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ORIGINAL RESEARCH

Effect of dietary inclusion of fermented pigeon pea (*Cajanus cajan*) meal on growth, apparent nutrient digestibility and blood parameters of cockerel chicks

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Abstract The effect of dietary inclusion of fermented pigeon pea meal (FPPM) on growth response, apparent nutrient digestibility, haematological indices and serum biochemistry of cockerel chicks was studied using 240-day-old cockerel chicks allotted to four dietary treatments consisting of 60 birds each. Four experimental diets were formulated to include FPPM at 0, 50, 100 and 150 g/kg inclusion levels, respectively. Each of the diets was fed to 60 birds replicated six times with ten birds per replicate. The feeding trial lasted for 56 days. Results indicated that final live weight (linear (L). quadratic (Q): $P<0.05$), weight gain (L.Q: $P<0.01$), feed intake (Q.: $P<0.05$) and coefficient of total tract apparent crude protein digestibility ($P<0.05$) were reduced with increasing dietary inclusion of FPPM. Similar improved feed-to-gain ratios were obtained for chicks fed the control and those fed a diet containing 50 g/kg FPPM. Coefficient of total tract apparent ether extract and ash digestibility were not affected ($P>0.05$) by the inclusion of FPPM. Haemoglobin and serum uric acid concentrations were also reduced ($P<0.05$) with increasing dietary inclusion of FPPM. Chicks fed with 150 g/kg FPPM had the least ($P<0.05$) packed cell volume, red blood cell and neutrophil count. It was concluded that dietary inclusion of up to 50 g/kg FPPM could be used in the ration for cockerel chicks without imposing any threat on the growth response, nutrient digestibility and blood constituents.

Keywords Growth response · Haematological indices · Nutrient utilisation · Serum biochemistry

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Introduction

The high cost and increasing demand for conventional oil seed cakes by livestock, man and agro-industries contributed to the huge cost of production incurred by poultry farmers in Africa. Pigeon pea (*Cajanus cajan*) is a grain legume of relatively low human preference and demand in the West African countries. The processed meal holds a lot of potential as unconventional feed ingredients (Etuk et al. 2002; Damaris 2007; Saeed et al. 2007). The crude protein content of the raw and boiled pigeon pea seed ranged between 210 to 300 g/kg (Amaefule and Onwudike 2000) and 200 to 255 g/kg (Amaefule and Obioha 2001), respectively. The toasted seed meal has a metabolisable energy content of 12.03 MJ/kg (Nwokolo 1987) while the mineral profile showed a high K, P, Ca, Mg and a low Fe, Zn, Cu and Mn content (Nwokolo 1987).

The use of pigeon pea as feed ingredients in poultry ration, like other oil seeds, is limited due to the presence of trypsin inhibitor, haemagglutinins and tannins in the seed (Udedibie and Carlini 2000). Previous literature reported successful inclusion of heat-treated (Onu and Okongwu 2006; Ani and Okeke 2011) and boiled (Amaefule et al.

2006; Iorgyer et al. 2009) pigeon pea meal in poultry nutrition. There is, however, a dearth of information on the use of fermented pigeon pea meal as potential oil seed meal in poultry nutrition. Fermentation of leguminous seeds has been reported to remove most of the deleterious factors contained in the seed (Sandberg 2002). This research was therefore conducted to investigate the effect of dietary inclusion of fermented pigeon pea (*C. cajan*) meal on growth, apparent nutrient digestibility and blood parameters of cockerel chicks

Material and methods

Fermentation of pigeon pea

Pigeon pea seeds were purchased from a local market and soaked in water under an air-tight environment at a ratio of 1 kg seed to 10 L of water (1:10, *w*/*v*) for 120 h at the prevailing ambient temperature (28.5°C). The plastic container used for fermentation was sealed throughout the duration of fermentation. At the expiration of 120 h, the sealed container was opened while the fermenting water was decanted. The fermented seeds were washed several times with clean water and sun dried (for about 4–5 days). The dried seeds were milled (2.5-mm sieve) to obtain the fermented pigeon pea meal (FPPM).

