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Nutritive value of pigeon pea (C*ajanus cajan*) seeds soaked in wood ash extract as protein feedstuff for broiler chicken production

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Abstract

An eight weeks feeding trial was conducted to assess the effect of feeding graded level of soaked pigeon pea meal (SPPM) in wood ash extract or solution on the growth performance, haematological and serum biochemical indices of broiler chickens. A total of 144 day-old chicks (Anak 2000) were randomly allotted to four treatment diets (1, 2, 3 and 4) in a completely randomized design. Each treatment group was replicated three times with twelve birds per replicate, thus, having thirty-six chicks per treatment group. Diet 1 contained 0% SPPM in wood ash extract for 72hours as the control diet, while diets 2, 3 and 4 were formulated to contain 25, 50 and 75% inclusions of SPPM in place of soybean meal. Results showed that the average live weight, daily weight gain and feed intake as well as protein efficiency ratio were significantly (P<0.05) influenced by the dietary treatments at the finisher phase. Broiler chickens fed 25% SPPM based diet had a higher (P<0.05) and comparable value of live weight, weight gain and protein efficiency ratio to that recorded in the control diet at the finisher phase. The haematological and serum biochemical indices revealed that albumin and total cholesterol were significantly (P<0.05) influenced by the treatment diets. The albumin value (2.2g/dl) was highest in birds fed 50% SPPM, whereas, the cholesterol value decreased (P<0.05) with increasing levels of inclusion of SPPM from 0 to 75%. It therefore implies that SPPM can be partially included in broiler diets up to 25% level with optimum growth performance and without any detrimental effect on the blood profile of broiler chickens.

Keywords: Broilers; growth, haematology, serum chemistry;

Introduction

Animal protein is essential for human health and the availability for human consumption especially in developing countries has become a mirage due to decreasing resources (Onilude and Oso, 1997). The level of animal protein consumption in Nigeria is estimated at 8.27g per *caput* per day as against the recommended 35g per *caput* per day (Amaefule *et al.,* 2009). Meanwhile, poultry products have been considered to be one of the options in Nigeria for reducing the incidence of malnutrition, particularly protein deficiency in diets of the populace. The exorbitant costs of commercial poultry feeds had been identified to be largely due to high cost of conventional feedstuffs, thus causing impediment on the increase in animal protein supply. Therefore, the best solution to this problem of inadequate protein supply to poultry is the intensification of research into cheaper alternatives that would support a commensurate performance of the birds. Research efforts have been geared towards the search for available substitutes for conventional plant protein sources that satisfy the nutrient requirements of balanced poultry rations (FAO, 1983). In this regard, an underutilized and unconventional legume such as pigeon pea seed comes to play because of its nutritive

value. Pigeon pea (*Cajanus cajan*) is one of the most widely grown agricultural legumes in the tropical and sub-tropical countries (Purdue, 2006) and has a low human food and industrial preference (Amaefule and Obioha, 2001). Being a perennial shrub, it has an advantage of producing peas for several years (Vickery and Vickery, 1979). It is a hardy crop that can be cultivated on a wide range of soils. The crude protein content of the seed ranges from 19-29% and it is low in methionine (Amaefule and Nwabara, 2004).

