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Assessing the Hematology and Serum Biomarkers of Broiler Birds Fed Graded Levels of Kapok (*Bombax costatum)*

H. B. Usman [a], K. M. Aljameel [a*], B. M. Musa [b], S. Ibrahim [b] and I. Bata [b]

[a] Department of Animal Science, Federal University Dutsin-Ma, Nigeria.
[b] Department of Animal Science, Usmanu Danfodiyo University, Sokoto, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study was conducted on broiler chickens to investigate the effects of graded levels of kapok seed meal fed on performance. A total of 288 broiler chickens were used. The broiler chickens randomly divided into four treatments groups (four experimental diets) T_1 (control), T_2 (0.5kg/100kg), T_3 (1Kg/100kg) and T_4 (1.5kg/100Kg). At the end of the feeding trial (eighth week), fifteen birds (5 per replicate) from each treatment group were randomly selected for blood collection. About 2ml of blood was collected per bird. The result showed a significant variation ($P<0.05$) in *WBC* and lymphocytes. The blood parameters were within the normal ranges for broiler chickens. The result showed that birds fed control diet have higher urea compared to those in treatment 3. It was concluded that the inclusion of kapok (*Bombax costatum*) seed meal up to 1.5kg/100kg in the diet of broiler birds had no adverse effect as indicated by hematological and serum chemistry of the birds.

Keywords: Hematology; serum chemistry; kapok seed meal; Broiler.

**Corresponding author: Email: muhdkjameel@gmail.com;*

1. INTRODUCTION

One of the objectives of any poultry producer is to feed the chickens with a balanced diet at least cost and also generate products that will attract premium prices in order to maximize profit. For many decades, farmers and feed manufacturers have been facing the challenge of effectively reducing the cost of poultry production and producing quality products. Several factors such as genotype, diet composition, digestible nutrient content, energy to protein ratio, feed form, feed processing, environment, and disease could affect the cost of production and poultry product quality through influencing feed intake, body weight gain and feed conversion ratio (FCR). In commercial poultry, broiler feed production contributes up to 70% of the total production cost. Due to increases in global feed prices, there is now a tendency in the poultry industry to move towards alternative or unconventional feed ingredients. However, this move is limited by several issues: high and low fibre and protein contents and the presence of anti-nutritional factors (ANF) in unconventional feed ingredients that can reduce feed digestibility [1].

Hematological studies have been found useful for disease prognosis and therapeutic and feed stress monitoring [2]. Hematological studies are important because the blood is the major transport system of the body, and evaluations of the hematological profile usually furnish vital information on the body's response to injury of all forms, including toxic injury [3-4]. Hematological studies represent a useful process in the diagnosis of many diseases as well as investigation of the extent of damage to the blood [5]. This is relevant since blood constituents' change about the physiological conditions of animals. The blood transports or conveys nutrients and materials to different parts of the body. Therefore, whatever affects the blood, either drugs, pathogenic organisms or nutrition will certainly affect the entire body adversely or moderately regarding health, growth, maintenance and, reproduction [6]. A readily available and fast means of assessing the clinical and nutritional health status of animals on feeding trials may be the use of blood analysis because ingestion of dietary components have measurable effects on blood composition [7] and may be considered as an appropriate measure of long-term nutritional status [8]. The examination of blood provides the opportunity to investigate the presence of several metabolites clinically and other constituents in the body, and it plays a vital role in the physiological, nutritional and pathological status of the animal [9]. It also helps distinguish the normal state from the state of stress, which can be nutritional [10]. Hematological parameters are precise indicators of the physiological status of animals. They are also an excellent medium for the measurement of potential biomarkers because their collection is relatively non-invasive, and it encompasses an enormous range of physiological processes in the body at any given time [10].

