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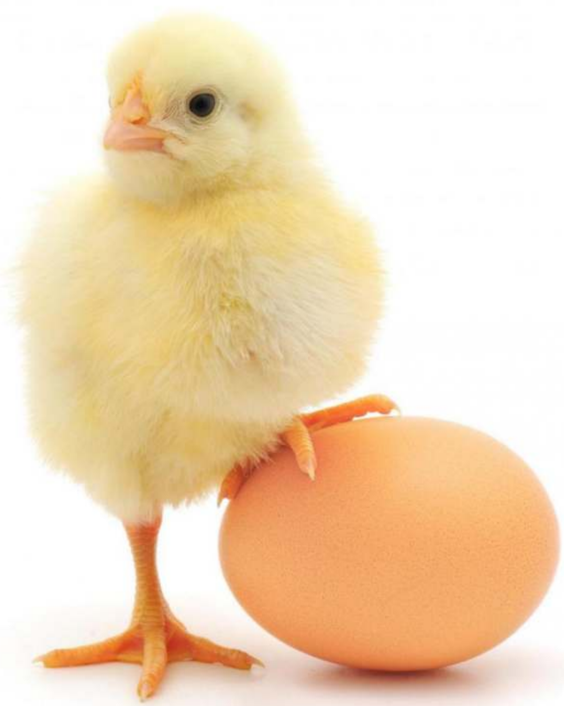

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# Equi-protein Replacement of Fishmeal with *Leucaena* Leaf Protein Concentrate: An Assessment of Performance Characteristics and Muscle Development in the Chicken

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**Abstract:** A batch of three hundred 7- day old Shaver broiler-chicks was used to assess the equi-protein replacement of fishmeal (FM) protein with *Leucaena* leaf protein concentrates (LLPC) in broiler-starter mash. Before the feeding trial, the LLPC was analyzed for its protein, amino acids, phytin and tannin contents. Thereafter, five diets were tested. The control (diet 1) had 5% FM protein whose protein was gradually replaced at 25, 50, 75 or 100% with LLPC in diets 2 to 5. Thus the inclusion level of LLPC was 2.24, 4.48, 6.72 or 8.96%, respectively for diets 2 to 5. The chicks were randomly assigned in triplicate of 60 chicks per dietary treatment. The average weight gain (AWG), average feed consumption (AFC) and feed conversion ratio (FCR) of chicks fed the control diet were significantly ($P<0.05$) higher than those fed the LLPC-based diets. The AWG of the broilers fed on the control diet decreased to 80.6% in chicks fed 2.24% LLPC-based diet and to 39.3% in chicks fed 8.96% LLPC based diet with a corresponding decrease of AFC by 88.6 and 62.9%, respectively. Nitrogen retention (NR) of chicks fed the control diet and those fed 2.24, 4.48 or 6.72% LLPC-based diets were similar. At the close of the feeding trial, the chicks were slaughtered for carcass traits, relative organ and muscle measurements as well as blood and serum analyses. Only the weights of the chest, belly fat, spleen, pancreas and gizzard were significantly ($P<0.05$) different. The weight of chest and belly fat decreased with increased FM protein substitution with LLPC while the relative weight of gizzard increased with increased LLPC substitution for FM protein. The relative weight, length and breadth of the *Supra coracoideus, Pectoralis thoracicus* and *Gastrocnemius* of chicks fed the control diet and those fed LLPC-based diets were similar. The blood variables and the total serum protein (TSP) of the chicks fed the control diet were not significantly ($P>0.05$) different from those fed the LLPC-based diets. In the main, the use of 2.24% LLPC in broiler-chick diets, especially in region with limited supply of fishmeal, in a complementary fashion with other ingredients rich in the limiting essential amino acids (EAAs) is suggested.

**Key words:** Fishmeal; *Leucaena* leaf protein concentrate

## Introduction

The current acute short supply of animal protein especially in the developing countries of the world has necessitated several investigations into the potentials of some novel feed resources for livestock. In particular, the quantities of fish that are available are not adequate for human consumption. Consequently, poultry feed manufacturers have been forced to seek alternative but cheaper protein resources, which can reasonably supplement or replace the inadequate supply of the more expensive fishmeal.

One of such alternatives can be found in green vegetables and in particular the leaf protein concentrates (LPCs) (Fellows, 1987; Oke, 1973; Fasuyi, 2000). For instance, cassava leaves, a byproduct of cassava root harvest, depending on the varieties are rich in protein (18-40% dry matter), minerals and vitamins (Aletor and Fasuyi, 1997; Fasuyi, 2000). Also, leaf protein concentrates fractionated from the leaves of *Glyricidia sepium* and *Leucaena leucocephala* contained high crude protein (48 and 34%, respectively) and their amino acid profiles were reported to be comparable with those of soybean and generally surpass FAO essential amino acid reference (Agbede, 2000).

