IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 10, Issue 12 Ver. II (December 2017), PP 01-07
www.iosrjournals.org

Fermented African Yam Bean (*Sphenostylis stenocarpa)* and Pigeon Pea (*Cajanus cajan)* Seed Meals: Effect of Residual Anti-Nutrients on the Blood Profile, Organ Weight and Carcass Characteristics of Broiler Chickens

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Abstract: *This study investigated the blood profile, organ weight and carcass characteristics of broiler chicken fed fermented African yam bean (sphenostylis stenocarpa) and Pigeon pea (Cajanus cajan) seed meals. A total of 120-d old broiler chicks were randomly allotted into five experimental groups of 3 replicates with 8 birds per replicate in a Completely Randomized Design. A maize-soybean based diet served as the control while 50% and 100% each of fermented Pigeon pea (FPP) and fermented African yam bean (FAYB) seed meal were used to replace soybean meal in diets 2-5 in a 42-d feeding trial. The results of this study showed that fermentation as a means of processing African yam bean and Pigeon pea meal reduced the contents of anti-nutritional factors (ANFs) marginally. The packed cell volume, haemoglobin, red and white blood cell counts were better enhanced in birds fed the dietary treatments. Birds fed the control diet and those that received 50% of the fermented meals had higher (P<0.05) dressing percentage, drumstick, thigh and breast meat compared to those on 100%, though, birds on 100%FAYB) had a heavier liver weight. Therefore, broilers chickens could tolerate up to 50% of the fermented meals.*

Keywords - *Afican Yam bean, Blood, Broilers, Carcass, Organ weight, Pigeon pea*

Date of Submission: 31-03-2017 Date of acceptance: 19-12-2017

I. Introduction

Pigeon Pea (*Cajanus cajan*) and African yam bean (*Sphenostylis stenocarpas*) have considerable potentials as feedstuffs for poultry [1-2]. The market and availability reveals that there has been a marked increase in pigeon pea production but there is a gradual decline and extinction of African yam bean production in Nigeria. At the same time, interest in non conventional feedstuffs for swine and poultry, in place of imported and often – expensive soybean meal has grown considerably. However, pigeon pea and African yam bean still remain underutilized in commercial feed production compared to soybean meal. Fasoyiro *et al.,* [3] reported that African yam bean possess high crude protein content between 22 and 30% while Pigeon pea has been reported by Damaris, [4] to have protein ranging from 18-26%. According to Khattab *et al*., [5], high nutritive value of legumes seeds is restricted by a few anti-nutritive factors (e.g trypsin inhibitors, tannins, phytic acid, flatulence causing oligosaccharides) which have been known to influence physiological status of animals including the blood profile, organ weight and carcass quality.

Blood is being increasingly studied in the area of toxicology and environmental monitoring [6], as the determination of blood component values provide reliable results and may also give inputs research studies on nutrition, physiology and pathology [7]. Blood parameters are a good diagnostic tool to examine the influence of feedstuffs or anti-nutrients on the physiological well-being of livestocks [8]. Haematological studies are important because the blood is the major transport system of the body and evaluation of the haematological profile usually furnishes vital information on body's response to injury of all forms including toxic injury [9]. Haematological parameters can be influenced by anti-nutritional constituents present in feed [10, 8]. Serum Parameters on the other hand provides information on the integrity of the internal organs while total serum protein is usually a reflection of the quality of the diets [11].

The weight of organs and carcass quality in broilers is known to indicate the response of birds to the feed intake and or anti-nutrients in relation to growth or the age of the birds [12]

This study therefore, examines the blood profile, organ weight and carcass characteristics of broilers fed fermented African yam bean and Pigeon pea seed meals.

II. Materials And Methods

2.1 Experimental site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. Ogbomoso is located on Longitude 4^{o}15’ East of the Greenwich meridian and latitude 8^{o}15’ North of the equator in the derived savanna zone of Nigeria.

