STUDIES ON THE PHYTOCHEMICAL, MEDICINAL AND NUTRITIONAL VALUES OF CONGRONEMA LATIFOLIA (UTAZI) IN RABBIT AND POULTRY PRODUCTION

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DECLARATION

We declare that this thesis on “STUDIES ON THE PHYTOCHEMICAL MEDICINAL AND NUTRITIONAL VALUES OF CONGRONEMA LATIFOLIUM (UTAZI) IN RABBIT AND POULTRY PRODUCTION” is an original research work, carried out by Ukorebi, Bassey A. Reg. No: 20044455448, in the Department of Animal Science and Technology, Post Graduate School, Federal University of Technology, Owerri, Nigeria.

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To my dear mother, Mrs. Affiong Ukorebi Asuquo, who provided the enabling environment for my academic pursuits and success.
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ABSTRACT

Experiments were conducted to evaluate the phytochemical medicinal and nutritional values of Congronema latifolia (utazi) leaf meal (CLLM) in rabbit and poultry production. The proximate analysis of the leaf meal (CLLM) yielded 8.04%, 14.25%, 60.39%, 6.26%, 2.84% and 2.84% moisture, crude protein, NFE, ash, ether extract and crude fibre respectively on dry matter bases. The methanolic extract of CLLM exhibited a minimum inhibitory concentration (MIC) of 15.62 mg/ml against Pseudomonas aeruginosa and E. Coli while Staphylococcus aureus, Klebsiella spp and Salmonella spp were all inhibited at a concentration of 250mg/ml. The minimum bactericidal concentrations (MBC) of the above five organisms tested were 31.25, 31.25, 250, 250 and 250 mg/ml, respectively, while the diameters of the zone of inhibition measured at 250mg/ml of the leaf meal extract in centimeters were 1.4, 1.5, 0.0, 0.0 and 0.3 for Ps. aeruginosa, E. Coli, S. aureus, Klebsiella spp, and Salmonella spp, respectively. This result suggests that the methanolic extracts of CLLM are very effective against Ps. aeruginosa and E. coli. In the rabbit experiment, 4 experimental diets were formulated such that diet 1 (control) contained 0% CLLM, while diets 2, 3 and 4 contained 10%, 20% and 30% CLLM, respectively. Each diet was fed to a group of 9 grower rabbits for 49 days. There were no significant differences (P>0.05) among the treatment groups for average final body weight, average body weight gain and average daily feed intake. The feed conversion ratio was also not affected by the treatments (P>0.05). The dressing percentage and internal organs (percentage hearts, lungs and pancreas weights) were not significantly affected (P>0.05) by the treatments. Dietary levels of 20% and 30% CLLM significantly (P<0.05) increased the percentage weight of liver and kidneys. Haematological parameter which showed significant increase (P<0.05) was the mean cell haemoglobin (MCH), at 20% and 30% levels of CLLM, while the white blood cell concentration decreased significantly (P<0.05) at 10%, 20% and 30% levels. Other indices (Hb, RBC, ESR, PCV, MCV and MCHC) were similar (P>0.05). Dietary levels of 10%, 20% and 30% CLLM, significantly (P<0.05) increased total serum protein, but other serum bio-chemical parameters were not affected by the treatments (P>0.05). There were no lesions of pathologic significance in the tissues (liver, kidney and pancreas) examined.

In the broiler experiment, 5 experimental diets were formulated such that diet 1 (control) contained 0% CLLM while diets 2, 3, 4 and 5 contained 2.5%, 5.0%, 7.5 and 10.0% CLLM respectively. Each diet was fed to a group of 30 broilers (one week old) for 49 days. The average final body weight, average body weight gain and feed conversion ratio indicated no significant (P>0.05) treatment effect. There was significant depression in average daily feed intake (P<0.05) at 10% dietary level. Ten percent dietary level also significantly (P<0.05) depressed RBC concentration but other haematological indices indicated no significant (P>0.05) treatment effect. Serum glucose was significantly (P<0.05) reduced at 7.5% and 10%; AST dropped significantly
(P<0.05) at 5%, 7.5% and 10%; bilirubin increased significantly (P<0.05) at 5%, 7.5%, 10% and 2.5% dietary levels of CLLM. Others serum parameters were not affected (P>0.05) by the treatments. Dietary levels of 2.5%, 5.0% and 7.5% CLLM (P<0.05) increased dressing percentage of the experimental birds. Percentage weights of necks, wings, thighs, drum sticks, breasts, hearts, liver, spleen, lungs and pancreas (expressed as percentage of live weights) were similar (P>0.05). However, dietary levels of 2.5% - 10.0% CLLM yielded significantly higher (P<0.05) percentage weights of proventriculus than the control diet. There were no lesions of pathologic significance in the liver, kidney, proventriculus and pancreas. It would therefore seem that rabbits can tolerate dietary levels of up to 30% CLLM while 7.5% inclusion level of the leaf meal can support normal broiler production.

**Keywords:** Poultry, Poultry Production, Phytochemical, Nutritional Values
CHAPTER ONE

INTRODUCTION

Livestock industry is of socio-economic and nutritional importance for nations worldwide. Apart from providing a means of livelihood for a significant population of the farming families, it contributes significantly to the Gross Domestic Product (GDP) of the nation (Duruna, 1996).

Worthy of note is the fact that the success of the industry hings basically on nutritional and health management of the animals. Over the years, poultry industry and swine production have been among the most lucrative sectors of agriculture in Nigeria. Indeed from inception, through the early 1980s, the industries have experienced a significant development and expansion. As a result, there was an appreciable national flock size and worthwhile income for farmers. This favourable trend was, however, short-lived. During the last two decades, the industry has witnessed a serious set back due largely to inadequate feed supply which stems from unavailability of protein concentrate and short supply of energy sources. Moreso, the problem has been aggravated by competitive demand for some of the existing feed ingredients like maize, millet and soybean for livestock feeding, and human consumption. This dismal trend has been responsible for the prohibitive cost of finished feeds.

Agreeably, feed cost accounts for 60 - 80% of the total production cost of commercial poultry and intensive livestock production in general. The
high feed cost relative to monogastric livestock products from the early 1980s to date has drastically slashed farmer’s profit margin. The obvious implication here is that revenue accruing from the sales of pork, poultry meat, eggs, and other commercial livestock products cannot adequately compensate for the cost of production of the same. The consequence of this has been far-reaching. Resource-poor farmers have been forced out of production while “bigger farmers” have reduced stock population to as low as 30 to 50%, while new entrants have been demoralized. In certain cases, sub-standard feeding has been resorted to, leading to poor production. In view of this limiting circumstance, it would seem reasonably important to search for cheaper, readily available, non-conventional feed sources that will be well suited for a sustainable monogastric production industry. Such feed should of course be low in competitive demand for human food. It is this line of thought that has generated this research interest in *Congronema latifolia*, known in Efik, Ibibio and Igbo languages as *utazi* to determine its value as a feed ingredient for poultry and rabbit diets.

The use of leaf meal of plants as feed ingredients as an alternative to conventional feed sources is a novel area of research in animal nutrition. A number of workers (Tewe, 2003; Adegbola, 2004) have shown that various alternative feedstuffs have been fed to poultry with remarkable results. Some of these alternative sources including the leaf meals of some tropical legumes and browse plants, rich in nutrients like vitamins,
minerals and oxycarotenoids have been reported (Vohra et al., 1972; Okoli et al., 2001 and 2003; Esonu et al., 2002, 2004 and 2005).

Considering the combinations of ingredients used by the traditional animal health practitioners, it is likely that additive, synergistic and nutritional effects of leaf meals might be involved in alleviating livestock maladies. Many phytochemicals have been shown to be bio-active, and have been useful as chemotherapeutic agents, pesticides, food/feed additives and other biologicals.

*Congronema latifolia* (*utazi*) is a wild tropical creeping plant with lush deep green vegetation. Where it grows in a swampy area or inland valley, the vegetation is perennial, otherwise, it is deciduous. The plant plays a significant role in ethnomedicine among the local populations where its leaf meal extract is used in treating malaria, stomach-ache and diarrhoea. Osuala *et al* (2005) reported that methanolic extract of *C. latifolia* exhibited marked activity against *Pseudomonas aeruginosa and E.coli* with both having a minimum inhibitory concentration (MIC) of 15.625 mg/ml in an *in vitro* study. Their investigations have new evidence to corroborate an earlier work by Dalxiel (1956), which reported the use of *C. latifolia* in the treatment of diarrhoea.

*Congronema latifolia* is abundantly available in Cross River State, Nigeria. It can readily be found in its southern part, up to areas beyond the central region of the state. This location lies along longitudes 8° and 9° East, and latitudes 6° and 7° North of the equator, with a warm weather and an ambient temperature range of 21 – 30°C. It experiences an annual rainfall of 500 – 1070mm (Mfam, 2002). The forest areas of
Akamkpa, Biase, Ugep, Mkpani, and Obubra among others are rich reservoirs of the plant.

So far, studies on *C. latifolia* have been basically *in vitro* investigations. It is worthy of note that the nutritional and pharmacological principles of the plants oftentimes behave differently in the animal’s physiological environment from what obtains in *in vitro* studies. This is because the factor(s) under investigation may be inactivated in the *in vivo* environment, or such factor(s) may form complexes which limit or inhibit its bio-availability. Sometimes it may be flushed out of the system so rapidly that it has no time to act. At other times active ingredients in the plant materials can be poisonous or toxic at certain concentrations or administration regimen.

There is paucity of information on the nutritional, phytochemical and toxicological properties of *C. latifolia*. It would therefore seem necessary to undertake a comprehensive preliminary investigation of its leaf to determine its nutritional significance as feed ingredient in poultry and rabbit diets, as well as its phytochemical composition and potentials.

**RESEARCH OBJECTIVES**

The objectives of the research were therefore to:

1. Determine the proximate chemical composition of the leaf meal of *C. latifolia*, including its mineral and amino acid profiles;
2. Test its bio-activity using common enteric bacterial organisms of poultry and rabbits.
3. Determine its phytochemical composition, and its
toxicological and histopathological effects using broiler chickens and crossbred rabbits;

4. Determine its effects on growth performance, organ weights, carcass weights, haematological and serum biochemical indices of the test animals and,

5. Provide reference data on its leaf meal as feed ingredient in poultry and rabbit diets.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Chemical Composition of Leaf Meals

The chemical content of feed ingredients is one of the most important criteria upon which their consideration and selection for livestock feeding programmes is based. Of particular interest in this regard is high protein and low fibre level. In assessing the potential of leaf meal in non-ruminant nutrition, a comparative review of the nutrient composition over other attributes such as palatability and texture must be a critical viewpoint. In general, leaf meals of leguminous species have higher crude protein content than those of cereal grains, with values relative to cassava leaf meal. Among the legumes studied are *Leucaena leucocephala* (D’Mello and Fraser, 1981), *Gliricidia sepium* (Chadhoker, 1982; Osei et al., 1990), *Sesbania sesban* (Brown et al., 1987), *Canavalia ensiformis* and *Canavalia gladiata* (Udedibie, 1988), *Cajanus cajan* (Udedibie and Igwe, 1989), *Microdesmis puberula* (Esonu et al., 2002; Azubuike, 2003). However, reports by Raharjo et al (1988) indicated that *Albizia falcata* does not seem to conform to this rule (Table 2.1). In an experiment to determine the optimum inclusion level of cassava leaf meal in the diet of growing rabbits, Adegbola and Okonkwo (2000) reported its nutrient composition as follows: Crude protein, 24.68%; crude fibre, 14.87%; ether extract, 7.93%; and gross energy (MJ/kg), 19.66.
The proximate composition of some multipurpose tree legume leaves have also been reported (Ayuk et al., 2002). Table 2.2 shows details of the data.

Sonaiya and Olori (1989) have also published the chemical composition of neem leaves as follows: Crude protein, 17.5%; ether extract, 42%; crude fibre, 12.3% and metabolisable energy (Kcal/kgDm), 752. This result is comparable with the proximate composition of neem leaf meal on dry matter basis as analysed by Esonu et al (2005).

2.1.1 Fibre Content of Leaf Meals

The fibre content of leaf meal is uppermost in the dry matter fraction. In most cases, it may equal and or even exceed the crude protein content (D’Mello, 1988). This tends to reduce the overall digestibility when there is significant proportion of leaf meal in the diet (Tangendjaja et al., 1990). The obvious consequence of this is depressed growth and egg production.

2.1.2 Essential Amino Acid Composition of Leaf Meals

Leaf meals in general are endowed with essential amino acids. Research publications (D’Mello and Fraser, 1981; Chadhoker, 1982; Brown et al., 1987; Lyan et al., 1988; Ash et al., 1992 and Ravindran, 1993) have shown that their levels vary. The variation might be due to differences within the cultivars and geographical locations. The differential levels were particularly notable for arginine, cystine, phenylalanine, tyrosine, leucine, methioine, cystine, glycine and threonine with samples from
Malawi generally containing higher concentrations of these amino acids than a sample from Thailand. Essential amino acid content of *Leucaena leucocephala* (D’Mello and Fraser, 1981), *Gliricidia sepium* (Cadhoker, 1982), *Sesbania sesban* (Brown *et al.*, 1987) *Prosopis chilensis* (Lyon *et al.*, 1988), *Sesbania grandiflora* (Ash *et al.*, 1992), and *Manihot esculenta* (Ravindran, 1993) have been published (Table 2.3).

D’Mello (1989) has reported that lysine concentrations of leaf meals are relatively higher than those of grains and some by-products such as oil extracted coconut meal. The researcher however noted that the level is not as high as to be compared with those of fish meal and soybean meal. Leguminous leaf meals are shown to be generally deficient in sulphur – containing amino acids but their use can be enhanced by supplementation with methionine. According to Eggum (1970), the biological value of cassava leaf meal ranges from 0.49 to 0.57 and this may be enhanced to 0.80 with methionine.

### 2.1.3 Mineral Composition of Leaf Meals

Data on mineral content of leaf meals are scanty. A set of data published by D’Mello and Fraser (1981) on *Leucaena leucocephala* indicated geographical differences in its mineral profiles. The researchers noted that a sample from Thailand had lower concentrations of phosphorus, iron and zinc when compared to three other samples of the same plant obtained from Malawi. The report also showed relatively wide variations among the samples in their contents of calcium, potassium, iron, and managanese.
Table 2.1: Proximate Composition of Leaf Meals (g/kg\(^{-1}\) Dm)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Leucaena leucocephala</th>
<th>Gliricidia sepium</th>
<th>Robina pseudoacacia</th>
<th>Cajanus cajan</th>
<th>Sesbania sesban</th>
<th>Prospios chilensis</th>
<th>Albizia falcata</th>
<th>Manihot esculenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>291</td>
<td>296</td>
<td>218</td>
<td>243</td>
<td>306</td>
<td>183</td>
<td>163</td>
<td>167</td>
</tr>
<tr>
<td>Crude Fibre</td>
<td>89</td>
<td>120</td>
<td>199</td>
<td>248</td>
<td>169</td>
<td>251</td>
<td>264</td>
<td>171</td>
</tr>
<tr>
<td>Ether Extract</td>
<td>48</td>
<td>30</td>
<td>72</td>
<td>52</td>
<td>53</td>
<td>65</td>
<td>-</td>
<td>79</td>
</tr>
<tr>
<td>Ash</td>
<td>70</td>
<td>99</td>
<td>93</td>
<td>57</td>
<td>102</td>
<td>45</td>
<td>51</td>
<td>125</td>
</tr>
</tbody>
</table>

Adapted from Opara (1996).
Table 2.2: Percentage Proximate Composition of some Multipurpose Tree Leaves (g/100g)

<table>
<thead>
<tr>
<th>Leaf Meal</th>
<th>Crude protein</th>
<th>Crude fibre</th>
<th>Ether extract</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterolobium cyclocarpum</em></td>
<td>14.45</td>
<td>14.76</td>
<td>2.21</td>
<td>10.11</td>
</tr>
<tr>
<td><em>Samanea siamea</em></td>
<td>10.07</td>
<td>22.80</td>
<td>4.05</td>
<td>8.03</td>
</tr>
<tr>
<td><em>Pterocarpus santalonoides</em></td>
<td>15.32</td>
<td>8.16</td>
<td>4.57</td>
<td>7.85</td>
</tr>
<tr>
<td><em>Gliricidia sepium</em></td>
<td>19.26</td>
<td>9.92</td>
<td>8.41</td>
<td>9.31</td>
</tr>
<tr>
<td><em>Leucaena leucocephala</em></td>
<td>26.27</td>
<td>13.08</td>
<td>3.95</td>
<td>8.15</td>
</tr>
</tbody>
</table>

Adapted from Ayuk et al. (2002).
Table 2.3: Essential Amino Acid Composition of Leaf Meals (g/kg⁻¹)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>L. leucocephala&lt;sup&gt;a&lt;/sup&gt;</th>
<th>G. sepium&lt;sup&gt;b&lt;/sup&gt;</th>
<th>S. sesban&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P. chilensis&lt;sup&gt;d&lt;/sup&gt;</th>
<th>S. granidiflura&lt;sup&gt;e&lt;/sup&gt;</th>
<th>M. esculenta&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>12.1</td>
<td>12.0</td>
<td>9.9</td>
<td>6.6</td>
<td>8.6</td>
<td>9.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>13.3</td>
<td>-</td>
<td>10.9</td>
<td>-</td>
<td>9.9</td>
<td>-</td>
</tr>
<tr>
<td>Valine</td>
<td>14.4</td>
<td>16.0</td>
<td>10.9</td>
<td>8.6</td>
<td>7.3</td>
<td>11.8</td>
</tr>
<tr>
<td>Cysteine</td>
<td>2.0</td>
<td>3.9</td>
<td>0.5</td>
<td>2.3</td>
<td>1.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Methionine</td>
<td>4.6</td>
<td>4.2</td>
<td>3.7</td>
<td>1.3</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>13.7</td>
<td>12.0</td>
<td>9.2</td>
<td>6.4</td>
<td>5.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Leucine</td>
<td>21.7</td>
<td>24.1</td>
<td>18.1</td>
<td>12.6</td>
<td>15.2</td>
<td>17.2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>12.5</td>
<td>11.2</td>
<td>6.9</td>
<td>-</td>
<td>6.7</td>
<td>-</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>14.8</td>
<td>15.4</td>
<td>10.5</td>
<td>8.8</td>
<td>8.8</td>
<td>11.3</td>
</tr>
<tr>
<td>Lysine</td>
<td>17.6</td>
<td>11.2</td>
<td>12.7</td>
<td>10.4</td>
<td>10.6</td>
<td>12.4</td>
</tr>
<tr>
<td>Histidine</td>
<td>5.4</td>
<td>5.1</td>
<td>4.4</td>
<td>3.6</td>
<td>4.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>15.1</td>
<td>15.9</td>
<td>11.0</td>
<td>8.4</td>
<td>9.5</td>
<td>11.1</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>3.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Sources:

<sup>a</sup>D’Mello and Fraser (1981); <sup>b</sup>Chadhoker (1982); <sup>c</sup>Brown et al (1987);
<sup>d</sup>Lyon et al (1988); <sup>e</sup>Ash et al. (1992); and <sup>f</sup>Ravindran (1993).
The mineral profiles of *Gliricidia sepium* (Smith and Van Houtert, 1987), *Robina pseudoacacia* (Horton and Christensen, 1981), *Cajan cajan* (Udedibie and Igwe, 1989), *Sesbania sesban* (Brown et al., 1988), *Prosopis chilensis* (Riveros, 1992) *Albizia falcata* (Raharjo, et al., 1998), and *Manihot esculenta* (Ravindran, 1993) have been published (Table 2.4). The publications reveal striking differences among the leaf meals in the concentrations of calcium and phosphorus. Apart from that, the available data also shows that zinc concentration in cassava leaf meal far exceeds those of *Leucaena leucocephala* and *Gliricidia sepium*.

Nwugo (2002) observed that growth dilutes minerals in leaf meal. This may be important in tropical climate, where fast growth causes a far-reaching depletion of minerals in leaves except for sodium and phosphorus.

**2.1.3 Metabolic Energy Content of Leaf Meal**

Comprehensive data on the metabolic energy content of leaf meals are limited. However, D’Mello and Acamovic (1982) have reported that excepting cassava leaf meal, the metabolic energy content of leaf meals is generally low.

**2.1.4 Carotenoids Content of Leaf Meals**

Leaf meals are well endowed with carotenoids, a class of compounds including the carotene and xanthophylls. Carotene is the precursor of
vitamin A, while xanthophylls do not possess vitamin activity but can be used by poultry as a source of skin and egg yolk pigmentation.

Agreeably, egg yolk and broiler skin pigmentation are becoming important criteria of quality. D’Mello and Taplin (1978) reported that *Leucaena leucocephala* leaf meal contains a generous concentration of β-carotene and xanthophylls, with the β-carotene content ranging from 227 mg to 248 mg/kg\(^{-1}\) dry matter (DM) and 741 mg to 766 mg total xanthophylls kg\(^{-1}\) DM for different cultivars of *Leucaena leucocephala*. Scott *et al* (1969) recorded xanthophylls concentrations of 400 to 550 mg kg\(^{-1}\) DM in dehydrated alfalfa leaf meal. Udedibie and Opara (1998) in their experiment with *Alchornea cordifolia* leaf meal observed that an inclusion level of only 5% of its leaf meal gave a score as high as 11 in the eggs yolk fan scale, showing its high pigmenting efficiency. Esonu *et al* (2004) also reported that *Microdesmis puberula* achieved an egg yolk pigmenting potential of up to 11 in egg yolk fan scale with an inclusion level of 150 kg per tonne of layers diets. The concentration of *carotenoids* in leaf meals will depend to a large extent on the method and duration of drying. Wood *et al* (1983) showed that the rapid sun drying of *Leucaena leucocephala* yielded a leaf meal with carotene and xanthophylls concentration of 484 and 932 mg kg\(^{-1}\) DM, respectively. On the contrary, they maintained that substantial losses occurred during oven drying at 60°C. Monthly losses of carotenenes and xanthophylls during storage of leaves were of the order of 19 to 40 mg/kg\(^{-1}\) and 29 to 53 mg/kg\(^{-1}\) respectively. The researchers also observed that carotenoids were more stable in sun dried leaf meal samples than in those oven
dried. They hinted that pelleting or inclusion of an anti-oxidant failed to arrest losses during processing or storage.

Table 2.4: Mineral Composition of Leaf Meals (dry matter basis)

<table>
<thead>
<tr>
<th>Mineral Constituents</th>
<th>L. leucocephala a</th>
<th>G. sepium b</th>
<th>Robina pseudoacacia c</th>
<th>Cajanus cajan d</th>
<th>Sesbania sesban e</th>
<th>P.chilensis f</th>
<th>Albizia falcata g</th>
<th>Manihot esculenta h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>18.1</td>
<td>13.0</td>
<td>27.0</td>
<td>12.0</td>
<td>21.5</td>
<td>21.3</td>
<td>6.5</td>
<td>14.5</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>2.5</td>
<td>1.8</td>
<td>2.0</td>
<td>2.5</td>
<td>3.3</td>
<td>3.9</td>
<td>1.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.0</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>8.0</td>
<td>33.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.8</td>
</tr>
<tr>
<td>Magnesium</td>
<td>5.1</td>
<td>3.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Trace elements (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>9.9</td>
<td>5.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12.0</td>
</tr>
<tr>
<td>Iron</td>
<td>239.9</td>
<td>207.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>259.0</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>21.2</td>
<td>26.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>149.0</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td>42.1</td>
<td>69.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>52.0</td>
<td></td>
</tr>
</tbody>
</table>

Sources:

aD’Mello and Fraser (1981); bSmith and Van Houtert (1987); cHorton and Christensin (1981); dMean of three values given by Udedibie and Igwe (1989); eBrown et al (1988); fMean of five values cited by Riveros (1992); gRabarjo et al (1988); hRavindran (1993).
2.2 Nutritive Value of Leaf Meals

The nutritive value of leaf meals has been based largely on their crude protein digestibility. To this end, studies done so far have been restricted to the determination of the digestibility of the crude protein fraction. Research with rabbits (Raharjo et al., 1986; Singh and Negi, 1986) showed wide variations in digestibility coefficients for the crude protein component of different leaf meals (Table 2.5). Biological value (BV) based investigations of this subject have been limited.

In general, leguminous sources were associated with higher coefficients than cassava tops. The low digestibility of the crude protein fraction of Robinia pseudoacacia leaf meal for rabbit corroborated an earlier observation with rats (Horigone et al., 1984). Raharjo et al (1986) reported that the digestibility coefficient of the crude protein of Leucaena leucocephala appears to be favoured when the legume constitutes the sole source of protein for rabbits. The researchers noted, however, that when the leaf meal forms a substantial part of a concentrate diet, digestibility of the crude protein component of the whole diet is markedly reduced. Tangendjadja et al (1990) also reported a depression of digestibility of crude protein from 0.73 with control diet to 0.41 on inclusion of Leucaena leucocephala leaf meal at 600g/kg diet. Raharjo et al (1986) also hinted that steam pelleting of the leaves of L. leucocephala
also resulted in a significant reduction in the digestibility of its crude protein.

Apart from the digestibility of its crude protein fraction, another important index of the nutritive value of feeds relates to the digestible energy (DE) and metabolisable energy (ME) contents. For poultry, limited apparent and true metabolisable energy data have been reported, all of which point to extremely low values in *L. leucocephala* and *Robina pseudoacacia* (D’Mello and Thomas, 1978; Takada *et al.*, 1980; D’Mello and Acamovic, 1982a). Gonzalez *et al.* (1982) also linked low metabolisable energy value for *L. leucocephala* to its poor digestibility. However, some workers (Ravindran *et al.*, 1983; Ravindran, 1990 and 1993) have shown that ME values of cassava leaf meal appears to be relatively higher for both poultry and pigs.

### 2.1 Leaf Meals in Non-ruminant Nutrition

Several studies have been carried out on leaf meals in non-ruminant nutrition to evaluate the growth performance of chicks, pigs, rabbits, as well as the performance of laying hens. In poultry nutrition, for instance, several foliage portions of different plants have been investigated. These include the leaf meals of *Microdesmis puberula* (Esonu *et al.*, 2002 and 2004); *Azadirachta indica* (Esonu *et al.*, 2005); *Adansonia digitat*, *Cynodon plectosaechyum* and giant star grass (Ogunmodede and Woga, 1976); Azola and Banana plants (Cambel, 1988a, b); lucaene (Taplin *et al.*, 1981; Dada *et al.*, 2000); baobab (Ojabo and Njoku, 1986); *Gliricidia*
*sepium* (Cheeke and Raharjo, 1987); cassava plant (Sanchez *et al.*, 1986; Ogbonna and Oredein, 1998; Eruvbetine *et al.*, 2003); duckweed

Table 2.5: Digestibility Coefficients of the Crude Protein Fraction of Some Leaf Meals

<table>
<thead>
<tr>
<th>Source of Leaf Meal</th>
<th>Rabbits&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rats&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Poultry&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leucaena leucocephala</em></td>
<td>0.69 - 0.73</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Robinia pseudoacacia</em></td>
<td>0.47&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.49</td>
<td>-</td>
</tr>
<tr>
<td><em>Sesbania sesban</em></td>
<td>0.84</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Albizia falcata</em></td>
<td>0.73</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Manihot esculenta</em></td>
<td>0.42</td>
<td>-</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Sources:
<sup>a</sup>Raharjo *et al* (1988); <sup>b</sup>Horigone *et al* (1984); <sup>c</sup>Ravindran *et al* (1983); <sup>d</sup>Singh and Negi (1986).
(Hanstein et al., 1994); wild sunflower (Odunsi et al., 1996); water hyacinth (Dairo, 1997) and mimosa (Nworgu and Fapohunda, 2002) which have been included in poultry diets. D’Mello and Thomas (1978) reported that high inclusion levels of leaf meals have growth depressing effect which may be attributed to their low digestibility and inadequate metabolisable energy contents.