Chemical composition and antinutritional factors of pigeon pea meal

Proximate composition of the ground samples (n=4) of the raw seed (before soaking) and fermented pigeon pea meal (FPPM) were determined according to the standard procedures of AOAC (1990). Gross energy of the samples was determined using a Gallenkamp Ballistic bomb calorimeter (Cam Metric Ltd, Cambridge, UK). The Ca and P content of the ground samples (of the raw seed and FPPM) were determined according to the standard procedures of AOAC (1995, ID 7.073). The extractable tannin contents of the ground samples were determined using the method of Hoff and Singleton (1977) while the trypsin inhibitor (expressed as amount (in milligrams) of pure trypsin inhibited per gram sample) and haemagglutinin content (expressed as reciprocal of minimum quantity (milligrams) of sample per millilitre of the assay medium which produced haemagglutination) were determined as described by Kakade et al. (1973) and modified by Liu and Markakis (1989).

Experimental birds, management and design

Two hundred and forty-day-old cockerel chicks were individually weighed and allotted based on weight equalization into 24 deep litter pens (each of 2.0×2.0 m in dimension). Ten chicks were housed in each pen. Chicks were allowed unlimited access to clean water and experimental diets. Four experimental diets were formulated to include FPPM at 0, 50, 100 and 150 g/kg, respectively (Table 2). Birds contained in each pen were assigned to one of the four dietary treatments such that there were six pens (representing replicate units) allocated to each treatment. The feed was offered in mash form. The birds were managed under a deep litter system of management with wood shavings used as beddings. The study lasted for 56 days. Records of feed intake, body weight gained and mortality of birds were measured and recorded weekly.

Measurement of apparent nutrient digestibility

At the expiration of 56 days, three birds per pen (making a total of 18 birds per treatment) were randomly selected and housed individually in metabolic cages. A 3-day acclimatization period was allowed prior to a 4-day collection period. Birds were fed with the quantity of feed which matched their daily feed intake. Daily excreta voided per bird were dried overnight (at 60°C for 12 h) and kept frozen (−20°C) till analysis. Prior to analysis, excreta samples were dried at 65°C in an air-draft oven to a constant weight and ground through a 1-mm screen for proximate analysis. Ground feed and faecal samples were used to determine their respective proximate constituents (AOAC 1990). Gross energy of the feed and faecal samples were determined using a Gallenkamp Ballistic bomb calorimeter (Cam Metric Ltd., Cambridge, UK).

Collection and analyses of blood samples

Blood samples were collected individually from three birds per pen via the wing vein using a sterilized syringe at the end of the trial. About 2.5 ml of blood sample was collected from each bird into vials containing ethylenediaminetetraacetate (EDTA) while another set was collected into heparinised tubes for serum biochemistry measurement. Plasma was harvested subsequently by centrifuging the whole blood samples at 3,000 rpm for 15 min.

Haemoglobin concentration was estimated using the cyanmethaemoglobin method (Cannan 1958). Packed cell volume (PCV), white blood cell (WBC) and total erythrocyte count of blood samples were determined in a Wintrobe haematocrit tube according to the method of Schalm et al. (1975). Plasma samples were analysed for total serum protein, serum albumin and globulin. Serum total protein (Biuret method, code. 391) and albumin (BCG dye method, code. 061) were analysed in the Automatic Blood Analyzer. Serum creatinine (Bousnes and Taussky 1945) and serum

Table 1 Chemical composition (in grams per kilogram) of raw and FPPM

Composition	Raw pigeon pea meal	FPPM
Dry matter	892.1±9.22	890.1±4.12
Crude protein	232.2±7.2b	267.9±5.9a
Crude fibre	75±0.89	74.90±0.95
Ether extract	11.25±0.73	11.00±0.40
Ca	4.13±0.73a	3.20±0.19b
P	2.10±0.22	1.50±0.72
Extractable tannin	2.45±0.64a	0.71±0.09b
Haemagglutinins $(mg/ml)^{-1}$ [a]	5.22±0.57a	2.01±0.55b
Trypsin inhibitor (mg/g)[b]	6.12±1.90a	1.92±0.66b
Metabolizable energy $(MJkg^{-1})$[c]	8.61±0.99	8.71±1.2
Gross energy $(MJkg^{-1})$	11.56±2.09a	10.91±1.77b

Means on the same row with different letters (a, b) are significantly ($P<0.05$) different

FPPM fermented pigeon pea meal

[a] Reciprocals of minimum quantity (milligrams) of sample per millilitre of the assay medium which produced haemagglutination

[b] Milligrams of pure trypsin inhibited per gram of sample

[c] Source: NRC (1994) (estimated using MEn=26.7% DM+77% EE–51.22% CF)

uric acid (standard kit—Quinica Clinica, Spain) were determined according to standard procedures.