Various unconventional vegetable protein resources such as soybean meal and cassava leaf protein concentrate (Aletor *et al*., 1989; Fasuyi, 2000) and more recently Glyricidia leaf protein concentrate (Agbede and Aletor, 2003) have been used to replace fishmeal in the diets of broiler-chickens with varying decree of success. However, information on the use of leaf protein concentrate especially from *Leucaena leucocephala*, a browse plant which have agronomical characteristic of been available in most time of the year, as possible replacement for fish meal in the diets of broiler-chicken appears scanty in spite of its nutritive potential.

Thus, this paper presents the study on equi-protein replacement of fish meal with *Leucaena* leaf protein concentrate in the diets of broiler-chicks with a view to

Table 1: Basal composition of experimental diets for broiler - chick (g/100g)

| | Diets | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| | % fish meal replaced with *Leucaena* leaf protein concentrate (LLPC) | | | | |
| Ingredients | 0 | 25 | 50 | 75 | 100 |
| Maize (11%) | 56.10 | 55.11 | 54.12 | 53.13 | 52.14 |
| Groundnut cake (42%) | 28.00 | 28.00 | 28.00 | 28.00 | 28.00 |
| Fish Meal FM (68%) | 5.00 | 3.75 | 2.50 | 1.25 | - |
| LLPC (37.92%) | - | 2.24 | 4.48 | 6.72 | 8.96 |
| Brewers Dried Grain (29%) | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Oil | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| Bone meal | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| Oyster shell | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| DL Methionine | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Premix* | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated | | | | | |
| % Crude Protein | 23.0 | 22.8 | 22.6 | 22.5 | 22.3 |
| ME ($MJkg^{-1}$) | 13 | 13 | 13 | 13 | 13* |

*Contained vitamins A (4,000,000iu); D (800,000iu); E (14000iu); K (760mg); $B_{12}$ (7.6mg); Riboflavin (2800mg); Pyridoxine (1520mg); Thiamine (880mg); D Pantothenic acid (4400mg); nicotinic acid (18,000mg); Folic acid (560mg); Biotin (45.2mg); and Trace elements as Cu (3200mg); Mn (25600mg); Zn (16,000mg); Fe (12800mg); Se (64mg); I2 (320mg) and other items as Co (160mg); Choline (190,000mg); Methionine (20,000mg); BHT (2,000mg) and Spiramycin (2,000mg) per 1.0kg.

ascertaining the effects of such intervention on the performance characteristics, carcass traits, relative organs weights, chest and hind muscles, haematology and biochemical variables.

## Materials and Methods

**Leaf Protein Production:** The *Leucaena* leaf protein concentrate (LLPC) was produced using a village-scale fractionation method (Fellows, 1987). Hot whey was siphoned using rubber hose while the protein coagulum was separated from other fractions by filtering through a muslin bag; followed by pressing with screw-press (Aletor, 1993). The LLPC was rinsed, pulverized, sun-dried and milled. Thereafter, the concentrate was used to formulate diets along with other ingredients purchased from Olukayode Farm, Ondo Road, Akure, Nigeria.

**Experimental Diets:** Five experimental diets were formulated with the basal as well as the proximate composition shown in Table 1 and 2, respectively. Diet 1 was the control with 5% fishmeal (FM). The fishmeal protein was substituted at 25, 50, 75 or 100% with LLPC in diets 2, 3, 4 and 5, respectively. The amino acid contents of the diets were calculated using the amino acid profile of the purchased ingredients from Feed Industry Red Book (1995) and the LLPC amino acid analyzed and presented as part of Table 2.

**Management of chicks and Experimental layout:** A batch of three hundred day-old broiler chicks (150 males + 150 females) was used for the experiment. The chicks were electrically brooded at the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria for the first week. They were brooded on the floor for the first 4 days and sexed as described by Laseinde and Oluyemi (1997), and later transferred to a metabolism cage for 3 days to allow the chicks adjust to the experimental conditions. During this period they were offered approximately $22.6gkg^{-1}$ crude protein commercial broilers-starter mash (Guinea Feed) and water *ad libitum*. Completely randomized design (CRD) was adopted for the trial with a total of 15 experimental units. At the end of the adjustment period, the chicks were weighed and 30 males and 30 females (total = 60) were assigned to each of the five triplicate dietary treatments (10 males + 10 females / replicate), such that the initial mean group weights per diet were identical (81.1±0.1g). The chicks were fed their respective experimental diets *ad libitum* for 21 days during which the daily feed consumption and group weight changes were measured. Faeces voided during the last five days were collected, weighed, dried at 55-60 $^{o}C$ in an air-circulating oven for 72 hrs and preserved while the corresponding feed consumed was recorded for the nitrogen studies.

