UTILISATION OF BRINE-DETOXIFIED *THEVETIA* (*Thevetia neriifolia* Juss) SEED FOR BROILER FEED PRODUCTION

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ABSTRACT

Conventional dietary plant protein sources for broilers are expensive thereby increasing cost of production. Cheaper but equally nutritive sources of plant protein are currently being explored. Thevetia Seed (TS), though rich in protein has limited use due to intrinsic antinutritional factors, which if detoxified could be harnessed. Information on detoxification and the use of TS in broiler production is scanty. Detoxification of TS and its utilisation in broiler feed production were therefore investigated.

One hundred grams of TS was soaked in water, ethanol (24 hours) or in 2.5, 5.0 and 7.5% brine solution for 2, 3 or 4 hours. The samples were either sun-dried for 3 days or toasted after soaking and thereafter analysed for chemical and residual glycoside composition. Treatment with the lowest residual glycoside was incorporated as TS Meal (TSM) to be a substitute for soyabean meal in broiler starter and finisher diets at 0, 25, 50, 75 and 100%. Two hundred 1- day old broilers were randomly allotted to five dietary treatments with four replicates of ten birds each. They were fed starter diets from day1-28 and finisher from day 29- 56. At weeks 4 and 7, blood samples from two birds per replicate were collected for serum biochemical and haematological analyses. In the last seven days of the experiment, 0.5% titanium dioxide indigestible marker was added to the diets to determine Apparent Ileal Digestibility (AID). Two birds per replicate were slaughtered for digesta collection and carcass characteristics. Other indices measured were Feed Intake (FI) and Weight Gain (WG). Data were analysed using descriptive statistics and ANOVA at p= 0.05.

Toasting significantly reduced crude protein in water and ethanol treated samples from19.35 in raw sample to 18.14 and 18.43%, respectively. Crude protein increased significantly in sun-dried water (22.1%) and brine treated (23.1%) samples. The TS soaked in 7.5% brine for 3 hours significantly increased crude protein from 23.1% to 44.87% and reduced glycoside content from 4.7% to 0.07% (98.51% reduction of glycoside). Variations in haematological and serum biochemical indices were not significantly different in values for birds on all treatments except in the levels of albumin, calcium, glucose and potassium ions at the finisher phase. The FI (105.8 ±0.7 g/bird) and WG (25.7 ±0.4 g/bird) at the starter phase were significantly reduced to 92.5 ± 0.9 g/bird and 21.2 ±0.4 g/bird for birds on 100% TSM ($p < 0.05$). Similar trend was observed for FI at the finisher phase which reduced from 176.4±5.7 to 118.6 ±1.1 g/bird. Birds on 50% TSM recorded highest values for live weight 1824.7 ±0.9 g/bird, dressed weight 1220.2 ±0.3 g/bird, breast 304.1 ±0.9 g, thigh 216.8 ± 0.6 g and AID coefficient of crude protein (0.79).

Detoxification with 7.5% brine for three hours best improved the nutritive value of *Thevetia* seed. *Thevetia* seed meal was well utilised and an enhanced apparent Ileal digestibility was achieved. *Thevetia* seed meal replaced up to 50% soyabean meal.

Keywords: Plant protein, Salt solution, Glycoside, Apparent Ileal digestibility.
Word count: **482**.

CERTIFICATION

I certify that this study was carried out under my supervision by Oluwayemisi Bosede AYINDE in the Department of Animal Science, University of Ibadan, Ibadan.

…… ……

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DEDICATION

This work is dedicated to

GOD ALMIGHTY

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1.0 INTRODUCTION

Livestock feed industry has enjoyed the attention of researchers over the years. This has resulted from the ever increasing price of conventional feed ingredients. Such a trend has drawn attention of stakeholders in animal production to the need for exploring the potentials of alternative but suitable feedstuff as substitute for the conventional ones.

Conventional feed ingredient can be defined as all those ingredients that have been established and proven to be generally acceptable in livestock production and commercial feed formulation. Examples of such feed ingredients are maize, soybean, groundnut cake cotton seed cake, fish meal and so on. Some of the feedstuffs are also of value in the diet of man thereby creating competition between human and his livestock. Hence there is a hike in the price of these ingredients, which becomes more severe by the seasonal nature of these agricultural products.

Over the years, a wide range of unconventional feed ingredients have been used to replace the expensive and more competitive conventional ones. They even enhance livestock performance and more cost effective than the conventional ones. (Tewe and Egbunike, 1992; Nwokolo, 1996; Amusa *et al., 2002*)

Animal Nutritionists have not relented in their quest to explore and establish the potential of other numerous and lesser known plant and animal products as feed ingredients in livestock industry. Plant and animal products such as pigeon pea, lima beans, cotton seed, sunflower, locust bean, cassava peel, feather meal, and blood meal have been elucidated through meaningful research to reduce or totally remove the toxins and anti-nutrient factors in these feedstuffs thereby making them of economic importance in livestock feed formulation. (Akande, 2009; Akande *et al*, 2010; Akintunde *et al*, 2010.).

In the bid to search for alternative feed ingredients, there is need to avoid feedstuff that will encourage keen competition between man and his livestock on one hand, and on the other hand to ensure that such material will be available in commercial quantities. They must be devoid of anti-nutrient factors, since there are not many plant materials that are

completely free of anti-nutrients or toxins. The quantity, lethal level and ease of removal of the toxin will determine the suitability of such material.

The plant *Thevetia neriifolia (Juss)* grows wild in the humid zone of West Africa including Nigeria. It is also known as yellow oleander, milk bush, trumpets flower or be-still tree which belongs to the family apocynaceae. It is a perennial shrub, 6m in height, native of Central and Tropical South America and named after the French monk F. Andre Thevet (1502 -1592) (Burkill,1985). It is an ornamental plant propagated for its flowers and used mainly as hedge plant. The seed contains 60- 65% oil while the defatted cake contains 30- 53% protein (Dutta, 1964; Ibiyemi et al, 2002; Oluwaniyi *et al*. 2007).

Work has been extensively carried out on the Pharmacological aspect of *T. neriifolia* while little has been documented on its nutritive value. Atteh *et al*. (1990) investigated the potentials of *Thevetia* oil as a replacement for palm oil in broiler chicks' diets and recommended further processing of the oil before it can be used effectively as a feed ingredient. Oderinde and Oladimeji (1990) analysed the composition of the oil and indicated a total unsaturated fatty acids of 75.4% of which oleic acid is 31.46% and linoleic acid 43.90%, it has the characteristic of a good edible oil, if the bitterness can be removed by alumina and alkali treatment.

Recently, work was directed towards detoxification of the seed for inclusion into the diets of one or two classes of livestock. (Ibiyemi and Oluwaniyi, 2003; Oluwaniyi et al., 2007). Such investigation met with some degree of success. The practicality of the methods of detoxification employed (in terms of cost and expertise) among the target clientele (local poultry farmers) leaves much to be desired. In a bid to detoxify *Thevetia* seeds, a method of detoxification that will increase the cost of production should not be employed as is the case with the alcoholic extraction (Oluwaniyi et al., 2007). Furthermore, the method should be practicable in terms of technicality and handling by local farmers which are the main beneficiaries of the findings of the research.

With recent global economic meltdown, animal protein from sources, such as, meat, fish, milk, egg are fast disappearing from the menu of most average and low income families due to relatively higher prices compared with maize and cassava. This has resulted in

poor nutrition among these people, which in turn resulted in ill health. Food and Agricultural Organisation (FAO, 2004) recommended daily minimum level of 30g of animal protein intake and 40g of plant protein intake per head. It is pertinent to know that an average Nigerian has not been able to consume this minimum recommendation as stated by Mayer (1976) who gave 5.5gof animal protein consumption per head per day.

The development of any nation and buoyancy of her economy is closely tied to the health of her work force and that of the population in general. In building a healthy and virile populace, there is need to balance the dietary protein intake from both plant and animal origin (Babatunde and Fetuga, 1975). This fact ensures that researchers in universities, government parastatals and private sectors have been geared toward finding alternative feed ingredients for the production of livestock products. This will make animal protein sources to be within the purchasing power of low-income and average Nigerians.

Broiler chicks are good converters of feed to muscle (flesh) and a cheap source of animal protein, if the cost of production can be brought down by sourcing for cheaper ingredients in formulating their ration without compromising their nutrient requirements. Feed accounts for seventy percent or more of the cost of production, the bulk of which emanates from the cost of dietary protein and energy (Olawumi, 2008). With a carefully chosen method of detoxification for Thevetia seeds, the meal, cake or oil can serve as a potential source of protein and or energy in broilers' diet, which may reduce the cost of production.

Various ways of eliminating toxic components and improving the nutritive value of feedstuff have been documented over the years. These include fermentation, ensiling, sun-drying, oven-drying, boiling, soaking in water or other solvents, autoclaving and so on depending on the nature of the substrate. (Verma, 2006; Olomu, 2011; Vasudevan *et al.*, 2011).

1.1 OBJECTIVES OF THE STUDY

The general objective of the study is to determine the best method of detoxifying *Thevetia* seed by employing three methods of detoxification (soaking in water, ethanol and brine). By monitoring the changes in their chemical properties, the best-treated seed will be milled and incorporated into broiler chicken diets.

1.2: SPECIFIC OBJECTIVES

Therefore, this study is aimed at determining the:

a, Appropriate method of detoxification of *Thevetia* seed.

b, Proximate composition of detoxified seed meal.

c, Residual quantity of glycosides (anti-nutritional factors) in detoxified seed.

d, Effect of detoxification on the amino acid profile of the seed meal.

e, Optimal level of inclusion of the seed meal into broiler starter and finisher diets

f, Performance of broilers fed detoxified *Thevetia* seed meal.

g, Apparent ileal digestibility of broilers fed detoxified seed meal.

1.3: JUSTIFICATION OF THE STUDY

a, The high cost of feed ingredients is a factor militating against the rapid expansion of the livestock industry.

b, Meeting animal protein intake requirement may continually be a mirage in developing countries due to food insecurity.

c, Cost of conventional feed ingredients has been on the increase in the last two decades.

d, The keen competition between man and his livestock on the use of these feed ingredients has foreclosed the downward review of their prices.

e, Thevetia seed is abundant and not competitive

f, Therefore the cake could serve as a useful source of protein in livestock (especially poultry) production if properly detoxified**.**

CHAPTER TWO

LITERATURE REVIEW

2.1 **THE PLANT *THEVETIA NERIIFOLIA* (JUSS)**

The plant *Thevetia neriifolia Juss.*, grows wild in the humid zone of West Africa including Nigeria. It is commonly known as Bush milk, Yellow oleander, Luckynut, Luckybean, Be-still plant, or Trumpet flower. Its local name in Yoruba is "Olomiojo". It is a perennial plant that belongs to the family Apocynaceae (Dutta, 1964). An ornamental plant , propagated for its flowers and used mainly as hedge row . It can grow to a height of about 5 metres or controlled at as little as 0.67 metres (Burkill, 1985). It has fine light-green shining leaves, which are spiral and linear in arrangement. The flowers which are yellow and trumpet-shaped are borne in terminal clusters. The tree leaves and fruits exude white latex when cut and it seeds profusely throughout the year. *T. neriifolia* has the ability to withstand drought and can produce seeds in commercial quantity. The plant is propagated either by stem cuttings or through the seeds. (Burkill, 1985). The fruit when unripe is hard and green but gradually turns black as it ripens. It has varying masses ranging from 2 to 6.1g (Ibiyemi and Oluwaniyi, 2007). The plant produces white latex which is highly poisonous. In spite of the toxicity of the plant, it has found useful applications in several spheres of life.

2.1.1 **POTENTIALS OF THE PLANT**

The potentials of different parts of the plant have been investigated. The extracts from the leaves and fruits are insecticidal in action (Satpathi *et al*., 1991; Guddeirar *et al*., 1992; Oji *et al*., 1993). The bark of T. neriifolia is used traditionally as an emetic and to treat malaria fever in West Africa. The oil-rich seeds are used to treat infected wounds and burns (Obasi and Igboechi, 1991; Oji *et al.*, 1993). Oji *et al*., (1993) demonstrated the rodenticidal properties of *T. neriifolia* when they fed lethal doses of ground bush milk seeds to non-fasted rats.

Latex from the plant is known to have nematicidal activity. It is very effective against the root-knot nematode *Meloidogyn incognita* (Ansari, 1990). The extracts from the leaves are also used as feeding repellants for slugs and it is said to be most effective against *Diplosolenoides occidentale* in *Phaseolus vulgaris* crops (Coto and Saunders, 1987).

Saxena and Jain (1990) worked on the oil in the seed of *T. neriifolia.* They observed its bactericidal activity most especially against *Bacillus subtilis* and *Staphylococcus anrens*. The oil was therefore recommended for the manufacture of bactericidal soaps. Obasi and Igboechi (1991) also assayed the bactericidal activity of *T. neriifolia* and reported its effectiveness against *Escheridia coli*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes.*

Atteh *et al*., (1990) investigated the potentials of *Thevetia* oil as a replacement for palm oil in broiler chicks' diets. They fed graded levels of the oil at 0%, 25%, 50% and 100% to broiler chicks from day old to week 3 of life. Their results showed a marked decrease in average daily feed intake and weight gain with increasing level of Thevetia oil and reduced protein, fibre and fat retention. However, their results showed that treatment effect on feed: gain ratio and mortality was not significant. They therefore recommended further processing of *Thevetia* oil before it can be used effectively as an ingredient in broiler feed. Atteh *et al*., (1995) also fed graded levels of *Thevetia* seed cake to broilers and recommended further processing of the seed cake for economical inclusion into broiler diets.

Oluwaniyi (2007) fed graded levels of ethanol- extracted *Thevetia* seed meal to cockerels and recommended up to 15% level of inclusion in cockerel diets.

2.1.2 **THE NUTRITIVE VALUE OF *THEVETIA NERIIFOLIA* SEEDS**

Little work has been documented on the nutritive value of T. neriifolia. The seed contains 60 – 65% oil while the defatted cake contains 30 – 53% protein (Ibiyemi *et al*., 2002). Information on the chemical composition of the seed showed that it contains up to 70% fixed oil (Obasi and Igboechi, 1991). Analysis of the composition of the oil

indicated a total unsaturated fatty acid of 75.4% of which Oleic acid is 31.46% and linoleic acid is 43.90% which is said to be a characteristic of good edible oil, if the bitterness can be removed by alumina and alkali treatment (Oderinde and Oladimeji, 1990). Oluwaniyi *et al*., (2007) reported a crude protein content of 42.79% in the raw seed to 53.60% in alcohol treated seed meal. They also reported the amino acid content of the seed meal before and after treatment with acid and alcohol and showed that *T. neriifolia* is limiting in lysine and methionine.

2.1.3 **TOXINS IN *THEVETIA NERIIFOLIA* SEEDS**

Toxins in *T. neriifolia* are not unique as there are not many seeds that are completely free of anti- nutrients or toxins of any type. The quantity and lethal level of the agent and ease of removal will determine how useful or otherwise the plant can be. Plants are chemical factories and rely upon elaborate chemical defense to ward off unwanted predators. For this reason they have in their arsenal an amazing array of thousands of chemicals toxic to bacteria, fungi, insects, herbivores and even man. (Ibiyemi and Oluwaniyi, 2007)

The toxicity of thevetia is due to the cardiac glycoside which is present in it. Cardiac glycosides are a class of a larger group of compounds known as **GYCOSIDES**. Glycosides are molecules or compounds formed from a monosaccharide bound by a glycosidic bond to either another monosaccharide or a non-carbohydrate residue in the same molecule. They are found in many drugs and spices and they are constituents of animal tissues (Vasudevan *et al*., 2011). Glycosides important in medicine because of their action on the heart are called **CARDIAC GYCOSIDES** (Melero *et al*., 2000; Desai, 2000). The main cardiac glycoside in *Thevetia* seed which has been reported to be water, ethanol and methanol soluble is known as **THEVETIN** (Chen and Chen, 1933). Other glycosides found in thevetia include thevetoxin, Peruvoside, Ruvoside and Nerifolin. Thevetin is made up of thevetin A and Thevetin B with molecular fomular and weight of $C_{42}H_{64}O_{19}$: 872.93 and $C_{42}H_{66}O_{18}$: 858.95 respectively, peruvoside has a molecular fomular and weight of $C_{42}H_{44}O_9$ and 548.65 (Arnold *et al*., 1935). Thevetia glycosides are soluble in methanol and ethanol but sparingly soluble in chloroform acetone (Merck

Index, 1976). Cardenolides in thevetia are not destroyed by drying or heating and they are very similar to digoxin from *Digitalis purpurea* (Wikipedia, 2011). The glycosides occur in higher concentrations in heart muscle than in blood. Cardiac glycosides can be investigated in the blood by competitive immunoassay. The serum potassium concentration should be monitored. Electrocardiography, frequent serum electrolytes (especially potassium concentration), and renal function tests are essential investigation (Ellenhorn and Barceloux, 1988).

2.1.4 **TOXICITY OF THEVETIA TO HUMANS**

Toxic exposure of humans to oleander cardenolides occurs throughout the geographical locations where this plant grows (Langford and Boor, 1996). Accidental poisonings occur throughout the tropics particularly in children (Shaw and Pearn, 1979; Brewster, 1986; Langford and Boor, 1996). The kernel of about ten (10) fruits may be fatal to an adult while the kernel of one fruit may be fatal to children (Saravanapavananthan, 1985). Generally, children and domestic livestock are at increased risk to *Thevetia* poisoning.

Deliberate self-poisoning with yellow oleander seeds is common in some parts of the world especially in Sri-Lanka and its environs (Eddleston *et al.*, 1999) It is associated with severe cardiac toxicity and a mortality rate of about 10% (Fonseka *et al*., 2002; de Silva *et al*., 2003). In Sri-Lanka, cases of attempted suicide with yellow oleander were extremely rare before 1980. During that year, the death of two girls who intentionally ate *Thevetia nerifolia* seeds was widely reported (Eddleston and Warrel, 1999). The practice suddenly became so popular that the number of cases admitted to Jaffna Hospital increased from zero in 1979 to 103 in 1983 (Saravanapavananthan and Ganeshamoorthy, 1988). Since then it has continued to gain popularity as a method of deliberate self-harm in Northern Sri-Lanka (Eddleston *et al*., 1998; Eddleston *et al*., 1999)

Thevetia neriifolia poisoning closely resembles digitalis poisoning with gastrointestinal and cardiac symptoms. Local irritation of mucous membrane is followed by nausea, vomiting and giddiness within hours. Other clinical features are severe diarrhea, abdominal pain, dilated pupils and occasionally convulsions (Fernando and Widyaratna,

1989). Samal *et al* (1992) reported additional symptoms to include weak and irregular pulse with varying degrees of heart block. This condition arises when the impulses generated from the atria fail to be transmitted to the ventricles. Jaundice and renal failure were also observed. Post mortem examination showed focal necrosis around central vein, patchy haemorrhages and dilation of central veins. Maringhini *et al.*, (2002) reported a case involving a 65-year old man who accidentally swallowed two seeds (non-fatal doses) of *thevetia*, about thirty minutes after ingestion of the seeds, the man came down with dizziness, giddiness, numbness and a burning sensation, diarrhea, sweating, vomiting and tremors.

