Haematological and serum biochemical indices of three different strains of pullets supplemented with *Khaya senegalensis* stem bark meal as anticoccidial in the diets.

Lala, A. O., Okwelum, N. Bello, K. O., Salami, W. A.
Institute of Food Security, Environmental Resources and Agricultural Research,
Federal University of Agriculture, Abeokuta, Ogun State
Corresponding Author: A. O., Lala funmilala02@yahoo.com

Abstract

The effects of supplementing different levels of Mahogany (khaya senegalensis) stem bark (KSSB) meal in the diet of pullets on some blood parameters was investigated. A total of 360 day-old chicks were arraigned in a 5 x 3 factorial design of 5 dietary levels (Diet 1 served as negative control without supplementation, Diet 2 was supplemented with 100 mg/kg of anti-coccidiostat (sulphaquinoxaline) , Diets 3 and 4 were supplemented with 100 and 200mg/kg KSSB respectively at intervals of 21st - 27th, 42nd – 48th and 112th – 118th day, while diet 5 was supplemented with 100mg/kg KSSB which was continuously fed from 1st -118th day) and 3 strains of chickens (Isa Brown, Nera Black and Oba Blue). Results showed (P<0.05) lowered packed cell volume (PCV) and higher white blood cell count in Isa brown and Nera black fed diet 1. Uric acid, creatinine, alkaline phosphatase and aspartate aminotransferase values were highest (P< 0.05) in pullets fed diets 1 and 5 irrespective of the strain. These results revealed that KSSB at 100mg/kg and 200mg/kg can be included in the diets of pullets at intervals without deleterious effect on the health of the birds. However, continuous usage of KSSB in the diet of the pullets showed some level of toxicity in the blood.

Keywords: haematological indices, khaya senegalensis, pullets, biochemical parameters.

Introduction

The use of phytogenic plants is on the rise among poultry producers because of the belief that all natural products are safe. However, these plants often contain toxins for their self-protection which could be dangerous to the animals and of deleterious effects to humans consuming them. Toxic effects of herbal medicine range from allergic reactions to cardiovascular, hepatic, renal, neurological and dermatologic toxic effects. Also, some herbal medicine are responsible for abnormal laboratory results in form of alternation in liver function tests, electrolyte disturbances and blood sugar level changes (Dasgupta, 2003).

One of the major constraints to production of chickens in developing countries is coccidiosis (Majoro, 1993). The maximum natural challenge of oocysts to commercially reared chickens usually occurs between 3 and 5 wk of age; thus, it is necessary to initiate immunity before this challenge occurs (Chapman *et al.*, 2005). Additionally, anecdotal evidence exists for late-breaking coccidiosis for broiler breeders and layer birds around 19 to 26 wk of age (Mc Dougald *et al.*, 1990; Soares *et al.*, 2004).

With the emergence of resistant strain of *Eimeria* to coccidiocidal drugs and inaccessibility of veterinarians by rural farmers has resulted to the use of available herbal plants in the treatment of coccidiosis (Abbas *et al.*, 2011). Hence, it is a well-recognised fact that treatment alone cannot prevent the economical losses. WHO reported that 80% of the world population especially in developing countries depends upon herbal medicines for prevention and cure (WHO, 2007).

Mahogany (*Khaya senegalensis*) tree bark had been reported to be useful in the treatment of coccidiosis in chickens (Fajimi and Taiwo, 2005; Maikai *et al.*, 2007). Several active compounds have been isolated from the *Khaya senegalensis* stem bark which includes saponins, tannins, alkaloids, glycosides, steroids, calicedrin (non-nitrogenous piero-resin), terpenoids, flavonoids, catechin and procyanidins (Makut *et al.*, 2008; Atawodi *et al.*, 2009). Some limonoids have also been separated from the *K. senegalensis* bark and leaves (Nakatani *et al.*, 2002; Yuan *et al.*, 2013).

The widespread use and popularity of herbal medicines do not guarantee their efficacy and safety (Shafaei *et al.*, 2011). Therefore, there is the need for adequate information on the toxicity of these herbal drugs. This study aimed to determine the health status of pullets fed diets supplemented with *Khaya senegalensis* stem bark as an anti-coccidiostat at the pre-laying phase.

