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A Study on Serum Biochemical Analysis "Broiler" Feed Diets Containing Graded Levels of Locust Beans (*Parkia biglobosa*) Seed Meal in Poultry Production and Research Unit of the Department of Animal Science, Usmanu Danfodiyo University Sokoto, Nigeria

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Authors' contributions

This work carried out in collaboration among all authors. Author AYK designed the study. Author MMI performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ABA and MSS managed the analysis of the study. Authors ABA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out evaluate the effects of feeding locust bean (*Parkia biglobosa*) seed at graded levels on the serum biochemistry of "broilers". Two hundred and forty broilers were used which were randomly allocated to four treatment groups, each replicated four times in a completely

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randomized design. The diets consisted of 0% level of LBSM which served as experimental control, while other three diets includes; 5, 10, and 15% levels of LBSM. The experiment was divided into two phases (starter and finisher) each of which lasted for 28 days. Broiler fed 5% LBSM had higher value for packed cell volume (PCV) of 33.25% and hemoglobin concentration (HC) (11.79 g/dl) which was significantly higher (P<0.05) than the PCV and the hemoglobin concentration (HC) of the broiler fed the other diets. The values of white blood cell (WBC) for broilers fed 5% and 10% were statistically similar (13.44 and 13.26 $x10^3/mm^3$) (P>0.05) but significantly higher (P<0.05) than values obtained from birds fed the Control Diet and 15% LBSM (11.54 $x10^3/mm^3$) (10.21 $x10^3/mm^3$). Red blood cell, (RBC) values obtained from the broilers fed the control diet, 10% and 15% LBSM (2.12, 2.43 and 2.32 respectively) $10^6/mm^3$ did not differ significantly from one another (P>0.05) but were significantly lower (P<0.05) compared to the value obtained from the birds fed 5% LBSM (3.55 $x10^6/mm^3$). There was no significant difference (P>0.05) in the mean corpuscular volume (MCV) of the broilers fed the experimental diets. However, the mean corpuscular hemoglobin concentration (MCHC) values obtained from broilers fed 5% LBSM (36.100%) was higher (P<0.05) than the values obtained from birds fed 10% LBSM.

Keywords: Treatment; park cell volume (PCV); white blood cell (WBC); diets and concentration.

1. INTRODUCTION

In Nigeria today, there is persistent short supply of animal protein for the populace, this is due to low levels of supply of the products and increase in human population which puts more pressure on every form of food supply [1]. Nigeria's population was estimated to be 142 million [2]. In order to avoid the occurrence of malnutrition among the people a well-balanced diet is required for consumption. Malnutrition has been the bane of healthy livelihood, mainly as a result of shortages in agricultural production. In order to achieve self-sufficiency and economic advancement, the country must be able to feed its population with adequate and nutritionally balanced diets. Protein is one of the classes of food that is needed by both man and animals to promote healthy growth at all stages of life. Most often, the level of protein intake by the populace is used as a yardstick in assessing the nutritional status of the society. The per capita protein consumption of Nigerians was estimated at between 48 and 60 g [3]. Between 1990 and 1992, 45 g of protein was supplied per capita per head in Nigeria out of which 38 g was from vegetable sources with only 7 g coming from animal sources [4]. According to ILCA [5]. Nigerians consumed only 6.8 g of protein of animal origin per person per day, which was one of the lowest animal protein intakes in the world. Despite this, [6] projected that the intake of protein of animal origin of Nigerians was expected to reduce to only 5.3 g per person per day even by the year 2010. Limited supply due to low level of production is the major reason for the short supply of animal proteins [7].

The domestic fowl compared to other animal species has the potential of providing Nigerians with adequate animal proteins. This is due to its short production interval [8]. It also takes lower quantity of feed for a layer (3.9 kg) for instance and broiler (1.9 kg) to produce one kg of product i.e. egg and meat, respectively [9] Acute shortage and high cost of feed ingredients have been identified as the major hindrances to the expansion of the poultry industry in Nigeria and other developing African countries [10].

