tree has other description as "nature's" gift to mankind, "the tree for many occasions", "the tree of the 21st century" and a tree for solving global problems (Ramesh, 2000).

According to (Ramesh, 2000) the botanic name *Azadirachta indica,* was derived from Farsi, "azad darachti hind" which literally means the "free or noble tree of India", suggesting that it is instrinsically free from pest and disease problems and it is benign to the environment.

Neem (*Azadirachta indica* A. Juss) tree is an attractive evergreen tree that can grow up to 20m in height. Its spreading branches form rounded crowns up to 10m in diameter. The short, usually straight trunk has a moderately thick, strongly furrowed bark that has a garlic-like

odour and bitter, astringent taste. Its leaves are imparipinate, 20-38cm long, crowded near the branch end, oblique, lanceolate, deeply and sharply serrated. The neem plant is rarely leafless and is usually in full foliage even during months of prolonged drought. It's small, white, bisexual and staminate (functionally male), flowers are born in auxiliary clusters. They have honey-like scent and attract many bees which act as pollinators. The fruit is a smooth ellipsoidal drupe; 1-2cm long which is yellow when matured and comprises a sweet pulp enclosing a seed (NRC, 1992).

The seed is composed of a shell and a kernel (sometimes two or three kernels), the latter having a high oil content. Neem tree will begin bearing fruit after 3-5years, become fully productive in 10years and can produce up to 50kg of fruits annually (NRC, 1992; Tewari, 1992; Ketkar and Ketkar, 1997; Gunasena and Marambe, 1998).

2.1.3. Origin and Distribution of Neem Plant

Two species of *Azadirachta* have been reported, *Azadirachta indica* A. Juss – native to Indian subcontinent and *Azadirachta excelsa* Kack – confined to Philippines and Indonesia (Jattan *et al*., 1995; Hedge, 1995). Neem plant is thought to have originated in Assam and Myammen in Asia where it is common throughout the central dry zone and the Siwalik Hills (NRC, 1992). However, the exact origin is uncertain and some authors suggest that it is a native to the dry forest region of South-East Asia including Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia (Ahmed *et al*., 1985).

Azadirachta indica A. Juss, commonly known as neem plant, has been well known in the Indian subcontinent for more than 2000 years. It grows in Himalayan tracks along with the forest plains and occurs evergreen. Nowadays, the plant is being grown in the tropical and subtropical regions of Asia, Africa, America, Australia and South Pacific Island widely in the

deciduous or as one of the most versatile medicinal plants possessing a wide spectrum of biological activities (Kumar *et al*., 2002).

It is in India that the tree is most widely used. It is grown from the southern tip of Kerala to the Himalayan hills, in tropical to subtropical regions, in semi arid to wet tropical regions, and from sea level to about 700 metres (NRC, 1992). Neem plant was introduced to Africa earlier this century. It is now well established in at least 30 countries, particularly; those in the regions along the Sahara's southern fringe, where it has become an important provider of both fuel and lumber (NRC, 1992).

In the continental United States, small plantings are prospering in Southern Florida, and exploratory plots have been established in Southern California and Arizona. The tree, most likely, made its way to Africa as a result of British colonialism at the start of this century and today it occurs in considerable numbers from Eritrea (an estimated 500,000 trees; NRC, 1992) and Somalia via Kenya and Tanzania northwards to Mozambique. Many neem trees do occur in some central regions of East Africa, e.g. in Uganda (25,000), Kenya, Tanzania and Malawi (Schmutterer, 1995). Neem tree (Strozok, 1992; Schmutterer, 1995) is frequently encountered in Sub-Saharan Africa as many million trees are located through the entire area from Ethiopia, Sudan, Senegal and Mauritania due to favourable hot climatic conditions with a precipitation level of 500-1200 mm/a: Nigeria record is of about 10 million trees followed by Senegal and Ghana having approximately 6 million trees with Mali, Burkina Faso and Niger recording about 2.5 million trees each (Girish and Shankara, 2008).

There is a general feeling to the people in West Africa these days that neem plant has been there since time immemorial. However, this tree is actually a recent addition to the African scene (NRC, 1992). It was Brigadier-General Sir Frederick Guggisberg who brought neem plant to Ghana. He was governor (of what was then known as the Gold Coast) from 1919 to 1927, and he introduced seeds or seedlings from India to Ghana sometime during that period.

The first seeds were planted in the Northern Territories. Today, as a result, neem tree is found everywhere in Ghana and the Sahelian region (NRC, 1992)

2.1.4 Sexual and breeding systems

The neem tree is andromonoecious, i.e. bisexual and staminate. Flowers occur on the same tree (Sigh and Raheja, 1996). The anthers start to dehisce around 8 hours in the closed flower, and the pollen matures before the stigma becomes receptive (protandry) (Gupta *et al*., 1996). The seeds are dispersed mainly by birds and mammals.

2.1.5 Habitat

The tree is said to grow "almost anywhere" in the lowland tropics. However, it generally performs best in areas with annual rainfalls of 400-1,200 mm. It thrives under the hottest conditions, where maximum shade temperature may soar past 50°C, but it will not withstand freezing or extended cold. It does well at elevations from sea level to perhaps 1,000 m near the equator. The taproot (at least in young specimens) may be as much as twice the height of the tree (NRC, 1992).

Neem tree grows in all types of soils, including clay, saline and alkaline soils, but does well in black cotton soils (NRC, 1992). It can tolerate dry, stony, shallow soil with a waterless sub-soil, or places where there are hard calcareous or clay pan near the surface (Anon, 2006). The species is also thought to be an efficient water user in comparison to some other trees. Neem plant does not tolerate water-logging, is fire-resistant and has unique property of calcium mining which neutralizes acid soils (Gunasena and Marambe, 1998). It coppices and pollards well, and also produces root suckers.

2.1.6. Propagation of Neem Plant

The tree is easily propagated—both sexually and vegetatively. It can be planted using seeds, seedlings, saplings, root suckers, or tissue culture. However, it is normally grown from seed, either planted directly on the site or transplanted as seedlings from a nursery.

2.1.7. Seed Collection and Processing

Naturally, ripened fruits drop on the ground and are to be collected within 1-2 days for further processing (NRC, 1992). First, the pulp is removed and the seeds washed clean. Seeds are air dried for 3-7 days in the shade or until the moisture content is about 30%. They can be then stored up to four months if kept at 15^0 C. Seeds will remain viable even longer if dried to 6-7% moisture content and refrigerated in sealed containers at 4^0C (NRC, 1992).

2.2.0. ANTI-NUTRITIONAL FACTORS

Anti-nutritional factors (ANF) are compounds which act to reduce nutrient utilization and or food intake and play a major role in limiting the wider use of many plants. They are natural compounds capable of precipitating deleterious effects in man and animals. The levels of toxic substances in plants vary with the specie of plant, cultivar and post-harvest treatment such as soaking, drying, autoclaving and seed germination (Osagie, 1998).

According to Barnes *et al*. (1984), they may occur as natural constituents of plant and animal feeds, as artificial factors added during processing or as contaminants of the ecosystem. They are highly biologically active (Zenk, 1991) and ingestion of feed containing such substances induces, in some cases, chronic intoxication and in others interfere with the digestion and utilization of dietary protein and carbohydrate and also interferes with availability of some mineral, thus feed efficiency and growth rate and consequently production of products (Barnes *et al.*, 1984). They include saponins, tannins, flavonoids, alkaloids, trypsin (protease)

inhibitors, oxalates, phytates, haemagluttinins (lectins), cyanogenic glycosides, cardiac glycosides, coumarins and gossypol (Sugano *et al*; 1993). Bone (1979) reported that if there are toxic elements in the feed, abnormalities in the weights of liver and kidney would be observed. The abnormalities will arise because of increased metabolic rate of the organs in an attempt to reduce these toxic elements or anti-nutritional factors to non toxic metabolites. Therefore (Soetan and Oyewole, 2009), ways and means of eliminating or reducing their levels to the bearest minimum should be discovered. Akinmutimi (2004) however, noted that most processing methods employed in improving feed value of non conventional or alternative feedstuff do not completely eliminate anti-nutritional substances but only reduce their concentration to tolerable levels in feedstuffs.

2.2.1.0. Some Anti-nutritional Factors or Metabolites of Neem

Chemical investigation of the products of neem tree was extensively undertaken in the middle of the twentieth century (Biswas *et al*., 2002). More than 135 compounds (Biswas *et al*., 2002) have also been isolated from different parts of neem tree and several reviews have also been published on the chemistry and structural diversity of these compounds. The compounds have been divided into two major classes; isoprenoids and nonisoprenoids (Devakumar *et al*., 1996).

The isoprenoids include diterpenoids containing protomelicians, liminoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin types of compounds and C-secomeliacians such as nimbin, salanim and azadirachtin. The nonisoprenoids include proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavenoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, and others.

2.2.1.1. Nimbidin

According to Biswas *et al*. (2002) nimbidin is a major crude bitter principle extracted from the oil from the seed kernels of *Azadirachta indica*. Several bioactivities of nimbidin on many organisms have been reported by many researchers. Pillai *et al*. (1981) indicated that oral administration of nimbidin demonstrated significant hypoglycaemic effect in fasting rabbits. It can also inhibit completely the growth of *Mycobacterium tuberculosis* invitro in many organisms and was thus found to possess bactericidal effect (Murthy *et al*., 1958). Nimbidin demonstrated antifungal activity by inhibiting the growth of *Tinea rubrum* in rats (Murthy *et al*., 1958).

2.2.1.2. Azadirachtin

It is highly oxygenated C-secomeliacin isolated from neem seed which was reported to possess anti-feedant activity (Kraus, 1995; Govindachari, 1992), and anti-malarial property (Jones *et al*., 1994) by inhibiting the development of malarial parasites. Azadirachtin is a complex, modified tetranortriterpenoid found in extracts of the neem tree and also one of the most powerful plant-derived insecticides known (Govindachari, 1992; Jacobson, 1989). A large part of the high level of enthusiasm for the use of azadirachtin as a natural pesticide is due to the compound's potential safety to humans and other warm blooded animals (Saxena, 1999). Aerts (1997), noted that azadirachtin related neem triterpenoids extracts may have beneficial effect on humans. A study conducted by Beard (1989) indicated that pure azadirachtin is not toxic to humans, while more recent study estimates at least 15mg of azadirachtin per kg of body weight could be taken safely by humans each day, which is well within range for use as a pesticide.

2.2.1.3. Salanin

Salanin is a third triterpenoid extractable from neem. Studies (NRC, 1992; Meisner *et al*., 1981) indicate that this compound powerfully inhibits feeding, but does not influence insects' molts and has strong anti-feedant deterrency against several insect species. The migratory locust, California red scale, striped cucumber beetle, houseflies and Japanese beetle have been strongly deterred in both laboratory and field test.

2.2.1.4. Nimbin

This compound has been found to have antiviral activity. It affects potato virus X, vaccinia virus and fowl pox virus. It could perhaps open a way to control these and other viral diseases of crops and livestock (NRC, 1992).

2.3. 0. METHODS USED TO REMOVE ANTI-NUTRITIONAL FACTORS IN PLANT PRODUCTS.

2.3.1. Autoclaving

Autoclaving entails cooking under pressure. The time of cooking is shortened by this method (Akande *et al*., 2010). Udedibie *et al.* (1988) reported that when jack beans were autoclaved for 30 minutes at 125^{o}C and 151b pressure, thermo-labile inhibitory substances such as cyanogenic glycosides, saponins, terpenoids and alkaloids could not be detected after autoclaving. Kessler *et al*. (1990) stated that there was little nutritional advantage in autoclaving for more than half an hour. They reported that autoclaving of Jack beans was a satisfactory technique for ensuring survival of birds receiving jackbeans diets.

Autoclaving for 10 minutes ameliorated the necrotizing effect but did not improve upon its feeding quality. However, combined heat treatment and water washing rendered the neem kernel as good protein supplement compared to groundnut cake (Uko and Kamalu, 2008).

Even though the processed neem kernel supported final weight of broilers similar to groundnut cake, growth rate of the experimental birds fluctuated throughout the period of feeding (Uko and Kamalu, 2008).

2.3.2. Urea Treatment

Urea is a very strong protein – denaturing agent and can achieve this by competing for hydrogen bonds with the peptide backbone, thereby breaking up the secondary structure of these native protein and disrupting their biologically active structures (Udedibie and Nkwocha, 1990).

Raw jackbean seeds were soaked in 3% solution of urea for 6 days at room temperature in plastic containers. During this period, strong ammonia gas odour was released from the solution. At the end of the period, the beans were rinsed with tap water and then cooked for one hour, dried in oven at 80^{o}C and then ground. Feeding trials with the resultant jackbean meal involving young broiler chicks demonstrated that jackbeans so proccessed could be tolerated by broiler chicks of up to 25% inclusion levels in the diet (Udedibie and Nkwocha, 1990).

2.3.3. Soaking

Soaking could be one of the processes used to remove soluble anti-nutritional factors, which can be eliminated with the soaking solution (Akande *et al*., 2010). However, some metabolic reactions can take place during soaking which will affect some of the constituent compounds (Vidal-valverde *et al*., 1992). Dhurandhar and Chang (1990) soaked navy and red kidney beans for 18 hours in water at ambient temperature and both showed insignificant decreases in typsin inhibitor activity. Nath *et al.* (1983) stated that the principal growth retarding factors of neem seed cake were believed to be water soluble. Odunsi *et al*. (2009) citing Nath *et al.* (1983), reported that soaking of neem seeds in water improves the crude protein content and

palatability of the cake. The higher crude protein content obtained (Odunsi *et al.*, 2009) in water-treated neem seeds than the untreated neem seeds after soaking seeds in water for 72 hours gave credence to the above assertion. Trugo *et al*. (1990) however, did not find any loss of activity when black beans were soaked in water for 16 hours.

2.4. Chemical Composition of Neem Seed and Neem Seed Cake.

The chemical composition of neem cake varies considerably depending on the type of processing such as solvents or expeller extraction and the type of kernel used; whether undecorticated or decorticated (Gowda and Sastry, 2000). According to (NRC, 1992) toxic compounds in neem seed are slightly soluble in water and are freely soluble in organic solvents such as hydrocarbons, alcohols and ketones. The problem of putting the seed cake to use still remains; this is because neem tree scattered around the world is genetically distinct and it's nutritional potentials are affected by climatic condition, method of processing and to a lesser extent the genetic make up of the animals (NRC, 1992). The residue left after the oil has been removed varies widely in composition. The results of amino acid and proximate composition of raw neem seed and neem seed cake from different processing methods by Bawa *et al.* (2007), Odunsi *et al.* (2009) and Uko and Kamalu (2006), are respectively shown in Tables 2.1, 2.2 and 2.3.

Table 2.1: Proximate composition of raw neem seed meal and neem seed cake from different processing methods

Parameter (%)	RNSM	HNSC	SNSC	ENSC
Dry matter	89.87	86.29	88.96	91.35
Crude Protein	23.19	22.69	23.06	22.50
Crude Fibre	9.35	4.14	7.80	12.60
Oil	38.61	21.02	33.44	22.22
Ash	9.33	7.16	8.26	8.95

RNSM: Raw neem seed meal, HNSC: Hydraulic press neem seed cake, SNSC: Solvent extracted neem seed cake, ENSC: Expeller neem seed cake.

Source: Bawa *et al.* (2007).

Table 2.2: Proximate composition of untreated neem seed cake and water soaked neem seed cake.