2.3.1 Leucaena leucocephala Leaf Meal

D’Mello and Thomas (1978) working on the nutritive value of dried Leucaena leucocephala leaf meal observed that graded levels of its leaf meal in chicks ration induced dose-related depression in their growth performance even when maize oil was used to compensate for the low metabolisable energy value in the leaf meal. Also studies with older broilers (D’Mello et al., 1987) showed that diets containing 100 gm leaf meal per kilogram diet significantly reduced growth without affecting dry matter intake. According to the researchers, although feed intake remained unaltered, amount consumed was insufficient to contain digestible nutrients, particularly protein and energy required to sustain rapid growth. This result contradicts an earlier report by D’Mello and Acamovic (1982b) which showed that there were no appreciable
differences in weight gain or efficiency of feed utilization between control chicks fed a maize-soybean meal diet and those offered diets containing *L. leucocephala* leaf meal. This indicates that some batches are unique with usually low anti-nutritional factors like mimosine and reduced

Table 2.6: Energy Contents of Selected Leaf Meals (Mcal/kg)

<table>
<thead>
<tr>
<th>Constituents</th>
<th><em>L. leucocephala</em></th>
<th><em>R. pseudoacacia</em></th>
<th><em>S. sesban</em></th>
<th><em>M. esculenta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross energy</td>
<td>4.684</td>
<td>4.47</td>
<td>4.254</td>
<td>4.493</td>
</tr>
<tr>
<td>Digestible energy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbits</td>
<td>2.342&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>3.752&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metabolisable energy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>0.813&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>1.84&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>(apparents)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry (True)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.984&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pigs</td>
<td>1.267&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>2.32&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Sources:
<sup>a</sup>Raharjo *et al* (1988); <sup>b</sup>D’Mello and Acamovic (1982a); <sup>c</sup>Takada *et al* (1980); <sup>d</sup>Ravindran (1983); <sup>e</sup>Gonzalez *et al* (1982).
concentrations of tannins and fibre as compared to other samples from another geographical location. However, the reason for the differential concentration of inhibitors is yet to be resolved. It is reasoned that seasonal factors, soil condition, and perhaps the variations in the leaf to stem ratio of the harvested samples may be implicated. In all trials in which *Leucaena leucocephala* leaf was included, maize oil was added to raise the dietary metabolisable energy concentration to levels present in the control diets. Malyniez (1974) working on commercial rations for growing pigs showed that pigs placed on diets containing *L. leucocephala* leaf meal at 100g per kg grew at faster rates than control animals. The researcher however observed that weight gain declined sharply with diets containing the leaf meal at 500g per kg. In the case of rabbits, Tangendjaja (1990) observed that graded additions of the leaf meal of the legume to a maize–soybean meal based diet induced severe dose-related depressions in growth of the animals. At the highest inclusion rate of 600g/kg diet, growth was less than 25% of the control value. On the basis of these results, it was concluded that *L. leucocephala* leaf meal could play no major role in rabbits production. However, Onwudike (1995) recommended that *L. leucocephala* should be used as green feed
for rabbits provided it does not exceed 70% dry matter. However, earlier study on the subject (Malynicz, 1974) showed a significant feed efficiency with inclusion of *L. leucocephala* at the rate of 200g/kg diet. Also Cheng *et al* (1981) indicated no difference in feed to gain ratios between the control diet and diets containing up to 160g/kg *L. leucocephala* for rabbits.

### 2.3.2 *Gliricidia sepium* Leaf Meal

From trials conducted by Mishra *et al* (1977) and Ranaweera *et al* (1981) with *Gliricidia sepium* leaf meal fed to growing chicks at inclusion rate of 50g and 100g/kg diets respectively, it was observed that the diets resulted in satisfactory growth rate relative to the control birds. The workers pointed out that a striking increase in these ratios occurred on inclusion of the leaf meal at 150g/kg. However, D’Mello *et al* (1987), D’Mello and Acamovic (1989), Ash *et al* (1992), and Opara (1996) have indicated that at higher leaf meal inclusion levels in poultry diets, growth is depressed. This is in line with the observations of Mishra *et al* (1977) and Ranaweera *et al* (1981) that chicks fed the higher concentration of leaf meal (500g/kg) had marked reduced growth rates.

### 2.3.3 *Sesbania species* Leaf Meal

Leaf meal derived from *Sesbania sesban* and *Sesbania grandiflora* are highly toxic to chicks even at inclusion rates as low as 100g/kg diet (Shqueir *et al*., 1989a; and Ash *et al*., 1992). At higher inclusion rate of 300g/kg, mortality of 100 per cent was recorded, whereas chicks fed
alfalfa leaf meal at this level gained 442g over a three week period and all birds survived the experiment (Brown et al., 1981).

2.3.4 Microdesmis puberula Leaf Meal
Azubuike (2003) observed that at 12.5% inclusion of Microdesmis puberula leaf meal for broilers, the birds’ performance compared favourably with those of the control group at 0% level. The researcher suggested that the improved body weight gain of the birds could be attributable to the lower concentration of inhibitory or anti-nutritional factors in M. puberula leaf meal as compared to other leaf meals. In a more recent study on the subject, Esonu et al (2004) observed depressed body weight gain of broilers fed M. puberula leaf meal at the level of 150g/kg of diet. The investigators figured out that the weight depression might be due to the fact that apparent digestibility and nutrient utilization particularly of protein and energy required to sustain rapid growth was low in the leaf meal. Iheukwumere et al (2005) working on rabbits performance, recorded that up to 30% inclusion of M. puberula leaf meal in the diet of grower rabbits did not adversely affect their growth performance as evidenced by their average daily weight gain which remained within the range reported for tropical conditions. they noted that increase in the inclusion level of M. puberula leaf meal tended to influence improvements in numerous blood parameters such as the Hb concentration, MCV, MCH and MCHC. The report also recorded increase in serum albumin and total blood protein values with increases in the leaf meal inclusion, suggestive of the high quality of the leaf meal protein.
2.3.5 *Cajanus cajan* (pigeon pea) Leaf Meal

Investigation by Udebibie and Igwe (1989) on the subject showed a decrease in feed conversion efficiency in hens fed pigeon pea leaf meal at 100g/kg dietary inclusion. The work further showed that at an inclusion level of 75g and 100g/kg of diet, hens incurred body weight losses over the 16 week experimental period.

2.3.6 *Vernonia amygdaliana* (bitter leaf) Leaf Meal

Fasina et al (2004) reported that addition of *V. amygdaliana* leaf meal in starter broiler diet at 50% and 100g/kg of ration significantly reduced feed intake and weight gain of experimental birds. The effect of the diet was most pronounced on birds fed diet containing 100g *V. amygdaliana* leaf meal per kg basal diets incorporated at the expense of groundnut cake (GNC) in which treatment mortality was also recorded. The researchers also showed that haematological parameters of birds were severely affected by the diet. In this regard, there were significant decreases in the values of red blood cell, packed cell volume, white blood cell and percentage neutrophils, and a significant increase in percentage lymphocytes. The report also showed that all the serum biochemical indices significantly increased in the blood serum of the birds. The presence of anti-nutritional factors in the leaf meal was implied for its growth depression effects, and the depressed blood parameters were attributed to its toxicity.
2.3.7 *Jacaranda mimosifolia* Leaf Meal

A study by Okorie (2006a) showed that inclusion of *Jacaranda mimosifolia* leaf meal at 2.5%, 5%, and 7% of ration induced dose-related depression in growth of finisher broilers, while average daily feed intake of birds was significantly affected at above 5% inclusion level of the leaf meal. He noted that the growth response was in line with the observations of Udedibie and Opara (1998), Esonu *et al.* (2002), and Okorie (2005), which revealed that at an inclusion rate of leaf meals above 50kg per tonne of feed, the weight of broilers is bound to drop. He suggested that the low feed intake at 7% inclusion of the leaf meal could be attributed to high fibre content coupled with caramel odour of the leaf meal which might have dissuaded the birds from taking the diets at higher inclusion levels. Similar reports on leaf meal studies with broilers have been published (Udedibie and Opara, 1998; Esonu *et al.*, 2002; Okorie, 2005). It is, however, interesting to note that the haematological and serum biochemical indices of broilers fed up to 7.5% *Jacaranda* leaf meal were within normal range and tended to improve with increasing inclusion rate of the leaf meal.

2.3.8 Cassava Leaf Meal

Studies on feeding potentials of cassava leaf meal with non-ruminants have been published. Adegbola and Okonkwo (2002) reported that rabbits fed on diets containing 15% and 30% cassava leaf meal gained more weight than those on other diets. Graded replacement of soybean
meal and maize with cassava leaf led to progressive depression in growth rates of chickens (Ross and Enriquez, 1969) and pigs (Ravindran, 1990). As compared to alfalfa meal, Ross and Enriquez (1969) indicated that cassava leaf meal elicits greater reductions in growth performance of chickens than alfalfa meal at all inclusion rates from 50 to 200g/kg. However, Ravindran et al (1986) showed that if the leaf meal diet was used to replace poor quality ingredients such as coconut meal, the inclusion rates of cassava leaf meal of up to 150g/kg diet might be employed without growth depression of broilers. In a similar report, Ravindran et al (1987) also confirmed earlier report by Ross and Enriquez (1969) that cassava leaf meal depresses feed conversion efficiency particularly when it is used to replace soybean and maize. The researchers still maintained that when however cassava leaf meal was employed in replacing poor quality feed resources like coconut oil meal, feed conversion efficiency was not depressed until the leaf meal inclusion rate reached 300g/kg in chicken diets. This new ceiling was striking when compared to publication by Ravindran et al (1986). The growth responses of non-ruminant animals to graded levels of leaf meal are summarized in table 2.7. Leaf meals are characterized by adverse effects on egg production and other indices of performance in laying hens. This is particularly so with leaf meals derived from tropical legumes. D’Mello and Taplin (1978) observed that in general, the inclusion of Leucaena leucocephala leaf meal in diets for laying hens reduced egg production. Sandoval (1955) had earlier reported similar observations in which egg production was depressed at both inclusion levels of 50 and 100g/kg diet.
Vohra et al (1972) showed that when hens were fed diets containing 50, 100 and 200g/kg *Leucaena leucocephala* leaf meal and maintained at two environmental temperatures (10°C and 22°C), *egg production* was depressed at both temperatures in hens fed the highest level of *L. leucocephala*, but effect became significant in hens at 10°C. Egg production was depressed despite high feed intake of hens relative to those maintained at 20°C.

The report also indicated that dietary inclusion rate of 100g/kg recorded moderate increases in egg production whereas incorporation of the leaf meal at the rate of 50g/kg marginally depressed egg production. Such discrepancies were attributable to the short-term study period of five weeks.

From limited data available, it is generally observed that *Leucaena leucocephala* leaf meal has deleterious effects on the body weight of laying birds. Springhall and Ross (1965) reported lower total body weight gains of hens fed meal at the rate of 100g/kg diet at the end of six months study.

According to Springhill and Ross (1955) fertility and hatchability of eggs were observed to be unaffected when hens were fed diets containing ferrous sulphate-treated *Leucaena leucocephala*. They, however, noted that pure mimosine injected into incubating eggs embryo caused high mortality. D’Mello and Acamovic (1982b) reported that virtually all the mimosine in the leaf meal incorporated in chickens diets appear in their
droppings, hence un-absorbed. This may account for its non-deleterious effects in the fertility and hatchability of eggs.

Various leaf meals affect the laying performance of hens variously. Osei et al. (1990) have shown that graded incorporation of *Gliricidia sepium* leaf meals in the diets of laying hens causes significant linear depression in egg production at 25, 50 and 75 g/kg inclusion rates. On the contrary, leaf meal derived from pigeon pea (*Cajanus cajan*) is somewhat more acceptable than other leguminous leaf meals as a feed component for laying hens. According to Udedibe and Igwe (1989), egg production in hens fed the leaf meals at 100g/kg diet declined by only 4.5% relative to that of hens fed the control diet. The researchers noted however that the feed conversion efficiency in the trial declined in all groups receiving the leaf meal in addition to body weight losses which occurred over the 16 weeks trial in hens on inclusion rates of 75 and 100g/kg diet. Literature reviewed so far has shown that leaf meal inclusion rates as high as 200g/kg diet and above have deleterious effects on chicken performance. It would seem that longer studies than the ones already carried out so far will be required to proffer a more worthwhile elucidation of the effects of leaf meals on laying hens.

### 2.4 Leaf Meal as a Source of Pigmenting Xanthophylls

Egg yolk and broiler skin colour are becoming important criteria of quality. In many countries, there is a consumer preference for egg yolk and chicken carcass skin colour of a characteristic yellow colour. Another interesting feature of leaf meals is their content of carotenoids, a class of
Compounds including carotene and xanthophylls. Carotenoid pigment consumed in the feed has been known to be primary source of the yellow skin and shank colour or broilers as well as that of the egg yolk. D'Mello

Table 2.7: Growth Responses of Non-Ruminant Animals to Graded Levels of Leaf Meals

<table>
<thead>
<tr>
<th>Source of Leaf Meal</th>
<th>Animal</th>
<th>0</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leucaena leucocephala</em></td>
<td>Chicks&lt;sup&gt;a&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>24.5</td>
<td>-</td>
<td>-</td>
<td>10.3</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chicks&lt;sup&gt;b&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>24.4</td>
<td>25.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chicks&lt;sup&gt;c&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>54.8</td>
<td>47.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pigs&lt;sup&gt;d&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>281</td>
<td>404</td>
<td>-</td>
<td>390</td>
<td>299</td>
<td>218</td>
<td>154</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rabbits&lt;sup&gt;e&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>166</td>
<td>-</td>
<td>-</td>
<td>122</td>
<td>-</td>
<td>66</td>
<td>-</td>
<td>41</td>
</tr>
<tr>
<td><em>Gliricidia sepium</em></td>
<td>Chicks&lt;sup&gt;f&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>7.7</td>
<td>7.6</td>
<td>5.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Chicks&lt;sup&gt;g&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>22.7</td>
<td>19.6</td>
<td>15.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Robinia Pseudoacacia</em></td>
<td>Chicks&lt;sup&gt;h&lt;/sup&gt; (final wt(g))</td>
<td>8.17</td>
<td>-</td>
<td>-</td>
<td>578</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rabbits&lt;sup&gt;i&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>28.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Rabbits&lt;sup&gt;j&lt;/sup&gt;(gwk&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>341</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>42</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Sesbania sesban</em></td>
<td>Chicks&lt;sup&gt;k&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>25.4</td>
<td>4.8</td>
<td>-</td>
<td>4.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Chicks&lt;sup&gt;l&lt;/sup&gt; (final wt(g))</td>
<td>394</td>
<td>166</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Sesbania grandiflora</em></td>
<td>Chicks&lt;sup&gt;m&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>16.3</td>
<td>3.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Manihot esculenta</em></td>
<td>Chicks&lt;sup&gt;n&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;) (final wt(g))</td>
<td>211</td>
<td>196</td>
<td>191</td>
<td>186</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Pigs&lt;sup&gt;o&lt;/sup&gt;(gd&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>560</td>
<td>480</td>
<td>420</td>
<td>370</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Sources:
<sup>a</sup>D'Mello and Acamovic (1982a); <sup>b</sup>D'Mello and Acamovic (1982b); <sup>c</sup>D'Mello <i>et al</i> (1987); <sup>d</sup>Maynicz (1974); <sup>e</sup>Tangendja <i>et al</i> (1990); <sup>f</sup>Mishra <i>et al</i> (1977); <sup>g</sup>Ranaweera <i>et al</i> (1981); <sup>h</sup>Cheeke <i>et al</i> (1983); <sup>i</sup>Harris <i>et al</i> (1984); <sup>j</sup>Raharjo <i>et al</i> (1990); <sup>k</sup>Shquier <i>et al</i> (1989a); <sup<l>Shquier <i>et al</i> (1989b); <sup>m</sup>Ash <i>et al</i> (1992); <sup>n</sup>Ross and Enriquez (1969); <sup>o</sup>Ravindran (1990).
Table 2.8: Response of Laying Hens Fed Leaf Meals Derived from *Leucaena leucocephala*, *Cajan cajan*, *Microdesmis puberula*, *Alchonia cordifolia* and *Gloricida sepium*

<table>
<thead>
<tr>
<th>Leaf Meal Sources and Parameter measured</th>
<th>Dietary leaf meal levels (g/kg) and measured parameter responses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>L. leucocephala</strong></td>
<td></td>
</tr>
<tr>
<td>Egg production (%)</td>
<td>57.3</td>
</tr>
<tr>
<td>Body weight gain (g 5wks⁻¹)</td>
<td>62.4</td>
</tr>
<tr>
<td><strong>G. sepium</strong></td>
<td></td>
</tr>
<tr>
<td>Egg production (%)</td>
<td>61.0</td>
</tr>
<tr>
<td>Feed: Egg ratio</td>
<td>03.0</td>
</tr>
<tr>
<td>Body weight gain (gw⁻¹)</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Cajan cajan</strong></td>
<td></td>
</tr>
<tr>
<td>Egg production (%)</td>
<td>67.1</td>
</tr>
<tr>
<td>Feed: Egg ratio</td>
<td>02.8</td>
</tr>
<tr>
<td>Body weight gain (g 16wks⁻¹)</td>
<td>80</td>
</tr>
<tr>
<td><strong>Alchonia cordifolia</strong></td>
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</tr>
<tr>
<td>Egg production (%)</td>
<td>62.0</td>
</tr>
<tr>
<td>Feed: Egg ratio</td>
<td>03.69</td>
</tr>
<tr>
<td>Final Body weight Gain (kg/bird)</td>
<td>02.01</td>
</tr>
<tr>
<td><strong>Microdesmis Puberula</strong></td>
<td></td>
</tr>
<tr>
<td>Egg production (%)</td>
<td>57.7</td>
</tr>
<tr>
<td>Feed: Egg ratio</td>
<td>02.3</td>
</tr>
<tr>
<td>Final Body weight Gain (kg/bird)</td>
<td>01.82</td>
</tr>
</tbody>
</table>

Sources:

⁹Vohra *et al* (1972); b Osei *et al* (1990); c Udedibie and Igwe (1989); d Udedibie and Opara (1998); e Esonu *et al* (2004).
et al (1987) have reported that broilers fed diets containing leaf meal of *Leucaena leucocephala* at 50g and 100g/kg showed a marked improvement in pigmentation at both levels of leaf meal inclusion when compared to the control. D’Mello and Acamovic (1987) suggested restricting *L. leucocephala* leaf meal inclusion rate to 50g/kg since growth performance was unaffected at this level and pigmentation only marginally improved by higher inclusion rates of the leaf meal. Udedibie (1987) has shown that leaf meals from sword bean and jack bean impart yellow skin and shank on broilers.

### 2.5 Egg Yolk Pigmentation

According to Ewing (1963) there has been a great deal of interest with regard to the matter of standard egg yolk pigmentation. The yellow colour of the egg yolk affects the acceptability of the egg by consumers without regard to its nutritive value. Statham (1984) reported that a deep yellow colour is more desirable than a pale yellow colour. Janky et al (1980) reported some degree of egg yolk pigmentation using feed containing green plants such as sea weed, lake weeds, algae, broccoli meal and kernaf tops. Okonkwo and Audikpe (1988) evaluated diets supplemented with 2% and 4% *Leucaena leucocephala* leaf meal and reported improved egg yolk coloration. Okonkwo and Alhassan (1990)
asserted that *Leucaena leucocephala* leaf meal supplemented at 1%, 3% and 7% affected egg yolk coloration. Sandoval (1955) also observed that *Leucaena leucocephala* leaf meal added to a basal diet induced intense pigmentation of egg yolks. Springhall and Ross (1965) in a subsequent study to determine the maximum level of hens’ ability to absorb xanthophylls from *Leucaena leucocephala* concluded that hens may attain maximum level when inclusion rate of 50g/kg diet is employed. Osei *et al* (1990) and Udedibie and Igwe (1989) reported that graded dietary concentration of leaf meal from *Gliricidia sepium* and *Cajanus cajan* induce progressive pigmentation of egg yolks.

According to Udedibie (1988), there is a progressive increase in yolk colour change on the inclusion of *Canavalia gladiata* leaf meal. He showed that the leaf meal dietary levels of 0, 20, 30, 40 and 50g/kg gave egg yolk colour score of 0, 4, 5, 8 and 12, respectively. Udedibie and Opara (1988) also observed that *Alchornea cordifolia* leaf meal markedly affected the colour of egg yolk, increasing the scores on Roche yolk colour fan from 0.8 for the control diet to 5.2 at 25g/kg\(^{-1}\), 11 at 50g/kg\(^{-1}\) and 13.2 at 75g/kg\(^{-1}\) dietary levels. Similar observations have been made by Esonu *et al* (2002) and Azubuike (2003) in their studies on *Microdesmis puberula*. Also Esonu *et al* (2004) reported egg yolk colour score of 4, 5, 7, 10 and 11 when laying hens were fed *Microdesmis puberula* leaf meal at dietary inclusion rates of 0.25, 50, 75, 100, 125 and 150g/kg, respectively.
Oxycarotenoids responsible for egg yolk pigmentation are consumed by laying hens in their diets. After absorption these pigments are deposited \textit{in situ} in their egg yolks. The other carotenoids like β-carotene which are not used in this manner, are converted to vitamin A. According to Karunajeewa \textit{et al} (1984), the molecular structure of the carotenoids determines the colour of the oxycarotenoids and egg yolk colour is influenced mainly by the yellow and red oxycarotene. It is important to note that the pigmenting efficiency of natural oxycarotenoids is a function of their functional groups. Karunajeewa \textit{et al} (1984) also maintained that the pigmentation of egg yolks could only be achieved by those oxycarotenoids, which possesses functional groups containing oxygen, for example, hydroxyl group.

2.6 Sources of Carotenoids

Research has identified several natural sources of carotenoids for egg yolk coloration. These include clover meal, algae meal such as \textit{Chlorella} \textit{species} and \textit{Spongio caecum} species, grass meal, \textit{Leucaena leucocephala} (Karunajeewa \textit{et al.}, (1984). Others also reported (D'Mello and Taphlin, 1978; Udedibie and Igwe, 1989) are \textit{Trifolium alexandrium}, \textit{Cajanus cajan}, \textit{Gliricidia sepium} and crustaceans. Middendorf \textit{et al} (1980) reported yellow maize and alfalfa as sources of carotenoids. Studies by Janky \textit{et al} (1980) also show that green plants such as sea weed, lake weed, algae, broccoli meal and kernaf tops are sources of egg yolk pigmentation.
Apart from the natural sources of carotenoids, synthetic forms are also available including carithaxanthin (CHX), citranaxanthin (CTX), β – apo – 8’ Carotenal (BAC) and β–apo-8’ carotenoic acid ethyl ester (BACE).

In developed countries, these compounds are manufactured on commercial scale. The use of synthetic forms of egg yolk pigments has been limited by the problem of global acceptability and cost constraints. Besides, they are not easily obtainable particularly in our local markets. For these reasons, it has become necessary to harness and utilize egg yolk pigments which are acceptable, less expensive, locally and readily available. Leaf meals of some common tropical plants, particularly the legumes have been tried as sources of egg yolk pigmentation. Among those studied are the leaf meal of jack bean, sword bean, pigeon pea and pawpaw (Udedibie, 1988), tomato pomace (Reddy, 1975), *Stylosanthes gracilis* (Onwudike and Adegbola, 1977), alfalfa meals, corn gluten meal (Ralph *et al*, 1973) and neem leaf (Obikaonu, 2009). Some of these plants have been reported to be good and cheap egg yolk pigments. Udedibie (1988) reported that sword bean leaf meal is a good egg yolk pigmenter, scoring 4, 5, 8 and 12 on the Roche colour fan scale at 2%, 3%, 4%, and 5% dietary levels, respectively. He also showed that jack bean and pigeon pea leaf meals gave a moderate score on the Roche fan scale at 5% dietary inclusion level. According to Coon and Couch (1976), unavailability of carotenoids in plant material tested for egg yolk pigmentation may be due to their existence in esterified forms.
2.7 Limitations to Leaf Meal Utilizations By Monogastrics

Despite their great potentials as feed sources the use of leaf meals in monogastric feeding programme has continued to face considerable set-

Table 2.9: Roche Fan Scores of Egg Yolks vis – a – vis Graded Levels of Leaf Meals in the Diets of Laying hens

<table>
<thead>
<tr>
<th>Source of leaf meal</th>
<th>Dietary Levels of Leaf Meal (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Leucaena leucocephala&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>6.6</td>
</tr>
<tr>
<td>Gliricidia sepium&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td>Cajanus cajan&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Achornia cordifolia&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.8</td>
</tr>
<tr>
<td>Canavalia gladiata&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>Microdesmis puberula&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Sources:
<sup>a</sup>Springhall and Ross (1965); Gill (1977);<sup>b</sup>Osei <i>et al</i> (1990); Odunsi <i>et al</i> (2002);<sup>c</sup>Udedibie and Igwe (1989);<sup>d</sup>Udedibie and Opara (1998);<sup>e</sup>Udedibie (1988);<sup>f</sup>Esonu <i>et al</i> (2004).
backs in its development. This is due largely to factors such as fibre content, presence of anti-nutritional factors and deficiencies of certain essential amino acids.

2.7.1 Anti-Nutritional Factors in Leaf Meal

Anti-nutritional factors constitute substances contained in feed which under practical circumstances can impair some aspects of animal or human metabolism and produce adverse biological or economic effects. Anti-nutritional factors in leaves are those characteristically associated with phytochemicals contained in plants. Phytochemicals are products of secondary metabolism defined variously as a metabolic cul-de-sac or peripheral metabolic pathways leading off from the universal (or primary) metabolism. Phytochemicals range from cell wall substances through photosynthetic pigments, terpenes and terpenoids, the alkaloids, plant phenolics to plant hormones, plant non-proteins, amino acids and cyanogenic glycosides (Harborne, 1973). Plants in general contain a diverse combination of these compounds and in varying proportions. A major factor limiting the wider utilization of many tropical plants is the ubiquitous occurrence in them of these natural substances capable of precipitating deleterious effects in man and animals. Manifestations of toxicity range from severe reduction in food (feed) intake and nutrient
utilization to profound neurological effects and even death (Osagie, 1998).

Pytochemical analysis is a routine investigation in biochemical studies of plant extracts and in nutritional studies on feedstuff of plant origin. Some of the photochemical substances that are usually tested for in plant extracts include alkaloids, terpenoids, tannins, flavonoids and saponins among others (Onwuliri and Umezurumba, 2003). The toxic factors of plants may be divided into two major categories: the proteins (such as lectins and protease inhibitors) which are sensitive to normal processing temperatures, and other substances which are stable or resistant to these temperatures and which include among others, polyphenolic compounds (mainly condensed tannins), non protein amino acids and galactomannan gums.

2.7.1.1 Protease Inhibitors

Perhaps the most studied of toxic factors of legume plants is the proteinase inhibitors. “Proteinase inhibitors” is a term used to describe those proteins, which associate with proteinases in a definite ratio to form complexes which are devoid of proteolytic activity (Laskowski and Laskowski, 1954; Laskowski, 1955; Liener, 1969; Kakade et al., 1973). These inhibitors” are unique in nature because of their ability to denature enzymes which have the capacity to degrade proteins under the same conditions (Feeney, et al., 1969). Generally, they are called “trypsin inhibitors since all are known to inactivate trypsin as well as other proteinases to varying degrees. Liener and Kakade (1980) reported that
trypsin inhibitors have a molecular weight of 20,000 – 25,000 with relatively few disulphide bonds but possessing a specificity which is directed primarily towards trypsin. They maintained that chymotrypsin inhibitors have a molecular weight of only 6,000 – 10,000 with high proportion of cystine residues and are capable of inhibiting trypsin as well as chymotrypsin at independent binding sites but possessing a specificity which is directed primarily at chymotrypsin.

