GROWTH PERFORMANCE, BLOOD PROFILES AND EGG PRODUCTION OF *COTURNIX JAPONICA* (TEMMINCK AND SCHLEGEL, 1849) FED DIETS SUPPLEMENTED WITH *TITHONIA DIVERSIFOLIA* A. GRAY LEAVES

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***Received** May 22, 2020; **Revised** July 18, 2020; **Accepted** July 20, 2020*

ABSTRACT

This study investigated the growth performances, egg production and blood profiles of Coturnix japonica fed concentrate diets supplemented with Tithonia diversifolia leaf meal (TDLM). One hundred (two-week old) C. japonica chicks were allocated to five dietary treatments. The experimental birds were allowed to acclimatize for one week, before feeding trials lasted for twelve weeks. Diets were formulated with TDLM inclusions at 0 % (A), 2.5% (B), 5 % (C), 7.5 % (D) and 10 % (E) to the standard dietary formulations. Diets and water were provided for the chicks, ad-libitum. Data collected on growth performance, blood profiles, serum biochemistry and egg production, were analyzed using descriptive and inferential statistics. Inclusion of TDLM had a significant influence on growth performance, blood profiles, and egg production (P<0.05). After 12 weeks, Diet C had the highest body weight (148.37 ± 0.76 g), while control diet had least (144.93 ± 1.05 g). The control diet had 20% mortality, while Diet D had 5% mortality due to injuries from other birds' attack. Egg weight varied from 9.60 ± 0.14 g (control) to 11.39 ± 0.12 g (Diet E).The blood profiles responded positively to TDLM inclusion with the birds developing stronger immunity when compared to those fed the control diet. Serum biochemistry did not significantly differ indicating that there were no negative effects due to TDLM inclusion. TDLM at 2.5 and 5.0 % inclusions had the highest positive effects on growth performance, haematological parameters, egg production and serum biochemistry of the quails.

Keywords: Blood profiles, *Coturnix japonica,* Digestibility, Growth performance, *Tithonia diversifolia*

INTRODUCTION

The daily protein intake in most developing countries is below recommended levels. This is because most conventional animal protein sources are expensive and above the reach of majority of the people (FAO, 2004). Hence, the need for alternative sources of animal protein in human diets. This has led to the successful domestication and rearing of wild birds such as Japanese quail (*Coturnix japonica* Temminck and Schlegel, 1849) for human consumption (FAO, 2011). This bird is tractable, prolific and widely accepted for consumption. The production of this bird species with short generation interval could be a viable option in ameliorating the shortage of protein in diets of people in developing countries like Nigeria (Dauda *et al.,* 2014). Japanese quail has a fast growth, early sexual maturity, high rate of egg production, small floor space requirements in both litter and cage systems, low feed requirements, short egg incubation period, and less susceptibility to poultry diseases (Aygun and Sert, 2013; Jatoi *et al.,* 2013).

Quail farming, therefore is an alternative to poultry production with social acceptability, preferred by many and has no religious taboo. Quail has good nutritive value, delicious taste and flavour, and tender meat which is low in calories but high in dry matter. The meat is rich in protein, vitamins, essential amino acids, fatty acids and phospholipids (Putra *et al.,* 2015; Moraes *et al.,* 2016; Da Silva *et al.,* 2018). However, awareness on the potentials of meat, egg and other by-products obtainable from the highly productive *Coturnix japonica* is at a low level in most parts of Nigeria (Dauda *et al.,* 2014).

Tithonia diversifolia (Hemsl. A. Gray) commonly known as wild or Mexican sunflower is a fast-growing invasive green forage plant that can be utilized as feed stuff for animals such as cows, goats and fish, due to its high protein content, especially in the leaves (Table 1).

Table 1: Proximate composition of plant parts of *Tithonia diversifolia*

Proximate composition (%)	Plant Parts		
	Leaves	Stem	Root
Moisture	9.27 ± 0.09	8.87 ± 0.03	9.43 ± 0.03
Total ash	8.10 ± 0.10	6.27 ± 0.03	12.53 ± 0.09
Crude fibre	13.47 ± 0.07	53.57 ± 0.14	31.33 ± 0.09
Crude fat	2.47 ± 0.09	0.27 ±0.03	0.83 ± 0.03
Crude protein	14.43 ± 0.12	0.80 ± 0.58	2.73 ± 0.03
Carbohydrate	52.27 ± 0.32	30.13 ± 0.09	43.13 ± 0.15

Source: Olayinka et al. (2015)

The leaves, soft branches and yellow flowers are edible and the species has been shown to be highly nutritious (Dutta *et al.,* 1993; Oyewole *et al*., 2008; Olayinka *et al*., 2015). The plant emerges annually towards the end of rainy season and thrives well on a wide variety of habitats with soil types ranging from sandy to loamy soils. It grows in dense populations among crop fields, in wastelands and along roadsides in the rainforest and coastal regions of Nigeria (Baruah *et al.,* 2000).