Constituents (%)	Untreated neem seed cake	Water soaked neem seed cake
Dry matter	89.26	89.74
Crude Protein	14.24	17.48
Crude fibre	17.26	19.59
Ash	8.21	9.82
NFE	45.69	38.73
Ether Extract	3.66	4.12
Gross energy (kcal/kg)	3252	3148

Source: Odunsi *et al.* (2009)

Table 2.3: Nutritional value of raw full-fat neem kernel (g/kg)

Constituents	**Composition (g/kg)**
Crude Protein	302.1
Crude Fat	453.2
Crude Fibre	103.6

Mineral (Ash)	46.1
NFE	91
Amino Acids	
Alanine	6.01
Arginine	1.70
Aspartic acid	8.66
Cystine	0.76
Glycine	9.66
Glutamic acid	13.06
Histidine	2.72
Isoleusine	3.50
Leusine	7.72
Lysine	4.06
Methionine	0.58
Phenlalanine	4.60
Proline	1.44
Serine	6.50
Threonine	4.08
Tyrosine	4.30
Valine	2.99

Mean of two determinations: on air dry basis; NFE: Nitrogen free extract

Source: Uko and Kamalu. (2006)

2.5. Chemical composition of soya bean meal

Soya bean meal is by far the most important protein used in poultry feeding today. It is produced by removing the oil from soyabean by a solvent extraction process (Austic *et al*., 1990). If soyabean meal contains most of the soyabean hull, it usually contains 44% protein, whereas dehulled soyabean meal normally contains 48% protein (Austic *et al*., 1990). Tisch (2006), however, gives the range of protein content of dehulled soyabean meal at between 44 and 49%. The dehulled meal is most commonly used in poultry feeding because it has a higher energy value than the meal containing 44% protein (Austic *et al*., 1990).

In their unextracted state (Smith, 2001) soyabean meals are high in fat (18%), low in fibre (5%), and have 38% protein. Soyabeans are rather low in calcium (0.25%) and phosphorus (0.60%).

The nutritive value of soyabeans and soya oil meal for poultry is increased by cooking. Cooking greatly increases the value and availability of protein for poultry and also destroys trypsin inhibitors contained within the seed. This substance depresses the growth of non-ruminants and prevents the action of proteins digestive enzymes. Raw soyabean protein has therefore a low feeding value for poultry but properly cooked whole soybean or soyabean oil meal provides protein which is nearly equal in value to the processed meal (Austic *et al*., 1990)

Table 2.4: Crude protein and essential amino acids in soya bean meal (DM basis)

Composition (%)	**Soya bean meal, solvent extracted**
Dry matter	88.4
Crude protein	47.5
Essential amino acids	
Arginine	3.5
Cysteine	0.7
Histidine	1.3
Isoleusine	2.1
Leusine	3.7
Methionine	0.7
Lysine	3.0
Phenylalaline	2.3
Threonine	1.9
Tryptophan	0.7
Valine	2.2

Source: NRC, (1994)

2.6.0 Effect of nutrition on haematological and biochemical blood components of broilers

Generally, both the haematological and biochemical components of the blood are influenced by the quantity and quality of feed and also the level of anti-nutritional elements or factors

present in the feed (Akinmutimi, 2004). Biochemical components are sensitive to elements of toxicity in feeds. Esonu *et al*. (2006) noted that haematological parameters are good indicators of the physiological status of the animal and its changes are of value in assessing the response of animals to various physiological situations. It has been observed by many research workers (Gilbert, 1968, Khan *et al*., 1987; Abdel-Hameed *et al*., 1972) that, there is a definite change in the profile of the blood cell throughout life. Available information indicates that haematological values of avian species are also significantly influenced by poultry diseases including fowl typhoid (Kokosharov *et al*., 1987), mycoplasmolysis (Branton *et al*., 1997), avian coccidiosis (Koinarski *et al*., 2001) and Newcastle disease (Galindo-Muniz, 2001).

2.6.1.0: Haematological factors of the blood

2.6.1.1. Red blood cell (RBC)

The major function of the red blood cell (RBC) is to transport haemoglobin (Hb), which in turn carries oxygen from the lungs into the tissues (Waugh *et al*., 2001). According to Moss (1999), reduction in the haemoglobin may be accompanied by a fall in the RBC and packed cell volume (haematocrit) and added that very low readings for RBC, Hb, and haematocrit can indicate anaemia.

2.6.2.0. Biochemical factors of the blood

2.6.2.1. Total Protein (TP)

Total serum protein retained has been reported as an indication of the protein retained in animal's body (Akinola and Abiola, 1991; Esonu *et a*l., 2001), while total blood protein and

creatinine contents have been shown to depend on the quality and quantity of dietary protein (Esonu *et al.,* 2001).

2.6.2.2. Globulins

The globulins are composed of three fractions, designated alpha, beta and gamma globulins. Alpha-globulins are a group of proteins manufactured almost entirely by the liver. Normally, these proteins increase with acute nephritis, severe active hepatitis, active, usually systemic inflammation, malnutrition and in nephrotic syndromes (Margaret, 2001).

Globulin level has been used as indicator of immune responses and source of antibody production (Abdel-fatta *et al*., 2008). According to Griminger (1986), high globulin level and low A/G (Albumn/ Globulin) ratio signify better disease resistance and immune response. Albumin serves as the major reservoir of protein and is involved in colloidal osmotic pressure, acid-base balance, and it acts as a transport carrier for small molecules such as vitamins, minerals, hormones and fatty acids (Margaret, 2001).

Lumeij (1997) also submitted that in acute or chronic conditions, a rise in total protein caused by elevated globulin fraction may occur. Often albumin concentrations are decreased in these situations. The combined effect of these changes is a decrease in the albumin/globulin ratio. Often the total protein concentration is within the reference range, while the albumin/globulin ratio is decreased; therefore the albumin/globulin ratio is often of greater clinical significance than the total protein from globulin.

2.7. Normal physiological ranges of biochemical and haematological components for broilers

Table 2.5: Normal physiological ranges of biochemical and haematological components for broilers

Haematological Components	
Parameter	Reference Range
Haemoglobin (g/dl)	7.0-13.0
Total Red Blood Cell ($x10^6$µl)	2.5-3.5
PCV (%)	22-35
MCV (fl)	90-140
MCH (pg)	33-47
MCHC (g/dl)	26-35
Total White Blood Cell ($x10^6$µl)	1.2-3.0
Biochemical Components	
Total Protein (mg/dl)	3-4.9
Cholesterol (mg/dl)	129-297

Source: Jain (1993) as cited by Aeangwanich *et al*., (2004).

2.8. Effect of neem products on haematological and biochemical components of the blood of broilers

In an experiment to evaluate the physiological response of laying birds to neem (*Azadirachta indica*) leaf meal based diet; body weight, organ characteristics and haematology, Esonu *et al.* (2006) reported that the RBC levels of the birds on the test diets was higher than the normal count 3.0 x 100/L for birds in the temperate environment. The conclusion was that the birds were affected by relative polycythaemia (a disease stage in which the proportion of blood volume that is occupied by the red blood cells increases due to an increase in the red blood cells (absolute polycythaemia) or decrease in the volume of plasma caused by a loss of body fluids such as through burns, dehydration and stress. Ogbuewu *et al.* (2010) reported that the pre-puberal rabbits fed different levels of neem leaf meal were not rendered anaemic by the diets since the RBC level was within the normal range for rabbits.

James *et al.* (2009) performed an experiment on biochemical and histological effect of dietary substitution with solvent extracted neem seed cake on albino rats (wistar strain). The results indicated that there was no significant ($p>0.05$) difference in the PCV and Hb of animals fed methanol-processed neem seed cake (MNSC) diet compared to the animals fed with standard protein diet. All the animals fed with the neem seed cake, however, showed significant ($p<0.05$) increases in albumin and protein composition compared to the standard diet. The conclusion was that, the processed neem cake for the experiment contained a quality protein.

Bawa *et al*. (2007) fed rabbits with processed neem seed cake and raw neem seed meal; the results (Table 2.6) revealed that rabbits fed the raw neem seed meal diet (RNMSD) had significantly ($p<0.05$) lower values than those fed the control and processed neem seed cake diets for packed cell volume (PCV), total protein (TP) and haemoglobin (HB). This, they noted, was an indication of negative effects of bitter principles of neem on the animals.

Table 2.6: Blood profile of rabbits fed experimental diets.

Parameter	1	2	3	4	5	SEM	S.L

	Control	RNSM	HNSCD	SNSCD	ENSCD		
PCV	42.0[a]	27.5[b]	38.0[a]	45.0[a]	43.5[a]	3.51	*
Total protein	70.0[a]	57.5[c]	75.0[a]	67.5[b]	70.0[a]	0.23	*
Haemoglobin	13.95[a]	9.51[b]	12.64[a]	13.45[a]	14.55[a]	1.18	*

a,b,c Means values in the same row with different superscripts differ. *Significantly ($p<0.05$) different.S. L: Significant level. RNSM-raw neem seed meal, HNSCD-hydraulic pressed neem cake diet, SNSCD-solvent extracted neem seed cake diet, ENSCD- expeller neem seed cake diet

Source: Bawa *et al*. (2007).

2.9. Carcass Characteristics of Birds Fed Neem Products.

In a 35-day feeding trial with cockerels fed raw full-fat neem kernel (RFK) and autoclaved full-fat neem kernel (AFK) at inclusion levels of 75, 150, 225 g/kg to determine the protein quality and toxicity of full-fat neem (*Azadirachta indica* A Juss), Uko and Kamalu (2006)

reported that eviscerated carcass weight decreased significantly ($p<0.05$) with increased levels of neem kernel (Table 2.7). The relative weights of the organs were not influenced by the dietary treatments except that of the lungs, liver and kidneys. Whereas only 225g AFK diet increased ($p<0.05$), the weights of the lungs, kidneys and liver of chicks on all AFK diets were markedly heavier ($p<0.01$) than those on the control diet. The reason for the relative increase in weight of the liver, kidneys and lungs was attributed to a compensatory reaction to inactivate and excrete the absorbed toxic neem substances from the body.

Table 2.7: Carcass and organ measurements of cockerels offered control or neem diets for 35 days

Diet	Control	AFK	AFK	AFK	RFK	SEM
Measurements	-	75.0	150.0	225.0	75.0	
Carcass weight (g/chick)	187.1^{a}	167.7^{b}	144.4^{b}	110.6^{c}	166.8^{b}	11.57**
Carcass yield (%)	73.5^{b}	73.6^{b}	76.9^{b}	81.8^{a}	74.7^{b}	1.69**
Organ weight (g/kg carcass weight)						
Lungs	8.5^{b}	8.5^{b}	9.3^{b}	11.8^{a}	11.0^{ab}	0.90*
Heart	10.4	10.9	10.3	11.8	10.8	1.38
liver	41.2^{b}	49.0^{a}	48.4^{ab}	55.8	41.3^{b}	3.10**
Proventriculus	9.6	11.1	10.9	12.0	9.7	1.50
Gizzard	42.6	52.8	44.7	52.7	47.4	4.0
Intestine	318.9	408.8	403.3	440.8	346.6	20.70
Pancreas	5.4	7.2	6.6	6.7	6.0	0.08

Kidney	16.9^{b}	17.0^{b}	18.1^{b}	23.7^{a}	19.9^{ab}	2.40*

$^{a\,b\,c}$ Means in a row with different superscripts differ significantly *p<0.05 **p<0.01

Source: Uko and Kamalu (2006).

2.10: Effect of the use of urea-containing diets on the blood profile of broilers

Urea is a white crystalline deliquescent solid substance which contains 42-45% nitrogen. Pure protein contains only 16 % nitrogen, thus urea has the protein equivalent of 261 - 281% as to its nitrogen content (Kornegoy *et al.*, 1970; Okumura *et al*. 1976). The toxicity of urea occurs due to local and generalized effects of ammonia released in sufficient amounts. Haematological changes appear in the form of decreased total erythrocytes, haemoglobin, packed cell volume and erythrocyte sedimentation rate. Differential leukocytic counts revealed lymphopenia, heterophilia and monocytosis in birds kept in diets containing urea (Chandra *et al*., 1984a). Urea ammoniated diets may be responsible for depression of growth, feed intake and efficiency of nutrient utilization (Abdou *et al*., 2006). Decrease in total leukocytic count, low alanine aminotransferases activities and increase in blood urea were observed in chicks fed urea containing diet (Nagalakshmi *et al*., 1999).

2.11. The use of neem products (neem seed meal, neem seed or kernel cake and neem leaf meal) as feed ingredients

Neem seed cake is a by-product of neem oil industry. It is a non-conventional feed ingredient which has a great potential for livestock feeding (Bawa *et al.,* 2005). Feeding neem seed cake in raw form to livestock is generally discouraged due to the presence of bitter triterpenoids (Musalia *et al.,* 2000). A feeding trial with neem seed meal (2.5%) on chicks (Jacobson, 1985), indicated mild to severe changes in weight of kidney, liver, spleen, intestine and heart. Retardation of spermatogenesis was also observed by feeding neem seed cake to rats

(Jacobson, 1985). Calves fed with neem seed cake showed reduced haemoglobin content in the blood, along with depression (Jacobson, 1985).

However, several works conducted on neem cake by other researchers indicated that feeding neem products (neem seed meal, neem seed or kernel cake and neem leaf meal) to animals could be encouraged. Kabeh *et al.* (2007) and Girish and Shakara (2008) stated that despite the bitter components, livestocks consume diets containing varied percentage of neem cake.

Uko and Kamalu (2008) observed that autoclaved and non extracted neem kernel produced the highest feed conversion efficiency and concluded that its use may, therefore, be popularised in developing countries where use has not yet been found for neem seed cake.

Odunsi *et al*. (2009) fed untreated neem seed cake (UNSC), charcoal-treated neem seed cake (CNSC) and water-treated neem seed cake (WNSC) to cockerels at 10% and 20% inclusion levels. The results on the performance of the cockerels fed untreated and treated neem seed cake diets showed that feed intake, weight gain and feed cost per kilogram weight gain were significantly ($p<0.05$) different across the dietary treatments. Feed gain ratio however did not show any significant difference (Table 2.8). Improved weight gain and feed consumption were evident ($p<0.05$) in WNSC at 10, 20 and 10% of CNSC. Feed cost per kilogram weight gain of the improved techniques revealed that soaking of neem seed cake in water was most cost effective followed by CNSC in addition to their practicality and simplicity. The marginal rise in feed cost noted with WNSC was due to labour costs accrued from soaking and sun drying. The conclusion was that soaking of the neem seed in water and charcoal supplementation were better and cost effective improvement technique even at 10 and 20% soyabean meal replacement levels.

Table 2.8: Performance of cockerels fed treated and untreated neem seed cake based diets

	UNSC (%)		WNSC (%)		CNSC (%)		SEM
Parameters	10	20	10	20	10	20	
Feed intake (g)	42.6^{ab}	41.^{4b}	44.8^{a}	42.5^{ab}	42.6^{ab}	40.7^{b}	0.91
BWG (g/b/d	7.03^{ab}	6.83^{b}	7.66^{b}	7.35^{a}	7.37^{a}	7.00^{ab}	0.14
Feed/gain ratio	6.06	6.06	5.84	5.71	5.87	5.83	0.14
Feed cost/kg (₦)	40.4	40.0	40.6	40.2	40.5	40.1	0.14
Feed cost /kg BWG (₦kg)	245.1^{a}	242.4^{a}	237.2^{ab}	229.4^{b}	237.7^{ab}	233.5^{b}	4.6

^{ab}Means bearing different superscript in the same row differ significantly ($p<0.05$). SEM: Standard Error of Means.

Source: Odunsi *et al.*, (2009).

In 8 weeks of feeding trial to determine the trend of food consumption and feed efficiency of broilers with raw or heat-treated neem kernels at 15 and 22.5% inclusion levels to replace groundnut cake, Uko and Kamalu (2008) reported that broiler chicks offered control, raw neem or toasted neem diets consumed similar quantities of feed and gain weight at similar rate. Feed efficiency were also similar ($p>0.05$) among the dietary groups (Table 2.9). The conclusion was that the similarity in weight gain and intake was an indication that the residue (neem cake) can replace groundnut cake in conventional diets for broilers chicks. It was also, noted that the poorer performance of birds on raw neem diets was, perhaps, due to neem toxicity. This was further supported by the non-significant differences in feed efficiency. Other conclusion drawn on this was that the in- appetence explains lower weight of birds on raw neem diets and not indigestion of the diets

Table 2.9: Growth performance of broilers on control and neem kernel diets (mean± *s.d*)

Diets	**Performance**

	Weight Gain (g/bird/day)	Feed intake (g/bird/day)	Feed Efficiency (gain/food)
Control	23.2± 2.4[b]	51.8 ± 3.2[b]	0.48± 0.12
15.5% raw neem	21.9± 1.3[b]	48.3± 2.5[b]	0.47± 0.08
22.5% raw neem	18.1± 1.4[c]	42.0± 2.5[c]	0.42± 0.9
15.0% autoclaved neem	25.2± 2.09[a]	51.6± 3.0[b]	0.50± 0.12
22.5% autoclaved neem	27.6± 1.6[a]	57.4±. 3.2[a]	0.47± 0.14
15.0% toasted neem	23.8± 1.9[a]	51.00± 3.0[b]	0.43± 0.45
22.5% toasted neem	21.6± 2.6[a]	53.6 ± 3.5[b]	0.40± 0.09

[a b c] Means in column bearing different superscripts differ significantly ($p<0.05$)

Source: Uko and Kamalu (2008).