The presence of trypsin inhibitor activity in uncooked animal feed has long been known to cause diminished growth in rats, chickens and other experimental animals. Cooking, hot soaking and traditional method of processing cause significant reduction in trypsin inhibitor activity (Egbe and Akinyele, 1990). The most logical explanation for the growth inhibition produced by protease inhibitors would be that they inhibit intestinal proteolysis in animals. There seem to be little doubt that hypertrophy of the pancreas represents one of the primary physiological effects produced by feeding raw soybeans (Booth et al., 1964), or the isolated inhibitor (Neshiem et al., 1962). Booth et al (1964) was of the opinion that pancreatic hypertrophy leads to an excessive loss of endogenous protein secreted by the pancreases. Since this protein consisting largely of pancreatic enzymes, is rich in cystine, the resulting effect is a net loss of sulphur – containing amino acids from the body.

2.7.1.2. Lectins and Their Nutritional Significance
Lectins are proteins which are characterized by their unique ability to bind specific sugars or glycoprotein. This reaction is manifested in vitro by the agglutination of red blood cells from various species of animals, and so lectins are also called haemagglutinins (Osagie, 1998). Most lectins are glycoproteins containing 4 to 10% carbohydrate and have molecular weights ranging from 100,000.00 to 150,000 (Liener, 1983).

From a nutritional view point is the important fact that lectins bind to the epithelial cells lining the small intestine. A series of complex events ensue which culminate in severe growth depression and ultimately in the death of the animals. Lectin toxicity is also due to their production of intestinal lesions and histopathological changes of some organs particularly the kidney (Ikegwuonu and Bassir, 1977). Lectins cause an increase in tissue invasion by normal innocuous gut bacteria due to reduction of body defenses. Among the consistent pathological manifestations of injected toxic lectins are zonal necrosis and fatty infiltration of the liver, oedema, marked distension of capillary vessels and haemorrhages in various tissues (Kakade et al., 1965). Ikegwuonu and Bassir (1976a) noted that pigeon pea seed has a total lectins yield of 1.28g/kg, with soybeans and cowpeas yielding 0.95g/kg and 2.17g/kg, respectively. Of these three legume seeds investigated, the specific activity of the pigeon pea lectin was lowest (500,000 Hu/g) whereas soybean had the highest activity (1,231,000 Hu/g), making it the most toxic legume evaluated. A dose of soybean lectin 200mg/kg killed a rat in 10 hours, whereas pigeon pea required the highest dose of 1147mg/kg.
and 18 hours for death to occur. It was suggested that lectins from immature seeds may be more toxic than those from mature seeds (Ikegwuonwu and Bassir, 1976a).
Table 2.10: Examples of Anti-nutritional Factors in Plants

<table>
<thead>
<tr>
<th>Class</th>
<th>Distribution</th>
<th>Physiological Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Most legumes, roots and tuber crops</td>
<td>Depressed growth, pancreatic hypertrophy/hyperplasia; acinar nodules.</td>
</tr>
<tr>
<td>Lectins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylase inhibitors</td>
<td>Most legumes, roots and tuber crops</td>
<td>Interference with starch digestion</td>
</tr>
<tr>
<td>Glycosides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>Cassava roots, some legumes, sorghum</td>
<td>Respiratory failure, goiter</td>
</tr>
<tr>
<td>Oligosaccharides</td>
<td>Most legumes</td>
<td>Flatulence</td>
</tr>
<tr>
<td>Saponins</td>
<td>Most legumes and tuber crops</td>
<td>Affects intestinal permeability</td>
</tr>
<tr>
<td>Phenolics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>Most legumes, root crops and fruits</td>
<td>Interference with protein digestion.</td>
</tr>
<tr>
<td>Gossypol</td>
<td>Cotton</td>
<td>Interferes with protein digestion</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phytate</td>
<td>Most legumes and root crops</td>
<td>Interference with mineral availability</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Some yam species and kola nuts</td>
<td>Depressed growth</td>
</tr>
</tbody>
</table>

Adapted from Liener (1989) in Osagie (1998)
2.7.1.3 Amylase Inhibitors

Amylase inhibitors found in most legumes inhibit the action of pancreatic and salivary amylases. This results in increased amount of indigested starch in the faeces and a subsequent decrease in the nutritional value of the foodstuff (feedstuff) (Osagie, 1998).

2.7.1.3 Cyanogenic Glycosides in Plants and Their Biochemical Properties

It has been known for a long time that wide variety of plants are potentially toxic because they contain glycosides from which HCN may be released on hydrolysis. Cyanogenic glycosides are a group of O-glycosides formed from decarboxylated amino acids. The cyano group arises from the alpha-carbon atom and the amino group (Fig. 2.1). The occurrence of cyanogenic glycosides in crop plants such as cassava, (*Manihot esculenta* Crantz) (Nartey, 1968, 1978), some cereals (Erb et al., 1980) and legumes (Okolie and Ugochukwu, 1989) is well known.

![Chemical Structure](image.png)

**FIG. 2.1 Decarboxylation of Amino Acid to Form Cyanogenic Glycoside**

Conn (1973) showed that cyanogenic glycosides will release prussic or hydrocyanic acid (HCN) upon treatment with dilute acids, usually at elevated temperatures. The phenomenon of cyanogenesis is usually due
to the rupture of the tissue of cyanophoric plants and the action of the enzymes are initiated by crushing or otherwise destroying the cellular structures of the plants. The spontaneous release of HCN from the plant depends on the presence of specific glycosidase and water.

Table 2.11: Types of Cyanogenic Glycosides formed from Different amino acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Cyanogenic Glycoside</th>
<th>$R^1$</th>
<th>$R^2$</th>
<th>Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-valine</td>
<td>Linamarin</td>
<td>-CH$_3$</td>
<td>-CH$_3$</td>
<td>Glucose</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>Lotaustralatin</td>
<td>-CH$_2$CH$_3$</td>
<td>-CH</td>
<td>Glucose</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>Prunacin</td>
<td>-C$_6$H$_5$</td>
<td>-H</td>
<td>Glucose</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>Amygdalin</td>
<td>-C$_6$H$_5$</td>
<td>-H</td>
<td>Gentiobiose</td>
</tr>
<tr>
<td>L-tyrosine</td>
<td>Dhurrin</td>
<td>-C$_6$H$_4$CH</td>
<td>-H</td>
<td>Glucose</td>
</tr>
</tbody>
</table>

Adapted from Osagie (1998).

The deleterious effects exhibited by a lethal dose of cyanide is characterized by its rapid reaction with metal ions of iron and copper in the blood, leading to a series of reactions to form cyanohaemoglobin which is not an oxygen carrier (Peter and Van Syke 1931). In experimental animals, the principal manifestation of toxicity is stupor or convulsion (Montgomery, 1980). Large doses of cyanide cause death by inhibition of cell respiration. Small does are changed on ingestion into thiocynate, a well known goitrogen. In the body, the major pathway of detoxification of small doses of cyanide is through reaction with
thiosulphate to form thiocyanate and sulphite, a reaction which is catalysed by rhodanase, an enzyme otherwise called sulphur transferase (Lange, 1933). Rhodanase, which is widespread in living tissues, is mostly concentrated in the liver, kidney, thyroid glands and the pancreas (Rosenthal, 1948). The resulting thiocyanate which is slowly oxidized to sulphate but does not appear to be an important factor in the body tissue is excreted in the urine (Clemedson et al., 1960). In a second pathway to thiocynate, which has been demonstrated in experimental animals (Fieldler and Wood, 1956), cyanogens react with 3–mercaptopyruvate with the aid of another sulphur transferase. These reactions require the presence of adequate amounts of cystine as a sulphur donor.

Cassava contains the cyanogenic glycosides, linamarin and lotaustralin, in relative amounts of about 93 and 7% respectively (Okigbo, 1980). The extra cellular enzyme linamarase present in cassava gains access to the cyanogenic glycosides after physical disruption of the cell, whence it catalyses their hydrolysis to glucose and corresponding cyanohydrin. The cyanohydrin breaks down rapidly to give HCN and acetone in alkaline solution at ambient temperature, but under slightly acidic condition (pH6), only 50% had broken down in 60 minutes (Cooke, 1978). According to Osagie (1998), the cyanide present in cassava can be of two types: (i) bound cyanide present as cyanogenic glycosides and (ii) free cyanide present as the cyanohydrin, a free HCN and as CN. It is much easier to remove free cyanide from cassava by cooking. The use of sorghum as human food or for livestock feed is seriously limited by the
presence of dhurrin in its seeds, shoots and roots. The level of this poisonous natural product depends among other factors on the varieties of sorghum, sprouting and component part of the sorghum sprouts (Dada and Dedy, 1987; Ikediobi et al., 1988).

2.7.1.5 Oligasaccharides

A major reason for avoiding consumption of legumes is the difficulty experienced in digesting them. Flatulence is the most common symptom associated with pulse consumption. Frequent belching, abdominal distention, weakness, and release of nauseating gas can accompany flatulence. Abdominal pain and diarrhoea are often experienced by susceptible individuals especially children (Ndubuaku et al., 1988). The production of flatus by monogastric animals is due to colonic fermentation of carbohydrates which escape breakdown in the stomach and small intestine. Osagie (1998) suggests that the oligosaccharides rafinose, stachyose and verbascoside which are common in legume seeds may be the major producers of flatulence when these foods are consumed. These saccharides are characterized by the presence of alpha-D-galacto pyranose residues bound to the glucose moiety of sucrose. Monogastric animals and man are not able to digest such oligosaccharides because of the absence of alpha-1, galactosidase in their intestinal mucosa. Consequently, the raffinose oligosaccharide pass into the wall, are fermented by intestinal bacteria with considerable production of gas, mainly carbon dioxide and hydrogen (Wagner et al., 1976; Fleming, 1981). It is clearly desirable to decrease the
oligosaccharide content of legumes if they are to be most effectively exploited as inexpensive sources of protein (Muzquiz et al, 1992; Philips and Abey, 1989).

2.7.1.6. Saponins

Saponins are steroid or tri-terpenoid glycosides which are characterized by their bitter or astringent taste, foaming properties and their hemolytic effects on red blood cells. They are widely distributed in the plant kingdom being found in over 500 genera (Agarwa and Rastogi, 1974; Chandell and Rastogi, 1980; Mahato and Nandy, 1991). Among plants grown for food, the presence of saponins in legumes such as soya and pea is particularly important. Saponins have been shown to possess both beneficial (cholesterol–lowering) and deleterious (cytotoxic; parmeabilization of the intestine) properties and to exhibit structure dependent biological activities (Prince et al., 1987; Oakenful and Sidhu, 1989).

2.7.1.7 Phenolics

According to Osagie (1998), the phenols and bio-chemically related substances of natural origin can be divided roughly into two groups:

i. The botanically widespread and structurally common ones, e.g. p-coumaric, caffeic, ferulic, sinapic, and gallic acids or their derivatives, flavanoids, lignin, hydrolysable and condensed tannins, ellagic acid etc.
Those with exotic structures and limited specific occurrence – include most of the few dozen phenolic derivatives known to have high toxicity or potent physiological activity in animals, e.g. gossypol and phlorhizin. Phenolic compounds are highly associated with the seed coat, thus dehulling is a simple processing method for reducing their content in foods. Structurally tannins represent a very diverse group of plant products. They are widely distributed in the plant kingdom, more especially the dicotyledonous plant families, and often they are present in surprisingly high concentrations. The ability of tannins to form complexes with protein may reduce the nutritive value of food stuffs (Harlam, 1977).

Goldestein and Swain (1963) proposed the mechanism of nutritional significance of tannins as a precipitation and/or immobilization of protein, which include enzymes by virtue of their molecular structures. This forms the basis of toxic and nutritional influences of tannins. One of the symptoms of continued intake of tannins is gastritis as well as oedema of the intestines. Under such conditions, (Kirby, 1960; Korpassy, 1961; Bichel and Bach, 1968), it appears tannins are absorbed. According to Freudenberg (1920) the range of compounds collectively designated as vegetable tannins are those plant phenols having molecular weight values ranging between 300 and 3,000 and containing a sufficiently large number of phenolic groups to enable them form effective cross linkages with protein and other macromolecules. The
author continued that tannins have therefore been categorized into two groups: hydrolysable and condense tannins. Hydrolysable tannins are expected to be more toxic than condensed tannins since the former tends to break down easily. The growth depressing effects of tannins in which 1.0% dietary level of tannin retarded the growth of chicks and rats, have been reported (Vohra et al., 1966). According to the authors, a decreased nitrogen retention in chicks fed 0.5% dietary tannic acid, and an overall 5% mortality was observed. Condensed tannins are however found to be less detrimental than hydrolysable tannins. Glick and Joslyn (1970a) also reported an increased excretion of protein in the faeces of rat fed 2.0% or more tannic acid in the diet. Weight loss when tannic acid was fed was more serious for smaller, younger animals and seemed to be at least one reason that larger animals are more tolerant of high tannin diet (Glick and Joslyn, 1970b).

Advancing reasons for growth – depressing property of tannins, Glick and Joslyn (1970b) proposed that the high nitrogen excretion resulted largely from the binding of dietary protein by tannin into an indigestible form. Any protein added in excess of the amount required to bind the tannin was utilized by the animal, resulting in greatly improved growth. The residual nitrogen in the faeces remained almost as it was before addition. They further proposed that the protein – tannin complex appeared to be formed by multiple hydrogen bonding between phenolic hydroxyl groups of the tannins and carboxyl groups of the protein peptide bonds of enzymes; part of the high nitrogen excretion is endogenous enzyme
protein. Jambunathan and Mertz (1973) were in support of these points. They showed that high tannin containing sorghum varieties exhibited poor response while the contrast was the case for low tannin containing varieties. Apart from growth depressing effects, tannins interfere with amino acid metabolism. Vohra et al (1966) reported that tannin is responsible for reduced digestibility of proteins in chicks. This clearly shows that legume tannins bind dietary protein and inhibit proteolytic enzyme activity in the gastrointestinal tracts, leading to a negative correlation between protein digestibility and tannin content in a material.

2.7.1.8 Phytate and Its Nutritional Significance

According to Osagie (1998), phytic acid, a hexaphosphate derivative of inositol, is an important storage form of phosphorus in plants. He asserted that phytic acid has 12 replaceable hydrogen atoms with which it could form insoluble salts with metals such as calcium, iron, zinc, and magnesium. The formation of these insoluble salts renders the metals unavailable for absorption into the body. Studies by Nwokolo and Bragg (1977) have shown that in the chicken, there is a significant inverse relationship between phytic acid and the availability of calcium, magnesium, phosphorus and zinc in feedstuffs like soybean, palm kernel seed, rapeseed and cotton seed meals. Phytate can also affect digestibility by chelating with calcium or by binding with substrate or proteolytic enzymes (Osagie, 1998).
The phytate content in some Nigerian legumes has been reported (Ologhobo and Fetuga, 1983a, 1984; Akinyele, 1989; Farinu and Ingrao, 1991). The phytic acid contents in foods and feedstuffs commonly consumed in Nigeria have been reported (Eka, 1977; chakraborty and Eka, 1978; Marfo et al., 1990; Osagie et al., 1996). Osagie (1998) noted that the phytate content of these foods (feeds) differ widely. He also observed that some of the data on the subject have been overestimated. Fifteen leguminous browse plants harvested in South West Nigeria were evaluated with respect to their oxalate phytin and phytin-phosphorus content (Aletor and Omodara, 1994). Phytin and phytin-p levels were generally not as high as those reported for legume seeds (Table 2.11 and 2.12).

2.7.1.9 Oxalate and Its Nutritional Significance

According to Osagie (1998) Oxalate C₂ dicaboxyclic acid anion is produced and accumulated in many crop plants and pasture weeds. Oxalate may be present in plants as the soluble salts, potassium, sodium or ammonium oxalate; as oxalic acid or as insoluble calcium oxalate. Oke (1965c) has shown that on dry weight basis, some of the Nigerian vegetables might be superior to milk as gross sources of calcium, except that the calcium is not available due to the presence of oxalic acid, but is bound as insoluble calcium oxalate. Osagie (1988) maintained that oxalate is a concern because high oxalate diets can increase the risk of renal calcium absorption. However, the detailed studies by Munro and Bassir (1989) revealed that the possibility of oxalate poisoning in Nigeria
from consumption of local fruits and vegetables is as remote as it is in other parts of the world. They have shown that oxalate poisoning is not considered to be a hazard in any of the foods examined (except possibly spinach).

2.7.1.10 Alkaloids and Their Nutritional Significance

Osagie (1998) reported that alkaloids are basic natural products occurring primarily in plants. They contain one or more heterocyclic nitrogen atoms, and are generally found in the form of salts with organic acids. He suggests that 10 to 20% of all higher plants contain alkaloids. Several thousands of alkaloids are known. Of particular nutritional concern are aristolochic acid and pyrrolizidine alkaloids because of their toxicity.

Adewumi (2004) has shown that pyrrolizidine can be divided into two categories based on their structure, namely those with an unsaturated nucleus (toxic), and those with a saturated nucleus (considered non-toxic).

According to him, these alkaloids are present notably in crotalaria, helitropium and senecio species. He maintained that these alkaloids are responsible for serious liver damage (hepatic veno-occlusive disease). The hepatotoxicity associated with their consumption is well documented and has been attributed to the pyrrolizidine alkaloid constituents (Mattocks, 1986). Over 100 hepatotoxic pyrrolizidine alkaloids are found
within species of *Asteraceae, Borginaceae* and *Fabaceae*. These plants are consumed as food, for medicinal purposes or as contaminants of other agricultural crops (FDA/CFSAN AEMS, 2002). Pyrrolizidine alkoids are harmful to the liver and lungs, causing veno – occlusive disease (Yff, et al., 2002). *Atawodi et al* (1993) in their studies on preformed volatile nitrosamines in some Nigerian foodstuffs observed that *Vernonia amygdalina* (bitter leaf) contained the lowest detectable level of nitrosodimethylamine. The authors concluded that Nigerians eating *V. amygdalina* may be exposed to chronic but very low levels of carcinogenic nitrosamines in their foods. Certain observations in this study suggest that the ethanolic extract of *Khaya senegalensis* exerted more deleterious effects on the kidney when administered continuously over a prolonged period than a short one. This adversely affects the functioning of the kidney (Adebayo et al., 2003).

2.8 Ethno – Veterinary Potential of Leaf Meals

Considering the combinations of ingredients used by the traditional animal health practitioners, it is likely that additive, synergistic and nutritional effects (Adewumi, 2004) of leaf meal might be involved in
Table 2.12: Oxalate, Tannic acid and Phytic acid Contents of some Wild under-utilised Crop seeds

<table>
<thead>
<tr>
<th>Crop – Seed</th>
<th>Family</th>
<th>Tannic Acid (g/100g DM)</th>
<th>Phytic Acid (g/100g DM)</th>
<th>Total oxalate (g/100g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Carapa procera</em></td>
<td>Meliaceae</td>
<td>0.89</td>
<td>330</td>
<td>14.0</td>
</tr>
<tr>
<td><em>Cderalla odorata</em></td>
<td>Meliaceae</td>
<td>0.46</td>
<td>1200</td>
<td>13.2</td>
</tr>
<tr>
<td><em>Azadirachta</em></td>
<td>Meliaceae</td>
<td>0.60</td>
<td>350</td>
<td>7.60</td>
</tr>
<tr>
<td><em>Terminalia catappa</em></td>
<td>Comretacea</td>
<td>0.40</td>
<td>1580</td>
<td>5.77</td>
</tr>
<tr>
<td><em>Vitex deniana</em></td>
<td>Comretacea</td>
<td>0.67</td>
<td>630</td>
<td>7.32</td>
</tr>
<tr>
<td><em>Xylopia aethiopica</em></td>
<td>Anonaceae</td>
<td>0.66</td>
<td>530</td>
<td>5.34</td>
</tr>
<tr>
<td><em>Mondora termifolia</em></td>
<td>Anonaceae</td>
<td>0.17</td>
<td>650</td>
<td>11.0</td>
</tr>
<tr>
<td><em>Rouvalia vonitoria</em></td>
<td>Apocynaceae</td>
<td>0.34</td>
<td>351</td>
<td>5.81</td>
</tr>
<tr>
<td><em>Bixa oreliana</em></td>
<td>Bixaeceae</td>
<td>0.51</td>
<td>333</td>
<td>8.44</td>
</tr>
<tr>
<td><em>Spondias mambin</em></td>
<td>Anacardiaceae</td>
<td>0.98</td>
<td>375</td>
<td>12.1</td>
</tr>
<tr>
<td><em>Adansonia digitata</em></td>
<td>Bambaceae</td>
<td>0.98</td>
<td>375</td>
<td>12.1</td>
</tr>
<tr>
<td><em>Lophira alata</em></td>
<td>Ochnaceae</td>
<td>0.15</td>
<td>725</td>
<td>9.85</td>
</tr>
<tr>
<td><em>Eucalyptus deaulota</em></td>
<td>Mythaceae</td>
<td>0.33</td>
<td>976</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Adapted from Osagie (1998).
Table 2.13: Oxalate, Phytin–Phosphorus Contents of air–dried leguminous browse plants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oxalate (mg 100g⁻¹ DM)</th>
<th>Phytin (mg g⁻¹ DM)</th>
<th>Phytin - P (mg g⁻¹ DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Accacia auricula</td>
<td>58.79</td>
<td>37.24</td>
<td>10.49</td>
</tr>
<tr>
<td>2. Centrosema pubesceas</td>
<td>70.17</td>
<td>26.85</td>
<td>7.56</td>
</tr>
<tr>
<td>3. Calapogonium mucunoides</td>
<td>126.04</td>
<td>82.26</td>
<td>23.18</td>
</tr>
<tr>
<td>4. Bauhinia thaningii</td>
<td>233.16</td>
<td>26.53</td>
<td>7.46</td>
</tr>
<tr>
<td>5. Caesalpina pulcharina</td>
<td>104.15</td>
<td>20.35</td>
<td>5.73</td>
</tr>
<tr>
<td>6. Cassia nodosa</td>
<td>97.20</td>
<td>13.12</td>
<td>3.69</td>
</tr>
<tr>
<td>7. Glyricidia sepium</td>
<td>90.89</td>
<td>16.18</td>
<td>4.55</td>
</tr>
<tr>
<td>8. Erythnna variegata</td>
<td>222.41</td>
<td>10.03</td>
<td>2.82</td>
</tr>
<tr>
<td>9. Delanic regia</td>
<td>ND</td>
<td>10.27</td>
<td>2.89</td>
</tr>
<tr>
<td>11. Glycine max</td>
<td>25.36</td>
<td>40.45</td>
<td>11.39</td>
</tr>
<tr>
<td>12. Vigna unguiculata</td>
<td>73.40</td>
<td>20.42</td>
<td>5.75</td>
</tr>
<tr>
<td>13. Cassia alata</td>
<td>62.70</td>
<td>5.07</td>
<td>1.43</td>
</tr>
<tr>
<td>14. Cassia fistula</td>
<td>36.51</td>
<td>6.87</td>
<td>1.93</td>
</tr>
<tr>
<td>15. Leucaena leucocephala</td>
<td>88.16</td>
<td>10.27</td>
<td>2.89</td>
</tr>
</tbody>
</table>

Means are for duplicate determinations

ND = Not Detected

Source: Aletor and Omodara (1994)
alleviating the problem of ill-health in animals. Many phytochemicals have been shown to be bioactive, that is, they exhibit pronounced biological activity in other living organisms (Harborne, 1973). Many of the bioactive phytochemicals have found usefulness as chemotherapeutic agents, pesticides, food (feed) additives, and other biologicals (Onwuliri et al., 2006).

The Fulanis have a traditional lifestyle and cattle management system and commonly use ethno-veterinary remedies. Gefu (2000) reported that these cattle rearers utilize local herbs and plants to treat disease conditions caused by flukes, worms and diseases such as dermatophilosis, trypanosomiasis, coccidiosis, vaginal and rectal prolapse and ephemeral fever. The recognition of ethno-veterinary knowledge as a viable option to enhance the health of the Karamojongs livestock is a positive development (Adewumi, 2004). According to Ole-Miraron (2003), a survey conducted on the ethno-veterinary knowledge of the Ilkisonko Maasai of Kenya showed that the folks use 18 medicinal plant species to combat livestock diseases diagnosed. A survey was carried out in Kaduna State of Nigeria to establish the indigenous knowledge system for treating trypanosomiasis in domestic animal. Data obtained revealed the use of several plants either alone or in combination, for the treatment and management of trypanosomiasis (Atawodi et al., 2002). The most common plants encountered were *Adansonia digitata*, *Terminalia avicennoides*, *Kaya senegalensis*, *Cissus populnea*, *Tamarindus indica*, *Lawsonia ninermis*, *Boswellia dalzielli*, *Psedocedrela kotschi*, *Syzium*
quinensis, Sterculia setigera, Afzelia africana, Prosopis africana and Lancea kerstingi.

Of striking importance is the use of a single remedy or plant extract in treating more than one disease, as well as a combination of various plant extracts for a broad–spectrum activity. For example, Adewumi (2004) indicated that extracts or ingredients from the mahogany tree (*Khaya senegalensis* A. tuss) are used to treat anthrax, diarrhea, dysentery, foot rot, helminthes infections and ringworm. He maintains that extracts from the mahogany tree are also used to improve appetite and fertility as well as to relieve animals in cases of gastric/emetic problems, poisoning and as a laxative. Another variety of mahogany tree (*K. Ivorensis*, known locally as “Senegal quinine” and obtainable largely in the southern part of Nigeria) is used as an anthelmintic and against diarrhoea in small ruminant owning householder (Alawa *et al*., 1996).

The anti-infective activities of some plant extracts have been published. For example, a 100ml extract obtained from 1.17g of Vernonia amygdaliana leaves pounded and soaked in water significantly reduced worm burden (*Bonustumum, Dichrocelium* and *Fasciola spp.*) in calves (Alawa *et al*., 2000). The anthelmintic properties of *V. amygdalina* and *Anonea senegalensis* also have been demonstrated in cattle (Chiezey, *et al*., 2002) and in chickens (Abdu and Faya, 2000; Igile *et al*., 1994). Abdu and Faya (2000) have also shown the anthelmintic activity of *K. senegalensis*. The methanolic extracts of *Casia alata* have bactericidal and fungicidal activity (Mattocks, 1986). Studies by Atawodi *et al* (2002)
also showed that coccidiosis and worm infestation in poultry were controlled with extracts from various plants that included *K. senegalensis*, *Solanum nodiflorum*, *Bozwellia dalzieli*, *Mimoudia balasamia*, *Vitex doniana*, *Striga spp* and *Butyrospermum paradoxum*. Abdu and Faya (2000) also reported the use of *Solanum incanum* fruits to treat coccidiosis in poultry and of *M. balsamia* to treat fowl pox and *Capsium frutenscens* for the treatment of Newcastle disease. The leaves of *V. amygdalina* were reported by Philipson et al. (1993) against *Plasmodium falciparum* in-vitro. Luteolin, luteolin 7-0-β-glucoside flavonoid compounds isolated from the leaves of *V. amygdalina* were reported for antioxidant activities (Igile et al., 1994).