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at the Teaching and Research Farm of the Department of Animal Science of Usmanu Danfodiyo University, Sokoto, Nigeria. The farm lies at longitude 5^{o} 27" E and latitude 13^{o} 08"N and an altitude of 266m above sea level; the readings were obtained from GNSS viewer software for androids. Average annual environmental temperature 28.3^{o}C (82.9^{o} F). However, the maximum daytime temperature for most of the year generally under 40^{o}C (104.0^{o}F). The low humidity of Sokoto state makes the heat bearable. Heat is more severe in the state in March and April, but the weather in the state is always cold in the mornings and hot in the afternoons except during the harmattan period [11]. The rainy season starts from late May to October. Rainfall starts late and ends early, with annual rainfall ranging between 500mm to 1,200mm [11]. There are two major seasons in the state, namely: wet and dry seasons. The dry season starts from October and last up to April and may extend to May or June. On the other hand, the wet season begins in most of the state in May and lasts up to September or October [11].

2.2 Experimental Design

2.2.1 Animals

A total of 288 apparently healthy broiler chickens were used in this study. The chickens were divided randomly into 4 treatments groups (totally 72 birds), each group divided into sub-groups consisting of 12 birds and replicated 6 times.

2.2.2 The experimental diets

The kapok (*Bombax costatum*) was used as an additive in this experiment. The kapok meal was used as additives in four (4) graded levels of 0,

0.5, 1 and 1.5kg/100kg to feed inclusion and very well mixed.

2.2.3 Experimental birds and management

The boiler chicks for this study were obtained from a reputable farm in Nigeria at the age of one day. The birds were transported to Sokoto under the cool evening hours through the night and arrived in the morning hours. The poultry house was cleaned, washed and disinfected before a week prior to the arrival of the birds. The birds were raised on deep litter in tropical house types, with open side walls and concrete floors. Litter materials (wood shavings and old newspaper were spread on the floor, feeding trays and small drinkers were used for the first 0-2 weeks (Starter phase), while conical feeders and plastic containers with wire guards were used at the final phase. Feed was given to the birds ***ad libitum*** on tray feeders for the first 10 days and the tray feeders were replaced with small conical feeders at second week of their age for proper feed management and efficiency. Freshwater was given to the birds every morning in small drinkers. Their health care was ensured by giving them routine vaccination and medication as at when due, proper sanitation and hygiene was ensured. The floor spacing was maintained at (4/9 ft) per replicate [12].

2.2.4 Experimental diet formulation

Maize, wheat offal, bone meal, Fish meal and salt were obtained from Sokoto central market. Soya bean meal, Groundnut cake, limestone and micro-ingredients such as Premix, Lysine, and Methionine were sourced from a vendor called Alkanchi farm ltd in the Sokoto Metropolis. The kopak seed was obtained from Achida village ground into powder form.

Feed ingredients used for this experiment, such as Maize, Groundnut cake (GNC), Soya bean meal and Bone meal, required crushing so that the particle size will suit the group of birds the feed is to be meant for. Feed ingredients that were in powdery form were weighed and mixed with the crushed ones. The feed compounding was done on a clean concrete floor and thoroughly mixed with a shovel to a uniform mix the feed compounding was done according to the formulation in Table 1.

Table 1. Gross, calculated and analyzed the chemical composition of experimental starter and finisher diets

Ingredient (kg)	Starter	Finisher
Maize	50.0	55.5
Soya beans meal	21.5	14.5
Groundnut cake	15.5	12.0
Wheat offal	8.0	11.0
Limestone	2.0	4.0
Bone meal	2.0	4.0
Premix	0.25	0.25
Salt	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
Total	100kg	100kg
Calculated Chemical Composition		
Crude protein (%)	23	19.05
Energy (kcal/kg)	2986	3352
Methionine	0.36	0.41
Lysine	1.0	0.9
Calcium	0.8	0.8
Phosphorus (av)	0.45	0.45
Fibre (%) (max)	6%	6

2.2.5 Blood collection and evaluation of blood parameters

At the end of the feeding trial (eighth week), fifteen birds (5 per replicate) from each treatment group were randomly selected for blood collection. About 2ml of blood was collected per bird. The collection was done by puncturing the brachial vein with a 5ml scalp vein needle and syringe; two separate blood samples were collected immediately, one sample into a set of sterile plastic bottles, containing ethylene diamine tetra acetic acid (EDTA) as the anticoagulant for determination of haematological parameters and the other into un-heparinized tubes for the determination of serum biochemical indices.