Eddleston *et al.*, (2000) studied 351 cases of patients with history of oleander ingestion, results showed that most of the patients had conduction defects affecting the sinus or atrioventricular (AV) nodes. Serious yellow oleander induced arrhythmias were associated with higher serum cardiac glycoside concentrations and hyperkalaemia. There were also serious electrolyte disturbances in many of the patients.

2.1.5: **TOXICITY OF THEVETIA TO ANIMALS**

The toxicity of *Thevetia* to animal has been well documented (Atteh *et al., 1990;* Atteh *et al., 1995;* Oji and Okafor, 2000). All parts of the plant have been implicated to be toxic to animals. The aqueous, alcohol and acetone extracts of *Thevetia* leaf was reported as a piscticide in shrimp farming, with the aqueous extract having a higher potency than the other extracts (Sambasivan *et al.*, 2003). Report (Singh and Singh, 2002) also showed that the dilute aqueous solutions of the leaf and stem bark of *Thevetia* were active in killing fishes. Toxicity studies on the stem bark, leaf and seed kernels of yellow oleander using albino rats as test animals confirmed that extracts from all parts of the plant were toxic and produced marked poisoning symptoms that culminated in death (Oji and Okafor, 2000).

Atteh *et al*., (1995) reported the toxicity of *thevetia* cake to broilers both at the starter and finisher phases. They recorded drastic reduction in feed intake and weight gain which resulted in death irrespective of the level of inclusion. Prior to death, symptoms shown by

the animals include loss of coordination, staggering, flexing of toes, paralysis, rolling of the body on the long axis, circular flailing of the tail, muscular twitch, titanic convulsions, tremors, collapse and finally death (Pahwa and Chartterjee, 1990; Atteh *et al.,* 1995).

Pahwa and Chartterjee, (1990) reported that rats fed 20% and 30% of the kernels of *Thevetia* seeds recorded 80% and 90% mortality respectively after ten days. Apart from mortality resulting from the ingestion of *Thevetia* seed cake or extract, the oil has also been implicated in causing drastic changes in the reproduction potentials of female mosquitoes (Hussein, 1999). Treatment of the larvae of mosquitoes with the fixed oil of *Thevetia* reduced the vitellogenesis, synthesis of protein, carbohydrate, lipid contents and DNA and RNA materials. Generally, the oil has the ability to kill the early stages, inhibit growth and development of ovaries and reduce the reproductive potentials of adult female mosquitoes, thereby making it a potential suppressant of mosquitoes.

2.2 **DETOXIFICATION**

The biochemical processes whereby the obnoxious substances are rendered less harmful and more water soluble are known as **detoxification.** Lipophilic toxicants are hard for the body to eliminate and can accumulate to hazardous levels. Biotransformation on the other hand is the process whereby a substance is changed from one chemical to another by a chemical reaction within the body. It is an important defense mechanism in that toxic substance and metabolites are converted into less harmful substances that can be excreted from the body (Vasudevan *et al*., 2011).

2.2.1 **METHODS OF DETOXIFICATION**

Detoxification simply means considerable reduction or total removal of toxic substances in a material through the application of one or a combination of processes. The type of detoxification process that will be employed for a particular material will depend largely on the type and level of anti-nutritional factors the material contains. Researchers all over

the world have successfully employed various methods of detoxification for a wide range of materials thereby improving the nutritional values of the materials treated. Cassava, Soyabean, Cotton seed, Pigeon pea etc. all contain anti-nutritional factors which have been eliminated or considerably reduced by various methods of detoxification like heating, autoclaving, fermentation, soaking in solvents, ensiling etc. (Liener, 1972; Schwenke *et al.*, 1990; Amaefule and Nwagbara, 2004; Babayemi *et al*., 2004b)

Toxins are grouped generally into heat-stable and heat-labile toxins. The heat-labile toxins can easily be removed by thermal treatment of the materials, while the heat-stable toxins oftentimes have to be subjected to chemical reactions. Those that are heat-labile are sensitive to standard processing temperatures and they include lectins, proteinase inhibitors and cyanogens while the heat-stable group include among others antigenic proteins, condensed tannins, quinolizidine alkaloids, glucosinolates, gossypol, saponins, non-protein amino acids etc. (Liener and Kakade, 1980; D'Mello, 2000).

2.2.2: DETOXIFICATION BY HEAT TREATMENT

Subjection of most legumes and other unconventional feeds to heat treatment results in the detoxification of many of these potential feed ingredients. Anti-nutrients such as protease inhibitors, haemagglutinin, tannin, cyanide, and gossypol are totally removed or considerably reduced by subjecting the materials to heat treatment.

Heat-labile toxins are easily removed at heat labile processing temperature such as cooking, roasting, toasting, boiling, dry-heating, autoclaving, oven-drying, sun-drying. Soyabean is effectively detoxified by either dry or wet heat treatment of the cake (Liener, 1972).

The time/temperature combination is an important factor in the effectiveness of heat treatment as a method of detoxification of anti-nutrients in feeds. Thirty minutes of dry heating had been found to have little effect on haemagglutinin activity of certain varieties of *Phaseolus vulgaris* and activity was still detectable after 18 hours of heating (Udedibie and Carlini, 1998; de Muelenaere, 1964).

Cooking involves the pouring of materials into hot water and allowing it to stay for a period of time. Cooking has been found to be an effective way of reducing tannin (Chang and Fuller, 1964). They reported the reduction of whole cowpea tannins by 38-76%, the cotyledons by 53-59% and the testa by 66-75%.

Toasting involves the spreading of seeds thinly on a hot pan placed on a source of heat and turning or agitating the seeds until a golden brown colour is obtained and crispy to the touch. Udedibie and Carlini (1998) observed that toasting of *Canavalia ensiformis* seeds appeared to be highly inefficient as a method of reducing the inhibitors since only 42% of the inhibitors could be inactivated by this treatment.

Autoclaving has to do with placing the material to be detoxified in an autoclave at a preset temperature and pressure. Ologhobo and Fetuga (1984) reported that haemagglutinin activities were completely destroyed when cowpea and lima bean were autoclaved at 105^0C and at a pressure of $1.2kgf/cm^2$ for 15 minutes. Siddhuraju and Visayakumari (1991) reported a reduction in the level of tannin in *Mucuna pruriens* of 72% and 28% by autoclaving and dry heat treatments respectively.

2.2.3: SOAKING

Soaking involves pouring the material in cold water and allowing a period of time to lapse. Overnight soaking in water was reported to remove as much as 23% of the protease inhibitor in lima bean (Ologhobo, 1981). Reports by de Lumen and Salamat (1980) stated the importance of soaking for the determination of the trypsin inhibitors activity (TIA). They observed in winged bean that without soaking, loss of TIA after boiling was 4% whereas soaking in water or 10% ash solution resulted in at least 95% total loss. Soaking also complements the effectiveness of other processing methods. Prolonged soaking in water prior to autoclaving and ensiling with ammonia and urea before autoclaving has been proposed by Montilla *et al.,* (1990) as the practical method of canavanine reduction/ and or removal from seeds.

2.2.4: SOLVENT EXTRACTION

This has to do with the removal of toxic substances from a material by soaking the material in a suitable solvent in which the toxins are soluble. The toxins go into solution leaving a toxin-free substrate. Montoro *et al.*, (2001) reported the extraction of glycosides from *Cyclanthera pedata* fruits using a mixture of $CHCl_3$ and methanol in ratio 9:1 for the extraction. Finnigan and Lewis (1988) also reported the ethanolic extraction of glucosinolates in rapeseed meal. Oluwaniyi *et al.*, (2007) investigated the extractability of *Thevetia peruviana* glycosides with alcohol mixture. Alcohol extraction of the glycosides was studied as a function of time, solvent to meal ratio and solvent composition. *Thevetia* seed meal was extracted with 10:1, 15:1, and 20:1 solvent to meal ratio, for 45min, 24, 48, and 72 hr. Varying concentrations (50 to 100% v/v) of aqueous alcohol were also used. A concentration of 70 or 80% aqueous alcohol resulted in the lowest glycoside content, while a solvent to meal ratio of 15:1, extracted over a period of 72hr gave the best compromise between glycoside extraction and cost of extraction solvent. They also observed an increase in the protein content of the samples treated.

Schwenke *et al.*, (1990) reported the detoxification of rapeseed flour by first soaking the seeds in citric acid or ammonium carbamate followed by drying the seeds and then dehulling, crushing and defatting the seed. The flours were then extracted with a mixture of alcohol and ammonia to give flours of increased water and oil absorption and of excellent foaming properties. The treatment did not change the amino acid composition of the flour.

2.2.4.1 Ethanol Extraction

Oluwaniyi *et al.*, (2007), extracted the glycosides of *Thevetia* seeds by acid hydrolysis followed by ethanolic extraction of the aglycones and reported 98.31% reduction of the glycosides.

2.2.4.2 Brine Extraction

Brine is a solution of salt and water. It is mainly used as a preservative for vegetables, fish, fruit and meat through a process known as brining. The high salt content in brine prevents the growth of bacteria and thus help to preserve food for a long time without creating any difference in taste. Brine acts as an excellent detoxification solution and improves the metabolism, maintains the body's pH Factor and eliminates heavy metals (Innovateus, 2012).

2.2.5: HYDROLYSIS

Hydrolysis is another method that has been reported for effective detoxification of toxic seed cakes. This method is often applied for the removal of toxins that are glycosides. The glycosides are hydrolysed to yield the aglycones which can then be extracted using organic solvents – usually alcohols. The hydrolysis can be by enzyme action or by using an acid or alkaline medium (Finnigan and Lewis, 1988).

2.3: BIOCHEMICAL INDICATION OF *THEVETIA* POISONING

Serum electrolytes especially serum potassium level is indicative of thevetia poisoning. Serum potassium levels higher than 6mmol/l is a sign of poisoning as a result of electrolyte imbalance (Ellenhorn and Barceloux, 1988). Other diagnostic procedures include immunoassay of the cardiac muscle (Wikipedia, 2011).

2.4: BROILER PRODUCTION

Broilers are male or female birds reared and marketed for meat. Broilers are ready for the market at any age between six and nine weeks. They are good converters of feed to flesh.

2.4.1: FEEDING BROILERS

Feed cost constitutes more than 60% of the cost of raising broilers to market weight (Olomu, 2011). Apart from the cost, the success of broiler production depends on adequate feeding regime for the birds. Lemme (2003) reported that a better conversion ratio can only be achieved when all essential amino acid in the form of protein are in balanced amount particularly during early starter phase. Until recently, two types of broilers diets are usually recommended. The two types of diets are broiler starter and broiler finisher diets. In recent times, these diets have been further grouped into three, namely – broiler starter (0-4 weeks), grower (4-6 weeks) and finisher (6 weeks and above). (Olawunmi, 2008).

2.4.2 BROILER STARTER DIETS.

The nutrient requirement of broiler chicks from weeks 0-4 of age according to National Research Council (NRC) of America (1995) is 23% crude protein, and 3000-3200 kcal/kg metabolisable energy on dry matter basis. According to Babatunde and Fetuga, 1975, broiler chicks require 22-23% crude protein. The requirement for any nutrient is the amount of that nutrient which must be supplied in the diet to meet the needs of an animal in an environment compatible with good health (Olomu, 2011). It is established that the requirement for certain nutrients is influenced by the concentration of energy in the diet (Verman, 2006) Energy is by far the most important among the several nutrients needed for growth and for this reason it usually determines the requirement for total feed (Olomu, 2011).

2.4.3: BROILER FINISHER DIETS.

Broiler finisher diets contain the nutrient density specified for broiler chickens from age 5- 9 weeks. The requirement for protein is 20-21% and energy is 2800-3200 kcal/kg

metabolisable energy. The requirements for other nutrients are as follows: Calcium 1%; Phosphorus 0.70%; Lysine 1% and methionine0.45% (NRC, 1994).

2.5 PERFORMANCE CHARACTERISTICS

Weight gain and feed consumption values are convectional indices used to evaluate the performance of broilers. When assessing the performance of a flock, feed conversion ratio is taken into consideration. This ratio takes into cognizance the feed consumed per unit weight gain and it is considered optimum when the value is low (Olomu, 2011). The carcass characteristics is also considered to be a good index of performance as lean meat is the objective of the producer, laying of fat is undesirable for most consumers and is indicative of poor performance.

For optimum performance in broilers, the floor, feeder and drinking spaces should be optimum. Feed ingredients have their maximum levels of inclusion in the diets. Inclusion of an ingredient beyond the maximum level may induce imbalance of other nutrients, difficulty in feed formulation and may reduce the performance of the birds due to the presence of anti-nutritional factors beyond tolerance level (Verma, 2006).

2.6: DIGESTIBILITY OF NUTRIENTS.

Nutrients present in feed stuffs are not completely available to the animal body. A large portion of the nutrients is excreted in the faeces because they are not digested in the gastro-intestinal tract. Digestibility is defined as that portion of a feed or any single nutrient of feed, which is not recovered in the faeces or that portion that is absorbed by the animal (Verma, 2006). When digestibility is expressed in percentage, it is known as digestibility coefficient. Nutrient digestibility increases as the chick become older (Batal and Parsons, 2002; Gracia *et al*, 2003).

2.6.1: Digestibility assay.

The nutritive value of protein in feed ingredients is determined by the total content and availability of amino acids. Bioavailable amino acids may be defined as amino acids

which can be released by digestion, absorbed and utilized by animals. While it is possible that an amino acid could be absorbed in a form not suitable for utilization under some situations, it is obvious that undigested amino acids (those appearing at the terminal ileum or in the excreta) make no contribution to the requirements of the animal. Describing the protein in feed ingredients in terms of digestible amino acid content is closer than total amino acid contents in reflecting the amount that actually becomes available for maintenance and production purposes (McNab, 1994).

2.6.2: Factors affecting digestibility measurements

Several factors have been studied in digestibility measurements. For example, effect of feed intake (Butts *et al.*, 1993; Hess and Seve, 1999; Stein *et al.*, 1999; Albin *et al.*, 2001; Moter and Stein, 2004), feed processing (Zuprizal *et al.*, 1991; Amornthewaphat *et al.*, 2005), enzyme supplementation (Sebastian *et al.*, 1997; Hew *et al.*, 1999; Ravindran *et al.*, 2001; Rutherford *et al.*, 2002; Rodehutscord *et al.*, 2004; Wang *et al.*, 2005), markers (Jagger *et al.*, 1992; Kadim and Moughan, 1997a; Fan and Sauer, 2003), feed particle size (Svihus and Hetland, 2001; Fastinger and Mahan, 2003), poultry species (Huang *et al.*, 2000; Kluth and Rodehutscord, 2006), feeding regime (Kadim and Moughan, 1997a; James *et al.*, 2002), anti nutrtional factors (King *et al.*, 2000; Wiseman *et al.*, 2003), age of poultry (Zuprizal *et al.*, 1992; Siriwan *et al.*, 1993; Batal and Parsons, 2002a; Batal and Parsons, 2002b; Thomas and Ravindran, 2005), dietary fat content (Li and Sauer, 1994; Danicke *et al.*, 2000) and dietary fibre content (Raharjo and Farrel, 1984; Souffrant, 2001) among others, have been studied previously. These references imply a wide range of digestibility measurements and the factors affecting it by different methodology.

2.6.3: APPARENT AND TRUE DIGESTIBILITY

The apparent dry matter digestibility is calculated by difference between the dry matter intake and the dry matter voided in faeces during a period of time. Apparent digestibility measures the digestibility of nutrients of both dietary and endogenous origins i.e. nutrients that were absorbed from the intestines and then re-secreted into the intestinal tract in the form of endogenous proteins such as mucin, sloughed cells, enzymes etc. True

digestibility on the other hand, includes a correction for endogenous secretions and is considered to be a fundamental characteristic of the feedstuff that is relatively constant across varying dietary protein levels (Thomas and Ravindran, 2005). The digestibility of protein is essentially that of the individual amino acids that make up the protein and since the amino acids of undigested dietary proteins entering the large intestine may be metabolised by hind-gut microbes before they are excreted in the fecal material, values for total tract digestibility of amino acids are not accurately predicting amino acid absorption by the animal. To avoid the manipulation by hind-gut microbes, the digestibility of amino acids by monogastric animals is most correctly measured at the end of the small intestine and is referred to as Ileal digestibility values (Sauer and de Lange, 1992). Apparent Ileal digestibility excludes the secretions from microbes in the hind-gut. According to Kadim and Moughan (1997), variations in food intake may affect apparent digestibility values by altering the relative contribution of exogenous materials in the total digesta.

However, it should be noted that there are some limitations in using apparent digestibility data in diet formulations. First, the additivity of apparent digestibility values of individual ingredients when combined in diet formulations remains questionable (Angkanaporn *et al.*, 1997a; Bryden and Li, 2004). Second, for feedstuffs with low protein content e.g cereals, grain legumes, the apparent digestibility values are underestimated relative to feedstuffs with high protein content because of the relatively greater proportion of endogenous amino acids in the digesta or excreta, especially those amino acids present at low levels in cereals or grain legumes (e.g lysine, threonine and trytophan) and those present in high levels in endogenous protein (e.g threonine) will be affected. Third, because of the way in which ideal protein ratios are determined, the patterns reflect true digestibility rather than apparent digestibility (Bryden and Li, 2004).

2.7: Methods used in poultry digestibility studies

Three types of methods can be used to estimate the bioavailability of nutrients in poultry. These are; in vitro methods, indirect and direct in vivo methods (Verma, 2006). The direct in vivo methods of estimation focus on three techniques: the growth assay, balance trial and nylon-bag techniques.

2.7.1: Growth assays

The common criteria used in the growth assay technique are animal body weight gain, body weight gain as a function of live weight, gain:feed or feed:gain ratio, and nitrogen retention. Independent variables used to establish response relationship include dietary amino acids (AA) concentration and AA intake (Sibbald, 1987). Furthermore, the same author indicated that the basic growth assay for an available AA involves several steps: a basal diet deficient in the AA of interest is supplemented with one or more levels of that AA and fed to animals to establish a relationship between the response and the AA level. Simultaneously, the same basal diet supplemented with one or more levels of the material to be assayed is fed to comparable animals under the same conditions.

2.7.2: Use of inert marker for digestibility determination

Inert markers are frequently employed in digestibility studies. They provide a means of calculating the digestibility of a nutrient when a detailed review has been made of the efficacy of various markers used in digestibility studies (Kotb and Luckey, 1972). Maynard *et al.* (1979) stated that an ideal marker for the determination of digestibility values should have the following properties: totally indigestible and unobservable, pharmacologically inactive within the digestive tract, readily determined chemically, pass through the tract at a uniform rate and easily recovered.