Statistical analysis

Determined chemical analyses of the raw and FPPM were done using SAS (1996). Data generated from the growth response, nutrient digestibility and blood parameters were laid out in a completely randomized design and analysed using one-way analysis of variance with the aid of SPSS (1999). Model sum of squares was partitioned to test for linear (L) and quadratic (Q) trends (Gomez and Gomez 1983).

Results

Chemical composition of pigeon pea meal

The chemical composition of the raw pigeon pea and FPPM is as shown in Table 1. Fermentation reduced ($P<0.05$) the gross energy, calcium content, tannin, haemagglutinin and trypsin inhibitor levels but increased ($P<0.05$) the crude protein level. FPPM contains a moderately high crude fibre and a low ether extract and calcium content (Table 2).

Table 2 Gross composition of experimental diets (grams per kilogram)

Ingredients	Inclusion level of FPPM			
	0 g/kg	50 g/kg	100 g/kg	150 g/kg
Maize	570.00	570.00	570.00	570.00
Soybean meal (SBM)	268.00	218.00	168.00	118.00
Fermented pigeon pea meal	–	50.00	100.00	150.00
Fish meal (720 g/kg crude protein)	10.00	10.00	10.00	10.00
Wheat offal	100.00	100.00	100.00	100.00
Bone meal	23.00	23.00	23.00	23.00
Oyster shell	15.00	15.00	15.00	15.00
Lysine–HCL	4.00	4.00	4.00	4.00
DL-Methionine	5.00	5.00	5.00	5.00
Premix[a] (starter)	2.50	2.50	2.50	2.50
Salt	2.50	2.50	2.50	2.50
Total	1,000	1,000	1,000	1,000
Determined analysis (g/kg)				
Dry matter	895.00	891.10	892.00	895.00
Crude protein	205.90	196.60	190.50	182.90
Crude fibre	30.10	33.41	37.68	42.30
Ether extract	31.50	32.10	31.90	30.9
Ca	12.40	12.20	12.12	12.10
P	6.10	5.99	5.85	5.83
Metabolizable energy[b] (MJ/kg)	10.37	10.27	10.31	10.08

FPPM fermented pigeon pea meal

[a] Mineral–vitamin premix to supply (per kilogram of diet) iodised salt, 2 g; calcium carbonate, 2 g; dicalcium phosphate, 5 g; manganese, 0.04 g; zinc, 0.034 g; iron, 0.023 g; copper, 0.0026 g; retinol, 2.48 mg; cholecalciferol, 0.003 mg; riboflavin, 5.55 mg; thiamin, 0.70 mg; pyridoxine, 0.70 mg; niacin, 2.80 mg; calcium pantothenate, 0.35 mg and cyanocobalamin, 0.70 mg

[b] Source: NRC (1994) (estimated using MEn=26.7% DM+77% EE–51.22% CF)

Table 3 Performance and apparent nutrient digestibility of cockerel chicks fed experimental diets

Parameters	Inclusion levels of FPPM				SEM	Probability[a]	
	0 g/kg	50 g/kg	100 g/kg	150 g/kg		L	Q
Initial weight (g/bird)	37.50	38.00	37.00	37.00	0.21	NS	NS
Final live weight (g/bird)	917.50	890.00	740.00	650.00	37.25	*	*
Weight gain (g/bird)	880.00	852.00	703.00	613.00	37.03	**	**
Daily feed intake/bird (g/bird/day)	38.90	37.60	36.50	32.50	1.19	NS	*
Feed-to-gain ratio	2.48	2.47	2.90	2.96	0.06	*	*
Mortality (%)	0.00	0.00	3.33	1.96	0.71	*	NS
Coefficient of apparent digestibility							
Crude protein	0.68	0.65	0.65	0.60	0.02	*	NS
Crude fibre	0.51	0.52	0.52	0.64	0.03	**	*
Ether extract	0.88	0.87	0.87	0.87	0.002	NS	NS
Ash	0.43	0.44	0.44	0.43	0.004	NS	NS

