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Haematological and Serum Biochemical Characteristics of Hacco Cocks Fed Cocoabean Shell Supplemented with Vitamin E and Beta-Glucan

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Abstract: A fifty-six (56) days feeding trial was conducted in a completely randomized design to determine the haematological and serum biochemical characteristics of Hacco cocks fed Cocoabean Shell (CBS) supplemented with vitamin E and beta-glucan. Forty (40) cockerels (aged twenty-five weeks) were used. Treatment diets comprised T_1 (control) (i.e. 0% CBS); T_2 (20% CBS); T_3 (20% CBS + Vitamin E) and T_4 (20% CBS + Beta-glucan). The haematological parameters determined were Packed Cell Volume (PCV), haemoglobin concentration (Hb), Red Blood Cell Count (RBC) and White Blood Cell Count (WBC). Others included the WBC differential counts-polymorphs, lymphocytes, monocytes and eosinophils. The serum biochemical parameters determined were serum enzyme activities namely Spartate Amino Transferase (AST), Alanine Amino Trasferase (ALT) and alkaline phosphatase. Other parameters were total protein albumin, globulin and cholesterol. Treatment had significant ($p<0.05$) effect on PCV, RBC, WBC and polymorphs, while Hb, lymphocytes, monocytes and eosinophils were not significantly ($p>0.05$) affected by the treatment. Cocks that were fed CBS and CBS + Beta-glucan had similar PCV, RBC and WBC and polymorphs with the control group, while those that were fed CBS + vitamin E had lower values for PCV, RBC and polymorphs than the control group. The groups fed CBS + vitamin E had higher ($p<0.05$) WBC values than other groups. All the serum parameters determined were not significantly affected by dietary treatment. It was concluded that 20% CBS in diets of cocks did not show adverse effect on the blood parameters of the cocks. When supplemented with β-glucan, there was no adverse effect either. However, when supplemented with vitamin E, some haematological parameters were adversely affected.

Key words: Blood characteristics, cocoabean shell, cocks, vitamin E, beta-glucan

INTRODUCTION

The high cost of poultry production has necessitated the search for the non-conventional feed ingredients for feed manufacturing. However in the recent time, the prices of some of the non-conventional feed ingredients such as wheat offal, corn bran, rice bran and the like are becoming expensive. There are quite a number of agro-industrial by-products that are less utilized due to the fear of the anti-nutritional factors which they contain. One of such by-products is Cocoa-Bean Shell (CBS).

The nutritional potential of CBS has been documented. Investigations by researchers (Menon, 1982; Ching and Wong, 1986; Yeong *et al*., 1989 and Abiola and Tewe, 1991) indicated that CBS contains between 13.2% and 17.7% crude protein, while the fibre content ranges between 13.0% and 16.1%. Hutagalung and Change (1980) observed that the amino acid profile of CBS compares favourably to palm kernel cake, suggesting that it could be utilized as a medium protein source to substitute grain proteins in livestock diets. However, it contains theobromine an alkaloid compound. Gohl (1981) reported that CBS is high in nutritive value but it is of limited use in animal feeds because of its theobromine content. Adverse effect of theobromine on body weight and reproductive characteristics of animals have been reported (Wang *et al*., 1992). Hutagalung and Change (1980) reported that 10% CBS increased body weight of broiler chicken by 20 g/day. According to Olubomiwa *et al*. (2000), raw CBS should not be substituted for more than 10% in layer's mash.

Vitamin E is a fat-soluble vitamin. It is composed of a group of compound called tocopherols. Vitamin E is an antioxidant which counters the oxidation of substances in the body. It prevents saturated fatty acids and vitamin E from breaking down and combining with other substances that may become harmful to the body. According to Smith (2001), vitamin E protects the red blood cells from destruction by poisonous substances such as hydrogen peroxide in the blood.

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Table 1: Gross composition and calculated nutrients of experimental diets

	Treatment			
Ingredient (%)	T1 (Control)	T2 (20% CBS)	T3 (20% CBS + Vitamin E)	T4 (20%CBS + β-glucan)
Maize	17.00	17.00	17.00	17.00
Groundnut cake (GNC)	12.00	12.00	12.00	12.00
Wheat offal	14.00	14.00	14.00	14.00
Corn bran	50.00	30.00	30.00	30.00
Cocoa bean shell	-	20.00	20.00	20.00
Palm kernel cake	2.00	2.00	2.00	2.00
Fish meal	1.00	1.00	1.00	1.00
Bone meal	1.80	1.80	1.80	1.80
Oyster shell	1.20	1.20	1.20	1.20
Methionine	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
Vit/Min premix*	0.30	0.30	0.30	0.30
Salt	0.30	0.30	0.30	0.30
Vitamin E	-	-	+	-
Beta-glucan	-	-	-	+
Total	100.00	100.00	100.00	100.00
Calculated nutrients				
Crude protein (%)	16.06	16.22	16.22	16.22
ME (kcal/kg)	2484.48	2464.48	2464.48	2464.48

