Haematological parameters and serum biochemistry of Japanese quail chicks (*Coturnixcoturnix japonica*) fed raw and processed pigeon pea (*Cajanus cajan*) seed meal based diets

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Abstract

An experiment was conducted with 720 one - week old Japanese quail chicks to evaluate the effect of feeding raw and processed pigeon pea (*Cajanus cajan*) seed meal based diets on the haematology and serum biochemistry of the Japanese quails in a completely randomized design. Birds were allocated to eight treatment groups with three replicates each containing 30 birds per replicate. The control diet (T1) had no pigeon pea seed meal, T2 was raw pigeon pea seed while other diets contained 30% of pigeon pea seed meal processed by soaking (for 24, 48 and 72 hours) (T3 – T5), fermentation for 72 hours (T5), boiling for 60 minutes (T6) and roasting for 30 minutes (T7). Blood samples were collected from the birds through the wing web vein at the 6th week of the feeding trial. The haematological parameters investigated were packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), white blood cells count (WBC), lymphocytes, neutrophils, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) while serum biochemical components were creatinine, cholesterol, albumin, urea and total protein concentrations. The results obtained in this study showed that all the haematological parameters and serum biochemistry of birds were significantly ($P < 0.05$) influenced by dietary treatments except albumin concentration (g/dL). The packed cell volume, haemoglobin concentration, red blood cells and total protein values were within the range of 41.00 – 52.17 %, 13.63 – 17.35 g/dl, 6.80 – 8.83 ($\times 10^6/\text{mm}^3$) and 5.62 – 7.70 g/dl respectively. The results suggest that raw and processed pigeon pea seed meal (PPSM) possess good dietary protein quality for optimal growth and had no adverse effect on haematological and biochemical profiles of quail chicks at 30 % dietary level of inclusion.

Keywords: Haematological, biochemical, quail, pigeon pea seed meal.

Introduction

The wide gap between the demand and supply of animal protein in Nigeria and many under developed countries of the world has informed the need to increase livestock production to satisfy their animal protein requirement. However, the problem of low per capital animal protein intake by Nigerians has been attributed to stiff competition for foodstuffs between human, industry and livestock particularly for the fast growing and prolific monogastric

species (Esonu *et al.*, 1999). Among the livestock of interest to poultry farmers is Japanese quails (*Coturnix coturnix japonica*) which are important animal protein source (Edache *et al.* 2005). This specie of poultry was introduced to Nigeria to expand the poultry subsector through meat and egg production (Ani, 2009). Quails being a fast growing species are very economical to maintain as they require less floor space (Haruna *et al.*, 1997), and less feed requirement of 20 – 25 g per day. They

have short generation interval, fairly disease resistant and produce meat and eggs of high quality protein with low body fat and cholesterol content. However, intensive approach to quail production would entail the use of alternative plant protein sources other than the conventional ones to enable keeper's produce meat at affordable price. One among such alternative plant protein sources currently investigated and explored for its nutritive value in the diets of poultry birds is pigeon pea (*Cajanus cajan*).

Pigeon pea (*Cajanus cajan*) is one of the widely grown legumes with a crude protein values of 20.6 – 27.7 % and is desirable due to its high nutrient profile (Apata and Ologhobo, 1993). Like most tropical legumes, the utilization of pigeon pea seeds for good growth and performance of poultry species may be limited due to the presence of anti-nutritional factors such as protease inhibitors (trypsin, chymotrypsin inhibitors), lecithin, tannins and non - starch polysaccharides (NSP) (D'Mello, 1992; Amaefule and Nwagbara, 2004). Hence, the need for processing through various methods such as boiling, roasting, soaking and germination, dehulling and chemical treatments which exerts beneficial effects by reducing or destroying the inherent anti-nutritional factors (Kaankuka *et al.*, 2000). Effective reduction of levels of anti-nutritional factors present in formulated rations and the quality of the feed therefore will be determined by the effectiveness of the processing methods employed. Processing methods thus influence quality of compounded rations or diets and thereby affect blood biochemical and haematological properties. Biochemical components of food are sensitive to elements of toxicity in feeds. They can also be used to monitor protein quality of feeds. Hematological components are also valuable in monitoring feed toxicity

especially with feed constituents that affect formation of blood (Oyawoye and Ogunkunle, 1998). The trial however aims at determining the effect of feeding raw and processed pigeon pea seeds on the haematological and serum biochemistry of Japanese quails chicks.