2.2 Sources of Test ingredients

Two different legumes seeds namely: African yam bean (*Sphenostylis stenocarpas*) and Pigeon pea (*Cajanus cajan)* were used in this study. African yam bean and Pigeon pea were purchased from local markets.

2.3 Processing Methods of Test Ingredients

The processing method employed on both legume seeds was unconventional (Traditional) fermentation as described by Achi, [13]. About 100kg each of both seeds were ground into granules and wet with water (10 litres of water to 100kg seed) and bagged in small pack of 5kg inside white-cellophane paper. They were then packed inside two different black pot drums and covered. The drums were well covered to prevent oxygen from penetrating. The drums were opened on the seventh day (7days) at room temperature [14] and the seeds were sun-dried for four days before milling.

The raw and the fermented seed meals were milled to pass through 0.5mm sieve and samples were stored separately in clean sealed cellophane papers until required for further analysis.

2.4 Experimental Diet

A total of five experimental diets were formulated while a maize-soybean diet served as the control diet. Fermented African yam bean and Pigeon pea seed meal were included in the diets at 50 and 100% at the expense of soybean meal in the control diet. The feed composition for starter and finisher broiler diets are shown in Tables 1 while Table 2 shows the determined proximate compositions of the experimental diets.

Table 1 Gross Composition of the Experimental Diets

Parameters (%)	D_1	D_2	D_3	D_4	D_5
Starter phase					
Maize	54.93	47.93	46.93	47.93	46.93
Soybean Meal	38.34	19.17	0.00	19.17	0.00
FPP	0.00	19.17	38.34	0.00	0.00
FAYB	0.00	0.00	0.00	19.17	38.34
Fish Meal	0.00	7.00	8.00	7.00	8.00
#Fixed Ingredients	6.73	6.73	6.73	6.73	6.73
Total (kg)	100.00	100.00	100.00	100.00	100.00
Calculated nutrient composition					
Crude Protein (%)	23.10	23.18	23.20	23.20	23.23
ME* (kcal/kg/DM)	3217.00	3212.00	3292.00	3281.00	3220.00
Phosphorus (%)	0.55	0.64	0.56	0.64	0.56
Calcium (%)	1.04	1.43	1.46	1.43	1.46
Finisher phase					
Maize	54.00	47.00	46.00	47.00	46.00
Soybean Meal Wheat Offal	30.00 9.58	15.00 9.58	0.00 9.58	15.00 9.58	0.00 9.58
FPP	0.00	15.00	30.00	0.00	0.00
FAYB	0.00	0.00	0.00	15.00	30.00
Fish Meal	0.00	7.00	8.00	7.00	8.00
#Fixed Ingredients	6.42	6.42	6.42	6.42	6.42
Total (kg)	100.00	100.00	100.00	100.00	100.00
Calculated nutrient composition					
Crude Protein (%)	21.10	21.18	21.18	21.10	21.16
ME* (kcal/kg/DM)	3053.51	3072.40	3090.11	3076.00	3080.11
Phosphorus (%)	0.88	1.28	1.31	1.26	1.31
Calcium (%)	0.53	0.64	0.58	0.64	0.58

FPP=Fermented Pigeon Pea; FAYB=Fermented African Yam Bean; ME=Metabolizable Energy.

#Fixed ingredients (Starter phase)=3.00% Vegetable oil, 1.50% Bone meal, 1.39% Limestone, 0.20% Salt, 0.12% Lysine, 0.25% Methionine, 0.25% Vitamin-mineral premix.

Fixed ingredients (Finisher phase)=9.58% Wheat offal, 3.00% Vegetable oil, 1.52% Bone meal, 1.00% Limestone, 0.30% Salt, 0.12% Lysine, 0.25% Methionine, 0.25% Vitamin-mineral premix.

