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ORIGINAL RESEARCH ARTICLE

Effects of *Albizia saman* seed meal on the haematological and biochemical indices of finisher broiler chickens

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ABSTRACT
Albizia saman seeds were collected, soaked in water for twenty-four hours, cooked for one hour, sundried for thirty minutes, toasted under fire till properly dried, toasted and milled to obtain the seed meal. The meal was used in formulating diets for broiler finishers at 0, 2.5, 5.0 and 7.5% inclusion rate tor treatments 1, 2, 3 and 4 respectively. The treatment diets were fed to a total of 120, 4-wk old Marshal broilers. The broilers were randomly assigned to the four treatment groups in a complete randomized design at 30 broilers per group which was replicated 3 times to give 10 broilers per replicate. The birds were raised in a deep litter pen. The experiment lasted for 28days. Blood samples were collected and analyzed using auto analyzer. The result for hematological indices showed a significantly different (P<0.05) reduction in the Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC) and the White Blood Cells with inclusion of the test ingredient. The Red Blood Cell (RBC), the White Blood Cell differentials and Erythrocytes Sedimentation Rate (ESR) were uniform. For the biochemical indices the proteins, the electrolytes and the liver enzymes were uniform while the kidney functions parameters and Cholesterol showed a significantly (P<0.05) lower results from that obtained from control. The parameters that showed a significant result however, did not indicate a negative influence on the broilers. It is therefore recommended that Albizia saman seed meal could be included up to 7.5% inclusion level in broiler finisher diet replacing 50% of the soyabean in a maize-soyabean diet.

Keywords: *Albizia saman,* Haematology, Biochemical indices, Broiler chickens

INTRODUCTION
The high cost of conventional feed ingredients for feeding poultry and other monogastric animals has made the cost of feeding this class of animals high (Ndubuisi, 2011). Feed alone accounts for over 70% of the total cost of poultry production and out of which 50% is expended in protein and energy source (Madubuike, 2012). This high cost has necessitated the need to discover alternative feed ingredients in feeding poultry.

Albizia samani also called S*amanea saman*, saman tree, rain tree, cow bean tree or monkey pod is one the shrubs whose seed can fit into, as protein source in the livestock industry. The mesocarp of the pod of *S. saman* contains a sweet nutritious pulp which smells of honey when the pods are broken and which contains 12-18% crude protein, highly digestible (40% digestibility), thus making the pods a good source of proteins, carbohydrates and minerals for livestock (Escalante, 1997). The pods can be dried and milled into a meal that makes an excellent animal feed, which is exported on a small scale in some South American countries (CABI, 2016). Dur, (2001) reported that the main utility of the tree, is in its pods which are produced in great quantity and are very good fattening forage for all types of grazing animals. Ukoha *et al.,* (2011) reported that in folklore medical practice, *Albizia saman* parts are used as remedy for cold, diarrhea, headache,

intestinal ailments, stomach ache and even cancer. This suggests that the plant can be an antimicrobial natural source. Though this plant as reported by CABI, (2016), makes an excellent animal feed, but its effect on performance indications of the animal is not the only indicator of its efficacy as feed ingredient. In evaluating unconventional feed resources, it is important, to also check the effects of such feed resources on the general status of the livestock (Esonu *et al.,* 2001). It is often very difficult to assess the current health status of animals without detailed examination of blood (Amakiri, *et al.,* 2009). Examination of blood provides the opportunity to clinically Investigate the presence of several metabolites and other constituents in the body and it plays a vital role in the physiological, nutritional and pathological status of the animal (Aderemi, 2004); Thus, considerations should be given to the types of ingredient, its effects on blood parameters, as well as the physiology of birds before feeding the ingredient to animals.

The study therefore, is aimed at investigating the effect of *Albizia saman* seed meal on the hematological and biochemical indices of finisher broilers.

MATERIALS AND METHODS

Study area

The study was conducted at the Teaching and Research Farm of the Imo State University Owerri. Owerri is within the rain forest belt of Nigeria and lies within latitude 5°45′ N and 7°15′N and longitude 6°50′E and 7°25′E with an annual rainfall range of 2400 - 2500mm (nimet.gov.ng/content/nimet-weather, 2015).

Collection and processing of A*lbizia saman* seed meal

The seeds were collected at Egbu Road in Owerri North Local Government Area of Imo State, South East Nigeria. The seeds were soaked in water for twenty-four (24) hours and the water discarded. The soaked seeds were boiled for twenty-five (25) minutes, dried in an oven to a moisture content of 9-10% and toasted on fire for 5 minutes to denature any possible anti-nutrient present in the seed. The seeds were then milled in hammer mill to obtain the seed meal.

