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# Haematology, biochemical, and egg quality analysis of ISA brown layers fed graded levels of *Faidherbia* albida leaves meal under a hot environment

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# **ABSTRACT**

Poor egg qualities during heat stress is a regular episode. The potential of Faidherbia albida leaves, a naturally occurring food nutraceutical at ameliorating heat-induced egg quality deterioration in laying birds is yet to be understood. This research aimed at assessing the outcome of serving processed Faidherbia albida leaves meal (FALM) to ISA brown layer chickens. Five different experimental diets were formulated having 0% (control), 5%, 10%, and 15% FALM of GNC labeled as T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub> respectively. One hundred eighty 18 weeks old ISA brown layer (IBL) chickens were served these diets in a completely randomized design. The data collection commenced at the 22<sup>nd</sup> week old, for seven weeks. The inclusion of FALM non-significantly (p>0.05) affected all the haematological parameters analyzed. These feeding trials had no effect (p>0.05) on serum TBARS of experimental birds, however, it was highest in  $T_1$  (0.29  $\pm$  0.269 M<sup>-1</sup>cm<sup>-1</sup>), but decreased with increasing levels of dietary FALM from  $0.28 \pm 0.269$  M<sup>-1</sup>cm<sup>-1</sup>in T<sub>2</sub> to  $0.25 \pm 0.233$  M<sup>-1</sup>cm<sup>-1</sup> in T4. The results of dietary FALM on serum vitamins C and E of experimental IBL chickens were significant (p≤0.05), and the serum vitamin C was highest in  $T_2$  (1.00  $\pm$  0.362 mg/dl) and the lowest in  $T_1$  (0.58  $\pm$  0.124 mg/dl). Contrarily, this feeding trial significantly decreased the serum concentration of vitamin E against the control. It was observed that dietary FALM non-significantly affected the egg quality traits evaluated except for the egg albumin, where dietary FALM at 5% significantly (p $\leq$ 0.05) increased albumen (T<sub>2</sub>: 14.97  $\pm$  2.412 g). Also, a nonsignificant increase in the egg albumin was noted in T<sub>3</sub> and T<sub>4</sub> when compared with T<sub>1</sub>. In conclusion, the present findings showed that FALM had no negative effect on the haemopoiesis of IBL chickens, is a potent antioxidant at ameliorating heat stress-induced egg quality deterioration in IBL chickens.

Keywords: Egg quality, Faidherbia albida, Heat stress, Layer chickens, Serum biochemistry

#### 1. INTRODUCTION

Daily consumption of animal protein such as poultry eggs and meat in many tropical countries of the world is considerably lower compared to that of developed countries. According to Daghir [1], the average daily consumption of animal protein in developing countries is below 25 g, when matched with above 45 g in most advanced countries. This might be connected to some constraints such as high cost of production, diseases, poor biosecurity, etc. facing poultry production in the developing nations. Furthermore, the prevailing economic situation i.e. economic downturn joined with the present-day inflationary course, scarceness, as well as the covid-19 pandemic, had contributed to the short supply of animal protein [2, 3]. Additionally, among the constraints to poultry production in the tropics; extremely high ambient temperature (HAT) or heat stress (HS) is one of the leading constraints, as it leads to high mortality [4]. Although HS is a universal challenge to poultry production [4], the tropical sections of the world are more predisposed to thermal effects due to their peculiar HAT and relatively high humidity.

The Savanna zones of Nigeria are classified into: Guinea, Sudan, and the Sahel Savannas, which are typical of a tropical environment characterized by hot-dry, cold-dry (Harmattan), and rainy seasons. For the duration of the hot-dry season, the meteorological feature of these zones is HAT (as high as  $40~^{\circ}\text{C} \pm 2.0~^{\circ}\text{C}$ ). When HAT combines with high relative humidity, it induces thermal discomfort in both human and animal inhabitants [5]. Likewise, livestock and poultry are also affected by HAT, as significantly negative impacts of heat on production efficiency, morbidity, mortality, egg production, egg quality, and shelf life of eggs are being attributed to the rise in global average temperature have been reported by Quinteiro-Filho et al. [6] and Lara & Rostagno [4].