Materials and Methods

Experimental site

This study was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, Ahmadu Bello University – Samaru – Zaria, Kaduna State. It is located within the Northern Guinea Savanna zone of Nigeria, Latitude 11° 12'N and Longitude 7° 23'E, at an altitude of 610m above sea level. The climate is relatively dry, with a mean annual rainfall of 700-1400mm, occurring between the Months of April and September. The dry season begins around the middle of October, with cold weather that ends in February. This is followed by relatively hot – dry weather from March to April, before the onset of rain. The mean maximum temperature ranges between 14°C to 24°C during the cold season while the mean maximum daily temperature is from 19°C to 36°C during the hot season. The relative humidity varies between 19 to 35% during the hot season, and 63 to 80% in the wet season as reported by Ovimaps (2012).

Source of seeds and processing

The Pigeon pea seeds used for this study were purchased from Samaru Market in Sabon Gari Local Government Area of Kaduna State, Nigeria.

Processing of pigeon pea seeds

Four processing methods of pigeon pea seeds were used.

Raw pigeon pea sample

For the raw sample, 30 kg of well stored pigeon pea seeds were milled and bagged. Soaking in water for different hours. Thirty

kilogram (30 kg) pigeon pea seeds were poured into 75 litres of cold clean water for different hours; 24, 48 and 72 hours at room temperature. The water was drained after each soaking duration and the seeds were sun dried for 4 days on a concrete floor. Seeds were then milled and bagged.

Fermentation

Thirty kilogram (30 kg) pigeon peas seeds were soaked in 75 litres of water for 24 hours at room temperature. The water was allowed to drain and the seeds bagged and put into an air – tight polythene bag and allowed to ferment for 3 days. After 72 hours, the seeds were then sun dried on a concrete floor for 4 days. Seeds were then milled and bagged.

Boiling for 60 minutes

Thirty kilogram (30 kg) pigeon pea seeds were boiled for 60 minutes. The 75 litres of water was allowed to boil at a temperature of 100°C using fire wood as the source of heat. The seeds were then poured into the boiling water, covered and then allowed to boil again for a specific duration of 60 minutes. The cooked sample was removed after draining the water and dried on a concrete floor for 4 days. Seeds were then milled and bagged.

Roasting

Thirty kilogram (30 kg) of raw pigeon pea seeds were roasted in a silver pot on a big tripod stand with fire wood supplying a steady flame. The seeds were constantly stirred with a spoon so as to have a uniform roast and prevent the seeds from burning. The roasting was done for 30 minutes. Seeds were then milled and bagged.

Milled pigeon pea seeds processed by the different methods above were incorporated into the dietary treatments at 30 % level of inclusion.

Experimental diets

Eight experimental diets were formulated based on the standard requirement (NRC,

1994) as shown in Table 1. Milled pigeon pea seeds processed by different methods as described above were incorporated into the dietary treatments at 30% of diet. Diet 1 formulated with soya bean seed meal as the main vegetable protein source served as the control. Diet 2 was formulated with raw pigeon pea seeds, while diets 3, 4, and 5 were formulated with pigeon pea seeds soaked for 24, 48 and 72 hours respectively. Diet 6 was formulated with pigeon pea seeds that were soaked and fermented for 3 days. Diet 7 contained pigeon pea seeds boiled for 60 minutes while Diet 8 contained pigeon pea seeds roasted for 30 minutes.

Design and management of experimental birds

A total of seven hundred and twenty (720) one – week old Japanese quail chicks purchased from National Veterinary Research Institute (NVRI) Vom, Jos were used for the study. Ninety birds were randomly assigned to each of the dietary treatments which had 30 chicks per replicate in a completely randomized design. The birds were raised in 75cm long × 75cm wide × 60cm high cages. Kerosene stoves were used to provide heat while electricity bulbs were installed in each pen to provide light and heat during the brooding period. Feed and water were provided *ad libitum*. Drugs were administered as at when necessary and all other routine management practices duly observed. The experiment lasted for five weeks.

