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ASSESSMENT OF SOME SERUM BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN BLOOD SAMPLES OF JAPANESE QUAILS FED DETOXIFIED JATROPHA SEED CAKE

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ABSTRACT

Jatropha curcas L. is a multipurpose shrub plant that grows wildly in the tropics and subtropics. The nutritional value of Jatropha seed cake (JSC), a by-product of *Jatropha curcas* seeds, compares favourably with conventional seed meals with a crude protein content of 58-64% and higher essential amino acids (excluding lysine) than soybean meal. However, the presence of anti-nutrients restricts the use of Jatropha seeds in poultry feeding. If properly detoxified, JSC can be utilised in livestock feeding. The detoxification and reuse of this seed cake are vital for adding economic value and also to reduce the potential environmental damage that may be caused by improper disposal of this by-product. The study was carried out to investigate the effect of heat-treated Jatropha seed cake on haematological and some serum biochemical parameters of growing Japanese quails. Diet 1 was a corn-soybean meal (basal diet) with no Jatropha seed cake (JSC). Diets 2, 3, 4 and 5 contained the basal diet and 5, 10, 15 and 20% JSC inclusion respectively, in a completely randomised design. Two hundred 14-day–old Japanese quails were grouped, weighed and randomly assigned to five treatments with five replicates of eight birds each in an experiment that lasted for two weeks. On day 28, blood samples were collected from the jugular vein of two birds per replicate into vials containing the anticoagulant ethylene diamine tetra-acetate (EDTA) for haematology and blood without EDTA to obtain serum for analysis. Diets had no significant effect on the haematological parameters of birds on the experimental diets except on the white blood cell (WBC) counts. White blood cell counts of birds fed 10% JSC diet was significantly ($P < 0.05$) higher than those fed 5% JSC diet but WBC counts of birds on both diets were similar to birds on other dietary treatments. Total proteins of birds fed the control, 5%, 10% and 15% JSC diets were similar but significantly ($P < 0.05$) higher than those of birds on 20% JSC diet. Serum urea, creatinine and aspartate aminotransferase (AST) of birds on the experimental diets were identical but significantly ($P < 0.05$) lower than those of birds on 20% JSC diet. Alanine aminotransferase (ALT) of birds on 15 and 20% JSC diets were similar to those on the control diet. It is concluded that 10% heat-treated jatropha seed cake could potentially be used in Japanese quail feeding because it reflects the optimal physiological state of the birds compared to other diets.

Key words: Jatropha seed cake, Japanese quails, heat treatment, blood metabolites

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INTRODUCTION

Conventional feedstuffs commonly used in formulating poultry rations are expensive, thereby, limiting the growth of the poultry industry in the tropics. Hence, it becomes imperative to identify cheap, locally available and less competitive alternatives to the conventional feedstuffs to widen feed resource in poultry nutrition [1]. Jatropha plant is a multipurpose drought resistant shrub, a native to tropical America but thrives in Africa, Asia and other tropical and subtropical countries. It also grows in some climatic zones including areas of low rainfall. Other attributes of Jatropha include easiness to establish, relatively rapid growth rate and ability to grow on marginal land and reclaim or restore eroded soils. Various parts of the plant hold potential for use as animal feed [2]. Jatropha plant produces a large quantity of seeds per hectare ranging from 0.1-8.0 tonnes/ha/year [3].

Preliminary studies on the nutritional value of Jatropha seeds showed that jatropha seed meal contains high crude protein of about 56.4%, which is higher than that of soybean meal (48%); the seed also has higher minerals and vitamins [4]. It is considered a potential protein source in livestock feeding. However, Makkar and Becker [5] reported that the presence of a cocktail of anti-nutritional factors like phorbol esters, saponins, tannins, phytates, lectins, hydrocyanides and oxalates prevent its use in animal feeding; phorbol ester is considered the most toxic compound. Therefore, to enhance utilisation of this protein-rich supplement, an appropriate detoxification process becomes imperative. Both physical and chemical treatments have been employed in detoxification of jatropha seeds and have been reported to reduce lectin, trypsin inhibitor, and phorbol ester compounds in jatropha seeds to a minimal level [6, 7].