It is a prolific seeder which germinates naturally and easily colonizes landscapes. *T. diversifolia* is highly medicinal with properties such as anti-plasmodia (Goffin *et al*., 2002), anti-leukemic and antiviral (Chiang *et al*., 2004), antidiabetic (Olukunle *et al.,* 2014), anti-diarrhoeal (Ezeonwumelu *et al*., 2012) and antimicrobial (Linthoingambi and Singh, 2013) properties. These activities have been attributed to the presence of active constituents such as sesquinterpene, lactones, diterpenoids and artemisinin acid analogs (Ajaiyeoba *et al.,* 2006; Chagas-Paula *et al.,* 2012). The leaves and other plant parts (Figure 1) have shown great promise in the treatment of ailments such as stomach pain, body pains and sore throat (Owoyele *et al*., 2004), indigestion (Tangjitman *et al.,* 2013) and liver disease (Di Giacomo *et al*., 2015). It has erosion control qualities and helps to improve soil conditions when planted as ornamental plants (Jama *et al*., 2000; Olabode *et al*., 2007).

Figure 1: *Tithonia diversifolia* plants growing in natural stands in Ibadan, south west Nigeria

Previous studies have revealed the potentials of *T. diversifolia* leaf meal (TDLM) as a cheap and readily available supplement in animal feed formulation (Togun *et al.,* 2006; Osuga *et al.,* 2012). For instance Olayeni *et al*. (2006) and Fasuyi *et al*. (2013) successful incorporated the leaves into the feed of weaner pigs at a maximum of 20 % inclusion in standard diets. Ramırez-Rivera *et al.* (2010) reported that the supplementation of browse foliage (Taiwan grass) fed to tropical sheep with *T. diversifolia,* increased the feed consumption and crude protein intake by 50 and 127 % respectively, with optimum growth performance observed at 20 % inclusion. Similarly, Funmilayo and Ayodele (2016) opined that 15 % TDLM inclusion in the diets of cockerels had no negative effects on their growth performance, haematological characteristics and serum biochemistry. However, Buragohain (2016) recommended that TDLM inclusion should not exceed 4 % when feeding broilers in order to sustain their growth, nutrient utilization and feed conversion efficiency in deep litter systems.

There is therefore a need to harness the nutritional and medicinal benefits offered by *T. diversifolia* in animal diet production for domesticated birds such as quail. This study determined the effects of diets supplemented with varying amounts of TDLM on the growth performance, blood profile, and egg production of *C. japonica*.

MATERIALS AND METHODS

Experimental Procedure: One hundred (100) two-weeks old Japanese quails (body weight: 36.92 – 39.18 g) were obtained from a quail farm located in Ibadan, Oyo State, Nigeria. All birds received standard routine vaccination to protect them against Newcastle disease (B1 Type, Lasota strain live virus). The animals were of both sexes (female: male = 3:1). The chicks were randomly allocated to five dietary treatment groups replicated four times with each replicate having five birds using a completely randomized experimental design. The animals were raised under an intensive management system using wooden cages covered with mesh nettings. Facilities for drinking, feeding and feacal collection were provided. The Japanese quails were fed for twelve weeks, while diet and water were provided *ad-libitum*.

All procedures and techniques reported in this study conformed to guidelines for the ethical conduct and reporting of research done with animals (Jarvis *et al*., 2005; Kilkenny *et al*., 2010). All adopted methods and protocols were carefully reviewed and authors adhered to ethical standards.

Preparation of Diets: Leaves of *T. diversifolia* were harvested from naturally growing stands in Ibadan, Nigeria. The leaves were identified using a taxonomic key (Beentje *et al.,* 2005) and authenticated by plant taxonomists at the University of Ibadan and Forestry Research Institute of Nigeria. They were air-dried under shade to a constant weight and milled to powder to produce TDLM and mixed with other dietary ingredients. The leaves of *T. diversifolia* have been shown to be non-toxic to animals with the median lethal dose (LD_{50}) being greater than 2000 mg/kg (Githinji *et al.,* 2018). Five experimental diets supplemented with 0, 2.5, 5, 7.5 and 10 % of *T. diversifolia* leaves were formulated and packed in feed bags labeled A, B, C, D and E, respectively (Table 2). Proximate analyses (Table 3) of the experimental feeds were done to determine: dry matter, moisture, crude protein, crude fibre, ether extract and nitrogen-free extract using the method described by AOAC (2005).

Data Collection: The formulated feeds were offered twice daily (at 9.00 am and 4.00 pm) to the Japanese quails. The leftover feed were collected and weighed at the beginning of the next day. The difference between the quantity of feed given and the leftover from the previous day was used to determine the daily feed intake (DFI). Weekly body weight measurements were used to monitor weight gain and feed conversion ratio (FCR).

Egg Production and Quality: Egg production from each treatment was recorded daily. The average number of eggs laid per week per treatment was determined. The average weight of eggs, total number of egg produced per bird and the age at egg laying peak were also determined.