Generally, stressors upturn the generation of free radicals by encouraging the formation of reactive oxygen species (ROS), resulting in increased lipid oxidant and induction of oxidative stress which is a state of overwhelming oxidative damage of macromolecules, cells, and tissues [7]. Antioxidants remove free radicals, activate endogenous antioxidant enzymes, and inhibit oxidative stress [8]. However, oxidative damage may be curtailed by antioxidant protection mechanisms that secure the cell from cellular oxidants, and different approaches have been used in the management of stress. The roles of vitamin A, vitamin C, and vitamin E and micro minerals Zn, and Se in the management of thermal stress have been attributed to their antioxidant properties [9, 10]. As advocacy for alternative medicine shift towards natural products due to their safety, availability, and cost-effectiveness, investigations into leaves that contain bioactive antioxidant components is becoming an area of research interest worldwide. Faidherbia albida (F. albida) leaves are natural, safe, and locally available containing antioxidant compounds [11]. Besides their high cost, adulteration of common dietary supplements is an evolving food safety issue [12]. Using functional leaves especially F. albida leaves in managing hot environmental stress in laying chickens is a new approach. Therefore, this research is designed to evaluate the potential of F. albida leaves meal in ameliorating the impacts of heat stress on haematology, serum biochemistry, and quality of egg in Institut de Sélection Animale (ISA) Brown layer chickens raised under a hot environment.

This will provide preliminary information on the antioxidant potentials of the *F. albida* leaves in laying birds in the study area and lay foundational knowledge for further studies.

#### 2. MATERIALS AND METHODS

# **Experimental location**

This study was conducted at the Poultry Research Farm of College of Agriculture and Animal Science Bakura, Zamfara State, Nigeria. Bakura is situated in the north-western part of Nigeria and located between latitude 12°42′37″N and longitude 5°52′23″E with characteristic semi-arid climatic conditions [13]. The State is climatically tropical having an atmosphere rising to and above 38°Ceric temperature (100.4°F) within March and May. It starts to rain in late May to September, while the dry season (Harmattan) lingers from December to April (2021 PHC Priority Tables – National Population).

# **Ethical approval**

The procedures for this experiment were evaluated and sanctioned by the Institutional Animal Care And Use Committee (IACUC) of the Usmanu Danfodiyo University Sokoto (UDUS) with the reference number UDUS/IACUC/AUP-R05/2020.

# Preparation of Faidherbia albida leaves meal (FALM) and experimental feeds

Faidherbia albida leaves were obtained from the forest around Bakura and were soaked in boiled water for two hours, then air-dried under shade and ground to smaller sizes with the aid of an electric grinding machine (CAPARCITOR START MACHINE) to make *F. albida* leaves meal (FALM). The FALM were stored at ambient temperature in airtight sacks until needed. Four diets were formulated and compounded (Table 1). FALM was included at a certain percentage of the crude protein (CP) of groundnut cake (GNC). Experimental diets 1, 2, 3, and 4contained 0, 5, 10 and 15% FALM respectively.

# Management of experimental animals and experimental design

For this study, one hundred and eighty (180) 18 weeks old Point of Lay (POL) *Institut de Sélection Animale* (ISA) Brown layer chickens were gotten from Zartech Farm, Ibadan, Oyo State, Nigeria. They were managed using a deep litter system at the Poultry Research Farm of College of Agriculture and Animal Science Bakura, Zamfara State, Nigeria. The experimental birds were served feed and water *ad libitum* throughout the study, they were vaccinated against infectious bursa disease, Newcastle disease, fowl pox, and typhoid accordingly. The birds were allowed to acclimatize for 14 days, and at the 20<sup>th</sup> week of age, the 180 ISA Brown layer (IBL) chickens were randomly spread into four treatment groups of 45 birds per treatment and were replicated thrice with 15 birds per replicate. Treatment one (T<sub>1</sub>) was the control group and was fed diet 1, while treatment two (T<sub>2</sub>), three (T<sub>3</sub>), and four (T<sub>4</sub>) were fed diets 2, 3, and 4 respectively (Table 2). The chickens were respectively and gradually introduced to experimental diets from the 20<sup>th</sup> week of age before the start of data collection at the 28<sup>th</sup> week of age. The experimental birds began to lay at the 22<sup>nd</sup> week of age and the egg production was supervised and noted to ascertain the percentage of egg production before data collection.