*To provide the following per kg of feed: Vitamin A, 10,000 iu; vitamin D_3, 2000 iu; vitamin E/5iu; vitamin K, 2 mg; riboflavin 4.2 mg; vitamin B12, 0.01 mg; pantothenic acid, 5 mg; nicotic acid, 20 mg; folic acid, 0.5 mg; choline, 3 mg; Mg, 56 mg; Fe, 20 mg; Cu, 1.0 mg; Zn, 5.0 mg; Co, 1.25 mg; iodine, 0.8 mg; ME = Metabolizable Energy

Beta-glucan is a primarily cultured extract of Baker's yeast cell wall and cereal fibres, such as oats, wheat and barley. It is used as an immunostimulant. Beta-glucan stimulates white blood cells called macrophages and neutrophils. These cells can recognize and kill tumor cells, remove cellular debris resulting from oxidative damage, speed up recovery of damaged tissue and further activate other components of the immune system (Di Renzo *et al*., 1991; Ross *et al*., 1999). Beta-glucan has also been identified as the key factor for the cholesterol-lowering effect of oat bran (Davidson *et al*., 1991; Braaten *et al*., 1994; Behall *et al*., 1997 and Bell *et al*., 1999). There is dearth of information on the effects of CBS with vitamin E and Beta-glucan supplementation on the blood profile of cocks. This study therefore aimed at evaluating the effect of CBS supplemented with vitamin E and Beta-glucan on the haematological and serum biochemical characteristics of hacco cocks.

MATERIALS AND METHODS

Experimental site: The experiment was carried out at the poultry unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Experimental birds and management: Forty 40 cocks (aged twenty-five weeks) were randomly selected from a batch of cocks previously maintained on standard commercial diet, which served as control in a previous experiment. The cocks were randomly allocated to four treatments with ten (10) birds per treatment and replicated into two with five birds per replicate in deep-litter pens. The birds were exposed to an acclimatization period of two weeks after which feeding trial commenced. Diets were formulated to contain 20% CBS except the control. Feeding trial lasted for 56 days. Feed and clean, cool water were given to birds *ad libitum*.

Diet preparation: The four (4) treatment diets were T_1 (0% CBS) which served as control, T_2 (20% CBS), T_3 (20% CBS + Vitamin E) and T_4 (20% CBS + Beta-glucan). Table 1 shows the gross composition of the experimental diets.

Vitamin E (∝-Tocopherol) tablets, EVITOL, by Teva Pharmaceutical Industries, Petach Tikva, were ground into powder form, dissolved in warm water and administered by gavage daily at a dosage of 13.5 mg/bird. Beta-glucan is an immune booster. It was administered intramuscularly at a dosage of 2ml/bird. It was given twice throughout the experiment (i.e. at the beginning and at 5th week). Feed samples were analyzed for proximate composition using AOAC (1990).

Blood collection and analysis: At the end of the 8th week, five (5) birds per treatment were randomly selected and bled. Blood was obtained from the main vein of the wing-web (vein puncture). The blood that was meant for haematological analysis was collected into plastic bottles coated with anticoagulant, Ethylene Diamine Tetra Acetic Acid (EDTA). The blood that was meant for serum biochemical analysis was collected into bottles that did not contain anticoagulant.

The haematological parameters determined were Packed Cell Volume (PCV), haemoglobin concentration

(Hb), Red Blood Cell (RBC) Count, White Blood Cell (WBC) count and the differential count of the WBC, namely the polymorphs, hymphocytes, monocytes and eosinophils. The serum parameters analyzed were aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP) total protein, albumin, globulin and cholesterol.

The haematological parameters were analyzed as follows. The PCV was determined by centrifuging the micro haematocrit capillary tubes containing blood samples in a haematocrit centrifuge for 5 minutes and read with haematocrit reader. The RBC, WBC and the differential counts were determined using improved Neubauer haemocytometer, while the Hb was determined using cyanmethaemoglobin method of Jain (1986).