Table 2: Experimental diets compounded with varying amounts of *Tithonia diversifolia* leaves and their calculated nutrient composition

Ingredients (%)	A	B	C	D	E
Maize	50.0	50.0	50.0	50.0	50.0
Soya Bean Meal	18.0	15.5	13.0	10.5	8.0
Wheat Offals	19.0	19.0	19.0	19.0	19.0
TDLM*	0.0	2.5	5.0	7.5	10.0
Oyster Shell	8.0	8.0	8.0	8.0	8.0
Bone Meal	4.0	4.0	4.0	4.0	4.0
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Premix**	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
TOTAL	100.0	100.0	100.0	100.0	100.0
Calculated Nutrients					
Crude Protein (%)	15.83[a]	16.35[b]	16.83[c]	17.30[d]	17.74[e]
Crude Fibre (%)	3.84[a]	4.18[b]	4.51[c]	4.82[d]	5.12[e]
Metabolizable Energy (Kcal/kg)	2556.55[a]	2541.00[b]	2526.18[c]	2512.05[d]	2498.56[e]
Available Ca (%)	5.36[a]	5.24[b]	5.12[c]	5.01[d]	4.90[e]

**TDLM: Tithonia diversifolia Leaf Meal, **Vitamin A = 8,000,000IU; Vitamin D = 1,500 IU; Vitamin E = 3 g; Vitamin K = 23 g; Calcium D Pantothenate = 3 g: Vitamin B6 = 0.3 g; Vitamin B12 = 8 mg; Mn = 10 g; Zn = 4.5 g; Cu = 0.2 g; 1 = 0.15g, Va = 0.02 g; Se = 0.01 g*

Table 3: Proximate composition of the experimental diets formulated with varying levels of *Tithonia diversiflora* leaf meal

Proximate Composition	A	B	C	D	E
Dry Matter (%)	77.58	77.51	77.44	77.36	77.29
Crude Protein (%)	14.91	14.72	14.51	14.39	14.26
Crude Fibre (%)	3.93	4.26	4.51	4.78	5.03
Ash (%)	45.21	46.51	46.70	46.89	47.11
Ether Extract (%)	3.30	3.24	3.17	3.11	3.05
Nitrogen-Free Extract (%)	10.23	8.78	8.55	8.19	7.84

Eggs were weighed using a digital weighing scale in grams, while egg length and diameter were measured in centimetres using a vernier mini caliper. The egg shape index was calculated as the egg diameter divided by the length.

Blood Biochemistry: The assessment of haematological and serum biochemistry of the Japanese quails were done by randomly selecting one bird per replicate at four, eight and twelve weeks. Blood samples were obtained from each bird and stored in separate glass test tubes to prevent contamination. Separate micro-capillary tubes were used each time blood was collected from the Japanese quails as a precautionary measure against transmission of infectious agents among the birds.

Bleeding was done from the jugular veins with a 5 ml scalp vein needle, and 2 ml of blood was collected from each bird into two sets of sterilized bottles, one set containing Ethylene Diamine Tetra Acetic Acid (EDTA) as the anti–coagulant, while the other set, plain bottles were used to collect blood samples for the determination of serum biochemical indices. Pooled blood samples per replicate were used to determine haematological and serum biochemical indices. The following parameters were then determined: packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), cholesterol, albumin, globulin, high density lipoprotein, triglycerides and total protein concentrations following the methods of Schalm *et al.* (1975).

Data Analysis: All data collected were subjected to analysis of variance (ANOVA) using

SigmaPlot version 11 (Sigmaplot, 2008). Differences between the treatment means were separated using Holm-Sidak multiple comparison range F-test at 5 % level of significance.

RESULTS AND DISCUSSION

Proximate Composition of the Experimental Diets: There were slight decreases in the dry matter content of the formulated feed as TDLM increased across the dietary levels implying minor increase in moisture content (Table 3). Moisture content is an index of the water activity of the feed and is used to determine the stability as well as susceptibility of the feed to microbial attack (Aruah *et al.,* 2012). The moisture content was relatively stable which indicated that the feed stuff were stored with minimum possible susceptibility to microbial spoilage and deterioration. Similarly, the crude protein content (14.26 – 14.91 %) did not vary, suggesting that the mixing ratio did not reduce the nutrient quality of the feed. This is an indication that TDLM can be used to supplement conventional feed stuffs such as groundnut cake, soybean meal, dried brewers grain, maize offal and palm kernel cake. Other plant leaves that have been identified as potential substitutes for conventional feed ingredients include cassava (*Manihot esculenta*) (Ravindran, 1993), fluted pumpkin (*Telfaria occidentalis*) (Nworgu *et al*., 2007), banana (*Musa* spp.) (Marie-Magdeleine *et al.,* 2010) and wild tamarind (*Leucaena leucocephala*) (Adedeji *et al.,* 2013).

Ash represents the mineral content after oxidative combustion of food material (Enwereuzoh *et al.,* 2015). The ash content in the experimental diets were higher than the control diet and increased with increasing amounts of the TDLM (Table 3). These ash contents indicated that the TDLM had slightly higher mineral contents, and this could have implications on the nutritional value of the feeds (Lienel, 2002). Similarly, crude fibre content increased with increasing amounts of *T. diversifolia* leaves in the feed mix. Crude fibre is important because of its physiological effects on the gastro intestinal tract, where it assists animals in digestion and overcoming constipation (Effiong *et al.,* 2009).

