International Journal of Poultry Science 4 (5): 339-349, 2005
ISSN 1682-8356

Protein Replacement Value of Cassava (Manihot esculenta, Crantz) Leaf Protein Concentrate (CLPC) in Broiler Starter : Effect on Performance, Muscle Growth, Haematology and Serum Metabolites

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Abstract: Cassava (Manihot esculenta, Crantz) leaf protein concentrate (CLPC) was used to replace a known and conventional source of protein in broiler starter diets 2, 3, 4, 5, and 6 at 20, 40, 60, 80 and 100% respectively. The reference diet 1 was one where fish meal was the major source in the diet without CLPC. The inclusion level of CLPC was 1.61, 3.22, 4.82, 6.43 and 8.04 respectively for diets 2 to 6. A batch of 120 starter chicks was randomly assigned in triplicate to these dietary treatments (i.e. chicks/treatment). The final weight and average weight gain of diet 1 (0% FM replacement) was significantly higher than others ($P < 0.05$) even when the average feed consumption was similar for diets 1, 2, 3 and 4. The feed efficiency (FE) for diets 1 and 2 was also similar ($P = 0.05$). The nitrogen retention for diets 1, 2 and 4 was similar ($P = 0.05$). Diets 2, 3 and 4 also had similar values ($P = 0.05$). The dressed weight of chicks in diets 2 and 6 was similar ($P = 0.05$) while that of diets 5 and 6 was also similar ($P = 0.05$). Except for kidney, pancreas and lungs, all other organs measured showed similar values ($P = 0.05$). The weight of inner chest muscle (*supra coracoideus*) of birds on diets 1, 2, 3 and 4 were similar ($P = 0.05$). The weight of outer chest muscle (*Pectorialis thoracicus*) and thigh muscles (*Gastrocnemius*) were similar ($P = 0.05$) with diet 5 (80% FM replacement with CLPC) having the longest inner chest muscle length of $20.7 \pm 4.3 cmkg^{-1}$. Except for red blood cell (RBC) and mean cell volume (MCV), all other parameters measured were similar ($P = 0.05$). The values of total serum protein, albumin, globulin and albumin/globulin ratio were similar ($P = 0.05$). Also the values for liver protein, albumin, globulin and liver albumin/globulin ratio were similar ($P = 0.05$). It was concluded that CLPC as a replacement for FM as a protein source had no deleterious effect up till 60%. However, several parameters investigated strongly support a realistic replacement of 40% FM with CLPC in practical diets. Performance can still be enhanced with supplementation of essential amino acids (EAAs).

Key words: Reference diet, cassava leaf protein concentrate, *pectorialis thoracicus*

Introduction

It is well known that the supply of protein concentrates for non-ruminant feeding in the tropics is becoming more and more inadequate over the years. This is further accentuated by the rising competition between man and animal for these feed ingredients especially those of protein origin. Efforts have been channeled towards finding alternative sources to these feed ingredients in animal feed formulation. Nutrition researches along these lines have been on the increase as feed has been acknowledged to account for up to 75 - 85% of the recurrent production inputs in intensive monogastric animal production. Cassava plant has the peculiar advantage of easy adaptability to our extreme stress condition under which farming is practiced in Africa. Its efficient production of food energy, year-round availability, tolerance to extreme stress condition and suitability to present farming condition and food systems in Africa have contributed in our choice of cassava as a potent source of extracting leaf protein for the purpose of replacing other protein sources of animal and plant origins that are very expensive and thus economically unviable. In most regions where cassava is widely grown, it is planted for its tuberous root, leaving the leaves to wither and at best, serve as manure to the top soil. Apart from the highly balanced amino acid profile, cassava leaf protein (Rogers and Milner, 1963), have also been shown to contain a high level of crude protein, vitamins and nutritionally valuable minerals. (Roger and Milner, 1963; Aletor and Adeogun, 1995).

Given the desirability of the nutrient constituents of cassava leaf protein concentrates, it was conceived that they could provide suitable alternatives to the more expensive conventional protein concentrates such as fish meal. This research and development (R and D) need has largely informed the design of the present study.

Table 1:Composition of Experimental Diets (g/100g)

	Diets					
	1	2	3	4	5	6
	% replacement of FM with CLPC					
Ingredient	0	20	40	60	80	100
Maize (11.0%CP)	54.35	53.74	52.63	52.03	51.42	50.81
Groundnut Cake (45.0% CP)	29.50	29.50	30.00	30.00	30.00	30.00
Brewers dried grain (25.3% CP)	5.00	5.00	5.00	5.00	5.00	5.00
Fish Meal (68.0%CP)	5.00	4.00	3.00	2.00	1.00	0.00
Cassava Leaf Protein Conc. (42.3%CP)	0.00	1.61	3.22	4.82	6.43	8.04
Palm Oil	2.00	2.00	2.00	2.00	2.00	2.00
Bone Meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster Shell	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Premix *	0.50	0.50	0.50	0.50	0.50	0.50
Nacl	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0	100.0	100.0
Calculated :						
Crude Protein %	23.93	23.86	23.95	23.88	23.82	23.75
GE** (kcal/100g) DM	461.6	462.6	461.1	462.6	463.7	464.7

* contained vitamins A (10,000,000iu); D(2,000,000 iu); E (35000 iu); K (1900mg); B12 (19mg); Riboflavin (7,000mg); Pyridoxine (3800mg); Thiamine (2,200mg); D Pantothenic acid (11,000mg); Nicotinic acid (45,000mg); Folic acid (1400mg); Biotin (113mg); and Trace elements as Cu (8000mg); Mn (64,000mg); Zn (40,000mg); Fe (32,000mg) Se (160mg); I_2 (800mg) and other items as Co (400mg); Choline (475,000mg); Methionine (50,000mg); BHT (5,000mg) and Spiramycin (5,000mg) per 2.5kg.
GE** (kcal/100g) calculated based on 5.7kcal/g protein; 9.5kcal/g lipid; 4.0kcal/g carbohydrate. (Ng and Wee, 1989)

Materials and Methods

Cassava leaf protein production: The cassava leaf protein concentrate (CLPC) 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, 1993a,b). The CLPC was rinsed, pulverized, sundried and milled. The proximate analyses, amino acid profile, mineral content and antinutrients were determined to chemically evaluate the nutritional value of extracted CLPC. Thereafter, the CLPC was used to formulate diets along with other ingredients purchased locally.