Blood sampling

At the 6th week, three male birds selected per replicate were bled and 1.0 ml of blood samples were collected from each bird by puncturing the jugular vein using hypodermic needle and a 3 ml sterile syringe and allowing free flow of blood into labelled sterile universal bottles containing

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Table 1: Composition of Quail Starter Diets Containing Differently Processed Pigeon Pea Seed Meal

Ingredients	Treatments							
	1 Control	2 Raw PPSM	3 Soaked PPSM (24hrs)	4 Soaked PPSM (48hrs)	5 Soaked PPSM (72hrs)	6 Soaked+ Ferment ation	7 Boiled PPSM (60mins)	8 Roasted PPSM (30mins)
Maize	47.97	30.29	30.29	30.29	30.29	30.29	30.29	30.29
SBM	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
PPSM	0.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
GNC	37.53	25.21	25.21	25.21	25.21	25.21	25.21	25.21
Bone	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Common Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total (%)	100	100	100	100	100	100	100	100
Determined Analysis								
M.E (Kcals/Kg)	2933	2976	2971	2965	2881	2927	2975	2955
Crude Protein (%)	25.12	25.88	26.13	26.68	24.87	24.68	25.06	24.50
Crude Fibre (%)	5.73	5.64	5.00	5.45	6.70	6.00	5.94	6.25
Ether Extract (%)	4.54	4.16	3.92	4.00	4.34	3.85	3.77	4.08
Calculated Analysis								
Calcium (%)	1.39	1.40	1.40	1.40	1.40	1.40	1.40	1.40
Phosphorus (%)	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Lysine (%)	1.22	1.43	1.43	1.43	1.43	1.43	1.43	1.43
Methionine (%)	0.74	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Cysteine (%)	0.41	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Feed cost/kg (₹)	80.20	89.53	89.53	89.53	89.53	89.53	96.20	96.20

*Biomix chick premix provided per kg of diet: Vit A. 10,000 I.U.; Vit D₃ 32,000 I.U.; Vit E 23,000 mg; Vit K 2,000mg; Vit. B₁ 1,800 mg; Vit. B₂ 5,000mg; Pantothenic acid 7,500mg; Vit.B₁₂ 150mg; Folic acid 750mg; Biotin 100mg; Choline chloride; 300,000mg; Cobalt 3,000mg; Iodine 1,000mg; Iron 20,000mg; Manganese 40,000mg; Selenium 200mg; Zinc 30,000mg; Antioxidant 1250mg.

SBM = Soybean meal, PPSM = Pigeon Pea Seed Meal, ME = Metabolizable Energy, GNC = Groundnut cake

anti-coagulant Ethylene Diamine Tetra Acetic (EDTA) to determine the haematological component (Frandsen, 1986). Another 1.0 ml was collected inside labelled sterile sample bottles without anti-coagulant and used for serum biochemistry investigation. The samples were analyzed in the laboratory for haematological parameters which included packed cell volume (PCV), haemoglobin (Hb) concentration, white blood cell (WBC), red blood cells (RBC), lymphocytes, neutrophil, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular

haemoglobin concentration (MCHC). Serum biochemical indices investigated were urea, creatinine, total protein, cholesterol and albumin.

Statistical analysis

All data obtained in this study were subjected to analysis of variance using the general linear model procedure of Statistical Analytic System (SAS, 2008). Significant differences among treatment means were separated using Tukey model.

Results and Discussion

The results of the effect of feeding raw and processed pigeon pea seed meal diets on

haematological and serum biochemical indices of Japanese quail chicks are presented in Tables 2 and 3 respectively. Significant effects of dietary treatments were observed in all the parameters measured across all the dietary treatments except albumin concentration. Blood parameters is a reflection of the effects of dietary treatments on the animals in terms of the type, quality and amounts of the feed ingested and available for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola *et al.*, 2004; Olorode, 2007; Odetola, *et al.*, 2012). The packed cell volume (PCV), haemoglobin (Hb), and red blood cells (RBC) were

similar and significantly ($P < 0.05$) higher for birds on diets T1 (control), T2 (Raw PPSM), T3 (Soaked PPSM for 24 hours), T4 (Soaked PPSM for 48 hours) and T5 (Soaked PPSM for 72 hours) compared to those on other treatments T6 (Fermented PPSM), T7 (Boiled PPSM) and T8 (Roasted PPSM).