Table 2: Proximate composition and Amino acid contents (g/100g) of the experimental diets

| | Diets | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Dry matter | 90.2 | 90.8 | 90.9 | 91.3 | 91.8 |
| Crude protein | 22.1 | 22.2 | 22.1 | 22.2 | 22.1 |
| Ether extracts | 10.3 | 9.9 | 6.1 | 5.4 | 4.8 |
| Crude fibre | 7.4 | 8.1 | 8.3 | 9.8 | 10.5 |
| Ash | 3.1 | 3.5 | 3.8 | 4.6 | 5.0 |
| Nitrogen free extract | 56.6 | 55.7 | 55.0 | 52.6 | 50.3 |
| Amino acids | | | | | |
| Arginine | 2.11 | 2.09 | 2.09 | 2.08 | 2.08 |
| Histidine | 0.57 | 0.57 | 0.56 | 0.56 | 0.55 |
| Isoleucine | 1.08 | 1.07 | 1.07 | 1.06 | 1.06 |
| Leucine | 1.95 | 1.94 | 1.94 | 1.94 | 1.94 |
| Lysine | 1.31 | 1.20 | 1.12 | 1.03 | 0.95 |
| Methionine | 0.45 | 0.45 | 0.43 | 0.43 | 0.42 |
| Cystine | 0.16 | 0.15 | 0.14 | 0.12 | 0.11 |
| Methionine + cystine | 0.61 | 0.60 | 0.57 | 0.55 | 0.53 |
| Phenylalanine | 1.18 | 1.16 | 1.15 | 1.14 | 1.12 |
| Threonine | 0.86 | 0.86 | 0.86 | 0.86 | 0.85 |
| Tyrosine | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 |
| Valine | 1.26 | 1.25 | 1.26 | 1.26 | 1.26 |
| Glycine | 0.34 | 0.32 | 0.31 | 0.28 | 0.26 |
| Tryptophan | 0.72 | 0.72 | 0.71 | 0.70 | 0.70 |

**Blood Collection for Analysis:** At the close of the feeding trial, the chicks were starved overnight so as to empty the crop and weighed and sacrificed first by stunning followed by severing the jugular vein. The blood was then allowed to flow freely into labeled bijour bottles one of which contained a speck of EDTA while the others were without EDTA. The blood in the EDTA-containing bijour bottles was processed for haematology while those in bottles without EDTA were processed for serum analysis. The serum was kept deep-frozen prior to analysis and the analysis was done with minimum delay.

**Carcass Characteristics and Organs Measurements:** After slaughtering and bleeding the chicks, the carcasses were scalded at 65 °C in water bath for 30 seconds before defeathering. The dressed chicks were later eviscerated. Ten (10) eviscerated carcasses per replicate were used for the measurement of the carcass characteristics viz: dressed weight %, eviscerated weight %, thigh, drumstick, shank, chest, back, neck, wing, belly fat and head and organ measurements. The organs were dissected out and any blood blotted with tissue paper. The organs measured were the liver, kidneys, lungs, pancreas, heart, spleen, bursa of fibricus and gizzard. All the carcass characteristics as well as the organs measured were expressed as g $kg^{-1}$ body weight except the dressed and eviscerated weights, which were expressed as percentages of the live weights.

**Muscle measurement:** Ten eviscerated carcasses per replicate were weighed and cooled under the fan for 3 min. before dissection. The dressed and eviscerated weights were taken before dissecting out the chest as well as thigh muscles. The inner chest muscle (*Supra coracoideus*), outer chest muscle (*Pectoralis thoracicus*) and thigh (*Gastrocnemius*) were carefully dissected out from their points of origin and insertion. Measurements of the fresh weight, length and breadth of these muscles were taken and recorded.

**Haematological and Serum analyses:** The packed cell volume (PCV), was estimated by spinning about 75 $\mu^1$ of each blood sample in heparinized capillary tubes in a haematocrit micro centrifuge for 5 minutes while the total Red Blood Cell count (RBC) was determined using normal saline as the diluting fluid. The Haemoglobin concentration (Hbc) was estimated using cyanomethaemoglobin method while the Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were calculated as described by Lamb (1981). Similarly, the Erythrocyte Sedimentation Rate (ESR) of the blood as well as the total protein, albumin and globulin of serum were determined as described (Lamb, 1981).