Site preparation: Shortly before the arrival of the chickens, the poultry house was thoroughly disinfected, fumigated with 1 part of Potassium Permanganate pellets to 3 parts of formalin and the house was allowed to rest.

Experimental rations formulation: The cassava leaf protein concentrate (CLPC) was processed according to the methods earlier discussed. The feed ingredients were purchased from Ebun-Olu farm, Ondo Road, Akure, Ondo State, Nigeria. The results of the proximate compositions earlier determined were used in the eventual formulation of the different diets. The experimental diets were prepared in the nutrition laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure. There were six experimental diets (Table 1). All diets were compounded to contain identical crude protein content. Diet 1 was the control diet without the test CLPC included. Diets 2, 3, 4, 5 and 6 were formulated such that CLPC replaced fish meal at 20, 40, 60, 80 and 100% on a protein - for - protein basis, respectively. Other constant protein sources in all the diets were groundnut cake and brewers' dried grain. All diets were also supplemented with feed-grade methionine.

Management of experimental birds and experimental design: A total of 120 day-old broiler chicks were purchased from Tuns Farm, Km 9, Ikirun Road, Osogbo, Osun State. All chicks were electrically brooded at the Teaching and Research Farm, Federal University of Technology, Akure. They were fed a 24% protein broiler-starter commercial ration *ad libitum* for the first week after arrival prior to the commencement of the experiment. The chicks were also sexed the second day of brooding. Water was provided *ad libitum* with appropriate antibiotics and antistress particularly after arrival. The following medications were administered from day old:

i. Intraocular vaccination against Newcastle disease at day one.

ii. Neoceryl (Antibiotics) for a period of 4 days from 3 days of age.
iii. Coccidiostat for the treatment/control of coccidiosis and chronic respiratory diseases.
iv. Gumboro vaccine at 2 weeks of age
v. Lasota vaccine (New castle booster) administered in a day at the age of about 3 weeks.

The experimental design was the completely randomized type with a total of 18 experimental units. After the uniform brooding for 1 week, the sexed chicks (3 males and 3 females) were randomly distributed into the 18 experimental units. The chicks were assigned at the rate of 18 chicks/diet in 3 replications of 6 chicks/replicate such that the mean group weights were similar. The chicks were fed the experimental diets *ad libitum* for 21 days during which records on daily feed consumption and 3 days periodic weight changes were recorded.

Estimation of nitrogen retention, apparent nitrogen digestibility and "operative" protein efficiency ratio: Total faeces voided during the last 5 days were collected, weighed, dried at 65-70°C in an air circulating oven for 72hrs and preserved while the corresponding feed consumed was also recorded for nitrogen studies. The nitrogen contents of the samples were determined by the method of AOAC (1990). Nitrogen retained was calculated as the algebraic difference between feed nitrogen and feacal nitrogen (on dry matter basis) for the period. Apparent nitrogen digestibility was computed by expressing the nitrogen retained as a fraction of the nitrogen intake multiplied by 100. The operative protein efficiency was calculated as the ratio of weight gain to total protein consumed.

Blood collection for analysis: At the end of the feeding trial, a male chick per replicate was randomly selected, weighed and scarified by severing the jugular vein and blood allowed to flow freely into labeled bottles one of which contained a speck of EDTA while the other without EDTA was processed for serum. The serum was kept deep frozen prior to analysis. The packed cell volume (PCV%) was estimated by spinning about 75:l of each blood sample in heparinized capillary tubes in an haematocrit micro centrifuge for 5 minutes while the total red blood cell (RBC) count 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.

Total Serum and Liver Protein determination: After the separation of the liver, 0.5g of each liver sample was homogenized in 5ml of ice cold distilled water using a Ystral-GMbH D-7801 homogenizer (Dottingen Type x 1020). Then 1ml was taken from this and diluted with 19ml of cold distilled water. Thus making the concentration of homogenate to be 0.5g/100ml or 0.5%. The homogenate was then put in sample bottles and kept frozen prior to analysis. The serum and the liver homogenate were thawed before the total protein as well as the albumin and globulin of serum and liver were determined.

Carcass, muscle and organ measurements: After slaughtering, the carcasses were scalded at 75°C in a water bath for about 30 seconds before defeathering. The dressed chicks were later eviscerated. The measurement of the carcass traits: dressed weight %, eviscerated weight %, thigh, drumstick, shank, chest, back, neck, wing, bellyfat and head, were taken before dissecting out the organs. The organs measured were the liver, kidneys, lungs, pancreas hearth, spleen, bursa of fabricus and gizzard. The following muscles: 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. All the carcass traits, except the dressed and eviscerated weights, were expressed as percentages of the live weight while the organs and muscles were expressed in g/kg body weight, while length and breadth of the muscles expressed in cm/kg body weight.

Statistical analysis: Data collected were subjected to either coefficient of variation analysis and analysis of variance (Steel and Torrie, 1980). Where significant difference were found, the means were compared using the Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

Results

Performance: The performance data of the chickens on experimental diets are presented in Table 3.