2.5 Experimental birds and Management

A total of one hundred and twenty (120) 1-day old broiler chicks were obtained from a reputable commercial hatchery within the project area. The birds were weighed on arrival and were allocated to five dietary treatments in a Completely Randomized Design (CRD). Each treatment had twenty-four (24) birds which were further sub-divided into three (3) replicates of eight (8) birds each. Feed and water were provided *ad libitum* throughout the period of the experiment. Standard management practices and routine vaccination were strictly observed. The experiment lasted for 42 days. Birds were monitored daily for morbidity and mortality. Mortalities were recorded as they occurred throughout the duration of the experiment. Dead birds removed were weighed and feed intake and feed conversion were corrected for mortality. Weight gain was measured weekly while feed intake was recorded daily.

2.6 Data Collection

2.6.1 Collection of blood samples

On day 42, two chicken from each replicate were selected and starved for 12 hours to avoid temporal elevation of blood metabolites by feeding and then slaughtered post cervical dislocation. During this period, water was supplied *ad-libitum.* Blood samples for biochemical analysis were collected into Ethylene – Diamine Tetra – Acetic Acid (EDTA) free tubes and allowed to clot for the separation of the serum from the coagulum/clot by decantation. Another set of blood samples were collected into sterilized tubes in order to avoid coagulation for determination of haematological parameters.

2.6.2 Determination of haematological parameters

The haematological parameters were determined according to the procedure of Dacie and Lewis, (1991) including, packed cell volume (PCV), haemoglobin concentration (Hb) (Comparator chamber method), Red blood cell (RBC), White blood cell (WBC) count while Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated as follows:
MCH=Hb x 10/RBC (Pg), MCV= Haematocrite x 10/RBC (fl), Hb x 100/Haematocrite (g/dL)

2.6.3 Determination of Serum Biochemistry

Blood samples for serum chemistry were centrifuged at 1000rpm for 10 minutes and the serum was separated. Serum protein was analyzed colorimetrically using diagnostic reagent kits (Rend Diagnusztikal Reagent, Skeszlet, Hungary) based on total protein by biuret method. Serum albumin was determined using Bromocresol green method as described by Cheesbrough [15]. Serum globulin was calculated by subtracting serum albumin from total protein value. Serum calcium ions were determined by flame emission spectrometry as described by Cheesbrough [15].

2.6.4 Weights of Organs and Carcass Examination

Three birds per treatment were selected at the end of the 6th week of the experiment. The birds were fasted overnight and killed by cervical dislocation. The fresh carcasses and organs were carefully dissected and weighed using a sensitive scale and were expressed as percentage of live weight.

2.6.5 Determination of Anti-nutritional factor

Trypsin inhibitors were determined using the method of Kakade *et al.* [16] while Tannin was determined using the method of Swain [17]. Spectrophotometric method described by Hiai *et al.* [18] was used to determine the total saponins while Phytate was determined using the procedure recommended by Maga [19].

2.6.6 Statistical Analysis

All data collected were subjected to one-way analysis of variance (ANOVA) using SAS [20] and where significant differences were observed in the means, Duncan multiple range test of the same software was used to separate the means.

III. Result

3.1 Residual anti-nutritional factors in the tested Ingredients

The results presented in Table 2 shows the residual anti-nutrients (Trypsin inhibitor, Tannins, Saponin and Phytate) in the raw and fermented seed meals of African yam bean and pigeon pea. Raw African yam bean had higher trypsin inhibitor compared with Pigeon pea while the content of phytate, tannins and saponin were higher in raw Pigeon pea (PP) meal than in the African yam bean (AYB) meal. Fermentation marginally reduced the contents of trypsin inhibitor, phytate, tannin and saponin in the two legumes. 21.47%, 12.5%, 12.5%

and 1.47% reduction in trypsin inhibitor, phytate, tannin and saponin respectively was observed in FAYB unlike 0.14%, 6.45%, 9.75 and 3.61% reduction in FPP respectively for same anti-nutrients.