A study was conducted by Bawa *et al*. (2007), on the effect of different methods of processing neem (*Azdiractha indica*) seeds on performance of young rabbits. Diet 1 (control), was a maize-groundnut cake based diet without neem seed. Diets 2, 3, 4 and 5 had raw milled neem seed (RMNS), hydraulic press neem seed cake (HNSC), solvent extracted neem seed cake (SNSC) and expeller neem seed cake (ENSC) at 20% inclusion levels of the diets respectively. The result (Table 2.10.) showed that the final live weight was significantly ($p<0.05$) different across the dietary treatments. Animals on the control diet had the highest final live weight. Live weights of rabbits fed solvent extracted neem seed cake were significantly ($p<0.05$) different from those fed the raw neem seed meal, hydraulic press neem cake and the control diet.

Cost per kg gain in weight ranged from N67.67 – N87.67. The highest feed cost per kg weight gain was obtained on rabbits fed raw neem seed meal diet (T2) and this could be attributed to poor rate of feed conversion which may be due to the level of bitter principles in the diet. However hydraulic press neem seed cake diets had the least feed cost per kg gain among dietary treatments. There was no mortality across the dietary treatments. The

inference drawn from this was that solvent extraction as a method of processing should be employed in the processing of neem seeds to be included in rabbit diets.

Table 2.10: Performance of rabbits fed experimental (neem) diets

Parameter	Treatment					SEM	S.L
	1 Control	2 RNSMD	3 HNSCD	4 SNSCD	5 ENSCD		
Initial live weight (g)	543.75	600.00	550.00	566.67	543.75	52.29	NS
Final live weight (g)	1642.67[a]	975.00[c]	1125.00[c]	1325.00[b]	1300.00[b]	50.60	*
Daily feed intake (g)	34.62[a]	23.07[b]	28.53[b]	31.38[ab]	31.07[ab]	5.05	*
Daily weight gain (g)	11.31[a]	5.76[b]	7.99[ab]	8.46[ab]	8.43[ab]	1.20	*
Cost/kg gain (N)	72.11	87.67	67.67	72.67	70.26	8.07	

[a, b, c]: Means value the same row with different superscript differ significantly (P<0.05)

NS: Non significant difference * Significant (P<0.05) difference

Source: Bawa *et al*. (2007)

Uko and Kamalu (2006) fed cockerels with raw full-fat neem kernel (RFK) and autoclaved full-fat neem kernel (AFK) at inclusion levels of 75, 150, 225g/kg to determine the protein quality and toxicity of full-fat neem (*Azadirachta indica* A Juss) in a 35-days feeding trial. The results on growth performance showed that feed intake, water intake and live weight gain of cockerels on the reference diet were higher ($p<0.01$) than those fed the AFK or RFK diets (Table 2.11). Weight gain of chicks decreased as the AFK content of the diet increased. Autoclaving also, markedly ($p<0.05$) reduced the palatability of the neem diets with increasing inclusion levels of the neem kernel whereas consumption of the AFK diet did not differ from that of the reference diet. The influence of the diet on mortality was not significant ($p>0.05$), although increased content of the AFK tended to increase the incidence.

They concluded that heat treatment did not necessarily improve the feeding value of the neem kernel.

Table 2.11: Body weight, feed efficiency and survival rate of cockerel chicks fed experimental diets.

Performance traits	Control	AFK	AFK	AFK	RFK	SEM
	-	75.0	150.0	225.0	75.0	
Live weight (g/chick)	291.4[a]	229.8[b]	217.5[b]	149.7b	227.2[b]	21.24**
Weight gain (g/chick/d)	7.5[a]	5.8[b]	5.3[b]	4.1[c]	5.7[b]	0.50**
Food consumption (g/chick/d)	25.5[a]	16.4[b]	16.5[b]	16.3[b]	23.4[b]	2.4**
Water intake (ml/chick/d)	60.9[a]	41.8[b]	43.7[b]	41.0[b]	54.59[a]	2.75**
Gain: feed ratio	0.30[a]	0.34[a]	0.31[a]	0.25[b]	0.25[b]	0.037*
Gain: protein ratio	1.35[a]	1.53[a]	1.45[a]	1.16[b]	1.08[b]	0.222*
Gain: water ratio	0.12	0.13	0.12	0.09	0.10	0.015
Survivors (no/40)	38	39	37	36	38	-
Mortality (%)	5.0	2.50	7.5	10.0	5.0	0.16

[a, b, c] Different superscripts in row differ significantly *p<0.05; **p<0.01; SEM: standard error of mean

Source: Uko and Kamalu (2006).

James *et al.* (2009) conducted a research work to elucidate the performance of albino rats (wistar strain) at 3 weeks of age. They were fed neem seed cake (NSC) after treatment with solvents. Rats on treatments 1 and those on 2 and 3 were fed experimental diets containing water, 75% methanol, 75% ethanol processed neem cake respectively as a replacement for soya bean for 28 days. The results revealed a general decrease and increase in body weight of the rats on processed neem seed cake during the second and the third week respectively. The fourth week was a period of stunted growth, and a decrease in weight of animal fed water processed neem seed cake. The effect was attributed to a reduction of feed conversion with increase in the age of the animals. The water intake of rats fed processed neem cakes was significantly ($p<0.05$) lower than those on the standard diet. Increase in bulkiness of food or feed intake was said to be responsible for the increase in water intake whereas, the decrease

in the water intake of rats fed processed neem seed cake was blamed on the decrease in the feed intake.

The performance and economic indices of broilers fed varying dietary levels of sun dried neem leaf meal (NLM) were investigated by Onyimonyi *et al*. (2009) using ninety 'Ross' unsexed two weeks old broilers. The birds were randomly assigned to five treatment groups of eighteen birds each in which NLM was incorporated at 0, 0.5, 1.0, 1.5 and 2% for treatments 1, 2, 3, 4 and 5 respectively. The results showed that birds on the 0.5% NLM had significantly ($p<0.05$) superior AFBW, ADG and FCR. ADFI of birds on the 0.5% NLM was statistically the same with the control birds but differed ($p<0.05$) from the rest on NLM. Gross margin analysis revealed that a profit of N707.30 was made per bird on the 0.5% NLM as against N630.97, N620.73, N621.81 and N507.06 for birds on the control, 1.0, 1.5 and 2.0% NLM respectively. It was concluded that, inclusion of 0.5% NLM in the diets of broilers will support optimum performance and economic benefit.

2.12. INFERENCES FROM LITERATURE

Neem (*Azadirachta indica*) kernel meal, a protein rich source (CP: 34-38%) is an important oil industry by-product in India. Feeding raw neem seed to livestock is generally discouraged due to presence of bitter triterpenoids such as Azadirachtin, nimbin and salanin which restricts its inclusion levels in livestock feeds (Gowda and Sastry, 2000; Devakumar *et al*., 1993). However, increased nutritive value and palatability has been achieved through various methods such as water washing (Agrawal *et al*., 1987) alkali treatment (Katiyar *et al*., 1991) urea treatment (Musalia *et al*., 2000) and solvent treatment (Chand, 1987). According to Bawa *et al.* (2007) and James *et al*. (2009), feedindg neem seed cake and other non-conventional feedstuffs to farm animal results in significant ($p< 0.05$) depressed feed intake and weight gain with subsequent significant ($p<0.05$) reduced water intake. However (Uko

and Kamalu, 2008; Odunsi *et al*., 2009), reported that although neem diets depressed weight gain initially, birds developed tolerance to neem toxins overtime without negative effect on the overall weight gain, feed intake and feed efficiency.

Feeding neem cake and other neem products (e.g. neem leaf meal) to poultry and other livestock result in significant ($p<0.05$) increases in the organ weights of the heart, liver, gizzard (Esonu *et al.,* 2006; Uko and Kamlu, 2005; Uko and Kamalu, 2008) and the kidney (Bawa *et al*., 2007; Musalia *et al*., 2000). Results from some research works (James *et al*., 2009; Bawa *et al*., 2007; Esonu *et al*., 2006), also indicate that the incorporation of neem seed cake and neem leaf meal into livestock diets lead to significant ($p<0.05$) increases in some biochemical and the haematological components of the blood due the presence of the triterpenoids. A marginal rise in the cost of producing a kilogram live weight of animals fed diets containing neem products due to cost of processing, have been observed (Odunsi *et al*., 2009) by researchers.

The incorporation of neem products into livestock feed is being researched into in many parts of the World especially in India and Nigeria. However, the practice is not common in Ghana. The present study was therefore designed to investigate the performance of broiler chickens fed water and urea treated neem (*Azadirachta indica*) kernel cake as protein supplement.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location and Duration of the Experiment

The experiment was conducted at the Poultry Section of the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The site is located at latitude $06^0$41'N and longitude 01^0 33'W and is generally described as being in the hot humid forest zone. Rainfall averages 1510mm with temperature generally fluctuating between 21.5^0C and 31.0^0C and relative humidity between 67 and 89% on daily basis. The experiment lasted for eight (8) weeks, from 30th July to 24th September, 2010.

3.2 Collection and Preparation of Neem Seeds

The neem seeds were collected during the fruiting season from May to July, 2010 at Tamale in the Northern Region of Ghana. The collected seeds were sundried for six day and weighed after the drying period. The seeds were decorticated (dehulled) by using a heavy wooding structure to crush the seed coat and winnowed to separate the kernels from the hulls. Both the kernels and the hulls were weighed separately with a hanging scale to determine their respective percentage weights in the seeds. The kernels were divided into two equal parts and soaked separately in 250 litters open basin containing water for 72 hours. The kernels were poured into jute bags to drain the water after soaking. One part of the kernels was re-soaked in the basin containing a urea solution at 1kg: 10 litres concentration of urea and water respectively for 72 hours. All the kernels were sundried for five days after the treatments. The dried kernels were hammer milled to pass through a 2mm sieve and thereafter, sent for oil extraction.

3.3. Oil Extraction

The oil extraction of the neem kernel meal (NKM) was carried out at Technology Consulting Centre (TCC) at KNUST, Kumasi. Prior to the extraction, the NKM was packed in cotton (batik) bags with sides 30cm x 10cm and preheated in an oven for 10 minutes to facilitate the extraction process. The sacks containing the NKM were packed in batches into a silver container (tub) which had a screw rod on top. The screw rod was used to squeeze the NKM in the tub until a greater percentage of the oil was removed. A rubber container was used to collect the oil that was pressed out of the NKM. The volume of the oil (for the two treated NKM) was taken with a graduated rubber container. The neem kernel cake (NKC) obtained after the oil extraction was weighed, sundried for four days and hammer milled. These were then incorporated into the various treatment diets of the broilers as water treated neem kernel cake (WNKC) and water and urea treated neem kernel cake (WUNKC).

3.4. Experimental Birds and Management

A total of 300 day-old Cobb 500 broiler chicks were randomly allocated to five (5) treatment groups with three (3) replicates of twenty (20) birds each. The design used for the experiment was completely randomized designed (CRD). The birds were placed in a brooder house with wood shavings as litter throughout the 8 weeks of the experiment. Feed and water were given *ad libitum* from the brooding to the finishing stage.

3.5. Brooding and Rearing Stage

A total of 300 day-old Cobb 500 broiler birds purchased from Akate Farm in Kumasi were brooded for 3 weeks in 15 deep litter pens with a floor space of one bird per square feet. The initial weights (41.10g) of the day- old chicks were taken before placing them in the pens. Light was supplied using 100W onion bulbs to provide the heat needed to maintain the optimum temperature range for the birds during the brooding period. Kerosene lanterns were also provided to supply light to the birds in times of power failure. Fifteen (15) flat feeders and 4-litter drinkers (1 in each pen) were used to supply feed and water respectively to the birds. Feeding was carried out twice daily between the hours of 07.00 and 16.00 GMT. Feed was weighed with a 20kg top pan scale before it was offered to the birds. Level of feed in the feeder was kept half way to reduce wastage of feed by the birds. Feed left in each feeder was stirred regularly with the hand to prevent it from caking before another feed was added. Pieces of wood shavings, crumbled feed and droppings of the birds in the feeders were also removed regularly.

At the end of the third week, the wooden troughs were replaced by standard tube feeders. The four-litre drinkers were also replaced by 11-litre drinkers. Each of these was hanged in

each pen. The heights of both the drinkers and the feeders were adjusted regularly to the shoulder level of the birds to reduce water and feed wastage respectively.

3.6: Experimental Diets

Five (5) diets were formulated (Table 3.1). Diet 1, 0%NKC (control or reference diet), had no NKC inclusions. Diets two (2) and three (3), contained WNKC at 5% and 10% levels respectively, whilst diets four (4) and five (5), contained 5% and 10% levels of WUNKC respectively, partially replacing soya bean meal (SBM) and wheat bran (WB). Diets 2-5 were referred to as NKC or test diets.

Additionally, diets 1-5 contained maize, wheat bran fish meal, dicalcium phosphate, vitamin and trace mineral premix and common salt. All the experimental diets were isonitrogenous. Both the starter (Table 3.1) and finisher (Table 3.2) diets contained crude protein (CP) levels of 20-21% and 18-19% respectively. The maize for the experiment was purchased from Kumasi central market. The soyabean meal and the other ingredients were respectively purchased from Emmapab Enterprise and Akropong Farms in Kumasi.