Raw pigeon pea seeds contain anti-nutritional factors such as protease (trypsin and chymotrypsin) inhibitors and haemagglutinins (Udedibie and Carlini, 2002) that increase methionine deficiency and inhibit the enzymatic action of trypsin. These anti-nutritional factors inhibit the activities of digestive enzymes thereby causing digestive losses. They also exact a negative impact on the nutrient quality of the protein which could reduce the performance of poultry. Singh (1991) asserted that pigeon pea contains oligosaccharides such as stachyose, raffinose and verbascose which cause flatulence and these constitute 53% of the total soluble sugars inherent in the intact seeds. A range of 4.3 to 11.4% tannin concentration had been reported in dark seed coated pigeon pea (Jamunathan *et al.,*1988). Therefore, to inactivate these anti-nutrient substances in legume seeds, emphasis has been on different processing methods. In view of the above, raw pigeon pea seeds have been processed by either boiling (Akintunde *et al.,* 2010), boiling and dehulling (Amaefule and Obioha, 2001), toasting (Amaefule and Obioha, 2005), roasting (Akanji *et al.,* 2010) and, supplementation with enzyme (Akintunde *et al.,* 2010) as a means of eliminating the anti-nutritional factors. Amaefule *et al.* (2004) reported that processing of pigeon pea seeds for use in pullet diets improved the crude protein retention and other nutrient utilization especially boiling and toasting. 30% raw, toasted or soaked pigeon pea seed meal diet could be fed to point of lay pullets without adverse effect on egg production, external and internal egg quality characteristics (Amaefule *et al.,* 2009). Onu *et al.* (2006) asserted that boiling, boiling with potash and toasting were effective in reducing the anti-nutritional factors in pigeon pea seeds and improving the nutritive value of pigeon pea boiled with potash. Yisa *et al.* (2006) suggested that up to 30% boiled pigeon pea seed meal can be included in cockerel diets without adverse effect on the meat yielding components. Kperegbeyi and Ikperite (2009) opined that boiling and soaking pigeon pea seeds improved the feeding value of *Cajanus cajan* seed meal for broiler chicks than using the raw or toasted *Cajanus cajan* seed meal. Pigeon pea can replace up to 75% of maize in broiler finisher diets without negatively affecting performance and carcass yield (Lorgyer *et al.,* 2009). Amaefule *et al.* (2011) reported that starter broilers could be fed up to 40% raw pigeon pea seed meal diet without adverse effect on performance of starter birds. Roasted pigeon pea seed meal has been included in broiler starter and finisher diets up to 26 and 27% inclusions respectively without adverse effect on the birds (Ani and Okeke, 2011). Wood ash is the residual powder left after the combustion of wood, such as burning of wood in home fireplaces or in industrial power plants. Wood ashes are complex heterogeneous mixture of all the non-flammable and volatile minerals which remain after the wood and charcoal have been burnt, including burnt soil. It is an alkaline substance (Etiegni and Campbell,

1991) commonly used by local people of south-western Uganda to reduce tannins in some resistant sorghum varieties. According to Hume (2006), wood ash contains 25 to 45% calcium carbonate, less than 10% potash, less than 1% phosphate, some trace elements such as: iron, manganese, zinc, copper and some heavy metals. This same author reported that wood ash helps in the breakdown of anti-nutrient substances and improves the mineral content of legume seeds. Due to the presence of carbondioxide in the fire gases, many of the minerals are converted to carbonates (Hume, 2006). Ashes contain predominately sodium and potassium carbonates, sodium and potassium chloride, silica and calcium carbonates (Tarun *et al.,* 2001). When ash is added to water, the soluble potassium and sodium salts are dissolved, while the insoluble silica and calcium carbonate settle at the bottom (Tarun *et al.,* 2001). Against this background, the present study was focused on the use of pigeon pea seeds soaked in wood ash extract as a replacement for soyabean meal on the growth performance and blood profile of broiler chickens.

Materials and methods

Location and duration of the study

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, Ambrose Alli University, Ekpoma, Edo State of Nigeria. The farm lies between latitude 6.44^{0}N and longitude 6.80^{0}E in Esan West Local Government Area, Ekpoma, Edo State of Nigeria. Ekpoma is within the South-South geo-political zone of Nigeria and has a prevailing tropical climate with a mean rainfall of about 1556mm. The mean ambient temperature ranges from 26^{o}C in December to 34^{o}C in February, relative humidity ranges from 61% in January to 92% in August with yearly average of about 82%. The vegetation represents an interface between the tropical rainforest and the derived savanna.

Sources of ingredients and raw material

Pigeon pea seeds were purchased in Ekpoma open market, Esan West Local Government Area of Edo State, Nigeria. Other feed ingredients for the study were purchased from Ekpoma and its environs. Wood ashes from burnt wood were collected from the vicinity of the experimental area.

Preparation of wood ash extract

Wood ash of 2kg weight was dissolved in 20L of water and allowed to stay for 24 hours. The wood ash solution was then filtered and the pH adjusted to 8.5 using digital hand pH meter. The adjustment of the pH was done by adding 2moles of hydrochloric acid in a ratio of 2mole/2L of the extract to reduce the alkalinity of the medium.

Processing of raw pigeon pea seeds

Raw pigeon pea seeds of 15kg were soaked in wood ash extract for 72 hours, after which the seeds were sieved from the solution and sun dried on jute mats for 5 days at atmospheric temperature of between 26 to 29^{o}C to prevent fermentation and achieve a moisture content of 12.60%. The dried seeds were ground into a meal using a hammer mill to pass through a sieve of 0.5mm and stored in bags before it was used for feed formulation and proximate analysis.