Analysis of the seed meal

Sample of the seed meal was taken to the laboratory to analyze its proximate and phytochemical composition. The proximate composition was analyzed adopting the Association of Official Analytical Chemists (A.O. A.C) (2000) A.O.A.C. procedure while the phytochemical composition was analyzed adopting the methods of Harborne (1998).

Experimental Diets

The seed meal was used in formulating four experimental diets. Treatment 1 which is the control treatment, contained no *Albizia saman* seed meal while in treatments 2, 3 and 4 the *Albizia saman* seed meal replaced soya bean meal at 2.5, 5 and 7.5% inclusion rate respectively on weight to weight basis. The ingredient composition of the experimental diets is presented in table 3.

Experimental birds and design

A total of one hundred and twenty (120) 4-week old Marshal broilers were used for the experiment. The broilers were divided into four experimental groups of 30 broilers each which was replicated 3 times to give 10 broilers per replicate. The groups were randomly assigned to the experimental diets and raised on deep litter system of management. Feed and water were given *ad-libitum* and proper routine management practices and medication adopted. The experiment lasted for twenty-eight (28) days.

Data collection

At the end of the 28 days, three broilers were randomly selected from each of the replicate for hematological and biochemical assay.

Haematological assessment

Five milliliters of blood were collected from each of the broilers by puncturing the webal sub-clavicles vein with five milliliters scalp vein needle set. The blood was collected into a bottle containing ethylene diamine tetra-acetic acid (EDTA) as an anti-coagulant and the following parameters analyzed using Sysmex Auto Analyzer: Hemoglobin Concentration (HB), Packed Cell Volume (PCV), Total White Blood Cells ($WBC_{(t)}$), Red Blood Cells (RBC), Mean

Cell Volume (MCV), Mean Cell Hemoglobin (MCH) and Mean Cell Hemoglobin Concentration (MCHC). The $WBC_{(t)}$ was separated to obtain the Differential White Blood Cells (WBC- Differential) by Preparation of a thin film blood smear slides and staining with LEISHMAN'S stain and manual counting of the Heterophils (N), the Eosinophils (E), the Basophils (B) and the Lymphocytes (L). While the Erythrocytes Sedimentation Rate (ESR) was obtained using Westergreen ESR tubes and stand.

Biochemical assessment

Another 5 ml blood was collected into a test tube, with no anti-coagulant. The coagulated blood was subjected to standard method of serum separation and the harvested sera analyzed using Victors DTE 11 Auto analyzer. The vitros DTE 11 auto analyzer has an automatic DT pipette and makes use of test slide for its analysis. Each test slide is parameter test specific and was used in analyzing the kidney function (Urea and Creatinine), Cholesterol, Proteins (albumin and globulin), Electrolytes (sodium, potassium, carbonate and chlorine) and the liver enzymes (Alkaline phosphatase, Aspartate aminotransferase and Alanine aminotransferase).

Data analysis

The data obtained from this study, were subjected to statistical analysis using analysis of variance procedure and computed with IBM SPSS Version 22. Means with significant differences were separated using Duncan Multiple Range Test of the statistical software.

Results

The results of the proximate and phytochemical analyses of *Albizia saman* seed meal are presented in tables 1 and 2 respectively. The result of the proximate analysis showed that the seed meal contains 16% crude protein which showed a reduced crude protein level when compared with soya bean that has more than 40% crude protein.

Table 1. Proximate composition of *Albizia saman* seed meal

Nutrients	Amount (%DM)
Dry matter	90.1
Ash	5.1
Crude Protein	16.0
Crude Fiber	8.0
Ether Extract	7.2
Carbohydrate	52.8

DM = Dry matter

The phytochemical analysis showed that the seed meal contains reasonable quantities of the following in percentage dry matter (%DM): tannin 8.24, cardiac glycoside 5.31, saponins 2.14, alkaloids 2.82 and flavoids 3.51 while the quantities of phytates, phenols and oxalate were low (0.82% DM, 0.69% DM and 31860mg/ 100g respectively). The result of the hematological indices of finisher broilers fed diet containing different levels of *Albizia saman* seed meal is presented in Table 4. The result showed that the Hb, PCV, MCV, MCH, MCHC, and WBC were significantly lower ($P>0.05$) for broilers whose diet contain the test ingredient. The Hb for T_1 was 10.3, and significantly higher than the values of 7.9, 8.4 and 8.0 obtained for T_2, T_3 and T_4 respectively which were uniform. The PCV, MCV, MCH, MCHC and WBC of broilers had the same trend, being uniform for T3 and T4 but significantly ($P>0.05$) higher than broilers on T2 diet. The results for RBC, the WBC differentials (the heterophils, the Lymphocytes, the Monocytes, the Eosinophils and the

Basophils) and ESR showed no significant difference (P<0.05) at all the treatment level