Materials and methods

Test material: Stem bark of *Khaya senegalensis* was obtained from the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR) farm, Opeji village, Odeda Local Government Abeokuta Ogun State, Nigeria. The stem bark was dried and grounded into powdery form. The results obtained from the phytochemical screening of the stem bark of *K. senegalensis* showed it contained saponins, tannins, reducing sugars, aldehyde, phlobatannins, flavonoids, terpenoids, alkaloids, glycoside and anthroquinones.

Experimental location: the experiment was carried out at the Poultry unit of the Livestock Production Research Programme, IFSERAR, Federal University of Agriculture, Abeokuta.

Experimental animals: A total of 360 day-old chicks of three strains of pullets (120 chicks each of Isa Brown, Nera Black and Oba Blue) were used for the experiment. Isa Brown and Nera Black chicks were purchased from Chi Farms Ajanla Ibadan, Oyo State, while Oba Blue chicks were from Obasanjo Farms Abeokuta, Ogun State. They were reared in a deep litter, well ventilated and hygienic poultry house.

Experimental diets: Finely grounded *Khaya senegalensis* stem bark meal (KSSB) was incorporated into the basal chick and grower diets (Table 1).

Experimental design: The 360 day-old chicks were randomly allotted to 15 dietary treatments of 24 chicks each replicated thrice. The chicks were arraigned in a 5 x 3 factorial design of 5 dietary levels (Diet 1 served as negative control without medication. Diet 2 was supplemented with 100 mg/kg of conventional anti-coccidiostat (sulphaquinoxaline) , Diets 3 and 4 were fed 100 and 200mg/kg KSSB respectively while Diet 5 was supplemented with 100mg/kg KSSB which was fed continuously from 1st -118th day) and 3 strains of chickens (Isa Brown, Nera Black and Oba Blue). Diets 2, 3 and 4 were fed between 21st - 27th day, 42nd – 48th day and 112th – 118th day.

Data collection

Health status assessment: The health status of the pullets were examined through the changes in the haematology (Packed cell volume, haemoglobin, red blood cell, white blood cell and white blood differential counts) and serum biochemical (Total protein, glucose, cholesterol, uric acid, creatinine, alkaline phosphatase, aspartate

Table 1: Composition of experimental basal diet (%)

Ingredient	Chick (0 – 8 weeks)	Grower (9 – 20 weeks)
Maize	53.00	53.00
Groundnut cake	12.00	9.00
Soyabean meal	18.00	8.00
Fish meal (72% CP)	4.00	0.00
Palm kernel cake	0.00	5.00
Wheat offal	6.90	19.00
Bone meal	3.00	3.00
Oyster shell	2.00	2.00
Premix	0.25	0.25
Table salt	0.30	0.25
Lysine	0.30	0.25
DL-Methionine	0.25	0.25
Total	**100.00**	**100.00**
Crude protein (%)	22.81	16.93
Metabolizable energy (MJ/kg)	11.72	11.11

Premix for chick per kg of feed: Vitamin A 10,000 iu; vitamin D 3,900 iu; copper 0.1 mg; vitamin E, 50.0 mg; manganese 8.5 mg; vitamin K 2.0 mg; iron 75.0 mg; vitamin B_1 2.0 mg; folic acid 5.0 mg; vitamin C 26.0 mg; pantothenic acid 20.0 mg; vitamin B_6 2.0 mg; choline 1200 mg; vitamin B_{12} 0.01 mg; niacin 50 mg; zinc 70 mg; biotin 0.2 mg.

Premix for chick per kg of feed: Vitamin A 8,000 iu; vitamin D 1,200 iu; copper 2.0 mg; vitamin E, 31.0 mg; manganese
80 mg; vitamin B_2 10.0 mg; pantothenic acid 150.0 mg; iodine 1.2 mg; selenium 0.1 mg; cobalt 2 mg.

aminotransferase, sodium and potassium electrolytes) indices.

Blood sample collection: Blood samples were collected from the wing vein of the birds at the end of 118th day into vials containing ethylene diamine tetra-acetate for the determination of haematological indices while another set of blood were collected into heparinised tubes for the determination of biochemical parameters. The blood samples in heparinised tubes were centrifuged for 5 min and the supernatant was subsequently used for the assay of biochemical parameters. Haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method. Packed cell volume (PCV) was determined using Wintrobe's microhaematocrit kit; red blood cell (RBC) and white blood cell count (WBC) were determined using Neubauer haemocytometer as described by Baker and Silverton (1985). Differential leucocyte counts (heterophils, lymphocytes, eosinophils, basophils and monocytes) were determined using blood smears stained with May-Grunwald-Giemsa stain on glass slides.