Growth Performances, Egg Production and Quality of *Coturnix japonica* Fed Experimental Diets: The mean daily weight gain of quails was significantly influenced by the *T. diversifolia* experimental diets, with strong indications that the feed supplementation enhanced growth (Table 4). The highest mean daily weight gain was recorded in birds fed Diet C (2.27 ± 0.07 g), while the least was recorded in birds fed the control diet (2.16 ± 0.03 g). The age at which the quails began to lay eggs reduced with increase in the leaf meal in the diets. The feed intake also increased with age and body weight (Table 4). The result of this study was in agreement with previous researches that showed positive influence of the feed on the growth and development of the birds (Marks, 1993; Dauda *et al.,* 2014). However, the high FCR observed for quails fed Diets A – D could be attributed to wastage of feed during the early stages of the experiment when the chicks were adapting to the feeding environment (Dauda *et al.,* 2014). The mortality (20 %) of quails on control diet may have been due to pathogenic infections as some disease symptoms were observed before the death of the birds. The inclusion of *T. diversifolia* leaves in Diets B – E may have assisted in building immunity against microbial and pathogenic disease attack. Fasuyi *et al.* (2013) opined that TDLM could be a suitable diet for combating some fatty acid metabolic diseases at as low as 10 % inclusion in the diets of pigs. The 5% mortality observed in birds fed Diet D may be due to microbial attacks which resulted from injuries and led to eventual death of the infected birds.

The age at first egg-lay decreased as the concentration of TDLM increased in the diets, with birds fed Diet E producing eggs on the 60^{th} day, while the control diet resulted in egg production on the 71^{st} day. This suggests that *T. diversifolia* improved egg production. Similarly, the average daily lay per bird was highest for Diet D (0.91 ± 010 eggs).

Table 4: Growth performance of *Coturnix japonica* fed experimental diets formulated with varying levels of *Tithonia diversiflora* leaf meal

Parameters	A	B	C	D	E
Initial weight(g)	39.18 ± 0.76[a]	38.55± 0.88[ac]	36.92± 0.73[bc]	38.13±0. 59[ac]	37.82± 0.70[ac]
Final Body Weight(g)	144.93± 1.05[ad]	146.50±1.05[ac]	148.37±0.76[bc]	144.94±0.76[ad]	147.88±1.36[bc]
Average daily gain(g)	2.16 ± 0.03[a]	2.20 ± 0.03[ac]	2.27 ± 0.07[b]	2.18 ± 0.08[ad]	2.25 ± 0.09[bcd]
Average daily feed intake (g)	17.41±0.15[a]	17.27 ± 0.16[a]	17.19 ± 0.15[a]	17.16 ± 0.16[a]	16.54 ± 0.16[b]
Feed Conversion Ratio	13.31 ± 4.33[a]	11.58 ± 2.89[a]	11.02± 2. 52[a]	11.63 ± 2.78[a]	10.65 ± 2.35[a]
Mortality (%)	20	0	0	5	0
Age at first egg lay (Days)	71	68	68	66	60
Average daily lay per bird	0.73 ± 0.24	0. 67 ± 0.15	0.74 ± 0.17	0.91 ± 010	0.88 ± 0.11

Means with different superscript on the same row differed significantly (p<0.05)

Table 5: Egg quality parameters of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal

Parameters	A	B	C	D	E
Weight (g)	9.60 ± 0.14[a]	10.33 ± 0.14[b]	10.90 ± 0.12[c]	10.70 ± 0.09[c]	11.39 ± 0.12[d]
Egg length (mm)	30.44± 0.23[a]	30.99 ± 0.19[b]	32.47 ± 0.22[c]	31.83 ± 0.13[d]	33.11 ±0.22[e]
Egg Diameter (mm)	24.19± 0.13[a]	24.86 ± 0.12[b]	25.08 ± 0.10[bc]	25.02 ± 0.09[b]	25.34 ±0.08[c]
Egg Shape Index	0.08 ± 0.01[a]	0.08 ± 0.00[a]	0.77 ± 0.00[b]	0.08 ± 0.00[a]	0.08 ± 0.00[a]
Yolk Length (mm)	24.52± 0.32[a]	25.83±0.45[bc]	26.05± 0.47[bc]	25.58 ± 0.35[b]	26.63 ±0.26[c]
Yolk Breath (mm)	22.93 ±0.28[a]	23.72±0.37[acd]	23.99±0.40[bcd]	23.46±0.30[ac]	24.80±0.32[d]
Yolk Shape Index	0.94 ± 0.01[a]	0.92 ± 0. 00[ab]	0.92 ± 0.01[ab]	0.92 ± 0.01[b]	0.93 ± 0.01
Egg Shell Thickness (mm)	0.23 ± 0.01[a]	0.23 ± 0. 02[a]	0.23 ± 0.01[a]	0.26 ± 0.01[a]	0.29 ± 0.01[b]

Means with different superscript on the same row differ significantly (p<0.05)

The inclusion of TDLM in the diets of quail increased both the number of eggs produced and the egg traits. The values obtained for the egg weight, length and diameter, yolk length and diameter, as well as the egg shell thickness were higher for quails fed Diets B – E than birds fed the Control Diet (Table 5). It was observed that egg yolks of quails on diets supplemented with TDLM were more yellowish than those of the control diet. Odunsi *et al.* (1996) had demonstrated that the leaves of *T. diversifolia*, when used as supplements in the diets of egg laying hens improved the egg yolk colour. This corroborated with the findings in this study where egg quality parameters increased with TDLM addition to the birds' diets (Table 5).

Haematological Parameters and Serum Biochemistry of *Coturnix japonica* Fed Experimental Diets Supplemented with Varying Amounts of *Tithonia diversifolia* Leaf Meal: The PCV increased with increased inclusion of TDLM in diets fed to quails.