Table 3.1: The percentage inclusion levels and chemical composition of the starter diets fed to the broilers (as fed basis)

Ingredients (%)	**Dietary treatments**				
	0%NKC	5%WNKC	10%WNKC	5%WUNKC	10%WUNKC
Maize	61.00	60.00	61.00	60.00	61.00
Wheat bran	10.00	8.95	5.95	10.95	8.95
WNKC	_	5.00	10.00	_	_
WUNKC	_	_	_	5.00	10.00
Soya bean meal	16.95	14.00	11.00	12.00	8.00
Fish meal	9.00	9.00	9.00	9.00	9.00
Oyster shell	0.50	0.50	0.50	0.50	0.50
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Vit/tm premix	0.30	0.30	0.30	0.30	0.30
Common salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00

Calculated composition (%)					
Crude protein (CP)	20.18	20.27	20.19	20.13	20.22
Crude fibre (CF)	3.77	4.05	4.17	4.17	4.35
Ether extract (EE)	3.21	4.05	4.17	4.17	4.35
Calcium	1.03	1.02	1.00	1.01	1.00
Available phosphorus	0.52	0.50	0.49	0.50	0.49
ME (kcal/kg)	2783.69	2917.75	3092.05	2892.91	3051.97
Analysed composition (%)					
Dry matter (DM)	89.00	88.50	89.00	88.50	88.50
Crude protein (CP)	18.3	18.4	18.6	18.4	18.7
Crude fibre (CF)	3.57	3.59	3.68	3.11	3.13
Ether extract (EE)	2.00	5.00	6.00	4.50	4.50
Ash	5.50	5.50	5.00	5.50	4.50
*NFE	70.63	67.51	66.72	68.49	69.17
*ME (kcal/kg)	3312.75	3417.65	3521.20	3446.05	3480.95

***calculated**

NFE- Nitrogen Free Extract

ME-Metabolizable Energy

Table 3.2: Percentage inclusion levels and chemical composition of the finisher diets (as fed basis)

Ingredients (%)	**Dietary Treatments**				
	0%NKC	5%WNKC	10%WNKC	5%WUNKC	10%WUNKC
Maize	65.00	64.00	65.00	64.00	65.00
Wheat bran	11.10	10.05	7.05	12.05	10.05
WNKC	_	5.00	10.00	_	_
WUNKC	_	_	_	5.00	10.00
Soya bean meal	14.45	11.50	8.50	9.50	5.50
Fish meal	7.00	7.00	7.00	7.00	7.00
Oyster shell	0.45	0.45	0.45	0.45	0.45
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50
Vit/tm premix[1]	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition (%)					
Crude protein (CP)	18.31	18.40	18.33	18.26	18.35
Crude fibre (CF)	3.77	4.07	4.19	4.18	4.37
Ether extract (EE)	4.27	5.20	7.15	5.12	6.96
Calcium	0.823	0.812	0.799	0.810	0.795
Available phosphorus	0.423	0.410	0.393	0.412	0.395

ME (kcal/kg)	2824.64	2958.70	3133.30	2933.86	3082.92
***Analysed Composition* (%)**					
Dry matter (DM)	88.50	88.00	88.50	88.50	89.00
Crude protein (CP)	19.50	22.90	20.70	19.00	18.40
Crude fibre (CF)	3.46	4.02	4.67	5.10	3.59
Ether extract (EE)	1.00	0.50	0.50	0.50	0.50
Ash	4.50	5.00	4.50	4.00	4.50
*NFE	71.54	68.08	69.63	71.40	73.01
*ME (kcal/kg)	3307.20	3271.00	3243.85	3242.90	3277.05

*-Calculated

[1]Vit/tm premix provided the following per kg of the diet: Iron- 100mg, Manganese-110mg, Copper-20mg, Zinc-100mg, Iodine-2mg, Selenium-0.2 Cholecarciferol-25mg, Cobalt-0.6mg, Sanoquine-0.6mg, Retinal-2000mg, Alpha-tocopherol-23000mg, Menadione-1.33mg, cobalamin-0.03mg, Thiamin-0.83mg, Riboflavin-2mg, Folic acid-0.33mg, Biotin-0.03mg, Pantothenic acid-3.75mg, Niacin 23.3mg, Pyridoxine-1.33mg.

3.7.0 PARAMETERS MEASURED

3.7.1: Feed intake

The amount of feed for each day was noted before it was given to the birds. The amount left in the feeders was deducted from the feed offered in the previous day as feed intake for the day. Feed intake was recorded on replicate basis and values recorded were used to compute the average weekly feed intake. The average weekly intake was divided by the number of birds and further divided by seven for average feed intake per bird per day.

3.7.2. Water intake

Volume of water for each day was noted before it was offered to the birds. The volume of water left in the drinkers was measured and recorded as water refused. This was deducted from the quantity offered in the previous day as water intake for the day. The figures obtained were summed up and divided by the number of birds per each replicate for the weekly average. This figure was again, divided by seven for average water intake per bird per day.

3.7.3. Body Weight Gain

The weight of the birds in each replicate was taken at the beginning of each weighing week with the aid of a 20kg top pan scale. This was recorded as the initial body weight. The body weight for the ensuing week was recorded as the final body weight. The initial body weight was deducted from the final body weight to obtain the body weight gained for the week.

3.7.4. Feed Conversion Ratio

The feed conversion ratio of the birds was calculated as the ratio between feed intake and body weight gain.

3.7.5 Carcass Analysis

Nine (9) birds were randomly selected per treatment (i.e 3 birds per replicate) for carcass cuts and organ examination at the end of the experiment. Water and feed were withdrawn for 6 hours before the cervical cut of the birds. Each bird was tagged and weighed before and after slaughtering to determine the live and bled weight respectively. Other parameters taken included: defeathered weight, dressed weight, shank, intestine, head, liver, and gizzard. These weights were expressed as the percentage of the final live weight of the birds. The dressing percentages were calculated as:

$$\text{Dressing percentage} = \frac{\text{Dressed weight} \times 100\%}{\text{Live weight}}$$

3.7.6. Blood Analysis

On days 28 and 56, blood samples meant for the haematological - packed cell volume (PCV), haemoglobin concentration (HB) red blood cell (RBC) white blood cell (WBC) etc. and

biochemical - high density lipoprotein (HDL), low density lipoprotein (LDL) albumin etc., analysis were drawn from the wing veins of 9 birds randomly selected from each treatment. The blood samples were collected in Ethylene Diamine Tetra Acetate (EDTA). The Haematological parameters of the blood were determined by 'The Sysmex KX-2IN Autoanalyzer' and the biochemical factors with 'Flexor Jnr. Autoanalyser'.

3.8. Medication and Vaccination

The vaccination programme was planned and strictly followed in accordance with the immunoprophylactic and preventive guide for broilers recommended by the Veterinary division of the Ministry of Food and Agriculture in Ghana until the 5th week that the birds were attacked by Newcastle disease. Dosages were given according to the specifications of the manufacturers.

3.9. Medication and Vaccination Schedules

Weeks	Days	Treatments
1	1	Glucose in water
	2-5	Antibiotics and vitamins
	6	Plain water
	7	1st Newcastle vaccine (HB1)
2	8	Plain water
	9-11	Coccidiostat
	12-13	Plain water
3	14-16	Antibiotics/vitamins
	17	Plain water
	18	Gumboro vaccine
	19	Plain water
4	20-22	Coccidiostat
	23-27	Plain water
	28	2nd Newcastle (Lasota)
5	29	Plain water
	30-34	Coccivit dex
	35	Plain water
6	36-38	Antibiotics/vitamins
	39	Plain water

7	40-44	Coccivit dex
	45	Plain water
	46-48	Antibiotics/vitamins
	49	Plain water
8	50-52	Coccivit dex
	53-56	Plain water

3.10. Culled birds

Birds which got paralysed were culled and recorded separately against their respective replicates as and when they occurred throughout the experimental period. Percentage of the number of culled birds was determined as;

$$\text{Percentage of culled birds} = \frac{\text{Number of culled birds} \times 100}{\text{Number of birds per treatment}}$$

3.11. Mortality

Mortalities were recorded against the respective replicates as and when they occurred throughout the experimental period. Percentage mortality was calculated as;

$$\text{Percentage mortality} = \frac{\text{Number of dead birds} \times 100}{\text{Number of birds per treatment}}$$

3.12. Post-mortem Examination and medication

Dead and sick birds were taken to the Veterinary Laboratories at KNUST and Amakom, in Kumasi for post mortem examinations and diagnosis respectively. Some signs of infected live birds were: coughing, sneezing, paralysis, and sudden death. Ulcerative enteritis, intestinal lesions and thicken air sacs with haemorrhages in the proventriculus were among the clinical signs of post-mortem examination. The signs were attributed to Newcastle disease infection by the Technicians, and upon advice, the birds were placed on coccivit dex (a sulphur based vaccine) and antibiotics intermittently from the fifth week till the end of the experiment to reduce the effect of the disease.

3.13. Economics of Production

The cost of feed per kg and the cost of feed consumed per bird to gain a kg body weight were the basis for the economics of production. The cost was calculated based on the prevailing market prices for the feed ingredients at the time of the experiment (Table 3.3). The cost of feed required to produce a kg weight gain was determined by multiplying the feed conversion ratio by the cost per kg weight of feed for the various treatments. Selling price was determined by the prevailing market price (GH¢5.20) per kg dressed weight of the birds at the time of the experiment multiplied by the number of dressed birds in each treatment.

Table 3.3: Feed ingredients and their prices per kg

Ingredients	Price per kg (GH¢/kg)
Maize	0.40
Soya bean meal	0.86
Fish meal	1.25
Wheat bran	0.30
Dicalcium Phosphate	3.00
Salt	2.00
Oyster shell	1.20
*WNKC	1.86
*WUNKC	2.20

*The feed cost/kg WNKC and WUNKC was computed from the price of 100kg of dehulled seeds as well as labour and processing cost at the time of the experiment

3.14. Chemical and Statistical Analysis

Proximate composition of the experimental diets and the treated neem products were determined by standard method of Association of Official Analytical Chemist (AOAC) (1990), at the Animal Science Department of KNUST. The data obtained were subjected to (two-way) analysis of variance (ANOVA) in a completely randomized design using SPSS (1999). Where analysis of variance indicated significant treatment effect, the means were compared using Duncan's Multiple Range Test (1995).

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

4.1: Proximate composition of neem kernel cake (NKC), from different processing methods

The result on the proximate composition of the NKC is shown in Table 4.1.

Table 4.1: Proximate composition of NKC from different processing methods

PARAMETER	RNSM (%)	WNKC (%)	WUNKC (%)
Dry matter	88.50	89.50	87.50
Crude protein	26.50	32.70	41.40
Crude fibre	3.92	12.88	13.60
Ether extract	40.00	40.50	38.00
Ash	5.00	2.00	2.00
NFE	24.58	11.92	5.00

*ME (kcal/kg)	5112.80	4940.00	4815.20

*-Calculated

RNSM - Raw neem seed meal. WNKC-Water treated neem kernel cake

WUNKC-Water and urea treated neem kernel cake

The crude protein levels of the processed neem seed cakes (Table 4.1) were higher than the RNSM with the WUNKC having the highest crude protein content. The crude protein figures obtained in this study were higher than the 17.48% and14.2% respectively recorded by Bawa *et al.* (2007) and Odunsi *et al*. (2009). The differences observed here could be attributed to the differences in the genetic constitution of the neem plant and the various processing methods used. This finding corroborates with that of Gowda and Sastry (2000), who reported that the chemical composition of neem cake varies considerably depending on types of processing such as solvents or expeller extraction of undecorticated or decorticated seed. NRC (1992) also reported that, the problem of putting the seed cake to use still remains. This is because neem tree scattered around the world are genetically distinct and its nutritional potentials are affected by climatic condition, method of processing and to a lesser extent, the genetic make up of the animals.

The crude protein content of the WUNKC being higher than the RNSM and the WNKC could be due to the addition of the urea for the detoxification process which might have raised the nitrogen content with the subsequent increase in the crude protein level of the diet. According to Kornegoy *et al.* (1970) and Okumura (1976), urea contains about 42-45 percent nitrogen and since the protein content of feeds is determined on the basis of the nitrogen content (AOAC, 2000), the crude protein content of the urea-containing feed, will in anyway, be higher than the feed without the urea.

4.2.0: PERFORMANCE OF BROILER BIRDS FED NKC DIETS

4.2.1: Average Final live weight

Significant ($p<0.05$) differences in average final live weight of the birds were observed at the end of the study (Table 4.2.) The values ranged from 2.63 to 1.52kg. Birds on the control diet had the highest weight (2.63kg) with birds on 10%WNKC, registering the least average final live weight (1.52kg) which was also stastistically ($p>0.05$) similar to the weight of the birds on 10%WUNKC. Comparable average final live weight values were also recorded for the birds on the 5% inclusion levels of WNKC and WUNKC.

The result also indicated that the average final live weight of the birds decreased as the inclusion levels of the NKC in the diets increased. The effect could be as a result of the increasing intensity of the bitterness of the neem triterpenoids which led to the reduced feed intake and the subsequent effect on average final live weight of the birds. This observation contradicts the findings of Uko and Kamalu (2008) but is similar to the observation made by Uko and Kamalu (2006) when cockerels were fed autoclaved neem kernel cake at 75g/kg and 150g/kg inclusion levels. The differences observed here could be attributed to the differences in the detoxification methods used in the research works. The toasting and the autoclaved methods used by Uko and Kamalu (2008) might have reduced the level of toxicity in the neem to a more tolerable level which led to an increased feed intake and a subsequent live weight gain than that of the water and urea soaking adopted in this experiment. Akinmutimi (2004) noted that most processing methods employed in improving feed value of non conventional or alternative feedstuff do not completely eliminate anti-nutritional substances but only reduce their concentration to tolerable levels in feedstuffs.

4.2.2: Average Daily Feed Intake.

Birds fed the control diet ingested significantly ($p<0.05$) higher quantity of feed than the birds on the NKC diets (Table 4.2). Feed consumption among birds fed the NKC diets was, however, not significantly ($p>0.05$) different from each other.

The significant difference observed in ADFI between the birds fed the control diet and those on the NKC diets could be attributed to the presence of the bitter triterpenoids, in the neem which reduced the palatability of the feed (Musalia *et al.*, 2000; Elangovan *et al.*, 2000).

According to Fairchid *et al*. (2005), birds have taste sensors for salt and bitterness. It may therefore, be natural for the birds to consume less feed if it has a bitter taste. This finding is in harmony with that of Elangovan *et al*. (2000) who reported that despite its high protein content of 300-400g/kg, neem seed cake could not earn favour as a good livestock feed due to its pungent smell and bitter taste imparted by the presence of toxic triterpenoids; azardirachtin, azardirone, nimbin and salanin. Similar results were also obtained by Atteh *et al*. (1995), Uko and Kamalu (2006) and Bawa *et al*. (2007).

The average daily feed intake of the birds reduced as the inclusion levels of NKC in the diets increased (Fig.1). The result showed that those on 5% inclusion levels of WNKC and WUNKC had feed intake values (76.52g, 77.79g) which were significantly ($p<0.05$) higher than the birds on the 10% inclusion levels of WNKC (66.07g) and WUNKC (68.96g). The effect observed here may be an indication of increasing intensity of the bitterness of the feed caused by the neem triterpenoids. Uko and Kamalu (2008) made similar observation of progressive reduction in feed intake when they fed broilers with raw neem kernel cake at 15.0% and 22.0% inclusion levels.

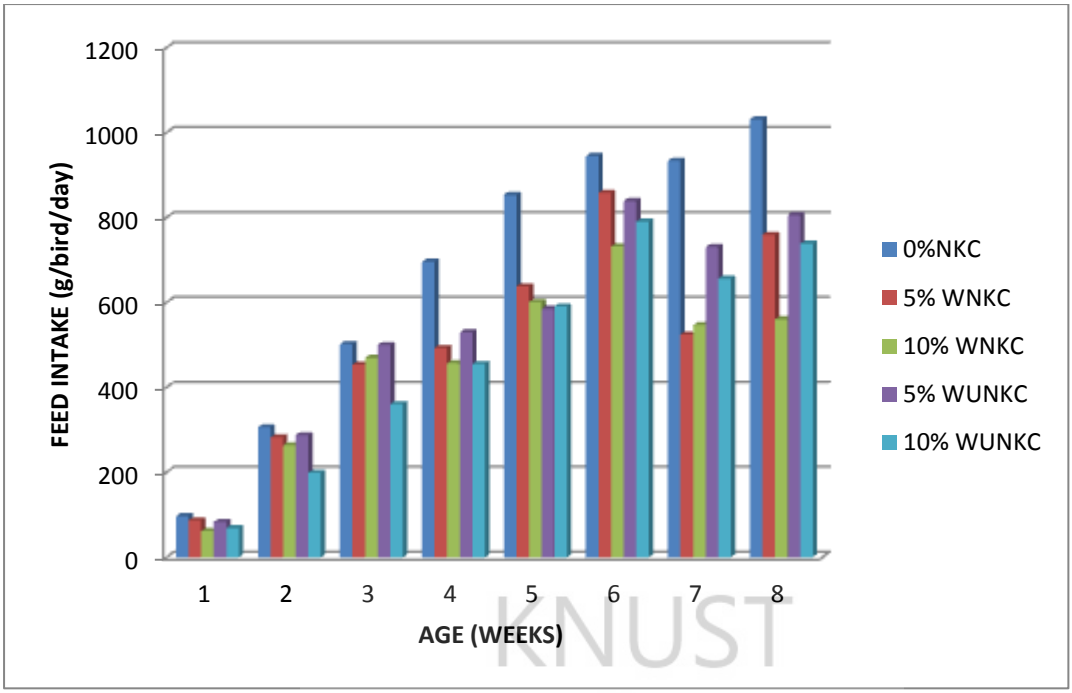

Fig. 1: The effect of feeding different levels of NKC on the average weekly feed intake (g/bird/day) of broiler chickens

Table 4.2: Performance of broilers fed different levels of neem kernel cake

Parameters	**Dietary treatments**					**LSD.**	**S.L**
	0% NKC	5% WNKC	10% WNKC	5% WUNKC	10% WUNKC		
Average Initial live weight (g/bird)	41.18	41.10	41.05	41.18	41.10	2.73	NS
Average final live weight (kg/bird)	2.63^{a}	2.16^{b}	1.58^{c}	2.11^{b}	1.52^{c}	0.150	*
Average Daily feed intake (g/bird/day)	95.78^{a}	76.52^{b}	66.07^{b}	77.79^{b}	65.96^{b}	14.39	*
Average daily weight gain (g/bird/day)	37.78^{a}	28.09^{b}	20.95^{cd}	27.44^{bc}	19.14^{d}	7.45	*

Feed conversion ratio	2.50	2.91	3.13	2.93	3.72	1.22	NS
Average daily water intake (ml/bird/day)	177.90[a]	135.66[b]	98.92[c]	125.07[b]	92.66[c]	25.48	*
Water /feed intake ratio	1.86	1.77	1.65	1.70	1.53	0.54	NS
Mortality (%)	13.33	11.67	20.00	8.33	13.33	-	-
Birds culled (%)	8.33	13.33	8.33	9.33	13.33	-	-

[a, b c.] Means bearing different superscript in the same row differ significantly ($p<0.05$)

NS:- not significantly different LSD: least significant difference; SL: significant level

*- Significant ($p<0.05$) difference.