Chiezey et al (2002) reporting on the evaluation of some medicinal plants for anthelmintic activity in young cattle in Northern Nigeria indicated that *K. senegalensis*, *V. amygdalina*, *Aloe barteri*, *Terminalia avicenninoids*, *Annona senegalensis*, and *Cassia occidentalis* produced various efficacy levels against different species of worms. In another investigation carried out at the National Animal Production Research Institute (NAPRI), Nigeria, Alawa et al., (2000) reported on the use and efficacy of *Vernonia amygdalina* as an anthelmintic in young cattle. Asuzu and Chineme (1990), Azuzu and Anaga (1991) and Nok et al., (1993) reported on the trypanocidal properties of the leaf and bark extracts of *Morinda lucidal*, *Histonia boonei* and *Azadirachta indica*, respectively. Methanol and aqueous extract from 43 plants were also screened for anti/protozoal and catatonic activities, out of which a few were active below 10µg/ml (Atindehou et al., 2004, Camacho et al., 2003; Hoct et al., 2004). Also
reported in Nigeria were anti-malarial activities of some plants (Agbedahunsi et al., 1998; Agbedahunsi and Elujoba, 1998; Agbedahunsi et al., 2001). Both in vitro and in vivo methods were used in the screening. The n-hexane fraction of *K. grandifoliola* stem bark containing a novel gradifoliola characterized by spectroscopic methods has potent anti-malarial property (Agbedahunsi and Elujoba, 1998).

The screening of *Congronema latifolia* (*utazi*) for anti-bacterial activities in respect of common enteric organisms of poultry and rabbits will give new information to corroborate an earlier study by Osuala et al (2005) which showed that methanolic extract of utazi exhibited marked activity against *Pseudomonas aeruginosa* and *E. coli* both at a minimum inhibitory concentration (MIC) of 15.625mg.ml in an in vitro study.
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Location of Study

The research was carried out in the Teaching and Research Farm of the School of Agriculture and Agricultural Technology (SAAT), of the Federal University of Technology (FUT), Owerri. The biochemical and haematological aspects of the research were done at the Federal Medical Centre, Owerri; the bio-activity and histopathological aspects were carried out at the laboratories of the Imo State Environmental Protection Agency (ISEPA) and the Department of Anatomy, College of Medicine, University of Calabar, respectively, while the amino acid assay and phytochemical analysis were done at the Department of Biochemistry laboratory, University of Jos, Nigeria, and the central laboratory of the National Root Crops Research Institute (N. R. C. R. I.), Umudike, respectively.

3.2 Preparation of *Congronema latifolia* Leaf meal (CLLM)

Fresh leaves of *C. latifolia* were harvested from the forest area of Ovonum in Obubra Local Government Area of Cross River State, Nigeria. Harvested leaves were dried under shade to prevent inactivation of its chemical constituents by direct sunlight. The drying process which was done in the months of December and January was carried out for 5 - 7 days until the leaves became crispy to touch. The dried leaves were milled to particle sizes that would pass through a 2mm sieve, using a
hammer mill. The *C. latifolia* leaf meal (CLLM) so prepared was stored in air-tight plastic containers under cool dry conditions prior to use.

### 3.3 Proximate Analyses and Metabolisable Energy Determination of CLLM

Samples of the leaf meal were subjected to proximate analysis using the methods described by A.O.A.C (1995) to determine the following proximate fractions:

a. Moisture content
b. Percentage dry matter (DM)
c. Crude protein (CP) as a percentage of DM
d. Crude fibre (CF), as % DM
e. Ether extract (EE), as % DM.
f. Ash, as a Percentage of dry matter (DM)
g. Nitrogen free extractives (NFE), as % DM

The metabolisable energy (ME) of the leaf meal was calculated, using the methods of Pauzenga (1985), using data from proximate analysis.

### 3.4 Mineral Analysis

The following minerals were determined using the standard procedures (AOAC, 1995).

1. Calcium (Ca)
2. Magnesium (mg)
3. Potassium (k)
3.5 Amino Acid Analysis

The analysis of CLLM protein to determine its constituent amino acids was carried out in the Department of Biochemistry laboratory, University of Jos, Nigeria, using methods described by Speckman et al (1958). The leaf meal sample was dried to constant weight, defatted, hydrolyzed, and evaporated in a rotary evaporator and loaded into the Technicon sequential Multy-Sample Amino Acid Analyzer (TSM).

3.5.1 Defatting of sample

A known weight (5.0g) of the dried CLLM sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol mixture at the ratio of 2:1 using soxhlet extraction apparatus as described by AOAC (2006). The extraction lasted 15 hours.

3.5.2 Nitrogen Determination

A small amount (200mg) of ground sample was weighed, wrapped in whatman filter paper (No. 1) and put in Kjeldhal digestion flask. Concentrated sulphuric acid, H₂SO₄, (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na₂SO₄), copper sulphate (CuSO₄) and selenium oxide (SeO₂) in the ratio of 10:5:1 was also added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.
The flask was then put on Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10ml) of the diluted solution with 10ml of 45% sodium hydroxide were put into the Markham distillation apparatus and distilled into 10ml 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected.

The distillate was then titrated with standard 0.01N hydrochloric acid to gray coloured end point. The percentage nitrogen in the original sample was then calculated using the formula:

\[
\text{Percentage Nitrogen} = \frac{(a - b) \times 0.01 \times 14 \times v \times 100}{W \times C}
\]

Where:
- \(a\) = Titre value of the digested sample
- \(b\) = Titre value of blank sample
- \(v\) = Volume after dilution (100ml)
- \(w\) = Weight of dried sample (mg)
- \(C\) = Aliquot of the sample used (10ml)
- \(14\) = Nitrogen constant in mg
- \(100\) = Conversion factor percentage

3.5.3 Hydrolysis of the Sample

A known weight (0.5g) of the defatted sample was weighed into glass ampoule. Thereafter 7ml of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this was to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and
cystine). The glass ampoule was then sealed with bunsen burner flame and put in an oven preset at 105°C ± 5°C for 22 hours. The ampoule was allowed to cool before breaking open at the tip and the content was filtered to remove the humus. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic bottles, which were kept in the freezer.

3.5.4 Loading of the hydrolysate into the TSM analyzer
The amount loaded was between 5 to 10 microlitres. This was dispensed into the cartridge of the analyzer. The TSM analyser is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of analysis lasted for 76 minutes, at the end of which the TSM produces a chromatogram which represented corresponding values of the different amino acids.

3.6 Phytochemical screening of CLLM
The phytochemical screening of C. latifolia leaf meal extract was carried out at the Central laboratory of the National Root Crops Research Institute (N.R.C.R.I.), Umudike. Factors screened included alkaloids, flavonoids, tannins, saponins, cyanogenic glycosides and phenols.

3.6.1 Determination of Alkaloid
Five grams (5g) of sample was analyzed using the alkaline precipitation method (Harbone, 1973). The weighed sample was soaked in 100ml of
10% acetic acid and ethanol solution. The mixture was allowed to stand at room temperature for four (4) hours before it was filtered with a Whatman filter paper.

The filtrate was reduced to a quarter of its original volume by evaporation over a steam bath. Alkaloid in the extract was precipitated by a drop-wise addition of conc. NH₄OH solution until full turbidity was obtained. The precipitate was recovered by filtration using a previously weighed filter paper. The precipitate was then washed with 1% NH₄OH solution, dried in the oven at 100°C for an hour. It was then cooled in a desiccator and re-weighed. The weight of alkaloid was determined by difference and expressed as a percentage of the sample analysed using the formula:

\[
% \text{Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100
\]

Where:

\( W_1 \) = Weight of empty filter paper

\( W_2 \) = Weight of paper+alkaloid precipitate

### 3.6.2 Determination of Flavonoids

The ethyl acetate precipitation method (Bohm and Kocipai, 1994) was used. A weighed sample (5g) was hydrolysed by boiling in 100mls of 2 molar HCl solution for about 35 minutes. The hydrolysate was filtered to the extract. The filtrate was treated with ethyl acetate drop-wise until in excess.
The precipitated flavonoid was recovered by filtration using a weighed filtered paper. After drying in the oven at 100°C for 30 minutes, it was cooled in a desiccator and reweighed. The difference in weight gave the weight of flavonoid expressed as a percentage of sample analyzed using the formula:

\[
\% \text{ Flavonoids} = \frac{W_2 - W_1}{W_1} \times 100
\]

Where:

\[
W_1 = \text{Weight of empty filter paper}
\]

\[
W_2 = \text{Weight of filter paper + flavonoid precipitate}
\]

**3.6.3 Determination of Tannins**

The Follins – Dennis spectrophotometric method (Pearson, 1976) was used in the determination. One gram (1g) of CLLM sample was dispensed in 50mls of distilled water and shaken for 30 minutes in a mechanical shaker. The mixture was filtered and the filtrate used for the analysis. Five millilitres (5mls) of the extract was measured into 50mls volumetric flask and diluted with 35mls of distilled water. Similarly, 5mls of standard tannic acid solution and 5mls of distilled water were measured into separate flasks to serve as standard and blank, respectively. Each of these was also separately diluted with 35mls of distilled water. One milliliter (1ml) of Follins – Dennis reagent was added to each of the flasks followed by 2.5mls of saturated sodium carbonate solution. The content of each flask was made up with distilled water and incubated for 90
minutes at room temperature. The absorbence of the developed colour was measured at 760nm wavelength, with the reagent blank at zero. The analysis was done in triplicate and the average was obtained. Tannin content was calculated as shown below:

\[
\text{% Tannins} = \frac{100 \times \text{Au} \times \text{C} \times \text{Vf} \times \text{D}}{\text{W} \times \text{As} \times 1000 \times \text{Va}}
\]

Where:

\(W\) = Weight of sample analysed
\(As\) = Absorbance of standard tannic solution
\(Au\) = Absorbance of test sample
\(C\) = Concentration of standard in mg/ml
\(D\) = Dilution factor
\(Vf\) = Volume of filtrate (extract)
\(Va\) = Volume of filtrate analyzed

### 3.6.4 Determination of Saponin

The content of saponin in the sample was determined by double solvent extraction gravimetric method (Harbone, 1973). Two grams (2g) of the powdered sample was mixed with 50mls of 20% aqueous ethanol solution. The mixture was heated with periodic agitation in water bath for 90 minutes at 55\(^\circ\)C. It was thereafter filtered through a Whatman filter paper number 40. The residue was extracted with 50mls of 20% ethanol and both extracts were pooled together. The combined extract was reduced to about 40mls at 90\(^\circ\)C and transferred to a separating funnel where 40mls of diethyl ether was added and shaken vigorously.
Separation was done by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partition was done repeatedly until the aqueous layer became clear in colour. The saponin was extracted with 60mls of normal butanol. The combined extract was washed with 5% aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish. It was dried at 60°C in the oven and re-weighed. The analysis was done in triplicate and the average obtained.

The saponin content was determined by difference and calculated as a percentage of the original sample as follows:

\[
\% \text{ saponin} = \frac{W_2 - W_1}{W_0} \times 100
\]

Where:

\( W_1 \) = Weight of dish + sample

\( W_2 \) = Weight of evaporating dish

### 3.6.5 Determination of phenols

The Follins-Dennis method described by Pearson (1979) was used to determine phenol content of CLLM. A small quantity (0.2g) of CLLM sample was dispensed into a test tube. 10mls of methanol was added and shaken thoroughly. The mixture was left to stand for 5 minutes before it was filtered using Whatman filter paper number 4. One milliliter (1ml) of the extract was placed in a test tube containing 5ml of distilled water. The colour was allowed to develop for about 3 to 4 hours at room temperature. The absorbance of colour was thereafter measured at
760nm. The analysis was carried out in triplicate and the average obtained.

Phenol content was calculated as:

\[
\% \text{ Phenol} = \frac{100 \times \text{Au} \times \frac{C}{1000} \times \frac{V_f}{V_a} \times D}{W} \times 1000
\]

Where:
- \( As \) = Absorbance of standard solution
- \( Au \) = Absorbance of test sample
- \( C \) = Concentration of standard (mg/ml)
- \( D \) = Dilution factor
- \( Va \) = Volume of filtrate analyzed
- \( Vf \) = Volume of filtrate (total)
- \( W \) = Weight of sample analyzed

### 3.7 Bio-activity Studies of CLLM

Bio-activity-assay of CLLM was carried out according to procedures described by Osuala et al (2005), in the Imo State Environmental Protection Agency (ISEPA) Laboratory, Owerri. Accordingly, 30g of the powdered leaf meal sample was measured into a clean flask containing 100ml of 78% methanol, corked and stored for five days at room temperature and thereafter filtered. The filtrate was evaporated in a water bath at 70°C to paste-like residue. This was stored in a refrigerator until needed for use.

Sensitivity tests were carried out on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella spp* and *Klebsiella spp*
Isolates. These were selected because they are common enteric organisms in poultry and rabbits and have been used in phytochemical sensitivity tests (Osuala et al., 2005; Osuala and Allison-Onyechere, 2006; Onwuliri et al., 2006). Isolates were obtained from ISEPA laboratory. Before use, they were subjected to a confirmatory culture overnight at 37°C in a nutrient broth.

Nutrient broth used in the study was prepared according to manufacturer’s instruction. The anti-microbial activity was determined using Minimum Inhibitory Concentration (MIC), Minimum Bactericidal concentration (MBC) and the Zone of Inhibition Tests (ZIT) tests.

3.7.1 Preparation of Nutrient Broth

Before use, eight grams (8g) of the nutrient broth was suspended in 1litre of distilled water. Heat was applied to dissolve the medium completely. Thereafter it was dispensed into final containers (test tubes) and sterilized by autoclaving at 15Lbs pressure and 121°C for 15minutes. The medium was then allowed to cool to about 47°C before use. The bacterial susceptibility test was carried out using minimum inhibitory concentration, minimum batericiadal concentration and zone of inhibition tests

3.7.2 Minimum Inhibitory Concentration (MIC) Tests

For the MIC tests, one gram of the plant extract was dissolved in four milliliter (4 ml) of nutrient broth to obtain a standard solution. Serial dilution was then carried out to obtain 250, 125, 62.5, 31.25 and 15.625mg/ml solutions. Five sets of such dilutions were prepared for the
five test isolates. The tubes containing the mixtures of the extract and broth were inoculated with about 0.1ml of test organisms and incubated for 24 hours at 37°C. The samples were thereafter examined for bacterial growth by observing for turbidity. The lowest concentration(s) of extracts that inhibited the growth of test organisms were recorded as the MIC. Control tests without the extracts were also assayed simultaneously. All samples were tested in duplicates.

### 3.7.3 Minimum Bactericidal Concentration Tests

Tubes showing no turbidity from the MIC tests were sub-cultured onto sterile nutrient agar plates and incubated again for 24 hours at 37°C. The plates were subsequently examined for growth, and the lowest concentration(s) of the extracts that yielded no growth were recorded as the MBC.

### 3.7.4 Zone of Inhibition Tests

For the Zones of Inhibition Tests (ZIT), a lawn of each of the five isolates was made on a separate sterile nutrient agar plate. Thereafter holes of 0.5cm were bored into the inoculated plated using the open end of a sterile Pasteur pipette. The holes were afterwards filled with standard solution (250 mg/ml) prepared from the extract and nutrient broth. The plates were then incubated at 37°C for 24 hours, after which they were observed for zones of inhibition, which were measured in centimeters and diameters recorded. Procedures described by Osuala *et al* (2005) were adopted for the experiment.
3.8 Feeding Trial with Rabbits

This experiment was carried out in the School of Agriculture and Agricultural Technology (SAAT) Teaching and Research Farm, Federal University of Technology, Owerri.

3.8.1 Experimental Diets

Four grower rabbit experimental diets were formulated to contain C. *latifolia* leaf meal at 0.00 (control), 10.00, 20.00 and 30.00 per cent inclusion levels, for treatments 1, 2, 3, and 4 respectively. The experimental diets composition is shown in table 3.1.

3.8.2 Experimental Rabbits and Design

A total of thirty-six (36) crossbred rabbits (hybreed of Dutch and Chinchilla) aged between seven and nine weeks were used for the study. The animals were procured from the Nigerian Veterinary Research Institute (NVRI), Vom, Jos.

After seven days of stabilization with conventional concentrate diet, they were distributed into four treatment groups of nine rabbits each, on weight equalization basis and randomly assigned to the four experimental diets in a completely randomized design (CRD). Each treatment was subdivided into three replicates of three rabbits each. Animals in each replicate were housed in a separate wooden cage equipped with wire mesh floors. The animals were fed for forty-nine (49) days. During this period, animals in all the treatments were offered feed and water *ad libitum*, while all necessary prophylactic medications were also carried out.
3.8.3 Data Collection

The rabbits were weighed at the beginning of the trial and thereafter on a weekly basis. Daily feed intake per treatment was measured. This was determined by the difference between the weight of feed offered and that of the left-over the next morning. On the whole, data collected included: initial body weight, weekly body weight, final body weight and mortality; total feed intake, weekly body weight gain, total body weight gain and feed conversion ratio (g feed/g gain) were calculated at the end of the trial.

3.8.4 Haematological Studies

These studies were carried out at the Federal Medical Center (FMC) Owerri. At the end of the feeding trial period of 49 days, twelve rabbits were bled between 9.00am and 10.30am from a punctured ear vein. The choice of animals for bleeding was by random selection, and triplicate blood samples were collected from three rabbits per experimental group. Twelve milliliter (12ml) of blood was aspirated from each of them. Two milliliter (2ml) of each blood sample was discarded into ethylene di-amine tetra acetic acid (EDTA) treated bijou bottles for haematological assay. The remaining ten milliliters (10ml) of each blood sample was allowed to coagulate to produce sera for blood chemistry measurements.

Blood samples were analysed within three hours (3hours) of their collection for total erythrocyte (RBC) and leukocyte (WBC) counts,
hematocrit (PCV), hemoglobin concentration (HC) and erythrocyte sedimentation rate (ESR).

Other haematological indices (Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC)) were calculated from results obtained. Erythrocyte sedimentation rate (ESR) was determined within six hours of sample collection.

3.8.4.1 Estimation of Haemoglobin Concentration

The concentration of blood haemoglobin in the test samples were estimated according to the cyanomethaemoglobin method of Alexander and Griffiths (1993).

**Principle**

The haemoglobin in the test samples were converted to cyanomethaemoglobin through methaemoglobin by the Drabkins reagent.

**Procedure**

Three test tubes were cleaned, dried and labeled B (blank), T (test) and S (standard). Into the tubes were pipetted 0.02ml of distilled water, haemoglobin standard and heparinised blood (test) samples, respectively. Thereafter, 5ml of Drabkin’s reagent was pipetted into each of tubes (B, S and T). The solutions obtained were mixed and incubated for 5 minutes at room temperature for the colour to be well developed,
Table 3.1: Ingredient and Chemical Composition of Experimental Diets (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CLLM 0</th>
<th>CLLM 10</th>
<th>CLLM 20</th>
<th>CLLM 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize (white)</td>
<td>52.00</td>
<td>47.00</td>
<td>42.00</td>
<td>37.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>CLLM*</td>
<td>-</td>
<td>10.00</td>
<td>20.00</td>
<td>30.00</td>
</tr>
<tr>
<td>Wheat offal</td>
<td>20.00</td>
<td>15.00</td>
<td>10.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Palm kernel cake</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Blood meal</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Bone meal</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Common salt</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vit./Trace Mineral Premix**</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Calculated Chemical Composition (%dm)

<table>
<thead>
<tr>
<th></th>
<th>CLLM 0</th>
<th>CLLM 10</th>
<th>CLLM 20</th>
<th>CLLM 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>18.86</td>
<td>19.42</td>
<td>19.88</td>
<td>17.73</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>4.63</td>
<td>4.39</td>
<td>4.15</td>
<td>3.91</td>
</tr>
<tr>
<td>Ether extract</td>
<td>3.84</td>
<td>3.75</td>
<td>3.66</td>
<td>3.57</td>
</tr>
<tr>
<td>Ash</td>
<td>3.37</td>
<td>3.61</td>
<td>3.85</td>
<td>4.09</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.49</td>
<td>1.48</td>
<td>1.48</td>
<td>1.48</td>
</tr>
<tr>
<td>Phosphorus (available P)</td>
<td>0.77</td>
<td>0.79</td>
<td>0.81</td>
<td>0.83</td>
</tr>
<tr>
<td>L-methionine</td>
<td>0.30</td>
<td>0.366</td>
<td>0.422</td>
<td>0.488</td>
</tr>
<tr>
<td>L-lysine</td>
<td>0.88</td>
<td>1.28</td>
<td>1.56</td>
<td>1.87</td>
</tr>
<tr>
<td>ME(kcal/kg)</td>
<td>2765.98</td>
<td>2781.12</td>
<td>2816.26</td>
<td>2841.40</td>
</tr>
</tbody>
</table>

** To provide the following per kilogram of diet: Vit.A, 10,000 iu; Vit.D3, 2000 iu; Vit.E, 5 iu; Vit.K, 2mg; Riboflavin, 4.20mg; Vit.B₁₂, 0.0mg; panthotenic acid, 5mg; Nicotinic acid, 20mg; folic acid, 0.5mg; choline, 3mg; Mg, 56mg; Fe, 20mg; Cu, 10mg; Zn, 50mg; Co, 125mg.

*C. latifolia Leaf Meal
after which the absorbence of test samples and standard were read against blank at 540nm.

**Calculation**

\[
\text{Hbc (g/dl)} = \frac{A_{\text{sample}} \times C_{\text{std}}}{A_{\text{std}}}
\]

Where

\begin{align*}
A_{\text{sample}} &= \text{Absorbence of sample} \\
A_{\text{std}} &= \text{Absorbence of standard} \\
C_{\text{std}} &= \text{Concentration of standard}
\end{align*}

**3.8.4.2 Red blood cell (Erythrocyte) count**

The blood samples were diluted 1:200 in a solution of 10ml of 40% formalin in a litre of 32g/l trisodium citrate. The diluents and samples were mixed and carefully loaded into the counting chamber (Petri dish with a small piece of damp blotting paper). This was left for 2 - 3 minutes for the cells to settle before they were counted using improved Neubauer haemocytometer at magnification of x 40. Sufficient number of cells was counted to minimize errors due to variable cell distribution. To obtain a variance of 2% it is necessary to count about 2500 cells. All the cells in the entire central square (1mm²) were counted.

**Calculation**

\[
\text{Cell count (/l)} = N \times (D/A) \times 10 \times 10^9
\]

Where

\begin{align*}
N &= \text{Total number of cells counted}
\end{align*}
\( D \) = Dilution factor of blood
\( A \) = Total area counted (in mm\(^2\))
\( 10 \) = Factor to convert area to volume (in µl)
\( 10^9 \) = Factor to convert count per µl to count per litre

### 3.8.4.3 White blood cell (Leucocyte) count

Before counting the number of white blood cells, a 1-in-20 dilution of the blood samples was made by adding 0.02ml of whole blood to 0.38ml of diluting fluid (2% acetic acid lightly coloured with 1% crystal violet) and mixed for 2 - 3 minutes. By this dilution, the red cells are lysed but the leucocytes remain intact. Before filling the counting chamber, the fluid was insected to ensure that it was clear. The chamber was then filled and left for 2 - 3 minutes for the cells to settle. Thereafter, the chamber was laced on the microscope stage and, using the x25 objective, the number of cells seen in the four large (1mm\(^2\)) corner squares was counted while observing the criteria for inclusion and exclusion of cells touching the borders.

**Calculation**

Cell count (/l) = \( N \times (D/A) \times 10 \times 10^9 \)

\( N \) = Total number of cells counted
\( D \) = Dilution factor
\( A \) = Total of area counted (in mm\(^2\))
\( 10 \) = Factor to convert area to volume (in µl)
\( 10^9 \) = Factor to convert count per µl to count per litre
3.8.4.4 Erythrocyte sedimentation rate (Westergren technique)

**Principle:** When citrated blood in a vertically positioned westergren pipette is left undisturbed, red cells aggregate, stack together to form rouleaux, and sediment through the plasma. The Erythrocyte sedimentation rate (ESR) is the rate at which this sediment occurs in 1 hour as indicated by the length of the column of clear plasma above the red cells measured in mm.

**Test method**

0.4 ml of sodium citrate anticoagulant was pipetted into a small container, 1.6 ml of venous blood or EDTA anticoagulated blood was added thereto and well mixed. The cap of the container was then removed and the sample placed in the ESR stand with a Westergren pipette inserted and properly vertically positioned.

Using a safe suction method, the blood was drawn to the 0 mark of the Westergren pipette, avoiding air bubbles. It was ascertained that the ESR stand was level by ensuring that the bubbles in the spirit level was central.

The timer was then set for 1 hour; it was ensured that the ESR stand and pipette were not exposed to direct sunlight during the period.

After exactly 1 hour, the level at which the plasma met the red cells was read in mm.

3.8.4.5 Estimation of Packed Cell Volume (PCV – haematocrit)

This was estimated from heparinized blood samples using the haematocrit method of Alexander and Griffiths (1993). This permitted the
determination of the volume occupied by the red cells in the blood. A capillary tube is filled with whole blood and spurred in a haematocrit centrifuge to pack the red blood cells. The haematocrit was then determined as % PCV, using a PCV reader.

**Procedure**

Haematocrit (capillary) tubes were filled by capillary action to the mark with whole blood. The bottom end of the capillary tubes were sealed with plastacine and the tubes centrifuged in a haematocrit centrifuge for 4 minutes. The PCV was subsequently determined by measuring the height of the red cell column and expressing it simultaneously as a ratio of the height of the total blood column using a PCV reader.

**Calculation**

\[
\text{% PCV} = \frac{\text{Height of red cell column}}{\text{Height of total blood column}} \times 100
\]

3.8.4.6 Mean Cell Volume (MCV)

This is the mean volume of the red cells expressed as fento litres.

It was calculated from the PCV and red cell count values thus

\[
\text{MCV} = \frac{\text{PCV}}{\text{RBC}} \times 10^{12}/L
\]

3.8.4.7 Mean Cell Haemoglobin (MCH)

This measures the weight of haemoglobin present in the average red cell, expressed in pictogram (pg). It was calculated from the haemoglobin and red cell count thus:

\[
\text{MCH (pg)} = \frac{\text{Hb}}{\text{RBC}}
\]
### 3.8.4.8 Mean Cell Haemoglobin Concentration (MCHC)

This is the amount of haemoglobin in 100ml of packed red cells. It was calculated from the haemoglobin concentration and PCV and expressed in grams per 100ml thus:

\[
MCHC = \frac{\text{Hb}}{\text{PCV}} \text{ g/100ml}
\]

### 3.8.5 Serum Biochemistry

The bottles of coagulated blood were centrifuged at 3000 rpm for ten minutes for serum separation. Thereafter, the harvested sera were used for evaluation of total serum protein (TSP), serum albumen (SA) and globulin.

Cholesterol (determined from fresh blood), and other biochemical assays such as creatinine and urea concentration were also assayed.

The standard flame photometry using Gallenkamp analysis was used to determine serum sodium ion (Na\(^+\)) and potassium ion (K\(^+\)), while Calcium ion (Ca\(^{2+}\)) was determined by atomic absorption spectrophotometry; while serum phosphate ion (HPO\(_4^{2-}\)) was measured using trichlotoacetic acid, ammonium molybdate and metol to develop blue colour and read thereafter in a Buck 205 spectrophotometer.

Other serum parameters monitored included aspartate aminotransferase (AST), alanine aminotransferase (ALT) (enzymes normally present in the liver and are released into the blood when the organ is damaged), and alkaline phosphatase (ALP). The activities of these enzymes were determined using spectrophotometric methods. Also assayed alongside were total or unconjugated bilirubin (TB) and direct or conjugated
bilirubin (DB). Bilirubin is derived mainly from the haem moiety of haemoglobin molecules following the destruction of red blood cells.

3.8.5.1 Estimation of total serum protein by Biuret Method

This assay was based on Tietz (1999)

**Principle**

In alkaline solution, cupric ions react with all compounds with two amide or peptide bonds linked either directly or through an intermediate carbon atom to form a violet coloured complex, where colour intensity is directly proportional to the protein concentration in sample

**Reagents**

Reagent 1:

- Sodium hydroxide 80mmol/L
- Potassium sodium tartrate 12.8mmol/L

Reagent 2

- Sodium hydroxide 100mmol/L
- Potassium sodium tartrate 16mmol/L
- Potassium iodide 15mmol/L
- Copper sulphate 6mmol/L

Working reagent (sample start procedure): To 4 parts of reagent 1, one part of reagent 2 was mixed to constitute the working reagent
**Test procedure**

Three test tubes were labeled, blank, standard and sample respectively: thereafter, the serum sample, distilled water and working reagent were dispensed into them in the scheme shown below.