Total protein was determined using the Biuret method, albumin was determined using the method described by Grant (1987), creatinine was determined by colorimetric method of Henry (1974), uric acid was determined using enzymatic colorimetric method by Fossati *et al.* (1980), glucose was determined according to the method of Barham and Trinder (1972), cholesterol was determined using the method of Roeschlau *et al.* (1974), alkaline phosphatase (ALT) and aspartate

Aminotransferase (AST) were determined using enzymatic colorimetric method as described by Reitman and Frankel (1957). Sodium (Na) and potassium (K) were measured using precipitation method (Henry, 1994) and tetraphenylboron formulated reagent (Tietz, 1976) respectively

Statistical analysis

All data were subjected to general linear model of analysis of variance using SAS (2000) and differences in means were separated at ($P< 0.05$) using the Tukey's Studentized Range Test of the same package.

Results

Effects of different dietary levels of KSSB on haematological and electrolyte indices of three different strains of pullets at the pre-laying stage are presented in Table 2. PCV, WBC and K were affected ($P< 0.05$) by dietary supplementation of KSSB in the diets of the pullets, while RBC, neutrophil, lymphocyte, monocyte and eosinophil were not affected. Isa brown and Nera black pullets fed diets without supplementation recorded significant ($P = 0.001$) lower PCV values, though the least value was recorded from Isa brown pullets. Similar values were obtained from pullets in other treatment groups. The highest WBC value ($P = 0.001$) was from Isa brown pullets fed control un-supplemented diets. WBC counts showed the least ($P = 0.001$) values in Isa brown fed KSSB continuously in the diet, Nera black fed 200mg/kg KSSB supplemented diet and Oba blue fed conventional anti-coccidiostat (diet 2) and 100 mg/kg KSSB supplemented diets at intervals. KSSB has elevating action on K concentration of Nera black fed 200 mg/kg KSSB supplemented diet while Isa brown pullets fed diet without supplementation, supplemented with sulphaquinoxaline and

Table 2: Haematological and electrolyte indices of pullets fed khaya senegalensis supplemented diets at the pre-laying phase

Breed	Treatment	PCV (%)	RBC $x10^6/\mu L$	WBC $x10^9/L$	WBC differential counts (%) N	L	M	E	Na mmol/L	K mmol/L
Isa brown	1	15.00^{b}	2.98	10.50^{a}	29.00	68.00	1.67	1.00	137.00	2.97^{b}
	2	24.67^{a}	3.27	5.03^{ab}	30.67	65.67	2.67	1.00	139.33	3.17^{b}
	3	24.00^{a}	3.40	5.63^{ab}	30.67	67.00	1.00	1.67	135.33	3.20^{b}
	4	25.67^{a}	3.49	5.00^{ab}	28.00	67.00	3.33	1.33	127.67	3.87^{ab}
	5	24.33^{a}	3.43	3.53^{b}	19.00	77.33	2.67	1.33	138.67	4.23^{ab}
Nera black	1	22.00^{ab}	3.55	6.80^{ab}	25.33	70.00	3.00	1.67	137.00	4.33^{ab}
	2	25.33^{a}	3.63	4.30^{ab}	31.00	65.33	2.33	1.33	138.00	4.23^{ab}
	3	23.33^{a}	3.23	3.23^{b}	32.00	62.67	3.00	2.33	128.67	4.27^{ab}
	4	25.33^{a}	3.70	4.97^{ab}	33.33	61.00	3.33	2.23	133.00	4.90^{a}
	5	23.00^{a}	3.67	4.40^{ab}	28.33	69.33	1.67	0.67	135.00	3.60^{ab}
Oba blue	1	23.80^{a}	3.33	4.03^{ab}	33.00	63.00	2.67	1.33	141.00	4.17^{ab}
	2	23.33^{a}	2.93	3.57^{b}	27.33	68.67	1.67	2.33	131.00	4.00^{ab}
	3	25.67^{a}	3.56	3.40^{b}	34.33	62.67	1.00	2.00	134.33	3.70^{ab}
	4	27.33^{a}	4.12	4.00^{ab}	32.33	63.67	1.67	2.33	130.33	3.90^{ab}
	5	25.67^{a}	2.37	4.03^{ab}	30.00	67.00	1.33	1.67	132.67	4.20^{ab}
SEM		0.515	0.098	0.449	1.201	1.045	0.253	0.199	1.245	0.095
P – values										
Treatment		0.001	0.338	0.011	0.515	0.102	0.673	0.632	0.319	0.337
Breed		0.101	0.431	0.017	0.498	0.085	0.289	0.411	0.844	0.002
Treatment x Breed		0.001	0.312	0.001	0.847	0.296	0.843	0.961	0.841	0.002