4.2.3: Body Weight Gain

Average daily gain (ADG) of the birds increased with age throughout the period of the experiment with the exception of week 8 in which all the birds lost weight (Fig.2). Weight gain of the birds on the NKC diet did not follow any particular pattern (Table 4.2). Birds on the control diet recorded a significantly ($p<0.05$) higher ADG value over the birds on the test diets. High weight gain of animals normally results from increased feed intake. This was evident in this study. Result on feed intake, showed that birds on the control diet had the highest average daily feed intake. It is therefore expected that their daily weight gain would be higher than the other birds on the test diets which recorded lower feed intake. Tabler (2008) reported that flocks with the highest feed intake will almost always have the highest average daily gain and weigh most at processing. The difference in weight gain here could also be attributed to better protein utilization by the birds fed the control diet.

The depressed weight gain observed across the dietary treatments of birds fed on the NKC diets could be as a result of the comparatively low feed intake recorded, which might have been caused by the toxic principles in the NKC. These principles might have interfered with the digestion and utilization of the essential amino acids and carbohydrates needed for growth. Barnes and Amega (1984) stated that ingestion of feed containing such substances

(anti-nutrients) induces in some cases, chronic intoxication and in others interfere with the digestion and utilization of dietary protein and carbohydrates and also interfere with the availability of some mineral and, therefore, affect feed efficiency and growth rate. The finding here is in conformity with the results obtained by Reddy *et al*. (1988) and Uko and Kamalu (2006).

Average daily weight gain of birds on the 5% inclusion levels of WNKC (28.09) and WUNKC (27.44) were statistically similar but were significantly ($p<0.05$) higher than the birds on the 10% inclusion levels of WNKC (20.95) and WUNKC (19.14). The reason for the difference might be that the levels of anti-nutritional factors in the diets containing 10% NKC were too high to promote growth. Oresegun and Alegbeleye (2001) noted that anti-nutritional factors negate growth and other physiological activities at higher inclusion levels. Uko and Kamalu (2006) had weight gain figures which were significantly ($p<0.05$) lower than the control diet when cockerels were fed raw full-fat neem kernel (RFK) and autoclaved full-fat neem kernel cake (AFK) at 75.0, 150.0, 225.0g/kg inclusion levels. The result, however, is in contrast with the findings of Uko and Kamalu (2005) who reported of similar feed ingestion and body weight gain after feeding cockerels with autoclaved neem seed kernel at 150g/kg. The reason for the difference here could be due to the genetic difference in the birds used (NRC, 1994).

A significant loss in ADG observed in week 8 on all the experimental birds could be due to a sub-clinical effect caused by the attack of the New castle disease. It is possible that the birds used a greater part of the ingested feed nutrients for maintenance rather than converting the feed into growth. The feed conversion ratio figures for all the birds in that week gave credence to this observation. Braton *et al*. (1997) reported that an unhealthy bird is obviously, likely to have poor feed efficiency. The main reason for this is that feed intake is reduced, and so proportionally more feed is directed to maintenance. Also with enteric diseases, there

can be more subtle changes in feed utilization because various parasites and microbes can reduce efficiency of digestion and absorption of nutrients. It is also possible that the birds had reached their peak and hence recorded a declined ADG.

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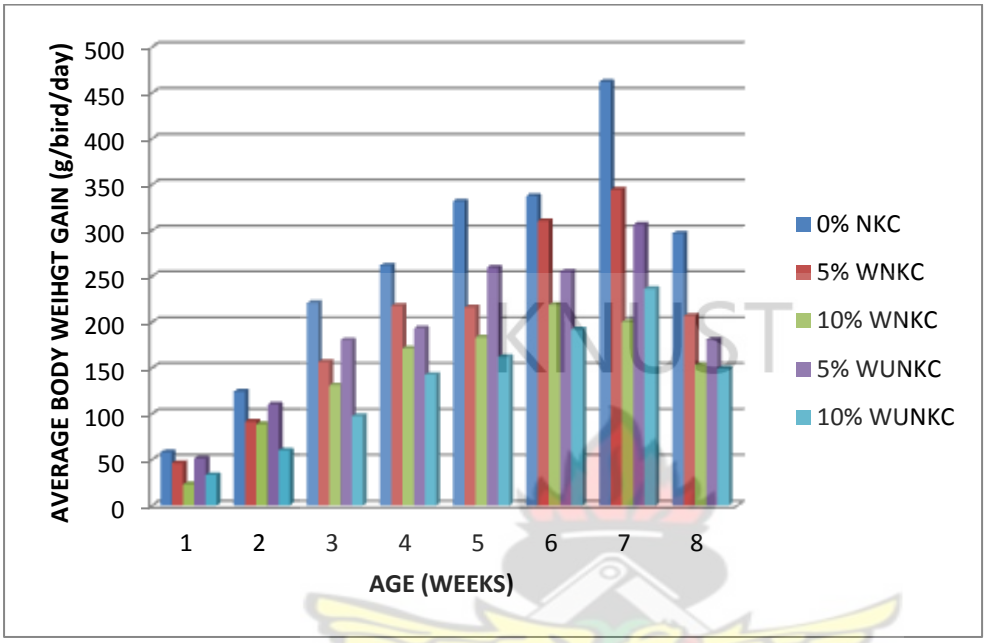

Fig.2: Effect of NKC on the average weekly weight gain (g/bird/day) of broiler chickens

4.2.4: Feed Conversion Ratio

The feed conversion ratio (FCR) of the birds on the control diet did not differ significantly ($p<0.05$) from that of the birds on the test diets (Table 4.2). The FCR value for the birds on the control diet was however, numerically lower than the values recorded by birds on the test diets.

The observed difference here could be as a result of inefficient nutrient utilization by the birds on the test diets due to the presence of the anti-nutrients (Azadirachtin, salanin nimbin and nimbidin) in the NKC. The values obtained here were above the optimum value of 2.0 for

broilers noted by Prabakaran, (2003) but were however better than the range (4.0-5.0) reported by Uko and Kamalu (2008).

4.2.5: Water intake

Water consumption increased progressively in all the treatment diets with the exception of the birds on the 10%WNKC which witnessed a marginal decrease in week 8 (Fig.3). The values recorded here (Table 4.2) were lower than the average water in take figure of 182.244 ml. per bird per day given by NRC, (1994).

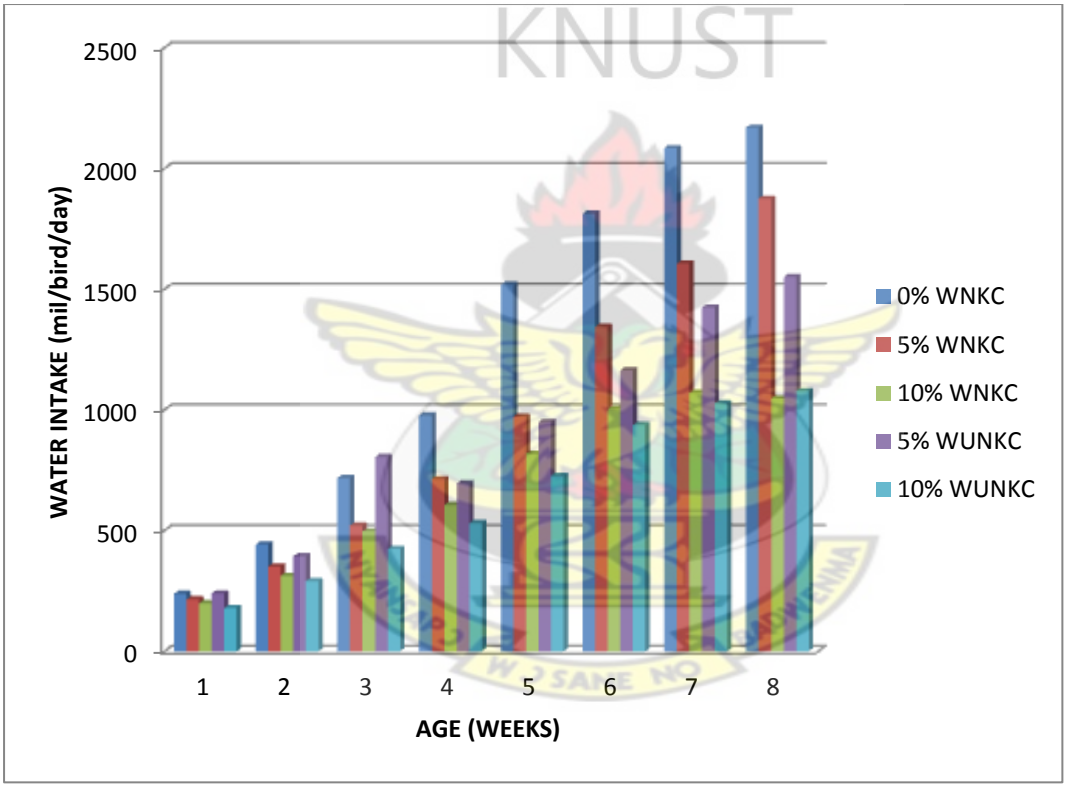

Fig. 3: Effect of NKC on weekly water intake (ml/bird/day) of broiler chickens

The results showed that birds on the control diet recorded the highest water consumption value which differed significantly ($p<0.05$) from the birds on the test diets. Birds on the 10%WNKC, consumed the least volume of water. In general, the broilers on 5% inclusion levels consumed significantly ($p<0.05$) higher volume of water than the birds on 10%

inclusion levels of WNKC and WUNKC. The observed difference in water intake between the birds on the control diets and those on the test diets could be due to the differences in feed intake and body weight gain figures registered by the birds. According to Medway and Kare (1959) and Ferket *et al*. (2006), the amount of water required by poultry depends on the animal's size, growth stage, environmental temperature and relative humidity, feed composition, rate of growth or egg production, and efficiency of kidney resorption of water in individual birds. Carter *et al*. (1997) also indicated that there is a close correlation between feed intake and water consumption. Thus, with higher feed intake, poultry would consume relatively higher amount of water to facilitate digestion to meet other physiological needs. This finding confirms what is reported by Uko and Kamlu (2006) and James *et al.* (2009).

4.2.6: Water/Feed Intake Ratio

The results of the ratio of daily feed intake to water consumption (Table 4.2) showed that the birds on the control diet recorded the highest ratio though the values obtained were not significantly ($p>0.05$) different from each other. The values, however, were within the range recorded by William *et al.* (2001) who stated that as a rule of thumb, for water intake, birds will consume 1.5 to 2 times feed intake on weight basis. This means that the inclusion of NKC in the diets did not have any deleterious effect on water intake of the birds fed the test diets.

4.2.7: Mortality

High mortality rate was recorded for all the dietary treatments (Table 4.2.). Birds on the 10%WNKC recorded the highest mortality (20%) with those on the 5%WUNKC, recording the least (8.33%). Percentage mortality for the birds on the control diets and the 10% WUNKC was equal (13.33%) but was however, higher than the birds on the 5%WNKC

(11.67) diet. These levels of mortality were above the ideal 2.0% and 2.5%, reported by Smith (2001) and Scane *et al.* (2004) respectively. The reason for the difference could be due to the attack of the Newcastle disease. Results on post mortem examination revealed that the cause of the mortality was due to the attack of the Newcastle disease (ND) rather than the NKC in the diets. This was also evident from the percentage mortalities recorded on the birds fed the control and the NKC diets.

According to Branckaert *et al*. (2001), Newcastle constitutes the most serious epizootic poultry disease in the world, especially in the developing countries. Newcastle disease occurs every year and kills an average of 70 to 80% of the unvaccinated birds. Analysis of mortality in family poultry flocks in Thailand (Thitisak, 1992), showed that the first four months of life are critical for the growing chicks. The mortality of chicks during this period often rose to 60% (Mathewman, 1977) even in flocks vaccinated for ND.

4.3.0: Carcass characteristics of broiler birds fed NKC diet

4.3.1: Defeathered weight

Values for the defeathered weight of the experimental birds were comparable across the dietary treatments (Table 4.3). The results indicated that the NKC did not significantly ($p>0.05$) influence the production of feathers since the removal of the feathers did not cause any significant ($p>0.05$) difference in the defeathered weights of the birds fed the control and the test diets. Uko and Kamalu (2008) conversely, reported of a marginal increase in feather production elicited by the neem diets. They observed that birds fed the neem diets had thicker feather cover than those on the control diet.

Table 4.3: Carcass characteristics (as % average final live weight) of broilers fed NKC diets.

Parameters (%)	Treatments					LSD	SL
	0% NKC	5% WNKC	10% WNKC	5% WUNKC	10% WUNKC		
Bled	96.57	97.31	96.43	96.54	96.91	1.194	NS
Bleeding	3.42	2.69	3.57	3.46	3.11	1.95	NS
Defeathered	89.72	90.66	86.97	88.04	90.21	5.480	NS
Dressed	83.50[a]	79.04[b]	77.44[b]	78.96[b]	76.43[b]	3.904	*
Heart	1.82[b]	2.26[a]	2.07[a]	2.31[a]	2.36[a]	0.694	*
Shank	11.89[b]	13.83[a]	12.89[a]	13.48[a]	15.43[a]	3.070	*
Liver	6.32[b]	7.91[b]	8.23[a]	7.68[b]	7.35[b]	1.720	*
Full intestine	17.17[b]	23.33[a]	23.91[a]	23.61[a]	23.48[a]	1.901	*
Empty gizzard	7.39[b]	7.96[b]	8.83[a]	9.49[a]	9.68[a]	1.291	*

[a, b c.] Means bearing different superscript in the same row differ significantly ($p<0.05$)

NS:- not significantly different LSD: least significant difference; SL: significant level

*- significant ($p<0.05$) difference

4.3.2: Dressed weight

The dressed weight of the birds on the control diet was significantly ($p<0.05$) higher than those on the NKC diets. Birds on the 10%WUNKC recorded the least percentage dressed

weight (76.43). The results showed that the weights of the heart, intestine, liver, and gizzard for the birds on the NKC diets were significantly ($p<0,05$) higher than those on the control diets. These might have reduced their percentage dressed weights obtained since these organs are not usually added to the dressed weight. The observation here is different from what was made by Uko and Kamalu (2006), who had the organ weights mentioned, here for cockerels on the test diets similar to those on the control diets and therefore did not have effect on the dressed weights

4.3.3: The heart weight

The weight of the heart expressed as percentage of the average final live weight ranged from 2.36 to 1.82%. The birds on the control diet had the least heart weight (1.82). The NKC significantly ($p<0.05$) increased the heart weight of the birds. The significantly higher values observed with heart weight could probably be due to higher physiological activities triggered by the presence of anti-nutritional factors in the neem and their concomitant effects as was also reported by Uchegbu *et al.* (2004).