NS not significant, *FPPM* fermented pigeon pea meal

*$P<0.05$; **$P<0.01$

[a]Probability for linear (L) and quadratic (Q) trends

Growth response and apparent nutrient digestibility

The growth response and coefficient of apparent nutrient digestibility of cockerel chicks fed experimental diets is as presented in Table 3. The final live weight (L.Q: $P<0.05$) and weight gain (L.Q: $P<0.01$) were reduced with increasing dietary inclusion of FPPM. Chicks fed with the control diet and those fed the diet containing 50 g/kg FPPM had similar feed-to-gain ratio. The dietary inclusion of more than 50 g/kg FPPM resulted in a worsened feed-to-gain ratio ($P<0.05$). Feed intake was reduced quadratically ($P<0.05$) while the coefficient of apparent crude protein digestibility was reduced linearly ($P<0.05$) with increasing dietary inclusion of FPPM. Birds fed with 150 g/kg FPPM had the highest (L: $P<0.01$; Q: $P<0.05$) coefficient of apparent crude fibre digestibility.

Haematological indices and serum biochemistry of cockerel chicks

Dietary inclusion of FPPM showed no effect ($P>0.05$) on eosinophil, basophil, total serum protein, serum globulin and serum albumin of the chicks (Table 4). Haemoglobin values were reduced quadratically ($P<0.05$) with increasing dietary inclusion of FPPM. The least ($P<0.05$) PCV, haemoglobin (Hb), red blood cells (RBCs) and neutrophil count were noticed with chicks fed with 150 g/kg FPPM. Serum

Table 4 Haematological indices and serum biochemistry of cockerel starters fed experimental diets

Parameters	Inclusion levels of FPPM				SEM	Probability[a]	
	0 g/kg	50 g/kg	100 g/kg	150 g/kg		L	Q
Haematological indices							
Packed cell volume (%)	39.08	37.00	37.22	36.75	0.20	NS	*
Haemoglobin (g/dl)	12.19	12.02	11.29	10.49	2.60	NS	*
Red blood cells ($\times10^6$/mm^3)	8.73	8.75	7.99	7.21	0.13	NS	*
White blood cells ($\times10^3$/mm^3)	6.00	6.11	6.51	6.30	0.07	NS	*
Neutrophils ($\times10^9$/l)	0.21	0.20	0.20	0.19	0.005	NS	*
Eosinophils ($\times10^9$/l)	0.30	0.35	0.30	0.29	0.02	NS	NS
Basophils ($\times10^9$/l)	0.11	0.10	0.13	0.11	0.006	NS	NS
Platelets ($\times10^9$/l)	216.50	219.10	228.00	227.00	4.03	*	NS
Serum metabolites							
Total serum protein (g/l)	60.00	61.20	59.00	61.46	0.5	NS	NS
Serum albumin (g/l)	40.00	40.20	39.00	41.00	0.69	NS	NS
Serum globulin (g/l)	20.00	21.00	20.00	20.46	0.70	NS	NS
Uric acid (mg/dl)	32.91	32.43	32.06	31.70	0.21	*	NS
Creatinine (mg/dl)	0.45	0.44	0.47	0.79	0.05	*	NS

NS not significant, *FPPM* fermented pigeon pea meal

*$P<0.05$; **$P<0.01$

[a]Probability for linear (L) and quadratic (Q) trend

uric acid was reduced linearly with increasing dietary inclusion of FPPM. Birds fed with 150 g/kg FPPM recorded the highest ($P<0.05$) serum creatinine level.

Discussion

The low calcium content of FPPM following fermentation of the pigeon pea seed was due to gradual leaching of the mineral in the fermenting water. This agreed with Dulhan et al. (2002) who reported a similar reduction in calcium content of pigeon pea seeds soaked in water. Fermentation of legume seed increased legume enzyme activity which results in loss of nutrients (Sandberg 2002). The reduction in antinutritional factor (ANF) content following fermentation of the raw seed agreed with Mubarak (2005) who reported reduced trypsin inhibitor and haemagglutinin following fermentation of mung bean. Processing of pigeon pea prior to inclusion was reported to improve its utilisation when included in diets for poultry (Amaefule and Nwagbara 2004; Ahmed et al. 2006).