The recovery of the marker, which is the quantity collected from the digesta or total collection of faeces expressed as a proportion of that consumed, is an important indication of its efficiency. Ideally, the recovery value should be 100%, Chromic oxide is the most widespread marker used in studies with pigs (Low, 1982). However, it has been associated with many problems. It has variable recovery rates (Moore, 1957; Payne *et al*., 1968b), and can oxidize unsaturated fats (Steel and Clapperton, 1982). Other metal oxides have been employed in digestibility studies.

The use of titanium dioxide ($Ti0_2$) has been explored in recent years as an alternative to commonly used digestible marker, such as chromic oxide. Titanium dioxide has advantages over Cr_2O_3 in that it can be legally added to an animal's diet (Titgemeyer *et al.*, 2001), and its use poses no concern as regards carcinogenic properties of Cr_2O_3 (Peddie *et al.*, 1982). Titanium dioxide has been reported to be a viable marker for total tract digestibility in studies with rats (Krawielitzki *et al*., 1987), pigs (Jagger *et al.*, 1992), chicken (Short et al., 1996), dairy cow (Hafez *et al*., 1998) and beef steers (Titgemeyer *et al*., 2001).

2.8: HAEMATOLOGICAL AND BIOCHEMICAL INDICES

Blood is an important index of physiological and pathological changes in the organism. Hematological and biochemical values are indicative of the state of the animal and they are tools for diagnostic exercises. Reference values aid decisions in diagnosing and controlling diseases/infections (Mitruka *et al*., 1977).

Analysis of normal hematological parameters of chickens is very essential in diagnosing the various pathological and metabolic disorders and can be used as a tool to assess the health status of a flock. Changes in the hematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and pathological factors.

Hematological values of poultry are influenced by age, sex, breed, climate, geographical location, season, day length, time of day, nutritional status, life habit of species, present status of individual and other factors (Duke, 1955).

CHAPTER THREE

DETOXIFICATION AND NUTRIENT EVALUATION OF THEVETIA SEED

3.1 INTRODUCTION

Presently, much emphasis is being placed on processing of legume seeds either by toasting, boiling, boiling and dehulling or soaking as a means of eliminating anti-nutritional factors. (Amaefule and Nwagbara, 2004)

Due to the high level of toxin in *Thevetia neriifolia* seeds it is necessary that it must undergo detoxification if it is going to find a place in livestock feed industry. The method to be employed will to a large extent depend on the physical and chemical properties of the toxin.

According to Chen and Chen (1933), *Thevetia neriifolia* glycosides are soluble in water and alcohol. Several attempts have been made in the past to detoxify the seed. These attempts have met with little success. Some of the methods employed included dry heating, autoclaving, fermentation, acid-hydrolysis, alcoholic extraction and alcohol mixture (Odetokun *et al*., 1999; Taiwo *et al*., 2004; Oluwaniyi *et al*., 2007; Oluwaniyi and Ibiyemi, 2007). Among these methods, alcoholic extraction and alcohol mixture met with appreciable degree of success in reducing the cardiac glycosides of the seed. The high cost of the solvent coupled with the technicality of handling, had placed a limitation on the extent to which the methods can be employed by farmers who are the target clientele.

The cost of detoxification should not be significant and the process should be readily undertaken by any farmer so that the use of *Thevetia* seed meal in feed formulation can be justified. It is in the light of these arguments that a cheaper and less technical method of detoxification is being advocated and evaluated. Therefore, processing techniques which are simple and inexpensive but still make detoxification of *Thevetia* seed worthwhile in terms of nutrient availability and digestibility should be pursued.

Desai, (2000) reported the affinity of *Thevetia* glycosides for sodium and potassium ions, stating categorically 'that "Thevetin binds specifically to Na^+ and or K^+ ATPase". This

binding tends to remove the glycosides from the substrate, thereby leaving it free of toxins. The extent to which the substrate will be free, depend on the concentration of the solvent, time of extraction and surface area exposed to the solvent among other variables.

In a bid to save cost and reduce the level of expertise required as outlined (Oluwaniyi *et al*., 2007), without destroying the nutritional components of *Thevetia* seeds, the present study employed simple methods of detoxification using cheaper and readily available solvents. This required no technical expertise to achieve the same if not higher level of success as the alcoholic extraction.

3.2 MATERIALS AND METHODS

3.2.1 SOURCE OF *THEVETIA NERIIFOLIA* SEEDS

Thevetia neriifolia fruits which have fallen off plants and turned black by reason of decomposition of the pericarp, were hand- picked and manually cracked between two stones to remove the soft seeds. The choice of fruits that were fallen off plant was to be sure that matured fruits were picked. The picking took place in Otta and Igbesa both in Ado-Odo/Ota Local Government Area of Ogun State. Picking and cracking continued simultaneously until 250 kg of seed was stored.

3.2.2 DETOXIFICATION OF *THEVETIA NERIIFOLIA* SEEDS

Preliminary studies were carried out to determine the conditions that would be most effective for detoxifying Thevetia Seed (TS) with little or no negative effect on the nutrient composition of the treated seed. Weighed whole seed (400g) was divided into 4 portions of 100g each and soaked in water and fine-grade ethanol for one day (24 hours) as described [Oluwaniyi *et al.*, 2007]. The extracts were then drained and the residues were either sun-cured or toasted and stored for proximate analysis and determination of residual glycoside content.

In another preliminary study, one kilogram of *Thevetia* seeds was divided into 10 portions of 100g each. Each of the portions was soaked in brine solution with varying concentrations of 2.5%, 5% and 7.5% for 2, 3 and 4 hours concurrently. Extraction was done at room temperature with intermittent stirring and at the end of the allocated time, the extracts were drained consecutively and the residues sun-dried until friable and stored for analysis.

One hundred grams of untreated seeds was also stored for analysis. This served as the control in these preliminary studies.

3.3 CHEMICAL ANALYSIS

3.3.1 PROXIMATE ANALYSIS

At the expiration of the experimental time, the samlpes were drained thorougly and air dried samples were taken for proximate analysis and the level of glycoside also monitored using the same methods as in the first set of experiment. Based on the results from these two sets of studies, samples of raw, ethanol-extracted and brine- extracted (C3) *Thevetia* seed meal were taken for amino acid analysis. Data collected were statistically analysed using descriptive analysis and analysis of variance and significant means were compared by Duncan's Multiple Range Test as outlined by Steel and Torrie (1980) with the aid of Statistical Analysis Software (1999) package.

Proximate analyses of all the samples were done according to the procedure of the Association of Official Analytical Chemist (AOAC, 2000). The moisture content of each sample was determined by drying to constant weight at 105^0C. Crude protein (CP) was determined by the Kjeldahl digestion method (N* 6.25). Crude Fibre was determined using the Trichloroacetic Acid (TCA) method. Determination of Ether Extract (Oil) was done using the Soxhlet extractor. Ash was determined by charring the residue from moisture determination over a Bunsen burner flame and igniting in the muffle furnace until the ash was grey or nearly white. The Nitrogen Free Extract (NFE) was estimated as follows: NFE = 100 – (% Crude Protein + %Crude Fibre + % Ether Extract + % Ash).

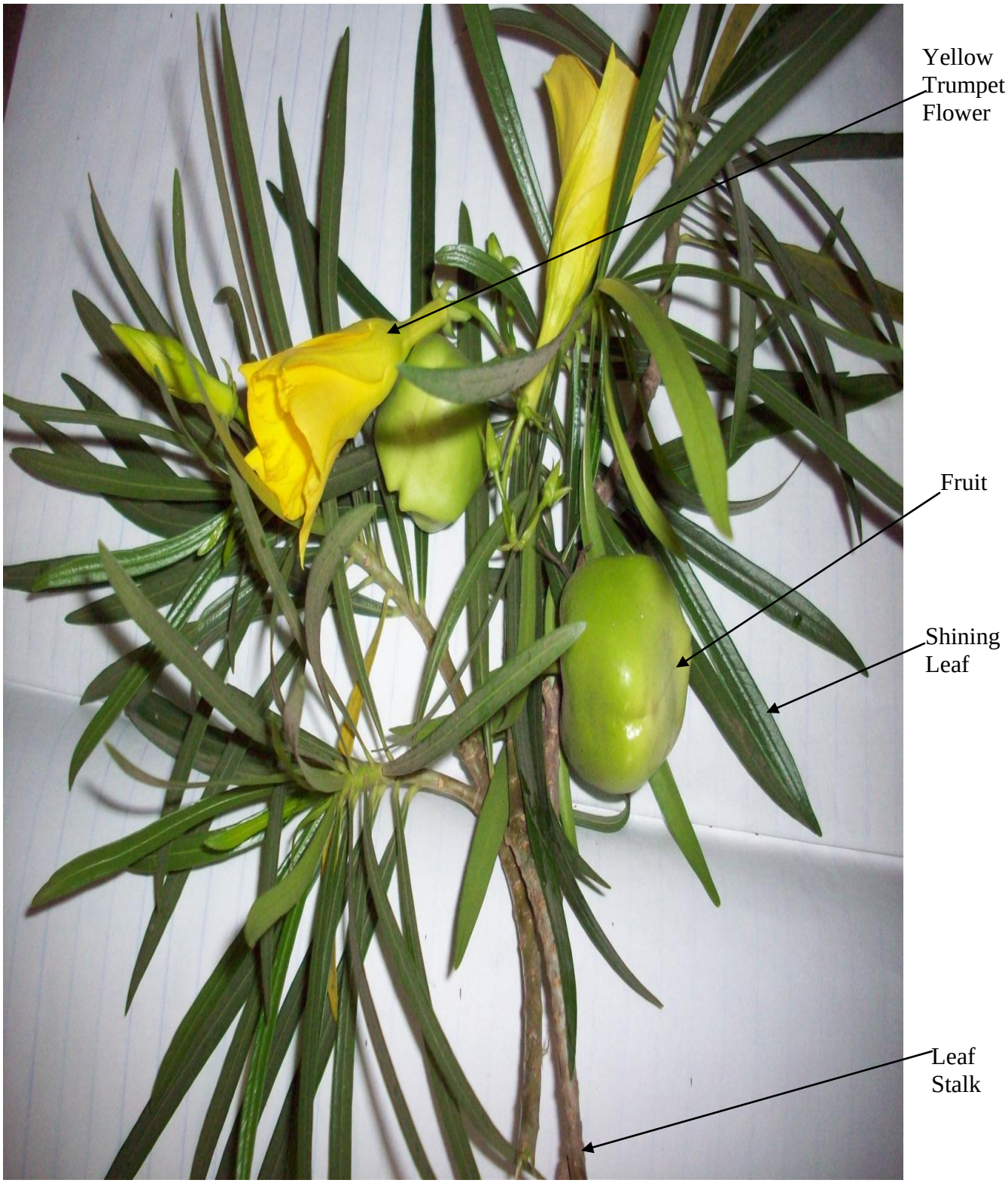

FIG 3.1: THEVETIA NERIIFOLIA PLANT

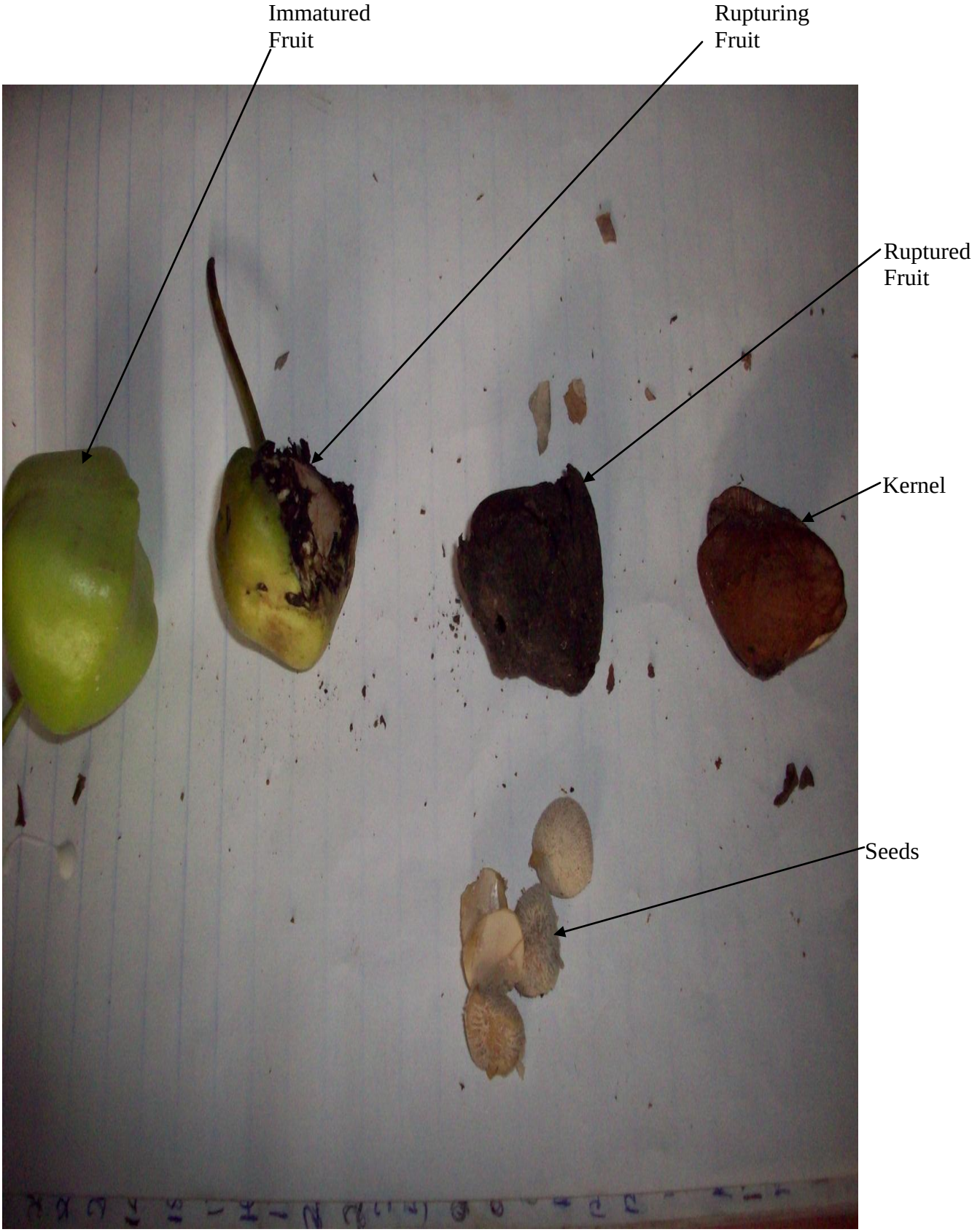

FIG 3.2: THEVETIA FRUIT, KERNEL AND SEEDS

3.3.2 GLYCOSIDE CONTENT DETERMINATION

The quantity of cardiac glycosides in the raw and treated samples and in the extracts was evaluated using a modification of the method described by El- Olemy *et al*. (1994). One (01) gram of each sample was soaked in 10ml of 70% ethanol and shaken with an orbital shaker for one hour. The extracts were purified using lead acetate and Na_2HPO_4 solutions before adding freshly prepared Baljet's reagent (95ml aqueous picric acid + 5ml 10% aqueous NaOH). The intensity of the colour produced was measured with a spectrophotometer at a wavelength of 495nm. A blank was prepared with distilled water and Baljet's reagent. Standard glycosides (mg/ml) 1.0, 1.5, 2.0, 2.5, 3.0 were also processed and the absorbance determined to facilitate the concentration of the glycoside.

3.3.3 EXTRACTION OF OIL FROM *THEVETIA* SEED MEAL (TSM)

Oil was extracted from the best detoxified TSM using two methods of extraction namely hydraulic press and expeller. The cake from both extractions was analysed for proximate composition.

3.3.4 AMINO ACID PROFILE DETERMINATION

The efficiency of protein utilisation depends to a large extent on the amino acid composition of the diet. Therefore, a good knowledge of the quality of the raw materials is the basis of successful modern feed compounding (Rodehutscord *et al*., 2004).

Quantitative analyses of amino acids were carried out using High Performance Liquid Chromatography (HPLC) after hydrolysis with 6M HCl for 18 hours at 110^0C as described by Pearson, (1991).

3.4 ENERGY CALCULATION

3.4.1 GROSS ENERGY CALCULATION

Gross energy was calculated based on the procedure of Ekanayeke *et al.*, (1999) as follows:

Gross Energy (KJ/100g DM) = (% Protein x 16.7) + (% Lipid x 37.7) + (% Carbohydrates x 16.7)

% Carbohydrates was estimated as described (Muller and Tobin, 1980)

% Total Crude Carbohydrate = 100 – (Crude Protein + Crude Lipid + Crude Fibre + Ash)

3.4.2 METABOLISABLE ENERGY CALCULATION

In calculating the metabolisable energy value of the samples, the procedure of Pauzenga, (1985) was employed as follows:

Metabolisable Energy (Kcal/kg DM) = 16.7 x % Protein + 37.7 x % Fat + 16.7 x % Nitrogen Free Extract

3.5 STATISTICAL ANALYSIS

The data (replicated three times) in a completely randomised design were analysed using descriptive analysis and analysis of variance of Statistical Analysis Software (1999) at p=0.05. When variations were significant, means were compared with Duncan Multiple Range Test of the same software.

3.6 RESULTS

3.6.1 PROXIMATE COMPOSITION OF RAW AND DETOXIFIED *THEVETIA* SEED (TS)

The proximate composition of raw, water and ethanol-detoxified TS is summarized in Table 3.1.

3.6.1.1 Crude Protein (CP).

Crude protein decreased significantly ($P<0.05$) from19.35% in the raw sample to 18.14% in T_1 (TS soaked in water and toasted) and 18.43% in T_3, but T_2 (TS soaked in water and sun-dried) had significantly ($P<0.05$) higher value of 22.11%.

3.6.1.2 Crude Fibre (CF).

A similar trend was observed for crude fibre, with a general drop from 6.56% in T_R to 5.62% in T_1. However the variations observed for CF in T_2, T_3 and T_4 were not significant ($P>0.05$).

3.6.1.3 Ether Extract (EE).

The trend continued for Ether Extract. The lowest ($P<0.05$) value of 35.20% for Ether extract was observed for T_3 (TS soaked in fine-grade ethanol and toasted) but this value was not significantly ($P>0.05$) different from the value observed for T_4 (TS soaked in fine-grade ethanol and sun-dried). However, the variations observed for ether extract for T_1, T_2, and T_3 were not significant.

3.6.1.4 Ash.

There were significant ($P<0.05$) variations observed in the composition of ash. Mean values observed ranged from 13.0% in the raw meal (T_R) to 11.04% in T_4 (TS soaked in fine-grade ethanol and sun-dried). Treatments 1and 2 (T_1 and T_2) however, showed no significant differences.