**Chemical and Statistical analysis:** The proximate composition of the ingredients, diets and faecal

Table 3: Performance characteristics and N-utilization broiler-starter fed with fishmeal protein substituted with *Leucaena* Leaf Protein Concentrate

| % Fish meal protein replaced with LLPC | Initial weight (g) | Final weight (g) | Average weight gain (g/chick/day) | Average feed consumption | Feed conversion (g/chick/day) | N-retention (g/chick/day) ratio |
|---|---|---|---|---|---|---|
| 0 | 81.0+0.4 | 502.6+9.1$^{a}$ | 20.1+0.4$^{a}$ | 43.7+1.5$^{a}$ | 2.2+0.0$^{a}$ | 0.5+0.2$^{a}$ |
| 25 | 81.1+0.2 | 420.3+39.9$^{b}$ | 16.2+1.9$^{b}$ | 38.7+5.3$^{ab}$ | 2.4+0.2$^{ab}$ | 0.6+0.3$^{a}$ |
| 50 | 81.2+0.2 | 360.0+40.6$^{bc}$ | 13.3+1.9$^{bc}$ | 35.9+3.8$^{ab}$ | 2.7+0.1$^{ab}$ | 0.4+0.2$^{ab}$ |
| 75 | 81.0+0.8 | 317.6+18.8$^{c}$ | 11.3+0.9$^{c}$ | 34.9+0.7$^{b}$ | 3.1+0.2$^{b}$ | 0.4+0.3$^{ab}$ |
| 100 | 81.3+0.0 | 247.5+23.0$^{d}$ | 7.9+1.1$^{d}$ | 27.5+4.9$^{b}$ | 3.5+0.2$^{b}$ | 0.3+0.3$^{b}$ |

Means with differing superscripts in the same vertical column are significantly (P<0.05) different

Table 4: Carcass traits of broiler - chicks fed with fishmeal protein substituted with *Leucaena* Leaf Protein Concentrate

| Traits | Diets | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| | % fish meal replaced with LLPC | | | | |
| | 0 | 25 | 50 | 75 | 100 |
| Dressed weight % | 91.9±2.3 | 91.5±2.0 | 92.5±2.3 | 92.0±2.2 | 86.3±9.0 |
| Eviscerated weight % | 85.1±3.3 | 80.9±4.9 | 78.4±3.9 | 79.4±4.0 | 50.0±43.3 |
| Thigh (g/kg body wt) | 40.8±9.6 | 45.9±2.3 | 41.2±3.8 | 40.0±4.2 | 40.6±0.6 |
| Drum stick " | 95.6±12.8 | 94.4±3.1 | 87.5±1.1 | 9.3±8.5 | 78.6±2.9 |
| Shank " | 28.8±3.5 | 30.6±1.1 | 32.1±5.3 | 27.8± 2.2 | 30.7±9.0 |
| Chest " | 118±15.4$^{a}$ | 98.5±8.4$^{ab}$ | 95.9±8.2$^{ab}$ | 92.8±4.4$^{ab}$ | 81.0±16.8$^{b}$ |
| Back " | 80.7±18.0 | 66.7±10.3 | 60.2±16.4 | 55.6±10.6 | 53.0±9.1 |
| Belly fat " | 10.1±2.3$^{a}$ | 6.1±2.0$^{ab}$ | 4.7±0.6 $^{ab}$ | 3.3±1.5$^{b}$ | 3.1±4.0 $^{b}$ |
| Head " | 41.8±1.4 | 51.4±7.8 | 51.0±9.1 | 56.2±2.5 | 55.0±6.0 |
| Neck " | 56.2±4.1 | 49.2±2.7 | 53.2±17.3 | 54.3±14.1 | 66.0±22.7 |
| Wing " | 36.5±2.5 | 38.3±3.1 | 38.9±2.8 | 34.4±0.5 | 36.3±4.4* |

Means with differing superscripts in the same column are significantly different (P<0.05). LLPC = *Leucaena* Leaf Protein Concentrate

samples were determined by the method of AOAC (1990). Nitrogen retained was calculated as the algebraic difference between feed nitrogen and faecal nitrogen (on dry matter basis) for a period of seven day towards the close of the feeding trial. The amino acid contents of the LLPC were analyzed as described by Agbede and Aletor (2003). The determination of some anti-nutritional factors typified by phytin as described (Makkar and Goodchild, 1996) and tannin by the methods (Young and Greaves, 1940; Makower, 1970; Wheeler and Ferrel, 1971) were done. Data collected on the performance indices, muscle growth, haematology and total serum protein were subjected to analysis of variance (Steel and Torrie, 1980). Where significant differences were found, the means were compared using the Duncan's Multiple Range Test (DMRT) (Duncan, 1955). However, data on muscle growth when found not to be significant were also subjected to coefficient of variation analysis (Snedecor and Cochran, 1973).

## Results

**Amino Acids, Phytin and Tannin contents of leaf protein concentrate:** The LLPC contained: lysine 5.99, histidine 2.11, arginine 5.54, aspartic acid 9.52, threonine 4.61, serine 4.25, glutamic acid 10.62, proline 5.07, glycine 5.51, alanine 5.88 cystine, 1.04 methionine 2.25, valine 5.75, isoleucine 9.11, tyrosine 4.18, phenyalaline 5.76 and tryptophan 1.85 g/16gN. It also contained 2.5 g/100g DM tannin as tannic acid equivalent and 6.9-mg/100mg phytin.