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample (serum)</td>
<td>-</td>
<td>20µL</td>
<td>20µL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>20µL</td>
<td>10µL</td>
<td>-</td>
</tr>
<tr>
<td>Working Reagent</td>
<td>1000µL</td>
<td>1000µL</td>
<td>1000µL</td>
</tr>
</tbody>
</table>

These tubes were mixed, incubated for 5 minutes at 25°C/37°C and absorbance read against reagent blank at 540nm.

**Calculation**

Total protein (g/dL) = \( \frac{\text{Absorbance (sample)} \times \text{Conc. (standard)}}{\text{Absorbance (standard)}} \)

**3.8.5.2 Serum albumin/globulin assay (BCG method)**

**Principle**

Albumin bonds with bromocresol green (BCG) to produce a blue–green colour whose intensity is directly proportional to the concentration of albumin in the sample.

**Materials and Reagents**

Sample: Serum

Reagent: Single, ready-to-use working reagents, composed of

- Bromocresol Green (BCG) 0.26mmol/L
- Citrate Buffer, pH 4.2 30mmol/L
**Test procedure**

With reagent and sample brought to room temperature, three test tubes labeled blank, standard and sample respectively were set, into which the working reagent, serum sample and standard were dispensed as shown in the scheme below.

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Reagent</td>
<td>1000µL</td>
<td>1000µL</td>
<td>1000µL</td>
</tr>
<tr>
<td>Sample(serum)</td>
<td>-</td>
<td>-</td>
<td>10µL</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10µL</td>
<td>-</td>
</tr>
</tbody>
</table>

The tubes were mixed, incubated for 10 minutes at 25°C/37°C and absorbance read at 546nm against reagent blank.

**Calculation**

\[
\text{Albumin (g/dL)} = \frac{\text{Absorbance (sample)}}{\text{Absorbance (standard)}} \times \frac{\text{Conc. (standard)}}{
\]

Serum globulin = Total protein - Albumin (g/dL)

**3.8.5.3 Creatinine assay**

The method adopted for this analysis was based on WHO’s guidelines on Standard Operating Procedures in clinical chemistry (SOP, 2005).

**Principle**

Creatinine reacts with picric acid in alkaline medium to form a red/yellow coloured complex (creatinine complex) which is measured at 490nm.

**Procedure**

Stage 1
To a centrifuge tube was added 1.5ml of distilled water, 0.5ml of serum, 0.5ml 5% sodium tungstate and 0.5ml of 2/3NH$_2$SO$_4$. These were mixed and spun at 1000 rpm for 5 minutes. This constituted the working reagent.

Stage 2

Three test tubes were labeled: Test, Standard and Blank respectively. To the test (tube) was added 2ml of clear supernatant from the working reagent, 1ml of 0.75N NaOH, 1ml of picric acid and 2ml of distilled water. To the standard (tube) was added 2ml of creatinine standard, 1ml of 0.75N NaOH, 1ml of picric acid and 2ml of distilled water. To the blank (tube) was added 2ml of distilled water, 1ml of 0.75N NaOH, 1ml of picric acid and 2ml of distilled water (for the second time). The contents of the various tubes were properly mixed and allowed to stand for 20 minutes at room temperature, after which absorbances were read at 490nm.

Calculations

Creatinine content (mg/dl) = \( \frac{\text{Abs. of test} \times \text{Conc. of Std. (4mg/dl)}}{\text{Abs. of Std.}} \)

3.8.5.4 Estimation of serum total cholesterol (Kits based)

Principle:

Cholesterol esterase catalyses the hydrolysis of cholesterol esters into free cholesterol and fatty acids. The free cholesterol through a sequence of reactions produces hydrogen peroxide which is quantified using a quinoneimine indicator, formed from 4-aminoantipyrine, phenol and H$_2$O$_2$. 
in the presence of POD (peroxide). This shows a pink/red colour, the intensity of which is proportional to the cholesterol concentration in the sample.

**Materials and Reagents**

**Sample:** Fresh serum

**Reagent:**

A single ready-to-use reagent comprising:

- Good’s Buffer, PH 6.7 50mmol/L
- Phenol 5mmol/L
- 4-Aminoantipyrine 0.3mmol/L
- Cholesterol esterase ≥200µ/L
- Cholesterol oxidase ≥50µ/L
- Peroxidase ≥3µ/L

**Test procedure**

Both reagent and samples were brought to room temperature and into three test tubes (Blank, Standard and Sample), the following were pipetted as shown in the scheme below:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1000µL</td>
<td>1000µL</td>
<td>1000µL</td>
</tr>
<tr>
<td>Sample/Standard (Std.)</td>
<td>-</td>
<td>10µL</td>
<td>10µL</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10µL</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

These tubes were mixed, incubated for 20 minutes at 25°C, after which absorbance of sample and standard were read at 500nm against reagent blank within 60 minutes.
Calculation

Cholesterol (mg/dl) = Absorbance (sample) x Conc (Std.)

Absorbance (Std.)

3.8.5.5 Determination of urea (based on W.H.O., 2005)

Principle:
Under strong acidic condition and at 100°C, diacetyl monoxime is hydrolysed to diacetyl and hydroxyl anions (monoxine). The diacetyl group which is very reactive couples with urea to form reddish pink colouration (Diazine), which is read at 540nm.

Materials and Reagents
Sample: Serum
Reagents: Ready-to-use acid reagent, Ready-to-use colour reagent, Urea standard

Analytical procedure
Three test tubes were labelled (Test, Standard and Blank) respectively.
To the Test tube was added 2.5ml of acid reagent, 2.5ml of colour reagent and 25μL (0.025L) of serum.
To the Standard tube was added 2.5ml of acid reagent, 2.5ml of colour reagent and 25μL of urea standard.
To the Blank tube was added 2.5ml of acid reagent, 2.5ml of colour reagent and 25μL of distilled water.
All tubes were well mixed, capped with cotton wool and placed in boiling water for 10 minutes. Thereafter they were cooled and read at 540nm.
**Calculations**

Urea (mg/ml) = \( \frac{\text{Abs. of Test} \times \text{Conc. of Std. (50mg/ml)}}{\text{Abs. of Std.}} \)

**2.8.5.6 Determination of Glucose (Glucose Oxidase Reagent kit)**

**Principle:**

\( \beta \)-D-glucose is oxidized by glucose oxidase to produce D-glucoronic acid and hydrogen peroxide. The hydrogen peroxide is then oxidatively coupled with 4-aminoantipyrine and phenol substitute, p-HBS, in the presence of peroxidase to yield a red quinoneimine dye. The amount of coloured complex formed is proportional to glucose concentration and measured photometrically.

**Materials and Reagents**

Sample: Fresh serum

Reagents:

Glucose reagent:- A single ready-to-use reagent consisting of glucose oxidase 15µl/ml, peroxidase 1.2µl/ml, mutarotase 4.0µl/ml, 4-aminoantipyrine 0.38mM, p-Hydroxybenzenesulfonate 10mM, and non-reactive ingredients.

Glucose standard: (100mg/dl \( \beta \)-D-glucose).

**Analytical procedure**

1. Test tubes were labeled: blank, standard, sample

2. 1.5ml of working reagent was pipette into all tubes and placed in 37°C heating bath for at least five (5) minutes.
3. 0.01ml (10µl) of sample was added to sample tubes, mixed, and incubated at 37°C for exactly ten (10) minutes.

4. After incubation, spectrophotometer was brought to zero with the reagent blank and absorbence of all tubes were read and recorded at 500 nm.

**Calculation**

\[
(A = \text{Absorbance})
\]

\[
\text{Glucose (mg/dl)} = \frac{A \text{ (sample)}}{A \text{ (standard)}} \times \text{Conc. of standard}
\]

**3.8.5.7 Determination of serum calcium (kit based)**

**Principle**

Calcium reacts with cresolphthalein complexone in 8-hydroxyquinoline to form a coloured complex (purple colour) that absorbs at 570 nm (550 – 580 nm). The intensity of the colour is proportional to the calcium concentration.

**Analytical procedure**

Test tubes were labeled, Test, Standard (Std.), and Blank respectively.

Into the Test (tube) was pipetted 1 ml of buffer, 1 ml of colour reagent and 40 µl of serum sample.

Similarly to the Test (tube), 1 ml of buffer, 1 ml of colour reagent was added while 40 µl of standard reagent was pipetted into Std. tubes.

Finally, into Blank tubes were pipetted 1 ml of buffer, 1 ml of colour reagent and 40 µl of distilled water.
The contents of each tube were well mixed and allowed to stand for 5 – 10 minutes for full colour development before being read at 540 nm with spectrophotometer.

**Calculations**

\( A = \text{Absorbance} \)

Calcium (Mg/dl) = \( A \text{ Sample} \times \text{Conc. (std.)} \)

\[ A \text{ (std.)} \]

### 3.8.5.8 Serum sodium (colorimetric method)

**Principle:** The present method is based on modifications of a previous procedure in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen increased.

**Procedure**

Test tubes: blank, standard, control, were labeled. 1.0ml of filtrate reagent containing 2.1 mM uranyl acetate and 20 mM magnesium acetate in ethyl alcohol was pipetted to all tubes. 50ul of sample was added to all tubes and distilled water to the blank. All tubes were shaken vigorously and then centrifuged at high speed (1500G) for 10 minutes. The absorbance was read at 550nm setting the spectrophotometer to zero.
Calculations

Abs = Absorbance
S = Sample

Na (Mmol/l) = \frac{Abs. of Blank – Abs of S \times \text{Conc. of STD}}{Abs. of Blank - Abs of STD}

3.8.5.9 Serum potassium determination (colorimetric method)

Principle

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension, the turbidity of which is proportional to potassium concentration in the range of 2 – 7 mEq/L.

Procedure

Test tubes: standard, control, blank, were labeled. 1.0ml of potassium reagent containing sodium tetraphenylboron 2.1 mM, preservatives and thickening agents was pipetted to all tubes. Also 0.01ml (10ul) of samples was added to respective tubes, mixed and left at room temperature for 3 minutes. The absorbance was read at 500nm after setting the spectrophotometer to zero with reagent blank.

Calculation

\[ \text{K concentration} = \frac{\text{Abs. of unknown}}{\text{Abs. of STD}} \times \text{Conc. of STD (Mmol/L)} \]
3.8.5.10 Aspartate aminotransferase (SOP, 2005 method)

**General principle**

Transamination is the process in which an amino group is transferred from amino acid to α-keto acid. The enzymes responsible for transamination are called transaminases. The substrates in the reaction are α-ketoglutaric acid (a-KG) plus L-aspartate for AST, and α-KG plus L-alanine for ALT. The products formed from the enzyme action are oxaloacetate for AST and glutamate and pyruvate for ALT. Addition of 2,4-dinitrophenyl hydrazine results in the formation of hydrazone complex with the keto acids. A red colour is produced on the addition of sodium hydroxide, the intensity of which is related to enzymatic activity.

**Specimen: serum**

**Method: colorimetric end-point**

**Procedure**

Test tubes were labeled, **test**, **standard** and **blank**.

To the **test** was added 0.5ml of AST substrate, 0.1ml of sample and incubated for 30 minutes in a water bath at 37°C. Thereafter, 0.5ml of 2,4 DNPH was added and incubated at room temperature for 15 - 20 minutes. Subsequently, 5.0ml of 0.5N NaOH was added and the content of test tube well mixed and read with a spectrophotometer at 510nm wavelength.

To the **standard** test tube was added 0.5ml of AST substrate, 0.1ml of standard reagent and incubated in a water bath at 37°C for 30 minutes. Subsequently, 0.5ml of 2,4 DNPH was added and the unit incubated at
room temperature for 15 – 20 minutes. Thereafter 5.0ml of 0.5N NaOH mixed and read with a spectrophotometer at 510nm wavelength.

To the **blank** test tube was added 0.5ml of AST substrate, 0.1ml of distilled water and incubated in a water bath at 37°C for 30 minutes. Thereafter, 0.5ml of 2,4-DNPH was added and incubated at room temperature for 15 – 20 minutes. Subsequently, 5.0ml of 0.5N NaOH was added, mixed and read with a spectrophotometer at 510nm wavelength.

### 3.8.5.11 ALT determination (SOP, 2005 method)

**Specimen:** Serum

**Reagents:** Phosphate buffer (PH 7.4), AST substrate, ALT substrate, Pyruvate standard (2Mmol/ml), 2, 4 Dinitrophenylhydrazine (2, 4 DNPH), 0.5N NaOH.

**Method:** Colorimetric end-point

**Procedure**

Test tubes were labeled Test, Standard and Blank. 0.5ml of ALT substrate was measured into all the three tubes. Thereafter, 0.1ml of sample was added to the test (test tube), while 0.1ml of Standard reagent and 0.1ml of distilled water respectively was added to the Standard Test tube and Blank test tube. All tubes were left in a water bath at 37°C for 25 minutes. Thereafter, 0.5ml of 2, 4-DNPH was added to all tubes (Test, Standard and Blank), after which they were allowed for 10 minutes at room temperature. 5 minutes of 0.5N NaOH was then added to the three
test tubes, and content of individual test tube were thoroughly mixed and red with a spectrophotometer at 510nm wavelength.

3.8.5.12 ALP determination (based on SOP, 2005)

*Specimen:* Serum

*Reagents:* Phosphate buffer, ALP standard (phenol 1/100 dilution), 0.5N NaOH, 0.5N NaHCO₃, 4–aminotipyrene, potassium ferrocynide

*Principle:* ALP at PH of 9.8 acts on substrate liberating phenol and phosphoric acid. The phenol is estimated by its reaction with 4–aminoantipyrene to produce a colour in the presence of potassium ferrocynide. The intensity of the colour which is read at 500nm is proportional to the concentration of enzyme present.

*Procedure*

The test tubes were label Test, Standard and Blank. 1.0ml each of ALP substrate and ALP buffer was added to all three test tubes, after which there were incubated for 5 minutes at 37°C. Thereafter, 0.1ml of serum sample, 0.1ml of ALP standard and 0.1ml of distilled water was added to the Test, Standard and Blank test tube respectively. This was followed by incubation of all test tubes at 37°C for 15 – 20 minutes. Thereafter, 0.8ml of 0.5 NaOH, 1.2ml of NaHCO₃, 1.0ml of 4–aminoantipyrene and 1.0ml of potassium ferrocynide was each measured into the Test, Standard and Blank test tube respectively. Individual tubes were then properly mixed and red with a spectrophotometer at 500nm wavelength.
**Calculations**

\[
\text{ALP (IU/L)} = \frac{\text{Abs. of Test} \times \text{Conc. of STD}}{\text{Abs. of STD}}
\]

**3.8.5.13 Determination of bilirubin (based on SOPs, 2005)**

**Specimen:** Serum. Clear and non-haemolysed serum samples

**Reagents:**
1. Methanol
2. Diazo 1 (sulphanalic acid in con. HCl)
3. Diazo II (sodium nitrite)
4. Mixed Diazo reagent (10ml of Diazo 1 to 0.3ml of Diazo II)
5. Diazo blank (1.5\%/ V/V HCl)
6. Bilirubin standard (methyl red)

**Principle**

Sulphanalic acid is diazotized by nitrous acid produced from the reaction between NaNO\(_3\) and HCl. Bilirubin reacts with diazotized sulphanalic acid to form azobilirubin which is read at 540nm.

**Procedures**

(a) Total bilirubin: Test tubes were labeled test and blank. Into the test (tube) was measured 0.2ml of serum sample, 0.5ml of mixed Diazo reagent, 2.5ml of methanol and 2.0ml of distilled water.

Into the blank was measured 2.0ml of distilled water, 0.2ml of serum sample, 0.5ml of Diazo blank and 2.5ml of methanol.

Individual tubes were well mixed and incubated in a dark compartment for 20 minutes and read at 540nm with a spectrophotometer.
Conjugated bilirubin: Into the test (tube) was measured 2.0ml of distilled water, 0.2ml of serum sample, 0.5ml of mixed diazo reagent and 2.5ml of distilled water (for the second time). For the blank test, 2ml of distilled water was measured into the test tube, followed by 0.2ml of serum sample, 0.5ml of diazo blank and 2.5ml of distilled water (for the second time). All tubes were thoroughly mixed and incubated in a dark compartment for 20 minutes, after which each absorbance was read at 540nm with the spectrophotometer.

**Calculation**

\[
\text{Abs. of test} - \text{Abs. of blank} \times \text{Conc. of Std.} = \text{Conc. of Bilirubin (mg/dl)}
\]

\[
\text{Abs. of std.} - \text{Abs. of blank}
\]

3.9 Internal Organ Evaluation

At the end of the feeding trial, three rabbits per treatment group were randomly selected for relative organ weights determination. The animals were starved of feed only for twenty-four hours. Thereafter they were weighed, slaughtered and thoroughly bled. The carcasses were cleaned, dissected and eviscerated, after which the lungs, heart, kidney, liver/ gall bladder and spleen were harvested and weighed.

The weights of the dressed carcasses and the internal organs were expressed as percentages of the respective live weights of the animals. Thereafter the organ samples were quickly transferred to formalin solution in preparation for histopathological study. Information generated
from organ weight evaluation complemented others from blood studies in monitoring the feed stresses or otherwise on the experimental animals.

3.10 Histopathological Study of Rabits
This study was executed at the Department of Anatomy Laboratory, College of Medical Sciences, University of Calabar. After organ weight evaluation, some internal organs used for the procedure (liver, kidney) and pancreas were cut and fixed in bouin’s fixative for 24 hours, then hydrated, cleared and infiltrated in molten paraffin. Thereafter, the tissues were embedded in pure paraffin wax and sectioned at 5 - 6 micron in a microton, after which they were stained with haematoxilin and eosin (H and E) and subsequently examined by light microscopy for histopatological changes. Details of the histopatological processing of the tissues and staining methods are shown in the appendix.

3.11 Statistical Analyses
Data on feed intake, body weight gain, feed conversion ratio, haematology, serum biochemistry, and organ weights evaluation were subjected to one-way analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1978). Where ANOVA detected significant treatment effects, means were separated using the Duncan’s New Multiple Range Test (DNMRT) as outlined by Little and Hills (1978)
3.12 Feeding Trial with Broilers

Study locations for this experiment remained the same as those for rabbit studies.

3.12.1 Experimental Diets

The experiment was in two phases, the starter and finisher phases respectively. Five broiler starter experimental diets were formulated such that inclusion of CLLM was at 0.0, 2.5, 5.0, 7.5 and 10% levels, respectively. White maize was used as the major energy source for the rations. At the second phase of the trial (finisher phase), the experimental diets were adjusted to broiler finisher diets. For a preliminary investigation such as this, 10% inclusion level of the leaf meal was considered moderate. The ingredients composition of the experimental diets is shown in tables 3.2 and 3.3.

3.12.2 Experimental Birds and Design

A total of one hundred and fifty (150) day-old Anak broiler chicks used in the trial was acquired from a reputable distributor. They were raised on commercial broiler starter diet for one week, after which random distribution of the birds was made into five groups of thirty birds each, and randomly assigned to the five experimental diets in completely randomized design (CRD). Each group was further sub-divided into three replicates of ten birds each and housed in separate pens. Normal brooding was carried out for three weeks. Throughout the experimental period, feed and water were provided ad libitum for all
treatment groups. This was accompanied by necessary prophylactic medication and vaccination. The starter phase of the experiment lasted four weeks (28 days).

By the fifth week of the experiment when the second phase of the trial commenced, the number of birds per treatment and average weight of each treatment group was recorded. During this phase, the birds were placed on the experimental finisher diet, and given feed and water ad libitum for another four week period. On the whole the broiler feeding experiment lasted for a total of eight weeks (56 days).

### 3.12.3 Data Collection

The birds were weighed at the beginning of the trial and weekly thereafter throughout the eight week duration of the trial. Daily feed intake per group was recorded. This was calculated from the weight of feed offered and that of the left-over the next morning. Data collected included: initial body weight, weekly body weight and final body weight. Others were daily feed intake, total feed intake, weekly body weight gain, total body weight gain, feed conversion ratio (g feed / g gain) and mortality. Visual observation on the skin, shank and beak colours was also made.

### 3.12.4 Haematological and Serum Biochemical Studies of the Broilers.

At the end of a feeding trial period of 56 days, four birds were randomly selected for bleeding. This was done between 9.00am and 10.30am from
punctured webal sub-clavical vein with a 5ml scalp vein needle. A total of 7ml of blood was aspirated from each bird. 2ml of each blood sample was discarded into ethylene di-amine tera acetic acid (EDTA) treated Bijou bottles for haematological assay, while the remaining 5ml was allowed to coagulate in vial bottles (without anti-coagulant) to produce sera for blood chemistry measurements. All paramenters were determined in triplicates. The haematological and serum biochemical indices were determined as in the rabbit trial.

3.12.5 Carcass and Organ Weights Evaluation

Four birds per treatment were randomly selected, starved overnight of feed only, weighed and sacrificed by cervical bone dislocation. Thereafter, their jugular veins were cut and the carcasses thoroughly bled. Before defeathering, the carcasses were scaled in hot water of about 80°C for about a minute and the feathers plucked manually after. The carcasses were then eviscerated by cutting through the vent and the viscera removed. Thereafter the dressed carcass weights were obtained. The neck, wings, thigh drum stick and breast were sectioned out and weighed using a sensitive electronic scale, and their weights expressed as percentages of respective live-weights of birds used. In addition, weights of the internal organs (heart, liver/gall bladder, spleen and gizzard), and abdominal fat were also recorded. These and the dressed carcass weights were also expressed as percentages of the respective live weights.
Table 3.2: Ingredient Composition of the Experimental Diets (Broiler Starter)

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>0.0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize (white)</td>
<td>50.00</td>
<td>48.00</td>
<td>46.00</td>
<td>44.00</td>
<td>42.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>28.00</td>
<td>28.00</td>
<td>28.00</td>
<td>28.00</td>
<td>28.00</td>
</tr>
<tr>
<td>CLLM*</td>
<td>0.00</td>
<td>2.50</td>
<td>5.00</td>
<td>7.50</td>
<td>10.00</td>
</tr>
<tr>
<td>Wheat offals</td>
<td>8.00</td>
<td>7.75</td>
<td>7.25</td>
<td>6.75</td>
<td>6.25</td>
</tr>
<tr>
<td>Palm kernel cake</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Fish meal</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Blood meal</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Bone meal</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
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<tr>
<td>Common salt</td>
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<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin/trace mineral premix**</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Calculated Chemical Composition**

<table>
<thead>
<tr>
<th></th>
<th>0.0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>22.72</td>
<td>22.79</td>
<td>22.86</td>
<td>22.93</td>
<td>23.0</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>4.15</td>
<td>4.11</td>
<td>4.11</td>
<td>4.12</td>
<td>4.13</td>
</tr>
<tr>
<td>Ether extract</td>
<td>3.68</td>
<td>3.65</td>
<td>3.63</td>
<td>3.60</td>
<td>3.57</td>
</tr>
<tr>
<td>Ash</td>
<td>3.46</td>
<td>3.56</td>
<td>3.66</td>
<td>3.75</td>
<td>3.85</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.33</td>
<td>1.32</td>
<td>1.32</td>
<td>1.32</td>
<td>1.32</td>
</tr>
<tr>
<td>Phosphorus (available P)</td>
<td>0.76</td>
<td>0.77</td>
<td>0.78</td>
<td>0.78</td>
<td>0.79</td>
</tr>
<tr>
<td>L-methionine</td>
<td>0.62</td>
<td>0.63</td>
<td>0.65</td>
<td>0.66</td>
<td>0.69</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>1.60</td>
<td>1.67</td>
<td>1.79</td>
<td>1.88</td>
<td>1.97</td>
</tr>
<tr>
<td>ME(Kcal/kg)</td>
<td>2873.45</td>
<td>2868.01</td>
<td>2856.56</td>
<td>2857.12</td>
<td>2851.67</td>
</tr>
</tbody>
</table>

** To provide the following per kilogram of feed: Vit. A, 10,000 iu, Vit. D₃, 2000 iu, Vit. E, 5iu; Vit. K, 2mg; Riboflavin, 4.20mg; Vit. B₁₂, 0.01mg; Panthotenic acid, 5mg; Nictotnic acid, 20mg; Folic acid, 0.5mg; choline, 3mg; Mg, 56mg; Fe, 20mg; Cu, 10mg; Zn, 50mg; Co.125mg.

*C. latifolia* leaf meal
### Table 3.3: Ingredient Composition of the Experimental Broiler Finisher Diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Dietary Levels of CLLM(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>Maize (white)</td>
<td>60.0</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>16.0</td>
</tr>
<tr>
<td>CLLM*</td>
<td>-</td>
</tr>
<tr>
<td>Wheat offals</td>
<td>8.0</td>
</tr>
<tr>
<td>Palm kernel cake</td>
<td>7.0</td>
</tr>
<tr>
<td>Fish meal</td>
<td>2.0</td>
</tr>
<tr>
<td>Blood meal</td>
<td>3.0</td>
</tr>
<tr>
<td>Bone meal</td>
<td>3.0</td>
</tr>
<tr>
<td>Common salt</td>
<td>0.25</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.25</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin/trace mineral premix**</td>
<td>0.25</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

**CALCULATED CHEMICAL COMPOSITION**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>3.81</td>
<td>3.80</td>
<td>3.79</td>
<td>3.78</td>
<td>3.76</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>3.78</td>
<td>3.75</td>
<td>3.73</td>
<td>3.70</td>
<td>3.67</td>
</tr>
<tr>
<td>Ether extract</td>
<td>2.96</td>
<td>3.06</td>
<td>3.16</td>
<td>3.26</td>
<td>3.36</td>
</tr>
<tr>
<td>Ash</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
<td>1.30</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.70</td>
<td>0.71</td>
<td>0.72</td>
<td>0.72</td>
<td>0.73</td>
</tr>
<tr>
<td>L-methionine</td>
<td>0.57</td>
<td>0.59</td>
<td>0.60</td>
<td>0.62</td>
<td>0.64</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>1.31</td>
<td>1.40</td>
<td>1.49</td>
<td>1.58</td>
<td>1.67</td>
</tr>
<tr>
<td>ME(Kcal/kg)</td>
<td>936.35</td>
<td>2930.91</td>
<td>2925.46</td>
<td>2920.02</td>
<td>2914.56</td>
</tr>
</tbody>
</table>

**To provide the following per kilogram of feed: Vit. A, 10,000iu; Vit. D₃, 2000iu; Vit. E, 5iu; Vit.K, 2mg; Riboflavin, 4.20mg; Vit. B₁₂, 0.01mg; Panthotenic acid, 5mg; Nictotnic acid, 20mg; Folic acid, 0.5mg; choline, 3mg; Mg, 56mg; Fe, 20mg; Cu, 10mg; Zn, 50mg; Co, 125mg.**

*C. latifolia Leaf Meal.*
3.12.6 Histopathological Study

The histopathological methods described in rabbit experiment was also adopted for the study in broilers. Specimen used were some internal organs of the birds harvested for organ weight analysis (Liver, pancreas, proventriculus and kidney).

Tissues of these organs were examined by light microscopy after a standard histological processing and staining described in the appendix.

3.12.7 Statistical Analyses

Data generated from the study on feed intake, body weight gain, feed conversion ratio, haematology, serum biochemistry and carcass/organ weight evaluation were subjected to one-way analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1978). Where significant treatment effects were detected by ANOVA, the means were separated using the Duncan’s New Multiple Range Test (DNMRT) as outlined by Little and Hills (1978).
CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Experiment 1: Determination of Chemical Composition, Amino acid profile, Phytochemical Composition and Bio-activity studies of CLLM

4.1.1 Chemical Composition of CLLM

The chemical composition of CLLM as determined by proximate/mineral analysis is shown in Table 4.1.