PCV is an index of toxicity, any reduction in its concentration in the blood usually suggests presence of toxic factors (haemagglutinins) which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). The PCV values obtained in this study fell within the normal range for avian species.

Table 2: Effect of diets containing differently processed pigeon pea seed meal on haematological parameters of Japanese quail chicks

Parameters	TREATMENTS								SEM
	1 (Control)	2 Raw PPSM	3 Soaked PPSM (24hrs)	4 Soaked PPSM (48hrs)	5 Soaked PPSM (72hrs)	6 Soaked + Fermentation	7 Boiled PPPSM (60mins)	8 Roasted PPSM (30mins)	
PCV (%)	49.37 ^a	51.17 ^a	50.00 ^a	51.60 ^a	52.17 ^a	41.00 ^b	44.72 ^b	47.11 ^b	3.55 [*]
Hb (g/dl)	16.40 ^a	16.92 ^a	16.63 ^a	17.17 ^a	17.35 ^a	13.63 ^b	14.83 ^b	15.64 ^b	1.20 [*]
RBC ($\times 10^6/\text{mm}^3$)	8.27 ^a	8.50 ^a	8.30 ^a	8.57 ^a	8.83 ^a	6.80 ^b	7.41 ^b	8.05 ^b	0.61 [*]
WBC ($\times 10^3/\text{mm}^3$)	7.30 ^a	7.50 ^a	9.23 ^a	6.12 ^b	6.93 ^a	8.29 ^a	6.89 ^a	8.58 ^a	1.53 [*]
MCV (%)	53.76 ^b	60.17 ^a	60.24 ^a	60.22 ^a	59.20 ^a	60.31 ^a	60.35 ^a	58.47 ^a	2.09 [*]
MCH (pg)	19.87 ^a	19.88 ^a	20.03 ^a	20.03 ^a	19.69 ^a	20.05 ^a	20.00 ^a	19.41 ^b	0.25 [*]
MCHC (%)	33.24 ^a	33.04 ^b	33.26 ^a	33.26 ^a	33.25 ^a	33.25 ^a	33.15 ^a	33.20 ^a	0.09 [*]
Lymphocytes (%)	87.33 ^b	88.49 ^a	88.67 ^a	92.17 ^a	88.50 ^a	87.78 ^a	90.45 ^a	90.11 ^a	2.01 [*]
Neutrophil (%)	12.33 ^a	11.28 ^a	10.83 ^a	7.83 ^b	11.17 ^a	12.00 ^a	9.11 ^a	9.66 ^a	1.90 [*]

abc: Means with different superscripts on the same row are significantly different ($P < 0.05$). PPSM : Pigeon Pea Seed Meal, SEM = Standard

Error of Means, PCV = Packed Cell Volume, HB = Haemoglobin, RBC = Red Blood Cells, WBC = White Blood Cells,

MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration

Table 3: Effect of diets containing differently processed pigeon pea seed meal on serum biochemistry of Japanese quail chicks

Parameters	TREATMENTS								SEM
	1 (Control)	2 Raw PPSM	3 Soaked PPSM (24hrs)	4 Soaked PPSM (48hrs)	5 Soaked PPSM (72hrs)	6 Soaked + Fermentation	7 Boiled PPPSM (60mins)	8 Roasted PPSM (30mins)	
Total Protein (g/dl)	7.70 ^a	6.57 ^b	5.90 ^b	6.01 ^b	6.10 ^b	5.68 ^b	5.88 ^b	5.62 ^b	0.50 [*]
Cholesterol (mg/dl)	4.42 ^b	5.23 ^{ab}	5.02 ^{ab}	4.32 ^b	5.82 ^a	4.47 ^b	4.49 ^b	4.19 ^b	0.37 [*]
Albumin (g/dl)	34.00	34.33	33.22	34.56	32.89	35.56	30.56	31.99	3.22 ^{ns}
Urea (mg/dl)	4.44 ^b	5.31 ^b	5.06 ^b	5.91 ^b	5.06 ^b	4.98 ^b	7.56 ^a	4.53 ^b	0.92 [*]
Creatinine (mg/dl)	66.78 ^b	72.00 ^b	67.19 ^b	69.55 ^b	60.45 ^b	66.67 ^b	89.11 ^a	57.45 ^b	8.61 [*]

abc: Means with different superscripts on the same row are significantly different ($P < 0.05$), NS = Non - significantly different ($P > 0.05$) PPSM :