**General Observation:** The chicks fed the LLPC-based diets had yellow pigmentation on their skin, beaks and shanks. The degree of pigmentation increased with increasing dietary level of leaf protein concentrate.

**Performance of Chicks and N-utilization:** Table 3 shows that the final weight, Average Weight Gain (AWG), Average Feed Consumption (AFC) and Feed Conversion Ratio (FCR) were significantly (P<0.05) influenced by the

Table 5: Relative Organ weight (gkg$^{-1}$ body wt) of broiler starter fed with fishmeal protein substituted with *Leucaena* Leaf Protein Concentrate

| | Diets | | | | |
|---|---|---|---|---|---|
| Organs | 1 | 2 | 3 | 4 | 5 |
| | % fish meal replaced with LLPC | | | | |
| | 0 | 25 | 50 | 75 | 100 |
| Liver | 22.4±4.0 | 22.7±3.0 | 26.0±3.7 | 29.0±5.1 | 24.1±3.6 |
| Kidney | 7.2±2.5 | 11.2±1.3 | 11.4±1.7 | 12.1±3.0 | 12.0±1.5 |
| Heart | 6.3±0.6 | 6.9±0.4 | 8.7±1.4 | 7.4±0.7 | 9.6±3.5 |
| Spleen | 1.0±0.4$^{b}$ | 1.5±0.5$^{ab}$ | 1.1±0.4$^{b}$ | 1.3±0.4$^{ab}$ | 2.4±0.7$^{a}$ |
| Pancreas | 2.4±1.3$^{b}$ | 3.8±0.6$^{ab}$ | 5.3±0.2$^{a}$ | 4.3±0.6$^{ab}$ | 4.6±0.9$^{a}$ |
| Bursa | 2.3±0.8 | 2.5±0.6 | 2.3±0.4 | 2.6±0.4 | 3.0±1.7 |
| Gizzard | 45.0±5.5$^{b}$ | 52.5±3.9$^{b}$ | 54.9±7.9$^{b}$ | 61.4±5.5$^{ab}$ | 69.8±5.7$^{a}$ |
| Lungs | 7.9±1.0 | 8.1±0.5 | 7.2±1.6 | 9.0± 2.8 | 8.5±1.3 |

*Means with differing superscripts in the same column are significantly different ( P < 0.05), LLPC = *Leucaena* Leaf Protein Concentrate

dietary treatment. The AWG of chicks fed the control diet was significantly (P<0.05) higher than those fed the test or LLPC-based diets. Among the test diets, the AWG was highest (16.2±1.9 g/chick/day) in chicks fed 2.24% LLPC-based diet and lowest (7.9±1.1 g/chick/day) in chicks fed 8.96 LLPC-based diet. Also the AWG of chicks fed 2.24-4.48% LLPC-based diets were identical but those fed 2.24% LLPC-based diet was significantly (P<0.05) higher than those fed 6.72-8.96% LLPC-based diets. Generally, the AWG decreased with the increased FM protein substitution with LLPC.

The AFC of chicks fed on the control diet and those fed on diets containing 2.24-4.48% LLPC were not significantly (P>0.05) different but was significantly (P<0.05) higher than those fed 6.72-8.96% LLPC-based diets. However, the AFC for all the chicks fed the test diets was identical. Like the AWG, the AFC decreased progressively with increased FM protein substitution with the LLPC. The trend observed for the FCR was similar to those observed for the AFC with the highest value (2.2±0.0) in chicks fed the control diet and decreased progressively to 3.5±0.2 in chicks fed the 8.96% LLPC-based diet (Diet 5).

The Nitrogen Retention (NR) of the chicks fed the control diet and those fed 2.24-6.72% LLPC-based diets were similar (Table 3). Furthermore, the NR of chicks fed 4.48, 6.72 or 8.96% LLPC levels was also similar.

**Carcass Traits and Relative Organ Measurement:** Of the entire carcass traits measured only the chest and belly fat were significantly (P<0.05) influenced by the dietary treatment (Table 4). While the weight of the chest of the chicks fed the control diet (118.3±15.4g kg$^{-1}$ body weight) was not significantly (P>0.05) different from those fed 2.24-6.72% LLPC-based diets, it was significantly (P<0.05) higher than those fed 8.96% LLPC-based diet. The weight of the chest decreased with increased LLPC in the diets. The belly fat of chicks fed the control diet was similar to those fed 2.24-4.48% LLPC-based diets but significantly (P<0.05) higher than those fed 6.72-8.96% LLPC-based diets. Also, the observed values for the belly fat decreased with increased LLPC levels in the diets.