3.6.1.5 Nitrogen Free Extract (NFE).

Values observed for Nitrogen free extracts ranged from 12.56% in T_1 to 28.31% in T_4 . These values varied significantly ($P<0.05$) among the groups except in T_2 (19.47%) and T_3 (20.09%) where the variations were not significant ($P>0.05$).

3.6.1.6 Dry Matter (DM).

Dry Matter significantly ($P<0.05$) increased in the toasted samples (T_1 and T_3) from 88.60% in the raw sample (T_R) to 90.40% in T_1 and 91.02% in T_3.

3.6.1.7 Gross Energy

There was a general decrease in gross energy from 5.94 kcal/g DM in the raw sample T_R to 5.02 kcal/g DM in T_4 but the apparent differences observed between T_R and T_2 were not significant ($P>0.05$).

TABLE 3.1: PROXIMATE COMPOSITION (g/100g) OF RAW AND DETOXIFIED TS.

Nutrients	T_R	T_1	T_2	T_3	T_4	SEM
Crude protein (%)	19.35^b	18.14^d	22.11^a	18.43^d	$18..86^c$	0.08
Crude fibre (%)	6.56^a	5.62^c	6.16^b	6.04^b	6.12^b	0.06
Ether extract (%)	44.01^a	42.02^a	40.12^a	35.20^b	35.67^b	1.21
Ash (%)	13.0^a	12.06^b	12.14^b	11.26^c	11.04^c	0.08
Nitrogen free extract (%)	17.08^c	12.56^d	19.47^b	20.09^b	28.31^a	0.17
Dry matter (%)	88.60^b	90.40^a	87.11^c	91.02^a	86.22^d	0.23
Gross energy (kcal/g DM)	5.94^a	5.20^b	5.89^a	5.11^b	5.02^b	0.10

abcd- Means along the same row with any identical superscripts are not significant (P>0.05); **T_R =** untreated TS; T_1 = *Thevetia* seed (TS) soaked in water and toasted; T_2 = TS soaked in water and sun-dried; T_3 = TS soaked in fine-grade ethanol and toasted; T_4= TS soaked in fine-grade ethanol and sun-dried. SEM = Standard Error of Means; DM = Dry matter.

3.6.2 GLYCOSIDE CONTENT.

The results of the level of glycoside in treated and untreated TS are as presented in Table 3.2. The level of glycoside significantly reduced from 47.2g/kg in the raw sample (T_R) to 0.08g/kg in T_3 (TS soaked in fine-grade ethanol and toasted) which was about 98.3% reduction in glycoside composition. The reduction in glycoside composition in water treated samples T_1 (TS soaked in water and toasted) and T_2 (TS soaked in water and sun-dried) was 41.28% and 45.74% respectively. Differences observed in values for water treated and ethanol treated samples were significant ($P<0.05$). The values observed for ethanol treated samples T_3 (TS soaked in fine-grade ethanol and toasted) and T_4 (TS soaked in fine-grade ethanol and sun-dried) were 0.08 and 0.09 about 98.30% and 98.09% respectively.

TABLE 3.2. GLYCOSIDE CONTENT OF RAW AND DETOXIFIED *THEVETIA* SEED (TS).

Sample	Total glycoside (%)	Reduction in glycoside level (%)
T_R	4.70 [a]	0.00 [c]
T_1	2.76 [b]	41.28 [b]
T_2	2.55 [b]	45.74 [b]
T_3	0.08 [c]	98.30 [a]
T_4	0.09 [c]	98.09 [a]
sem	0.56	3.45

Values are means of three determinations. a,b,c means along the same row with any identical superscript are not significant ($p>0.05$). SEM = Standard error of means.

3.6.3: PROXIMATE COMPOSITION OF BRINE- TREATED *THEVETIA* SEED (TS_B)

The proximate composition of brine-treated Thevetia seed is shown in Table 3.3.

3.6.3.1 Crude Protein (CP).

There were significant ($p<0.05$) differences in the mean values observed for crude protein. Crude protein, significantly ($p<0.05$) increased in all brine-treated samples. The observed mean values for crude protein ranged from 23.20% in B_4 (TS soaked in 5.0% brine for 4 hours) to 19.36% in the raw sample T_R. The apparent variations observed for brine-treated samples A_3 (TS soaked in 2.5% brine for 3 hours), A_4 (TS soaked in 2.5% brine for 4 hours), B_2 (TS soaked in 5.0% brine for 2 hours), B_3 (TS soaked in 5.0% brine for 3 hours), B_4 (TS soaked in 5.0% brine for 4 hours), C_2 (TS soaked in 7.5% brine for 2 hours), C_3 (TS soaked in 7.5% brine for 3 hours) and C_4 (TS soaked in 7.5% brine for 4 hours) were not significant ($p>0.05$).

3.6.3.2 Crude Fibre (CF).

Observed values for crude fibre ranged from 6.56% in raw sample T_R to 5.25% in B_4 (TS soaked in 5.0% brine for 4 hours). These variations were however not significant ($p>0.05$).

3.6.3.3 Ether Extract (EE).

Composition of ether extractives varied significantly ($p<0.05$) from 44.01% in raw sample to 32.75% in A_4 (TS soaked in 2.5% brine for 4 hours). The variations observed in A_4 (TS soaked in 2.5% brine for 4 hours), B_4 and C_4 were not significant ($p>0.05$). Similarly, the variations observed for A_2, A_3, B_2, B_3, C_2 and C_3 were not significant ($p>0.05$).

3.6.3.4 Ash

Observed values for ash ranged from 15.20% in C_4 (TS soaked in 7.5% brine for 4 hours) to 13.00% in raw sample $T_{R..}$ Apparent variations observed in values for A3, A4, B2, B3 and B4 were not significant (P>0.05). Similarly, the apparent variations observed in values for C2, C3 and C4 were not significant (p>0.05), but significantly (P<0.05) higher than values observed for A2 (TS soaked in 2.5% brine for 2 hours) and TR (raw sample) which are 13.89% and 13.00% respectively.

3.6.3.5 Nitrogen Free Extract (NFE).

Observed values for Nitrogen free extractives ranged from 24.35% in B4 (TS soaked in 5.0% brine for 4 hours) to 15.56% in C2 (TS soaked in 7.5% brine for 2 hours). Values observed for A4, B4 and C4 (24.04%, 24.35% and 23.13% respectively) were not significantly (P>0.05) different among these groups but significantly (P<0.05) higher than those observed for other treatments.

Table 3.3: Proximate Composition of Brine-treated TS

Nutrients (%)	A2	A3	A4	B2	B3	B4	C2	C3	C4	Raw	SEM
Crude protein	20.1^{b}	23.1^{a}	23.2^{a}	23.1^{a}	23.1^{a}	23.2^{a}	23.0^{a}	23.1^{a}	23.2^{a}	19.4^{b}	2.5
Crude fibre	6.1	5.7	5.8	5.8	5.6	5.3	5.9	5.7	5.7	6.6	2.1
Ether extract	42.9^{b}	38.4^{b}	32.8^{c}	40.7^{b}	38.4^{b}	32.8^{c}	40.6^{b}	38.4^{b}	32.8^{c}	44.0^{a}	1.1
Ash	13.9^{c}	14.3^{b}	14.3^{b}	14.4^{b}	14.4^{b}	14.4^{b}	15.0^{a}	15.2^{a}	15.2^{a}	13.0^{c}	2.1
NFE	17.0^{b}	18.7^{b}	24.0^{a}	16.1^{c}	18.5^{b}	24.4^{a}	15.6^{c}	17.6^{b}	23.1^{a}	17.1^{b}	2.1
Dry matter	88.8	88.8	88.9	88.8	88.9	88.9	88.9	88.9	88.9	88.6	1.1
Moisture	11.3	11.2	11.1	11.2	11.1	11.1	11.2	11.1	11.0	11.4	0.9

Abcd = means along the same row with any identical superscript are not significant (p>0.05)

Raw = Untreated *Thevetia Seedl,* A_2 = Solution A + 100g *Thevetia Seed* soaked for 2 hours

A_3 = Solution A + 100g *Thevetia Seed* soaked for 3 hours ,A_4 = Solution A + 100g *Thevetia* Seed soaked for 4 hours

B_2 = Solution B + 100g *Thevetia Seed* soaked for 2 hours, B_3 = Solution B + 100g *Thevetia Seedl* soaked for 3 hours

B_4 = Solution B + 100g *Thevetia Seed* soaked for 4 hours, C_2 = Solution C + 100g *Thevetia Seed* soaked for 2 hours

$_{C3}$ = Solution C + 100g *Thevetia Seed* soaked for 3 hours, C_4 = Solution C + 100g *Thevetia Seed* soaked for 4 hours

A = 2.5% Sodium Chloride Solution., B = 5.0% Sodium Chloride Solution,. C = 7.5% Sodium Chloride Solution.

Figures 2, 3 and 4 stand for time in hours. NFE= Nitrogen Free Extract.

SEM = Standard error of means.

TSM = Thevetia seed meal.

3.6.4: GLYCOSIDE LEVEL OF BRINE-TREATED TS

The level of cardiac glycoside (measured as digitoxin) in the samples is summarised in Table 3.4. Glycoside content of brine-treated samples significantly ($P<0.05$) reduced from 4.70% in the raw meal to 0.07% in C_3 (TS soaked in 7.5% brine for 3 hours). There was also a marked reduction in glycoside level in samples A_3 (TS soaked in 2.5% brine for 3 hours) and B_2 (TS soaked in 5.0% brine for 2 hours), (0.21% and 0.41% respectively).The level of glycoside reduction observed for samples A_3, B_2 and C_3 were not significant but the value observed for C_3 was the lowest, even below what was observed for the ethanol treated samples T3 (TS soaked in fine-grade ethanol and toasted) and T4 (TS soaked in fine-grade ethanol and sun-dried). Percentage reduction in glycoside composition ranged from 63.40% in C4 (TS soaked in 7.5% brine for 4 hours) to 98.51% in C3 (TS soaked in 7.5% brine for 3 hours). The highest value of 98.51% observed for C3 was closely followed by 95.53% and 91.28% for A3 and B2 respectively. The observed value for percentage glycoside significantly ($P<0.05$) dropped from 75.53% in C2 (TS soaked in 7.5% brine for 2 hours) to 63.40% in C4, however values observed for B4 (TS soaked in 5.0% brine for 4 hours) and C2 were not significantly different ($P>0.05$)

Table 3.4: Glycoside Level of Brine-detoxified TS

Sample	Glycoside level (%)	Reduction of glycoside (%)
Raw	4.70^{a}	0.00
A2	0.48^{d}	89.79
A3	0.21^{f}	95.53
A4	0.66^{d}	85.96
B2	0.41^{f}	91.28
B3	0.68^{d}	85.53
B4	1.09^{c}	76.81
C2	1.15^{c}	75.53
C3	0.07^{f}	98.51
C4	1.72^{b}	63.40

Abcd = means along the same row with any identical superscript are not significant ($p>0.05$); **Raw = Untreated *Thevetia* seed , A_2 = Solution A + 100g *Thevetia Seed* soaked for 2 hours. A_3 = Solution A + 100g *Thevetia Seed* soaked for 3 hours, A_4 solution A + 100g *Thevetia Seed* soaked for 4 hours. B_2 = Solution B + 100g *Thevetia Seed* soaked for 2 hours; B_3 = Solution B + 100g *Thevetia Seed* soaked for 3 hours; B_4 = Solution B + 100g *Thevetia Seed* soaked for 4 hours; C_2 = Solution C + 100g *Thevetia* seed for 2 hours; C_3 = Solution C + 100g *Thevetia Seed* soaked for 3 hours; C_4 = Solution C + 100g *Thevetia* seed soaked for 4 hours. The alphabet stands for the concentration of brine and the number stands for time in hours. A = 2.5% Sodium Chloride solution; B = 5.0% Sodium Chloride solution; C = 7.5% Sodium Chloride solution.**

3.6.5 PROXIMATE COMPOSITION OF DEFATTED TSM

Table 3.5 shows the summary of the proximate composition of TSM after extracting oil from it. The two methods of oil extraction employed gave significant variations (P>0.05) in the values recorded for all the nutrient components except for CF, ash and dry matter.

3.6.5.1 Crude Protein (CP).

Crude protein significantly (P<0.05) increased from 27.20%, with hydraulic extraction to 44.8%, with expeller extraction.

3.6.5.2 Ether Extract (EE).

Ether extract significantly reduced from 28.5% with hydraulic extraction to 5.1% with expeller.

3.6.5.3 Crude Fibre (CF).

The observed variations in the mean value for CF, were not significant (P>0.05). CF increased from 13.8% with hydraulic extraction to 14.2% with expeller.

3.6.5.4 Ash

The composition of ash dropped (P>0.05) from 8.0% with hydraulic extraction to 7.7% with expeller.

3.6.5.5 Nitrogen Free Extract (NFE).

Percentage NFE significantly (P<0.05) increased from 18.5% with hydraulic extraction to 255.3% with expeller.

3.6.5.6 Dry Matter (DM).

The method of oil extraction had no significant (P>0.05) effect on the percentage DM.

Table 3.5: Proximate Composition (g/100g DM) of Defatted *Thevetia* Seed Meal.

Nutrient (%)	Hydraulic	Expeller	SEM
Crude protein	27.2[b]	44.8[a]	2.16
Ether extract	28.5[a]	5.1[b]	2.05
Crude fibre	13.8	14.2	0.34
Ash	8.0	7.7	0.41
NFE	18.5[b]	25.3[a]	1.94
Dry matter	96.0	97.2	3.85

NFE= Nitrogen Free Extract; SEM = Standard error of means

3.6.6. AMINO ACID PROFILE OF RAW AND TREATED TSM

The amino acid profile of raw and treated TSM is given in Table 3.6. The table shows the summary of the amino acid profile for raw, ethanol-extracted and brine-extracted (7.5% brine) TSM. There was a general drop in the level of the different amino acid in the brine-treated sample as compared to the ethanol- extracted sample but the decreases were not significant.

Table 3.6: Amino Acid Analysis (g/100g) of Raw and Detoxified TSM

Amino Acid	TSM_R	TSM_E	TSM_B (C_3)	Defatted TSM_B	*SBM	**Egg
Alanine	2.03	1.37	2.06	3.82	2.01	5.7
Arginine	2.03	1.92	2.35	4.34	3.69	5.9
Aspartic acid	8.98	9.89	9.20	17.03	5.22	9.2
Cysteine	0.76	0.75	0.76	1.41	0.70	
Glutamic acid	6.43	9.09	7.09	13.12	8.48	15.7
Glycine	1.64	1.01	1.67	3.10	2.10	3.20
Histidine	0.73	0.63	0.75	1.38	1.80	2.41
Isoleucine	1.33	0.95	1.34	2.49	2.20	7.1
Leucine	2.48	2.21	2.53	4.68	3.93	9.9
Lysine	2.02	1.80	2.55	4.73	3.54	6.4
Methionine	0.40	0.29	0.41	0.75	0.66	5.4
Phenylalanine	1.53	1.46	1.67	3.10	2.60	7.5
Proline	1.92	1.74	2.03	3.76	2.44	3.8
Serine	1.78	1.41	1.81	3.35	2.50	8.5
Threonine	1.18	0.92	1.21	2.24	1.93	4.0
Tyrosine	1.13	0.88	1.13	2.08	1.84	3.75
Valine	1.81	1.61	1.81	3.36	2.30	8.8

TSM_R = Raw *Thevetia* Seed Meal; TSM_E = Ethanol-extracted TSM; TSM_B = Brine-extracted TSM; SBM = Soyabean meal.

*source: Olomu, 2011; ** source: Lewis *et al.*, 2010.

3.7 DISCUSSION

The processing methods used in this study resulted in a decrease in the crude protein content of the treated samples except for T_2 and the brine-treated samples where CP significantly increased from 19.35% in the raw sample to 22.11% in T_2 (soaked in water and sun-dried) and 23.2% in C_3 (7.5% brine). The changes observed especially in the sun-cured samples must have been due to the activities of micro organisms during soaking (fermentation) and sun-curing which could have resulted in biochemical changes and significant modification of food quality. This agrees with the report of Campbell and Laherrere (1998) which showed that fermentation could improve the nutritive qualities of food in both plant and animal tissues. The decrease in percentage CP for the toasted samples could be attributed to the negative impact of heat on proteins as reported elsewhere. [Vasudevan *et al*, 2011]. The percentage composition of nutrients especially crude protein recorded in this study was below the values reported by Oluwaniyi *et al.*, 2007. This could be due to differences in the location of the source of *Thevetia* seeds and the methods of detoxification employed.

The reduction in the level of cardiac glycoside measured as percentage digitoxin in all treatments agreed with other reports [Chen and Chen, 1933 and Bai and Koshy, 1999] in which glycosides of *Thevetia* are water and alcohol soluble. These affirmed that glycosides are more soluble in ethanol than in water. In the present study, water-extracted TS recorded 45.74% reduction in the level of glycoside as compared to the raw sample, while ethanol extraction was 98.30%. The undesirable cost associated with the use of ethanol in Study 1 gave rise to Study 2 in which brine was used as the solvent. Brine-treatment recorded a higher percentage (98.51%) reduction of glycoside in C_3 (7.5% brine soaked for three hours) as compared to ethanol-treatment. This agrees with the report of Desai, 2000 who stated that cardiac glycosides have affinity for Na^+ and/or K^+ contained in brine. The reversal of the reaction after three hours could be attributed to lack of Na^+ ions in the solution to combine with thevetin in the presence of ATPase resulting in a relapse of the reaction.

Oil extraction increased the percentage CP of detoxified TSM. The CP of full-fat TSM increased from 23.12% in C_3 to 27.2 and 44.8% in the hydraulic and expeller- extracted

samples respectively.This is in line with other reports [Finnigan and Lewis, 1988 and Lutz and Pryztulski, 2008] in which the crude protein of oilseeds tend to increase when the oil content is reduced considerably. The differences observed in the nutrient composition of the cake from the two methods employed could be as a result of the fact that the proteins got more concentrated as more oil was extracted by the expeller method. This therefore suggested the superiority of electrical power over the hydraulic press.

The amino acid profile of brine-detoxified TS compared favorably with that of other oilseeds like soyabean and groundnut. The values observed for the essential amino acids of defatted TSM were higher than those of SBM except for histidine in which TSM had 1.38% and SBM had 1.80%. This suggests that the protein quality of TSM could be better than that of SBM. The protein of TSM is limiting in lysine and methionine as do other oil seed cakes. The observed values for some amino acids like glutamic acid, glycine, aspartic acid and proline in TSM compared favourably with the standard reference protein of egg and even higher in some cases as in the case of aspartic acid which had a value of 17.03g/100g as compared to 9.2g/100g in egg.