There were significant differences (P < 0.05) between the mean values obtained for weight gain, average feed consumption, feed efficiency (FE) and 'operative' protein efficiency ratio (OPE). With respect to weight gain, diet 1 (0% FM replacement) was significantly higher than the other diets. Duncan's Multiple Range Tests indicated that diets 2, 3 and 4 (at 20, 40 and 60% FM replacement with CLPC respectively) had mean values that were not significantly different from one another (P > 0.05). Diets 5 and 6 (at 80 and 100% FM replacement with CLPC respectively) had the lowest weight gains which were not significantly different from each other (P > 0.05). The average feed consumption for chicks fed diets 1, 2, 3 or 4 (0, 20, 40 and 60% FM replacement with CLPC respectively) were not significantly different (P > 0.05). Diet 5 (80% FM replacement with CLPC) also had a

Table 2: Proximate composition (g/100g DM) and energy value (kcal/g) of experimental diets

	Diets					
	1	2	3	4	5	6
	% replacement of FM with CLPC					
	0	20	40	60	80	100
Dry Matter	92.0	92.4	91.6	92.1	91.9	92.1
Crude protein	23.9	23.9	24.0	23.9	23.8	23.8
Crude fibre	2.8	2.6	2.5	2.5	2.5	2.4
Ether extract	3.4	3.0	3.1	3.4	3.3	3.0
Ash	5.7	5.7	5.2	6.0	6.0	5.9
Nitrogen free extract	40.4	39.8	39.5	40.0	40.2	39.5
Gross energy (Kcal/g)	3.5	3.8	3.9	3.9	3.9	3.9

CLPC = Cassava Leaf Protein Concentrate; FM = Fish Meal

Table 3: Performance of broiler chicks fed CLPC - based diets from age 7 - 28 days

	Diets					
	1	2	3	4	5	6
	% replacement of FM with CLPC					
Parameters	0	20	40	60	80	100
Initial Weight (g/chick)	105.3	106.0	106.7	105.8	105.5	105.3
Final Weight (g/chick)	660.3±80.8	573.5±31.3	529.6±69.7	492.0±45.5	431.1±18.7	346.1±27.5
Average Weight Gain (g/chick)	554.9^{a}±80.0	467.5^{b}±27.2	422.9^{b}±63.3	399.2^{bc}±20.8	325.6^{cd}±13.2	240.7^{d}±23.0
Average Feed Consumption (g/chick/day)	49.3^{a}± 7.1	44.5^{ab}± 2.0	42.1^{ab}± 5.7	44.0^{ab}± 2.7	37.6^{bc}± 1.8	34.5^{c}± 5.4
Feed Efficiency (FE)	1.9^{a}± 0.1	2.0^{ab}± 0.1	2.1^{b}± 0.1	2.3^{c}± 0.1	2.4^{c}± 0.1	3.0^{d}± 0.2
'Operative' Protein Efficiency Ratio (OPE)	2.2^{a}± 0.1	2.1^{b}± 0.1	2.0^{b}± 0.1	1.8^{c}± 0.1	1.7^{c}± 0.1	1.4^{d}± 0.1
Mortality (%)	-	-	-	-	-	-

Means are for 18 chicks/diet (± s.d). Means with different superscripts in the same horizontal row are significantly different (P < 0.05); FM = Fish Meal; CLPC = Cassava Leaf Protein Concentrate

mean value that was not significantly different from that of diets 2, 3 and 4. Interestingly, diets 5 and 6 (100% FM replacement with CLPC) also had mean values that were not significantly different (P > 0.05). The feed efficiency (FE) for diets 1 and 2 (at 0 and 20% FM replacement with CLPC respectively) were the best and not significantly different from each other. Expectedly, diets 2 and 3 (at 40% FM replacement with CLPC) also did not show any significant difference in their mean values. Diets 4 and 5 (at 60 and 80% FM replacement with CLPC respectively) also did not show significant difference in their mean values. However, diet 6 (100% FM replacement with CLPC) had the least efficiency which was significantly different from the others (P < 0.05). The 'operative' protein efficiency ratio for chicks fed diet 1 (at 0% FM replacement with CLPC) was significantly higher (P < 0.05) than for other diets. Diets 2 and 3 (at 20 and 40% FM replacement with CLPC respectively) followed diet 1 with their mean values which were not significantly different from each other. Diets 4 and 5 (at 60 and 80% FM replacement with CLPC respectively) had mean values that were not significantly different (P > 0.05). Diet 6 (at 100% FM replacement with CLPC) had the lowest protein efficiency ratio which was significantly different from all other diets.

Nitrogen utilization: Table 4 shows data on nitrogen utilization of the experimental birds. The nitrogen intake and nitrogen retention were significantly (P < 0.05) affected by the dietary treatments while the apparent nitrogen digestibility was not. The mean nitrogen intake for diet 1 (i.e 0% FM replacement with CLPC) was the highest followed by diet 4 (at 60% FM replacement with CLPC). These two diets (1 and 4) had mean values that were not significantly different (P > 0.05) from each other. However, diets 2, 3, 4 and 5 (at 20, 40, 60 and 80% FM replacement with CLPC respectively) also had mean values that were not significantly different (P > 0.05). Only diet 6 (at 100% FM replacement with CLPC) had a mean value that was significantly lower than all the other diets (P < 0.05). The mean nitrogen retention for diets 1, 2 and 4 (i.e 0, 20 and 60% FM replacement with CLPC respectively) showed the highest values which were not significantly different from one another (P > 0.05). However, diets 2, 3 and 4 (i.e 20, 40 and 60% FM