Chemical analysis

Feed and processed pigeon pea seeds samples were analyzed for proximate composition according to the standard described by AOAC (1990) as reflected in Determined analyses. The metabolizable energy was estimated using the formula as expressed by Ichaponani (1980).

Table 1: Mean values for proximate composition of raw and soaked pigeon pea seeds

Proximate fractions (%)	Raw	Soaked
Moisture	11.50	12.60
Dry matter	88.50	87.40
Crude protein	21.88	22.75
Crude fibre	5.00	9.00
Ether extract	3.00	5.00
Ash	4.00	6.00
Nitrogen free extract	54.62	44.62
Metabolizable energy (kcal/kg)	2991	2751

Experimental diets

Four experimental broiler starter and finisher diets were formulated. Diet 1 which served as the control was formulated to contain 100% soybean meal as a plant protein source. Diets 2, 3 and 4 were formulated to contain soaked pigeon pea meal in wood ash extract for 72 hours (SPPM) at 25, 50 and 75% levels of inclusion respectively. All diets were formulated to be isonitrogenous (23% and 21%) and isocaloric (2800 and 3000 MEkcal/kg) at both starter and finisher phases (Table 2).

Table 2: Percentage composition of broiler starter and finisher diets

	Inclusion levels of soaked pigeon pea meal (SPPM) (%)							
	0	25	50	75	0	25	50	75
	Starter Diets				Finisher Diets			
Ingredients (%)	1	2	3	4	1	2	3	4
Maize	46.50	46.50	41.50	41.50	53.79	43.07	33.66	26.14
Soybean meal	42.96	32.25	25.00	23.00	30.70	25.25	15.50	7.75
SPPM (72hrs)	0.00	13.04	25.00	26.50	0.00	19.15	41.56	57.83
Fish meal	1.00	1.00	2.50	3.00	1.00	1.00	1.00	2.02
Wheat bran	5.46	4.11	3.00	3.00	10.00	4.11	15.18	3.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Methionine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analyses								
Crude protein	23.00	23.00	22.80	22.60	21.00	21.00	21.00	21.00
M.E kcal/kg	2801	2864	2864	2872	2900	2900	2900	2900

Experimental design

A total of 144 day-old Anak 2000 broiler chickens were used for the experiment. Thirty six chicks were randomly selected based on their average initial weights to each of the four treatment diets. Each treatment group contained three replicates with twelve birds per replicate and they were assigned to the four treatment diets in a complete randomized design. The chicks were fed commercial starter diet for one week acclimatization period. The birds were allowed to have free access to the treatment diets and water *ad-libitum* throughout the duration of the experiment. Routine medication, vaccination, ventilation and adequate spaces were provided.

Performance study

During the feeding trial, the broiler

chickens were weighed at the beginning of the experiment and subsequently on a weekly basis. Weight changes and feed consumption were recorded weekly, where weight gain, feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER) were estimated to assess the growth performance of the birds. Weight gain was calculated as final weight minus initial weight, feed conversion ratio (FCR) and protein efficiency ratio were calculated using the formulae:

$$\text{Feed Conversion Ratio} = \frac{\text{Feed Intake (g)}}{\text{Weight gain (g)}}$$

Protein Efficiency Ratio = Weight Gain of bird (g) /Protein Consumed (g)

Haematological and serum biochemical studies

On the 56th day of the feeding trial, the birds were starved overnight and three birds per treatment were randomly picked and each was decapitated and bled. Blood samples were collected into sterilized tubes containing ethylene diamine tetra-acetic acid (EDTA) labeled bottles, while another set of blood samples were collected from the same birds into heparinized tubes for serum chemistry determination. Packed cell volume (PVC), red blood cell (RBC), white blood cell (WBC) and haemoglobin (Hb) were determined using improved Neubuars haemacytometer after dilution, and cyanomethamoglobin methods as described by Dacie and Lewis (1991). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and serum metabolites such as: total protein, albumin, creatinine and urea were determined by the method of Hyduke (1975), while globulin was estimated by the subtraction of albumin value from serum total protein value (Dacie and Lewis 1991).