For the proximate fractions, the moisture content was 8.04%. The percentage composition of other proximate fractions on dry matter basis was crude protein, 14.25; ether extract 2.84 and ash, 6.26. Others were crude fibre, 2.84 and nitrogen free extractives, 60.39.

The five minerals analysed for yielded the following results (mg/100g): calcium 10.8 magnesium, 45; potassium, 486; sodium, 3.86 and phosphorus, 395mg/100g.

4.1.2. Amino Acid Composition

The result of analysis of CLLM protein to determine its amino acid profile is shown on Table 4.2. Accordingly the concentrations of lysine, histidine, arginine and aspartic acid (g/100g protein) were 3.97, 2.19, 4.42 and 7.75 respectively. Others were threonine, 3.02; serine, 2.41; glutamic acid, 9.27; proline, 2.97; glycine, 3.60 and alanine, 4.09. Cystine
concentration was 0.86, valine, 4.01 methionine, 0.86 while yields of 3.39, 6.70, 2.90 and 3.97 were recorded for isoleucine, leucine, tyrosine and phenylalanine, respectively.

4.1.3. Phytochemical Composition of CLLM

The result of the quantitative phytochemical analysis of CLLM is presented in Table 4.3.

The percentage composition of alkaloid, flavonoid, saponin and tannin were 1.03, 0.37, 0.47, and 0.55%, respectively. Others were phenol, 0.17; phytate, 0.12 and cyanogenic glycoside, 7.07 (all in mg/100g).

4.1.4. Bio–activity studies of CLLM

The results of the Bio–activity studies of CLLM are summarized in tables 4.4 (a) to (c).

The methanolic extract of CLLM exhibited anti–microbial activity against the organisms tested.

The minimum inhibitory concentration (MIC) for pseudomonas aeruginosa and E. coli was 15.625mg/ml, while S. aureus, klebsilla spp and Salmonella spp were all inhibited at a concentration of 250mg/ml.

The minimum bactericidal concentration (MBC) of the five organisms listed were 31.25, 31.25, 250, 250, 250 mg/ml, respectively; the diameters of zone of inhibition measured at 250mg/ml in centimeters were 1.4, 1.5, 0.0, 0.0 and 0.3 for pseudomonas aeruginosa, E. coli, S. aureus, klebsilla spp and salmonella spp., respectively.
4.2 Experiment 2: Rabbit Trial

The calculated nutrient composition of the treatment diets is shown in table 3.1.

The metabolisable energy values of the diets increased with increasing levels of CLLM. The diet containing 0% CLLM (control diet) had the lowest energy level of 2765.98kcal/kg, while the 10.0%, 20.0% and 30.0% percent levels were 2781.12, 2816.26 and 2841.40kcal/kg, respectively. These energy values indicated the high energy profile of CLLM. On the other hand, the diet with the highest crude protein content (19.88%) was treatment diet which contained 20.0% CLLM. This was followed by the T2 (10.0% CLLM) which had 19.42% crude protein. The control treatment and T4 (30% CLLM) contained 18.86% and 17.73% crude protein, respectively.

The effects of the different dietary levels of CLLM on the performance of grower rabbits is summarized in table 4.5. The initial average body weights of the rabbits assigned to the different treatments were 716.7, 765.6, 777.8 and 788.9 grams for 0% (control), 10.0% (T2), 20.0% (T3) and 30.0% (T4), respectively. There were no significant differences (P>0.05) among the treatment groups in respect of initial average body weights.

4.2.1. Average Final Body Weights

The average final body weights of the rabbits after eight weeks of feeding trial were 1.54, 1.42, 1.33 and 1.3kg, respectively for T1 (control), T2
Table 4.1: Chemical Composition of CLLM

<table>
<thead>
<tr>
<th>Nutrients (%)</th>
<th>Concentration</th>
</tr>
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<tbody>
<tr>
<td>Moisture</td>
<td>8.04</td>
</tr>
<tr>
<td>Dry matter</td>
<td>91.96</td>
</tr>
<tr>
<td>Crude protein (%Dm)</td>
<td>14.25</td>
</tr>
<tr>
<td>Ether extract (%Dm)</td>
<td>2.84</td>
</tr>
<tr>
<td>Ash (%Dm)</td>
<td>6.26</td>
</tr>
<tr>
<td>Crude fibre (%Dm)</td>
<td>2.84</td>
</tr>
<tr>
<td>Nitrogen free extractives (%Dm)</td>
<td>60.39</td>
</tr>
<tr>
<td>Metabolizable energy (Kcal/kg)</td>
<td>2903.41</td>
</tr>
<tr>
<td>Calcium (mg/100g)</td>
<td>10.8</td>
</tr>
<tr>
<td>Magnesium (mg/100g)</td>
<td>45.0</td>
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<tr>
<td>Potassium (mg/100g)</td>
<td>486.0</td>
</tr>
<tr>
<td>Sodium (mg/100g)</td>
<td>3.86</td>
</tr>
<tr>
<td>Phosphorus (mg/100g)</td>
<td>395.3</td>
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</table>
Table 4.2: Amino Acid Profile of CLLM

<table>
<thead>
<tr>
<th>Amino acids (g/100g)</th>
<th>Concentration</th>
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</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>3.97</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.19</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.42</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>7.75</td>
</tr>
<tr>
<td>Threonine</td>
<td>3.02</td>
</tr>
<tr>
<td>Serine</td>
<td>2.41</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>9.27</td>
</tr>
<tr>
<td>Proline</td>
<td>2.97</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.60</td>
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<tr>
<td>Alanine</td>
<td>4.09</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.86</td>
</tr>
<tr>
<td>Valine</td>
<td>4.01</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.86</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.39</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.70</td>
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<tr>
<td>Tyrosine</td>
<td>2.90</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.97</td>
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</table>
Table 4.3: Phytochemical Composition of CLLM

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids (%)</td>
<td>1.03</td>
</tr>
<tr>
<td>Flavonoids (%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Saponins (%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Tannin (%)</td>
<td>0.55</td>
</tr>
<tr>
<td>Phenols (mg/100g)</td>
<td>0.17</td>
</tr>
<tr>
<td>Phytates (mg/100g)</td>
<td>0.12</td>
</tr>
<tr>
<td>Cyanogenic glycosides (mg/100g)</td>
<td>7.07</td>
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</table>
Table 4.4 (a): Minimum Inhibitory Concentration (MIC) of Methanolic extracts of CLLM

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Concentration of CLLM Extracts</th>
<th>250mg/ml</th>
<th>125mg/ml</th>
<th>62.5mg/ml</th>
<th>31.25mg/ml</th>
<th>15.625mg/ESC MIC(mg/ml) (Ctrl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps. aeruginosa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Klebsilla spp</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: (-) = No growth, (+) = Growth, ESC = Extract sterility control
Table 4.4 (b): Minimum Bactericidal Concentration (MBC) of Methanolic extract of CLLM

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Concentration of CLLM Extracts</th>
<th>MBC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250mg/ml 125mg/ml 62.5mg/ml 31.25mg/ml 15.625mg/ESC (Ctrl)</td>
<td></td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>-      -      -      -      +      +      -</td>
<td>31.25</td>
</tr>
<tr>
<td>E. coli</td>
<td>-      -      -      -      +      +      -</td>
<td>31.25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>-      +      +      +      +      +      -</td>
<td>250</td>
</tr>
<tr>
<td>Klebsilla spp</td>
<td>-      +      +      +      +      +      -</td>
<td>250</td>
</tr>
<tr>
<td>Salmonella Spp</td>
<td>-      +      +      +      +      +      -</td>
<td>250</td>
</tr>
</tbody>
</table>

Key: (-) = No growth, (+)= Growth, ESC = Extract sterility control
Table 4.4 (c): Diameters of zone of inhibition (cm) of CLLM Extracts using a single extract concentration of 250mg/ml

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Diameters of Zone of inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps. aeruginosa</td>
<td>1.4</td>
</tr>
<tr>
<td>E. coli</td>
<td>1.5</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.0</td>
</tr>
<tr>
<td>Klebsilla spp</td>
<td>0.0</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>0.3</td>
</tr>
</tbody>
</table>
(10% CLLM), T₃(20% CLLM) and T₄(30% CLLM). There were no significant differences (p>0.05) among the treatment means.

4.2.2. Average Body Weight Gain
Data on average body weight gain of the rabbits measured in grammes were 0.83kg (T₁), 0.67kg (T₂), 0.55kg (T₃), and 0.51kg (T₄). The weight gains were not affected (p>0.05) by the treatments.

4.2.3 Average Daily Feed Intake
The average daily feed intake of the rabbits were 71.9gm (T₁), 74.9gm (T₂) 71.3m (T₃) and 70.6m (T₄). There were no significant differences (p>0.05) among the treatments.

4.2.4. Average Daily Body weight Gain
The average daily body weight gains of the groups were, 14.7, 11.9, 9.8 and 9.1 grams, respectively for T₁, T₂, T₃ and T₄. There were no significant differences (p>0.05) among the groups. 4.2.5 Feed Conversion Ratio (FCR)

The feed conversion ratio were 5.62 (T₁), 6.42 (T₂), 7.42 (T₃), and 8.08 (T₄). There were no significance differences (P>0.05) among the treatment.

4.2.6 Haematological indices of Grower Rabbits
The effects of the different levels of Congronema latifolia leaf meal on the haematological parameters of grower rabbits are summarized in table 4.6.
4.2.6.1 Haemoglobin (HB) Concentration
The values for haemoglobin concentration were 12.83, 13.13, 12.87 and 12.53 g/dl, respectively for control (0%), T₂ (10%), T₃ (20%) and T₄ (30%), dietary levels of CLLM. There were no significant differences (p>0.05) among the treatment means.

4.2.6.2 Red Blood Cell (Erythrocyte) Concentration
The concentrations of the red blood cells (Erythrocytes) were 4.37 and 4.1 x 10⁹/mm for T₁ and T₂ respectively. T₃ recorded 3.7 x 10⁹/mm while the value for T₄ was 3.67 x 10⁹/mm. there were no significant differences (P>0.05) among the treatment means.

4.2.6.3 White Blood Cell (Leucocytes) concentration.
The total white blood cell (Leucocytes) concentration were 19000, 15,633.33, 10,466.67 and 8,833.33 X 10³ mm³, respectively for T₁, T₂, T₃ and T₄. Treatment 1 (0%CLLM) and treatment 2 (10% CLLM) differed significantly (P<0.05) from treatments 4 and 5 (20% and 30%) dietary levels of CLLM, respectively.

4.2.6.4 Erythrocyte Sedimentation Rate (ESR)
The values for erythrocyte sedimentation rate stood at 0.33 and 1.00 mm/hr for T₁, and T₂, respectively. Others were 0.67 mm/hr for treatments 3 and 0.33 mm/hr for T₄. There were no significant differences (p>0.05) among the treatment groups.
Table 4.5 The Effects of Different Dietary levels of CLLM on the Performance of Grower Rabbits

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Dietary Levels of CLLM</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁ (0%)</td>
<td>T₂ (10%)</td>
<td>T₃ (20%)</td>
<td>T₄ (30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Av. Initial body weight (g)</td>
<td>716.7</td>
<td>755.6</td>
<td>777.8</td>
<td>788.9</td>
<td>21.16</td>
<td></td>
</tr>
<tr>
<td>Av. Final body weight (g)</td>
<td>1542.2</td>
<td>1422.2</td>
<td>1328.8</td>
<td>1300.0</td>
<td>73.39</td>
<td></td>
</tr>
<tr>
<td>Av. Body weight gain (g)</td>
<td>825.6</td>
<td>666.7</td>
<td>550.0</td>
<td>511.1</td>
<td>82.51</td>
<td></td>
</tr>
<tr>
<td>Av. Daily feed intake (g)</td>
<td>71.9</td>
<td>74.9</td>
<td>73.3</td>
<td>70.8</td>
<td>1.72</td>
<td></td>
</tr>
<tr>
<td>Av. Daily weight gain (g)</td>
<td>14.7</td>
<td>11.9</td>
<td>9.8</td>
<td>9.1</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>Feed conversion ratio (g-feed/g-gain)</td>
<td>5.62</td>
<td>6.42</td>
<td>7.42</td>
<td>8.08</td>
<td>0.58</td>
<td></td>
</tr>
</tbody>
</table>
4.2.6.5  Packed Cell volume (PCV)

Values recorded for packed cell volume of experimental rabbits (PCV) were 37.67, 38.33, 37.33 and 37 %, respectively for T₁, T₂, T₃, and T₄. There were no significant differences (P>0.05) among the treatment means.

4.2.6.6  Mean Cell Volume (MCV)

The mean cell volume values were 86.6, 95.3, 101.67 and 101.33 Fl for T₁, T₂, T₃, and T₄, respectively. There were no significant differences (P>0.05) among the treatment groups.

4.2.6.7  Mean Cell Haemoglobin (MCH)

The values for Mean Cell Haemoglobin were 29.57 Pg (T₁), 30.73pg (T₂), 34.9pg (T₃) and 34.3pg (T₄). MCH values were significantly (P<0.05) high at T₃ (20%) and T₄ (30%) dietary levels of CLLM.

4.2.6.8 Mean Cell Haemoglobin Concentration (MCHC)

The values for Mean Cell Haemoglobin Concentration (MCHC) were 34.07, 34.23, 35.4 and 33.87g/dl for T₁, T₂, T₃ and T₄, respectively. There were no significant differences (P>0.05) among the treatment groups.

4.2.7 Serum Biochemical Indices of Grower Rabbits

The effects of different dietary levels of Congronema latifolia leaf meal on the serum biochemical parameters of grower rabbits are summarized in Table 4.7.
4.2.7.1 **Total Serum protein (TSP)**

The total serum protein values were 6.4, 6.98, 7.44, and 7.26 g/dl for T₁, T₂, T₃ and T₄, respectively. There were significant differences (P<0.05) among the treatment groups. TSP values were significantly higher at T₂, T₃ and T₄ than the control group (T₁).

4.2.7.2 **Serum Albumin**

The values for serum albumin were 4.83, 5.61, 5.62 and 6.16 g/dl for T₁, T₂, T₃ and T₄, respectively. There were no significant differences (P>0.05) among the treatment means.

4.2.7.3 **Serum Globulin**

The values for serum globulin were, 1.55 g/dl, 1.35 g/dl, 1.8g/dl and 1.10g/dl for T₁, T₂, T₃, T₄, respectively. There were no significant differences (P>0.05) among the treatment groups.

4.2.7.4 **Cholesterol**

Values obtained for cholesterol were 59.92, 65.01, 56.75 and 60.89 mg/dl for T₁, T₂, T₃ and T₄, respectively. There were no significant differences (P>0.05) among the treatment groups.

4.2.7.5 **Creatinine**

The serum creatinine levels were 0.72, 0.72, 0.93 and 1.06 mg/dl, for T₁, T₂, T₃ and T₄, respectively. There were significant differences (P>0.05) among the treatment groups.
Table 4.6: The Effects of Dietary Levels of CLLM on Haematological Indices of Grower Rabits

<table>
<thead>
<tr>
<th>parameters</th>
<th>Dietary Levels of CLLM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁ (0%)</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.83</td>
</tr>
<tr>
<td>RBC (10⁹/mm)</td>
<td>4.37</td>
</tr>
<tr>
<td>WBC (X10³/mm)</td>
<td>19,000ᵃ</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>0.33</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>37.67</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>86.60</td>
</tr>
<tr>
<td>MCH(pg)</td>
<td>29.57ᵇᵇ</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>30.07</td>
</tr>
</tbody>
</table>

Means in the same row bearing different superscripts are significantly different (P<0.05).
4.2.7.6 Urea Concentration
The concentrations of urea were 46.86, 46.93, 54.68 and 53.62(mg/dl), for T1, T2, T3 and T4, respectively. There were no significant differences (P>0.05) among the treatment means.

4.2.7.7 Glucose
The serum glucose levels were 64.0 mg/dl for T1 and T2 respectively. T3 recorded a concentration of 66.0g/dl while the value for T4 was 69.3mg/dl. Treatment means were not significantly different (P>0.05).

4.2.7.8. Serum Sodium (Na)
The values for sodium concentration were 149.0, 152.0, 150 and 147.0 mmol/l for T1, T2, T3, and T4 respectively. The values were statistically similar (P>0.05).

4.2.7.9 Serum Calcium (Ca) Concentration.
The serum calcium levels were 10.33mg/dl(T1), 10.80mg/dl (T2), 10.79mg/dl (T3) and 10.95mg/dl (T4). There were no significant differences (P>0.05) among the treatment means.

4.2.7.10 Serum Potasium (K) Concentration
The potassium concentrations were 5.8mmol/l for T1, 6.27mmol/l, T2, 6.43mmol/l for T3, and 6.1mmol/l for T4. The values were not significantly different (P>0.05).
4.2.7.11 Aspartate Transaminase (AST)
The serum Aspartate Transaminase (AST) concentrations were 22.33µl for T₁ and 23.0 µl for T₂ while T₃ and T₄ each recorded a value of 24µl. There were no significant differences (P>0.05) among the treatments.

4.2.7.12 Alanine Transaminase (ALT)
The values for serum Alanine transaminase were 6.0µl for T₁, 7.6 µl for T₂, 6.67µl for T₃ and 6.33µl for T₄. There were no significant differences (P>0.05) among the treatment groups.

4.2.7.13 Alkaline Phosphatase (ALP)
The alkaline phosphatases concentrations were 184.19, 179.52, 117.25 and 113.06 (µ/l) for T₁, T₂, T₃, T₄, respectively. The treatment means were not significantly different (p>0.05).

4.2.7.14 Total (Unconjugated) Bilirubin
The total (unconjugated) serum bilirubin values were 0.25 mg/dl for T₁, 0.26 mg/dl for T₂, 0.34 mg/dl for T₃ and 0.29 (mg/dl) for T₄. The values were not affected by treatments (p>0.05).

4.2.7.15 Direct (conjugated) Bilirubin
Values for direct serum bilirubin were 0.125, 0.069, 0.052 and 0.047 (mg/dl) for T₁, T₂, T₃ and T₄, respectively. There were no significant differences (p>0.05) among the treatment groups.
4.2.8 Carcass and organ weight characteristics

The effects of different dietary levels of *Congronema latifolia* leaf meal on the carcass and organ weights (% of live weights) of grower rabbits are summarized in table 4.8.

The live weights of the rabbits were 1.63, 1.63, 1.56, and 1.38 grams for T1 (0.0%) T2 (10.0%) T3 (20%) and T4 (30%) dietary levels of CLLM, respectively. There were no significant differences (p>0.05) among the treatment groups.

The dressed carcass weights (dressing %) were 62.03, 58.02, 58.26 and 56.66% for T1, T2, T3 and T4, respectively. Treatment means were not significantly different (p>0.05).

The weights of liver (% of live weights) were 1.97%, 2.48%, 3.31% and 3.85% for T1, T2, T3 and T4, respectively. Treatment groups differed significantly (p<0.05). The weights increased with increase in dietary levels of CLLM.

Hearts’ weights (% of live weights) were 0.23, 0.19, 0.22 and 0.21 percent for T1 (control), T2, T3 and T4, respectively. There were no significant differences (p>0.05) among the treatment means.

The weights of kidneys (% of live weights) were 0.48, 0.49, 0.5 and 0.59% for T1, T2, T3 and T4, respectively. The means were significantly different (p<0.05). The weights of the kidney increased with increase in CLLM levels in the diets.
Table 4.7: The Effects of Different Dietary levels of CLLM on the Serum Biochemical indices of Grower rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T_1(0%)</th>
<th>T_2(10%)</th>
<th>T_3(20%)</th>
<th>T_4(30%)</th>
<th>SEM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>6.40</td>
<td>6.98</td>
<td>7.44</td>
<td>7.26</td>
<td>0.18</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.83</td>
<td>5.61</td>
<td>5.62</td>
<td>6.16</td>
<td>0.48</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>1.55</td>
<td>1.35</td>
<td>1.84</td>
<td>1.10</td>
<td>0.45</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>59.92</td>
<td>65.01</td>
<td>56.75</td>
<td>60.89</td>
<td>7.53</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.72</td>
<td>0.72</td>
<td>0.93</td>
<td>1.06</td>
<td>0.29</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>46.86</td>
<td>46.93</td>
<td>5.46</td>
<td>53.62</td>
<td>3.38</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>64.00</td>
<td>64.00</td>
<td>66.00</td>
<td>69.3</td>
<td>3.06</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>149.00</td>
<td>152.00</td>
<td>150.00</td>
<td>147.00</td>
<td>2.24</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>10.33</td>
<td>10.20</td>
<td>0.79</td>
<td>10.95</td>
<td>0.47</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>5.80</td>
<td>6.27</td>
<td>6.43</td>
<td>6.10</td>
<td>0.69</td>
</tr>
<tr>
<td>AST (µ/l)</td>
<td>22.33</td>
<td>23.00</td>
<td>24.00</td>
<td>24.00</td>
<td>2.13</td>
</tr>
<tr>
<td>ALT (µ/l)</td>
<td>6.00</td>
<td>7.67</td>
<td>6.67</td>
<td>6.33</td>
<td>1.0</td>
</tr>
<tr>
<td>ALP (µ/l)</td>
<td>184.19</td>
<td>179.52</td>
<td>117.25</td>
<td>113.06</td>
<td>19.33</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>0.25</td>
<td>0.26</td>
<td>0.34</td>
<td>0.29</td>
<td>0.08</td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dl)</td>
<td>0.125</td>
<td>0.069</td>
<td>0.052</td>
<td>0.047</td>
<td>0.02</td>
</tr>
</tbody>
</table>

^ab^ Means in the same row with different superscripts are significantly different (P<0.05).
The weights of lungs (% of live weights) were 0.42%, 0.41%, 0.51% 0.51% for T1, T2, T3 and T4, respectively. There were no significant differences (p>0.05) among the treatment groups.

Pancreas’ weights (% of live weights) were 0.036%, 0.036%, 0.028% and 0.033% for T1, T2, T3 and T4, respectively. Treatment groups did not differ significantly (p>0.05).

4.2.9 Histopathological Studies (Rabbits)
The effects of graded levels of CLLM in the diets of grower rabbits on the histological integrity of livers, kidneys and pancreas tissues of the experimental rabbits are shown in table 4.9.

The photomicrograph of liver tissues from all treatment groups presented a liver lobule with its central vein at the center. The hexagonal shaped hepatocytes are shown radiating from the central vein. The hepatocytes had the normal open faced nuclei. Some of the hepatocytes had more than one nucleus. The radiating patterns of the hepatocytes were well outlined.

The kidney tissues showed venal cortex with rounded glomeruli. The endothelial cells were intact. The glomerular capsules were outlined and their nuclei prominent. The distal and proximal convoluted tubules were also differentiated with the nuclei well stained.
Pancreas tissues examined in $T_1$ animals showed acinar tissues in a background of congested and oedematous matrix with focal areas of haemorrhage, and the islet cells were not well stained. Tissues of $T_4$ animals showed poorly stained cytoplasm and nucleus, but no inflammatory cells were seen. However, tissues from $T_2$ and $T_3$ showed no degenerative changes.

### 4.3 EXPERIMENT 3 (BROILER TRIAL)

#### 4.3.1 Average Final body weights

The average final body weights of the birds (in Kg) were 1.90, 1.71, 1.62, 1.61 and 1.60 for the control $T_1$, $T_2$, $T_3$, $T_4$ and $T_5$, respectively. There were no significant differences ($P>0.05$) among the treatment means.

#### 4.3.2 Average Body Weight Gain

The average body weight gain of the birds were 1.76Kg ($T_1$), 1.61Kg ($T_2$), 1.51Kg ($T_3$), 1.50Kg ($T_4$) and 1.50Kg ($T_5$). The treatment groups were not significantly different ($P>0.05$).

#### 4.3.3 Average Daily feed intake

The average daily feed intake of the birds were 90.82g, 85.51g, 79.80g, 73.88g and 73.06g for $T_1, T_2, T_3, T_4$ and $T_5$, respectively. Average daily feed intake was significantly ($P<0.05$) affected by the treatments. Average daily feed intake dropped significantly ($P<0.05$) at 7.5% and 10% dietary levels.
Table 4.8: Effects of Different Dietary levels of CLLM on Carcass and Organ Weights of Grower rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary levels of CLLM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_1$ (0%)</td>
</tr>
<tr>
<td>Live weights (LW) Kg</td>
<td>1.63</td>
</tr>
<tr>
<td>Dressed carcass (% of LW)</td>
<td>62.03</td>
</tr>
<tr>
<td>Liver (% of LW)</td>
<td>1.97$^b$</td>
</tr>
<tr>
<td>Heart (% of LW)</td>
<td>0.23</td>
</tr>
<tr>
<td>Kidney (% of LW)</td>
<td>0.48$^{ab}$</td>
</tr>
<tr>
<td>Lungs (% of LW)</td>
<td>0.42</td>
</tr>
<tr>
<td>Pancreas (% of LW)</td>
<td>0.036</td>
</tr>
</tbody>
</table>

$^{ab}$Means within the same row with different superscripts are significantly different ($p>0.05$).
Table 4.9: Effects Different Dietary Levels of CLLM on Histopathological Structures of the kidneys, livers and pancreas of Rabbits

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary levels of CLLM (%)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Necroses (pancreas)</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oedematous cytoplasm (pancreas)</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Intercellular reaction (kidney)</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic necrosis (liver)</td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fatty change (liver)</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal fibrosis (liver)</td>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mononuclear cellular reaction (liver)</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(+) Relative presence of histopathological structure
(-) Absence of histopathological structure
4.3.4 Average Daily Body Weight Gain

The average body weight gains of the experimental birds were 36.63g, 32.80g, 30.84g, 30.68g and 30.63g, respectively for T1, T2, T3, T4, and T5. There were no significant differences (P>0.05).

4.3.5 Feed Conversion Ratio (FCR)

The feed conversion ratios (g feed / g gain) of the birds were 2.48 (T1), 2.61 (T2), 2.61 (T3), 2.41(T4) and 2.56 (T5). They were similar (P>0.05) across the treatments.

4.3.6 Haematological indices of experimental Broiler Birds

The effects of different dietary levels of Congronema latifolia leaf meal on the haematological indices of broiler birds are summarized in Table 4.10.

The values for haemoglobin concentration were 9.27, 8.87, 9.77, 8.97 and 8.9 g/dl respectively for T1(control), T2(2.5%), T3 (5%), T4 (7.5%) and T5 (10.0%) dietary levels of CLLM. There were no significant differences (P>0.05) among the treatment groups.