Table 4: Nitrogen Utilization of Broiler Chicks Fed CLPC Based Diets

	Diets					
	1	2	3	4	5	6
	% Replacement of FM with CLPC					
Parameters	0	20	40	60	80	100
Nitrogen Intake (gN/chick/day)	2.9±1.9[a]	2.6±0.3[b]	2.4±1.3[bc]	2.6±0.2[ab]	2.1±0.6[bc]	2.0±1.5[c]
Nitrogen Retention(gN/chick/day)	2.3±1.1[a]	2.0±0.1[ab]	1.9±1.1[bcd]	2.0±0.3[ab]	1.6±0.2[cd]	1.6±1.2[d]
Apparent Nitrogen Digestibility (%)	79.9±4.0	78.1±1.6	77.7±4.9	78.1±1.5	75.7±3.1	80.00±2.1

Means with different superscripts in the same horizontal row are significantly different (P < 0.05).

replacement with CLPC respectively) also did not vary significantly in their mean values (P > 0.05).

Carcass characteristics: Carcass characteristics are presented in Table 5. The result showed that only the dressed weight (% of live weight) and the back (gkg^{-1} body weight) showed significant difference in their treatment means. Dressed weight (%) of chicks fed diet 5 (i.e 80% FM replacement with CLPC) was highest at 91.39% of liveweight but this was not significantly different (P > 0.05) from values obtained for diets 5 and 6. Control diet 1 (at 0% FM replacement with CLPC) had a mean value that was not significantly different (P > 0.05) from the mean values obtained for diets 2 and 3 (at 20 and 40% FM replacement with CLPC). The relative weight of the back also showed a significant difference (P<0.05) across the various treatments. Diet 2 (at 20% FM replacement with CLPC) had the highest value of 87.25gkg^{-1} body weight while diet 6 had the least (50.3gkg^{-1} body wt). This value was significantly different from every other mean value (P < 0.05). This value was closely followed by mean values of diets 4 and 1 (at 60% and 0% FM replacement with CLPC). These two mean values were not significantly different (P > 0.05) from each other. Diet 1 treatment mean was also not significantly different (P > 0.05) from diet 3 (at 40% FM replacement with CLPC) while diets 5 and 6 (at 80 and 100% FM replacement with CLPC) had mean values that were lower and significantly different (P < 0.05) from each other and from the rest of the treatment mean values.

Relative organ weights: The relative weights of the organs measured are presented in Table 6. Except for the kidney, pancreas and lungs that showed significant differences in their respective mean values across the diets (P < 0.05), the relative weights of the other organs were not significantly different (P > 0.05). The mean kidney weights of chicks fed diets 1, 3 and 5 (i.e 0, 40 and 80% FM replacement with CLPC respectively) were not significantly different (P > 0.05) from one another. However, chicks fed diet 6 had the highest kidney weight (P<0.05).The mean pancreas weight of chicks fed diet 4 (i.e 60% FM replacement with CLPC) was lowest and significantly different from the other diets (P < 0.05). The relative weights of the lungs were not significantly different (P > 0.05) except for diet 5 (i.e 80% FM replacement with CLPC).

Relative weight, length and breadth of some muscles: Table 7 shows the data on relative weight (gkg^{-1} body weight) of chest and thigh muscles while Table 8 shows the relative length and breadth (cmkg^{-1} body weight) of chest muscle in the experimental chicks. The weight of the inner chest muscle (*Supra coracoideus*) showed that a significant difference existed in their treatment mean values (P < 0.05). The inner chest muscle weight of birds on diet 3 (i.e 40% FM replacement with CLPC) had the highest mean value (9.7 ± 0.8gkg^{-1} body weight) although this was not significantly different (P > 0.05) from the mean values obtained from control diet 1, diets 2 and 4 (i.e 0, 20 and 60% FM replacement with CLPC respectively). Interestingly, treatment mean value of diet 5 (i.e 80% FM replacement with CLPC) was not significantly different (P > 0.05) from that obtained for diet 2 (i.e 20% FM replacement with CLPC). The weight of the outer chest muscle (*Pectoralis thoracicus*) and thigh muscles (*Gastrocnemius*) were not significantly different (P>0.05) over their treatment means. The length of the inner and outer chest muscles showed significant differences in their treatment mean values. Diet 5 (i.e 80% FM replacement with CLPC) had the longest inner chest muscle length of 20.7±4.3cmkg^{-1}, although this was not significantly different (P > 0.05) from the mean values obtained for diets 2 and 4 (at 20 and 60% FM replacement with CLPC respectively). Values for diets 2 and 4 were also not significantly different (P > 0.05) from that obtained for control diet 1 (at 0% FM replacement with CLPC). Diet 3 (at 40% FM replacement with CLPC) also had mean value that was similar (P > 0.05) to diet 6 (at 100% FM replacement with CLPC). The overall pattern was generally inconsistent. The length of the outer chest muscle also varied significantly (P < 0.05) due to dietary treatments. Diet 5 (at 80% FM replacement with CLPC) also had the longest outer chest muscle at 24.20cmkg^{-1} but this showed no significant variation (P > 0.05) from values obtained for diets 2, 4 and 6 (at 20, 60 and 100% FM replacement with CLPC respectively).