Examination of blood for their constituents has been used to monitor and evaluate disease prognosis of animals [8]. Various reports of blood profile in animals have been well documented [9, 10, 11]. The blood contains a myriad of metabolites and other constituents, which provide a valuable medium for clinical investigation and nutritional status of animals. According to Church [12], dietary components have measurable effects on blood constituents such that significant changes in their values could be used to draw inference on the nutritive value of feeds offered to the animals. It was, therefore, the aim of this study to determine the effect of heat treated Jatropha seed cake supplemented diets on some haematological and biochemical parameters of growing Japanese quails.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Oyo State, Nigeria. The University is located at latitude 7^{o} 10^{o} N and longitude 3^{o} 2^{o} E and lies in the South-western part of Nigeria with a prevailing tropical climate with a mean rainfall of about 1037mm per annum. The mean monthly ambient temperature ranges from 28^{o}C in December to 36^{o}C in February with an average yearly humidity of about 82%. The vegetation in the University represents an interphase between the tropical rain forest and the derived savannah. The project complied with the University of Ibadan ethics requirements for animal handling. The study was

carried out within the University of Ibadan Teaching and Research Farm and did not include any activity that contravenes animal welfare and humane handling in animal husbandry.

Preparation of Test Ingredient

The Jatropha seeds were sourced from a reliable Jatropha plantation in Ibadan, Oyo state, Nigeria. The seeds were toasted (it was done by spreading out the seeds in an even layer, heating them over medium heat and shaking often until the seeds were golden brown and removed from pan to cool) for about 20 minutes before oil extraction was done with the use of a mechanical screw press (a palm oil extraction press). The press residue which was the seed cake was immersed in water and heated in an open fire to about 90^{o}C for 45 minutes for further oil removal. Cooling was allowed to take place for separation of oil moisture and the residue. The separated seed cake was further sun dried for 5 days until a significant amount of moisture was removed [13]. The cake was milled using a hammer mill. Other feed ingredients like maize, soybean meal, dicalcium phosphate (DCP), salt, limestone, vitamin premix, DL-methionine and lysine were purchased and mixed at the University of Ibadan feed mill.

Management of Birds and Experimental Diets

Two hundred (200) two-week–old Japanese quails were used for this study. The birds were reared and housed in a 25-unit compartment cages placed in a well-ventilated and illuminated poultry house. They were randomly allotted to five dietary treatments consisting of five replicates of eight birds in a completely randomised design using Experimental Animal Allotment Programme (EAAP) [14]. Diets were formulated to meet the requirements of growing quails according to National Research Council (NRC) [15]. Diet 1 was the control diet (a corn-soyabean meal diet) without Jatropha seed cake while diets 2, 3, 4 and 5 contained the control diet with varying levels of heat-treated jatropha seed cake substituted for soybean meal at 5, 10, 15, and 20%, respectively (Table 1) [13]. Experimental diets were given *ad libitum* and birds had free access to clean water.

Blood Collection and Determination of Blood Parameters

On day 28, blood samples were collected from the jugular veins of 2 birds per replicate into two vaccutainer tubes for each poult, one containing the anti-coagulant Ethylene Diamine Tetra acetic Acid (EDTA) for haematological analysis and the other sterile vaccutainer tubes without EDTA. The blood in second set of tubes was allowed to clot; the tubes were covered and centrifuged to separate the serum in each tube. Each serum was then decanted into another labeled sterile tube that was kept deep-frozen till required for the biochemical analyses.

