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Supplementation with aqueous extract of Talinum triangulare and effect on the hematology and serum biochemistry in growing pullets

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Abstract: The effects of administration of Talinum triangulare aqueous extract on the hematology and serum biochemistry of growing pullets was studied. Ninety ISA Brown chicks purchased at 1 day old were randomly assigned into three groups: PA, PB, and PC. Pullets in groups PA and PB received 1000 mg of extract from dried T. triangulare and from freshly harvested T. triangulare per liter of water, respectively. Pullets in group PC served as the unsupplemented control. Hematology and serum biochemistry were evaluated at 2-month intervals within the 6-month period of the experiment following standard procedures. Feed and water were administered ad libitum. Supplementation with T. triangulare extract led to significant (P < 0.05) increase in serum high-density lipoprotein concentration and significant (P < 0.05) decrease in serum low-density lipoprotein concentration. Aspartate aminotransferase activity was significantly (P < 0.05) lower in the supplemented groups. Serum albumin was significantly (P < 0.05) higher at month four of pullet life. It was concluded that dietary supplementation with aqueous extract of Talinum triangulare had no deleterious effect on hematology and serum proteins and enzymes, and it had a positive effect on lipid profile. It is therefore recommended for use as a nutraceutical for healthy rearing of growing pullets.

Key words: Talinum triangulare, pullets, nutraceutical, supplementation, lipid profile

1. Introduction

The overreliance on grain-based animal feed in industrial food animal production has negative implications for animal health, the environment, and human health [1,2]. Moreover, alternatives to fish oil and fish meal (from fishes obtained from the wild) in the likes of terrestrial agricultural crops are increasingly used to meet most of the new demand for compounded or complete feed [3]. As in farmed fish, use of "complete feed" and intensive rearing methods and facilities make animals reach harvest weight more quickly than animals raised on forage [4]. As is the case with humans, the synergetic effect of enhanced feed intake and reduced physical activity may possibly promote obesity [5] and its attendant consequences for the health of animals and the quality of animal products such as eggs, meat, and milk.

Plant-based food sources generally contain lower levels of cholesterol than animal-based sources [6]. Some plants contain phytosterols, which in some cases displace cholesterol during absorption in the intestine, thereby promoting cholesterol excretion from the body [6]. Herbal preparations, otherwise known as phytobiotics, are used traditionally for the improvement of poultry health [7].

The choice of Talinum triangulare for this study was based on the reported positive impact of the vegetable on the lipid profile and cholesterol status of humans and animals [8,9] and its merits in terms of availability all through the year, acceptability among animals and humans, and its nutritional potency [8-10].

The general aim of this study was to evaluate the use of varied forms or presentations of T. triangulare as a nutraceutical to improve health and survivability of pullets. The specific objectives of the study were to compare the performance of pullets treated with a 1000 mg/L dose of the aqueous extract of T. triangulare from dried/pulverized forms and the freshly harvested form of T. triangulare to evaluate the effects of supplementation on the hematology and serum biochemistry of treated pullets.

2. Materials and methods

2.1. Experimental animals and design

A total of 90 ISA Brown 1-day-old chicks were used for the research work. The pullet chicks were procured from

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Kosy Veterinary Consult, Enugu, Nigeria. The chicks were randomly assigned into three experimental pullet groups (PA, PB, and PC) with three replicates per group. Thus, 10 chicks were included in each replicate for 30 chicks per experimental group (Table 1).

Experimental birds were treated uniformly in terms of feeding, vaccinations, and other management practices in accordance with standard production procedures except for the test extracts, which were administered according to the specific groups in drinking water. The pullets were fed chick mash with energy of 27500 ME kcal/kg and protein of 20% from 1 day old to 8 weeks of age, grower mash with energy of 2700 ME kcal/kg and protein of 15.50% from 8 to 18 weeks of age, and prelayer mash with energy of 2725 ME kcal/kg and protein of 16.5% from 18 to 24 weeks (i.e. 6 months) of age. Drinking water and feed were provided ad libitum. A dose of 1000 mg/L of T. triangulare extract in drinking water was used, based on our earlier findings on the efficacy of this dose [9]. The study lasted for 6 months and the parameters investigated included hematological indices, serum lipid profile, liver enzymes, and serum proteins.

2.2. Experimental plant, collection, identification, and preparation

Talinum triangulare was harvested weekly from a vegetable farm attached to the Animal Health and Production Departmental Students' Research Poultry House, where waterleaf (*T. triangulare*) was planted for the purpose of this research. Identification of *T. triangulare* was done by a taxonomist with the herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

The dried *T. triangulare* sample was obtained from aerial parts (leaves and succulent stems) of matured plants harvested from the farm, with were washed and destalked. It was further shade-dried at room temperature on a laboratory table with frequent turning, a process that lasted for about 6 weeks during the rainy season and 4 weeks in the dry season in Nsukka, in the southeast

of Nigeria. The plant was air-dried in the shade to avoid photolysis and thermal degradation [9]. The dried plants were pulverized using an electric grinding machine (SFP-2210, Sonik, by Sonik Japan Electrical Appliances Co. Ltd.) to obtain a powdered form of the sample, which was stored in the refrigerator at 4 °C until used.

The extract was obtained by subjecting 500 g of the pulverized leaves to cold maceration in 1400 mL of distilled water. The mixture was kept for a period of 48 h. Thereafter, it was stirred for 2 h and then sieved with a domestic sieve cloth. The filtrate was collected and stored in the refrigerator for use.