Table 5: Carcass Traits of Broiler Chicks Fed CLPC Based Diets from age 7 - 28 days

	Diets					
	1	2	3	4	5	6
	% replacement of FM protein with CLPC					
Parameters	0	20	40	60	80	100
Live Weight (g)	629.3±66.3	481.0±90.8	515.3±72.2	469.7±45.4	368.3±41.9	333.7±36.0
Dressed Weight (%)	89.7±0.4 [a]	90.7±1.3[ab]	89.5±1.0[ab]	90.9±0.1[bc]	91.4±0.8[c]	91.3±0.7[c]
Eviscerated Weight (%)	82.0±0.8	80.9±2.5	81.0±2.3	83.6±0.4	80.7±1.4	80.6±1.5
Thigh (g/kg body wt)	47.3±4.8	45.1±5.3	45.3±6.3	45.8±4.7	46.8±3.5	45.8±3.3
Drumstick (g/kg body wt)	104.2±2.7	94.6±11.5	104.8±5.2	96.5±2.7	92.4±3.8	95.6±5.1
Back (g/kg body wt)	81.0±0.53[ab]	87.3±0.9[c]	78.9±0.3[b]	83.8±0.3[a]	60.6±0.1[d]	50.3±4.4[e]
Backfat (g/kg body wt)	2.7±0.7	2.0±0.5	2.3±1.1	2.6±0.5	2.6±0.4	3.0±0.8
Shank (g/kg body wt)	29.9±3.7	32.2±4.3	32.9±2.1	30.7±3.6	33.7±2.5	30.9±1.5
Wing (g/kg body wt)	38.3±3.3	40.3±5.7	41.3±7.4	39.2±3.3	38.2±3.9	47.1±1.6
Head (g/kg body wt)	42.9±5.4	46.1±7.4	44.2±4.8	44.9±3.1	49.1±2.8	54.0±2.6
Neck (g/kg body wt)	63.7±2.6	56.5±16.4	52.9±7.1	62.5±1.0	58.9±6.5	63.0±2.8

Means with different superscripts in the same horizontal row are significantly different (P < 0.05); FM = Fish Meal; CLPC = Cassava Leaf Protein Concentrate

However, values obtained for diets 1, 2, 3 and 4 (at 0, 20, 40 and 60% FM replacement with CLPC respectively) did not show any significant difference (P > 0.05). The breadth of the inner and outer chest muscles did not show any significant difference across the dietary treatments.

Haematological indices of experimental birds: Some haematological indices measured are presented in Table 9. Except for the red blood cell (RBC) and the mean cell volume (MCV) that showed significant variations in their treatment mean values (P < 0.05), all other parameters measured did not exhibit significant variation in their mean values due to dietary treatment. The treatment mean values for red blood cells (RBC) had the highest values in diets 3 and 5 (at 40 and 80% FM replacement with CLPC respectively). These two treatment means (diets 3 and 4) were not significantly different (P > 0.05). Diets 1, 2 and 6 (i.e 0, 20 and 100% FM replacement with CLPC respectively) did not show significant variation in their treatment mean values (P > 0.05). Diets 2 and 4 (i.e 20 and 60% FM replacement with CLPC respectively) also did not show significant difference in their mean values (P > 0.05). The same followed for diets 4 and 6 (at 60 and 100% FM replacement with CLPC respectively) which did not also show any significant difference in their mean values. The MCV treatment mean values for diets 1,2, 4, 5 and 6 (at 0, 20, 60, 80 and 100% FM replacement with CLPC respectively) were not significantly different (P >0.05) which ranged from 122.63 μm^3 in diet 5 to 137.45 μm^3 in diet 6. Only diet 3 (i.e 40% FM replacement with CLPC) had a mean value that was significantly different from all others (P < 0.05) at a value of 89.43 μm^3.

Some serum metabolites of broiler chicks fed CLPC based diets: Table 10 shows some serum metabolites measured in the experimental birds. Total serum protein, albumin, globulin and albumin / globulin ratio determined were not significantly affected by the dietary treatments.

Some liver metabolite of broiler chicks fed CLPC based diets: Table 11 shows some liver metabolites measured. Total liver protein, albumin, globulin and liver albumin/globulin ratio determined were also not significantly influenced by the dietary treatments.

Discussion

The performance parameters such as weight gain, feed consumption, feed consumption efficiency and 'operative' protein efficiency ratio all demonstrated a consistently better performance in the control diet (i.e. 0% FM replacement with CLPC) than all others. This was not surprising as fish meal has long been recognized as a one of the reference protein sources with regard to its well balanced amino acid profile. The average weight gain of birds on diet 1 was highest at 554.94gchick^{-1}. Although this value was significantly different from other diets, diets 2, 3 and 4 (i.e. 20, 40 and 60% FM replacement with CLPC respectively) showed remarkable average weight gains that were similar or close to the control diet. However, diets 5 and 6 (at 80 and 100% FM replacement with CLPC) had the lowest values for weight gains. The weight gain values for diets 2, 3 and 4 were still in agreement with the normal weight gain bracket predicted for broiler strains of such category (Oluyemi, 1979). The average feed consumption of birds on diet 1 (i.e 0% FM replacement with CLPC) predictably

Table 6: Relative Organs Weights (g/kg body weight) of Broiler Chicks Fed CLPC Based Diets

	Diets					
	1	2	3	4	5	6
	% replacement of FM with CLPC					
Parameters	0	20	40	60	80	100
Liver	19.2±1.6	24.0±3.3	22.1±2.3	20.3±4.0	23.7±3.7	22.1±1.7
Kidney	7.2±0.1[a]	5.0±0.1[bcd]	6.8±1.7[ab]	4.3±1.2[cd]	5.4±1.3[ad]	9.7±1.0[e]
Heart	6.4±0.9	8.2±1.0	7.1±1.0	6.9±1.1	6.5±1.1	6.8±0.8
Spleen	1.1±0.2	1.2±0.3	1.1±0.2	1.2±0.1	1.0±0.5	1.0±0.5
Pancreas	2.9±0.5[a]	3.2±0.5[a]	2.9±0.2[a]	1.8±0.5[b]	3.1±0.3[a]	3.1±0.1[a]
Bursa	2.9±0.3	2.5±0.4	2.4±0.7	2.4±0.6	2.6±0.8	2.6± 0.5
Gizzard	36.8±3.8	47.2±6.9	39.0±2.5	7.1±0.6	7.3±0.8	9.3±1.4
Lungs	6.7±1.0[a]	6.7±1.0[a]	7.1±0.6[a]	7.3±0.8[a]	9.3±1.4[b]	5.8±1.3[a]