The relative weights of organs are presented in Table 5. Only the relative weights of the spleen, pancreas and gizzard were significantly (P<0.05) influenced by the dietary treatments. The relative weight of spleen of chicks fed the control diet were the least (1.0±0.4 g kg$^{-1}$ body weight) and this was significantly (P<0.05) lower than those fed 9.86% LLPC level but identical to those fed 2.24, 4.48 or 6.72% LLPC-based diets. The relative weight of the pancreas did not follow a particular trend but was lowest (2.4±1.3g kg$^{-1}$ body weight) in chicks fed the control diet and highest (5.3±0.2 g kg$^{-1}$ body weight) in chicks fed on 4.46% LLPC-based diet. The relative weight of the gizzard increased with increased FM protein substitution with LLPC in the diets with chicks fed the control having the lowest value (45.0±5.5 g kg$^{-1}$ body weight) and chicks fed on 8.96% LLPC level having the highest value of 69.8±5.7 g kg$^{-1}$ body weight.

**Relative Weight, Length and Breadth of muscles:** Table 6 shows that there were no wide variations in the relative weights of *Supra coracoideus, Pectoralis thoracicus* and *Gastrocnemius* of chicks fed FM or LLPC proteins as evident in their relatively low coefficient of variations (CVs) of 15.2, 8.0 and 6.9%, respectively. However, the relative weights of the *S. coracoideus* and *P. thoracicus* deceased with increased FM protein substitution with LLPC. Also, Table 6 shows that the CV of the relative length and breadth of *S. coracoideus* and *P thoracicus* varied from 7.4 to 10.3%.

Table 6: Effect of varying levels of dietary LLPC on relative weight ($gkg^{-1}$ body weight), length and breadth ($cmkg^{-1}$ body weight) of Some muscles in broiler-starter

| | Weight | | | Length | | Breadth | |
|---|---|---|---|---|---|---|---|
| Diets | *Supra coracoideus* | *Pectoralis thoracicus* | *Gastrocneminus* | *Supra coracoideus* | *Pectoralis thoracicus* | *Supra coracoideus* | *Pectoralis thoracicus* |
| 1 | 7.8±0.3 | 23.9±5.8 | 27.3±2.1 | 13.3±3.3 | 17.7±3.5 | 3.8±0.4 | 7.8±1.0 |
| 2 | 7.2±1.5 | 21.1±3.1 | 29.9±0.3 | 17.3±1.1 | 21.6±3.1 | 5.0±1.8 | 10.3±0.1 |
| 3 | 6.8±0.3 | 21.2±1.9 | 27.6±3.7 | 19.5±6.4 | 23.6±2.8 | 4.4±0.9 | 10.9±2.3 |
| 4 | 5.8±1.6 | 20.5±2.8 | 27.4±0.9 | 15.6±3.3 | 28.9±5.7 | 3.9±0.3 | 10.9±1.5 |
| 5 | 5.4±1.2 | 19.2±2.6 | 31.8±7.0 | 19.9±1.7 | 25.1±7.5 | 3.2±0.7 | 7.6±3.8 |
| MEAN | 6.6 | 21.2 | 28.7 | 17.6 | 20.7 | 3.9 | 8.4 |
| STD | 1.0 | 1.7 | 2.0 | 1.31 | 2.0 | 0.4 | 0.7 |
| CV% | 15.2 | 8.0 | 6.9 | 7.4 | 9.7 | 10.3 | 8.3 |

CV; Coefficient of Variation. Means with differing superscript along the same column are significantly ($P\leq0.05$) different

**Haematological Variables and Serum Metabolites:** Table 7 reveals that the PCV, RBC, Hbc, MCHC, MCH, MCV and ESR of the chicks fed the control diet were not significantly (P>0.05) different from those fed the test diets. While the PCV varied from 28.3±0.6% to 30.0±1.7% the RBC varied from 2.1±0.1 to 2.8±0.6 X $10^6/mm^3$. The Hbc was lowest (1.4±0.3 g/100ml) in chicks fed 2.24% and 6.72% LLPC-based diets and highest (1.7±0.1 g/100ml) in chicks fed 8.96% LLPC-based diet. The MCHC, MCH, MCV and ESR ranged: 4.9±0.6-6.9±1.4%, 5.2±0.7-8.0±1.1pg, 106.5±21.7-135.9±11.3 $\mu^3$m and 5.0±0.0-7.3±3.1 mm/hr, respectively. Also Table 8 shows that the Total Serum Protein (TSP), albumin, globulin and albumin/globulin ratio were not significantly (P>0.05) influenced by the dietary treatments. The globulin values were higher than their corresponding albumin values.