CHAPTER FOUR

4.0 PERFORMANCE CHARACTERISTICS OF BROILERS FED GRADED LEVELS OF *THEVETIA* SEED MEAL

4.1 INTRODUCTION

The gap between the required dietary animal protein intake and the actual intake among Nigerians can be conveniently bridged. This can be achieved by reducing the cost of feed through sourcing for high quality but unconventional feed ingredients that are cheaper than the conventional ones. According to Longe (2006), poultry production represents the fastest means of correcting shortage of dietary animal protein because of their faster rate of production and turn over of investment than cattle, sheep and goats.

Feed alone accounts for over seventy percent of total cost of producing birds (Atteh, 2002). The quality and quantity of each feed ingredient in a diet determine the performance of the birds. Whether a ration is balanced or not is a function of the active ingredient contained in the feed stuff. Birds can only perform economically well and profitably if they consume on daily basis the appropriate amount of energy, protein, vitamins and minerals (Oluyemi and Roberts, 2000). Various studies have revealed that broilers fed with high quality rations perform better than their counterparts reared on low quality or badly produced feeds (Oluyemi and Roberts, 2000).

The use of cheaper unconventional feed sources to replace conventional but expensive ones in livestock feed formulation, would bring about considerable success in reducing the high cost of production. Some of these feed resources that have been used successfully in monogastric feed formulation include Agro-industrial by products such as cassava flour and peels (Omole, 1992; Onabowale, 1992; Idahor *et al*., 2010), leucerna leaf meal in broiler diets (Dada *et al*, 1998), and pigeon pea seed meal (Amaefule and Ukpanah, 2010). In some cases, these sources have been supplemented with other substances to boost their nutritional values.(Iyayi and Aderolu,2004)

Thevetia seed meal has also been used in cockerel ration in recent times (Oluwaniyi, 2007), after alcoholic extraction of the glycosides and a 15% level of inclusion

recommended. The present study explores the use of brine-extracted seed meal in broilers' diets at a level of inclusion that can bring about justifiable reduction in the cost of production and make animal protein more affordable and accesible to the low and average income populace.

4.3.0 MATERIALS AND METHODS

4.2.1 Experimental Site.

The experiment was carried out at the pullet unit of the Teaching and Research Farm, University of Ibadan. A total of twenty pens were used to accommodate two hundred (200) birds on five dietary treatments with four replicates per treatment and ten birds per replicate.

4.2.2 PREPARATION OF THEVETIA SEED

Thevetia seed (TS) used in this study was soaked in 7.5% brine solution for 3 hours and drained at the end of the time. The seed was rinsed with freshly prepared 7.5% brine solution to remove residual glycosides before draining with a sieve. The seed was sun dried until friable, milled and the oil was extracted by expeller method. The substrate was stored and incorporated into broiler starter and finisher diets at graded levels (%) 25, 50, 75 and 100 in both phases.

4.2.3 EXPERIMENTAL DIETS

Thevetia seed meal with other feed ingredients was used to formulate diets to meet the NRC (1994) nutrient requirement of broilers for both starter and finisher phases as shown in Tables 4.1 and 4.2 respectively. Soya bean meal was used as the protein source in the control diets. Equivalent quantities of Soya bean meal in the control diets were replaced with thevetia seed meal (TSM) at levels 25, 50, 75 and 100% (weight for weight substitution). The diets were iso-nitrogenous and iso-caloric.

TABLE 4.1. GROSS COMPOSITION OF EXPERIMENTAL DIETS (STARTER PHASE)

	DIETS				
Ingredients	**T1**	**T2**	**T3**	**T4**	**T5**
	0%	**25%**	**50%**	**75%**	**100%**
Maize	60.0	60.0	60.0	60.0	60.0
Soyabean Meal (SBM)	31.0	23.25	15.5	7.75	-
***Thevetia* Seed Meal (TSM)**	-	7.75	15.5	23.25	31.0
Fish Meal (72%)	5.0	5.0	5.0	5.0	5.0
Bone Meal	2.0	2.0	2.0	2.0	2.0
Oyster shell	1.0	1.0	1.0	1.0	1.0
Broiler Premix	0.25	0.25	0.25	0.25	0.25
Common Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.40	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00	100.00
Calculated					
Crude Protein (%)	22.62	22.84	23.06	23.29	23.5
ME (Kcal/kg)	3040.40	3028.78	3030.33	3025.29	3019.85

T1: Control; T2: 25%TSM; T3: 50%TSM; T4: 75%TSM; T5: 100%TSM; ME = Metabolisable energy

TABLE 4.2. GROSS COMPOSITION OF EXPERIMENTAL DIETS (FINISHER PHASE)

Ingredients	T1	T2	T3	T4	T5
	0%	**25%**	**50%**	**75%**	**100%**
Maize	50.0	50.0	50.0	50.0	50.0
Soyabean Meal (SBM)	30.0	22.5	15.0	7.5	-
***Thevetia* Seed Meal (TSM)**	-	7.5	15.0	22.5	30.0
Fish Meal (72%)	0.5	0.5	0.5	0.5	0.5
Wheat Bran	15.0	15.0	15.0	15.0	15.0
Bone Meal	2.0	2.0	2.0	2.0	2.0
Oyster Shell	2.0	2.0	2.0	2.0	2.0
Common Salt	0.25	0.25	0.25	0.25	0.25
Broiler Premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.1	0.1	0.10	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1
Total	100.2	100.2	100.2	100.2	100.2
Calculated					
Crude Protein (%)	20.4	20.6	20.8	21.0	21.2
ME (Kcal/kg)	2836.1	2831.2	2826.3	2821.5	2816.6

T1: Control; T2: 25%TSM; T3: 50%TSM; T4: 75%TSM; T5: 100%TSM.; ME= Metabolisable energy.

4.2.4 MANAGEMENT OF EXPERIMENTAL BIRDS

A total of two hundred unsexed day-old Arbor-acres broiler chicks were used for the study. There were five groups of forty birds per treatment with four replicates of ten birds per replicate randomly assigned to the five dietary treatments. The chicks were reared on deep litter and were all fed the control diet for one week before being allotted to treatment groups. Two weeks prior to the arrival of the chicks, the brooder house, feeders and drinkers were properly disinfected with Morigad. The house was partitioned into pens according to the design of the experiment. Wood shaving was used as litter materials and spread at the height of 2.5cm at the beginning of the starter phase. Flat trays and fountain drinkers were used for the chicks from day-old to week 3 of age. Then hanging feeders replaced tray feeders and bowl drinkers replaced fountain drinkers. Electric bulbs (200 watts) and coal pots were provided as source of heat for brooding. Ventilation was adequate and routine medications were administered as at when due (Table 4.3).The birds were fed broiler starter diets from week 1 to 4 and finisher diets from week 5 to 8.

4.3 DATA COLLECTION.

4.3.1 Feed Intake

Records of feed consumption were taken on daily bases using a sensitive scale. This was obtained as the difference between the quantity offered and the left over in a period of twenty-four hours. Average Daily Feed Intake (ADFI) was calculated by dividing daily feed intake by the number of birds in the replicates.

4.3.2 Weight Gain

Birds were weighed at the start of the experiment and subsequently on a weekly basis. Weight gain was calculated as the final live weight minus initial live weight. Average daily live weight gain (ADG) was obtained by adding together the weight of birds in the replicate (W2), subtracting from the previous week's value (W1) and dividing the result by the number of days in a week (7) as illustrated by the equation below.

ADG = W2 – W1

7

4.3.3 Feed: Gain Ratio

Feed to gain ratio (FCR) was calculated from the records of feed intake and weight gain of experimental birds as shown below.

Feed: Gain ratio = Feed intake (g)

Weight gain (g)

4.3.4 Protein Efficiency Ratio (PER)

Protein efficiency ratio (PER) was estimated from the data collected by dividing average weight gain by the protein intake of the birds in each treatment. Protein intake was calculated by multiplying average feed intake by percentage crude protein in each experimental diet.

PER = Average weight gain (g)

Protein Intake (g)

Protein Intake = Average feed intake (g) x Crude protein (%) in diet.

TABLE 4.3. MEDICATIONS (DRUGS AND VACCINATION)

Age/ Date of Administration	Vaccine and Drugs used
Day1/ 3/02/2011	NDV (occular)
Day 1-5/ 3-7/02/2011	Antibiotics (oral)
Day 10/12/02/2011	Gumboro 1 (oral)
Day 14/16/02/2011	NDV (Lasota 1, oral)
Day 17/ 19/02/2011	Gumboro 2 (oral)
Week 3	Coccidiostat (oral)
Week 4	NDV (Lasota 2, oral)
Week 5	Antibiotics (oral)
Week 6	Coccidiostat (oral)

NDV = Newcastle Disease Virus

4.3.5 APPARENT ILEAL DIGESTIBILITY OF CRUDE PROTEIN

At day 49, 0.5% titanium dioxide (TiO_2) was incorporated into the diets as indigestible dietary marker for digestibility study. The birds were given food and water *ad libitum* till the last day of the experiment. Two birds per replicate were slaughtered and the abdominal part cut open to remove the distal part of the ileum (portion of the small intestine from Meckel's diverticulum to approximately 1cm proximal to ileo-cecal junction). The contents were gently flushed with distilled water into plastic containers. Ileal digesta from each pen was pooled and immediately stored in a freezer until processed. The length of the ileum from which digesta was flushed was measured with a tape rule.

Digesta was flushed with distilled water into well labeled small round bowls with tight lids to prevent spillage during freezing and freeze-drying. Freeze-drying was done at the International Institute for Tropical Agriculture (IITA), Ibadan in Oyo State, Nigeria. Freeze-dried samples were then analyzed for crude protein at the Central Nutrition Laboratory in the Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan.

4.3.5.1 Digestibility Calculations

Apparent ileal digestibility of crude protein was calculated using the following equation:

$$D_{CP}\ (\%) = 1 - \left[\frac{TiO_{2\ diet}}{TiO_{2digesta}} \times \frac{CP_{digesta}}{CP_{diet}}\right] \times 100$$

Where:

D_{CP} = percentage apparent ileal crude protein digestibility

TiO_{2diet} = concentration of titanium dioxide in the diet in percentage

$TiO_{2digesta}$ = concentration of titanium dioxide in the digesta in percentage

$CP_{digesta}$ = concentration of crude protein in the digesta (%)

CP_{diet} = concentration of crude protein in the diet (%)

The digestibility values of crude protein (CP) in the diets were used to calculate the amount of CP (g/d) digested up to the terminal ileum. The CP (g/d) intake was calculated from the data of feed intake and CP concentration in the diets.

4.3.6 CARCASS QUALITY EVALUATION

At the end of week 8 of the experiment, two birds per replicate (totaling eight birds per treatment group) that had their weights close to the mean of the group were selected from each group, fasted overnight and slaughtered. Feathers were removed after scalding and the birds were cut opened and eviscerated. The live weight, bled weight and dressed weight were taken. Eviscerated carcass was cut into prime cuts and each cut weighed and expressed as percentage of live weights. The internal organs and abdominal fat were also weighed and the weights were expressed as the percentage of the live weights of the birds.

4.4 CHEMICAL ANALYSIS

4.4.1 Proximate Composition

The proximate composition of the feeds and digesta was determined according to AOAC (2000). Three determinations were carried out on each sample and the mean values estimated.

4.4.2 Concentration of Titanium dioxide

The concentrations of titanium dioxide in samples were estimated by photometric technique of Brandt and Allam (1987). Standard concentrations of titanium dioxide (0.1, 0.2, 0.3, 0.4 and 0.5 mg) were prepared to standardize the spectrophotometer and the

values obtained used to obtain a gradient for the estimation of the concentration of titanium dioxide.

4.5 EXPERIMENTAL DESIGN

The design of the experiment was Completely Randomized Design (CRD).

4.6 STATISTICAL ANALYSIS

Data collected were analyzed using Descriptive statistic and Analysis of Variance (ANOVA) of the Statistical Analysis Software (1999). Significant differences between treatment means were compared at P = 0.05, using Duncan's Multiple Range Test of the same software.

4.7 RESULTS

4.7.1 PROXIMATE COMPOSITION OF EXPERIMENTAL DIETS (STARTER)

Proximate composition of experimental diets fed to broilers at starter phase is presented in Table 4.4. The diets were iso- nitrogenous and iso- caloric.

Diet T5 (100% TSM) had the highest ($p>0.05$) percentage dry matter (91.29) while the control diet (T1) had the lowest (90.13). Percentage crude protein increased ($p>0.05$) with increasing level of TSM, diet T5 had the highest (24.37) and T1 (control diet) had the lowest (23.48). The crude fibre content increased with increasing levels of TSM up to 50% (3.81) after which there was a decline and diet T5 had the lowest ($p>0.05$) percentage crude fibre (3.58). Percentage soluble ash increased from 4.35 in the control diet (T1) to 4.74 in T2 (25% TSM), declined (4.56) in T3 (50% TSM) and then increased, T5 (100% TSM) had the highest value of 6.37. A similar trend was observed for percentage ether extract. There was a general decrease ($P>0.05$) in percentage Nitrogen Free Extract with increasing levels of TSM in the diets.

Table 4.4 Proximate composition (g/100g DM) of broiler starter diets.

Nutrient (%)	**T1 (0%)**	**T2 (25%)**	**T3 (50%)**	**T4 (75%)**	**T5 (100%)**
Dry matter	90.13	90.35	90.29	91.18	91.29
Crude protein	23.48	23.69	23.94	24.08	24.37
Crude fibre	3.64	3.76	3.81	3.61	3.58
Soluble ash	4.35	4.74	4.56	6.29	6.37
Insoluble ash	1.18	1.38	1.46	1.13	1.08
Ether extract	3.59	3.71	3.65	3.76	3.73
NFE	53.89	53.07	53.87	52.31	52.16
ME(Kcal/kg DM)	3040.4	3028.8	3030.3	3025.3	3019.6

TSM= *Thevetia* seed cake; NFE= Nitrogen free extract; ME= Metabolisable energy

4.7.2 PROXIMATE COMPOSITION OF EXPERIMENTAL DIETS (FINISHER)

The proximate composition of the experimental diets at the finisher phase is as shown in Table 4.5. The diets were also iso-nitrogenous and iso-caloric.

There was a general increase ($p>0.05$) in percentage dry matter with increasing levels of TSM in the diet except in diet T3 (50% TSM) where a slight decrease (90.03) was recorded.

The values observed for crude protein ranged from 19.89% in T4 (75% TSM) to 20.42% in T3 (50% TSM). The observed values were within the NRC, 1994 recommendation for broiler finisher diets. Percentage crude fibre increased ($p>0.05$) from 3.95 in the control diet (T1) to 4.21 in diet T5 (100% TSM). Observed values for soluble ash ranged from 4.17% in T5 to 4.46% in T3, T5 had the lowest value. There was a general increase in percentage nitrogen free extract with increasing levels of TSM and T4 (75% TSM) had the highest value of 57.45%. Metabolisable energy (Kcal/kg DM) ranged between 2816.6 in T5 to 2836.1 in the control diet (T1).

Table 4.5 Proximate composition (g/100g DM) of broiler finisher diets.

Nutrients (%)	T1 (0%)	T2 (25%)	T3 (50%)	T4 (75%)	T5 (100%)
Dry matter	90.06	90.15	90.03	90.18	90.21
Crude protein	20.27	20.15	20.42	19.89	19.97
Crude fibre	3.95	4.15	4.07	4.02	4.21
Soluble ash	4.21	4.33	4.46	4.28	4.17
Insoluble ash	0.96	0.89	1.05	0.93	1.02
Ether extract	3.56	3.68	3.64	3.61	3.59
NFE	57.09	56.95	56.39	57.45	57./25
ME(Kcal/kg DM)	2836.1	2831.2	2826.4	2821.5	2816.6

TSC= *Thevetia* seed cake; NFE= Nitrogen free extract; ME= Metabolisable energy; F=finisher

Diets were isonitrogenous and isocaloric.

4.7.3 PERFORMANCE CHARACTERISTICS OF BROILERS (Starter Phase)

Summary of the performance characteristics of broilers fed graded levels of TSM at the starter phase is presented in Figures 4.1 and 4.2.

4.7.3.1 Weight Gain

The highest final weight gain of 667.5g/bird was observed for birds on T2 (25% TSM). The variations observed for birds on T1, T2, T3 and T4 were significant ($p<0.05$). Birds on T5 (100% TSM) recorded the lowest final weight gain of 547.50g/bird which was significantly different ($p<0.05$) from the values recorded for birds on all the other diets.

4.7.3.2 Feed Intake

Feed intake significantly reduced from 105.75 g/b/d for birds on the control diet (T1) to 92.48g/b/d for birds on T5. Birds on T2, T3 and T4 recorded 101.98, 99.05 and 101.80 respectively which were significantly different from value observed for birds on diet T5.

4.7.3.3 Feed: Gain Ratio

Feed conversion ratio followed the same trend as feed intake. Birds on T5 had the highest ($p<0.05$) value of 4.37 and birds on T2 had the lowest value of 3.86.

4.7.3.4 Protein Efficiency Ratio (PER)

Birds on diet T2 had the highest ($p<0.0$) value of 95.83 for protein efficiency ratio (PER) while birds on the control diet (T1) had the lowest value of 62.37. The variations observed in values for PER for birds on diets T2, T3, T4 and T5 were not significant ($p>0.05$) but the variation observed for birds on the control diet was significant ($p<0.05$).