[ab]Means on the same column with the different superscript are significantly different ($P<0.05$) Treatment 1= negative control without supplementation. Treatment 2 = 100 mg/kg of conventional anti-coccidiostat (sulphaquinoxaline), Treatments 3 and 4 = 100 and 200mg/kg *K. senegalensis*, Treatment 5 were continuously fed *K. senegalensis* at 100 mg/kg in the diets. PCV = packed cell volume, RBC = red blood cell, WBC = white blood cell; N= neutrophil, L = lymphocyte, M = monocyte, E = eosinophil, Na = sodium, K = potassium and SEM = standard error of the mean

Table 3: Serum biochemical of pullets fed khaya senegalensis supplemented diets at the pre-laying phase

Breed	Treatment	Total protein g/L	Albumin g/L	Globulin g/L	Glucose mg/dL	Uric acid mg/dL	Creatinine mg/dL	ALP iμ/L	AST iμ/L
Isa brown	1	3.43^{e}	1.93	1.50	113.00	5.27^{abc}	1.35^{a}	458.00^{a}	52.77^{ab}
	2	3.63^{cde}	1.80	1.83	110.67	3.77^{cd}	1.12^{abc}	374.33^{def}	41.89^{abc}
	3	3.47^{de}	1.90	1.57	122.67	4.03^{bcd}	1.14^{abc}	374.00^{def}	41.46^{abc}
	4	3.73^{cde}	2.13	1.60	131.33	4.63^{cd}	1.06^{bc}	395.00^{cdef}	40.26^{abc}
	5	3.63^{cde}	2.10	1.53	113.00	6.50^{a}	1.25^{ab}	448.33^{ab}	52.46^{ab}
Nera black	1	4.33^{bc}	2.00	2.33	105.00	5.47^{ab}	1.20^{abc}	454.67^{ab}	52.79^{ab}
	2	4.60^{ab}	2.67	1.93	120.00	4.60^{bcd}	1.07^{bc}	367.67^{ef}	45.19^{abc}
	3	4.77^{ab}	2.43	2.33	126.00	3.63^{cd}	1.01^{bc}	364.33^{ef}	39.37^{bc}
	4	4.23^{bcd}	2.27	1.97	123.00	4.50^{bcd}	1.06^{bc}	382.00^{cdef}	41.79^{abc}
	5	4.27^{bc}	2.20	2.07	111.67	6.30^{a}	1.14^{abc}	424.00^{abc}	47.43^{abc}
Oba blue	1	4.37^{abc}	2.40	1.97	118.33	5.20^{abc}	1.11^{abc}	416.67^{abcd}	53.39^{ab}
	2	4.73^{ab}	2.67	2.07	121.67	4.17^{bcd}	1.00^{bc}	387.33^{cdef}	42.62^{abc}
	3	4.30^{bc}	2.40	1.90	109.67	3.40^{d}	0.95^{c}	356.00^{f}	35.22^{c}
	4	4.97^{ab}	2.67	2.30	110.67	3.77^{cd}	1.06^{bc}	350.33^{f}	46.22^{abc}
	5	5.13^{a}	2.80	2.33	111.00	6.37^{a}	1.09^{bc}	409.33^{bcde}	54.32^{a}
SEM		0.087	0.062	0.063	1.665	0.173	0.02	5.56	1.062
P – values									
Treatment		0.252	0.395	0.999	0.252	<0.001	0.003	<0.001	<0.001
Breed		< 0.001	< 0.001	< 0.001	0.616	0.060	0.004	0.009	0.839
Treatment x Breed		< 0.001	0.011	0.600	0.166	<0.001	<0.001	<0.001	<0.001

^{abcd}Means on the same column with the different superscript are significantly different (P<0.05)
Treatment 1= negative control without supplementation. Treatment 2 = 100 mg/kg of conventional anti-coccidiostat (sulphaquinoxaline), Treatments 3 and 4 = 100 and 200mg/kg *K. senegalensis*, Treatment 5 were continuously fed *K. senegalensis* at 100 mg/kg in the diets
ALP = Alkaline phosphatase, AST = aspartate aminotransferase and SEM = standard error of the mean.