Means with different superscripts in the same horizontal row are significantly different ($P < 0.05$); FM = Fish Meal; CLPC = Cassava Leaf Protein Concentrate

Table 7: Relative Weight (g/kg body weight) of Chest and Thigh Muscles of Broiler Chicks Fed CLPC - Based Diets

	Diets					
	1	2	3	4	5	6
	% Replacement of FM with CLPC					
Muscle type	0	20	40	60	80	100
Inner Chest muscle (*Supra coracoideus*)	8.5±0.3[ac]	8.1±2.0[abc]	9.7±0.8[a]	8.6±2.1[ac]	7.2±1.1[cd]	6.0±0.6[bd]
Outer Chest muscle (*Pectoralis thoracicus)*	27.4±2.8	23.0±5.9	23.2±7.1	21.3±2.7	22.8± 3.0	24.9±2.0
Thigh Muscle(*Gastrocnemius)*	31.2±1.7	29.4±5.1	31.5±3.2	34.7±3.7	37.7± 4.0	33.0±1.5

Means with different superscripts in the same horizontal row are significantly different ($P < 0.05$); FM = Fish Meal; CLPC = Cassava Leaf Protein Concentrate

was the highest as a result of the palatability of the control diet. Cassava leaves from which CLPC was obtained had appreciable levels of antinutritional factors notably cyanide, phytin and tannin. Even after different processing methods, it was found out that ample levels (although not deleterious) still existed in the leaves of cassava varieties tested (Aletor and Fasuyi, 1997). Poor palatability of CLPC may be a factor militating against its adequate consumption in diets containing CLPC as was evident in chicks fed diets 5 and 6 relative to the control. However, the average feed consumption values for chicks on diets 2, 3 and 4 (at 20, 40 and 60% FM replacement with CLPC) were still in agreement with the standard values obtained from many studies (Oluyemi, 1979) indicating that these values may still be within the acceptable order. Feed efficiency which is a basis for assessing feed utilization i.e. the conversion of feed into body tissues was best in the control diet 1 (i.e 0% FM replacement with CLPC). Diet 2 (at 20% FM replacement with CLPC) was not significantly different from diet 1 and diet 3 (at 40% FM replacement with CLPC). This result showed that FM replacement with CLPC at 40% as in diet 3 still demonstrated a favourable feed efficiency value. This result agrees with an earlier report by Lindner (1978) to the extent that FM, as high quality animal protein, can be replaced by vegetal protein, provided the limiting amino acids, usually methionine and lysine, are supplemented. The present study also corroborates an earlier work by Oke (1973) that the cassava supplemented with leaf protein concentrate showed a high digestibility value of 94.77% in feeding trials with rats. This result agreed with earlier study by Ross and Enriquez (1969) who reported a decrease in weight gain and feed efficiency when the diet fed to poultry had more than 5 percent cassava leaf meal. The absence of mortality throughout the experimental period indicated that the CLPC had been sufficiently detoxified to harmless level and its antinutritional factors considerably reduced to a tolerable level. This is in agreement with Tewe (1976) that processing effects such as sun curing or dehydration of the cassava leaves completely liberated the HCN content and no toxic effects were therefore found when the leaves were fed to animals. The daily nitrogen intake values showed some significant variations among the treatment diets. Diet 1 (at 0% FM replacement with CLPC) had the highest value at 2.94gN/chick/day. This value was however not significantly different ($P > 0.05$) from the values obtained for diets 2, 3 and 4 (at 20, 40 and 60% FM replacement with CLPC respectively). This result suggested that FM replacement with CLPC up to 60% in broiler diets had no effect on the nitrogen intake. The nitrogen intake can

Table 8: Relative Length and Breadth (cm/kg body weight) of Chest Muscle of Broiler Chicks Fed CLPC Based Diets

	Diets					
	1	2	3	4	5	6
	% Replacement of FM with CLPC					
Parameters	0	20	40	60	80	100
Length of Inner chest muscle(*Supra coracoideus*)	13.1±2.9[a]	16.1±1.4[abc]	14.9±0.8[ad]	16.1±2.6[abc]	20.7±4.3[bc]	19.1±3.1[cd]
Length of Outer chest muscle*(Pectoralis thoracicus)*	17.8±2.8[a]	21.3±1.9[ab]	18.3±2.5[a]	17.3±7.3[ab]	24.2±4.8[b]	24.0±2.4[b]
Breadth of Inner chest muscle*(Supra coracoideus*)	3.4±0.2	3.5±0.2	3.6±1.0	3.6±0.5	2.7±0.3	4.5±1.7
Breadth of Outer chest muscle *(Pectoralis thoracicus*)	6.9±0.8	7.2±0.6	7.1±2.0	7.6±1.8	7.2±2.6	9.5±1.7