4.3.4: Shank weight

The NKC diets influenced the shank weight of the birds. Values recorded for all the birds on the NKC diets were significantly ($p<0.05$) higher than that of the control diet. Uchegbu *et al*. (2004) had a similar observation and attributed the effect to poor growth performance caused by the *Napoleona imperialis* seeds fed to the broilers. This finding however, differed from what was reported by Odunsi *et al*. (2009).

4.3.5: Liver weight

The results (Table 4.3) indicated that the liver weight of the birds on the NKC diets were numerically higher than the birds on the control diets but the differences were not significant ($p>0.05$) with the exception of the birds on 10%WNKC which was significantly ($p<0.05$)

higher than the rest of the birds on all the treatment diets. The higher weights of liver observed between the birds on the control and NKC diets indicate that there was an increase in the metabolic rate of the liver of the birds fed the neem diets in an attempt to reduce the effect of the toxic triterpenoids in the neem. Bone *et al*. (1979) submitted that if there are toxic elements in the feed, abnormalities in the weights of liver and kidney would be observed. The abnormalities will arise because of increased metabolic rate of the organs in an attempt to reduce these toxic elements or anti-nutritional factors to non toxic metabolites. This finding agrees with the work of Uko and Kamalu (2005) and Uko and Kamalu (2008). Again, Babatunde *et al*. (1987) stated that visceral organ hypertrophy is common when monogastrics are fed insufficiently processed plant protein. This is usually associated with increased enzyme secretions by the organ in response to presence of enzyme inhibitors from plants.

4.3.6: Weight of Intestine

The results on the weight of the intestine (Table 4.3), showed that the NKC significantly ($p<0.05$) increased the intestinal weights of the birds fed the NKC diets. The value obtained for the control diets (17.17%) was significantly ($p<0.05$) lower than the values for 5%WNKC (23.33%), 10%WNKC (23.61%), 5%WUNKC (23.61%) and 10%WUNKC (23.48%). The intestinal weights of all the birds on the test diets were however, similar. The increase in weight of intestine observed among the birds fed the experimental diets could be attributed to the inflammatory response to the neem toxins (Uko and Kamalu, 2008) and also due to additional bulk and greater volume of digester staying in the gastrointestinal tract during enzymatic digestion. Bawa *et al.* (2007), reported of a significantly ($p<0.05$) higher intestinal weight after feeding rabbits with raw neem seed meal diet for 63 days. Uchegbu *et al*. (2004) also reported of increased weights of the proventriculus and intestine of all the broilers fed on *Napoleona imperialis* seed meal.

4.3.7: Weight of Empty Gizzard

The weight of the empty gizzard for the birds expressed as percentage average final live weight for the control (0%NKC) diet was similar to the gizzard weights of the birds on 5%WNKC diet but was significantly ($p<0.05$) lower than the rest of the birds on the other NKC diets (Table 4.3). The gizzard weight of the birds on 10%WUNKC was the highest (9.68%) but did not differ ($p>0.05$) significantly from the birds on 10%WNKC (8.83%) and 5%WUNKC (9.49%).

The higher percentage weight of gizzard observed on birds fed the neem diet might be as a result of the development of the muscularized gizzard in order to handle some extraneous component of the diet. This observation agrees with the findings of Oloyede *et al*. (2010) who fed broilers with raw and processed bambara groundnut seed as a component of poultry feed to evaluate their growth and haematological characteristics. The gizzard weights observed in this study were, however, lower than what was reported by Odunsi *et al*. (2009) who had no significant ($P>0.05$) difference in the in gizzard weight of cockerels fed water-treated neem seed cake and those on the control diet. The difference could be due to the differences in the breed of animals used for these various studies.

4.4.0. The Haematological and Biochemical Characteristics of Broilers fed NKC diets

The results on the haematological and biochemical components of the experimental birds fed the NKC diets are presented in Table 4.4 There were no significant ($p>0.05$) differences in the values recorded on Haemoglobin (Hb), Packed cell volume (PCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) Mean corpuscular volume (MCV) and white blood cell (WBC) between the birds on the reference diet and those on the test diets.

Table 4.4: Haematological and biochemical analysis of broilers fed neem kernel cake

Parameters	Treatments					LSD	S.L
	0%NKC	5%WNKC	10%WNKC	5%WUNKC	10%WUNKC		
Haemoglobin (g/dl)	9.3	9.62	9.73	9.85	9.17	0.846	NS
PCV (%)	29.5	30.95	31.30	31.67	30.38	2.923	NS
MCH (Pg)	38.17	35.58	33.54	39.60	39.19	7.647	NS
MCHC (g/dl)	30.77	30.57	31.52	30.65	30.48	1.413	NS
MCV (fl)	129.17	131.67	131.50	132.00	132.17	4.088	NS
RBC ($x10^{12}$/L)	2.30^{b}	2.54^{b}	2.42^{b}	2.45^{b}	2.85^{a}	0.291	*
WBC ($x10^{9}$/L)	2.481	2.540	2.572	2.582	2.550	0.1351	NS
	BIOCHEMICAL ANALYSIS						
Albumin (g/l)	16.67	15.17	17.17	15.67	17.17	3.3405	NS
Cholesterol (mmol/l)	3.35	3.45	3.59	3.62	3.49	0.6999	NS
Globulin (g/l)	16.32^{b}	16.00^{b}	20.83^{a}	21.67^{a}	21.17^{a}	4.3250	*
HDL (mmol/l)	1.55	1.55	1.63	1.65	1.39	0.5483	NS
LDL (mmol/l)	1.42	1.54	1.58	1.60	1.70	0.4437	NS
Total protein (g/l)	32.99^{a}	31.17^{b}	38.00^{a}	37.34^{a}	38.34^{a}	6.396	*
TGS (mmol/l)	0.80	0.83	0.83	0.83	0.80	0.2323	NS

[a, b.] Means bearing different superscript in the same row differ significantly ($p<0.05$)

NS:- not significantly different ; LSD: least significant difference; SL: significant level(99.5%)

*significant difference ($p<0.05$)

4.4.1: Red Blood Cell (RBC)

The values recorded on the RBC for the experimental birds ranged from 2.30-2.85 ($x10^{12}$/L). Birds on the 10%WUNKC recorded the highest value ($2.85x10^{12}$/L) which was significantly ($p<0.05$) different from the lowest value ($2.30x10^{12}$/L) recorded by the birds on the control diet. The values recorded by the birds on the other NKC were not statistically different ($p>0.05$) from those on the control diet.

The red blood cell levels observed for the birds on the test diets being generally higher than those on the control diet are indication that the neem cake improved the oxygen carrying capacity of the birds and did not in anyway render the birds anaemic. This finding agreed with that of Ogbuewu *et al*. (2010) but differed from what was reported by Esonu *et al*. (2006). There was, however, no appropriate explanation for the statistically ($p<0.05$) higher value in RBC level recorded by the birds on the 10%WUNKC diet.

4.5.0: Biochemical characteristics of broiler birds fed NKCdiets

The results on the biochemical composition of the blood of the birds indicated that there was no significant ($P>0.05$) difference in the cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) triglyceride (TGS) and albumin counts between the birds on the control diet and those on the NKC diets. However, significantly ($P<0.05$) different values were registered on the globulin and total protein composition of the blood of the experimental birds.

4.5.1: Globulin Levels

The globulin values of the birds on the 10%WNKC (20.83), 5%WUNKC (21.67g/l), 10%WUNKC (21.17g/l) diets were significantly ($p<0.05$) higher than the birds on the control diet. The value (16.00g/l) recorded by birds on the 5%WNKC diet, on the other hand, was statistically similar ($p>0.05$) to those on the control diet (16.32g/l). The rise in the globulin levels with subsequent significant rise in the total protein levels observed here showed that

the birds on the NKC diets had better resistance and immune response to disease infection than the birds on the control diet. According to Abdel-Fatal (2008), globulin levels have been used as indicator of immune response and source of antibody production. Griminger (1986) stated that high globulin levels and low A/G ratio signify better disease resistance and immune response.

Lumeij (1997) also noted that in acute or chronic conditions, a rise in total protein caused by elevated globulin fraction may occur. Often albumin concentrations are decreased in these situations. The combined effect of these changes is a decrease in the albumin/globulin ratio. Often the total protein concentration is within the reference range, while the albumin/globulin ratio is decreased; therefore the albumin/globulin ratio is often of greater clinical significance than the total protein from globulin.

4.5.2: Total Protein

The figure registered by the birds on the 5%WNKC diet was significantly ($p<0.05$) lower than those on the control and the other NKC diets. This could be an indication of the negative effect of the bitter principles of neem on the animals as a result of increase in size of the heart, liver and the intestine which was also observed by Bawa *et al*. (2007), after feeding broiler birds with raw neem seed meal diet (RNSMD). Why the birds on the other neem diets behaved otherwise could not be explained.

4.6: Economics of Production

The cost of producing a kilogram of feed was influenced by the inclusion of the NKC in the diets. Feed cost/kg (Table 4.5). The result showed that the cost of feed increased with the inclusion levels of the NKC. In all, it was cheaper to produce a kilogram of the control diet (GH¢1.16). The cost of producing a kilogram of the WUNKC diets was higher than that of

WNKC at the two inclusion levels used in this study. Again, it was most expensive to produce a kilogram of the 10%WUNKC diet (GH¢1.45). The feed cost of the test diets being generally higher than the cost of the control diet was due to the inclusion of the NKC which attracted an additional cost of transportation and processing. Similar marginal increase in the cost of diets containing neem seed cake than the control diet which did not contain neem seed cake was observed by Odunsi *et al*. (2009). The cost of WUNKC diets being higher than those on the WNKC could be due the further additional cost incurred on the urea used for the treatment of the seeds.

Table 4.5: Economics of production for broilers fed neem kernel cake

Parameters	**Dietary treatments**					**LSD.**	**S.L.**
	0%NKC	5%WNKC	10%WNKC	5%WUNKC	10%WUNKC		
Feed cost/kg (GH¢)	1.16	1.28	1.41	1.31	1.45	-	-
Feed cost/kg live weight (GH¢)	2.87[a]	3.73[b]	4.41[b]	3.85[b]	5.09[b]	1.68	*
Total feed intake /bird (kg)	5.36[a]	4.09[b]	3.49[b]	4.36[b]	3.86[b]	1.16	*
Cost of total feed intake /bird (GH¢)	6.22	5.49	5.22	5.90	5.05	1.23	NS
Selling price /bird (GH¢)	11.42	8.88	6.36	8.66	6.04	-	
Profit/bird (GH¢)	5.20	3.39	1.14	2.76	0.99	-	

[a b]Means bearing different superscripts in the same row differ significantly ($p<0.05$).

NS:- not significantly different LSD:- least significant difference; SL:- significant level

*significant ($p<0.05$) difference

Birds on 10%WUNKC recorded the highest feed cost per kilogram live weight of GH¢ 5.09 with those on the control registering the lowest (GH¢2.87). Feed cost per kilogram live

weight gain of 5%WNKC, 10%WNKC and 5%WUNKC were; GH¢3.73, GH¢4.41 and GH¢3.85 respectively. Feed cost/kg live weight gain of birds on the NKC diets being generally higher than the birds on the control diet could be blamed on the high FCR recorded by the birds on the NKC diets as well as the high feed cost of the NKC diets.

CHAPTER FIVE

5.0. Conclusions and Recommendations

The study revealed that:

- The crude protein content of the neem kernel can be improved by soaking the seeds in water and urea solution for 72 hours.
- The methods of detoxification (urea and water treated) employed in this study did not improve the feeding value of the neem kernel. Birds fed the reference diet performed better than those on the NKC diets.
- The inclusion of NKC in the diets did not have any deleterious effect on water intake of the birds.
- Replacing soya bean meal with NKC at the inclusion levels employed was not cost effective considering the cost involved in producing a kg live weight of a broiler bird fed the NKC diets.
- The inclusion of NKC at 5% level will significantly ($p<0.05$) improve average final body weight and water intake of the birds than the 10% level.

It is recommended that lower inclusion levels of NKC diets rather than the 5% and 10% used in this study be employed. Further work should also be done to reduce the level of the bitter principles in the seed to enhance feed intake and its utilization by extending the water

soaking period of the seeds. Alkali (Sodium hydroxide) treatment of the seed to reduce the level of bitter triterpenoids could also be another alternative.

REFERENCE

Abdel-Fattah, S. A., El-Medway, N. M. and Abdel-Abdel A. F. (2008). Thyroid Activity, Some Blood Constituents, Organs Morphology and Performance of Broiler Chicks fed Supplemental Organic Acids. International Journal of Poultry Science, 7(3): 215-222

Abdel-Hameed, M. F. and Neat, H. J. (1972). Study on erythrocyte cell volume and number in normal and triploid inter sex chicken. Poultry Sci. 51:1376-1382.

Abdou, K. A., Mubarak, K. and Sharkawy, A. A. (2006). Toxo-pathological effects induced by urea in broiler chicks, BS. Vet. Vol. 16(1):75-84

Aeangwanich, W., Simarakas, S. and Chinrasri, O. (2004). Haematological Erythrrocyte and Serum Biochemical Value of the Thai Indigenous Chicken (*Gallus domesticus*) in North-eastern Thailand. Songklanakarian J. Sci.Technol. 26 (3): 425-430.

Aerts, R. J. M. (1997). Feeding Deterrence and toxicity of neem triterpenoids.J.chem. Ecol. 23, 2117-2132.

Agrawal, D. K., Garg, A. K. and Nath, K. (1987). The use of water washed neem (*Azadirachta indica*) seed kernel cake in feeding of buffalo calves. J. Agric Sci. (Camb.) 108: 497-499.

Ahmed, S. (1985). Utilizing Indigenous plant resources in resources in Rural Development: Potential of the Neem Tree East-West Center, University of Hawaii, and Honolulu, Hawaii.

Akande, K. E. and Fabiyi, E. F. (2010). Effect of Processing Methods on Some Anti-nutritional Factors in Legume seeds for Poultry Feeding. Int. J. Poultry Sci. 9 (10): 996-1001.

Akinmutimi, A. H. (2004). Evaluation of Sword bean (*Canavalia gladiata*) as an alternative feed resource for broiler chickens. PhD. Thesis, Department of Non-ruminant Animal production, Michael Okpara University of Agriculture, Umudike, Nigeria.

Akinola, A. O. and Abiola, S. S. (1991). Blood Chemistry and carcass yield of cockerels fed melon husk diets. Trop. J. Anim. Sci: 2:39-44.

Anonymous (2006). Neem: Growing neem, organic farming, health, animal health, environmental use, home uses, economic potential, patents, new bazaars, research papers, world neem conferece. Neem foundation (internet) Mumbai, India. Available from: http://www.neemfoundation.org/

Association of Official Analytical Chemists (1990). Official Methods of Analysis 15th Edition, A.O.A.C. Arington VA.

Atteh, J.O. and Ibeyimi, S. A. (1995). Response of broilers to dietary levels of *Thevitia* seed cake. J. Agric. Sci. 125: 307-310

Austic R. E. and Nestheim, Malden, C. (1990). Poultry Production, Lea and Febiger, 600 Washington Square Philadelphia. PA. 19106-4198. USA.13th edition Pp. 172-259.

Babatunde, G.M, and Pond, W.G. (1987). Nutritive value of Nigerian rubber seed (*Hevea brasiliensis*) 1-Rubber seed meal. Nutr. Rp. Int.36: 617-630.

Barnes, A.R. and Amega, W.K. (1984). Utilization of cocoa pod husk meal by growing - finishing pigs. Proc. 9thinternational Cocoa conference, Lome, Togo, Pp: 449-454.

Bawa, G. S., Orunmuyi, M. and Onabanjo, O. A. (2005). Effects of dietary inclusion levels of mechanically extracted neem seed cake on performance of young rabbits. Nig. J. Anim. Prod. 32:233-239.