The serum enzyme activities, AST. ALT and ALP were determined using spectrophotometric methods as described by Rej and Hoder (1983) and Hoder and Rej (1983). Serum total protein was determined using Biuret method as described by Kohn and Aleen (1995), while albumin was determined using Bromocresol Green (BCG) method of Peters *et al*. (1982). The serum globulin was determined by subtracting albumin value from total protein value. Serum total cholesterol was determined using enzymatic-colorimetric method (according to Randox diagnostic reagent Kit-CHOD-PAP®).

Statistical analysis: Data obtained were subjected to Analysis of Variance (ANOVA) using completely randomized design of SAS (1999). Separation of means was done by Duncan's multiple range test option of same statistical software.

RESULTS AND DISCUSSION

The results of this study are presented in Tables 2-4. The proximate composition of experimental diets and CBS is presented in Table 2. The crude protein content of the CBS was within the range reported by Menon (1982); Ching and Wong (1986) and Yeong *et al*. (1989). The crude fibre content of the CBS was also in agreement with the report of earlier workers. Probably environmental variations and analytical methods did not have appreciable influence on the CBS constituents as these reports represent observations from works done at different locations and times. The crude protein content is however higher for CBS than that of cocoa husk (6.5%) reported by Smith and Adegbola (1985).

The results of the haematological analysis are presented in Table 3. Treatment had significant effect on PCV, RBC, WBC and polymorphs. The PCV value for T_2 (20% CBS) and T_4 (20% CBS + Beta-glucan) was similar to that of the control group, whereas the PCV value for T_3 (20% CBS + vitamin E) was significantly ($p<0.05$) lower than that of the control. Red blood cell count for the cocks fed 20% CBS and 20% CBS + Beta-glucan were also similar to that of the control, while the cocks fed 20% CBS + vitamin E had significantly ($p<0.05$) lower RBC than the control. Haemoglobin concentration was not significantly ($p>0.05$) different among treatments, although 20% CBS + vitamin E tended to be lower than other treatments. WBC for the birds that were fed 20% CBS + vitamin E was significantly ($p<0.05$) higher than for other treatments. Eosinophils for the group fed 20% CBS + vitamin E were also significantly ($p<0.05$) higher in T_3 birds than for other treatment groups. However, polymorphs count was significantly ($p<0.05$) lower for 20% CBS + vitamin E group than for other groups.

Table 2: Proximate composition of experimental diets and cocoa bean shell

Nutrient (%)	T1 (Control)	T2 (20% CBS)	T3 (20% CBS + Vitamin E)	T4 (20%CBS + β-glucan)	Cocoa bean shell
Dry matter	92.00	91.00	91.00	91.00	90.00
Crude protein	15.05	16.09	16.09	16.09	16.08
Crude fibre	4.00	6.00	6.00	6.00	13.00
Ether extract	23.00	21.00	21.00	21.00	17.00
Ash	19.00	17.00	17.00	17.00	10.00
NFE	30.50	30.00	30.00	30.00	37.00
Theobromine	-	-	-	-	0.42

NFE = Nitrogen Free Extract

Table 3: Haematological parameter s of cock fed 20% CBS supplemented with vitamin E and β-glucacan

Parameter	T1 (control)	T2 (20% CBS)	T3 (20% CBS+Vitamin E)	T4 (20%CBS + β-glucan)
PCV (%)	54.60±2.01^{a}	51.80±4.22^{ab}	45.80±0.92	49.60±2.46^{ab}
Hb (g/100 ml)	18.00±0.55	17.10±1.51	15.10±0.26	16.98±0.92
RBC (x10^6/µl)	9.08±0.33^{a}	8.66±0.58^{ab}	7.66±0.09^{b}	8.26±0.41^{ab}
WBC (x10^3/µl)	8.40±0.50^{b}	8.48±0.29^{b}	10.02±0.25^{a}	7.76±0.29^{b}
Polymorphs	40.40±1.03^{a}	38.80±1.43^{a}	31.20±0.66^{b}	38.60±1.86^{a}
Lymphocytes	47.60±1.33	47.60±1.78	50.20±0.58	48.40±1.50
Monocytes	1.60±0.24	1.80±0.37	1.80±0.20	2.00±0.55
Eosinophils	10.40±1.81^{b}	10.60±1.12^{b}	17.00±0.32	11.40±1.44

^{a,b}Means within row with different superscripts differ significantly ($p<0.05$); PCV = Packed Cell Volume; Hb = Haemoglobin concentration; RBC = Red Blood Cell Count; WBC = White Blood Cell Count

Table 4: Serum biochemical parameters of cocks fed cocoabean shell supplemented with vitamin E and β-glucan