However, there was a drop in the PCV for quails fed Diets A, D and E as the experiment progressed from 8 to 12 weeks, while those fed Diets B and C continued to increase (Figure 2).

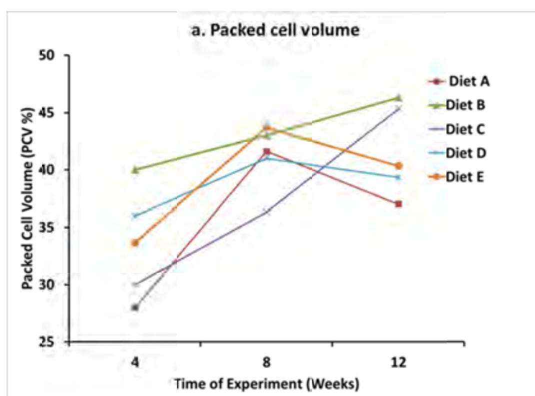

Figure 2: Packed cell volume of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

Fasuyi *et al.* (2013) also reported that the supplementation of the diets of pigs with 10, 20 and 30 % TDLM inclusion levels resulted in increased PCV. After 12 weeks, the highest PCV was observed for Diet B (46.33 ± 3.53 %), while the least was for those fed Diet A (37.00 ± 1.00 %) (Table 2). The observed increase in PCV values indicated better transportation of oxygen and absorbed nutrients by the birds. Indigenous chicken have also shown an increase in PCV as they grew from 90 to 150 days (Addass *et al.,* 2012).

The haemoglobin increased with an increase in the amount of TDLM (Figure 3) with Diet B having the highest, while Diet C (15.57 ± 0.03 g/dl) consistently increased up to the 12th week, and Diet A (12.27 ± 0.28 g/dl) was least.

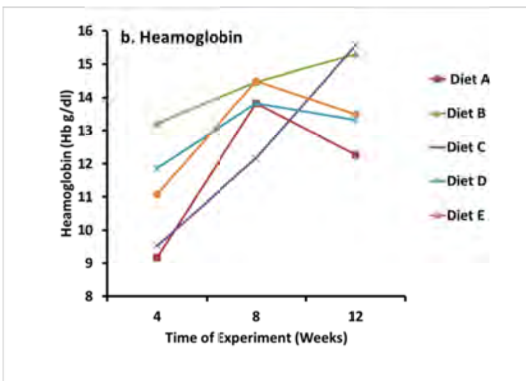

Figure 3: Haemoglobin concentration of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

However, there was a slight drop in the haemoglobin content of other diets by the 12th week (Figure 3). Higher values of haemoglobin recorded at weeks 8 and 12 indicated that the oxygen carrying capacity was high in adult quails (Egbe-Nwiyi *et al.,* 2000). There was a significant increase in the RBC counts of quails across all diets from the 4th to 8th week, which then significantly decreased in the 12th week. All diets followed this trend for the RBC counts. However, there was no significant difference in the RBC among the diets within each time period (Figure 4). Egbe-Nwiyi *et al.* (2000) and Daramola *et al.* (2005) opined that age has a significant influence on the haemoglobin, red blood cell and mean corpuscular hemoglobin concentrations of livestock such as sheep and goats.

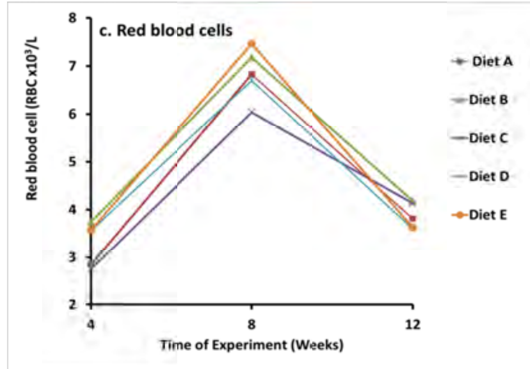

Figure 4: Red blood cell counts of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

The WBC continued to increase for Diet A over the 12 week period, while those fed Diet B decreased over time (Figure 5).

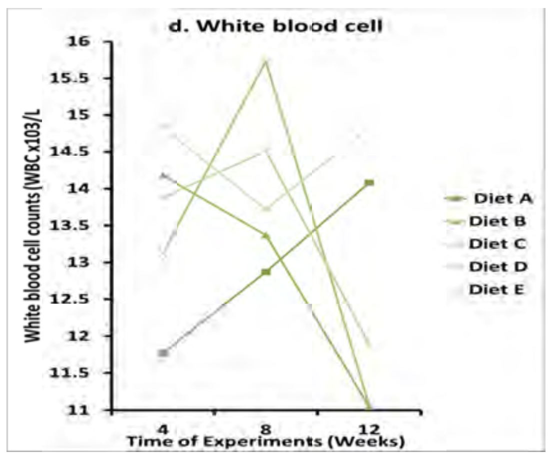

Figure 5: White blood cells count of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

The WBC of Diet C and D increased in the 8th week before a sharp decrease by the 12th week, while that of Diet E decreased in the 8th week but increased by the 12th week. The WBCs help animals to fight infections, defend their body system against invasion by microorganisms and produce as well as distribute antibodies in immune responses (Etim *et al.,* 2014), however high levels of WBC indicates infection. The decrease in the level of WBC in this work further emphasizes the healing properties of TDLM.