Table 2: Phytochemical composition of *Albizia saman* seed meal

Component	Qualitative value	Quantitative value
Tannin	+++	8.24
Saponins	++	2.14
Alkaloids	++	2.82
Flavonoids	++	3.51
Cardiac glycosides	++	4.31
Oxalates	+	3186 mg/100g
Phytates	+	0.82
Phenols	+	0.69

Table 3. Ingredient composition of the experimental diets (%)

	Treatment levels in %			
Ingredients	**T_1(0.00)**	**T_2(2.50)**	**T_3(5.00)**	**T_4(7.50)**
Yellow maize	55.00	55.00	55.00	55.00
Soyabean meal	15.00	12.50	10.00	7.50
Albizia saman seed meal	0.00	2.50	5.00	7.50
Groundnut Cake	10.00	10.00	10.00	10.00
Brewer's Spent Grain	5.00	5.00	5.00	5.00
Palm kernel Cake	5.00	5.00	5.00	5.00
Fish meal	3.00	3.00	3.00	3.00
Blood meal	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00
Common salt	0.25	0.25	0.25	0.25
*Vit/mineral premix	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**
Calculated nutrients composition of the diets				
Crude Protein	22.61	21.91	21.21	20.51
Crude Fiber	4.03	4.06	4.10	4.14
Ether Extract	4.60	4.69	4.78	4.87
Metabolizable Energy (kcal/g)	2863.68	2853.43	2863.31	2822.93

** To provide the following per kilogram of feed; vit A 10,000IU; vt. D_3 1,500iu; vit. E 2mg; riboflavin 3mg; pantothenic acid 10mg; nicotinic acid, 2.5mg; choline 3.5mg; folic acid 1mg; magnesium 56mg; lysine 1mg; iron 20mg; zinc 50mg; cobalt 1.25mg.*

The result of biochemical indices is presented in table 5. The results for kidney function parameters showed that for urea, the result obtained for control was significantly (P>0.5) higher than the T_2 (24), while T_2, T_3 and T_4 were uniform being 20, 18 and 20 respectively. The result for the other kidney function parameter, the creatinine, showed that T2 was uniform with control and significantly (P>0.05) higher than T3 and T4 while T3 and T4 were uniform. The results for the proteins, the electrolytes and the liver enzymes parameters showed no significant difference (P<0.05) among the broilers at all the treatment levels. There was also a significant reduction in the value for cholesterol of broilers

fed with the test ingredient.

Table 4. Hematological indices of finisher broilers fed different levels of *Albizia saman* seed meal

	Treatment levels (%)				
Parameters	**T1(0.00)**	**T2(2.50)**	**T3(5.00)**	**T4(7.50)**	**SEM**
Hb(g/dl)	10.3^{a}	7.9^{b}	8.4^{b}	8.0^{b}	6.32
PVC (%)	35.3^{a}	245^{b}	28^{b}	27.5^{b}	1.23
$WBC_t(x10^{-3}/mL)$	7227^{a}	4988^{c}	5799^{b}	5599^{b}	249
WBC-Differential (%)					
Heterophils (%)	49	50	49	49	1.26
Eosinophils (%)	3	4	5	5	0.35
Basophils (%)	0	0	0	0	Nil
Lymphocytes (%)	44	42	42	42	0.36
Monocytes (%)	4	4	4	4	0.25
$RBC(x10^{-6}mL)$	4.8	4.66	5.01	4.9	5.91
ESR(mm/1stHr)	4	9	6	6	0.59
MCV(*fl*)	66^{a}	45.6^{c}	52^{b}	51.1^{b}	2.28
MCH(pg)	22.7^{a}	15.6^{c}	17.9^{b}	17.9^{b}	0.78
MCHC(g/dl)	25.5^{a}	17.6^{c}	20.1^{b}	19.8^{b}	0.87

Without subscript= *Not Significant* a,b,c, means with different superscript means significantly (P<0.05) different.

SEM= standard error mean

Table 5: Biochemical indices of finisher broilers fed different levels of *Albizia saman* seed meal