In Nigeria in particular, the high cost of animal protein has made the intake to fall below 10g per capita compared to the recommended daily intake of 35 g [11]. The expansion of the nation's commercial poultry holds great promise in bridging the animal protein consumption gap in the country within the shortest possible time [12]. Feed, which has always been a major limiting factor in the growth of the poultry industry accounts for over 70% of total cost of producing poultry intensively [13, 14]. In order to arrest this situation, several workers have proposed the use of the little known non-conventional products as feed ingredients [15, 16].

1.1 Aim and Objectives of the Study

The aim of the study is to evaluate the effects of feeding locust bean (*Parkia biglobosa*) seeds at graded levels on the blood chemistry of broilers at both starter and finisher phase.

The specific objectives are;

1. To determine the chemical composition of locust bean seed (*Parkia biglobosa*).

2. To assess the effect of locust bean seed meal on the haematology and blood serum of the bird (broiler).

2. MATERIALS AND METHODS

2.1 Study Area

The eight weeks experiment was conducted at the poultry production and Research unit of the Department of Animal Science, Usmanu Danfodiyo University, Sokoto. Sokoto State is located between latitudes 12^0 and 13^0 N and longitudes 4^0 and 6^0E in the Northern part of Nigeria at an altitude of 350 m above sea level [17]. The state falls within the Sudan savannah vegetation zone with alternating wet and dry seasons. The hot dry spell extends from March to May and some time to June in the extreme northern part. A short, cool, dry period (Harmattan) occurs between late October and late February [18].

The mean annual rainfall is about 700mm. The rainy season starts from June to early October but some time ends in september with a peak in August Potential evapotranspiration has been reported to be 162 mm. Maximum temperature of 41^OC has been reported in April and minimum of 13.2^OC in January [19].

Commercial poultry production is gradually gaining acceptance in Sokoto State. However, there are more commercial poultry farming activities in the capital city of the State than in other parts of the State. Most households and major restaurants and fast food centers within the Sokoto metropolis depend on the commercial broiler poultry farms in Sokoto metropolis for broilers poultry meat supply [20]. The populace now cherish broiler meat for daily consumption and during festivities and ceremonies unlike in the past when people considered broiler meat as being too tender and costly.

2.2 Source of Locust Bean Seeds and Other Feeding Stuffs

The locust bean seeds used in the feeding trial were obtained from Kaiama in Kaiama Local Government Area of Kwara State. The seeds were removed from the pods and washed clean of the yellow pulp and dried as described by [21]. The seeds were boiled for eight hours in large pots on open fire to reduce or destroy the anti-nutritional factors, dried in an open air for five days before they were ground into meal and transported to Sokoto for storage before the commencement of the experiment. Other ingredients for the experimental diet formulation which include maize, groundnut cake, wheat offal, bone meal and limestone were sourced from Kara market in Sokoto town while methionine, lysine and premix were sourced from established vendors within Sokoto metropolis.

2.3 Experimental Design and Diets

Four experimental diets were formulated; the first diet contained zero level of locust bean seeds meal and served as the control. The three remaining diets were formulated to contain locust bean seed meal at 5, 10 and 15% level of inclusion, respectively. The diets served as the experimental treatments that were fed to broilers during the feeding trial at starter and finisher phases, respectively. Each of the phases lasted for four weeks making a total of eight weeks.

Two hundred and forty (240) day-old chicks were randomly allotted to four experimental treatment groups each replicated four times. The gross and chemical composition of the starter and finisher diets are shown in Tables 1 and 2 respectively.