Bawa, G.S., Orunmuyi, M., Agbaji, A. S. Ladan, Z. and Okekeifi, U. O. (2007). Effect of Different Methods of Processing Neem (*Azadirachta indica),* seeds on performance of young Rabbits. Pakistan Journal of Nutrition 6 (3): 212-216.

Beard, J. (1989). Tree may hold the key to curbing chagas' parasite. New Scientist Pg 124.

Biswas, K. I, Chattopadhyay, Ranajit, K. B. and Uday, B. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*), *Current Science.* 82 (11): 1336 – 1345.

Bone, F. J. (1979). Anatomy and Physiology of farm Animals. Printice-Hall, Inc. New Jersey, USA

Branckaert, R. D. S., Gaviria, L., Jallade, J. and Seiders, R. W. (2000). Transfer of technology in poultry production for the developing countries. In: FAO Sustainable Development Dimensions. October 2000 and proceedings WPC2000 Montreal, Canada (WWW.fao.org/ag/AGAP/LPA/fampo1/links.htm)

Branton, S. L., Lott, B. D., Deaton J. W., Maslin, W. R., Hustin,F. W., Pote, L. M., Keirs, R. W., Latour, M. A. and Day, E. J. (1997). The effect of added complex carbohydrates or added dietary fiber on necrotic enteritis lesions in broiler chickens. Poultry Sci.76:24-78

Branton, S. L., May, B. D. and Maslin, W. R. (1997). Various blood parameters in commercial hens acutely and chronically infected with Mycoplasma synoviae. Avian Dis., 41: 540-547.

Carter, T.A. and Sneed R.E. (1997). Drinking Water Quality for Poultry and Techonology Guide 42. Extension Poultry Science; north Carolina State. University of Raleigh, North Carolina.

Chamberlain, J. R. (1999). Improvement of neem *(Azadirachta indica*) and its potential benefits to poor farmers in developing countries.Reports on a visit to India.CNRD, Green College, Oxford UK. 13pp.

Chand, S. (1987). Nutritional evaluation of neem seed meal to chicks.Ph. D. Thesis, Rohilkhand Univ., India. Pp: 66-106.

Chandra, M., Sigh, S. Gupta, P. P. and Ahuja, S. P. (1984a). Comparative pathogenesis of nephritis in poultry induced by high protein, high calcium, urea and vitamin A deficient diets. Acta Veterinaria 34: 113-134.

Chaudhary, O. P. (1997). New record of a shoot borer on neem. Insect Environments 3:8

Devakumar, C. and Dev, S. (1996). In: Neem, Randhawa and Parmar B.S (Eds) 2nd Edition, Pp. 77-110

Devakumar, C., D. Sukh-Dev (1993): Chemistry. Neem Res. Develop. Pub. No. 3, Soc. Pesticide Sci., India, pp. 63-96.

Dhurandhar, N. V. and Chang, K. C. (1990). Effect of cooking on firmness, trypsin inhibitors, lectins and cystine/cysteine content of navy and red kidney beans (*Phaseolus vulgaris*). J. Food Sci. 55:470-474.

Elangovan, A.V., Verma, S. V. S. Sastry, V.R. B and Sigh, S. (2000). Effect of feeding neern (*Azadirchta indica*) kenel meal on growth, nutrient utilization and physiology of Japanese quals, (*Cortunix cortunix japonica*). Asian-Aust. J. Animal Prod; 30:3-8.

Esonu, B. O. Emenalom, O., Udedibie, A.B.I., Herbert U. Ekpor, C. F., Okoli, I. C. and Iheukwumere, F. C. (2001). Performance and blood chemistry of weaner pigs fed raw mucuna bean (velvet bean) meal. Trop. Anim. Prod. Invest., 4: 49-54.

Esonu, B. O., Opara, M. N., Okoli I. C., Obikaonu H. O., Udedibie C. Iheshiulor, O. O. M. (2006). Physiological Response of Laying Birds to Neem (*Azdirachta indic*a). Leaf Meal –Based Diets: Body Weight organ characteristics and Haematology. Online J. Health Allied Sci. Pp 2,4

Fairchid, B. D., Batal, A. B., Ritz, C. W. and Vendrell, P.F. (2005). Drinking Water iron concentration impact on broiler performance. *Poultry Sci*: 84, 103-194

FAO. (2008). Report on African Farming September/October, 2008. Pp 13-14

Ferket, P. R. Gernat, A. G. (2006). Factors that affect feed intake of meat birds: A review. International Journal of Poultry Science 5 (10): 905-911.

Galindo-Munz, F., Calderno, N. L., Charles, M. N., Telez, I. G. and Fortoul, T. I. (2001).Haematological and histological findings in experimental Newcastle disease.Acta Vet. Bmo, 70:185-189.

Ganguli, S. (2002). Neem; 'A therapeutic for all seasons'. Current Sci. 82 (11): 1304.

Genstat Statistical Package. (2008). Genstat Discovery Edition, Version 7.2 VSN International Limited

Gentle, M. J. (1975). Neural and Endocrine Aspects of Behaviour in bird, Wright. P.G., Caryl, P.G and Vowels, D.M. Editors. Amsterdam, Elsevier Pp: 305-318.

Gilbert, A.B. (1968). The relationship between the erythrocyte sedimentation rate and packed cell in the domesticfowl. Br. Poultry. Sci.9:29-299.

Girish, K. and Shankara B, S. (2008). Neem – A green Treasure. Electronic Journal of Biotechnology and Biochemistry. 4(2): 179-183

Govindachari, T. R. (1992). Chemical and Biological Investigations on *Azadirachta indica* (the neem tree). *Current sciences*. 63, 117-122

Gowda, S. K. and Sastry, V. R. B. (2000).Neem, (*Azadirachta indica*) seed cake in animal feeding scope. Rev. Asian-Aus. J. Animal Sci. Pg. 5

Gowda, S. K. and Sastry, V. R. B. (1998). Neem (*Azadirachta indica*), study on the utilization of Neem Kernel Meal as a Protein supplement for growing rabbits. Ind. Vet. J. 75: 281-282.

Griminger, P. (1986). Lipid Metabolism in 'Avian Physiology'.Edited by Sturkie.4th Edition Springer- Verlag, Inc., New York. USA.

Gunasena, H. P. M. and Marambe, B. (1998). Neem in Sri Lanka: a monograph,University of Peradeniya, Sri Lanka. Pg. 62.

Gupta, V.K., Solanki, K.R. Gupta, R. Kumar, R.V. and Datta, A. (1996). Reproductive biology of neem (*Azadirachta indica* A.Juss). Range Management and Agroforestry 17(2): 187-192

Hegde, N. G. (1995). Neem and small farmers constraints at grass root level. Indian For., 121: 1040-1048

Jacobson, M. (1985). In: The neem tree. Source of Unique natural Product for Integrated Pest Management, Medicine, Industry and other purposes. Pp.484-489.

Jacobson, M. (1989). *The neem Tree* CRC Press: Boca Raton, Florida, Pgs 4 and 178.

Jain, N.C. (1993). Essentials of Veterinary Hematology, Lea & Febiger, Philadelphia

James, D.B. Ameh, D.A. Agbaji, S.A and Ibrahim, N. D. G. (2009). Biochemical and Histologic Effect of Dietary Substitution with Solvent Extracted Neem seed cake of Albino Rats (Wistar Strain). International journal of Animal Veterinary Advances 1(1):15.

Jattan, S. S., Shashikumar, M. and Pujar, G. (1995) .Perspectives in intensive management of neem plantations. Indian For., 121: 981-988.

Jones, I., Ley, S.V., Denholm, A. A., Lvell, H., Wood, A. and Sinden, R.E. (1994). Sexual development of malaria parasites is inhibited in *in vitro* by neem extract and its semi synthetic analoques. FEMS, Microbiol. Lett., 120: 267 273.

Kabeh, J. D. and Jalingo, M.G. D.S.S. (2007). Exploiting neem (*Azadirachta indica*) Resources for improving the quality of Life in Taraba State, Nigeria. Int. J. Agric. And Biology, 560-853

Katiyar, R. C. Verma, S. V. S, Nagalakshmi, D. Sastry, D. K, and Agrawal, R. C. (1996). Performance of broiler chicks fed on alkali –treated neem kernel (*Azadirachta indica*) as protein supplement. British Poultry Science 37:809-818.

Kellems Richard, O. and Church, D.C. (2002). Livestock Feeds and Feeding. Fifth Edition.Pearson Educaton Inc., Upper Saddle River, New Jersey, 07458. Pp 297-310.

Kessler, M., Belmar, R. and Ellis, N. (1990). Effects of autoclaving on the nutritive value of seeds of *Canavalia ensiformis* (jackbeans) for chicks. Trop. Agric. (Trinidad), 67:116-120.

Ketkar, C. M and Ketkar, M. S. (1997). Botany. In: Neem in sustainable Agriculture, S.S. Narwal, P. Tauro and S.S. Bisla (Eds), Scientific Publishers. Jodhapur, India. Pp. 1-12.

Khan, M. and Wassilew, S.W. (1987). In Natural Pesticides from the Neem Tree and other Tropical Plants (H.Schmutterer and Asher, K.R.S Eds), GTZ Eschborn, Germany, Pp. 645-650.

Koinarski, V. Angelov, G. and Lalev. (2001). Haematologic and blood electrolyte changes in broiler chickens experimentally invaded with Eimeria tenella. Bulgarian J.Vet. Med. Nauki., 24: 70- 74.

Kokosharov, T. and Todorova, T. (1987). Changes in the iron content, erythrocytes and haemoglobin in the blood of poultry with acute experimental fowl typhoid. Vet. Med. Bmo, 70:185-189.

Kornegoy, E. T., Mosanghini, V. and Sneed, R. D. (1970). Urea and amino acid supplementation of swine diets. J. Nutr., 10:330-340.

Kraus, W. (1995). In: The Neem Trees, edited by Schmutterer H. VCH, Verlag sgeselle schaft, Weinheim (FRG), 35-88.

Kumar, A. R. V. (2002). Neem for the Industry or for the common man: where does India stand? Curr. Sci. 84: 265-267.

Lumeij, J. T. (1997). Avian Clinical Biochemistry. In: Kaneko, J. J., J. W. Harvey and M. BBL. Bruss (Eds.). Clinical Biochemistry of Domestic Animals. Academic Press, SanDiego, Pp: 857-883.

Margaret, A. W. (2001). Avian plasma proteins.http://www.exoticpetvet.net.

Margraf, V. J. (1990). Trees and shrubs of the Sahel, their characteristics and uses.Pp.173-176.

Mathewman, R. W. (1977). A survey of small livestock at the village level in derived Savanna and Lowland Forest Zones of South West Nigeria. Study No. 4, University of reading.

Medway, W. and Kare, M. R. (1959). Water metabolism of growing domestic fowl with special reference to water balance. Poultry Sci. 38:631.

Meisner, J., Ascher, K. R. S., Aly, R. and Warthen, J. D. (1981).'Response of *Spodoptera littrals* and *Erias insulana* Lavea to Azadirachtin and Salannin'.

Mitruka, B. M, Rawnsley, H. M. (1997). Clinical biochemical and haematological reference values in normal experimental animals. Masson Publ. Co. New York, pp: 102-

Moss, P. P. (1999).Blood Banking, Concepts and Applications. W. B. Saunders Co. Philadelphia. Pp.12-34

Muhammad, N. O. and Oloyede, O. B. (2009). Haematological Parameters of Broiler Chicks fed *Aspergillus niger* and Fermented *Terminalia Catappa* Seed Meal-Based Diet. Global Journal of Biotechnology and Biochemistry 4(2): 179-183. IDOSI Publications.

Murthy, S. P and Sirsi, M. (1958). Pharmacological studies on *Melia azadirachta. Ind. J. Physiol. Pharmacol*, 2:387-396.

Musalia, L. M. Anandan. S. Sastry V. R. B. and Agrawal, D. K. (2000). Urea-treted neem (*Azadirachta indica*) seed kernel cake as a protein supplement for lambs, Small ruminants Res, 35:107-116.

Nagalakshmi, D., Sastry, V. R., Ktiyar, R. C., Agrawal, D. K. and Verma, S. V. (1999). Performance of broiler chicks fed on diets containing urea ammoniated neem (*Azadirachta indica*) kernel cake. Br. Poult. Sci., 40(1): 77-83.

Nath, K. Rajagopal, S. and Garg, A. K. (1983). Water washed neem (*Azadirachta indica*) seed kernel cake as cattle feed. J. Agric. Sci. Camb.101: 323-326.

National Research Council (1992). Neem, A Tree for Solving Global Problem. National Academy Press. Washington D.C. Pp. 23-113

National Research Council, NRC (1994).Nutrient Requirements of Poultry.9th Edition National Academy Press. Washington, D. C. Pp 1-114

Odunsi, A. A., Akande, T.O., Yusuph A.S. and Salami, R.I. (2002). Comparative utilization of high inclusion rates of four agro-industrial by products in the diet of egg type chickens. Arch. Zootec, 51:465-468.

Odunsi A. A. Adegbile, S. A. Akande, T. O Olayeni T.B. (2009). Neem (*Azadirachta indica*) seed cake in the diets of cockerel chickens.International Journal of Poultry Science.(8 (1): 47-55.

Ogbuewu, I. P., Uchegbu, M. C., Okoli, I. C. and Iloeje, M. U. (2010). Assessment of blood chemistry, weight gain and linear body measurements of pre-pubertal buck rabbits fed different levels of neem (*Azadirachta indica* A Juss) leaf meal. Chilean Journal of Agricultural Research, 70 (3):515-520.

Okumura, J., Hewitt, D., Salter, D.N. and Coates, M.E. (1976). The role of gut microflora in the utilization of dietary urea by the chicks. Br. J. Nutr. 36: 265-272

Oloyede, O.B, Minari, J.B and Muhammad N.O. (2010). Evaluation of Growth characteristics and Haematological indices of Broilers chicks Fed raw and Processed Bambara Groundnut seed as a component of Poultry feed. International journal of Poultry Science 9 (7): 652-655. ISSN. 1682-8356. Asian Network for scientific information 2010

Onyimonyi, A.E. Adeyemi Olabode and Okeke, G.C. (2009). Performance and Economic characteristics of Broilers fed Varying Dietery Levels of Neem Leaf Meal (*Azadirachta indica)*. International Journal of Poultry Sci. 8(3):256-259.

Oresegun, A. and Alegbeleye, W. D. (2001). Serum and tissue thiocynate concentration in tilapia (*Oreochromis niloticus*) fed cassava peels based diets supplemented with D. L-methionie, In: Eyo, A.A. (Ed.) Nutrition and Fish feed. Published by FISON, Nigeria.107-115.

Osagie, A. U. (1998). Anti-nutritional factors. In: Nat. Qual. Plant Foods. Pp 857-883.

Paul, B.N. Haque, N and Garg, A.K. (1996). Effect of feeding neem seed kernel cake (*Azadirachta indica*) on rumen enzyme of buffalo Ind. Vet. J. 73:720-728.

Pearson, T. C. (1991). 'Apparent Polycythaemia'. Blood Reviews, 5(4): 205-213

Pillai, N.R. Santhakumari, G. (1981). Planta Med. 43: 59-63

Prabakaran, R. (2003). Good Practices in planning and management of integrated commercial poultry production in South Asia, FAO Animal Production and Health paper 159:12-24.

Radwanski, S. (1977). Neem tree 2: uses and potential uses. World crops and Livestock. 29:111-113.

Ramesh, K. and Padhya, M. A. (2000). Neem for sustainable development and environmental conservation. Pp. 17-19

Ranga, N. G. (2002). Detoxification of certain toxins (triterpenoids) in neem (*Azadirachta indica*) seed cake.Indian Journal of Animal Science. 63:120-125

Reddy, V. R. Rao, P. V. and Reddy, C. V. (1988). Utilization of Chemically Treated Neem Seed for Broller Chickens. Journal of Animal science; 58:830-834.

Sathe, S. K., Desphande, S. S. and Salunkhe, D. K. (1984). Dry beans of phaseolus: A review: Part 1. Chemical Composition: Proteins. CRC, Rev.Food Sci. Nutr. 20: 1-46.