Means with different superscripts in the same horizontal row are significantly different ($P < 0.05$); FM = Fish Meal; CLPC = Cassava Leaf Protein Concentrate

therefore be said to be sufficient at levels not exceeding 60% FM replacement with CLPC. The same pattern as obtained for nitrogen intake was similar to the nitrogen retention where diet 1 (at 0% FM replacement with CLPC) had the highest nitrogen retention value at 2.34 closely followed by diets 2, 4 and 3 (at 20, 60 and 40% FM replacement with CLPC respectively) in that order. The retention of nitrogen correlated positively with nitrogen intake suggesting that the higher the nitrogen intake, the higher the nitrogen retained subject to requirement levels since any nitrogen consumed in excess of requirement would be degrade and voided in faeces. As the dietary inclusion of CLPC increased, the nitrogen utilization indices (nitrogen intake, nitrogen retention and protein efficiency ratio) also decreased in values indicating poorer nitrogen utilization with the increasing dietary CLPC. The apparent nitrogen digestibility values for the treatment showed no significant variation indicating a uniform nitrogen digestibility rate ranging from 75.73% in diet 5 (at 80% FM replacement with CLPC) to 79.98% in diet 6 (at 100% FM replacement with CLPC). This result suggested that CLPC as a nitrogen source may not have a poor digestibility index and if properly consumed with other veritable protein sources as supplementation may produce a comparable nitrogen utilization with conventional nitrogen sources of acceptable standards. This observation is in line with earlier work on cassava (Maust *et al*., 1972) that the protein deficiency as typified by amino acid imbalance demands higher protein supplementation in animal rations. Apart from dressed weight (%) and the relative weight of the back (gkg^{-1} body wt) which is a percentage of the body weight and the back all other carcass traits were not significantly different ($P > 0.05$) among treatment mean values. Although diets 4, 5 and 6 did not have the highest live weights per chick, they manifested remarkable dress weights as a percentage of live weights indicating that all diets supported a proportional cumulative weight gain. Incidentally, treatment diet 5 had the highest mean value for dressed weight at 91.39% of live weight followed by diet 6 at 91.29% of live weight. This observation therefore implies that the dressed weights of chicken were not directly proportional to the weight gain or performance traits as such. A high weight gain value does not imply a concomitant increase in the dressed weight value as a percentage of live weight. It therefore appears that the CLPC diets do not fully support feathering in the experimental birds. The back weight (gkg^{-1} body weight) also showed a significant variation in their treatment means with diet 2 having the highest mean value of 87.25 gkg^{-1} body weight followed by diets 4, 1, 3, 5 and 6 in that order. This result showed a significant decrease in the back formation of chicken fed diets 5 and 6 at 80% and 100% FM replacement with CLPC suggesting that the growth of some body parts of the chicken may not be well supported by the replacement levels of above 80% of fish meal with CLPC. The kidney, pancreas and lungs all showed significant variations among their treatment mean. However, other organs measured (liver, heart, spleen, bursa, and gizzard) did not vary among treatment mean values. The pancreas although showing a significant variation among treatment mean values also did not have a particular relationship with the treatment diets. Diet 2 (i.e 20% FM replacement with CLPC) had the highest mean value for pancreas followed by diet 6 (i.e 100% FM replacement with CLPC) although these values were not significantly different from values obtained for diets 1, 3 and 5 (i.e 0, 40 and 80% FM replacement with CLPC respectively). Only diet 4 (i.e 60% FM replacement with CLPC) showed a significantly lower mean value from other diets. This result apparently may not have any relationship with the treatment diets indicating that other factors other than the effect of replacing FM with CLPC was responsible. It can generally be surmised that the control diet and the test diets enhanced identical carcass development. The above explanation equally suffice for the result obtained for the significant variation among treatment mean values for the lungs. Diet 5 (at 80% FM replacement with CLPC) had the highest and significantly different value from every other diet. As the results have shown (Table 7 and 8) only the weight of the inner chest muscle, length and breadth of the inner chest muscle showed significant differences in their treatment means. The

Table 9: Haematological Indices of Broiler Chicks Fed CLPC Based Diets

	Diets					
	1	2	3	4	5	6
	% replacement of FM with CLPC					
Parameters	0	20	40	60	80	100
PCV %	28.7±1.5	27.7±2.1	25.3±1.5	27.0±2.0	28.3±1.5	29.3± 0.6
RBC count (x 10^6/mm^3)	2.2±0.2[a]	2.3±0.3[ab]	2.8±0.1[c]	2.0±0.2[bd]	2.3±0.1[c]	2.1±0.1[ad]
Hbc (g/100ml)	2.1±0.2	2.6±0.1	2.3±0.3	2.4±0.2	1.9±0.7	2.6±0.78
MCHC (%)	7.4±0.9	7.4±2.9	9.2±0.8	9.1±1.5	6.9±2.7	8.8±2.7
MCH (pg)	9.5±0.3	9.0±2.9	8.3±1.5	12.1±0.7	8.4±3.3	10.6±2.3
MCV (Fm^3)	129.2±13.8	123.6±10.5[a]	89.4±8.9	135.5±25.7[a]	122.6±10.8[a]	137.5±7.4[a]
ESR (mm)	4.3±0.6	4.3±0.6	5.5±0.5	4.8±1.0	4.8±0.8	4.7±0.6