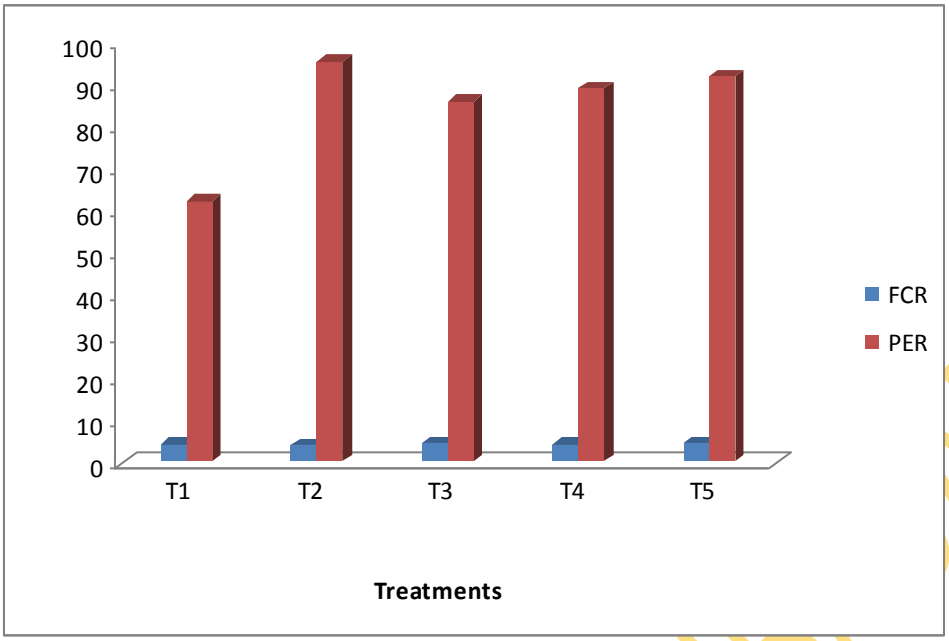

FIGURE 4.1: PERFORMANCE CHARACTERISTICS OF BROILER (STARTER PHASE)

T1 =0% Thevetia seed meal (TSM); T2 = 25% TSM; T3 = 50% TSM; T4 = 75% TSM; T5 = 100% TSM. X axis = dietary treatments; Y axis = feed conversion ratio (FCR)/ protein efficiency ratio (PER)

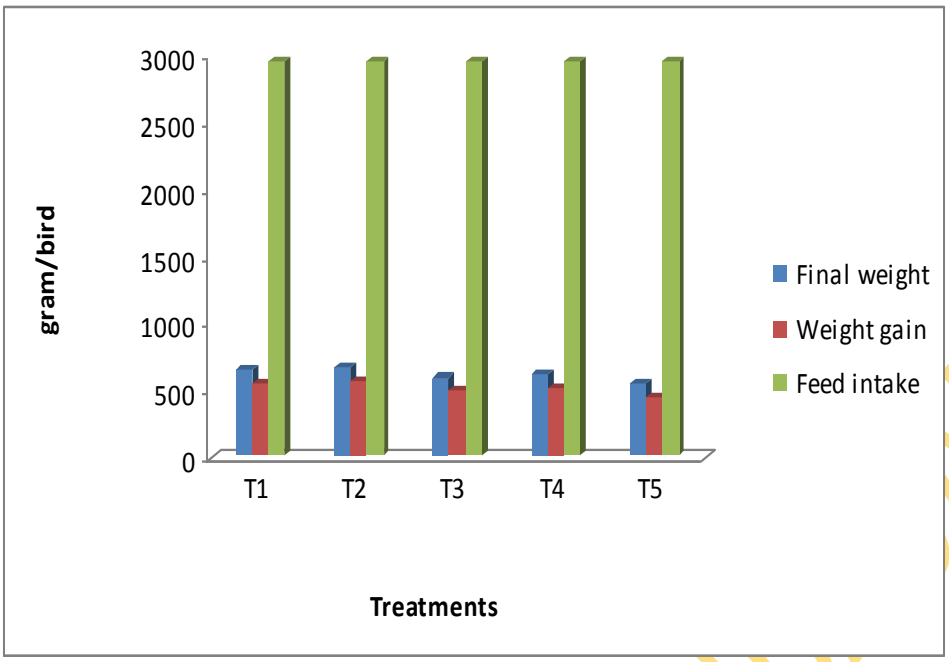

FIGURE 4.2: PERFORMANCE CHARACTERISTICS OF BROILER (STARTER PHASE)

T1 = 0% Thevetia seed meal (TSM); T2 = 25% TSM; T3 = 50% TSM; T4 = 75% TSM; T5 = 100% TSM.

4.7.4 PERFORMANCE CHARACTERISTICS OF BROILERS (FINISHER PHASE)

Summary of the performance characteristics of broilers fed graded levels of detoxified TSC at the finisher phase is as shown in Figure 4.3 and 4.4.

4.7.4.1 Weight Gain

Final weight ranges from 1897.5g/bird in T1 to 1562.3g/bird in T5. The variations observed in values for birds on T1, T2, T3 and T4 were not significant ($p>0.05$) but differ significantly for birds on T5.

4.7.4.2 Feed Intake

The highest value of 176.41g/b/d for feed intake was observed for birds on the control diet (T1) while birds on T5 had the lowest value of 118.65g/b/d.

4.7.4.3 Feed: Gain Ratio

Birds on T5 had the lowest value of 3.27 for feed conversion ratio (FCR) followed by birds on T3 (3.33) with the highest value of 4.11 recorded for birds on the control diet.

4.7.4.4 Protein Efficiency Ratio (PER)

Protein efficiency ratio (PER) at the finisher phase was generally lower than values observed at the starter phase with birds on T5 having the highest value of 43.01 followed by 41.68 for birds on T3. The lowest value of 35.21 was observed for birds on the control diet (T1). However, the variations observed in values for birds on T1 and T5 were significant ($p<0.05$).

4.7.4.5 Mortality

Substitution of soyabean meal with thevetia seed meal in broiler diet had no adverse effect on the birds irregardless of the level of substitution as there was no mortality recorded throughout the duration of the experiment.

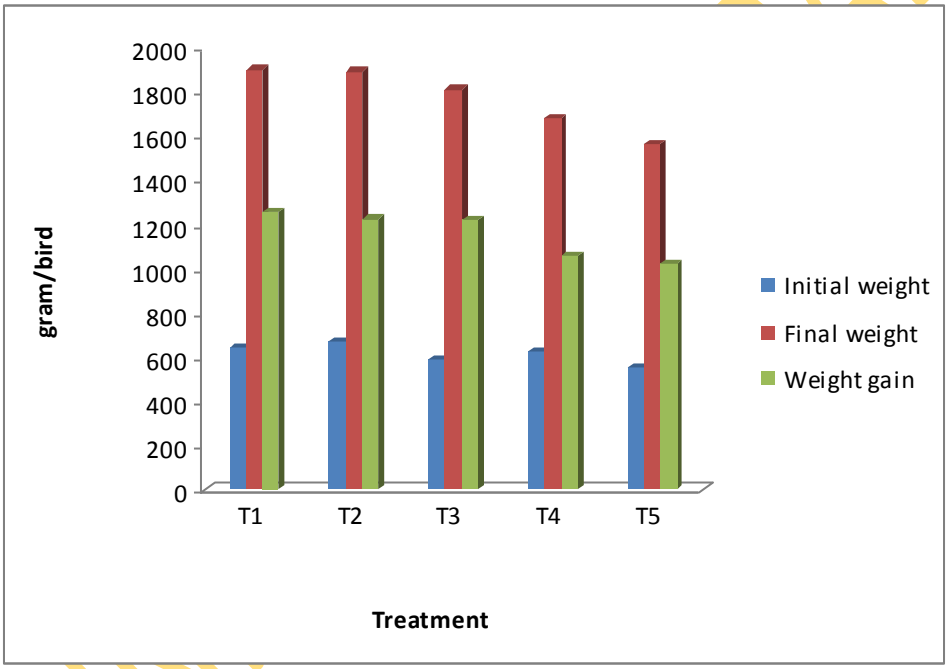

FIGURE 4.3: PERFORMANCE CHARACTERISTICS OF BROILER (FINISHER PHASE)

T1 =0% Thevetia seed meal (TSM); T2 = 25% TSM; T3 = 50% TSM; T4 = 75% TSM; T5 = 100% TSM.

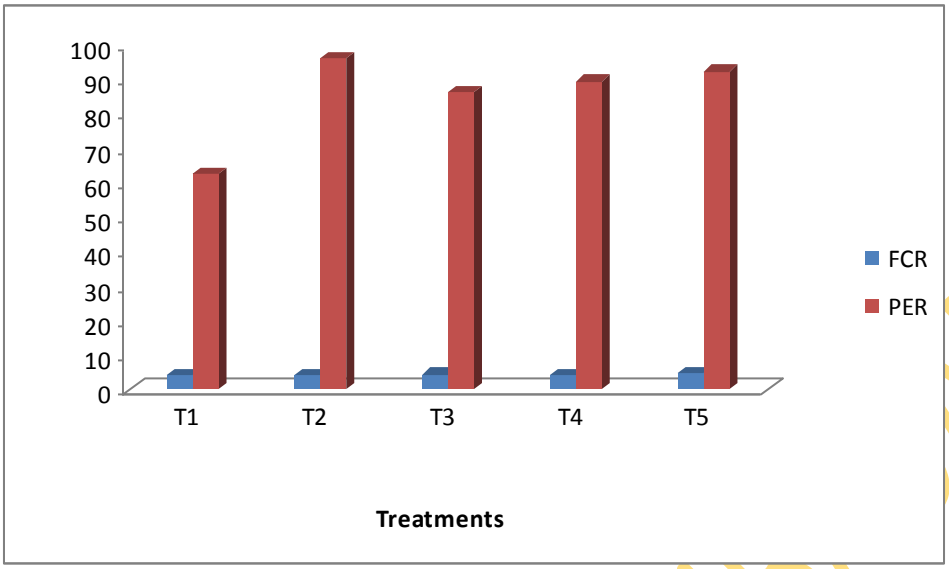

FIGURE 4.4: PERFORMANCE CHARACTERISTICS OF BROILER (FINISHER PHASE)

T1 = 0% Thevetia seed meal (TSM); T2 = =25% TSM; T3 = 50% TSM; T4 = 75% TSM; T5 = 100% TSM. FCR = feed conversion ratio; PER = protein efficiency ratio

4.7.5 APPARENT ILEAL DIGESTIBILITY

The Apparent ileal crude protein digestibility of birds fed graded levels of TSC ranged from 69.00% for birds on T5 to 79.00% for birds on T3. The apparent differences in the mean values were not significant ($p<0.05$). Summary of the Apparent Ileal crude protein digestibility is presented in Figure 4.5.

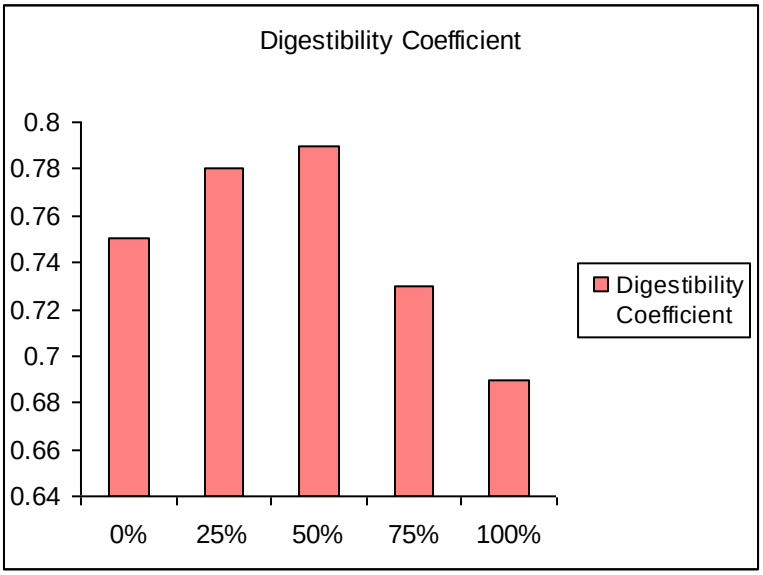

FIG 4.5 SUMMARY OF APPARENT ILEAL DIGESTIBILITY COEFFICIENT

Y axis = Digestibility Coefficient; X axis = Graded levels of Thevetia seed meal.

4.7.6 CARCASS CHARACTERISTICS

The summary of the carcass characteristics of broilers fed graded levels of TSM is as shown in Table 4.6.

4.7.6.1 Live Weight

Substitution of soyabean meal (SBM) for thevetia seed meal (TSM) in broiler diets had no significant effect (P>0.05) on the final weight of the birds on diets T1, T2, T3 and T4. However, birds on diet T5 (100% TSM) showed significant decrease (P<0.05) in final weight. The average final weight ranged from 1824.8g for birds on diet T3 (50% TSM) to 1492.8g for birds on diet T5. Birds on diet T3 had higher (P>0.05) average final weight than those on the control diet (1775.8g) though the observed differences were not significant.

4.7.6.2 Dressed Weight

The observed differences in the dressed weight of birds fed diets in which TSM replaced SBM followed a similar trend as in the live weight as birds on diet T3 had the highest (P<0.05) dressed weight (1220.3g) while birds on diet T5 had the lowest (992.4g).

4.7.6.3 Wings

Expressed as a percentage of dressed weight, birds on diet T3 had the highest (P<0.05) average wing dressing percentage (14.82) as compared to birds on diet T5 (100% TSM) with an average dressing percentage of 07.03. Birds on the control diet had an average wing dressing percentage of 14.52.

4.7.6.4 Thigh

Dressing percentage of thigh followed a similar trend as observed for wings. Birds on diet T3 recorded the highest thigh dressing percentage of 17.76 which was higher (P>0.05) than the value observed for birds on the control diet T1 (16.93%). Birds on T2 had the lowest (P>0.05) average thigh dressing percentage of 16.88.

4.7.6.5 Breast

Average breast dressing percentage ranged from 27.26 for birds on diet T2 (25% TSM) to 23.81 for birds on diet T4 (75% TSM). Birds on the control diet had an average breast dressing percentage of 26.85.

4.7.6.6 Back

Birds on diet T5 (100% TSM) had an average back dressing percentage of 24.01 which is closely followed by an average of 23.88 recorded for birds on diet T4 (75% TSM). The lowest ($P<0.05$) average back dressing percentage (22.09) was observed for birds on diet T3 (50% TSM). Birds on the control diet had an average back dressing percentage of 22.74.

4.7.6.7 Drum Stick

Average dressing percentage of drum stick for birds on all dietary treatments ranged from 15.87 for birds on diet T4 to 16.59 observed for birds on the control diet. Birds on diet T3 recorded an average of 16.41. The variations observed were not significant ($P>0.05$).

4.7.6.8 Head

As the level of TSM in the diets increased, the dressing percentage of head increased. Birds on the control diet in which TSM was not included had the lowest ($P>0.05$) average head dressing percentage of 03.61 after which there were increases and birds on diet T4 (75% TSM) recorded the highest percentage of 04.46. Birds on diets T2 and T3 had 03.66 and 03.84 respectively.

Table 4.6 CARCASS CHARACTERISTICS OF BROILERS FED GRADED LEVELS OF TSM (g/bird)

Parameters	T1	T2	T3	T4	T5	SEM
TSM level	**0%**	**25%**	**50%**	**75%**	**100%**	
Live weight	1775.8[a]	1729.6[a]	1824.8[a]	1714.3[a]	1492.8[b]	0.05
Bled weight	1709.0[a]	1672.0[a]	1775.1[a]	1548.4[a]	1358.5[b]	0.05
Dressed wgt	1207.6[a]	1185.0[a]	1220.3[a]	1175.9[a]	992.4[b]	0.04
Wings	175.36[a]	169.36[a]	180.88[a]	169.50[a]	69.75[b]	6.35
% DW	14.52	14.29	14.82	14.41	07.03	
Thigh	204.50[a]	200.00[a]	216.75[a]	199.63[a]	170.25[b]	20.11
% DW	16.93	16.88	17.76	16.98	17.16	
Breast	324.28[a]	323.00[a]	304.13[a]	280.00[ab]	239.50[b]	14.54
% DW	26.85	27.24	24.92	23.81	24.13	
Back	274.63[a]	275.00[a]	269.60[a]	280.86[a]	238.29[b]	8.48
% DW	22.74	23.21	22.09	23.88	24.01	
Drum stick	200.38[a]	189.00[a]	200.25[a]	186.63[a]	159.38[b]	6.79
% DW	16.59	15.95	16.41	15.87	16.06	
Head	43.57[b]	43.38[b]	46.88[ab]	52.50[a]	40.63[b]	2.57
% DW	03.61	03.66	03.84	04.46	04.09	

TSM = Thevetia seed meal (TSM); T1 = 0% TSM; T2 = 25%TSM; T3 = 50% TSM; T4 = 75% TSM; T5 = 100% TSM. SEM = Standard error of means: DW = Dressed weight: wgt = weight.

4.7.7 ORGAN WEIGHTS

The summary of organ weights of birds fed graded levels of Thevetia seed meal substituted for soyabean meal (expressed as a percentage of live weight) is as shown in Table 4.7. The organs weighed included heart, liver, gizzard, abdominal fat and ileum length.

4.7.7.1 Heart

Heart weights of birds fed diets in which SBM was substituted for TSM in broiler were not significantly (P>0.05) affected by dietary treatments. The values ranged from 0.45 for birds on diet T2 (25% TSM) to 0.53 for birds on diet T5 (100% TSM). Birds on the control diet recorded an average of 0.51%.

4.7.7.2 Liver

Average percentage liver weights increased (P>0.05) with increasing level of TSM in the diets. The percentage ranged from 1.49 for birds on the control diet to 2.33 for birds on diet T5. Birds on diet T3 had an average of 1.74%.

4.7.7.3 Gizzard

On the average, birds on the control diet and diet T5 recorded 1.95%. The highest (P>0.05) percentage of gizzard weight was observed for birds on diet T2 (25% TSM; 2.04).

4.7.7.4 Abdominal fat

The average percentage of abdominal fat increased ($P<0.05$) with increasing level of TSM in the diets. The lowest percentage was observed for birds on the control diet (1.00) while the highest value ($P<0.05$) was observed for birds on diets T4 and T5 (2.05%). Variations observed for birds on T2 (1.37) and T3 (1.47) were not significantly ($P>0.05$) different from the value recorded for birds on the control diet.

4.7.7.5 Ileum length

Dietary treatment had significant ($P<0.05$) effect on ileum length. Birds fed 50% TSM had the longest (80.13cm) ileum on the average and the shortest (69.88cm) ileum length was observed for birds on the control diet ($P<0.05$).

Table 4.7 INTERNAL ORGANS (g/100g live weight)

Parameters	T1	T2	T3	T4	T5	SEM
Heart	0.51	0.45	0.50	0.48	0.53	0.04
Liver	1.49	1.88	1.74	2.06	2.23	0.18
Gizzard	1.95	2.04	1.99	1.90	1.95	0.17
Abdominal fat	1.00^{b}	1.37^{ab}	1.47^{ab}	2.05^{a}	2.05^{a}	0.6
Ileum length (cm)	69.88^{b}	73.88^{ab}	80.13^{a}	77.75^{b}	77.88^{ab}	3.16

abcd means along the same row with any identical superscript are not significant (p>0.05)

T1 = 0% TSM; T2 = 25% TSM; T3 = 50% TSM; T4 = 75% TSM; T5 = 100% TSM: SEM = Standard error of means.

4.8 DISCUSSION

4.8.1 PROXIMATE COMPOSITION OF EXPERIMENTAL DIETS

The proximate composition of the experimental diets both at the starter and finisher phases showed that the diets were isonitrogenous and iso-caloric as the diets were similar (P>0.05). The diets also met the recommended nutritional requirements (NRC, 1994; Babatunde and Fetuga, 1975) for both starter and finisher broilers.