**Table 1.** Composition of the experimental feed (layers mash)

Ingredients (kg)	Diet 1 (0% FALM)	Diet 2 (5% FALM)	Diet 3 (10% FALM)	Diet 4 (15% FALM)
Maize	57.98	57.98	57.98	57.98
GNC	12.28	10.98	8.76	6.66
FALM	0.00	2.10	4.20	6.30
Soybeans	3.24	3.24	3.24	3.24
Wheat offal	15.00	15.00	15.00	15.00
Blood meal	2.00	2.00	2.00	2.00
Limestone	4.00	4.00	4.00	4.00
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Methionine	3.00	2.20	2.32	2.32
Lysine	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00
		Calculated		
Crude protein (%)	16.00	16.02	16.10	15.78
Energy (kcal/kg)	2624.27	2633.26	2622.00	2600.71

Key: FALM: Faidherbia albida leaves meal, GNC: groundnut cake

Premix: Minerals and vitamins (Avitech, 2020)

Table 2. Experimental Design

Grouping	Level of boiled-water processed FALM (%)			
$T_1$	0% FALM (Control)			
$T_2$	5% FALM			
T <sub>3</sub>	10% FALM			
T <sub>4</sub>	15% FALM			
KEY: FALM: Faidherbia albida leaves meal				

The feeding trial lasted for 7 weeks from  $23^{rd}$  of March, 2020 to  $11^{th}$  of May, 2020 during which time HAT is projected to be prevalent. At the beginning of data collection, the chickens were at the  $28^{th}$  week of age and  $\approx 60\%$  production level. The average minimum and maximum diurnal temperatures and relative humidity of the experimental poultry pen were observed using digital temperature and the hygrometer (BIOBASE® China), placed in the poultry pen.

### Some haematological and biochemical indices

At the end of the experimental feeding, six IBL chickens were randomly chosen from each treatment, they were sacrificed using the Halal slaughtering method and 2 ml of the blood samples were dispensed into labeled EDTA sample containers for haematological analyses using methods described by Jelalu [14]. Another 3 ml was put inside labeled plain sample bottles; it was left to clot, centrifuged at 4000 rpm for 15 minutes, and then the clear unhaemolysed serum was harvested. Using pipettes, the sera gotten were drawn out into 3 labeled tubes for the valuation of antioxidant enzymes and vitamins C and E. The sera were stored at -20 °C at the Chemical Pathology Laboratory, Usmanu Danfodiyo University Teaching Hospital, Sokoto, until the time of analysis. The formation of thiobarbituric acid reactive substances (TBARS), is an indication of lipid peroxidation and was determined according to Niehans & Samuelson [15], while serum vitamins C and E were assessed according to Natelson [16] and Hashim & Schuttringer [17] respectively.

# Egg quality traits

Six eggs were randomly selected per treatment weekly and each egg was examined for external and internal quality indices as follows:

For external egg quality traits; egg weight (EW) was determined by weighing individual eggs from each treatment weekly using an electronic balance. Shape index (SI) was determined by measuring the width (circumference) and length (from the middle of the sharp end to the middle of the air-space end) of each egg in millimeters using a vernier caliper. Then the widths were divided by their corresponding lengths to obtain the SI of the egg. Eggshell weight (ESW) was determined by gently cleaning, cracking, and opening the individual egg and then all the content (yolk and albumin) were harvested in a clean petri dish. The eggshell was air-dried for 3 hours at room temperature and then weighed using an electronic balance. Eggshell thickness (EST): from the air-dried eggshell, the thickness of the eggshell was determined at three different points on the eggshell (the air-space, equator, and sharp end) using a micrometer screw gauge, to the nearest of 0.01 mm.

For internal egg quality traits: Egg yolk and albumin: from the earlier harvested yolk and albumin, the yolk and albumin were carefully separated into dry, already weighed, labeled, and clean two glasswares and then each of them was weighed separately with their contents (the yolk in one and the albumin in the other) using electronic balance to determine the weights of egg yolk and albumin.

#### Statistical analysis and data presentation

Data generated in this experiment were analyzed using one-way analysis of variance (ANOVA), on SPSS software version 20 and the results were presented as mean  $\pm$  standard

deviation of mean in tables. Differences between mean marks of different treatments were considered statistically significant at p $\leq$ 0.05, using Tukey HSD as the post hoc test.