The concentrations of the red blood cells (erythrocytes) were 2.28 and 2.35 x 10^9/mm for T1 and T2, respectively. T3 recorded 2.44 x 10^9/mm, while the values for T4 and T5 were 2.43 and 2.27 x 10^9/mm, respectively. There were significant differences (P<0.05) among the treatment groups. Erythrocyte concentration was significantly depressed both at 0% and 10% dietary levels.
Table 4.10: The Effects of Different Dietary Levels of CLLM on Performance of Broilers Birds from the Starter to Finisher Phases.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary levels of CLLM</th>
<th>T1 (0%)</th>
<th>T2 (2.5%)</th>
<th>T3 (5.0%)</th>
<th>T4 (7.5%)</th>
<th>T5 (10%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. initial body weight (g)</td>
<td></td>
<td>105.0</td>
<td>103.3</td>
<td>105.0</td>
<td>104.3</td>
<td>104.0</td>
<td>0.36</td>
</tr>
<tr>
<td>Av. final body (Kg)</td>
<td></td>
<td>1.90</td>
<td>1.71</td>
<td>1.62</td>
<td>1.61</td>
<td>1.60</td>
<td>0.08</td>
</tr>
<tr>
<td>Av. body weight gain (Kg)</td>
<td></td>
<td>1.80</td>
<td>1.61</td>
<td>1.51</td>
<td>1.50</td>
<td>1.50</td>
<td>0.08</td>
</tr>
<tr>
<td>Av. daily feed intake (g/day)</td>
<td></td>
<td>90.8a</td>
<td>85.5b</td>
<td>79.8b</td>
<td>73.9b</td>
<td>73.1b</td>
<td>0.13</td>
</tr>
<tr>
<td>Av. daily body weight gain (g)</td>
<td></td>
<td>36.6</td>
<td>32.8</td>
<td>30.8</td>
<td>30.7</td>
<td>30.6</td>
<td>1.70</td>
</tr>
<tr>
<td>Feed conversion ratio (g feed/g gain)</td>
<td></td>
<td>2.5</td>
<td>2.6</td>
<td>2.6</td>
<td>2.4</td>
<td>2.6</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*abMeans in the same row with different superscripts are significantly different (P<0.05)
The total white blood cell (leucocytes) concentrations were 4198.33, 52394.67, 39296.00, 36430.67 and 26602.67 x 10^2 mm^3 respectively for T₁, T₂, T₃, T₄ and T₅. There were significant differences (P<0.05) among the Treatments groups. Treatments 2 (2.5% dietray level) recorded the highest lecucyte concentration, this was followed by T₁ (0% dietary level). For T₃, T₄, and T₅, the concentration decreased with increase in CLLM dietray level.

The values for erythrocyte sedimentation rate (ESR) stood at 2.0, 3.0, 2.33 (mm/hr) for treatments 1, 2 and 3, respectively. Others were 3.67 mm/hr (T₄) and 4.67 mm/hr (T₅). The treatment groups were not significantly different. (P>0.05).

Values recorded for packed cell volume (PCV) as a percentage of whole blood samples were 26.67, 27.67, 27.67 and 27.0 %, respectively for T₁, T₂, T₃, T₄, and T₅. There were no significant differences (P>0.05) among the treatment groups.

The mean cell volums (MCV) were 116.63, 117.77, 118.70, 122.37 and 111.07 for the five treatments, respectively. There were no significant differences (P>0.05) among the treatment groups.

The Mean cell haemoglobin (MCH) values were 40.61pg (T₁), 37.75pg (T₂), 39.96pg (T₃), 39.65pg (T₄) and36.61pg (T₅). There were no significant differences (P>0.05) among the treatment groups.
The Mean Cell Haemoglobin Concentration (MCHC) were 34.67g/dl (T₁), 32.05g/dl (T₂), 33.62g/dl (T₃), 32.47g/dl (T₄) and 33.34g/dl (T₅). There were no significant differences (P>0.05) among the treatment groups.

### 4.3.7 Serum Biochemical Indices of the Broiler Birds

The effects of different dietary levels of *Congronema latifolia* CLLM leaf meal on the serum biochemical parameters of the broiler birds are summarized in table 4.11.

The total serum protein (TSP) values were 2.99, 3.31, 3.21, 3.13 for T₄ and 2.99g/dl for T₁, T₂, T₃, T₄ and T₅, respectively. There were no significant differences (P>0.05) among the treatment means.

The values for serum albumin (SA) were 1.79, 1.89, 2.02, 1.96 and 1.57 (g/dl) respectively for T₁, T₂, T₃, T₄, and T₅. There were no significant differences (P>0.05) among the treatment means.

The serum globlin values were 1.27g/dl (T₁), 1.42g/dl (T₂), 1.20g/dl (T₃), 1.25g/dl (T₄) and 1.33g/dl (T₅), respectively. There were no significant differences (P>0.05) among the treatment groups.

Values obtained for cholesterol were 74.86mg/dl, 77.30mg/dl, 80.40mg/dl, 76.30mg/dl and 77.52mg/dl for T₁, T₂, T₃, T₄ and T₅, respectively. There were no significant differences (P>0.05) among the treatments.
The serum creatinine levels were 0.14, 0.18, 0, 0.31, 0.21 and 0.21 mg/dl. for \( T_1, T_2, T_3, T_4 \) and \( T_5 \), respectively. There were no significant differences (\( P>0.05 \)) among the treatment groups.

The concentrations of urea were 15.57, 18.48, 24.62, 22.4 and 21.71 (mg/dl), respectively for \( T_1, T_2, T_3, T_4 \) and \( T_5 \). There were no significant differences (\( P>0.05 \)) among the treatment means.

The values of glucose were 118.83, 104.79, 105.49, 88.63 and 83.61 mg/dl for \( T_1, T_2, T_3, T_4 \) and \( T_5 \), respectively. Treatment means were significantly different (\( P<0.05 \)). Serum glucose dropped with increasing dietary levels of CLLM.

Serum sodium (Na) concentrations (mmol/l) were 127.0 (\( T_1 \)), 133.87 (\( T_2 \)), 136.87 (\( T_3 \)), 138.70 (\( T_4 \)) and 137.20 (\( T_5 \)), respectively. There were no significant differences (\( P>0.05 \)) among the treatments.

The serum calcium (Ca) levels were 9.81 mg/dl (\( T_1 \)), 10.86 mg/dl (\( T_2 \)), 10.05 mg/dl (\( T_3 \)), 9.19 mg/dl (\( T_4 \)) and 10.79 mg/dl (\( T_5 \)). The levels were not affected by treatments (\( P>0.05 \)).

The Potassium (K) concentrations (mmol/l) were 3.03, 3.77, 3.53, 3.43 and 3.33 for \( T_1, T_2, T_3, T_4 \) and \( T_5 \), respectively. The values were not significantly different (\( P>0.05 \)).
Table 4.11 The Effects of Different Dietary Levels of CLLM on Haematological indices of Broiler birds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary Levels of CLLM</th>
<th>T1 (0%)</th>
<th>T2 (2.5%)</th>
<th>T3 (5.0%)</th>
<th>T4 (7.5%)</th>
<th>T5 (10%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td></td>
<td>9.27</td>
<td>8.87</td>
<td>9.77</td>
<td>8.97</td>
<td>8.90</td>
<td>0.52</td>
</tr>
<tr>
<td>RBC (10^9/mm)</td>
<td></td>
<td>2.28ab</td>
<td>2.35a</td>
<td>2.44a</td>
<td>2.43a</td>
<td>2.27ab</td>
<td>0.04</td>
</tr>
<tr>
<td>WBC (X10^3/mm^3)</td>
<td></td>
<td>41984.33ab</td>
<td>52394.67a</td>
<td>39296.0b</td>
<td>36430.67b</td>
<td>26602.67c</td>
<td>7969.55</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td></td>
<td>2.00</td>
<td>3.00</td>
<td>2.33</td>
<td>3.67</td>
<td>4.67</td>
<td>0.68</td>
</tr>
<tr>
<td>PCV (%)</td>
<td></td>
<td>26.67</td>
<td>27.67</td>
<td>29.00</td>
<td>27.67</td>
<td>27.00</td>
<td>1.57</td>
</tr>
<tr>
<td>MCV (FI)</td>
<td></td>
<td>116.83</td>
<td>117.77</td>
<td>118.70</td>
<td>122.37</td>
<td>111.07</td>
<td>6.39</td>
</tr>
<tr>
<td>MCH (g/dl)</td>
<td></td>
<td>40.61</td>
<td>37.75</td>
<td>39.96</td>
<td>39.65</td>
<td>36.61</td>
<td>2.02</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td></td>
<td>34.67</td>
<td>32.05</td>
<td>33.62</td>
<td>32.47</td>
<td>35.34</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Means in the same row with different superscripts are significantly different (P<0.05)
The serum aspartate transaminase (AST) concentrations were 76.0µl for T₁, 65.33µl for T₂, 65.0µl for T₃, 51.33µl for T₄ and 40.33µl T₅, respectively. The compound dropped significantly (P<0.05) at 7.5% and 10% dietary levels.

The values of serum alanine transaminase (ALT) were 4.33µl (T₁), 5.67µl (T₂), 4.67µl (T₃), 5.0µl (T₄) and 6.67µl for T₅, respectively. There were no significant differences (P>0.05) among the treatment groups.

The total (unconjugated) serum bilirubin values were 0.091mg/dl (T₁), 0.21mg/dl (T₂), 0.37mg/dl (T₃), 0.32mg/dl (T₄) and 0.31mg/dl (T₅). The treatment groups were significantly different (P<0.05). Total bilirubin was highest at 5%, 7.5% and 10% dietary levels. This was followed by 2.5% level while the control recorded the lowest value.

Values for direct (conjugated) bilirubin (mg/dl) were 0.03 (T₁), 0.06(T₂), 0.17(T₃), 0.15(T₄) and 0.15(T₅) respectively. There were significant differences (P<0.05) among the treatment groups. The concentrations of the compound followed the same trend recorded for total bilirubin.

### 4.3.8 Carcass and Organ Weights Evaluation

The effects of different dietary levels of *Congronema latifolium* leaf meal on the carcass and organ weights (% of live weights) of the broiler birds are summarized in table 4.12.

The live weights of the birds were 1.70, 1.65, 1.70, 1.72 and 1.52 Kg for T₁ (0.0%), T₂ (2.5%), T₃(5.0%), T₄(7.5%) and T₅(10.0%), respectively.
There were no significant differences (P>0.05) among the treatment groups.

The percentage dressed carcass weights (% of live weights) were 81.30, 88.01, 91.22, 88.21 and 84.62 for \(T_1\), \(T_2\), \(T_3\), \(T_4\) and \(T_5\), respectively. Treatment groups differed significantly (P<0.05). The dressing percentage was significantly high at \(T_2\) (2.5%), \(T_3\) (5.0%) and \(T_4\) (7.5%) dietary levels.

The weights of the neck (% of live weights) were 6.0, 6.4, 6.31, 6.26 and 5.76 (percent) for \(T_1\) (control), \(T_2\), \(T_3\), \(T_4\) and \(T_5\) respectively. There were no significant differences (P>0.05) among treatment groups.

Weights of the wings (% of live weights) were 8.48% (control), 8.71% (\(T_2\)), 8.58% (\(T_3\)), 8.60% (\(T_4\)) and 8.19% (\(T_5\)), Treatment groups were not significantly different (P>0.05).

Weights recorded for the thighs (% of live weights) were 21.61% for \(T_1\) (control), 22.53% (\(T_2\)), 22.41% (\(T_3\)), 21.94% (\(T_4\)) and 21.10% (\(T_5\)). There were no significant (P>0.05) treatment effects among the treatment groups.

The drum sticks had the following weights (% of live weights): 3.56%, 4.16%, 3.49%, 3.56% and 3.62% for \(T_1\) (control), \(T_2\), \(T_3\), \(T_4\), and \(T_5\), respectively. Treatment groups did not differ significantly (P>0.05).
Average weights of breasts (% of live weights) were 20.87%, 19.64%, 19.45%, 19.84% and 18.99% respectively for T_1 (control), T_2, T_3, T_4 and T_5. There were no significant differences (P>0.05) among treatment groups.

Weights of the hearts (% of live weights) were 0.51, 0.42, 0.42, 0.46, 0.48 and 0.57 for T_1 (control), T_2, T_3, T_4 and T_5, respectively. Treatment means showed no significant (P>0.05) treatment effects.

The weights of liver/gall bladder (% of live weights) were 1.67% (T_1), 1.72% (T_2), 1.82% (T_3), 1.70 (T_4) and 1.77 (T_5). There were no significant differences (P>0.05) among the treatment groups.

The weights of Spleen (% of live weights) were 0.16%, 0.14%, 0.14%, 0.16% and 0.14% for T_1 (control) T_2, T_3, T_4 and T_5 respectively. The means were not significantly different (P>0.05).

The gizzards had the following weights (% of live weights): 2.08%, 2.09%, 2.31%, 2.10% and 2.37% for T_1 (control), T_2, T_3, T_4 and T_5, respectively. Treatment groups did not differ significantly (P>0.05).

Weights of the lungs (% of live weights) were 0.79%, 0.92%, 0.94%, 0.95% and 1.0%, for T_1 (control), T_2, T_3, T_4 and T_5 respectively. There were no significant differences (P>0.05) among the treatment groups.
Table 4.12: The Effects of Different Dietary Levels of CLLM on the Serum Biochemical indices of Broiler birds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary levels of CLLM</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁(0%)</td>
<td>T₂(2.5%)</td>
<td>T₃(5.0%)</td>
<td>T₄(7.5%)</td>
<td>T₅(10.0%)</td>
<td>SEM</td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>2.99</td>
<td>3.31</td>
<td>3.21</td>
<td>3.13</td>
<td>2.99</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>1.79</td>
<td>1.89</td>
<td>2.02</td>
<td>1.96</td>
<td>1.57</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Globulin (mg/dl)</td>
<td>1.27</td>
<td>1.42</td>
<td>1.20</td>
<td>1.25</td>
<td>1.33</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>74.86</td>
<td>77.30</td>
<td>80.40</td>
<td>76.30</td>
<td>77.52</td>
<td>5.43</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.14</td>
<td>0.18</td>
<td>0.31</td>
<td>0.21</td>
<td>0.21</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>15.57</td>
<td>18.48</td>
<td>24.62</td>
<td>22.04</td>
<td>21.76</td>
<td>1.97</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>118.33</td>
<td>104.79</td>
<td>105.49</td>
<td>88.63</td>
<td>83.61</td>
<td>6.35</td>
<td></td>
</tr>
<tr>
<td>Sodium (Na)(mmol/l)</td>
<td>127.0</td>
<td>133.87</td>
<td>136.87</td>
<td>138.70</td>
<td>137.20</td>
<td>2.69</td>
<td></td>
</tr>
<tr>
<td>Calcium (Ca)(mg/dl)</td>
<td>9.81</td>
<td>10.86</td>
<td>10.05</td>
<td>9.19</td>
<td>10.79</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Potassium (K)(mmol/l)</td>
<td>3.03</td>
<td>3.77</td>
<td>3.53</td>
<td>3.43</td>
<td>3.33</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>76.0</td>
<td>65.33</td>
<td>65.0</td>
<td>51.33</td>
<td>40.33</td>
<td>7.40</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>4.33</td>
<td>5.67</td>
<td>4.67</td>
<td>5.0</td>
<td>6.67</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>0.09</td>
<td>0.21</td>
<td>0.37</td>
<td>0.32</td>
<td>0.31</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Direct Bilirubin (mg/dl)</td>
<td>0.03</td>
<td>0.06</td>
<td>0.17</td>
<td>0.15</td>
<td>0.15</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

<sup>ab</sup>Means in the same row with different superscripts are significantly different (P<0.05).
Weights recorded for the pancreas (% of live weights) were 0.21%, 0.26%, 0.21%, 0.22% and 0.24%, respectively for T₁ (control), T₂, T₃, T₄ and T₅. There were no significant treatment effects (P>0.05).

The proventriculus had the following weights (% of live weights): 0.39%, 0.46%, 0.47%, 0.49% and 0.58% for T₁ (control), T₂, T₃, T₄ and T₅, respectively. Treatment means differed significantly (P<0.05). The weights increased with increasing dietary levels of CLLM.

4.3.9 Histopathological studies (Broilers)

Effects of CLLM on histopathological structure of livers, kidneys proventriculus and pancreas tissues of the experimental birds are presented in Table 4.14.

The liver lobules across the treatments showed a central vein from where the hepatocytes radiated out in hexagonal pattern. The hepatocytes were well stained. Some hepatocytes had more than one nucleus; the nuclei had the normal open faced conformation.

Kidney tissues showed renal cortex which indicated a tuft of capillaries of the rounded glomerulus. Its endothelial cells were outlined. There was a narrow clearance between the renal (glomerular) capsule and the glomerulus, thus the urinary space was outlined. The proximal and distal convoluted tubules were differentiated and their nuclei were prominent.
Tissues of $T_1$ birds showed normal proventricular mucosal tissues. However, the sub-mucosa and muscularis externa indicated degenerative changes. Muscle tissues were atrophic. In $T_5$ birds proventricular tissues showed mildly distorted cytoarchitecture in the mucosal layer. However, the muscularis were intact. Other treatments indicated no marked degenerative changes in their tissue sections.

Pancreas tissues of $T_1$ birds showed diffused acinar tissue necrosis with islet cells degeneration. No inflammatory cells were seen. Tissue sections in $T_5$ also showed similar degenerative changes.

### 4.4.1 Proximate Composition of CLLM

The proximate composition of CLLM (table 4.1) showed that crude protein and nitrogen free extractive (NFE) values were 14.25 and 60.39 percent of dry matter respectively. These values are quite reasonable for a leaf meal. The protein content is higher than those of cereal grains while the NFE value is higher than those of most leaf meals and compares favourably with those of some alternative local feedstuffs like jackbean, Pigeon pea, bread fruit (pulp), etc. (Esonu, 2006). The crude fibre content of 2.84% is amazingly low for a leaf meal, far less than those of other leaf meals already investigated and compares favourably with those of cereal grains like maize, guinea corn, wheat, rice and barley (Esonu, 2006). The low fibre content of CLLM makes it well suited for monogastric feeding programs. Its metabolisable energy (ME) value of 2903.41 Kcal/kg compares favourably with those of conventional energy
concentrates such as maize, guinea corn, millet, wheat, etc., while its ash and mineral contents are comparable to those of most leaf meals.

### 4.4.2 Amino Acid Analysis

The analysis of CLLM protein detected 17 amino acids, made up of both essential and non-essential amino acids, almost in the ratio of 1:1. The most abundant amino acids were glutamic acid and aspartic acid. These were followed by leucine, arginine, alanine and valine respectively. Apart from arginine, other amino acids in this second group rank with those of oil seeds such as peanuts, soybean, buffalo gourd and colocynthis in value (Sawaya et al, 1986). The concentration of aspartic acid was comparable to those of the oil seeds earlier mentioned. The values of the sulphur containing amino acids, methionine and cystein were generally low. This trend is common to most leaf meal proteins already studied.

On the other hand, the value of lysine compared with those of the oil seeds except for Soyabean (Ukorebi, 2004). This implies that CLLM protein could be of a better quality than cereal proteins which are generally deficient in lysine. The significance of the amino acid profile of a protein cannot be over emphasized, as protein quality and its utilization in the animal’s physiological environment depends much on it, especially in monogastric nutrition.
Table 4.13: Effects of Different Dietary Levels of CLLM on Carcass and Organ Weights of Broiler birds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary Levels of CLLM</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_1 (0.0%)$</td>
<td>$T_2 (2.5%)$</td>
</tr>
<tr>
<td>Live weight (Lw)(Kg)</td>
<td>1.70</td>
<td>1.65</td>
</tr>
<tr>
<td>Dressing % (% of Lw)</td>
<td>81.30$^b$</td>
<td>88.01$^a$</td>
</tr>
<tr>
<td>Weight of neck (% of Lw)</td>
<td>6.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Weight of wings (% of Lw)</td>
<td>8.48</td>
<td>8.71</td>
</tr>
<tr>
<td>WeightHig thigh (% Lw)</td>
<td>21.61</td>
<td>22.53</td>
</tr>
<tr>
<td>Weight of drum stick (% of Lw)</td>
<td>3.56</td>
<td>4.16</td>
</tr>
<tr>
<td>Weight of breast (% of Lw)</td>
<td>20.87</td>
<td>19.64</td>
</tr>
<tr>
<td>Weight heart (% of Lw)</td>
<td>0.51</td>
<td>0.42</td>
</tr>
<tr>
<td>Weight of liver/gallbladder (% of Lw)</td>
<td>1.67</td>
<td>1.72</td>
</tr>
<tr>
<td>Weight of Spleen (% of Lw)</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>Weight of Gizzard (% of Lw)</td>
<td>2.08</td>
<td>2.09</td>
</tr>
<tr>
<td>Weight of lungs (% of Lw)</td>
<td>0.79</td>
<td>0.92</td>
</tr>
<tr>
<td>Weight of pancreas (% of Lw)</td>
<td>0.21</td>
<td>0.26</td>
</tr>
<tr>
<td>Weight of proventriculus(% of Lw)</td>
<td>0.39$^{bc}$</td>
<td>0.46$^b$</td>
</tr>
</tbody>
</table>

$^{abc}$ means in the same row with different superscripts are significantly different (P<0.05).
Table 4.1: Effect of different Dietary Levels of CLLM on Histopathological Structure of the Kidneys, Livers, Pancreas and Proventriculus of the Experimental broilers.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dietary levels of CLLM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>Diffused acinar tissue necrosis (pancreas)</td>
<td>+++</td>
</tr>
<tr>
<td>Islet cells degeneration (pancreas)</td>
<td>++</td>
</tr>
<tr>
<td>Muscularis externa degeneration (proventricular)</td>
<td>+</td>
</tr>
<tr>
<td>Nephritis (kidney)</td>
<td>-</td>
</tr>
<tr>
<td>Intercellular reaction (kidney)</td>
<td>++</td>
</tr>
<tr>
<td>Hepatic necrosis (liver)</td>
<td>+</td>
</tr>
<tr>
<td>Mononuclear reaction (liver)</td>
<td>+</td>
</tr>
<tr>
<td>Fatty liver change</td>
<td>+</td>
</tr>
</tbody>
</table>

+ Relative presence of histopathological structure  
- Absence of histopathological structure.
4.4.3 Phytochemical Composition of CLLM

The quantitative phytochemical analysis of CLLM for anti-nutritional factors presented in table 4.3 showed that alkaloid had the highest concentration of 1.03%, followed by tannins, saponins and flavonoids. Among the compounds measured in mg/100g, cyanogenic glycoside had the highest value (7.07mg/100g), followed by phenols and phytates.

Of the several thousands of alkaloids known, two of them (aristolochic acid and pyrroloidizidine) forms are of particular nutritional concern because of their toxicity (De Smet, 1992). Aristolochic acids are nephrotoxic, carcinogenic and mutagenic, whereas pyrroloidizidine is often implicated in hepatotoxicity and cause damage to the lungs and kidney (Adewumi, 2004; Mattoeks, 1986; Yff et al., 2002; Atawodi et al., 1993; Adebayo et al., 2003).

The result of this study, however, suggest that the alkaloids contained in CLLM are none of the toxic forms described above, as no pathological effects characterizing them were observed in the histological integrity of the internal organs of the experimental animals.

Tannins are reputed for their capacity to bind dietary proteins thereby reducing the nutritive value of feeds. However, the serum biochemical studies of the experimental animals showed a consistently high serum protein in the CLLM fed animals when compared to the control animals. This phenomenon suggests that the 0.55% content of tannins in CLLM is a tolerable level or that the bulk of the compound (tannins) is the condensed form which is less toxic than the hydolysable ones.
Flavonoids are classified into several groups that perform functions of pigmentation and defense in plants. Griffiths and Thomas (1981) reported that such groups include anthocyanins that attract insects, flavones and flavonols that protect the plant from excessive ultra violet radiation and iso-flavonoids that provide antifungal and antibacterial defenses to the plant. This indicates that flavonoids may contribute to the antibacterial properties of CLLM.

Saponins are bitter and reduce the palatability of livestock feeds. This may account for the significantly (P<0.05) low feed intake of broiler chickens fed graded levels of CLLM in their diets, and the slight depression in their growth rate.

The HCN value of CLLM (7.07mg/100g) recorded in this study is higher than those of some legume seeds reported by Liener (1977).

4.4.4 Bio-activity Studies of CLLM
The results indicated that the methanolic extract of CLLM is bio-active against all the organisms tested. The findings are in agreement with the work of Osuala (2005). This suggests that the methanolic extracts of CLLM could be used as feed additive in livestock and poultry feeding programmes. It is important to note that the poverty devastating most of the developing countries of Africa and Asia has continued to place the costly input driven Western medicaments out of the reach of these countries (Sofowora, 1962, Fajimi and Taiwo, 2004).
Okoli et al. (2002) also pointed out that scientists’ interest in the search for local remedies to most endemic ailments over the years is due partly to their affordability and sustainability as against the prohibitive cost of most synthetic drugs. Many workers (Fajimi and Taiwo, 2004; Janovska, et al., 2003; Olukoya et al., 1993; Otshudi et al., 2000; Itelim and Onwuliri, 2003; Onwuliri and Umezurumba, 2003; Onwuliri 2004) have reported the medicinal potentials of plants. Furthermore, the bacterial resistance to most conventional antibiotics (Okoli, 2004) also gives credence to the studies on herbal remedies as an alternative to conventional drugs.

4.4.5 Rabbit Trial

Table 4.5 summarises the performance of grower rabbits fed graded levels of CLLM in their diets.

4.4.5.1 Body Weight Gain

There were no significant differences (P>0.05) among treatment groups for average final body weights, average body weight gain and average daily body weight gain. However there was a general dose-related depression in growth of the rabbits induced by dietary levels of CLLM. The mean daily weight gain range of beween 9.1 to 14.7g obtained in this study is similar to 10.1g reported by Adama and Nma (2002) when groundut leaves were fed to rabbits, 12.0g reported by Omok and Ajayi (1976) in a feeding trial with rabbits involving dried brewers grain and the result of 10.2 to 12.8g obtained by Ayoade et al. (2007) in a study on the evaluation of the feed value of sugar cane scrapping meal for weaner
rabbits. Also comparable to daily growth rates pattern of rabbits in this experiment is the result obtained by Udedibie et al (2005) which reported a mean daily growth rate of 9.06 to 10.31g per rabbit in a study which compared the performance of young growing rabbits fed diets containing cracked and cooked jackbean and Jackbean soaked in water prior to cooking, but lower than 17.65 to 18.80g recorded by Agunbiade et al (1999) on cassava leaf and peel meals.

4.4.5.2 Average Daily Feed Intake

There were no significant differences (P>0.05) among the treatment groups for average daily feed intake. However, apart from T2 (10.0% CLLM) which recorded the highest daily feed intake, the feed consumption trend indicated a general decline with increasing level of CLLM. This feed intake pattern is similar to the report of Ekenyem (2006) which showed a dose related drop in feed intake of grower pigs fed graded levels of Ipomoea asarifolia leaf meal diets; Oduns et al (2002) in which a decline in feed consumption rate of laying birds was observed when Gliricidia sepium leaf meal diet was compared with a control ration and Esonu et al (2002) which observed poor feed intake and feed utilization of broilers fed Microdesmis puberula leaf meal at high inclusion level. However, the decreases in feed consumption in the reports cited above were all at significant levels (P<0.05).

Of a better comparison with the result of this study is the work of Olabanji et al (2007) which reported that there was no difference (p>0.05) among the treatments in the daily feed intake of weaner rabbits.
fed different levels of wild sunflower (*Tithonia diversifolia* Hemsb A.Gray) leaf-blood meal mixture.

The lower feed intake of the diets containing higher CLLM inclusion levels might be attributable to the combined effects of low palatability, higher energy contents and perhaps the antinutritional factors (ANFS) content of the diets. The consumption of feed by animals is a function of its palatability, energy, fibre and ANFS contents among others. It is important to note that *C.latifolia* leaves have a strong bitter taste which could discourage their consumption by animals. It will also be recalled that the calculated analysis of the treatment diets for the experiment indicated that their energy values increased linearly with the level of CLLM, while the fibre content followed the opposite trend. It is well known that rabbits have preference for diets with reasonable fibre content than those very low in fibre. This might account for their higher consumption of the control diet which was richer in fibre than the test diets.

**4.4.5.3 Feed Conversion Ratio**

There were no significant differences (*p*>0.05) in feed to gain ratios among the treatment groups. The range of values (5.62 - 8.08) obtained in this study is comparable to 4.63 - 7.09 reported by Ortserga *et al* (2008) on garlic (*Allium sativum*), but higher than 3.45 - 5.12 reported by Adejumo (2002) on gliricidia leaf meal. It is also higher than 4.43 - 5.25 reported by Iheukwumere *et al* (2002) on rice milling waste, but superior to the value of 11.27 - 16.35 reported by Nweze (2005) on some grass and legume species supplemented with compounded diet.
This shows that the test ingredient (CLLM) can compete with some non-conventional feed ingredients.