4.8.2 PERFORMANCE CHARACTERISTICS OF BROILERS FED GRADED LEVELS OF TSM (STARTER PHASE)

The performance characteristics of broilers fed graded levels of TSC at the starter phase showed significant differences (p<0.05) in values observed for feed intake, weight gain, feed conversion ratio (FCR) and protein efficiency ratio (PER). The lowest value for feed intake (92.48g/b) was observed for birds on T5 (100% TSC) while the highest value of 105.75g/b was recorded for birds on the control diet (T1). This could be attributed to the fact that diet T5 might not be as acceptable as the soybean-based diet T1 because of the bitterness associated with TSC as reported (Odetokun *et al*, 1999). This reduction in feed intake which was beyond a desirable level further resulted in poor productive performance of the birds. Weight gain and feed conversion ratio of birds on T2, T3 and T4 were not significantly different from values observed for birds on the control diet. The highest weight gain of 26.61g/bird/day was observed for birds on T2 which was not significantly different from 23.16 recorded for birds on T3 with lower feed intake. This could be attributed to better protein utilisation by the birds on these diets. Significantly lower feed intake observed for birds on diet T5 could also be responsible for the reduction in weight gain and subsequently lighter live and dressed weights. This agrees with the works of Oluwaniyi, 2007 and Atteh *et al.*, 1995 who recorded undesirable weight gain in cockerels and broilers fed graded levels of *thevetia* seed cake with increasing level of the cake in their diets.

4.8.3 PERFORMANCE CHARACTERISTICS OF BROILERS FED GRADED LEVELS OF TSM (FINISHER PHASE)

Productivity significantly increased (P>0.05) for birds on diet T5 at the finisher phase with the lowest FCR of 3.27 and the highest PER of 43.01. The result in this study indicated that broilers could efficiently utilize dietary inclusions of TSC above 50% at the finisher phase but undesirable at the starter phase. This agrees with the report of Batal and Parson, (2002) and Gracia *et al*, (2003) that older birds tend to utilize or digest nutrient better than younger birds. At the finisher phase, the productivity of birds on diet T5 (100% TSC) was the best with the lowest feed: gain ratio of 3.27. This advantage was however, marred by undesirable reduction in feed intake. If feed intake for birds on this diet had been optimal, then their overall performance would have been the best going by the values observed for FCR and PER. This was further confirmed by the amino acid profile of TSC having higher values than that of SBM for all the essential amino acids except histidine.

4.8.4: APPARENT ILEAL DIGESTIBILITY

Digestibility is one of the most essential parameters with which the nutritive value of a particular feedstuff can be assessed. However, components of nutrients cannot be said to be beneficial unless such nutrients are capable of being properly digested and assimilated by the animal. Nutrient digestibility is the ratio of the nutrient retained to the total intake expressed in percentage.

The results obtained from this experiment showed no significant (p>0.05) differences in the apparent ileal digestibility coefficient of birds on the different dietary treatments. However, AID coefficient of birds on diet T3 (50% TSC) was higher (0.79) than those observed for birds on other diets, the lowest value (0.69) was observed for birds on T5 (100% TSC). This could be attributed to the complimentary effects of the mixture of plant protein sources used in compounding the experimental diets. According to Olomu, (2011), the mixture of two or more protein brings about complementary effects when

they compensate for their deficiencies. The complimentary effect of mixing SBM with TSC could be said to be optimal in diet T3 though it was also high in T2. This effect declined at TSC dietary levels higher than 50% with T5 having the lowest. However, the apparent differences observed were not significant. This could be indicative of the superior quality of the protein of TSC.

4.8.5: CARCASS CHARACTERISTICS

Carcass characteristics of broilers suggested the superiority of diet T3 over other diets as higher values of live weight, dressed weight and prime cuts were observed for birds on this diet. This could be attributed to better utilization of dietary protein in the formation of muscle. Treatment effects on all prime cuts were not significant (P> 0.05) for diets T1 through T4 but significant for birds on diet T5. This is suggestive of an inhibitory effect on the utilization of protein which could be due to the effect of residual toxins. This agrees with the work of Oluwaniyi, *et al.*, (2007) who reported growth depression in cockerels fed TSM. The residual glycoside in TSC though below the lethal dose, could be responsible for growth depression observed for birds on 100% TSC diet.

There were no significant differences (p>0.05) in the weight of the heart, liver and gizzard of birds fed graded levels of TSC and those on the control diet but the abdominal fat and ileum length of birds on the control diet were significantly lower than values observed for birds on the other diets. The differences observed in values for abdominal fat could be attributed to the very oily nature of TSC, but the benefit of weighty prime cuts like the thigh, breast drum stick and dressing percentage far out weighs abdominal fat deposition.

CHAPTER FIVE

5.0 HEMATOLOGICAL AND SERA BIOCHEMICAL INDICES OF BROILERS FED GRADED LEVELS OF *THEVETIA* SEED MEAL (STARTER PHASE)

5.1 INTRODUCTION

Blood is an important index of physiological and pathological changes in the organism. Hematological and biochemical values are indicative of the state of the animal and they are tools for diagnostic exercises. Reference values aid decisions in diagnosing and controlling diseases/infections (Mitruka and Rawnsley, 1977).

The possibility of using alternative plant protein source like *Thevetia* seed meal in broiler production will depend on its effective detoxification which will be reflective not only in the overall physical performance of the animal but also in body fluids like the blood. Nutrients are digested, metabolized and absorbed into the body and utilized for various biological functions going on in the body. These metabolites are transported by the blood to the various sites where they are needed (Vasudevan *et al*., 2011).

Blood as well as serum parameters are useful tools in diagnosing abnormalities in the body of an organism. Since this study had to do with the use of a feed ingredient that had anti nutritional factor, it was expedient and pertinent to carry out studies on body fluids (blood/ serum) to identify the effect (s) of the test ingredient on physiological and pathological changes in the experimental animals.

One of the diagnostic tests for Thevetin poisoning according to Desai, (2000) is the level of K^+ ion in the serum. The level of potassium ion in the serum higher than 6mmol/l is said to be indicative of Thevetin poisoning. Hematological indices like Packed cell volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), basophiles, Mean Corpuscular Haemoglobin, Concentration (M.C.H.C), Mean Corpuscular Volume (M.C.V) and Mean Corpuscular Haemoglobin (M.C.H) are all important tools in identifying the state of the animal that will not pose health hazards to consumers.

5.2 MATERIALS AND METHODS

5.2.1 EXPERIMENTAL SITE

The experiment was carried out at the Pullet house in the Poultry unit of the Teaching and Research Farm of the University of Ibadan and in the Department of Animal Science within the Physiology laboratory.

5.2.2 EXPERIMENTAL DIETS

Thevetia seed meal with other feed ingredients were used to formulate diets to meet the NRC(1994) nutrient requirement of broilers for both starter and finisher phases as shown in Tables 5.1and 5.2 respectively. Soyabean meal was used as the protein source in the control diets.

5.2.3 HAEMATOLOGICAL INDICES

Blood for haematological analysis was collected from eight birds per dietary treatment (two birds per replicate) with the aid of needle and syringe into sterile sample tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA). The samples were properly shaken and immediately cooled and stored in a refrigerator until they were required for analysis. The packed cell volume was determined using microhaematocrit centrifugation method by Jain (1986). Haemoglobin concentration was measured by the cyanomethaemoglobin method of Kelly (1979). The red blood cell count (RBC) and white blood cell count (WBC) were determined using the haemocytometer method as described by Jain (1986). Mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were calculated from the PCV, Hb concentration and RBC count using the method described by Jain (1986) as shown by the following equations:

$$\text{MCHC (\%)} = \frac{\text{Hb} \times 100}{\text{RBC}}$$

$$\text{MCH (pg)} = \frac{\text{Hb x 10}}{\text{PCV}}$$

$$\text{MCV (fl)} = \frac{\text{PCV x 10}}{\text{RBC}}$$

5.2.4 BLOOD/ SERUM COLLECTION

Blood samples for serum biochemical analysis were collected from two birds per replicate through the jugular vein with a needle and syringe for each bird at week four and seven of the experiment. A total of eight birds were sampled per dietary treatment. The blood samples were collected into sterile sample tubes. The set of tubes without anticoagulant was kept in a wooden rack slanted at an angle of 45^0 for ease of separation of serum. Clotted blood was centrifuged for 15 minutes at 3500 revolutions per minute to separate the serum from the plasma. The clear fluid (serum) was pipette into clean and sterilized tubes for biochemical analysis.

5.2.5 BLOOD/SERA ANALYSIS

The serum protein and albumin were analyzed using sigma assay kit. The serum globulin was determined by deducting the values of serum albumin from those of total protein. Glucose was determined immediately by o-Toluidine method using acetic acid (Cooper and McDaniel, 1970), and cholesterol was determined by the method of Roschlan *et al.*, (1974). Flame photometer was used for the determination of potassium while calcium and phosphorus were determined using a Perkin Elmer atomic absorption Spectrophotometer.

5.3: EXPERIMENTAL DESIGN

The design of the experiment is Completely Randomised Design (CRD).

5.4 STATISTICAL ANALYSIS

All data collected were analyzed statistically using Descriptive statistic and Analysis of Variance of Statistical Analysis Software (SAS, 1999). Significant means were compared using Duncan Multiple Range Test of the same software (Duncan, 1955).

5.5 RESULTS

5.5.1 HEMATOLOGICAL INDICES OF BROILERS (STARTER)

Summary of the hematological indices of broilers fed graded level of TSC at the starter phase is given in Table 5.1. The treatment effects on the birds for all hematological parameters investigated were not significant (P>0.05).

5.5.1.1 Packed Cell Volume (PCV)

Substitution of soyabean meal with thevetia seed meal in broiler starter diets had no significant (P>0.05) effect on the packed cell volume of the birds in all treatment groups. Birds on diet T3 (50% TSM), however, recorded the highest value of 31.13% on the average while the lowest value of 25.13% was observed for birds on diet T5 (100% TSM).The values observed for birds on the control diet (26.63%) served as the reference value.

5.5.1.2 Haemoglobin Concentration (Hb).

Haemoglobin concentration was not significantly (P>0.05) affected by dietary inclusion of TSM in the birds diets. The highest value (9.13mg/dl) was observed for birds on diet T3 (50% TSM) this was closely followed by a value of 8.88mg/dl observed for birds on the control diet, which served as the reference value in this study. The lowest value of 8.38mg/dl was recorded for birds on 100% TSM (T5).

5.5.1.3 Red Blood Cell (RBC) Count.

Dietary inclusion of TSM in broiler starter diets had no significant (P>0.05) effect on the Red Blood Cell counts of the birds. The highest (P>0.05) value of 189.88mg/dl was observed for birds on diet T2 (25% TSM) while the lowest (P>0.05) value of 181.88mg/dl was recorded for the group fed the control diet T1.

5.5.1.4 White Blood Cell (WBC) Counts.

The observed variations in the White Blood Cell counts of broilers fed graded levels of TSM substituted diets were not significant (P>0.05). Birds on the control diet recorded the highest (P>0.05) value of 16612.5mg/dl while the group fed 25% TSM (T2) had the lowest value of 15487.5mg/dl.

5.5.1.5 Lymphocytes Counts.

Substituting soyabean meal with thevetia seed meal in broilers starter diet had no significant (P>0.05) effect on the lymphocytes counts. Birds on 50% substitution (T3) had the highest (P>0.05) counts (65.63mg/dl) while birds on diet T4 (75% TSM) recorded the lowest (61.13mg/dl) counts. Birds on the control diet (T1) and those on T2 (25% TSM) recorded similar values (64.25mg/dl).

5.5.1.6 Heterocytes Counts.

Dietary inclusion of TSM in broilers starter diets had no significant (P>0.05) effects on the heterocytes counts. The values ranged between 30.25mg/dl for the group fed 50% TSM (T3) to 33.13mg/dl for birds on 75% TSM (T4). Values observed for birds on the control diet and the group fed 50% TSM were close (30.63 and 30.25 respectively).

5.5.1.7 Monocytes Counts.

Values observed for monocytes ranged between 1.63mg/dl for birds on T3 (50% TSM) to 2.63mg/dl for birds on diet T2 (25% TSM). Birds on the control diet (T1) had a value of 2.13mg/dl which served as the reference value. The apparent variations were not significant (P>0.05).

5.5.1.8 Eosinophils Counts.

Replacing soyabean meal with thevetia seed meal at graded levels in broilers starter diets had no significant (P>0.05) effect on eosinophils counts. Observed values ranged from 2.13mg/dl for the group fed 25% TSM (T2) to 3.63mg/dl for birds on diet T4 (75% TSM). Birds on the control diet recorded a value of 2.75mg/dl on the average.

5.5.1.9 Basophils Counts.

Inclusion of TSM in the starter diets of broilers gave similar values irrespective of the levels of inclusion. Birds fed the control diet had a value of 0.25mg/dl on the average while birds on all other dietary treatments had 0.13mg/dl each. The variations were however, not significant (P>0.05).

5.5.1.10 Mean Corpuscular Haemoglobin Concentration (MCHC).

Dietary inclusion of TSM in broiler diets both at the starter and finisher phases had no significant effect (P>0.05) on the Mean Corpuscular Haemoglobin Conc`entration (MCHC). Values observed ranged between 4.54% for birds on diet T2 and 4.88% for birds on the control diet (T1).

5.5.1.11 Mean Corpuscular Haemoglobin (MCH).

Treatment effects on the MCH of broiler birds fed graded levels of TSM at both Starter and finisher phases were not significant (P>0.05). MCH values ranged from 2.93pg for birds on diet T3 to 3.33 for birds on all other diets. (T1, T2, T4 and T5).

5.5.1.12 Mean Corpuscular Volume (MCV).

Differences observed for MCV of birds on all dietary treatments were not significant (P>0.05). Observed values ranged between 1.36fl for birds on diet T2 and 1.70fl for birds on diet T3.

Table 5.1: HEMATOLOGICAL PARAMETERS OF BROILERS (STARTER PHASE)

DIETARY TREATMENTS

Parameters (mg/dl)	T1	T2	T3	T4	T5	SEM
Packed cell vol. (%)	26.63	25.88	31.13	26.50	25.13	1.94
Hemoglobin	8.88	8.63	9.13	8.83	8.38	0.34
RBC	181.88	189.88	183.63	183.13	183.50	8.84
WBC	16612.5	15487.5	16225.0	16487.5	16370.0	403.79
Lymphocytes	64.25	64.25	65.63	61.13	64.50	1.81
Heterocytes	30.63	31.25	30.25	33.13	32.00	1.84
Monocytes	2.13	2.63	1.63	2.00	2.25	0.42
Eosinophils	2.75	2.13	2.50	3.63	2.50	0.60
Basophils	0.25	0.13	0.13	0.13	0.13	0.13
MCHC (%)	4.88	4.54	4.97	4.82	4.57	0.26
MCH (pg)	3.33	3.33	2.93	3.33	3.33	0.52
MCV(fl)	1.46	1.36	1.70	1.45	1.37	0.33

RBC = Red blood cell; WBC = White blood cell; SEM = Standard Error of Means; MCHC = Mean Corpuscular Haemoglobin Concentration; MCH = Mean Corpuscular Haemoglobin; MCV = Mean Corpuscular Volume.

5.5.2: SERA BIOCHEMICAL INDICES OF BROILERS FED EXPERIMENTAL DIETS (STARTER)

The blood serum metabolites of broiler chicken as affected by replacing soyabean meal in their diets with TSM are summarized in Table 5.2.

5.5.2.1 Total Protein.

Replacement of soyabean meal with thevetia seed meal in broilers starter diets had no significant (P>0.05) effects on the total blood protein. The highest value of 2.93mg/dl was observed for birds on the control diet (T1) while the lowest value of 2.70mg/dl was observed for birds on 75% TSM diet (T4).

5.5.2.2 Albumin.

Substituting thevetia seed meal for soyabean meal in broilers diet had no significant (P>0.05) effect on serum albumin of the birds on all treatment groups. Values observed ranged between 1.20mg/dl for birds on the control diet and 1.44mg/dl for birds fed 100% TSM diet (T5).

5.5.2.3 Globulin.

There was no significant (P>.05) effects observed in the values obtained for serum globulin of broilers fed graded levels of TSM as compared to those on the control diet. Values observed ranged from 1.27mg/dl for the group fed 75% TSM based diet to 1.73mg/dl for birds on the control diet.

5.5.2.4 Calcium.

The apparent variations observed for serum calcium for birds in all dietary treatments were not significant (P>0.05). The observed values ranged between 8.94mg/dl for birds

on 25% TSM replacement and 11.11mg/dl for birds on 100% TSM diet. Birds on the control diet had 9.83 mg/dl on the average which served as the reference value in this study.

5.5.2.5 Phosphorus.

Dietary inclusion of thevetia seed meal (TSM) in broiler starter diets had significant ($P<0.05$) effect on the concentration of serum phosphorus. The observed values ranged from 4.51mg/dl for birds on 25% TSM diet to 5.67md/dl for the group on 100% TSM diet. Birds on diets T4 (75% TSM) and T5 (100% TSM) had significantly ($P<0.05$) higher serum phosphorus concentration than birds on the control diet. The value observed for birds on 50% TSM diet (T3) was not significantly ($P>0.05$) different from the value recorded for birds on the control diet (T1).

5.5.2.6 Potassium Ions (K^+)

Replacement of soyabean meal with thevetia seed meal in broilers starter diets significantly ($P<0.05$) affected the concentration of serum potassium ions (K^+) of the birds. The observed values ranged between 3.33mmol/l for birds on 75% TSM (T4) and 3.88mmol/l for birds on 50% TSM (T3). Birds on the control diet had a serum potassium ion (K^+) concentration of 3.56mmol/l which was not significantly ($P>0.05$) different from values observed for birds in all other dietary treatments.

5.5.2.7 Urea Nitrogen.

Thevetia seed meal had no significant ($P>0.05$) effect on the blood urea nitrogen. The observed values ranged between 0.59mg/dl for the control group and 1.28mg/dl for the group fed 50% TSM diet (T3).

5.5.2.8 Glucose.

The concentration of serum glucose was not significantly (P>0.05) affected by the inclusion of thevetia seed meal in broiler starter diets irrespective of the level of inclusion. The observed values ranged from 242.86mg/dl for birds on 100% TSM (T5) diet to 260.45mg/dl for birds on the control diet (T1). There was a progressive decrease (P>0.05) in the level of serum glucose with increasing level of TSM up to the 50% level (T3) after which there was an increase (254.21mg/dl) observed for the group fed 75% TSM diet (T4).

5.5.2.9 Triglyceride.

Dietary inclusion of TSM in broiler starter diets had no significant (P>0.05) effect on the concentration of serum triglyceride (TG). Observed values ranged between 133.58mg/dl for birds on 75% TSM (T4) and 156.42mg/dl for birds on the control diet.

5.5.2.10 Cholesterol.

The apparent values observed for the concentration of serum cholesterol of birds fed graded levels of TSM at the starter phase were not significant (P>0.05). The observed values ranged between 95.97mg/dl for birds fed 75% TSM diet and 107.71mg/dl for birds on 25% TSM diet (T2). Values recorded for birds on the control diet (105.32mg/dl) and that observed for birds on 50% TSM diet (105.40mg/dl) were relatively close.