#### 3. RESULTS

The result on the influence of dietary inclusion of FALM on haematological and differential parameters is presented in Table 3. This study showed that the dietary FALM impacted no significant (p>0.05) effect on any of the haematological parameters analyzed. However, it was observed that PCV, lymphocytes, and monocytes values numerically increased with an increasing level of FALM in this study. The PCV values ranged between 26.25 and 33.17%, the highest value of 33.17% was obtained in  $T_1$  (0% FALM). Haemoglobin and RBC values ranged among the groups between 7.56 and 13.03 g/dl and 1.22 and  $3.13\times10^6/\text{mm}^3$  respectively. WBC values were non-significantly (p>0.05) highest in  $T_2$  (12.02×10<sup>3</sup>/mm<sup>3</sup>), and  $T_3$  had the lowest value of  $8.03\times10^3/\text{mm}^3$ , while it was  $10.07\times10^3/\text{mm}^3$  and  $9.76\times10^3/\text{mm}^3$  in  $T_1$  and  $T_4$  respectively. The heterophil counts decreases with increasing levels of FALM ( $T_1$ : 17.17%,  $T_2$ : 20.50%,  $T_3$ : 20.00%, and  $T_4$ : 17.60%), lymphocyte counts increases with increasing levels of FALM across the groups ( $T_1$ : 73.67%,  $T_2$ : 79.25%,  $T_3$ : 80.33%, and  $T_4$ : 81.60%), while monocytes ranged from 0.25 to 0.83% in the experimental birds.

The result on serum TBARS and vitamins C and E levels of IBL chickens fed dietary FALM is obtainable in Table 3. It was observed that the dietary inclusion of FALM produced no significant (p>0.05) impact on the serum TBARS levels of birds in all the treatment groups, however, the highest level of serum TBARS was observed in  $T_1$  (0.29  $\pm$  0.269) and nonsignificantly decreased with increasing levels of dietary FALM from 0.28  $\pm$  0.269 in  $T_2$  to 0.26  $\pm$  0.240 in  $T_3$  and least in  $T_4$  (0.25  $\pm$  0.233). The result of dietary FALM on serum vitamins C and E of experimental IBL chickens revealed that feeding IBL chickens with FALM statistically (p≤0.05) affected the levels of vitamins C and E in the serum. It was observed that the level of vitamin C in the serum increased significantly (p≤0.05) for  $T_2$  (1.00  $\pm$  0.362 mg/dl) and the decrease in  $T_1$  (0.58  $\pm$  0.124 mg/dl). Contrarily, dietary inclusion of FALM at 5, 10, and 15% levels significantly decreased the serum concentration of vitamin E when compared with the control.

Effects of FALM on the quality traits of eggs of IBL chickens raised in a hot environment are presented in Table 4. It was observed that dietary inclusion of FALM non-statistically (p>0.05) affected the EW of IBL chickens in a hot environment. This study showed that birds in  $T_3$  (51.62  $\pm$  4.751 g) had similar EW with the control (51.90  $\pm$  4.419 g), it was 50.76  $\pm$  2.819 g and 48.48  $\pm$  6.664 g in  $T_4$  and  $T_2$  respectively. Data on egg SI showed that dietary inclusion of FALM had no effect (p>0.05) on egg SI of the experimental IBL chickens. However, it was highest in the control group (0.82  $\pm$  0.085), and least in  $T_4$  (0.77  $\pm$  0.037). The ESW of IBL chickens exposed to a hot environment and treated with FALM indicated that dietary FALM did not affect (p>0.05) the ESW.

In comparison with  $T_1$  (6.48  $\pm$  0.753 g), it was however observed that eggs from  $T_2$  (6.74  $\pm$  1.338 g) had the heaviest eggshell, while ESW decrease with increasing inclusion of FALM in  $T_3$  (6.40  $\pm$  1.551 g) and  $T_4$  (5.62  $\pm$  1.216 g). Results on EST revealed that nutritional FALM impacted no effect (p>0.05) on the quality of egg in terms of EST. Generally, when compared with the control,  $T_1$  (39.53  $\pm$  8.040 mm), the eggshell was thickest in  $T_2$  (43.74  $\pm$  3.857), while

a decrease in EST was noted with increased levels of FALM ( $T_3$ : 37.51  $\pm$  6.604 mm and  $T_4$ : 36.68  $\pm$  5.689mm).