4.4.5.4 Mortality
Two rabbits died from the control treatment (T1). The mortalities were traceable to mechanical injuries.

4.4.6 Carcass Characteristics and Organ Weight
The meat yield (inclusive of head, legs and skin) determined as dressing percent was statistically similar (p>0.05) among treatments but tended to decrease as the inclusion level of CLLM in the diets increased.

The dressing percentage values obtained in this study compared with the values reported by Ani (2006 and 2007) on growing rabbits fed graded levels of raw bambara nut waste and toasted bambara nut waste respectively. It is also similar to the values reported by Dairo et al (2005) when growing rabbits were fed varying levels of rumen content and blood-rumen content mixture.

The dressing percentage value (56.66%) recorded at 30% CLLM level is higher than the value (54.2%) reported by Arijeniwa and Igene (2002) for rabbits fed 30% raw bambara nut. There were significant differences (p>0.05) among the treatments in the weights of the liver and kidney. Both organs increased in weight as the inclusion levels of CLLM increased. This is suggestive of stress factor(s) in the diets. Bamgbose and Niba (1995) associated significantly heavier liver weights of birds fed
diets containing high levels of raw cotton seed meal with presence of toxic factors in the diets.

Other organs (heart, lungs and pancreas) did not show any significant treatment effects ($p>0.05$). This is an indication of the nutritional safety of the test diets.

### 4.4.7 Histopathological Observations

Results of the histopathological studies conducted were represented by presence or absence of lesions on the organs investigated. Lesions are described as structural or functional abnormalities in cells, tissues or organs which is an indication of static presentation of a dynamic process (Akpavie, 2008). When many cells in an organ or tissue undergo degeneration or necrosis, a visible lesion is produced which in turn interferes with homeostasis.

Table 4.9 shows observed histopathological state of the livers, kidneys and pancreas tissues of the grower rabbits fed graded levels of CLLM in their diets.

There were no lesions of pathologic significance in liver tissues of experimental animals. The increase in the number of nuclei in the hepatocytes showed that the cells were not necrotic but rather normally dividing. It would therefore seem that CLLM is not hypathotoxic.

Kidney tissues of the experimental animals showed no degenerative changes in all the treatments. The clearance between the glomerular
capsule and the glomerulus indicated the presence of a functional urinary space. In addition, the non-distortion of the entire tissue cyto-architecture is indicative of a normal renal integrity. Pancreas tissues of T₂(10% CLLM) and T₃(20% CLLM) experimental animals showed no degenerative changes. However, poor staining of tissues and oedematous cytoplasm were observed in T₁(0% CLLM) and T₄(30% CLLM). This condition might not be attributable to toxic factors in CLLM as the diet of T₁ animals did not contain the test material. The abnormalities could be traceable to poor histopathological processing of tissues prior to staining and microscopic examination.

4.4.8 Haematological Indices of the Grower Rabbits

There were no significant differences (P>0.05) among the treatment groups in haemoglobin concentration (Hb), red blood cell (RBC) value, packed cell volume (PCV), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC). The highest numerical haemoglobin (HB) value was recorded by T₂ (10.0% CLLM), followed by T₃ (20.0% CLLM). The control treatment (0.0% CLLM) was third in the rank, while the last and least in the order was T₄ (30.0% CLLM). This result suggests a better blood building potential of the CLLM at up to 20% dietary level for grower rabbits.

Lindsay (1977) reported that Hb falls gradually in animals on a low protein intake, parasitological infestation and / or liver damage or anaemia. This is in agreement with the report of Machebe et al (2009)
that dietary protein may affect the physiological process in erythropoietin production.

The highest numerical value of mean cell volume (MCV) was recorded by T₃ (20.0% CLLM). This was followed by T₄ (30% CLLM) and T₂ (10.0% CLLM), while the control treatment (0.0% CLLM) recorded the least value.

The mean cell haemoglobin (MCH) values of the rabbits on test diets (T₂ - T₄) were all significantly (P<0.05) higher than those in the control treatment. The highest value was recorded by T₃, followed by T₄ and T₂, while T₁ was the least in value. From this result, it would seem that the optimum inclusion level of CLLM in the diets of grower rabbits should be 20%.

In general, all values of the red cell indices obtained in this study indicated a healthy status of the experimental animals. This suggests that the diets were nutritionally safe and adequate.

The white blood cell (leucocyte) concentration showed a declining trend with increasing levels of CLLM in the diets. The highest concentration was recorded in the control treatment, while T₄ (30% CLLM) had the lowest. This indicates that there was an infection challenge in the research unit which triggered off the production of Leucocytes, the number of which corresponded to the level of clinical infection. This result is corroborative of the findings in experiment I, (Bacterial sensitivity study of this
research) and Osuala et al (2005) which showed that the methanolic extracts of CLLM exhibited a marked anti-microbial activity against bacterial organisms tested in an in-vitro assay.

4.4.9 Serum Biochemical Parameters of the Grower Rabbits
All test diets (T₂ - T₄) recorded significantly (P<0.05) higher serum protein levels than the control treatment. This suggests that CLLM protein is a high quality protein, otherwise it would have been removed from the system by the liver as urea or ammonium ion through the process of deamination. According to Beitz (1993), a specific amount of protein is required by the body to maintain protein homeostasis. The increase in total blood protein reflects the ability of the animals to store reserve protein when they have reached the maximum capacity for protein intake (Fasae et al., 2005, Ogunbanjo et al., 2009).

The serum urea levels obtained in this study were similar (P>0.05). This suggests that the compound was within the normal range. The concentration of urea in the blood is a function of the quantity and quality of the daily dietary protein as well as the renal and hepatic intergrity. When dietary protein quality is poor much of it will be converted to urea and excreted by the kidney. These vital processes are hampered in a situation of liver and / or kidney damage leading to either a drastically reduced or an accumulation of urea in the blood. According to Ogunbanjo et al (2009), when the amount of urea excreted is within normal range, it is an indication of proper functioning of the kidneys.
There were no significant differences (P>0.05) in the level of albumin among the treatment groups. However, there was a gradual numerical increase in the compound with increasing level of CLLM in the diets. It is known that albumen holds water in the blood and decreases in liver and kidney diseases. Ezekwesili (2005) reported that a case of severe malnutrition decreases albumin fraction in the blood. The result of this test indicated that CLLM has the potential of boosting albumin level in blood serum of grower rabbits.

Serum globulin levels from the different treatments were similar (P>0.05). Serum globulin level is also a function of dietary protein quality. This indicates that CLLM protein is of good quality. Globulin is a protein which, in combination with albumin, promotes normal water retention in the blood. Iyayi et al (1998) observed that globulin and albumin as well as urea concentrations are indications of the quality and quantity of protein supplied in the diets.

The values of serum creatinine across the treatments were not significantly different (P>0.05). However, apart from T₁ (control) and T₂ (10.0% CLLM), there was a dose-related numerical increase in the concentration of the compound. An abnormal increase in serum creatinine indicates renal disease or malfunction and / or myocardial infarction or congestive heart failure. Adebayo (2008) stated that the higher the values of urea and creatinine, the better the quality of protein of test feed stuff. Since creatinine values
from animals in the test diets were similar to those in the control, it would seem that the values were within the normal range. There were no significant differences (P>.05) among the treatments in the concentration of serum glucose. T₁ and T₂ recorded the same value. The highest level of glucose was obtained from T₄. This was closely followed by T₃. The level of glucose in the control treatment vis-à-vis the test groups, indicates that it was normal. The Cholesterol levels were similar (P>0.05) in all the treatments. No particular trend was established. Cholesterol test is normally used to evaluate the risk of heart disease. The values obtained from this study suggest that the levels were safe.

There was no significant (P>0.05) treatment effect on the serum electrolytes (sodium, calcium and potassium) studied. These ions are needed to maintain proper osmotic and electrolyte balance in the body fluid of animals (Machebe, et al., 2009). The similarity of serum electrolyte level of test groups to the control indicates that CLLM diets are capable of supplying adequate proportions of the ions. There were no significant differences (P>0.05) among the treatments in aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). The serum activity of the above listed enzymes are indicators of hepatocellular integrity and function.
Enemor et al. (2005) stated that AST and ALT are enzymes commonly found in the liver and leak out into the general circulation when liver cells are injured. It has also been observed that a high value of ALP suggests increased activity of the liver due to presence of toxic substance (Ologhobo et al., 1993, Onwukwe 2000 and Owen et al., 2009).

The findings of the study indicated that CLLM diets were not hepatotoxic to the grower rabbits up to 30% dietary inclusion level. There were no significant (p>0.05) treatment effects in the values of both total (unconjugated) bilirubin and direct (conjugated) serum bilirubin. According to Enemor et al. (2005), bilirubin is formed primarily from the breakdown of the haem fraction of haemoglobin. The researchers maintained that conditions which cause increased formation of bilirubin, such as destruction of red blood cells or decrease of its removal from the blood stream, such as liver disease, may result in an increase in its level. The similarities in values of unconjugated and conjugated serum bilirubin of the test groups to the control suggested that up to 30% dietary inclusion of CLLM in the diets of grower rabbits does not interfere with hepatic function and erythrocyte activities. This observation agrees with values and inference obtained for AST, ALT and ALP in this study.

4.4.10 Experiment 3: Broiler Trial

Table 4.9 summarises the performance characteristics of broilers fed graded levels of CLLM in their diets.
**4.4.10.1 Body weight gain**

The final body weights, average body weight gain were similar (P>0.05) among the treatment groups. However, there were progressive decreases in their numerical values with increasing dietary levels of CLLM. This result is different from observations made by Nworgu and Fapohunda (2002) when broiler chicks were fed graded levels of *Mimosa invisa* leaf meal; Nworgu (2004,2007) reported significantly (P<0.05) depressed performance of broiler chickens fed *Centrosera pubescens* leaf meal at 5% dietary level and above. The results obtained in this study might be attributable to lower fibre level of CLLM, its higher, digestibility, lower concentration of anti-nutritional factors and hence better utilization. This result appears slightly superior to the observations of Ngodigha (1994) with Centrosera leaf meal, which showed that broiler chickens fed 0.0, 5.0, 10.0 and 15% Centrosera leaf meal supplements had average final body weights of 1690, 1630, 1490 and 1280g/bird respectively. CLLM also seems to have a good potential in broiler chickens production when compared to the reports of Donkor *et al* (1999) which recommended 2.5% *Cnidoscotus aconitifolius* leaf meal for optimal performance of broiler chickens.

**4.4.10.2 Average Daily feed intake**

Average daily feed intake decreased significantly (P<0.05) with increasing dietary levels of CLLM. The result agreed with the findings of Esonu *et al* (2002) that feed intake and feed utilization of broiler chicks fed *Microdesmis puberula* leaf meal was significantly (P<.05) poor at high dietary level of inclusion and those of Odunsi *et al* (2002), which showed
a significant reduction in laying birds’ feeding rate when a test diet containing *Gliricidia sepium* was compared with control diet. Ekenyem (2006) also observed a similar drop in feed intake of grower pigs fed graded levels of *Ipomea asarifolia* leaf meal. The result also agrees with findings of other leaf meal studies (Mishara, *et al.*, 1977; Onwudike, 1995; Raharjo *et al.*, 1987; Herbert, 1998) among others.

Lower feed intake observed in this study is attributable to lower fibre and higher energy contents of CLLM as birds eat to satisfy their energy needs. The low palatability CLLM could also contribute to the low intake of the test diets.

**4.4.10.3 Feed Conversion Ratio (FCR)**

The feed conversion ratios (g feed/g gain) were similar (P>0.05) among the treatments. However, treatment 4 (7.5% CLLM) recorded the most superior feed-to-gain ratio.

The FCR values obtained in this study are similar to those reported by Nworgu (2007) with Centrosema leaf meal as protein supplement for broilers. The values are superior to others earlier reported (Uchegbu *et al.*, 2001; Adejinmi *et al.*, 2008; Fanimo *et al.*, 2007; Ekanyem *et al.*, 2008) when broiler chicks were evaluated on some non-conventional feed stuffs. This shows that the test ingredient (CLLM) compares favourably with other non-conventional ingredients for broiler diets such as *Centrosem* *pubescens* leaf meal, rice offal, cocoa bean shell, *Microdesmis puberula* leaf meal, cashew nut testa and *Napoleona imperialis* seed meal.
4.4.10.4 Body Pigmentation

Broiler chickens placed on the test diets (CLLM containing diets) developed strong yellowish colouration of the skin, beaks and shanks which clearly distinguished them from the birds on the control diet. This occurrence agrees with the observations of Udedibie and Opara (1998) on *Alchornea cordifolia* and D’Mello and Acamovic (1989) on *Leucaena leucocephala*. This could be a factor for consumer preference.

4.4.10.5 Mortality

Mortalities (8 birds) recorded were in treatment 1 (control treatment). They were as a result of mechanical injuries.

4.4.10.6 Carcass Characteristics

The dressing percentages were significantly (P<0.05) different among the treatment groups. T₃ (5.0% CLLM), T₄ (7.5% CLLM) and T₂ (2.5% CLLM) recorded the highest dressing percentage. These were followed by T₅ (10.0% CLLM) and T₁ (0.0% CLLM) which were statistically similar (P>0.05). In general, birds from the test groups had numerically higher dressing percentages than those from the control. Weights of necks, wings, thighs drum sticks, and breasts were similar (P>0.05) across the treatments. The findings suggest that CLLM diets were nutritionally adequate for the wholistic development of the birds, to achieve a desirable carcass value. Apart from proventriculus, there were no significant weight differences (P>0.05) among the treatment groups for organs (heart, liver/gallbladder, spleen, gizzard and lungs) studied. It
therefore appears that the test diets had no obvious deleterious effects which could clearly manifest in organ weights.

Weights of proventriculus showed significant (P<0.05) treatment effects among the treatment groups. The weights increased progressively with increasing level of CLLM in the diets. This is suggestive of slow gastric digestion of CLLM diets and consequent accumulation of the feed in the organ for a longer period of time than the control. The reason for this is unclear.

4.4.10.7 Histopathological observations of the organs

Data on the histopathological integrity of the livers, kidneys, proventriculus and pancreas of the experimental birds are presented in table 4.10.

No gross lesions of pathologic significance were observed in all the liver and kidney tissues studied. The normal structures and contours of the organs were maintained. The organs showed no discolouration, neither were there adhesions between them. The liver and kidney are the primary organs of biotransformation in animals. Clear lesion on these organs would be an obvious indication of the toxicity of the test material. The absence of the notable degenerative changes in these organs (for birds tested) is attributable to their normal roles in the elimination of metabolic wastes and toxins from the animal body.

However, there were some changes in focal mononuclear reactions of hepatocytes which were rather pronounced in control birds that had no
test material in their diet. Also, necrotic spots observed (in hepatocytes), had no trend. Furthermore, intercellular reaction noticed in the kidney was more pronounced in the control birds. These changes therefore might have been caused by factors other than the test material. Degenerative changes observed in the proventricular and pancreas tissues were common to both the control and T₅ birds. They were, however, not of pathologic significance. These observations suggest that the inclusion of CLLM in the diets of broiler birds (up to 10%) will not be toxic to them.

4.4.10.8 Haematological Indices of the Broilers

There were no significant differences (P>0.05) among the treatment groups in haemoglobin (Hb) concentration. The highest numerical Hb value was recorded by T₃ (5.0% CLLM), followed by T₁ (control), T₄ (7.5% CLLM), T₅ (10.0% CLLM) and T₂ (2.5% CLLM), respectively. The values were within literature range as reported by Mitruka and Ransley (1977). Erythrocyte (RBC) values were significantly (P<0.05) affected by the treatments. The highest RBC values were recorded by T₃ (5.0%), T₄ (7.5%) CLLM and T₂ (2.5%) CLLM, respectively, which were statistically similar. These were followed by T₁ (control) and T₅ (10.0% CLLM) which were within the range of standard values (Mitruka and Ransley, 1977). The higher RBC values recorded by T₂ to T₄ are suggestive of the blood building potential of CLLM up to 7.5% inclusion level for broiler birds. This agrees with the reports of Ezekwesili (2005).
Haematological indices (packed cell volume (PCV), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration) were statistically similar (P>0.05) across the treatments. There were also no significant differences (P>0.05) among the treatment groups in erythrocyte sedimentation rate (ESR). White blood cell (WBC) value was highest at 2.5% dietary level, followed by the control, T₃, and T₄, respectively. The values dropped significantly (P<0.05) at T₅(10%) dietary level. Proliferation of leucocytes in the system is body defence reaction against invading pathogenic microbes. Since the level of production of the anti bodies is commensurate to the magnitude of infection challenge, this result suggests that C. latifolia has anti-bacterial properties.

All haematological parameters studied showed that the test diets were nutritionally adequate and safe when compared to the control diets.

4.4.10.9 Serum Biochemical parameters of the Finisher Broilers

There were no significant differences (P>0.05) among the treatment groups in total serum protein. However, T₂ (2.5% CLLM), T₃(5.0% CLLM) and T₄(7.5% CLLM) recorded higher numerical values of this metabolite than T₁(control) and T₅(10.0% CLLM).

The similarity of serum protein values in the test diets to that of the control is an index of similarity in their qualities.

The serum urea levels of the treatments were not statistically different (P>0.05). This indicates that their concentrations were normal. Normal urea level in the blood portrays adequate dietary protein quantity and
quality. Elevated urea concentration in the blood, statistically above the normal range, is an indication of excessive deamination of serum protein as a result of its poor quality in terms of its amino acid profile and consequent inadequacy for normal protein metabolism in the animal system.

There were no significant differences (P>0.05) in the levels of serum albumin, serum globulin as well as serum creatinine across the treatments. Ezekwesili (2005) reported that severe malnutrition decreased albumin fraction in the blood. Globulin combines with albumin in the blood to promote normal water retention in the blood. Iyayi and Tewe (1998) observed that globulin and albumin as well as urea concentrations in the blood are indicators of the quality and quantity of proteins supplied in the diets.

The values of the parameters obtained in this study and the normal health status of the birds across the treatments indicate that CLLM diets were safe and nutritionally adequate.

Glucose levels obtained showed significant (P<0.05) treatment effects. T₁(control), T₂ (2.5% CLLM) and T₃(5.0%) CLLM recorded statistically higher glucose level than T₄ (7.5% CLLM) and T₅(10.0% CLLM). The cause of the significantly lower glucose concentration in T₄ and T₅ is unclear.

There were no significant differences (P>0.05) among the treatments in cholesterol levels. Cholesterol test is relevant in the evaluation of the risk
of heart disease. The similarity of the values obtained in the study is suggestive of normal and safe concentration.

Serum electrolytes (sodium, calcium and potassium) showed no significant differences (P>0.05) among the treatment groups. This is an indication of proper osmotic and electrolyte balance in the body fluid of the birds (Machebe et al., 2009) and the normal development of their skeletal system.

Aspartate transaminase (AST) dropped significantly (P<0.05) at 7.5% and 10% dietary levels. There were no significant (P>0.05) differences among the treatment groups in alanine transaminase (ALT) and alkaline phosphatase (ALP). The liver is a prime site for the destruction of toxic compounds found in the blood. However, when the challenge of such deleterious factors become excessive, the cells of the organ are damaged and the above listed enzymes which are contained in it leak into the general circulation. The serum concentration of the enzymes is therefore a function of hepatocellular integrity and function. The similarity of the values for the serum enzymes from the experiment suggests that whatever toxic factor(s) are contained in CLLM were of mild concentrations and were adequately tolerated by the birds up to 10% dietary inclusion level. This observation agrees with the reports of several authors (Enemor et al., 2005; Ologhobo et al., 1993; Onwukwe, 2000; Owen et al., 2009) on serum or liver enzyme activities.

The values of the total (unconjugated) bilirubin and direct (conjugated) bilirubin were significantly (P<0.05) different among the treatments.
Total bilirubin was highest at 5%, 7.5% and 10% dietary levels of CLLM. This was followed by 2.5% level while the control had the lower value. A similar trend was recorded for direct bilirubin. Bilirubin is primarily a product of the breakdown of the haem moiety of haemoglobin. The result of this study suggests that conditions which cause increased formation of bilirubin, such as destruction of red blood cells or decrease of its removal from the blood stream, such as liver disease occurred during the experiment. But since findings on the histopathological analysis of the livers of the experimental birds did not indicate damaged or diseased livers, the elevated bilirubin levels would be traceable to conditions which cause the destruction of the haem moiety of haemoglobin. The reason why the highest numerical value was recorded at 5% rather than 10% dietary level is unclear.
CHAPTER FIVE

5.0 Conclusions and Recommendations

5.1 Experiment 1: Bio-activity studies of CLLM

5.1.1 Conclusions

The results of this study have shown that:

1. the methanolic extract of CLLM could inhibit the growth of enteric bacteria of poultry and rabbits such as *E. coli* at a minimum inhibitory concentration (MIC) of 15.62mg/ml while *Staphilococcus aureus*, *Klebsiella spp* and *Salmonella spp* could be inhibited at a concentration of 250mg/ml;

2. *Pseudomonas aeruginosa, E. coli, Staphilococcus aureus, Klebsiella spp* and *Salmonella spp* could be killed at a minimum bactericidal concentration (MBC) of 31.25, 31.25, 250, 250 and 250 mg/ml, respectively and

3. the standard concentration of the methanolic extract of CLLM (250mg/ml) produced a zone of inhibition of 1.4, 1.5, 0.0, 0.0 and 0.3cm for the five organism listed respectively.

5.1.2 Recommendations

It is suggested that CLLM be also extracted using other media such as water and ethanol. Bio-activity studies with water extract of CLLM in particular will show whether its anti-bacterial property is as a result of certain compounds in the leaf meal or due to the methyl alcohol (methanol), the medium of extraction used in this experiment.
In addition, it would be necessary to characterise the active ingredients in CLLM that are responsible for its anti-bacterial activity so that it could be extracted and concentrated for possible use in the manufacture of “organic antibiotic” and as a feed additive.

5.2 Experiment 2: Rabbit Trial

5.2.1 Conclusions

Based on the result of this trial, it was concluded that:

1. up to 20% dietary level CLLM could support normal performance of grower rabbits;
2. kidneys and livers of grower rabbits were stressed at dietary levels of CLLM above 20%;
3. blood building of grower rabbits could be enhanced at 20% and 30% dietary levels of CLLM; in addition, CLLM also appeared to profer bacteriostatic/bacteriocidal effect at 10%, 20% and 30% dietary levels;
4. dietary levels of 10%, 20% and 30% CLLM could also enhance the total serum protein of grower rabbits, but had no effect on other serum biochemical parameters and
5. up to 30% dietary level of CLLM did not significantly affect the histological integrity (P<0.05) of the livers, kidneys and pancreas of grower rabbits.

5.2.2 Recommendations

It is recommended that experiments be carried out to ascertain the digestibility of CLLM in the diets of rabbits. In addition, the aspect of
histopathology should also include other internal organs such as the small intestine, the large intestine and the caecum of rabbits.

Furthermore, it would seem necessary to investigate the reproductive potentials of rabbits fed CLLM-based diet.

5.3 Experiment 3: Broiler Trial

5.3.1 Conclusions

The results of this study have shown the following:

1. that performance parameters of broiler birds such as the average final body weight, average body weight gain and feed conversion ratio were not significantly (P>0.05) affected by dietary inclusion of CLLM up to 10%;

2. that 10% dietary level of CLLM significantly (P<0.05) depressed the average daily feed intake of the birds;

3. that 10% dietary level also significantly (P<0.05) depressed the RBC concentration in the blood of the birds, but did not affect other haematological indices except the leucocyte concentration;

4. that CLLM could provide coverage against bacterial attack on the experimental broilers as shown by the WBC concentration in the blood of the birds in the different treatment groups;

5. that 7.5% and 10% dietary levels of CLLM caused a significant drop (P<0.05) in the serum glucose level of the broilers while AST was significantly (P<0.05) depressed at 5%, 7.5% and 10% levels, whereas serum bilirubin significantly (P<0.05) increased at 5%, 7.5% and 10% levels;
6. that dietary level of CLLM, up to 10% significantly (P<0.05) increased the dressing percentage of the experimental birds and
7. that the histological integrity of experimental birds were not significantly (P>0.05) affected by the treatments.

5.3.2 Recommendation

It is recommended that this research be also carried out with laying birds to study their production parameters.

5.4 General Conclusion and Recommendation

5.4.1 General Conclusions

The results of the experiment have shown that:

1. methanolic extract of CLLM is bio-active against the enteric bacteria of rabbits and poultry;
2. rabbits can perform normally at CLLM dietary level of up to 20% and
3. 7.5% inclusion level of the leaf meal can support normal broiler production.

5.4.2 General Recommendations

1. the use of CLLM in rabbits and poultry diet is hereby recommended in the tropics in view of its nutritional and medicinal values and
2. the medicinal potential of the leaves of C. latifolia should be further investigated and exploited through further research.
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effect of processing on the retention of Ca-rotenoid fraction of
*Leucaena leucocephala* during storage and the effects on mimosine

HISTOLOGICAL EXAMINATIONS

Materials and reagents

1. Rotatory microtome (Cambridge medical instruments)
2. Incubator (Hearson, England)
3. Tissue floating bath (Electrothermal, England)
4. Microscope (Lietz Dialux, 20)
5. Camera (Nikkon Japan)
6. Film (Kodak, Japan)
7. Absolute ethanol (BDH)
8. Chloroform (May and Baker)
9. Concentrated H$_2$SO$_4$ (May and Baker)
10. Eosin TD (East Anglia Chemicals)
11. Halmatoxylin EHRLICH (Raymond A. Lamb)
12. Xylene (May and Baker)

Procedure

1. Fixation

Tissues were fixed in Bouin’s fluid for twenty four hours.

2. Processing

i) Dehydration: Following fixation, tissues were dehydrated in ascending grades of alcohol (70, 95% and absolute alcohol), in two changes, one hour each.

ii) Clearing: The dehydrated tissues were then cleared in two changes of xylene, one hour each.

iii) Impregnation/Embedding: After dehydration, tissues were infiltrated in a molten paraffin wax at $60^\circ$C after which they were embedded in a pair of L-shaped piece of rust-proof metal containing pure paraffin wax. The paraffin wax embedding the
tissues was carefully trimmed to size and fixed to a wooden block.

3. Sectioning
The composite structures consisting of the tissues and accessories were fixed to a microtome and serial sections of 5-6 micron thickness were obtained.
After every 50 seconds, 3 sections per tissue were selected for mounting on slides. Selected sections were floated in a water bath at 50°C.
Cleaned, albuminized microscope slides (75x25mm) with a thickness of 1.2mm were used to place the tissues when picked from the water bath. The sections on the slides were dried; face down over a hot plate at 50°C for about 12hours.

Staining:
Haematoxylin and Eosin method was applied on the sections.

Steps:
i) After drying, the sections on the slides were deparaffinzed by passing the slides in xylene for one minute and then rehydrated by passing through descending grades alcohol (Absolute, 95% and 70% ethanol), for about 10 seconds in each, and finally distilled water.
ii) Sections were stained in haematoxylin for 10 minutes washed briefly in water and differentiated in 1% acid alcohol for 30 seconds. Thereafter, the slides were placed under running tap water until blue colouration appeared (within about 10 minutes).
iii) Sections were then counter stained in 1% alcoholic eosin for three minutes and rinsed briefly in water thereafter.
iv) Finally the sections were dehydrated by dipping several times in ascending grades of alcohol and cleared in two changes of xylene.
A drop of DPX mounting media was placed on a cover slide and each slide was inverted over it to pick it up. The slides containing the sections were left to dry and then viewed under the microscope. Suitable slides were photomicrographed with a camera mounted on a microscope with T-adapter. Nuclei appeared blue-black (violet), while cytoplasm, tissue fibres appeared pink.