2.4 Management of Experimental Birds

The day- old flock of broilers were sourced from Agric. Tech.farm (farm well) in Ibadan Oyo State and used for the experiment. The birds were raised on deep litter with open sided wall and cemented floor. The house was cleaned, washed, fumigated and disinfected a week to the arrival of the chicks. Litter materials (wood shavings) were spread on the floor two days before the arrival of the chicks. Charcoals were used as source of heat and light was supplied before the arrival of the chicks. Feeding trays and small drinkers were used for the chicks during the brooding period of 0-4 weeks, while conical feeders and plastic container drinkers with wire guard were used during the finishing stage.

Experimental birds were kept for three days after transport to take care of stress due to transportation. During the period anti-stress drugs such as Vitalyte were administered to the birds. After three days the birds were weighed and allotted to their treatment and replicate groups. Routine vaccination and medication were administered during the course of the trial as

recommended by Oluyemi and Roberts [22]. Feed and water were served to the chick's *ad-libitum*.

2.5 Laboratory Analysis of Locust Bean Seeds (*Parkia biglobosa*)

Proximate analysis was conducted in the Soil Science Laboratory of Usmanu Danfodiyo University Sokoto using the methods of [23] in order to determine the proximate composition of test ingredients. (Ca, Mg, P, K and Fe), Atomic Absorption Spectrometer (AAS) was used for Ca, Mg and Fe, determination, Spectrophotometer for P, and Plane Photometer was used for K determination Samples of the experimental feed were also analyzed for proximate and mineral content.

2.6 Determination of Anti-nutritional Factors

Determination of anti- nutritional factors was conducted in the biochemistry laboratory of Usmanu Danfodiyo University Sokoto.

The Oxalate content of parkia seeds was determined using the method described by A.O.A.C [24], Tannin content was determined using the standard method described by A.O.A.C [24-27] and [28] and Saponin content was determined using methods designed by Negi [28].

2.7 Determination of Oxalate

The oxalate content of Parkia seed was determined using the method described by A.O.A.C [24].

2.8 Determination of Tannins

The method of estimation of tannins content was according to the standard method described by [25-28].

2.9 Determination of Phytate

The method used for phytate analysis was as described by A.O.A.C [24].

2.10 Determination of Cyanide

Cyanide was determined by the method reported by Railes [29]. This employs the extraction of cyanide with water and its quantitative measurement by the alkaline picrate reagent.

Table 1. Gross composition of experimental diets fed to the broiler starter (0-4weeks)

		Experimental	Diets	
Ingredients	**Diet I (0%)**	**Diet II (5%)**	**Diet III (10%)**	**Diet IV (15%)**
Maize	50.00	50.00	45.80	45.00
Soya BM	19.00	16.10	14.00	14.90
LBSM	0.00	5.00	10.00	10.00
GNC	11.73	11.05	10.00	10.00
W/offal	13.50	13.75	15.00	15.00
Bone meal	1.50	1.20	2.50	2.50
Vitamin premix*	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Fish meal	2.97	1.85	1.65	1.50
Total	100.00	100.00	100.00	100.00
Calculated Chemical Composition				
Metabolizable Energy (Kcal/kg)	3000	3000	3041	3033
Crude protein (%)	23.00	23.10	23.00	23.40
Lysine (%)	1.30	1.20	1.10	1.10
Methionine (%)	0.60	0.50	0.50	0.50
Calcium (%)	0.40	0.40	0.20	0.60
Available P (%)	0.40	0.40	0.30	0.40
Ether Extract (%)	4.20	3.90	3.70	3.40
Crude fiber (%)	4.00	3.90	3.60	3.40
Feed cost ₦ / Kg	139	134	132	130

**Vitamin/mineral premix contained; Vitamin A, 1000 I.U Vitamin D1, 3000 I.U., Vitamin E8.0 I.U., Vitamin K, 2.0 mg; Vitamin B1, 2.0 mg; Vitamin B6, 1.2 mng; Vitamin B12, 0.2 mg; Pantothenic acid, 7.0 mg; Mg , 1000 mg; Cu, 8.0 mg and Se, 0.1 mg per of diet*