The WBC of quails differed significantly with the highest observed for those fed Diet C after 8 weeks. Nevertheless the highest WBC was observed for Diet E after 12 weeks (Table 6). Feeding trials involving plant based supplementation have shown fluctuations in WBC of the target livestock. For instance, Olayeni *et al.* (2006) attributed WBC fluctuations to the immune boosting minerals in wild sunflower leaf meal, which enhanced normal functioning of the blood cells in tissues and organs of weaner pigs. Also, Nworgu *et al.* (2007) reported an increase in WBCs and a boost in the immunity of broiler chicks served *Telfaria occidentalis* leaves extract supplement. Therefore, the noticeable elevation of WBC in quails fed Diet C at week 8, maybe an indication of a recent infection usually with bacteria (Ahamefule *et al.,* 2008). The MCV, MCH and MCHC followed the same pattern with a decrease in the 8th week before an increase in the 12th week. Only the levels of MCV and MCHC of quails fed Diet A differed from other dietary treatments (Figures 6, 7 and 8).

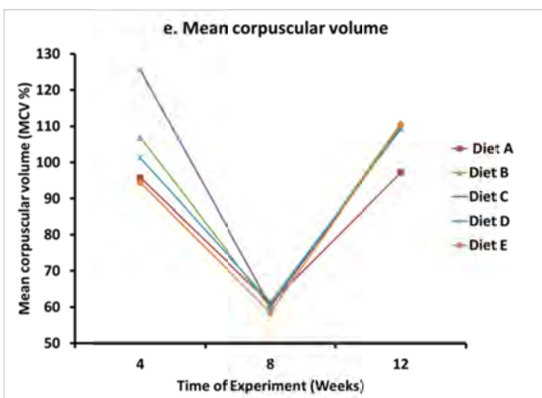

Figure 6: Mean corpuscular volume of ***Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks**

The MCV is important in the diagnosis of anemic conditions of animals. It was observed that Diets B and C had the highest values for most of the parameters by the 12th week. The significantly increasing effect of *T. diversifolia* on RBC, HB, PCV and MCV by the 12th week (Table 6) may be as a result of inherent nutritional quality of the feed (Ramirez-Rivera *et al.* 2010; Ezeonwumelu *et al.* 2012; Funmilayo and Ayodele 2016).

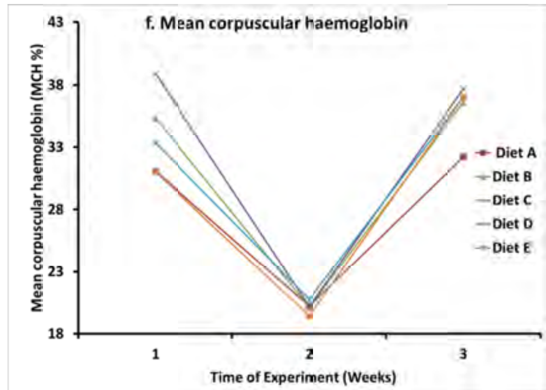

Figure 7: Mean corpuscular haemoglobin of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

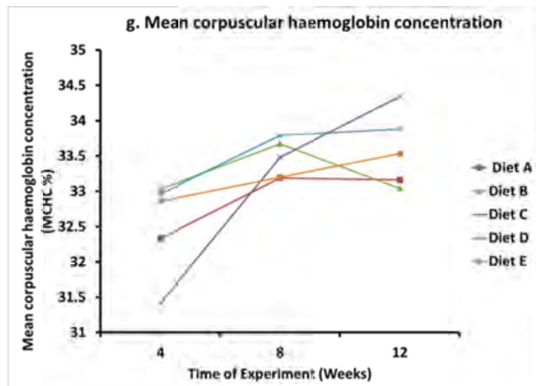

Figure 8: Mean corpuscular haemoglobin concentration of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

The improved value of PCV, RBC and HB indicates haematinic and blood enhancer attributes of the leaf meal on quails fed Diets B to E. This increase in the RBC and PCV may also be as a result of the level of the ash content in the diets containing *T. diversifolia* leaves. The platelets increased in the 8th week and then decreased by the 12th week except for Diet E which had very low platelet variation over time. Diet A had the highest platelets value after 12 weeks (Figure 9). The decrease in platelets suggests that the quails had less risk of hyposplenism and thrombocytosis which are caused by high levels of platelets (Adesehinwa *et al.,* 2011). This also has implications on the process of blood clot formation in the case of an injury, thus reducing blood loss (Nworgu *et al.,* 2007).