The egg yolk of experimental chickens showed that varying levels of dietary FALM did not affect (p>0.05) the quantity of egg yolk. It was observed that increasing the inclusion levels of FALM from 5% ( $T_2$ ) to 10% ( $T_3$ ) improved the quality of egg in terms of egg yolk quantity from  $30.27 \pm 4.128$  g to  $33.71 \pm 2.938$  g respectively, while further incensement from 10% ( $T_3$ ) to 15% ( $T_4$ ) decreased the quantity of egg yolk from  $33.71 \pm 2.938$  g to  $31.38 \pm 3.351$  g. Data on egg albumin showed a statistical effect (p $\leq$ 0.05), comparing with birds under  $T_1$  ( $12.19 \pm 1.352$  g), the inclusion of FALM in the diet at 5% ( $T_2$ :  $14.97 \pm 2.412$  g) significantly (p $\leq$ 0.05) caused the higher value of albumen. Also, a non-significant increase in the egg albumin was noted in  $T_3$  ( $13.72 \pm 1.510$  g) and  $T_4$  ( $13.26 \pm 1.496$  g) when compared with the control,  $T_1$ .

**Table 3.** Mean  $\pm$  SD haematology and biochemical analysis of IBL chickens fed graded levels of FALM under hot environment.

Parameter	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	P-value		
Haematology							
PCV (%)	$33.17 \pm 6.824$	$26.25 \pm 5.568$	$27.33 \pm 6.658$	$29.00 \pm 7.450$	0.153		
Hb (g/dl)	$13.03 \pm 2.268$	$7.56 \pm 4.812$	$11.86 \pm 3.115$	$11.13 \pm 3.379$	0.267		
RBC (×10 <sup>6</sup> mm <sup>-3</sup> )	$3.13 \pm 3.143$	$1.59 \pm 0.206$	$1.22 \pm 0.372$	$1.60 \pm 0.684$	0.368		
WBC (×10 <sup>3</sup> mm <sup>-3</sup> )	$10.07 \pm 2.779$	$12.02 \pm 1.721$	$8.03 \pm 1.193$	$9.76 \pm 2.256$	0.336		
HET (%)	$17.17 \pm 2.639$	$20.50 \pm 2.082$	$20.00 \pm 2.000$	$17.60 \pm 2.608$	0.429		
LYP (%)	$73.67 \pm 19.552$	$79.25 \pm 5.217$	$80.33 \pm 6.216$	$81.60 \pm 5.302$	0.627		
MNC (%)	$0.83 \pm 0.753$	$0.25 \pm 0.500$	$0.33 \pm 0.577$	$0.80 \pm 0.447$	0.369		
ESN (%)	0.00	0.00	0.00	0.00			
BAS (%)	0.00	0.00	0.00	0.00			
Serum biochemistry analysis							
TBARS (M <sup>-1</sup> cm <sup>-1</sup> )	$0.29 \pm 0.269$	$0.28 \pm 0.269$	$0.26 \pm 0.240$	$0.25 \pm 0.233$	0.998		
Vitamin C (mg/dl)	$0.58 \pm 0.124$ °	1.00 ± 0.362 a	$0.84 \pm 0.197$ b	$0.90 \pm 0.271$ b	0.036		
Vitamin E (mg/dl)	$1.37 \pm 0.506$ a	$0.37 \pm 0.140 \ ^{\mathbf{b}}$	$0.37 \pm 0.043$ b	$0.34 \pm 0.085$ b	0.0001		

Data within the same row with different superscripts <sup>a, b, c</sup> are significantly different (p≤0.05). Key: IBL: ISA Brown Layers; FALM: *Faidherbia albida* leaves meal; PCV: Packed Cell Volume; Hb: Hemoglobin; RBC: Red Blood Cell; WBC: White Blood Cells; HET: Heterophils; LYP: Lymphocytes; MNC: Monocytes; ESN: Eosinophils; BAS: Basophils