Table 2. Gross composition of experimental diets fed to the broiler finisher (5-8 weeks)

		Experimental	Diets	
Ingredients	**Diet I (0%)**	**Diet II (5%)**	**Diet III (10%)**	**Diet IV (15%)**
Maize	50.00	50.00	45.80	45.00
Soya bean meal	19.00	16.05	14.00	10.00
LBSM	0.00	5.00	10.00	15.00
GNC	11.73	11.05	15.00	10.00
W/offal	13.50	13.75	15.00	14.95
Bone meal	1.50	1.20	2.50	2.50
Vitamin premix*	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
G/oil	2.97	1.85	1.65	1.50
TOTAL	100.00	100.00	100.00	100.00
Calculated Chemical Composition				
Metabolizable Energy (Kcal/kg)	3000	3015	3022	3030
Crude protein (%)	20.00	20.00	20.00	19.98
Lysine (%)	1.16	1.29	1.45	1.57
Methionine (%)	0.54	0.60	0.67	0.74
Calcium (%)	0.49	0.40	0.75	0.94
Available P (%)	0.42	0.51	0.82	0.94
Ether Extract (%)	3.80	3.90	3.90	4.00
Crude fiber (%)	3.97	3.81	3.70	3.50
Cost of feed/gain₦/kg	289	284	282	280

**Vitamin/mineral premix contained; Vitamin A, 1000 I.U Vitamin D1, 3000 I.U., Vitamin E8.0 I.U., Vitamin K, 2.0 mg; Vitamin B1, 2.0 mg; Vitamin B6, 1.2 mng; Vitamin B12, 0.2 mg; Pantothenic acid, 7.0 mg; Mg , 1000 mg; Cu, 8.0 mg and Se, 0.1 mg per diet*

2.11 Data Collection

2.11.1 Heamatological and serum biochemical indices analysis

At the end of the eight week, three birds from each replicate were fasted overnight. Blood samples were collected in Ethylene Diamine Tetra Acetic Acid (EDTA) treated bottles for determination of packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and hemoglobin concentration (Hb) at the Usmanu Danfodiyo University Veterinary Teaching Hospital Sokoto. Mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH) and mean corpuscular volume (MCV) were later calculated according to the methods of [30].

Plain bottles were used for the collection for of blood samples for biochemical indices analysis. The serum biochemical indices measured were the Total Protein (TP), Total Bilirubin (TB), Direct Bilirubin (DB), Albumin (ALB), Uric acid and Glucose. The analysis was carried out in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto.

3. RESULTS AND DISCUSSION

3.1 Proximate and Phytochemical Analyses

3.1.1 Anti-nutritional factors

The ant nutritional factors of *P. biglobosa* seed obtained from the Laboratory analysis revealed that Oxalate had 0.0045 mg/100 g, phytate had 4.90 mg/100 g, tannin had 0.39 mg/100 g, Saponin had 1.14 mg/100 g and Cynanide had 0.010 mg/100 g phytochemical values. All the values obtained from this study did not agree with the values reported by Kehinde et al. [31], who reported 0.47 Oxalate, 1.30 Phytate, 0.51tannin, 0.92 Saponin and 0.00 Cyanide content. The differences may be due to the different geographical locations and the soil on which they were cultivated or as a result of method of processing as well as difference in cultivar.

The result of the Laboratory analysis obtained in this study showed that *Parkia biglobosa* seed has a high Crude protein (CP) content of 29.90 %, compared to the values available in

Literature. This high CP content of *Parkia seed* is not comparable with 33.50% reported by Kehinde et al. [31], 34.3% reported by [32] and 30. 00% reported by Fetuga et al. [33-37]. The Crude Fibre content of *P. biglobosa* seed was 1.0, which is not comparable with 2.00 reported by Eka [35], 4.66 reported by Kehinde et al. [31]. The fat content of the *P. biglobosa* seed was 17.00, which is lower than 49.20 reported by Kehinde et al. [31] but higher than 4.0 reported by Odunfa and Dawadawa [36,33]. The carbohydrate content of the *P. biglobosa* seed was 47.07, which is lower than 49.00 reported by Oke and Umoh [37]. The Ash content was found to be 5.0, which is higher than 4.81 reported by Kehinde et al. [31] and also 2.0 reported [37].