Table 6: Haematological parameters of *Coturnix japonica* after 12 weeks of feeding with diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal

Parameters	A	B	C	D	E
PCV (%)	37.00 ± 1.00[a]	46.33 ± 3.53[b]	45.33 ± 0.33[c]	39.33 ± 3.38[d]	40.33 ± 5.17[e]
HB (g/dl)	12.27 ± 0.28[a]	15.30 ± 1.11[a]	15. 57 ± 0.03[a]	13.33 ± 1.20[a]	13.50 ± 1.66[a]
RBC x 10^3/L	3.81 ± 0.04[ab]	4.18 ± 0.06[a]	4.13 ± 0.02[a]	3.60 ± 0.11[b]	3.63 ± 0.14[b]
WBC x 10^3/L	14.08 ± 1.29[a]	11.05 ± 0.65[b]	11.07 ± 0.35[b]	11.90 ± 1.23[c]	14.83 ± 0.45[a]
MCV (%)	97.17 ± 1.82[a]	110.78 ± 7.15[a]	109.68 ± 0.39[a]	109.19± 8.05[a]	110.40 ±10.30[a]
MCH (%)	32.22 ± 0.47[a]	36.58 ± 2.24[a]	37.66 ± 0.13[a]	37.01 ± 2.86[a]	36.97 ± 3.23[a]
MCHC (%)	33.16 ± 0.15[a]	33.04 ± 0.11[a]	34.34 ± 0.22[b]	33.88 ± 0.13[ab]	33.53 ± 0.26[ab]
Platelets ×10^3/µl	244.33 ±34.72[a]	140.67 ±23.13[a]	168.00 ±11.02[a]	185.67 ±4.48[a]	184.33 ±24.31[a]

Means with different superscript on the same row differ significantly (p<0.05)

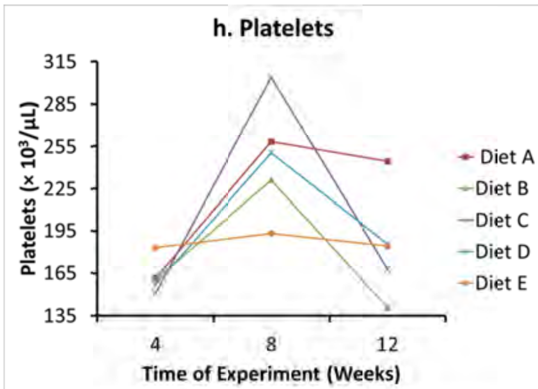

Figure 9: Platelets counts of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

There were no significant differences in the total protein of the blood of the quails fed the control (Diet A) and TDLM supplemented diets. Blood total protein increased by the 8th week in all diets and then decreased by the 12th week, with Diet C recording the lowest level of total protein throughout the study (Figure 10).

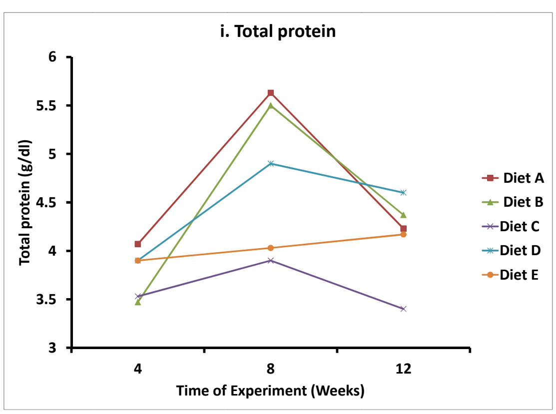

Figure 10: Total protein concentrations of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

This implies that *T. diversifolia* can supply the protein and amino acid requirements in feed formulation for quails. The birds were able to efficiently mobilize and utilize the protein with little or no negative effects (Nworgu *et al.,* 2007; Alagbaoso *et al.,* 2015). The triglycerides did not differ significantly (p>0.05) among the birds fed the different diets. The triglyceride of birds fed Diet C decreased and did not follow the observed pattern for other diets (Figure 11).

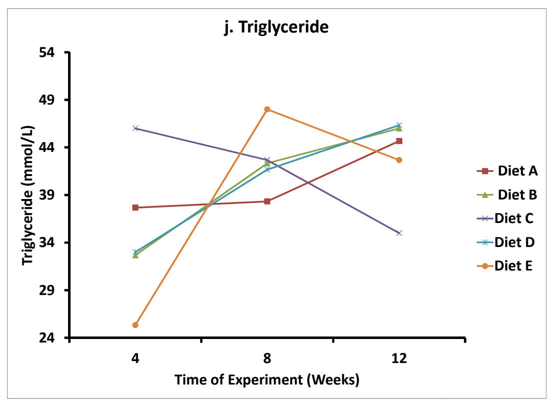

Figure 11: Triglyceride concentrations of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

After the 12th week, the triglyceride ranged from 35.00 ± 3.21 mmol/L (Diet C) to 46.33 ± 5.81 mmol/L (Diet D). These low serum triglycerides indicated that TLDM did not cause excessive fat accumulation in the blood (Putra *et al.,* 2015; Da Silva *et al*., 2018). The serum albumin followed the same trend as the blood total protein. The serum albumin of the quails had similar patterns of fluctuation except for Diet D and E, while only the globulin of Diet E differed from others (Figure 12 and 13).