Pigeon Pea Seed Meal, SEM = Standard Error of Means

Maxwell *et al.* (1990) reported 23 – 55 % for healthy birds which showed that the birds were not anaemic. The values obtained for Hb were also within the recommended haemoglobin concentration (7 – 18.6 g/dl) for healthy birds (Maxwell *et al.*, 1990). The aim of estimating the haemoglobin content

was to determine the oxygen carrying capacity of the bird's circulatory system (Mmereole, 1996). The values obtained for RBC of experimental birds may suggest nutritional adequacy of the diets and safety of the test ingredient (PPSM). The WBC and neutrophils were significantly ($P < 0.05$)

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lower in birds fed T4 compared to those on other treatments. The range of values ($6.12 - 9.23 \times 10^3/\text{mm}^3$) obtained for white blood cell counts in birds across the dietary treatments may suggest that there was no microbial infection or the presence of foreign bodies in the circulatory system of the birds.

The Mean corpuscular volume (MCV) and Lymphocytes were also significantly ($P < 0.05$) lower in birds fed the control diet compared to those on PPSM diets. The Mean Corpuscular Haemoglobin (MCH) was significantly ($P < 0.05$) lower in birds fed T8 while Mean corpuscular Haemoglobin Concentration (MCHC) was significantly ($P < 0.05$) lower in birds fed T2 compared to other treatments. The values obtained for MCV, MCH and MCHC in this study may suggest nutritional adequacy of the diets.

The high values (87.33 – 92.17 g/dl) of lymphocytes of birds across the dietary treatments suggests the resistance of the birds to disease conditions. The lymphocytes to neutrophil ratio in this study were similar to that reported by Fouad and Bynner (2008) of greater lymphocytes: neutrophil ratio observed in caged chicken compared to those reared in floor pens.

The result also indicated that PPSM diets had significant ($P < 0.05$) effect on total protein, cholesterol, urea and creatinine. However, PPSM diets had no significant ($P > 0.05$) effect on albumin of the experimental birds. The total protein was significantly ($P < 0.05$) higher in the control group compared to all other treatments. The values obtained for total serum protein of birds were within the normal range recommended for healthy birds as most normal birds have serum total protein values between 3 and 9 mg/Ld (Altman and Katz., 1979; Maxwell *et al.*, 1990). Values less than 2.5 mg/Ld indicates a grave

prognosis and birds with severe hypoproteinemia rarely survive (Altman and Katz., 1979). However, the non-significant difference in the total serum protein observed among birds on PPSM based diets also suggests nutritional adequacy of the diets and safety of the test ingredient. Urea and creatinine were also significantly ($P < 0.05$) higher in birds fed T7 compared to those on other treatments. The creatinine values observed in this study may suggest that the diets were of good protein quality and there was no muscular stress caused by anti-nutritional factors in birds.

Cholesterol levels of birds fed diets T5, T2 and T3 were similar but significantly ($P < 0.05$) higher compared to those on other treatments. However, the cholesterol content (4.19 and 5.82 g/dL) of birds in this study were within the normal range reported by Mitruka and Rawnsley (1977) and Akinola and Abiola (1999). The non-significant difference in albumin values observed in birds across the treatments may suggest nutritional adequacy of the diets and safety of the test ingredients.

Conclusion

This study has demonstrated that both raw and processed PPSM possess good dietary protein quality for optimal growth of quail chicks. However, the result revealed that feeding both raw and PPSM at 30 % inclusion level did not alter the haematological and serum biochemical variables measured as these were within the normal range and posed no adverse effect on the health status of the quail birds.

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