3.2 Haematological Indices of Broiler Fed Diet Containing Graded Levels of LBSM

The heamatological components of broiler chickens fed diets containing graded levels of LBSM is shown in Table 3.

Heamatological analysis results is shown in Table 3, showed that broiler fed 5% LBSM had higher value for packed cell volume (PCV) of 33.25% which was significantly higher (P<0.05) than the PCV of the broiler fed the other diets. PCV of birds fed 10% and 15% LBSM were statistically similar. While the PCV of birds fed10% LBSM was significantly higher (P<0.05) than the value obtained from birds fed the Control diet (28.25 and 20.50), the value obtained from the bird fed 15 % was also similar to that obtained from those in the Control group.

The hemoglobin concentration (HC) of broilers fed 5% LBSM (11.79 g/dl) was significantly higher (P<0.05) compared to the other treatment groups (6.65, 7.81 and 7.76) g/dl for those in the Control, 10% and 15% LBSM respectively. Which were statistically similar (P>0.05). Values of white blood cell (WBC) for broilers fed 5% and 10% were statistically similar (13.44 and 13.26 $x10^3/mm^3$) (P>0.05) but significantly higher (P<0.05) than values obtained from birds fed the Control Diet and 15% LBSM (11.54 $x10^3/mm^3$) (10.21 $x10^3/mm^3$). Red blood cell, (RBC) values obtained from the broilers fed the control diet, 10% and 15% LBSM (2.12, 2.43 and 2.32 respectively) $10^6/mm^3$ did not differ significantly from one another (P>0.05) but were significantly lower (P<0.05) compared to the value obtained from the birds fed 5% LBSM (3.55 $x10^6/mm^3$). There was no significant difference (P>0.05) in the mean corpuscular volume (MCV) of the broilers fed the experimental diets. Those fed the control diet had 9.73%, while those fed 5, 10 and 15% LBSM had 9.29, 11.79 and 10.55%, respectively. The values of mean corpuscular hemoglobin (MCH) followed the same trend as MCV, 3.15, 3.27, 3.16 and 3.31%, for broilers fed the control diet, 5, 10, and 15% LBSM, respectively.

However, the mean corpuscular hemoglobin concentration (MCHC) values obtained from broilers fed 5% LBSM (36.100%) was significantly higher (P<0.05) than the values obtained from birds fed 10% LBSM. The values obtained from birds fed the control diet, (32.48%) and 15% LBSM (31.83%) were similar and did not differ significantly from the values obtained from broilers in both 5 and 10% LBSM diets.

3.3 Blood Serum Analysis of Broilers Fed Diets Containing Graded Levels of LBSM

The blood serum indices of broilers fed graded levels of LBSM in their diets are shown in Table 4.

The analysis of blood serum obtained from the experimental birds indicated that significant difference was observed with respect to “Albumin”. (P>0.05) among the treatments. Total protein (TP) ranged from 2.73 to 3.03 mg/dl while albumin ranged from 1.03 to 1.25 g/dl. Total bilirubin (TB) and direct bilirubin (DB) values also ranged from 0.30 to 0.38 and 0.10 to 0.11 mg/dl, respectively. The levels of the uric acid and glucose in the blood serum samples also ranged from 5.15mg/dl for birds fed 15% LBSM to 6.60 mg/dl for those 10% LBSM and 8.99 mmo/dl to 9.49 mmo/dl for birds fed control diet and 15%, respectively.