**Table 4.** Mean ± SD egg quality of IBL chickens fed graded levels of FALM under hot environment

Parameter	T <sub>1</sub>	<b>T</b> 2	Т3	T4	P-value
EW (g/egg)	$51.90 \pm 4.42$	$48.48 \pm 6.66$	$51.62 \pm 4.75$	$50.76 \pm 2.82$	0.552
ESI (/egg)	$0.82\pm0.09$	$0.79 \pm 0.03$	$0.80 \pm 0.10$	$0.77 \pm 0.04$	0.531
ESW (g/egg)	$6.48 \pm 0.75$	$6.74 \pm 1.34$	$6.40 \pm 1.55$	$5.62 \pm 1.22$	0.362
EST (mm/egg)	$39.53 \pm 8.04$	$43.74 \pm 3.86$	$37.51 \pm 6.60$	$36.68 \pm 5.69$	0.178
EY (g/egg)	$32.81 \pm 4.68$	$30.27 \pm 4.13$	$33.71 \pm 2.94$	$31.38 \pm 3.35$	0.365
EA (g/egg)	12.19 ± 1.35 b	14.97 ± 2.41 a	$13.72 \pm 1.51$ a,b	$13.26 \pm 1.50$ a,b	0.048

Data within the same row with different superscripts <sup>a, b, c</sup> are significantly different (p≤0.05) Key: SD: standard deviation; IBL: ISA Brown Layers; FALM: *Faidherbia albida* leaves meal; DEP: daily egg production; EW: egg weight; ESI: egg shape index; ESW: eggshell weight; EST: eggshell thickness; EY: egg volk and EA: egg albumin

#### 4. DISCUSSION

From the results of the haematological studies of the experimental IBL chickens, it was observed that the dietary FALM did not change (p>0.05) all the investigated haematological parameters in all the chickens. This result agreed with the earlier reports of Mohammed et al. [11], and it is an indication that dietary inclusion of FALM in this study in IBL chickens raised in a hot environment had no adverse effect on the birds' haemopoiesis, hence it is safe. The values of PCV ranged from 26.25 – 29.00%, FALM treated birds had lower values for PCV when compared to the control. However, the range observed is within the values for stressed birds [18] and also within the range of 24.90 – 45.20% for healthy birds [19]. Haemoglobin values obtained in this study ranged from 7.56 – 11.13 g/dl and fell within the range of 7.40 – 13.10 g/dl as informed by Mitruka & Rawnsley [19] for healthy birds.

Also, values obtained for RBC ranged from  $1.22-1.60\times10^6/\text{mm}^3$  and fell below  $2.07-2.86\times10^6/\text{mm}^3$  obtained for normal birds [18]. The observed values suggested that the birds' health and haemopoietic capability were not compromised by the inclusion of FALM in the diets of the birds. Furthermore, these results suggest that the experimental birds received sufficient minerals from the diets, which enabled the synthesis of haemoglobin required for carbon dioxide and oxygen transportation in the blood.

Thiobarbituric acid reactive substances (TBARS) are made as a byproduct of lipid peroxidation which can be identified by the TBARS assay using thiobarbituric acid as a reagent.

It is frequently used to indicate the extent of oxidative stress within a biological sample [20]. In this study dietary inclusion of FALM did not affect the level of TBARS of birds in all the treatment groups.

However, the highest level of serum TBARS was observed in the untreated group ( $T_1$ :  $0.29 \pm 0.269~M^{-1}~cm^{-1}$ ) and non-significantly decreased with increasing levels of dietary FALM from  $0.28 \pm 0.269~M^{-1}~cm^{-1}$  in  $T_2$  to  $0.26 \pm 0.240~M^{-1}~cm^{-1}$  in

 $T_3$  and least in  $T_4$  (0.25  $\pm$  0.233 M<sup>-1</sup> cm<sup>-1</sup>). This is an indication that the degree of lipid peroxidation due to heat stress was higher in the untreated groups ( $T_1$ ), while lower values of TBARS in FALM treated groups ( $T_2$ ,  $T_3$ , and  $T_4$ ) signifies the antioxidant potential of FALM. This might be attributed to the presence of eight phenolic compounds in the leaves of F. albida such as  $\beta$ -amyrin (L-1),  $\beta$ -sitosterol (L-2),  $\beta$ -sitosterol-3-O- $\beta$ -D-glucopyranoside (L-3), Quercetin (L-5), Gallic acid (L-6), Rhamnocitrin (L-4), Afzelin (L-7) and (6S, 9S)- Roseoside (L-8) which have been reported for their strong antioxidant activities [11]. The antioxidant potential in the FALM may have no negative effect on the livers of the chickens. To the fact that the liver enzymes were not affected, as evident by the insignificant (p>0.05) improvements observed in the TBARS activities.