Saxena, R. C. (1984). Development of neem in Africa. Paper presented at the World neem Conference 99, UBC, Vancouver, Canada

Scane, C.G., Brant and Ensminger, M. E. (2004) .Poultry Science; 4th edition.Upper Saddle River, New York, Pearson Prentice Hall.

Schmutterer, H. (1988). Potential of Azadiractin containing pesticides for developing countries. Journal of insects Physiology, Vol. 34 (7): 713-717.

Schmutterer, H. and Singh, R.P. (1995). List of Insects Susceptible to Neem Products. In: The Neem Tree Sources of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes, Schmutterer, H. (Ed.). VCH, Publishers, New York.

Sigh, R, P. and Raheja, A. K. (1996). In: Neem and Environment Proc. World Neem Conference (Bangarolore, India, 1993), Oxford and IBH, New Delhi.

Smith, A. J. (2001). Poultry: Technical Centre for Agriculture and rural Co-operation, Post bus, 380. 6700 AS Wageningen, Netherlands. Pp 69-193

Soetan, K. O. and Oyewole, O. E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. African Journal of Food Science Vol. 3(9): 223-232.

Sonaiya, E.B. (1990). The context and prospects for development of small holder rural poultry production in Africa. Pp. 35-52

SPSS. (2007). Statistical package for social sciences.Procedure and facilities for Research. McGraw-Hill Book.Co. New York.

Strozok, P. (1992). Cost /benefit analyses of neem tree product technologies: South Asia, Haiti and the Sahelian Zone, West Africa USAID Contract. Minnea-polis, Minnesota, USA. Pp 63

Sugano, M., Goto, S., Yaoshida, K., Hashimoto, Y., Matsno, T. and Kimoto, M. (1993).Cholesterol lowering activity of various undigested fractions of soya bean protein in rats. J. Nutr., 120 (9): 977-985.

Tabler, G. T. (2008). Early Feed Intake and Bird Performance. Applied Broiler Research Unit, Center for Excellence for Poultry Science- AVIAN Advice Vol. 5, No.1

Tewari, D.N. (1992). Monograph on neem (*Azadirachta indica* A. Juss). International Book Distributors, Dehra Dun, India.Pg. 279.

Thitisak, W. (1992). Causes of death found in an epidemiological study of native chickens in Thai villages. Acta Veterinaria Scandinavia, 84 (Suppl): 200-202

Tisch David, A. (2006). Animal Feeds, Feeding and and Nutrition and Ration Evaluation with CD-ROM. Library of Cogress Ctaloging-in- Publication, USA. PP 29-63

Trugo, L. C., Ramos, L.A., Trugo, N. M. F. and Souza, M. C. P. (1990).Oligosaccharides composition and trypsin inhibitor activity *Phaseolus vulgaris* and the effect of germination on the alpha-galactoside composition and fermentation in the human colon. Food Chem., 36: 53-61.

Uchegbu, M. C. Okoli, T. C., Etuk, E. B, (2004). Preliminary Studies on *Napoleona imperialis* as livestock feed ingredient: proximate composition. Proceedings of the 36th Annual Conference of the Agricultural Society of Nigeria, FUT, Owerri, October 20 – 24, pp. 422 -424.

Udedibie, A. B. I. and Madubuike, F. N. (1988). Effect of dietary raw and cooked jackbean (*Canavalia ensiformis*) on the performance of laying hens. J. Arid Agric., 1: 47-54.

Udedibie, A. B. I. and Nkwocha, C. O. (1990). Comparative study of jackbean (*Canavalia ensiformis*) and sword bean (*Canavalia gladiata*) as protein supplements young broiler chicks. Nig. Agric J., 24:7-14.

Uko, J.O. and Kamalu, T.N. (2006). Protein Quality and Toxicity of full-fat Neem (*Azadirachta indica* A.Juss) Seed Kernel.Archivos de zootecnia, ano vol. 55.Numero 209. Universidad de cordoba, Espania Pp. 51-62

Uko, O.J. and Kamalu, T.N. (2005).Haematological Parameters and Weight Changes of Cockerels Fed Raw or Autoclaved Neem seed kernels in Diets.Nigerian Veterinary journal vol.26 (1). Pp. 10-17

Uko, O. J. and Kamalu.T.N. (2008). Trend of food consumption and efficiency of broiler production with raw or heat-treated neem kernels, Archzootec. 57 (220): 489-496.

Vidal-Valverde, C. J. and Valverde, S. (1992). Effect of processing on soluble carbohydrate content of lentils. J. Food Prod., 55: 301-304.

Waugh, A. and Grant, A. (2001).Anatomy and Physiology in health and illness; 9th ed. Churchil, Livingstone, an imprint of Elsevier Science Limited, Pp. 59-70.

William, A. Dozier, III, Mchael, P., Lacy Lary R. Vest. (2001). Extension Poultry Scientist Department of Poultry Science, Cooperative Extension Service. The University of Georgia, College of Agricultural and Environmental Sciences

Zenk, H. M. (1991). Chasing the Enzymes of Secondary Metabolism. Plant cell cultures as a pot of goal, Phytochemistry. 30 (12): 3861-3863.

LISTS OF APPENDICES

Appendix 1: Analysis of variance tables

Appendix 1A: ANOVA FOR AVERAGE DAILY FEED INTAKE

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	3.582	1.791	0.61	
Treatment	4	49.038	12.259	4.18	0.041
Residual	8	23.478	2.935		
Total	14	76.09			

Appendix 1B: ANOVA FOR AVERAGE WEEKLY FEED INTAKE

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	174.5	87.3	0.61	
Treatment	4	2397.7	599.4	4.71	0.041
Residual	8	1149.6	143.7		
Total	14	3721.8			

Appendix 1C: ANOVA FOR AVERAGE DAILY WATER INTAKE

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	49.11	24.56	2.20	
Treatment	4	163.42	40.85	3.66	0.056
Residual	8	89.24	11.16		
Total	14	301.77			

Appendix 1D: ANOVA FOR WEEKLY WATER INTAKE

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	2405.1	1202.6	2.20	
Treatment	4	8008.1	2002.0	3.67	0.056
Residual	8	4369.9	546.2		
Total	14	14783.2			

Appendix 1E: ANOVA FOR AVERAGE DAILY WEIGHT GAIN

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	1.1801	0.5901	1.36	
Treatment	4	40.8791	10.2198	23.63	<.001
Residual	8	3.4600	0.4325		
Total	14	45.5192			

Appendix 1F: ANOVA FOR AVERAGE WEEKLY BODY WEIGHT GAIN

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	97.67	48.83	1.34	
Treatment	4	2362.65	590.66	16.19	<.001
Residual	8	291.82	36.48		
Total	14	2752.13			

Appendix 1G: ANOVA FOR FEED CONVERSION RATIO

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	0.2712	0.1356	1.27	
Treatment	4	1.1542	0.2885	2.71	0.108
Residual	8	0.8526	0.1066		
Total	14	2.2780			

Appendix 1H: ANOVA FOR WATER/ FEED INTAKE RATIO

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	0.0446	0.0223	0.06	
Treatment	4	1.2170	0.3043	0.85	0.532
Residual	8	2.8622	0.3578		
Total	14	4.1237			

Appendix 1I: ANOVA FOR FEED COST/KG LIVE WEIGHT

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	0.4895	0.2447	1.10	
Treatment	4	4.2488	1.0622	5.17	0.24
Residual	8	1.6439	0.2055		
Total	14	6.3822			

Appendix 1J: ANOVA FOR BLEEDING PERCENTAGE

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	3.057	1.6528	4.10	
Treatment	4	1.5279	0.3820	0.95	0.484
Residual	8	3.2235	0.4029		
Total	14	8.0571			

Appendix 1K: ANOVA FOR BLED WEIGHT

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	2.3329	1.6664	4.14	
Treatment	4	1.5433	0.3858	0.96	0.479
Residual	8	3.2177	0.4022		
Total	14	9.0939			

Appendix 1L: ANOVA FOR DRESSED WEIGHT.

SOURCE OF	d.f	s.s	m.s	v.r	F.pr

VARIATION					
Rep Stratum	2	16.860	8.430	1.96	
Treatment	4	87.798	21.949	5.11	0.024
Residual	8	34.394	4.299		
Total	14	139.052			

Appendix1M: ANOVA FOR EMPTY GIZZARD

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	0.4763	0.2382	0.51	
Treatment	4	11.5481	2.8870	6.14	0.015
Residual	8	3.7599	0.4700		
Total	14	15.7843			

Appendix1N: ANOVA FOR FULL INTESTINE

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	4.962	2.481	0.46	
Treatment	4	95.981	23.995	4.43	0.035
Residual	8	43.380	5.423		
Total	14	144.322			

Appendix 1O: ANOVA FOR HAEMOGLOBIN

SOURCE OF	d.f	s.s	m.s	v.r	F.pr

VARIATION					
Rep Stratum	2	0.2253	0.1127	0.71	
Treatment	4	0.9333	0.2333	1.46	0.299
Residual	8	1.2747	0.1593		
Total	14	2.4333			

Appendix 1P: ANOVA FOR MEAN CORPUSCULAR VOLUME (MCV)

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	17.200	8.600	1.69	
Treatment	4	100.400	25.100	4.92	0.027
Residual	8	40.800	5.100		
Total	14	158.400			

Appendix 1Q: ANOVA FOR MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC)

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	5.3693	2.6847	5.43	
Treatment	4	1.8467	0.46	0.93	0.491
Residual	8	3.9573	0.4947		
Total	14	11.1733			

Appendix 1R: ANOVA FOR RED BLOOD CELL

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	0.00533	0.00267	0.09	
Treatment	4	0.05733	0.01433	0.49	0.745

Residual	8	0.23467	0.02933		
Total	14	0.29733			

Appendix 1S: ANOVA FOR ALBUMIN

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	0.400	0.200	0.04	
Treatment	4	14.267	3.567	0.66	0.634
Residual	8	42.933	5.367		
Total	14	57.600			

Appendix 1T: ANOVA FOR CHOLESTEROL

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	0.0840	0.0420	0.15	
Treatment	4	0.467	0.1067	0.39	0.811
Residual	8	2.1893	0.2737		
Total	14	2.2737			

Appendix 1U: ANOVA FOR TOTAL PROTEIN

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	1.10	0.55	0.02	
Treatment	4	92.76	23.19	0.97	0.475
Residual	8	191.72	23.97		
Total	14	285.58			

Appendix 1V: ANOVA FOR TRIGLYCERIDES

SOURCE OF VARIATION	d.f	s.s	m.s	v.r	F.pr
Rep Stratum	2	0.09733	0.04867	1.92	
Treatment	4	0.13733	0.03433	1.36	0.330
Residual	8	0.20267	0.2533		
Total	14	0.43733			

Appendix 2: Tables

Table 2a: Average Weekly Body Weight Gain (g)

WEEK	O%NKC	5% WNKC	10% WNKC	5%WUNKC	10%WUNKC	LSD.
1	58.0	46.30	23.10	51.10	33.30	11.37
2	124.20	91.60	88.80	110.10	60.10	20.64
3	220.80	156.70	131.10	180.30	97.50	30.46
4	261.70	217.70	171.20	193.20	142.60	45.60
5	331.60	216.00	183.20	259.40	161.80	63.04
6	377.50	310.40	218.8	255.00	192.00	53.04
7	461.90	344.60	200.20	306.30	236.20	69.32
8	297	207.00	53.00	181.00	116.00	192.60

Table 2b: Average Weekly Feed Intake (g)

Week	O% NKC	5%WNKC	10%WNKC	5%WUNKC	10%WUNKC	LSD.
1	96.70	86.70	61.70	83.20	68.00	22.57
2	305.80	282.30	263.30	287.30	198.90	58.12
3	502.00	453.00	470.00	500.00	361.00	97.6
4	696.00	492.00	457.00	530.00	455.00	113.00
5	853.00	638.00	601.00	585.00	591.00	109.70
6	945.00	858.00	732.00	835.00	791.00	165.70
7	934.00	724.00	547.00	731.00	656.00	139.60
8	1031.00	759.00	561.00	806.00	738.00	224.0

Table 2c: Average Weekly Water Intake (mls)

Week	O% NKC	5%WNKC	10%WNKC	5%WUNKC	10%WUNKC	LSD.
1	238.7	215.4	199.3	239.0	179.1	44.01
2	442.9	350.6	311.8	394.0	290.4	63.84
3	718	520	497	605	424	100.8
4	977	713	605	695	531	202.3
5	1519	972	817	949	726	158.3
6	1813	1344	1003	1164	937	197.2
7	2084	1606	1070	1424	1027	264.5
8	2169	1875	1046	1550	1078	394.8

Table 2d: Average Weekly Feed/Water Intake Ratio

Week	O% NKC	5%WNKC	10%WNKC	5%WUNKC	10%WUNKC	LSD.
1	2.47	2.52	3.23	2.96	2.71	1.13
2	1.45	1.25	1.13	1.40	1.62	0.67
3	1.45	1.14	1.06	1.21	1.25	0.37
4	1.40	1.45	1.39	1.27	1.16	0.24
5	1.78	1.55	1.35	1.47	1.23	0.32
6	1.92	1.57	1.41	1.40	1.20	0.24
7	2.25	2.23	1.69	1.95	1.57	0.66
8	2.13	2.47	1.95	1.95	1.50	0.70

Table 2e: Average Weekly Feed Conversion Ratio

WEEK	0% WNKC	5%WNKC	10%WNKC	5% WUNKC	10%WUNKC	LSD.
1	1.67	1.89	2.38	1.62	3.05	0.61
2	2.46	3.11	3.07	2.61	3.29	1.10
3	2.28	2.93	3.60	2.79	3.63	0.73
4	2.67	2.31	2.67	2.75	3.20	0.51
5	2.37	2.94	3.29	2.22	3.86	1.40
6	2.51	2.81	3.45	3.29	4.92	0.70
7	2.27	2.10	2.86	2.39	2.87	0.78
8	3.79	5.22	3.69	5.79	4.97	3.93

Table 2f: Average Feed Intake /Bird/Day (g)

WEEK	0% WNKC	5%WNKC	10% WNKC	5%WUNKC	10%WUNKC	LSD.
1	13.81	12.38	8.81	11.88	9.77	3.22
2	43.70	40.30	37.71	41.00	28.40	8.32
3	71.70	64.80	67.00	71.40	51.50	13.86
4	99.40	70.40	65.30	75.60	65.00	16.10
5	121.90	90.40	85.90	83.60	84.40	15.64
6	135.00	122.60	105.60	119.30	112.90	23.75
7	133.40	102.80	78.10	104.40	93.70	20.40
8	147.30	108.50	80.10	115.10	106.00	13.80

Table 2g: Average Water intake /bird/day

WEEK	0%WNKC	5%WNKC	10%WNKC	5% WUNKC	10%WUNKC	LSD.

1	34.09	30.77	28.47	34.14	25.59	6.289
2	63.30	50.10	43.10	57.20	41.50	11.10
3	102.50	74.30	71.00	86.40	60.50	14.40
4	139.60	101.80	86.50	96.00	75.40	27.57
5	217.00	138.90	116.80	135.50	103.70	22.61
6	259.00	192.10	143.20	166.30	133.90	28.19
7	297.70	229.50	152.90	203.50	146.70	37.79
8	310.00	267.80	149.40	221.50	154.00	56.33

Table 2h: Average Weekly Feed Cost/ Live Weight Gain (GH¢)

WEEK	0%NKC	5%WNKC	10%WNKC	5%WUNKC	10%WUNKC	LSD
1	1.93	2.41	3.36	2.12	2.97	0.85
2	2.86	3.98	4.34	3.43	4.77	1.55
3	2.64	3.75	5.09	3.66	5.27	1.03
4	3.09	2.95	3.76	3.61	4.63	0.67
5	2.74	3.76	4.62	2.96	5.55	1.97
6	2.91	3.6	4.86	4.29	6.16	1.40
7	2.38	2.69	4.03	3.13	4.16	0.94
8	4.4	6.69	5.2	7.59	7.21	5.02