Means with different superscripts in the same horizontal row are significantly different ($P < 0.05$); FM = Fish Meal; CLPC = Cassava Leaf Protein Concentrate.
CLPC = Cassava 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

inner chest muscle of chicks fed diet 3 (i.e 40% FM replacement with CLPC) had the highest value but this value was not significantly different ($P > 0.05$) from the mean values of the control diet 1, diets 2 o 4 (i.e 0, 20 and 60% FM replacement with CLPC respectively). Generally, it may be inferred that the relative weights of most muscles were unaffected by dietary increase in CLPC inclusion and when affected (as in the case of chest muscle weight), it had a negative correlation value indicating a decreasing lower muscle weight as CLPC dietary inclusion increased. The relative length of the inner and outer chest muscles were significantly different ($P < 0.05$) with positive correlation values with the varying dietary CLPC suggested that chest muscle length may not be seriously affected by varying dietary CLPC. Other intrinsic factors requiring microanatomy may be responsible for the slight deviations in the inner and outer chest muscle lengths. Of all the blood parameters examined, only the red blood cell (RBC) counts and mean cell volume (MCV) showed significant variations among their treatment mean values. This was in agreement with earlier reports by Ologhobo *et al*. (1986) that RBC counts is one variable that is most consistently affected by dietary influence. The RBC counts for diet 3 (at 40% FM replacement with CLPC) was highest but not significantly different from the value obtained for diet 5 (at 80% FM replacement with CLPC). However, the range of 2.02 x 10^6mm^{-3} in diet 4 (at 60% FM replacement with CLPC) to 2.84 x 10^6mm^{-3} in diet 3 was not a departure from values obtained for normal chicks (2.3 x 10^6mm^{-3}) or by (2.54 - 3.30 x 10^6mm^{-3}) by Aletor and Egberongbe (1992). Values for the mean cell volume (MCV) for diet 3 (i.e 40% FM replacement with CLPC) only showed a lower significant value from other values (89.43 μm^3). Other haematological values were in general agreement with normal values previously reported (Aletor, 1987; Ologhobo *et al*., 1986; Aletor and Egberongbe, 1992). The blood variables most often affected by dietary influences were identified as PCV, plasma protein, glucose and clotting time (Aletor, 1987; Ologhobo *et al*., 1986). These values in the experimental birds were found to be consistently higher than most values earlier reported and comparable with the report for chicks fed soya bean in place of fish meal (Aletor and Egberongbe, 1992). On a similar note, the MCHC, MCH and Hbc were not significantly affected by the dietary treatments suggesting similar haemoglobin contents. The ESR of the test diets were similar to the control diet indicating that the test diets did not predispose the birds on the test diets to any known general infection or malformation of any kind. Frandson (1986) reported that ESR are increased in cases of acute general infection, malignant tumors and pregnancy. The total serum protein, albumin, globulin and albumin/globulin ratio (Table 10) did not vary significantly from one another and from the control diet ($P > 0.05$). Also the total liver protein, albumin, globulin and albumin/globulin ratio (Table 11) were not significantly affected by the dietary treatments. The TSP and TLP are indirect indices for measuring the nutritional protein adequacy (Eggum, 1987; Tewe, 1985). The similar TSP and TLP values with the control diet indicate that the nutritional quality of CLPC as a protein source compares favourably with known conventional sources such as fish meal

Conclusion: The introduction of CLPC as a replacement for FM (protein for protein) had no obvious deleterious effect up till 60% FM replacement with CLPC The performance characteristics investigated relatively showed comparable values. The weight gain, average feed consumption, feed efficiency and ‘operative’ protein efficiency ratio as performance indices all showed

Table 10: Some Serum Metabolites of Broiler Chicks Fed CLPC Based Diets

	Diets					
	1	2	3	4	5	6
	% replacement of FM with CLPC					
Parameters	0	20	40	60	80	100
Total serum protein(g/100g)	9.8±0.4	9.2±1.4	9.0±0.6	9.2±0.7	9.0±1.6	8.6±0.7
Albumin (g/100g)	0.6±0.1	0.7±0.2	0.6±0.1	0.6±0.1	0.7±0.1	0.7±0.1
Globulin (g/100g)	9.2±0.5	8.5±1.5	8.4±0.5	8.6±0.7	8.3±1.5	7.9±0.8
Albumin/Globulin Ratio	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1

Means with different superscripts in the same horizontal row are significantly different. ($P < 0.05$); FM = Fish Meal; CLPC = Cassava Leaf Protein Concentrate

Table 11: Some Liver Metabolites of Broiler Chicks Fed CLPC Based Diets

	Diets					
	1	2	3	4	5	6
	% Replacement of FM with CLPC					
Parameters	0	20	40	60	80	100
Total Liver Protein(g/100g)	10.1±0.4	10.1±0.2	10.0±0.4	9.9±0.1	10.0±0.1	10.4±0.7
Albumin (g/100g)	2.6±0.1	2.7±0.4	2.7±0.1	2.5±0.2	2.4±0.1	2.4±0.1
Globulin (g/100g)	7.5±0.4	7.3±0.3	7.4±0.4	7.4±0.3	7.6±0.2	8.1±0.3
Albumin/Globulin Ratio	0.3±0.1	0.4±0.1	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.1

Means with different superscripts in the same horizontal row are significantly different. ($P < 0.05$); FM = Fish Meal; CLPC = Cassava Leaf Protein Concentrate.

values fairly comparable with the control diet in which there was no replacement of FM with CLPC. The drastic drop in performance as experienced by birds on 80% and 100% FM replacement with CLPC respectively may be as a result of the sudden drop in feed intake with a corresponding drop in nitrogen intake and nitrogen retention. Since the apparent nitrogen digestibility (AND) revealed no significant variation among all treatment means, it is implied that CLPC and FM proteins may be digested to a similar extent by broilers. Economic consideration suggests a realistic replacement of 40% fish meal in practical diet. Better performance can still be obtained with adequate supplementation of essential amino acids (EAAs) especially methionine and tryptophan which have been identified to be in marginal quantities in cassava leaf. It is imperative that efforts should be made to improve the palatability and hence consumption of poultry diets that must contain cassava leaf protein concentrate.

Acknowledgement

I am highly indebted to my academic mentor, Professor V. A. Aletor who designed this research work and Mr Mike Oguntokun who painstainkly carried out the laboratory analyses.

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