5.5.2.11 High Density Lipoprotein (HDL).

Replacement of soyabean meal in broilers starter diets with graded levels of thevetia seed meal had no significant (P>0.05) effect on the concentration of high density lipoprotein in the serum. Observed values ranged between 73.86mg/dl for birds on the control diet and 79.36mg/dl for birds fed 75% TSM diet.

5.5.2.12 Very Low Density Lipoprotein (VLDL).

Dietary inclusion of TSM in broilers starter diets had no significant (P>0.05) effect on the concentration of very low density lipoprotein in the blood of the birds. Observed values ranged from 28.17mg/dl for birds on 100% TSM diet to 33.47mg/dl for birds on 50% TSM diet. Birds on the control diet had 31.18mg/dl on the average.

5.5.2.13 Aspartate Aminotranferase (AST).

The apparent differences observed in the values of serum enzyme AST for the birds on the control diet and those fed diets in which TSM replaced soyabean meal were not significant (P>0.05). The observed values ranged from 54.37mg/dl for birds fed 100% TSM diet to 65.33mg/dl for birds on 25% TSM diet. Birds on the control diet had 54.73mg/dl which served as the reference value for this study.

5.5.2.14 Alanine Aminotranferase (ALT).

Dietary inclusion of TSM in broilers starter diets had no significant (P>0.05) effect on the blood concentration of ALT of the birds. Observed values ranged between 11.81mg/dl for birds on the control diet and 13.00mg/dl for birds fed 25% TSM diet.

5.5.2.15 Alkaline Phosphatase (ALP).

Values observed for the serum enzyme ALP of broiler chicken fed diets in which TSM replaced SBM ranged between327.85mg/dl for birds on the control diet and 337.32mg/dl for birds on 75% TSM diet. The apparent differences were however, not significant (P>0.05).

Table 5.2 BIOCHEMICAL INDICES OF BROILERS (STARTER)

	DIETARY TREATMENTS					
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
mg/dl	**0%**	**25%**	**50%**	**75%**	**100%**	
Total Protein	2.93	2.71	2.76	2.70	2.88	0.21
Albumin	1.20	1.21	1.22	1.43	1.44	0.08
Globulin	1.73	1.50	1.54	1.27	1.43	0.22
Calcium	9.83	8.94	10.87	9.59	11.11	0.87
Phosphorus	4.98^{b}	4.51^{b}	5.15^{ab}	5.63^{a}	5.67^{a}	0.31
K^+ mmol/l	3.56^{ab}	3.46^{ab}	3.88^{a}	3.33^{b}	3.53^{ab}	0.15
Urea	0.59	0.86	1.28	0.76	1.00	0.24
Glucose	260.45	245.37	244.64	254.21	242.86	7.93
Triglyceride	156.42	153.52	136.65	133.58	151.74	7.75
Cholesterol	105.32	107.71	105.40	95.97	97.33	5.38
HDL	73.86	78.50	77.20	79.36	74.77	3.86
VLDL	31.18	29.47	33.47	32.91	28.17	3.39
AST	54.73	65.33	55.03	55.02	54.37	4.67
ALT	11.81	13.00	12.39	12.16	12.29	`1.24
ALP	327.85	333.81	334.98	337.32	337.04	5.47

abc means along the same row with any identical superscripts are not significant (p>0.05) ; HDL = High Density Lipoprotein; VLDL = Very Low Density Lipoprotein; AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline Phosphatase

5.5.3 HEMATOLOGICAL INDICES OF BROILERS (FINISHER PHASE)

The summary of the hematological indices of broilers fed graded levels at the finisher phase is given in Table 5.3.

5.5.3.1 Packed Cell Volume (PCV).

Substitution of soyabean meal with thevetia seed meal in broilers finisher diets had significant ($P<0.05$) effect on the packed cell volume of the birds. The observed values ranged between 27.38% for birds on 75% TSM diet and 30.50% for the group on 50% TSM diet. The group on the 50% TSM diet (T3) had the highest ($P<0.05$) value of 30.50% which was apparently higher ($p>0.05$) than value observed for birds on the control diet (29.00%).

5.5.3.2 Red Blood Cell (RBC) Counts.

The concentration of red blood cell was not significantly ($P>0.05$) affected by the replacement of soyabean meal with thevetia seed meal in the finishers diets. The apparent variations ranged between 234.43mg/dl for birds on 25% TSM diet and 245.13mg/dl for the group on the control diet. The group on 50% TSM had a value of 242.50mg/dl which is closer to the value observed for birds on the control diet.

5.5.3.3 White Blood Cell (WBC) Counts.

Dietary inclusion of thevetia seed meal in broilers finisher' diet had no significant ($P>0.05$) effect on the white blood cell counts of the birds. Observed values ranged between 15507mg/dl for birds on 100% TSM diet and 19531mg/dl for birds on 50% TSM diet. Birds on the control diet had 18169mg/dl on the average.

5.5.3.4 Lymphocytes Counts.

Replacement of soyabean meal with thevetia seed meal in broilers finisher diet had no significant (P>0.05) effect on the lymphocytes counts of the birds. The variations observed ranged from 61.00mg/dl for birds on 50% TSM diet to 70.00mg/dl for the group of birds on the control diet. The closest lymphocytes counts to the value observed for the control group was observed for birds on 25% TSM diet (69.38mg/dl).

5.5.3.5 Heterocytes Counts.

Heterocytes counts was significantly (P<0.05) affected by the inclusion of thevetia seed meal in broiler' finisher diets. Birds fed 50% TSM diet had significantly (P<0.05) higher heterocytes counts (34.13mg/dl) than birds on diet T2 (25% TSM; 24.88mg/dl) which had the lowest heterocytes counts.

5.5.3.6 Monocytes Counts.

Dietary treatments had no significant (P>0.05) effect on the monocytes count of birds fed graded levels of thevetia seed meal diets. Observed values ranged from 1.50mg/dl for birds on the control diet to 2.63mg/dl for birds fed 50% TSM based diet.

5.5.3.7 Eosinophils Counts.

Eosinophils counts significantly (P<0.05) ranged between 1.63mg/dl for birds on the control diet and 3.50mg/dl for birds on 25% TSM diet. The variations observed for other dietary treatments were not significant (P>0.05).

5.5.3.8 Basophils Counts.

Basophils counts was not affected by dietary inclusion of thevetia seed meal in broilers finisher diets as values observed were 0.00mg/dl for birds in all dietary treatments.

TABLE 5.3 Hematological Parameters of Broilers (Finisher Phase)

	DIETARY TREATMENTS					
Parameters	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**
mg/dl	**0%**	**25%**	**50%**	**75%**	**100%**	
PCV (%)	29.00^{ab}	27.63^{b}	30.50^{a}	27.38^{b}	28.57^{ab}	0.81
RBC	245.13	234.43	242.50	236.00	237.38	5.37
WBC	18169	18219	19531	18119	15507	1.50
Lymphocytes	70.00	69.38	61.00	68.00	67.25	2.13
Heterocytes	27.50^{ab}	24.88^{b}	34.13^{a}	28.63^{ab}	29.38^{ab}	2.16
Monocytes	1.50	2.38	2.63	1.75	2.25	0.43
Eosinophils	1.63^{b}	3.50^{a}	2.25^{ab}	2.13^{ab}	2.25^{ab}	0.49
Basophils	0.00	0.00	0.00	0.00	0.00	0.00
MCV (fl)	1.18	1.18	1.26	1.16	1.20	0.36

abc means along the same row with any identical superscripts are not significant (p>0.05); PCV =Packed Cell Volume; RBC = Red Blood Cell; WBC = White Blood Cell; SEM = Standard Error of Means; MCV = Mean Corpuscular Volume.

5.5.4 Serum Biochemical Indices of Broilers (Finisher Phase)

The summary of the biochemical parameters of broilers fed graded levels of TSM is given in Table 5.4.

5.5.4.1 Total Protein.

Replacement of soyabean meal with thevetia seed meal in broilers finisher diets had no significant (P>0.05) effect on the concentration of total protein for birds in all dietary treatments. Observed values ranged between 6.49mg/dl for birds on 25% TSM based diet and 6.87mg/dl for the group fed 75% TSM based diet.

5.5.4.2 Albumin.

Serum albumin of broilers fed graded levels of thevetia seed meal diets at the finisher phase was not significantly (P>0.05) affected. Values observed ranged between 1.23mg/dl for birds on the control diet and 1.34mg/dl for birds on 75% TSM diet.

5.5.4.3 Globulin.

The globulin fraction of the serum protein was not significantly (P>0.05) affected by the dietary inclusion of thevetia seed meal in broilers finisher diets. Observed values ranged from 5.24mg/dl for birds on 25% TSM (T2) to 5.79mg/dl for birds fed 100% TSM based diet. The value observed for birds on the control (5.35mg/dl) served as the reference value in the present study.

5.5.4.4 Calcium.

The variations observed in the concentration of sera calcium of birds fed diets in which thevetia seed meal replaced soyabean meal in broiler finisher diets were significant (P<0.05). Dietary inclusion of thevetia seed meal in the diets significantly (P<0.05)

increased the concentration of calcium from 19.18mg/dl for birds on the control diet to 29.47mg/dl for birds fed 50% TSM diet (T3). However, the variations observed for birds on treatments T2, T3 and T5 were not significant (P>0.05).

5.5.4.5 Phosphorus.

Substitution of soyabean meal for thevetia seed meal in broilers finisher diet had no significant (P>0.05) effect on the concentration of phosphorus in the sera of the birds on all dietary treatments. Values observed ranged between 7.90mg/dl for birds on 25% TSM diet and 5.64mg/dl for birds on 75% TSM diet.

5.5.4.6 Potassium Ions (K^+).

Dietary inclusion of thevetia seed meal in broilers finisher diet significantly (P<0.05) reduced the concentration of K^+ in the sera of birds fed graded levels of TSM. The mean values significantly reduced from 3.50mg/dl for birds on the control diet to 3.01mg/dl for birds on 25% TSM diet. However, the variations observed for birds on diets T3, T4 and T5 were not significant (p>0.05).

5.5.4.7 Glucose.

The level of glucose in the sera of birds fed graded level of TSM in broiler finisher diets was significantly (P<0.05) affected. Observed mean values ranged from 204.26mg/dl for birds on 100% TSM diet to 233.26mg/dl for birds on 25% TSM diet. Birds on the control diet had an average of 212.00mg/dl.

5.5.4.8 Cholesterol.

Replacement of soyabean meal with thevetia seed meal significantly (P<0.05) affected the level of cholesterol in the sera of birds fed graded levels of thevetia seed meal diets. Observed mean values ranged between 146.08mg/dl for birds on the control diet and 193.64mg/dl for birds on diet T4 (75% TSM). The level increased with increasing levels of TSM in the diets, it reached the peak in birds on diet T4 and thereafter declined (P<0.05) in birds fed 100% TSM.

5.5.4.9 Aspartate Transaminase (AST).

Differences observed in mean values of AST, for birds on all dietary treatments were significant (P<0.05). The mean values for AST ranged from 60.78mg/dl for birds on T5 to 80.67mg/dl for birds on T3. Birds on the control diet (T1) had 65.89mg/dl on the average.

5.5.4.10 Alanine Transaminase (ALT).

The concentration of ALT in the sera of birds was significantly (P<0.05) affected by dietary inclusion of TSM in broiler finisher diets. Observed mean values ranged from 14.17mg/dl for birds on 100% TSM diet to 9.85mg/dl for the group on the control diet. The variations observed between birds on 25% TSM diet and those on the control diet was not significant (P>0.05).

5.5.4.11 Alkaline Phosphatase (ALP).

The apparent variations observed in the mean values of the enzyme ALP for birds on all dietary treatments were not significant (P>0.05). Mean values ranged between 326.54mg/dl for birds on the control diet and 339.35mg/dl for birds on 50% TSM diet.

5.5.4.12 High Density Lipoprotein (HDL).

The concentration of high density lipoprotein in the sera of birds fed graded levels of TSM was not affected by dietary treatments (P>0.05). Observed mean values ranged between 90.55mg/dl for birds on 50% TSM diet and 97.84mg/dl for birds on 25% TSM diets.

5.5.4.13 Very Low Density Lipoprotein (VLDL).

The apparent variations observed in the concentration of VLDL in the sera of broiler finishers fed graded levels of TSM was not significant (P>0.05). Mean values ranged from 28.70mg/dl for birds on 100% TSM diet to 35.06mg/dl for birds on 25% TSM diet.

Table 5.4 Sera Biochemical Parameters of broilers fed graded levels of *Thevetia* seed Meal (Finisher Phase)

DIETARY TREATMENTS

Parameters mg/dl	T1 0%	T2 25%	T3 50%	T4 75%	T5 100%	SEM
Total Protein	6.58	6.49	6.82	6.87	6.83	0.18
Albumin	1.23	1.25	1.30	1.34	1.27	0.08
Globulin	5.35	5.24	5.52	5.34	5.79	0.20
Calcium	19.18^{b}	24.02^{ab}	29.47^{a}	28.12^{ab}	25.29^{ab}	2.51
Phosphorus	5.98	7.90	6.73	5.64	6.51	0.82
K^{+} mmol/l	3.50^{a}	3.01^{b}	3.13^{ab}	3.16^{ab}	3.26^{ab}	0.12
Glucose	212.00^{ab}	233.26^{a}	197.82^{ab}	206.35^{b}	204.26^{b}	8.28
Cholesterol	146.08^{b}	146.82^{b}	165.55^{ab}	193.64^{a}	156.07^{b}	9.00
AST	65.89^{b}	72.05^{ab}	80.67^{a}	72.49^{ab}	60.78^{b}	4.25
ALT	9.85^{b}	10.90^{ab}	13.56^{a}	14.14^{a}	14.17^{a}	1.02
ALP	326.54	335.75	339.35	336.42	334.78	4.32
HDL	95.97	97.84	90.55	95.74	93.30	3.60
VLDL	32.70	35.06	31.48	32.82	28.70	2.89

abc means along the same row with any identical superscripts are not significant (P>0.05)
AST = Aspartate Transaminase; ALT= Alanine Transaminase; ALP =Alanine Phosphatase; HDL =High Density Lipoprotein; VLDL = Very Low Density Lipoprotein; SEM =Standard Error of Means.

5.6.0 DISCUSSION

The apparent variations observed in the mean values for hematological indices of broilers fed graded levels of TSC at the starter phase were not significant and were all within the reference values reported by Mitruka and Rawnsley, (1977). This could be attributed to effective detoxification of Thevetia seed meal which left no trace of pathological abnormality in the blood. Further more, there was no indication of anemic conditions as revealed by the absence of basophilic cells in the blood samples analyzed.

The values recorded for other haematological parameters like MCHC, MCH and MCV further confirmed the absence of any type of anemia as these values were within the normal range as given by Mitruka and Rawnsley, 1977. This could be attributed to the effective detoxification of *Thevetia* seeds used in this study.

Serum biochemical parameters are used in assessing the nutritional status of birds (Church *et al.* 1984). The total serum protein functions in defense mechanism and maintenance of osmotic pressure (Vasudevan *et al*., 2011). Though, the variations in the values observed for total protein, albumin and globulin were not significant, the mean values obtained for birds on diets T2, T3, T4 and T5 were comparable to values observed for birds on the control diet at both starter and finisher phases of the experiment. Furthermore the diets favoured the proliferation of the desirable lipoprotein (HDL) which carries cholesterol from the peripheral tissues to the liver where they are acted upon by bile salts. This is important as the consumption of broilers reared on TSM-based diets will not constitute heart related health hazards.

Glucose is the energy substrate for all living organisms. Digestibility of dietary carbohydrate, if impeded can limit the circulating level in the blood. It is also related to utilization of fatty acids as deficiency precipitates ketosis and acidosis. The variations in blood glucose values at the finisher phase were significant. Birds on diet T2 recorded the highest value 233.26mg/dl which compares well ($p>0.05$) with the figures for birds on the control and diets T3, T4 and T5. Although the values did not depict a consistent trend, the release of glucose was not inhibited by any adverse effect of digestibility as suggested

by the data. Variations in ileal digestibility which were not significant further buttressed this contention.

It can be inferred that the challenges that the birds had to contend with through the immune system were not appreciable.

5.7 SUMMARY AND CONCLUSION

Three experiments were carried out to determine the utilisation of detoxified *Thevetia* seed meal by broiler chickens. The first study involved the detoxification of Thevetia seed meal by subjecting it to three methods of detoxification and the determination of chemical composition and residual glycoside content. The second experiment determined the performance characteristics, apparent ileal digestibility and carcass characteristics of broilers fed diets in which soybean meal was replaced by graded levels of *Thevetia* seed meal. In the third trial, hematological and sera chemistry of broiler birds fed graded levels of TSM were assayed.

In experiment one, detoxification and chemical characterization of Thevetia seed meal were determined. The results indicated that:

1 The different treatments to which Thevetia seed meal was subjected to had significant effect ($P<0.05$) both on the chemical and glycoside compositions of the meal.

2 There was an increase in Crude Protein from 19.35% in raw seed to 44.87% in brine-detoxified and deffated seed meal.

3 Ether extract decreased with the increase in Crude Protein. Ether extract decreased from 44.01% in raw seed meal to 5.10% in defatted meal.

4 Glycoside reduced from 4.7% in raw seed meal to 0.07% in 7.5% brine-detoxified seed meal.

5 Effective detoxification of *Thevetia* seed meal was achieved with soaking for three hours with 7.5% brine solution.

6 The nutritive value of TSM was enhanced by soaking in brine and sun-cured.

In experiment two, 0%, 25%, 50%, 75% and 100% TSM replaced soybean meal in diets that were isonitrogenous and isocaloric. The results obtained indicated that:

1 There were significant differences ($p<0.05$) in feed intake of broiler birds fed graded levels of TSM.

2 The lowest feed intake and weight gain was observed for birds on diet T5 (100%)

3 Nutrient utilization of birds on the 50% TSM diets (T3) was better than that observed for birds on the control diet.

4 The apparent variations observed in weight of the internal organs were not significant ($P>0.05$).

5 Apparent ileal digestibility declined with increasing level of TSM after diet T3 (50% TSM)

In study three, hematological and serum biochemistry of birds fed graded levels of TSM were assayed. The results indicated that:

1 Hematological indices showed no significant difference ($P>0.05$) among treatment means both at the starter and finisher phases.

2 Serum concentration of cholesterol at the finisher phase was significantly ($P<0.05$) higher in diets T4 and T5.

3 Serum potassium ion concentration showed significant differences ($P<0.05$) at both starter and finisher phases, but values observed were within the normal range of 3.5- 5mmol/l.

4 Other serum biochemical parameters investigated showed no significant (P>0.05) differences among treatment means.

Therefore, it can be concluded that brine-detoxified defatted *Thevetia* seed meal can replace up to 50% of the more expensive defatted soybean meal in broiler ration without any deleterious effects.

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