Packed Cell Volume Estimation

The blood samples collected in bottles containing EDTA were gently mixed and drawn up in a micro haematocrit capillary tube to ¾ of its length. One end of the tube was sealed with plasticine. The capillary tube was placed in micro–haematocrit centrifuge ensuring that the plasticine end is outward. After closing, these were then centrifuged at 12,000 rpm for 4 minutes. The tubes were then read in the haematocrit reader. The reading expressed the packed red blood cells as a percentage (%) of the total volume of blood [16].

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Haemoglobin Concentration Determination
Haemoglobin concentration was determined by a cyanmethaemoglobin method using Drabkin's solution as diluent [16].

Red Blood Cell (RBC) and Platelets Counts
Properly mixed blood sample from bottle containing EDTA was drawn up to 0.5 mark of a red blood cell pipette. The tip of the pipette was immersed into normal saline and carefully drawn up to exactly 101 marks after which the dilute blood was mixed by shaking for about half a minute. About a quarter of the content was expelled before filling the haematocytometer counting chamber and was allowed to stand for about a minute to settle after filling. All the red cells were then counted using the x 40 objective lens and x8 eye piece of the microscope, with the aid of a counter [17]. Platelets were determined by phase microscopy method of Brecher and Cronkite [18].

White Blood Cell (WBC) and Differential Leukocytes Counts
The total leukocyte counts were determined using Neubauer haemocytometer after appropriate dilution, and differential leukocyte counts performed using the oil immersion objective examination of blood films stained with the modified Romanovsky's Giemsa stain [17].

Serum Parameters
The biuret method was used for the determination of total protein. Albumin was determined by the direct colorimetric method that uses Bromocresol Green (BCG) dye as described by Peters *et al.* [19]. Serum creatinine was determined using the principle of Jaffe reaction as described by Bonsnes and Tausslay [20], while serum urea was determined by the kit (Quinica Clinica Spam), the Uricase method as described by Wootton [21].

Aspartate and Alanine Aminotransferases Determinations
Aspartate amino transferase (AST) and Alanine aminotransferase (ALT) activities were determined using spectrophotometric methods as described by Rej and Hoder [22].

Chemical and Statistical Analyses
The proximate composition of diets was determined by the methods of Association of Official Analytical Chemists (AOAC) [23]. Data were analysed using descriptive statistics and Analysis of Variance, ANOVA ($P<0.05$) SAS [24]. Means differences were separated using Duncan's Multiple Range Test.

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RESULTS

The result of haematological indices of growing Japanese quails fed experimental diets is as shown in Table 2. There was no effect of dietary treatments on all the parameters measured except the white blood cell (WBC) counts. White blood cell counts of birds on 5, 10, 15 and 20% Jatropha seed cake-based diets were similar to those on the control diet; however, there was a significant difference between WBC counts of birds fed 5 and 10% Jatropha seed cake diets.

Table 3 shows the results of the analysis of some biochemical parameters in the serum of growing Japanese quails placed on experimental diets. The diets had significant ($P < 0.05$) effects on the total protein, urea, creatinine concentrations as well as AST and ALT activities. However, serum albumin concentration of birds on the treatment diets did not differ significantly. Total serum proteins of birds on the control, 5, 10 and 15% JSC diets were identical but were significantly ($P < 0.05$) higher than those of birds on 20% JSC diet. Serum urea, creatinine and AST of birds on the experimental diets were similar but significantly ($P < 0.05$) lower than those of birds on 20% JSC diet. Alanine aminotransferase (ALT) of birds on 15 and 20% JSC diets were similar to those of birds on the control diet.

DISCUSSION

The haematological parameters are important indices that reflect the physiological state of the individual animal, the ability to interpret the blood profile in healthy and diseased conditions is one of the primary objectives of haematological studies [25].