Table 2: Residual Anti – Nutritional Factors in Raw and Fermented African Yam Bean and Pigeon Pea meal

	African yam bean			Pigeon pea		
Parameters	**Raw**	**Fermented**	**Reduction (%)**	**Raw**	**Fermented**	**Reduction (%)**
TI(Tiu/mg)	46.75	36.71	**21.47**	21.23	21.20	**0.14**
Phytate (%)	0.24	0.21	**12.50**	0.31	0.29	**6.45**
Tannin (%)	0.32	0.28	**12.50**	0.41	0.37	**9.75**
Saponin (%)	0.68	0.67	**1.47**	0.83	0.80	**3.61**

TI=Trypsin inhibitor

3.2 Blood parameters

3.2.1 Haematological parameters

The results of the haematological parameters of birds fed fermented African yam bean and Pigeon pea seed meals are presented in Table 3. There were significant differences (P<0.05) in pack cell volume (PVC), haemoglobin (Hb) , red blood cells (RBC), white blood cell (WBC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH). Birds fed diets containing either African yam bean or Pigeon pea had higher PCV, Hb, RBC and WBC compared with those fed the control diets. However, the MCV and MCH values in the control diet compared favourably with those of diets 2, 3 and 5 and were significantly higher than the value obtained in birds fed diet 4. However, the Platelet counts and Mean Corpuscular Haemoglobin Concentration (MCHC) were not influenced by the dietary treatments (P>0.05).

3.2.2 Serum Biochemistry

Table 4 shows the result of serum biochemistry of broiler chickens fed diets containing fermented African yam bean and Pigeon pea meals. Dietary treatments significantly (P<0.05) influenced the Total proteins, Albumin, Globulin and Alanine aminotransferase. Birds fed D_2 (50% FPP) had the higher total protein (3.53g/dL) and the value was similar to the values in birds fed diet D_5 (100% FAYB) and the control diet. The least value was obtained in birds fed D_3. Similar trend was observed for Globulin. Nevertheless, Plasma calcium and phosphorus were not significantly different (P>0.05).

Table 3: Haematological parameters of broiler chicken fed African yam bean and Pigeon pea meals.

Parameters (%)	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**SEM**
Pack cell volume (%)	31.87^b	36.60^a	34.39^a	34.60^a	34.00^a	0.58
Haemoglobin (g/dl)	11.83^b	13.90^a	13.13^a	12.90^a	13.13^a	0.29
Red blood cell (10^6/mm^3)	2.29^b	2.73^a	2.54^a	2.62^a	2.48^a	0.04
White blood cell (10^3/mm^3)	151.83^b	170.57^a	164.90^a	168.37^a	163.00^a	1.80
MCV (fl)	139.33^a	134.63^a	135.17^a	132.43^b	137.30^a	0.92
MCH (Pg)	51.63^a	50.97^a	51.53^a	49.30^b	52.90^a	0.35
MCHC (g/dL)	37.13	37.93	38.17	37.23	38.60	0.19
Platelets (10^6/Nl)	9.33	6.33	7.33	7.00	7.00	0.35

^{ab} Means in the same row with different superscripts are significantly different (P<0.05)
SEM= Standard error of mean
D_1-100%SBM, D_2-50%PP & 50% SBM, D_3-100%PP, D_4-50%AYB & 50%SBM, D_5-100%AYB, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration

Table 4: Serum chemistry of chicken fed fermented African yam bean and Pigeon pea meals

Parameters (%)	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**SEM**
Total protein (g/dL)	3.38^a	3.53^a	3.01^b	3.09^b	3.44^a	0.09
Albumin (g/dL)	1.53^a	1.30^c	1.37^{bc}	1.10^c	1.59^a	0.05
Globulin (g/dL)	1.85^a	2.23^a	1.64^b	1.99^a	1.85^a	0.06
AST (u/l)	8.33	13.93	12.07	14.33	13.20	1.21
ALT (u/l)	34.67^b	37.33^a	29.80^c	39.57^a	33.37^b	1.00
Plasma Phosphorus (%)[3]	3.39	3.39	3.10	3.26	3.44	0.04
Plasma Calcium (%)[2]	4.05	3.36	3.40	3.89	3.17	0.16