Parameter	T1 (control)	T2 (20% CBS)	T3 (20% CBS+Vitamin E)	T4 (20%CBS + β-glucan)
AST (SGOT) (µ/l)	18.20±1.59	18.00±1.34	17.00±0.71	14.80±1.66
ALT (SGPT) (µ/l)	12.80±1.56	12.40±1.12	12.80±1.02	10.60±1.17
Alkaline phosphatase (ALP) (µ/l)	18.80±1.11	20.00±1.58	18.60±0.93	18.20±1.59
Total protein (g/dl)	4.00±0.21	3.96±0.21	4.60±0.26	4.30±0.25
Albumin (g/dl)	2.14±0.11	2.12±0.10	2.44±0.14	2.30±0.14
Globulin (g/dl)	1.86±0.10	1.84±0.11	2.16±0.12	2.00±0.11
Cholesterol (mg/dl)	255.40±12.17	266.40±12.43	262.80±8.09	233.00±10.98

AST: Aspartate amino transferase; ALT: Alamine amino transferase; SGOT: Serum glutamic oxaloacetate transferase; SGPT: Serum glutamic phosphate transferase

Lymphocyte and monocyte counts were not significantly ($p>0.05$) affected by treatment. The values obtained for the PCV, Hb and RBC were generally higher than those reported for normal male chickens by Mitruka and Rawnsley (1977). These high values indicate that none of the treatments elicited anaemia in the subjects. It also means that nutrients were available for adequate erythropoiesis in the cocks. The non-significant variations between the high values of Hb indicate that oxygen-carrying and releasing capacity of the blood was not adversely affected by the treatments. The haematological values observed in this study for PCV, Hb and RBC were generally higher than those reported by Odunsi and Longe (1995) for chick starters at 4 weeks of age when 20% cocoa bean cake was used.

The observation that the birds fed 20% CBS had similar values with the control for most of the haematological variables suggest that at this inclusion level, the theobromine in the CBS does not constitute a potential threat to the health of the birds. The fact that the treatment group that received 20% CBS + Beta-glucan did not differ from the control and 20% CBS group in all haematological variables demonstrates that supplementing CBS with Beta-glucan was unnecessary. The result that the group of birds treated with 20% CBS + vitamin E showed lower values for PCV and RBC and higher value for WBC suggests that supplementing CBS with vitamin E might stimulate theobromine in CBS in the body to depress erythropoiesis. It could also form complexes with metabolites that may be harmful to the body and to which WBC may respond by increasing in number. This is further explained by the same pattern followed as WBC by eosinophils which are known for phagocytosis of foreign bodies or particles. It follows then that supplementing 20% CBS with vitamin E in diets of cocks is unnecessary.

Table 4 shows the serum metabolites of cocks fed cocoabean shell supplemented with vitamin E and β-glucan. All the parameters analyzed, AST, ALT, ALP, serum total protein, albumin, globulin and cholesterol were not significantly ($p<0.05$) different among treatments. The similarities observed in the values of AST, ALT and ALP activities among treatments indicate that there was no tissue damage caused by the diets. This implies that at 20% CBS inclusion level in diets of cocks, theobromine contained in the CBS did not constitute a toxic factor that could damage the liver and other tissues in the cocks. The values obtained for AST and ALP in this study were lower than the minimum values for normal chicken reported by Mitruka and Rawnsley (1977). However, the values obtained in this study for ALT were within the normal physiological range documented by Mitruka and Rawnsley (1977). It has been reported (Ewuola *et al*., 2008) that elevated serum enzyme activities such as AST, ALT and ALP is an indication of heart, kidney and/or liver damage due to cellular destruction caused by toxins.

The non-significant ($p>0.05$) differences observed in the serum total protein, albumin and globulin indicate that the protein content of the diets was adequate and available and that neither vitamin E nor beta-glucan supplementation was necessary for the protein metabolism. While the total protein levels observed in this study fell below the minimum value reported by Mitruka and Rawnsley (1977), the values for both albumin and globulin fell within the range reported by same authors.

Cholesterol level was not significantly ($p>0.05$) different among treatments. Although supplementation of 20% CBS diet with β-glucan tended to lower serum cholesterol. This is in agreement with the findings of Davidson *et al*. (1991) and Braaten *et al*. (1994) that beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects.

It could be concluded from this study that feeding diet containing 20% CBS to cocks is not detrimental to the health and welfare of the cocks. Also, since 20% CBS did not have adverse effect on the haematological and serum metabolites of the cocks, supplementation with either vitamin E or β-glucan is unnecessary.

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