	Treatment levels (%)				
Parameters	**T1(0.00)**	**T2(2.50)**	**T3(5.00)**	**T4(7.5)**	**SEM**
Kidney function					
Urea(mg/dl)	24^{a}	20^{b}	18^{b}	20^{b}	1.7
Creatinine(mg/dl)	0.8^{a}	0.8^{a}	0.5^{b}	0.6^{b}	0.5
Proteins					
Total(g/dl)	6.9	6.5	6.6	6.6	0.51
Albumin(g/dl)	4.0	4.0	3.8	3.6	0.18
Globulin(g/dl)	2.9	2.5	2.8	3.0	0.14
Electrolytes					
Na+(mmol/l)	141	139	141	1.38	0.46
K+(mmol/l)	3.7	3.8	3.7	3.8	1.29
HCO_3*(mmol/l)*	30.0	29.0	31.0	28.0	2.14
CL-(mmol/l)	101	100	100	100	1.78
Liver enzymes					
Alkaline phosphate(iu/L)	1.2	1.3	1.2	1.3	0.21
Aspartate amino transferase(iu/L)	11.7	11.4	11.5	11.6	0.13
Alanine amino transferase(iu/L)	7.0	6.65	6.67	7.2	0.27
Cholesterol(mg/ml)	200^{a}	120^{c}	170^{b}	163^{b}	1.52

Without subscript= *Not Significant* a,b,c, means meal with different superscript and are significantly (P<0.05) different. SEM= standard error mean

DISCUSSION

The significant reduction in the values of Hb, PCV, MCV, MCH and MCHC for broilers at the inclusion of the test ingredient in the diet could be because of the phytochemical content of the *Albizia saman* seed meal. The phytochemical analysis showed that *Albizia saman* seed contains moderate level of tannins and saponins. Jansman (1993) reported that Tannins have the property of forming insoluble complexes with metal ions such as iron, thereby rendering them less available for absorption.

Iron is known to be a vital component of Hb, which according to (Maton *et al.,* 1993) is the iron containing oxygen-transport metalloprotein in the RBC of all vertebrates. A decrease in iron absorption therefore will lead to a decrease in the Hb. The result could be in line with the report of (Graham, 2010) that small sized cell is usually seen in Iron deficiency. The MCV is the expression of the average volume (size) of individual RBC, the MCH is the expression of the average hemoglobin content of a single RBC, the MCHC is the expression of the volume within the RBC occupied by the hemoglobin, while the PCV is the percent of blood volume filled by the red cell component of the entire blood (Jaime, 2005). All these parameters have connection to the Hb and could be the reason for their having the same trend. The uniform result obtains from RBC count which is a measure of how many red blood cells in a given blood quantity is an indication that the RBC of broilers fed on the test diet conforms with those on control diet and that the RBC is adequate. The significant reduction in WBCt could be as a result of the antimicrobial properties of tannin. Tannins-rich remedies are used as anti-helmintics, antioxidants, antimicrobials and antivirals (Buzzini et al., 2008). The primary role of the WBC is defense; it rises in event of issues it must fight (Graham, 2010). The test ingredient may have reduced the microbial load of the broilers which resulted to low WBC as there were less issues for the WBC to fight. However, the WBC differentials were uniform showing that the test diet did not affect them and so their functions were performed effectively. Also, the uniformity in the result of the ESR which is a measure of how far the red cells fall in a column of blood in 1 hour

(Gaham, 2010) is an indication that the test diet did not affect the parameter and that it conforms to the control. The reduction in the values of the kidney function parameters (urea and creatinine) of the broilers on the test ingredient could be because of the reduced protein level of their diets. Joshua (2015) reported that high-protein diet may result in an increased urea production. It has also been reported that Creatinine levels may be 10%-30% higher with the intake of diet high in protein (American Association for Clinical Chemistry (2016). This could be responsible for the reduction in the values of these parameters. The uniformity of the result for proteins however, suggests that the serum albumin and globulin of the broilers are adequate. Total serum protein concentration is an indicator of adequacy of protein in terms of quality and quantity in the diet and blood proteins depend on the quality of dietary proteins (Hellwing *et al.*, 2007). The result therefore is an indication of nutritional adequacy of the dietary proteins. The uniformity of the results obtained for electrolyte and liver enzymes is an indication that the regulatory functions of the electrolytes and the liver enzymes were not affected by the test ingredient. The decline in values of cholesterol with the increasing level of the test ingredient could be as a result of the phytochemical content of the test ingredient. It has been reported that Saponins which is one of the phytochemical substances present in the test ingredient, can form insoluble complexes with cholesterol in the digesta and inhibits the intestinal absorption of endogenous and exogenous cholesterol (Oakenfull *et al.*, 1990). Sofowora, (1993) also reported that Saponins have the properties of protein precipitation, cholesterol binding and haemolysis of red blood cells. The low cholesterol quality according to Ekenyem and Madubuike, (2005) is an advantage especially now that people are very conscious of reducing cholesterol content of animal protein, which has made some people to reject red meat.

CONCLUSION

The incorporation of *Albizia saman* seed meal up to 7.5% inclusion level, in the diet of broiler finisher, did not adversely affect the hematological and biochemical indices of the broilers. The seed meal however reduced the cholesterol level of the broiler, a quality that is desirable.

CONFLICT OF INTEREST

Authors have declared that no conflict of interests exists.

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