3.4 Hematology

The result of the heamatological analysis presented in this experiment revealed that MCV and MCH were not significantly different (P>0.05). However, there were significant

Table 3. Effects of graded levels of LBSM on blood composition of broiler chickens

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM
	Control	5% LBSM	10% LBSM	15% LBSM	
Packed Cell Volume (%)	20.50^{c}	33.25^{a}	28.25^{b}	25.00^{bc}	1.78
Hemoglobin Concentration (g/dl)	6.6550^{b}	11.7900^{a}	7.8125^{b}	7.76125^{b}	0.68
White Blood Cell(10^3/mm^3)	11.5375^{b}	13.4375^{a}	13.3250^{a}	10.2125^{b}	0.54
Red Blood Cell(10^6/mm^3)	2.1150^{b}	3.5500^{a}	2.4300^{b}	2.3175^{b}	0.17
Mean Cell Hemoglobin Conc. (%)	32.4750^{ab}	36.1000^{a}	27.6500^{b}	31.8250^{ab}	1.07
Mean Corpuscular Volume(%)	9.7325	9.2900	11.7875	10.5525	0.43
Mean Cell Hemoglobin(%)	3.1525	3.2650	3.1575	3.3050	0.09

Means along the same row with different superscript are significantly different (P>0.05)

Table 4. Blood serum indices of broilers fed diet containing graded levels of LBSM

Parameter	Diet 1	Diet 2	Diet 3	Diet 4	SEM
	Control	5% LBSM	10% LBSM	15% LBSM	
Total protein (g/dl)	2.8250	2.7250	3.0250	2.9000	0.11
Albumin (g/dl)	1.03^{b}	1.15^{ab}	1.25^{a}	1.15^{ab}	0.11
Total bilirubin(mg/dl)	0.3	0.38	0.32	0.37	0.36
Direct bilirubin (mg/dl)	0.11	0.10	0.1025	0.10	0.01
Uric acid (mg/dl)	6.00	5.60	6.60	5.15	0.34
Glucose (mmol/dl)	8.99	9.12	9.25	9.49	0.39

Means along the same row with different superscript are significantly different (P>0.05)

Differences (P<0.05) in PCV, HB, WBC and MCHC values. Esonu et al. [38] and Roberts et al. [39] reported that heamatological constituent reflects the responsiveness of animal to its internal and external environment, which include Feed and feeding, levels of antibodies production and bone marrow development. All parameters recorded in this study were within the normal range for healthy fowl. Schalm et al. [40,41] and [42] who found that there is a strong influence of diet on the heamatological traits with PCV and HG being strong indicators of the nutritional status of animals. Abnormal values may be an indication of anemia [43, 44] and [45]

3.5 Serum Biochemical Analyses

The analysis of blood serum obtained from the experimental birds indicated that all parameters tested for did not show any significant difference. It was well documented that serum biochemical indices has direct correlation to the quality of diet and feeding of an organism [46, 47]. The values of glucose obtained in the various Treatments were within the normal range of 9.90-11.00 as contained in [48]. The TP is lower than the 24.00 for broilers reported by Uchegbu et al. [49]. TP in the serum has been reported as an indication of the retained protein in animal body [50] and [38].

4. CONCLUSION

At the end of the experiment, it was concluded that LBSM containing 5% levels of inclusion performs significantly higher in terms of PCV, HC, WBC, RBC and MCH while 10% level of inclusion performed significantly higher in respect to Albumin among the treatments. All the other treatments can be included in the diet of broilers at starter and finisher phases from 5% – 15 %, level of inclusion, without any deleterious effect on the blood components of the broiler chickens. The heamatological and serum values obtained which were within the normal range, where significant difference (P<0.05) existed PVC, HB, WBC,RBC and MCHC showed that the dietary experimental diets were adequately utilized indicating the normal functioning of the internal organs and absence of anemia, stress related disease and muscle degeneration.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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