The crude protein content obtained in this study for FPPM was slightly higher than values reported in literatures (Amaefule and Obioha 2001; Etuk et al. 2002). The variations could be due to differences in the type of cultivars, processing methods, fermentation period, conditions and storage methods (Udedibie and Carlini 2000; Onu et al. 2001). The gross energy content of weight parts per million (WPPM) obtained in this study, however, agreed with the values obtained by Amaefule and Onwudike (2000) for toasted pigeon pea meal. The extractable tannin content recorded for WPPM in this study was lower than the dietary levels considered harmful for poultry birds (Ortiz et al. 1994). The haemagglutinins and trypsin inhibitor contained in pigeon pea seeds were reported to be removed when boiled with potash (Onu and Okongwu 2006) or using heat treatment (Akanji et al. 2003).

The reduction in weight gain of the chicks noticed with increasing dietary inclusion of FPPM could be linked to a poor nutritional profile created as FPPM inclusion increased. Nwokolo (1987) reported that the amino acid availability of pigeon meal was lower than soybean meal. Etuk et al. (2002) reported that the cystine and methionine content of pigeon meal is low. This low available amino acid accounted for the poorer nutritional profile created in the diets containing FPPM. Similar poor feed efficiency and reduced growth were obtained in birds fed diets containing raw pigeon pea seed (Udedibie and Carlini 2000).

The residual ANFs contained in the FPPM could be responsible for the reduced weight gain and feed intake of birds noticed with high inclusion of FPPM. Amaefule and Onwudike (2000) attributed the reduction ($P<0.05$) in feed intake and resultant low weight gain observed in poultry birds fed with pigeon pea-based diet to the accumulated tannin and bitter taste of the seed coat. Tannin forms a less digestible complex with dietary protein, binds and inhibits endogenous protein, such as digestive enzymes, and interferes with growth and production (Kumar and Singh 1984). Protein inhibitor affected the activity of digestive enzymes resulting in digestive losses.

The reduced crude protein digestibility noticed with increasing dietary inclusion of FPPM was as a result of the low amino acid availability created with FPPM inclusion (Nwokolo 1987; Etuk et al. 2002). However, a linear relationship between protein intake and digestibility has been postulated (Pond et al. 1995). Poor coefficient of apparent crude protein digestibility recorded for birds fed a diet containing 150 g/kg FPPM implied a poor protein utilisation. The decline in protein digestibility noticed with the increased dietary replacement value of soybean meal (SBM) in this study agreed with the earlier reports that pigeon pea negatively influences intestinal morphology and nutrient absorption in piglets (Mekbungwan et al. 2002).

The reduced Hb concentration and PCV obtained with increasing dietary replacement value of SBM could be a result of the poor nutritional profile created and increased dietary concentration of antinutritional factors arising from the inclusion of FPPM. Haemagglutinins are proteins which agglutinate RBCs (Liener 1985). Low Hb, PCV and RBC recorded with birds fed with 150 g/kg FPPM could be due to increased agglutination of the red blood cell. Similar antinutritive effects of concanavalin A (Con-A) agglutinating RBCs was reported (Liener 1985).

The slight increase in WBC values noticed with increasing dietary inclusion of FPPM could be attributed to the physiological efforts of the birds in increasing their body immunity following increased accumulation of deleterious factors in the feed. A similar trend was noticed in rabbits fed with pigeon pea meal (Ahamefule et al. 2008). Increased WBC values beyond normal have been found in infectious inflammatory conditions, under stress conditions, in association with allergy, parasitism and chronic tissue damage (Eggum 1989). High blood platelet levels and serum creatinine were observed with chicks fed 150 g/kg FPPM. A marked increase in thrombopoiesis has been linked with stress, occlusion of large vessels, chronic inflammatory disease and iron deficiency (MacWilliam et al. 1982).

In conclusion, the present study shows that dietary inclusion of FPPM above 50 g/kg for cockerel chicks poses threats on the growth response, nutrient digestibility and blood constituents.

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