Primarily vitamin C as an antioxidant serve to deactivate free radicals. Also, because vitamin C is water-soluble, it can work extracellular to mitigate damages due to free radicals. It is an exceptional source of electrons that can be donated to the free radicals hydroxyl and superoxide radicals to quench their reactivity [21]. This research noted that the serum level of vitamin C was highest ( $p \le 0.05$ ) in the groups treated with FALM ( $T_2$ ,  $T_3$ , and  $T_4$ ) when compared with the untreated group  $(T_1)$ . This is in accordance with the report of [10, 22], in their opinion, usually, non-enzymatic antioxidants, like vitamin C, are produced in the bird's kidneys, and are involved in the removal of excess free radicals or ROS from the body, but under stressful condition, they might become overwhelmed or exhausted; as observed in this study. Therefore, a decrease in serum vitamin C in the untreated group in this study might be attributed to the damaging effect of heat stress on the kidney [23, 24]. Since lipid peroxidation was lower in groups fed dietary FALM as indicated by biologically lower values of TBARS, higher (p≤0.05) levels of vitamin C in FALM treated birds is a pointer that FALM had no negative impact on the kidney [25, 26] of IBL chickens. Also, increased serum vitamin C could be because FALM supplies necessary nutrients that are capable of boosting vitamin C production in stressed chickens. This result further corroborates previous reports [9, 27] on the effectiveness of vitamin C in the management of negative impacts of heat stress in chickens. Feeding IBL chickens with FALM in the diet significantly ( $p \le 0.05$ ) decreased the serum levels of vitamins E when compared with the control. There is no obvious explanation for the decrease (p<0.05) in vitamin E as a result of including F, albida in the diet of IBL chickens.

It was also observed that graded inclusion of FALM non-significantly (p>0.05) decreased the EW of layers. This is an indication that dietary FALM has no negative effect on the EW of IBL chicken. The average EW of between 48.48 - 51.90 g observed in this study was lower than 54.44 g [28], 50.00 - 70.00 g [29], and 61.89 g [30], but falls within the range of 37.04 - 55.92 g reported by Kilic & Simsek [31]. This is because the values reported by Saki et al. [28], Nys & Guyot [29], and Ebeid et al. [30], were obtained from birds in the thermoneutral zone, while those of Kilic & Simsek [31] were from heat-stressed birds. In addition, this study showed that dietary inclusion of FALM had no effect (p $\le 0.05$ ) on egg SI, ESW, EST, and egg yolk and are found to be within normal ranges for laying hens [29]. These feeding trials revealed a significant (p $\le 0.05$ ) decrease in the weight of egg albumin [29] of all IBL chickens raised in the hot environment used in this study. These outcomes are in accord with the document of Melesse et al. [32], that heat stress usually induces a reduction in albumin quality. Moreover, it has been observed that white-egg strains are known to have better albumin quality than brown

eggs [33]. This might be the reason for the general reduction in albumin quantity in this study, as IBL chickens produced brown eggs. However, FALM at 5% improved (p<0.05) egg albumin above all other groups.

#### 5. CONCLUSIONS

It was observed that dietary FALM especially at 5% inclusion level limited lipid peroxidation due to oxidative stress, and boosted endogenous synthesis of vitamin C, with a consequent these are signals for improved egg production. Dietary inclusion of FALM, notably at 5% although non-significantly enhanced both the internal and external quality of an egg. The haematology and some of the biochemical analyses revealed that FALM in the diet of laying birds is nontoxic to the system. It could therefore be concluded that *Faidherbia albida* leaves meal (FALM) could be included in the diet of ISA Brown layer chickens up to 5% level to improve egg quality and potentially egg production of ISA Brown layer chickens raised in a hot environment. However, further study should be conducted on the standardization of the quantity of FALM for use as a feed ingredient for commercial purposes. Also, hepatoprotective and renoprotective potentials or otherwise of FALM demands further studies.

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