The results of the haematology parameters obtained in this study except for WBC are within the normal ranges reported for growing Japanese quails [26, 27]. However, observed packed cell volume (PCV) in this study was lower than the reported values (40.00 and 44.00%) by Al-Daraji *et al.* [28] and Akande *et al.* [27] when Japanese quails were fed with graded levels of cassava peel meal fortified with dried brewer's grains. The result of the red blood cell (RBC) counts was within the normal range (3.8 to 5.5 $x10^6/\mu L$) reported by Campbell [29]. Haemoglobin and PCV results were within the respective normal ranges (12. 0 to 15. 2g/dL and 37.0 to 69.0%). This implies that probably the concentration of residual anti-nutrients present in the test ingredient was insufficient to limit the bioavailability of iron (Fe) as Fe is a major constituent of haemoglobin [30].

Serum chemistry is routinely used to detect organ disease in domestic animals and the amount of available protein in the diets [31]. The range of total protein observed in this study (1.51 – 3.81g/dL) was below the range (5.87 – 6.55g/dL) reported by Akande *et al*. [27] when Japanese quails were fed with graded levels of cassava peel meal fortified with brewer's grain. The variation observed in this study when compared with the reported values could be attributed to the total protein intake [13]. Abdel Hameed *et al***.** [11] reported that the serum protein concentration at any given time is a function of hormonal balance, nutritional status, water balance and other factors affecting health. The least value observed in the total protein (1.51g/dL) and albumin (1.57g/dL) of the quails fed

with 20% jatropha seed cake could be attributed to the binding effects of residual metabolite (phorbol ester), in the test ingredient which may have prevented the proper utilisation of the protein in the diets [1, 7]

Eggum [32] noted that there was a relationship between diet protein and total serum protein and this has been used to measure protein quality. Serum proteins are important in osmotic regulation, immunity and transport of several substances in the blood [33]. High serum urea value was recorded in quails fed with 20% Jatropha seed cake diet compared to other treatments. This may probably have been due to persistent hypoglycemia according to Radostits *et al*. [34]. Catabolic activity is increased for gluconeogenesis thus resulting in higher serum urea levels.

According to Carola *et al*. [35], normal enzyme level in serum is a reflection of a balance between biosynthesis of the enzyme and its release, as a result of the different physiological processes in the body. Enzyme activities are useful indicators in nutrition study as both AST and ALT are connected with amino acid metabolism and serve as useful indicators of cell damage in the liver [36]. The highest AST and ALT values (146.85 and 5.84 U/L), respectively recorded in quails fed with 20% Jatropha seed meal were lower than the reported values by Al-Daraji *et al*. [28]. However, the values of ALT and AST were within the normal physiological range for quails as reported by Bounous and Stedman [37], which indicate normal functioning of liver.

CONCLUSION

The present study showed that heat-treated Jatropha seed cake-based diets in 14-day-old growing quails had no effect on the haematological parameters measured except the white blood cell counts. The total serum protein, urea, creatinine, AST and ALT of birds on dietary treatments were significantly influenced, though total serum protein and urea were negatively affected at 20% Jatropha inclusion. However, albumin levels of birds were not affected by the diets.

It can, therefore, be concluded that 10% detoxified Jatropha seed cake could potentially be used in feeding growing Japanese quails because it reflects the optimal physiological state of the birds compared to other diets.

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Table 1: Gross composition (g/100g DM) of experimental diets

Jatropha seed cake inclusion (%)					
Ingredient	**0**	**5**	**10**	**15**	**20**
Corn	56.20	56.20	56.20	56.20	56.20
Soybean meal	40.00	38.00	36.00	34.00	32.00
Jatropha seed cake	0.00	2.00	4.00	6.00	8.00
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50
Limestone (38% Ca)	1.20	1.20	1.20	1.20	1.20
Salt	0.25	0.25	0.25	0.25	0.25
*Vit-Min Premix	0.25	0.25	0.25	0.25	0.25
DL- Methionine	0.35	0.35	0.35	0.35	0.35
L-Lysine	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated Nutrient (%)					
Protein	22.4	22.5	22.7	22.8	22.9
Metabolisable energy (Kcal/kg)	3010	3020	3030	3039	3049
Calcium	0.84	0.84	0.83	0.83	0.83
Phosphorus	0.68	0.67	0.66	0.65	0.63
Lysine	1.53	1.52	1.52	1.51	1.51
Methionine	0.72	0.74	0.77	0.79	0.82