Statistical analysis

Data generated were subjected to a one-way analysis of variance and treatment means were compared using the Duncan's multiple range test using the SAS (1999) package.

Results and discussion

The inclusion of up to 75% wood ash treated pigeon pea meal did not affect growth and feed conversion in the starter phase ($P<0.05$) as reflected in Table 4, but in the finisher phase, inclusion levels above 25% depressed growth performance (Table 5). The improvement ($P<0.05$) in live weight, weight gain of birds placed on diet 2 at the finisher phase compared to other dietary treatments may be attributed to the nutrient availability and density which eventually translated to their improvement in growth rate. This is in agreement with the report of Ani and Okeke (2011). These authors reported that roasted pigeon pea meal can be included in broiler starter and finisher diets at 26% and 27% levels respectively without adverse effect on broiler chickens. In addition, Emenalom *et al.* (2011) reported that 10% inclusion of fermented *Alchornea cordifolia* seed meal significantly improved the live weight and weight gain of broiler chickens. The depression in growth in broilers fed 50-75% SPPM in the present study agrees with the report of Etuk and Udedibie (2003) who observed growth depression in broiler chickens fed 20-50% cooked pigeon pea meal. There was a significant ($P<0.05$) reduction in feed intake of birds as the level of inclusion of SPPM increased from 25-75% at the finisher phase. The reduction in feed intake may be due to high energy in the diets, because birds generally eat to satisfy their energy requirements and intake is reduced drastically as soon as this is satisfied (Jorgensen *et al.,* 1990). This observation is in line with the findings of Fafiolu *et al.* (2015), these authors reported that feed intake was significantly reduced in broiler

chickens fed palm kernel extraction by-products. At the finisher phase, the protein efficiency ratio of the broiler chickens was better in diet 2 and was similar to that recorded in the control diet. This could be due to the quality and availability of nutrient in pigeon pea seeds soaked in wood ash extract (Kperegbeyi and Ikperite, 2009). This may be responsible for the significant improvement in the growth performance of broiler chickens fed on these diets (1 and 2), which is suggestive of the fact that SPPM could be included up to 25% in the diets of broiler chickens.

Table 3: Performance characteristic of broiler starters fed graded levels of soaked pigeon pea meal (SPPM)

	Inclusion levels of SPPM (%)				
	0	25	50	75	
	Diets				
Indices	1	2	3	4	SEM±
Initial live weight (g/bird)	51.00	48.00	52.00	49.00	-
Live weight (g/bird)	946.67	906.67	930.00	873.33	31.36
Daily weight gain (g/bird)	38.78	36.82	35.78	36.78	0.84
Daily feed intake (g/bird)	43.91	43.50	44.15	44.25	2.20
Feed conversion ratio	1.13	1.18	1.23	1.20	0.02
Protein efficiency ratio (%)	1.69	1.60	1.56	1.60	0.04
Mortality (%)	0.00	0.00	0.00	0.00	-

SPPM: Soaked Pigeon Pea Meal; SEM: Standard Error of Mean

Table 4: Performance characteristic of broiler finishers fed graded levels of soaked pigeon pea meal (SPPM)

	Inclusion levels of SPPM (%)				
	0	25	50	75	
	Diets				
Indices	1	2	3	4	SEM±
Live weight (kg/bird)	2.41^{a}	2.44^{a}	2.18^{b}	1.98^{c}	0.04
Daily weight gain (g/bird)	65.61^{a}	70.37^{a}	55.20^{b}	55.34^{b}	2.37
Daily feed intake (g/bird)	83.02^{b}	84.28^{a}	71.55^{c}	71.56^{c}	0.06
Feed conversion ratio	1.27	1.20	1.30	1.29	0.08
Protein efficiency ratio (%)	3.12^{a}	3.35^{a}	2.63^{b}	2.64^{b}	0.11
Mortality	0.00	0.00	0.00	0.00	-

abc: Means in the same row with varying superscripts differed significantly (P<0.05)
SPPM: Soaked Pigeon Pea Meal; SEM: Standard Error of Mean