^{abc} Means in the same row with different superscript are significantly different (P<0.05)

AST=Aspertate aminotransferase, ALT=Alanine aminotranseferase, SEM= Standard error of mean
D_1-100%SBM, D_2-50%PP & 50% SBM, D_3-100%PP, D_4-50%AYB & 50%SBM, D_5-100%AYB

3.2.3 Carcass measurements

The result of carcass characteristics of broiler chickens fed fermented African yam bean and Pigeon pea seed meal are presented in Table 5. Dietary treatments significantly (P<0.05) influenced the Final live weight, dressing percentage, drumstick, thigh, wing, breast and back weights. Birds fed the control diet and those that received diets 2 and 4 had higher dressing percentage, final weight, drum stick, thigh and breast meat weight compared with those that were fed diets 3 and 5. Although, the dietary treatments did not influenced the weight of the Shank.

3.2.4 Relative organ weights

The result of the relative organs weight of broiler fed fermented African yam bean and Pigeon pea are presented in Table 6. Significant differences (P<0.05) were obtained in the weights of liver, proventriculus, spleen and gizzard. Birds fed diet 5 (100%AYB) had a heavier liver weight compared with the other dietary treatments, while birds fed diets 3 and 5 had heavier proventriculus compared with those fed other dietary treatments. Weight of the kidney, lung and heart were not significantly (P>0.05) influenced by the dietary treatments.

Table 5: Carcass weight of broiler chicken fed fermented African yam bean and Pigeon pea (% BW)

Parameters (%)	D_1	D_2	D_3	D_4	D_5	SEM
Final live weight (Kg)	1.62^a	1.77^a	1.33^b	1.52^a	0.95^c	0.06
Dressing percentage	66.91^a	66.67^a	54.12^b	62.63^a	54.46^b	2.56
Shank	4.61	5.04	4.47	4.37	4.49	0.09
Drumstick	10.16^a	9.70^a	8.43^b	9.08^a	8.90^{ab}	0.15
Thigh	12.30^a	10.59^a	9.36^b	10.59^a	9.40^b	0.23
Wing	8.50^a	8.81^a	7.01^c	8.47^a	7.40^b	0.15
Breast	18.50^a	15.17^b	13.95^c	16.97^a	12.55^d	0.46
Back	11.10^b	14.14^a	10.26^c	9.74^c	11.34^b	0.28

^{abcd} Means in the same row with different superscript are significantly different (P<0.05)

SEM= Standard error of mean

D_1-100%SBM, D_2-50%PP & 50% SBM, D_3-100%PP, D_4-50%AYB & 50%SBM, D_5-100%AYB

Table 6: Relative organ weights of broiler chicken fed fermented African yam bean and Pigeon pea (% BW)

Parameters (%)	D_1	D_2	D_3	D_4	D_5	SEM
Proventriculus	0.52^b	0.50^b	0.64^a	0.51^b	0.64^a	0.01
Liver	2.23^b	2.27^b	2.27^b	2.51^b	3.09^a	0.06
Kidney	0.72	0.69	0.62	0.64	0.68	0.03
Spleen	0.06^a	0.08^a	0.03^b	0.07^a	0.02^b	0.00
Heart	0.35	0.46	0.37	0.37	0.39	0.01
Lung	0.71	0.58	0.55	0.59	0.49	0.02
Gizzard	3.33^b	3.93^{ab}	4.80^a	4.30^a	4.18^{ab}	0.13

^{ab} Means in the same row with different superscript are significantly different (P<0.05)

SEM= Standard error of mean

D_1-100%SBM, D_2-50% PP & 50% SBM, D_3-100%PP, D_4-50%AYB & 50%SBM, D_5-100%AYB

IV. Discussion

The result of the present study shows that fermentation was not effective in the total elimination of the anti-nutrients in the fermented African yam bean and Pigeon pea seed meals. This observation is different from the report of Emiola [21], who reported that soaking could be an intermediate step prior to cooking for the complete elimination of the anti-nutritional factors (ANFs) in kidney beans. Nevertheless, the result of this study showed that fermentation marginally decreased the levels of ANFs unlike significant reduction of trypsin inhibitors activities through dehulling prior to cooking [21-24].