2.2.6 Hematological indices determination

The haematological parameters analyzed include packed cell volume (PCV), red blood cells (RBC) count, total white blood cells (WBC) count, leucocytes differential count and haemoglobin concentration (Hb) in accordance with the methods outlined by Bush [13].

Erythrocyte indices which include the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) was computed in accordance with the standard formula indicated by Jain [14] and [3-4] as shown below:

$$\text{MCV (fl)} = \frac{\text{PCV}}{\text{RBC Count In } 10^6/\text{Mm}^3} \times \frac{10}{1}$$

$$\text{MCH (pg)} = \frac{\text{Hb (G/Dl)}}{\text{RBC (In } 10^6/\text{Mm}^3\text{)}} \times \frac{10}{1}$$

$$\text{MCHC (g/dl)} = \frac{\text{Hb (G/Dl)}}{\text{PCV (\%)}} \times \frac{100}{1}$$

Where: MCV= mean corpuscular volume, PCV= parked cell volume, RBC= red blood cell, MCH = mean corpuscular haemoglobin, Hb = haemoglobin, MCHC = mean corpuscular haemoglobin concentration

2.2.7 Serum chemistry

The plasma total protein was measured using biuret reaction according to the procedure of Savory and Sunderman [15], while albumin was measured by colorimetric estimation using the sigma diagnostic kit according to the method described by Reinhold [16] Globulin was obtained by calculating the difference of total protein and albumin. The serum enzyme, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using photoelectric colorimeter as described by Duncan *et al*. [17]. Blood urea, nitrogen and creatinine levels were determined using photoelectric colorimeter as described by Gbore *et al*. [18]. Total cholesterol was evaluated as described by Baker *et al*. [19.

2.8 Statistical Analysis

Analysis of variance (ANOVA) was used to compare the aematology and serum biomarkers of broiler birds administered fed graded levels of kapok(*Bombax costatum*)seed meal, where significant differences (P<0.05) occur means were separated using Duncan New Multiple Range Test (DNMRT).

3. RESULTS AND DISCUSSION

The result of haematology of broiler birds fed graded levels of kapok *(Bombax costatum)* seed meal was presented in Table 2. Significant variation (P<0.05) was observed in WBC and lymphocytes. The blood parameters were within the normal ranges for broiler chickens and indicated that broilers adequately utilized the nutrients and posed no problem to the birds. Birds in treatment 1 (control) had the highest (P<0.05) concentration of WBC compared to treatments 2 and 3. The value obtained in this study for MCV is lower than 92.44-122.09 reported by Akinola and Etuk [20] but MCHC was within the range of 30.78-33.39 the same author. Lymphocytes are higher (P<0.05) in treatment 2 (0.5kg/100kg) kapok meal while compared to the other treatments. Control birds had lower lymphocytes (P<0.05). hematological traits, especially PCV and Hb, were correlated with the nutritional status of the animal [21] and agreed with Oyawoye and Ogunkunle, [22] who stated that PCV is an index of toxicity in the blood and high-level usually suggest the presence of toxic factors which has an adverse effect on blood formation. The packed cell volume observed was within the normal range as reported by Jain, [14] and Chinrasri and Aengwanich (2007) who reported a normal range for PCV 29.75-31.87%, an indication that the birds were anaemic, hence proving the presence of immune challenge. The absence of Monocyte in the result is an indication that birds fed kapok meal were not stressed during the experiment by nutritional or

environmental factors, since leucocytes responses are considered as better indicators of chronic stress [23].