*Vit-Min= Vitamin-Mineral, *Composition of Premix per Kg of diet: vitamin A, 12,500 I.U; vitamin D_3, 2,500 I.U; vitamin E, 40mg; vitamin K_3, 2mg; vitamin B_1, 3mg; vitamin B_2, 5.5mg; niacin, 55mg; calcium pantothenate, 11.5mg; vitamin B_6, 5mg; vitamin B_{12}, 0.025mg; choline chloride, 500mg; folic acid, 1mg; biotin, 0.08mg; manganese, 120mg; iron, 100mg; zinc, 80mg; copper, 8.5mg; iodine, 1.5mg; cobalt, 0.3mg; selenium, 0.12mg; Anti-oxidant, 120mg*
DM – *Dry matter*

Source: Agboola and Adenuga [13]

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Table 2: Haematological parameters of growing Japanese quails fed different inclusion levels of Jatropha seed cake

	Levels of Jatropha seed cake (%)					
Parameter	**0**	**5**	**10**	**15**	**20**	**SEM**
Packed Cell Volume (%)	38.70	38.70	36.80	37.13	37.00	1.13
Haemoglobin (g/l)	12.77	12.77	11.96	12.30	12.85	0.36
Red Blood Cell ($x10^{12/L}$)	4.02	4.16	3.90	3.72	3.86	0.18
White Blood Cell ($x10^{9/L}$)	18.64^{ab}	15.21^{b}	19.86^{a}	18.35^{ab}	17.73^{ab}	1.12
Lymphocyte ($x10^{9/L}$)	62.70	68.40	63.30	62.80	63.00	3.50
Heterophyl ($x10^{9/L}$)	31.00	25.80	31.30	32.50	31.00	3.67
Monocyte ($x10^{9/L}$)	2.40	2.70	3.00	2.38	2.50	0.53
Eosinophil ($x10^{9/L}$)	3.50	2.90	2.70	2.25	3.00	0.59
Basophil ($x10^{9/L}$)	0.40	0.20	0.00	0.13	0.50	0.17
Platelets ($x10^{9/L}$)	154.90	150.10	132.20	123.50	161.0	12.00

^{ab} *Means along the same row with different superscripts are significantly different (P < 0.05)*

SEM - *Standard Error of Mean*

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Table 3: Some biochemical parameters in the serum of Japanese quails fed varying levels of Jatropha seed cake

		Levels of Jatropha seed cake (%)				
Parameter	**0**	**5**	**10**	**15**	**20**	**SEM**
Total protein (g/dL)	3.33^{a}	3.81^{a}	3.66^{a}	2.82^{ab}	1.51^{b}	0.46
Albumin(g/dL)	1.72	1.80	1.84	1.60	1.57	0.10
Urea (mg/100ml)	6.68^{b}	6.15^{b}	5.07^{b}	4.59^{b}	12.83^{a}	0.77
Creatinine (mg/100ml)	0.60^{b}	0.63^{b}	0.43^{b}	0.74^{b}	1.15^{a}	0.15
AST (U/L)	127.42^{b}	119.21^{b}	119.00^{b}	115.24^{b}	146.85^{a}	8.75
ALT (U/L)	5.04^{ab}	3.71^{b}	4.05^{b}	4.38^{ab}	5.84^{a}	0.42

^{ab} Means along the same row with different superscripts are significantly different ($P < 0.05$)

SEM - Standard Error of Mean, AST – Aspartate aminotransferase, ALT - Alanine aminotransferase

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