The phytate was higher in raw and fermented Pigeon pea (0.31 vs 0.29) and lower in African yam bean (0.24 vs 0.21). This is similar to the report of Emiola [21] and Ajayi *et al*. [24]. The value reported for tannins in this study was slightly lower to the value reported for African yam bean but close to the value of Pigeon pea by Apata, [25] and Ajayi *et al*. [24]. However, the report of this study revealed that fermentation partially reduced the concentration of tannin in African yam bean and Pigeon pea seeds, just like the report of previous researchers that different processing procedure decreased tannin in animal feed. Nsa *et al*. [26] reported similar decreases when they worked with castor oil seeds.

The saponin content was higher in Pigeon pea than in African yam bean. The value of saponin obtained is slightly lower than the values reported by Ajayi *et al*. [27] but similar to values reported by Akinmutimi *et al*. (10) on African yam bean.

As observed in this study, significant increase in the packed cell volume, haemoglobin, red blood cell and white blood cell indicated that the birds were not anaemic, had a well balanced oxygen carrying capacity with a good immune system. This is unlike the report of Emiola, [21] where birds fed uncooked Mucuna diet, had significant reduced PCV, Hb and RBC, which suggest a dysfunction of blood haemopoesis.

Total serum protein is usually a reflection of the quality of the [28]. Observations on the total protein, albumin and globulin could be attributed to the ability of tannin to bind, coagulate and precipitate protein [29] thereby making it unavailable for body use. Moreover, the value obtained for albumin content in control and diet 5 indicated that African yam bean can replace soybean meal without affecting the serum albumin [30]
Weight of carcass in this study showed that soybean meal can be substituted with up to 50% fermented African yam bean and Pigeon pea in broiler feed without detrimental effects. The result of this study agreed with the report Iorgyer *et al*. [31] and Yisa *et al*. [32] when they fed fermented and boiled Pigeon pea seed meal respectively to finishing broiler and cockerels chicken. The quadratic response observed in the dressing percentage, drumstick, thigh, wing, breast and back may be attributed to the protein binding effect of trypsin inhibitor coupled with higher phytate, tannin and saponin in FPP and notable is the reduced weights at 100% inclusions. Birds on 50% FAYB compared favourably with the control except for the back weight. This may be attributed to the reduced anti-nutrients (phytate, tannin and saponin) in FAYB. Moreover, the difference in the weight of wings was in line with the report of Raji *et al*. [33]. In contrast, Bamigbose *et al*, [34] and Oyewola and Ewa, [35] reported no difference in the wing weight of broiler chicken on varying dietary plant protein. Birds fed 100% AYB and PP had an increased proventriculus which is attributable to the residual anti-nutrients. The results also revealed higher value of liver weight in diet containing 100% AYB. This could be attributed to the effect of the increased intake of residual anti-nutritional factors which further increased the metabolic activities of the liver being a major detoxification organ; this probably leads to the enlargement and increased weight of the liver. Emiola, [21] observed decreased organ weights when diets containing improperly processed AYB, kidney bean and mucuna bean were fed to broiler chicks.

V. Conclusion

It was observed that fermentation employed as a means of processing reduced the anti-nutritional factors (ANFs) marginally in both African yam bean and Pigeon pea seed meals such that 21.47%, 12.5%, 12.5% and 1.47% reduction in trypsin inhibitor, phytate, tannin and saponin respectively was observed in FAYB unlike 0.14%, 6.45%, 9.75 and 3.61% reduction in FPP respectively for same anti-nutrients. Nevertheless, broilers can tolerate up to 50% of fermented meal of these legumes in their feed without detrimental effects on blood parameters and some carcass yield.

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