The haematological indices assayed (Table 6) showed that the inclusion of varying levels of SPPM did not significantly (P>0.05) affect the haematological indices, which indicated the nutritional adequacy and safety of the test diets. Furthermore, the values obtained showed that the birds were well nourished as that they were able to provide essential amino acids and minerals necessary for the normal functioning of the haematopoetic tissues (Ezeagu *et al.,* 2002). The good physiological state of the birds during the experiment may not be unconnected to the non-significantly (P>0.05) variation in the packed cell volume (PCV) of the broiler chickens because PCV is an indicator of blood dilution. The similarities in the values of haemoglobin among the birds irrespective of the levels of inclusion implies that the

diets were adequate in critical nutrients such as; iron, copper, vitamins and amino acids. This is because levels of haemoglobin lower than that of Mitruka and Rawnsley (1977) are indicative of dietary deficiencies of these minerals, vitamins, and amino acids, (Frandson, 1981). In addition, haemoglobin measures the ability of an animal to withstand some levels of respiratory stress (Sainsbury, 1983). The values of WBC obtained in this study fell within the normal limit for broiler chickens (Ross *et al.,* 1978) which revealed that birds were undergoing normal physiological processes such as the production of antibodies which is associated with blood characteristics (Anon, 1980). Since, there was no variation in white blood cell counts; there was no fear of hazard that may be associated with the utilization of SPPM in broiler chickens. Esonu *et al.* (2001) reported that the quality of haematological indices is always a reflection of animal's responsiveness to both internal and external environments which include feeds and feeding. The values obtained for RBC, PCV, MCV, MCH and MCHC were not significantly different (P>0.05) despite the increasing levels of SPPM which suggest the good quality of the test diets. However, all the haematological values fell within the range for chickens (Maxwell *et al.,* 1990; CCAC, 1993).

Table 5: Haematological indices of broiler chicken as affected by the dietary treatments

	Inclusion level of SPPM (%)				
	0	25	50	75	
			Diets		
Indices	1	2	3	4	SEM ±
PCV (%)	37.40	39.20	37.20	36.70	1.65
WBC ($x10^3/\mu l$)	336.00	238.00	242.00	324.50	29.24
RBC ($X10^6/\mu/l$)	3.12	3.02	2.84	2.82	0.08
Hb(g/dl)	11.80	11.50	10.90	11.40	0.75
MCV(fl)	5.01	4.67	4.92	4.92	0.13
MCH(Δ/g)	1.61	1.50	1.66	1.64	0.04
MCHC(g/dl)	0.34	0.34	0.34	0.34	0.22

The serum biochemical indices revealed that only albumin and total cholesterol were significantly (P<0.05) influenced by the test diets, while total protein, globulin, urea and creatinine were not significantly (P>0.05) affected by the treatment diets. Birds on 0% SPPM (control) had the highest value (P<0.05) of cholesterol and the value decreased progressively as the inclusion levels of SPPM increased. This reduction in the cholesterol level of birds as the level of inclusion of SPPM increased may be due to the low level of oil in pigeon pea seeds compared to that in soybean meal. The non-significant (P>0.05) variation in the globulin values of all the test diets could be due to the adequate protein in all the treatment diets. Urea and creatinine values were not significantly (P<0.05) affected by the dietary treatments, which indicated that there was no obvious muscular wastage due to protein adequacy. This trend supports the reports of Omoikhoje *et al.* (2010), Animashahun and Omoikhoje (2014) who opined that cornflakes and bread waste meals can serve as alternative energy resources in broiler chicken rations without adverse effects on the blood profile of the birds.

Table 6: **Serum chemistry of broilers as influenced by the dietary treatments**

	Inclusion levels of SPPM (%)				
	0	25	50	75	
			Diets		
Indices	1	2	3	4	SEM±
Total protein (g/dl)	6.87	6.47	6.60	6.50	0.78
Albumin (g/dl)	1.80[b]	2.10[a]	2.20[a]	2.10[a]	0.06
Globulin (g/dl)	5.06	3.28	4.47	4.47	0.78
Urea (mg/dl)	5.50	5.00	4.00	4.50	0.61
Creatinine (mg/dl)	0.80	0.70	0.80	0.80	0.29
Cholesterol (mg/dl)	126.50[a]	112.00[b]	108.50[b]	107.60[b]	3.68

ab: Means in the same row with varying superscript differ significantly (P<0.05)

Conclusion

The overall results indicated that soaked pigeon pea meal in wood ash extract is a satisfactory protein source which can be included in the diets of broiler chicken up to 25% without any adverse effect on the growth performance.

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