## Discussion

The yellowish pigmentation observed on the skin, ear lobe, beaks and shanks, and the greenish pigmentation observed in the droppings of the chicks fed the test diets suggest the presence of high concentration of carotene in the leaf protein concentrate (LLPC), which increased as the levels of LLPC increased in the diets from 2.24 to 8.96%. This thus corroborated the previous reports (Oke, 1973; Fellows, 1987) that apart from having high crude protein content, leaf protein concentrates are also veritable sources of carotene, a precursor of vitamin A. In this study, the weight gain, feed consumption and feed conversion ratio of chicks fed the test diets decreased with increasing substitution of FM protein with LLPC. For instance, while the weight gain declined to 80.6% in chicks fed 2.24% LLPC-based diet it declined to 39.3% in chicks fed 8.96% LLPC-based diet. Also in like manner, the feed consumption declined from 88.6% in chicks fed 2.24% LLPC inclusion level to 62.9% LLPC inclusion level. The observed decline in these performance characteristics could be attributed to the concentration of some essential amino acids (EAAs) which tend to decrease with increase substitution of FM protein with LLPC in the diets. For instance, lysine, methionine, cystine phenyalanine and glycine decreased by 8.40-27.5%, 4.4-6.7%, 6.3-31.3%, 1.7-5.1% and 5.9-23.5%, respectively in the LLPC-based diets (Table 2). Thus the observed decline in the performance of the chicks on LLPC-based diets could be attributed, in part, to the progressive decrease in the amino acids contents of the test diets. This is inconsonance with the report by Agbede and Aletor (1997; 2003) that amino acid imbalance can adversely impair appetite and feed intake with a concomitant reduction in growth performance of chicks. Also, the poor performance of the chicks could in part be attributed to the effect of some anti-nutritional factors such as phytin and tannin (Agbede, 2000). For example, while the levels of the phytin due to the LLPC in the diets varied from 15.5 mg/100mg to 61.9 mg/100mg in the LLPC-based diets, tannin varied from 5.6 g/100g DM to 22.4 g/100g DM. Consequently, the decrease in the performance of the chicks fed on LLPC-based diets could be attributed to amino acid imbalances and the possible effects of some anti-nutritional factors.

The identical N-retained by the chicks fed the control diet and those fed on 2.24-6.72% LLPC-based diets could be attributed to similar dietary protein contents in their diets. The NR of the chicks fed diet containing 8.96% LLPC was the lowest and this might be properly be due to the high dietary fibre and some EAAs which are grossly limiting in the said diet (Table 2). However, the NR ($Y_1$) correlated negatively (P<0.05) with the levels of FM protein replacement with LLPC ($X_1$) with a prediction equation ($Y_1 = 0.56 - 0.002X_1$, r = -0.83), suggesting that as the quantity of LLPC increased in the diets NR decreased.

This study further showed that neither 5.0% FM protein-based diet nor the LLPC-based diets had significant influence (except the weight of the chest and belly fat) on the carcass 'fast food' cuts, implying that identical carcass traits could be attained by feeding either FM or LPC from *Leucaena leucocephala*. The weight of chest decreased with increased FM protein replacement with

Table 7: Haematological variables of broiler-starter fed varying levels of fishmeal substituted with LLPC

| DIETS | % FM Protein replaced with LLPC | PCV % | RBCx$10^6$ $mm^{-3}$ | Hbc g/100 ml | MCHC % | MCH pg | MCV $\mu^3m$ | ESR mm/hr |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 28.3±0.6 | 2.1±0.2 | 1.7± 0.3 | 6.0±1.3 | 8.0±1.1 | 134.9±12.8 | 7.0±1.7 |
| 2 | 25 | 28.7±0.6 | 2.1±0.1 | 1.4± 0.3 | 4.9±1.0 | 6.7±1.5 | 135.9±11.3 | 6.0±2.0 |
| 3 | 50 | 30.0±1.7 | 2.5±0.4 | 1.5± 0.3 | 5.1± 0.7 | 6.3± 2.2 | 122.9±27.9 | 5.0± 0.0 |
| 4 | 75 | 28.7±1.6 | 2.8±0.6 | 1.4±1.3 | 4.9± 0.6 | 5.2± 0.7 | 106.5±21.7 | 7.3±3.1 |
| 5 | 100 | 29.7±0.6 | 2.5±0.4 | 1.7±0.1 | 6.9±1.4 | 0.9±1.4 | 120.7±16.5 | 6.0±2.0 |

LLPC = *Leucaena* Leaf Protein Concentrate; PCV = Packed Cell Volume; RBC = Red Blood Cell; WBC = White Blood Cell; Hbc = Haemoglobin Concentration; MCHC = Mean Cell Haemoglobin Concentration; MCH = Mean Cell Haemoglobin; MCV = Mean Cell Volume; ESR = Erythrocyte Sedimentation Rate. Mean with differing superscipts in the same horizontal row are significantly (P<0.05) different