The result of serum biomarkers of broiler birds fed graded levels of kapok seed meal was presented in Table 3. difference (P<0.05) was observed only in urea. The result shows that birds fed the control diet have higher urea compared to those in treatment 3. Globulin, albumin and total protein are comparable between the treatments; this indicates that kapok (*Bombax costatum)* seed meal significantly influences the dietary treatment. The values obtained in this study were slightly higher than the normal range of serum protein (4.55-6.46g/dl) reported by Duwa*et al,* [24] A higher value indicated that there is enzyme hydrolysis of dietary proteins and explained that the blood pool serves as a major source of amino acids needed for protein synthesis (Scott, 1970; [25]. Cholesterol levels lower than 3.10-3.64 mg/dl were reported by Duwa*et al,* [24] and 2.77-3.90mol/l reported by Akinola and Etuk (2015). Urea were significantly higher in control and lower in treatment 3. The values where than lower the normal range of 9.9-11.1 mmol/l stated by Banerjee (2009) for urea. AST values obtained in this study were higher than the range of 13.72-15.65 Iu/c reported by Akinola and Etuk (2015) and the range of 17.36-29.41 i.u/l Duwa*et al,* (2012). The similarity in the levels of AST and ALT showed that there was no liver damage by the diets. This was in line with the finding of Ekpenyong and Biobaku [26] who stated that the values of AST and ALT are normally low in blood but become high when there is the occurrence of liver damage by toxic substances.

Table 2. Hematologic of the parameter of Broiler birds fed graded levels of kapok seed meal

Parameter	Treatments				SEM
	Control	0.5kg/100kg	1kg/100kg	1.5/100kg	
Haemoglobin (g/dL)	7.45	7.67	7.08	6.97	0.45
PCV (%)	22.28	22.95	21.33	20.97	0.93
RBC (x10^6/ul)	6.87	6.74	6.67	6.67	0.51
MCH (pg)	10.85	11.38	10.61	10.45	0.51
MCV (fl)	32.43	34.05	31.97	31.44	0.98
MCHC (g/dL)	32.84	29.92	30.13	30.09	1.21
WBC (x 10^9/L)	4.33^a	3.28^b	3.33^b	4.11^{ab}	0.29
Neutrophils (%)	21.5	20.00	21.00	21.5	0.09
Eosinophil (%)	3.5	3.0	3.0	2.5	0.20
Lymphocytes (%)	75.00^c	77.00^a	76.00^b	76.00^b	0.31

a,b,c means in the same row with different superscripts are significant (P<0.05) different. RBC- Red blood cells, MCV- Mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, WBC-white blood cells

Table 3. Serum Biomarkers of Broiler birds fed graded levels of kapok seed meal

Parameters	Treatments				SEM
	Control	0.5kg/100kg	1kg/100kg	1.5/100kg	
Albumin (g/dl)	2.87	2.70	2.85	2.97	0.53
Globulin (g/dl)	3.75	3.25	3.00	3.21	0.33
Total protein (g/dl)	6.62	5.95	5.85	6.18	0.61
Triglycerides (Mmol/L)	0.75	0.81	0.77	0.82	0.28
Chloride (Mmol/L)	98.53	99.05	98.76	97.32	0.69
Cholesterol (mmol/L)	2.08	1.96	1.93	1.95	0.06
Urea (Mmol/L)	4.55^a	3.28^{ab}	3.00^b	3.11^{ab}	0.50
AST (u/L)	30.85	31.70	30.50	32.00	2.31
ALT (u/L)	21.00	22.31	21.32	22.00	0.51

a,b,c means in the same row with different superscripts are significant (P<0.05) different.

4. CONCLUSION

The study concluded that the inclusion of kapok (*Bombax costatum*) seed meal up to 1.5kg/100kg in the diet of broiler birds had no adverse effect as indicated by hematological and serum chemistry of the birds.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather, it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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