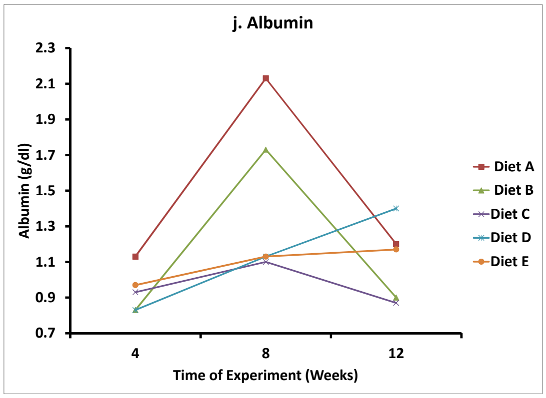

Figure 12: Albumin concentrations of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

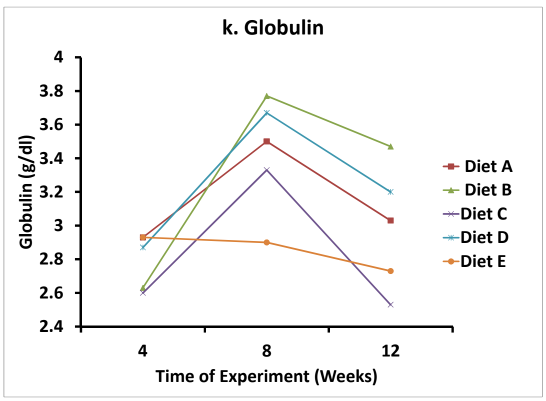

Figure 13: Globulin concentrations of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

The similarities among the TDLM dietary and control treatments signify normalcy because abnormal serum albumin indicates an alteration of normal systematic protein utilization and low dietary protein intake (Olayeni *et al.,* 2006).

The cholesterol levels of Diet C continued to increase when compared with other diets which increased from 4 to 8 weeks and then decreased by the 12th week (Figure 14). However, the control diet decreased and then slightly increased by the 12th week. The increase in the levels of blood cholesterol due to the introduction of TDLM could be attributed to high saponin content which has been shown to bind to serum lipids, especially cholesterol, thereby easing their removal from circulation in the animal's system (Olayeni *et al.,* 2006).

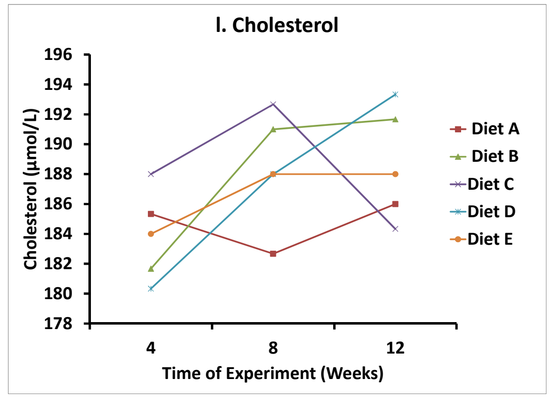

Figure 14: Cholesterol concentrations of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

The HDL in the blood of the quails fed Diet A did not differ significantly (p>0.05) from those fed TDLM supplemented diets. Serum HDL increased by the 8th week and then decreased by the 12th week, with birds fed Diet D recording the lowest HDL values (Figure 15).

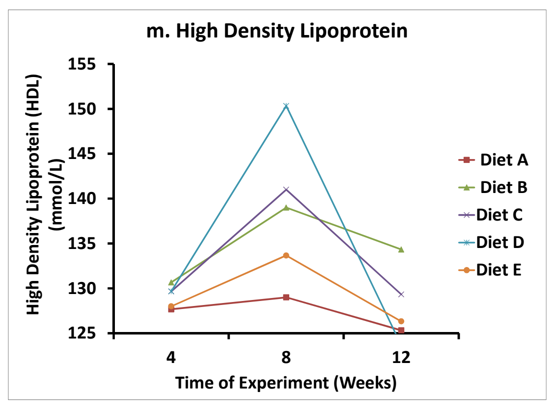

Figure 15: High density lipoprotein concentrations of *Coturnix japonica* fed diets supplemented with varying amounts of *Tithonia diversifolia* leaf meal for 12 weeks

The slight increase in HDL levels for birds fed TDLM supplemented diets suggests possible protection against diseases such as atherosclerosis (Adebayo *et al.*, 2009; Khalifa and Noseer, 2009). Moreover, the increased cholesterol and reduced triglyceride with the increasing inclusion levels of TDLM suggest that it has anti-lipidemic properties and it could help manage some metabolic conditions involving fatty acids and glucose imbalances in the birds (Etim *et al.*, 2014; Putra *et al.,* 2015; Da Silva *et al.*, 2018).

Conclusion: This study revealed the potential inclusion of the leaves of *T. diversifolia* as a nutritional supplement in the diets of Japanese quails. The inclusion of the leaf meal at 2.5 % (Diet B) and 5.0 % (Diet C) had the highest influence on growth performance, haematological parameters and serum biochemistry of the quails. The feed intake, digestibility and immune boosting qualities were also positive. The use of *T. diversifolia* as a feed ingredient in the diets of Japanese quail portends wealth creation and improved production. It is therefore recommended that TDLM be incorporated into animal feeds as supplements or replacement for protein source without any detrimental effect.

ACKNOWLEDGEMENTS

We would like to thank staff of the Wildlife Domestication Unit, Department of Wildlife and Ecotourism Management, University of Ibadan, Nigeria, and Dr. Olugbenga Ogunwole (Department of Animal Science, University of Ibadan) for their assistance during the study. We are also grateful to the reviewers, editor and editorial team, for their indelible contributions, which greatly improved the manuscript.

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