Table 8: Serum Metabolites of broiler–starter chicks fed varying levels of fishmeal substituted with LLPC

| DIETS | % FM protein replaced with LLPC | Total Serum protein (g/100ml) | Albumin (g/100ml) | Globulin (g/100ml) | Albumin/ Globulin Ratio |
|---|---|---|---|---|---|
| 1 | 0 | 3.5±0.5 | 1.6±0.1 | 1.9±0.5 | 0.9±0.3 |
| 2 | 25 | 4.8±1.9 | 1.7±0.1 | 3.1±1.8 | 0.7±0.3 |
| 3 | 50 | 6.1±1.4 | 1.7±0.2 | 4.4±1.6 | 0.4±0.2 |
| 4 | 75 | 5.3±1.6 | 1.7±0.1 | 3.5±1.5 | 0.6±0.2 |
| 5 | 100 | 4.6±1.5 | 1.7±0.1 | 2.9±1.4 | 0.7±0.3 |

LLPC = *Leucaena* Leaf Protein Concentrate; TSP = Total Serum Protein

LLPC. This tends to suggest that the chicks utilized the LLPC-based diets poorly in laying the chest muscle. Belly fat is an important component of the chicken carcasses. The deposition of the belly fat in this study appears not to be enhanced by the increased inclusion of LLPC in the diets. Conceivably, this may be due to the lower fat contents of the test diets (Table 2), which decreased with increased LLPC inclusion.

Of the entire organs measured only the relative weights of spleen, pancreas and gizzard were significantly influenced by the dietary treatments. The relative weight of spleen did not follow a definite pattern but the weights, were in most cases higher in the chicks fed the LLPC-based diets than those fed on the control diet. This may suggest that the growth of spleen was enhanced by the LLPC vis-à-vis its activities. Similarly, the relative weight of pancreas did not follow a definite pattern but it was in most cases significantly lower in chicks fed the control diet than the LLPC-based diets. However, it remains to be established whether the LLPC contains an inhibitor of trypsin. Trypsin inhibitors are growth inhibitor common in untreated soybean and have been implicated in the precipitation of pancreatic hypertrophy. The relative weight of the gizzard in the present study tends to increase with increased levels of LLPC in the diets. The test diets appeared more coarse and of high dietary fibre content (Table 2) than the control diet. This might have induced higher gizzard muscle development in the chicks fed LLPC-based diets in abide to evolve a highly muscularised grinding organ for the fibre component for proper digestion and/or utilization.

Previous reports (Butson and Cater, 1985; Aletor *et al.*, 1989; Schreurs, 2000) and more recently (Agbede and Aletor, 2003) showed that apart from changes in the nitrogen balance and biochemical parameters, nutrition or dietary manipulations exert several influences on the development of certain muscles in broiler. The relative weight, length and breadth *S coracoideus, P thoracicus* and *Gastrocnemius* of chicks fed the control diet and the test diets were similar as evident in their relatively low coefficient of variation values. *P thoracicus* has been described as the major component power for the down stroke while the *S coracoideus* is the major power for the up stroke in birds (Gretty, 1977). These two muscles are consequently very essential in the wild birds unlike in the domesticated birds such as broiler.

Blood parameters of nutritional importance are the RBC counts, PCV, MCHC, MCV, glucose level and clotting time (Olorede and Longe, 2000; Adeyemi *et al.*, 2000). The result of blood variables in this study suggests that neither the control diet nor the LLPC-based diets precipitated any severe effects on the health status of the chicks. However, the values observed for PCV and Hbc were lower than those recently reported by Agbede and Aletor (2003) for chicks of the same age fed Glyricidia leaf protein concentrate in place of fish meal protein but the values of PCV, Hbc, MCHC and MCV fell within the range reported by Fasuyi (2000); Olorede and Longe (2000). Also the blood physical properties with respect to the ESR were not significantly affected by the dietary treatments. This suggest that like the control diet, the LLPC-based diets did not give rise to acute general infections as high values of sedimentation rates could precipitate acute general infections and malignant tumor (Frandson, 1986). Generally, it is believed that the frictional resistance of the surrounding gravitational pull on the erythrocytes mostly determines the ESR.

The TSP, albumin and globulin syntheses were generally similar for all the dietary treatments. Though the values observed for the serum protein generally compared favourably with those reported by Fasuyi (2000) for chicks of the same age fed cassava LPC, they were consistently higher than those reported for birds fed LPC from glyricidia (Agbede and Aletor, 2003).

**Conclusion:** The study showed that the fishmeal protein is superior to the LLPC protein with regards to the performance characteristics. However, in most cases, the carcass traits, relative organ weights, muscle growth, haematology and the TSP of chicks fed the LLPC-based diets favourably compared with those fed the control diet. The poor performance of chicks on the test diets could be enhanced if the essential amino acids (EAAs) notably methionine + cystine and lysine which are limiting in LLPC are adequately supplemented. However, Research and Development (R&D) aimed at enhancing the nutritive value of LLPC by reducing the anti-nutritional factors effects on its utilization by broilers is currently receiving attention. In the main, the use of 2.24% LLPC in broiler-chick diets, which produced 80.6% of the weight gain of chicks fed 5% FM protein, hold promise for broiler-starter feeding, especially in the third world countries where fishmeal resource is scarce.

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