100 mg/kg KSSB obtained the least (P = 0.001) K values.

Table 3 shows the biochemical indices of pullets fed KSSB supplemented diets at the pre-laying phase. TP, uric acid, creatinine, ALP and AST were significantly (P< 0.05) affected by the dietary treatment. TP values were least in Isa brown pullets across the treatment levels while the highest value was obtained from Oba blue fed KSSB continuously in the diet. Uric acid, creatinine, ALP and AST values was highest (P< 0.05) in pullets fed the control diet without supplementation and those fed 100 mg/kg KSSB continuously in the diet irrespective of the strain. Highest creatinine and ALP values were obtained from Isa brown fed control un-supplemented diet and the least values from Oba blue fed 100 mg/kg KSSB supplemented diet. Isa brown and Nera black pullets fed diets 2 and 3 had similar creatinine and ALP values. RBC, albumin, globulin and glucose were not affected by dietary treatment.

Discussion

The low PCV values from Isa brown and Nera black fed the control diet is indicative of anaemia. The result might be attributed to breed stress during coccidiosis infection; however the addition of sulphaquinoxaline and KSSB improved the PCV. A number of natural compounds such as saponin, tannins and flavonoids have been reported for their antioxidant effect (Masood *et al.*, 2013). Tannins have capacity to act as powerful antioxidants, scavenge free radicals, terminating oxidative reactions and chelation of transition metals and inhibition of pro-oxidative enzymes (Yilmaz and Toledo, 2004; Molan *et al.*, 2009). This agrees with Sanni *et al.* (2005) that the aqueous extract of *Khaya senegalensis* stem bark reduces anaemia.

The high number of WBC in pullets fed the control diet and KSSB continuously in the diet might have reflected inflammation in defending the body against infectious microorganism and toxicity. Medicinal plants with their antioxidant properties mainly due to their polyphenolic compounds have been useful in prevention of diseases (Botsoglou *et al.*, 2002; Florou-Paneri *et al.*, 2006). Similarly saponins have been reported to prevent the growth of parasite by interacting with the cell membrane cholesterol contents which modify the cell membrane structure and function thus resulting in parasite death (Wang *et al.*, 1998). Proteins in the diets were more effectively utilized in pullets fed KSSB suplemented diets. Saponin has the ability to form pores in all membranes, reducing the superficial tension of fluids thereby allowing better absorption of nutrients by the intestinal epithelium (Plock *et al.*, 2001; Alfaro *et al.*, 2007).

Also, catechin and catechin-related compounds have been shown to be powerful antioxidants which can inhibit a number of tumour cell proliferation- and survival-related proteins (Kazi et *al.*, 2002). The increase in the level of potassium ions may suggest the inability of the kidney to properly regulate these electrolytes as a result of renal dysfunction (Oboh and Olumese, 2008).

The adverse effect of KSSB as reflected by high AST, ALP, creatinine and uric acid values on pullets fed un-supplemented diet and those on continuous supplementation of KSSB is an indication that the pullets in the control group were challenged and the extended administration of KSSB was detrimental to the health of the pullets and may have caused tissue damage. The extent of damage to the organ has been reported to be directly proportional to the degree of activities of AST and ALP (Nevin and Vijayammal, 2005; Aliyu *et al.* 2007). It has

been reported that KSSB contains active phenolic acid (catechin and procyanidins) compounds, which are known to inhibit free radicals generated in the cellular system. A dose-dependent effect of the stem bark extract of *Khaya senegalensis* is also well described in rats (Sule *et al*., 2008). The high uric acid and creatinine obtained from pullets fed control diet in all the strains may be an indication of kidney malfunction raising the level of blood urea and creatinine. Such deleterious effects of KS are well reported in rats treated with the stem bark extract of *Khaya senegalensis* (Adebayo *et al*., 2003; Abubakar *et al*., 2010; Kolawole *et al*., 2011).

Conclusion

It can be concluded that *K. senegalensis* exhibits opposing effects in the blood depending on the concentration, such that, usage at intervals acts as a feed supplement, but at higher doses, it induces liver and kidney toxicity.

K. senegalensis stem bark was effective in combating the high susceptibility to infections and organ toxicity observed in Isa Brown pullets and Nera Black. However, Oba Blue pullets seem to express some innate immunity.

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