The extract from freshly harvested leaves of *T. triangulare* was obtained as follows. The leaves and succulent shoots of *T. triangulare* were washed, drained, and cut into small pieces. These were blended with 1 kg of waterleaf to 1 L of distilled water. The mixture was left for 48 h and stirred for 2 h for complete mixing and extraction. Thereafter, the mixture was filtered with a sieve cloth. The filtrate, dark green in color and of a pasty consistency, was then collected and kept in a refrigerator for the experiment.

Fresh plant extracts from both freshly harvested and dried leaves of *T. triangulare* were produced weekly and respectively used to compare their efficacies as health and growth modulators in pullet production. The aqueous extract of shade-dried pulverized leaves and succulent stems of *T. triangulare* was used for treatment group A (labeled PA), while extract from freshly harvested leaves and succulent stems of the same plant was used for treatment group B (labeled PB). Group C (labeled PC) represented the control group that was not given any extract.

For the determination of percentage yield of extracts from both the dried and freshly harvested *T. triangulare* samples, three glass plates were washed, dried, and weighed with an analytical weighing balance (Metler H_3O , Switzerland). With a syringe (5 mL), 1 mL of the extract

Table 1. Experimental design for the dietary supplementation with 1000 mg/L of aqueous extract of *T. triangulare* in drinking water of growing pullets.

	Treatment groups				
Replicates	PA (dried <i>T. triangulare</i>)	PB (fresh <i>T. triangulare</i>)	PC (no T. triangulare)	Total number of pullets/ replicate	
R ₁	10	10	10	30	
R ₂	10	10	10	30	
R ₃	10	10	10	30	
Total number of pullets/group	30	30	30	90	

Each treatment group's replicates were treated uniformly.

was aspirated and deposited on each of the glass plates; the plates were placed in a vacuum rotary evaporator (Büchi, Germany) and allowed to dry to a constant weight. The weight of the empty glass plate was subtracted from the weight of the glass plate with dried matter from the extract and the difference was multiplied by 100 to give the percentage yield of the extract. The obtained percentage yield of extract of *T. triangulare* was used to determine the concentration and dosage of administration following the method of Anaga et al. [11]. This procedure was repeated weekly with each batch of extract.

2.3. Parameters analyzed

The following hematological and serum biochemical parameters were evaluated during the study: packed cell volume (PCV), hemoglobin concentration (HBC), red blood cell (RBC) count, total white blood cell (TWBC) count, serum total cholesterol (TChol), triacylglycerol (TAG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumins, and globulins.

2.4. Laboratory procedures

2.4.1. Blood sample collection

A total of three pullets per replicate were bled every 2 months beginning from day zero. Five milliliters of blood was collected from the right jugular vein of each of the birds using a hypodermic needle and syringe. Two milliliters of the blood was gently dispensed into a labeled 5-mL plastic sample bottle (Micropoint Diagnostics, UK) containing 2 mg of Na-EDTA for hematology determinations. The sample bottle was gently rocked to mix the blood with Na-EDTA to prevent coagulation. The hematological determinations were done immediately upon sample collection to avoid artifactual changes [12]. The remaining 3 mL of blood was dispensed into a prelabeled plain test tube containing no anticoagulant (Surgifriend Medical, UK). The tube was kept in a slanted position for 30 min and then centrifuged for 10 min at $3000 \times g$. The serum supernatant was aspirated into a clean labeled 2-mL sample bottle for serum biochemistry determinations.

2.4.2. Hematological determinations

The packed cell volume (PCV) was evaluated by the microhematocrit method [13] using a Haematospin 1400 microhematocrit centrifuge and a Hawksley Microhaematocrit Reader (Hawksley & Sons Ltd., West Sussex, UK). The hemoglobin concentration was determined by the cyanmethemoglobin method [14] using a CHEM5V3 semiautomated blood analyzer (Erba Diagnostics, Mannheim, Germany). The RBC and TWBC counts were done manually by the hemocytometer method using Natt and Herrick's diluting fluid [15], an improved Neubauer counting chamber (Hawksley & Sons Ltd.), and a light microscope (Leica Gallen, New York, NY, USA).

2.4.3. Evaluation of the serum lipid profile

The serum lipid profile was evaluated following standard procedures with test kits sourced from Quimica Clinica Aplicada (Spain) and a CHEM5V3 semiautomated blood analyzer (Erba Diagnostics). The enzymatic colorimetric method [16] was used to determine the serum total cholesterol while the glycerol-phosphate oxidase method [17] was used to evaluate the serum TAG levels. The dextran sulfate magnesium(II) precipitation method [18] was used in quantifying the serum HDL-C levels, while the serum LDL-C levels were calculated using Friedewald's formula [19].

2.4.4. Evaluation of the serum enzyme activity and serum protein levels

Test kits sourced from Quimica Clinica Aplicada were used for the evaluation of the serum enzyme activity and serum protein levels and the assay was done using the CHEM5V3 semiautomated blood analyzer (Erba Diagnostics). The determination of serum ALT and AST activity was based on the Reitman–Frankel colorimetric method [20]. Total serum protein determination was performed by the biuret method [21], while serum albumin levels were determined based on the bromocresol green method [22]. The serum globulin levels were obtained by subtracting the albumin levels from the total protein levels.

2.5. Data analysis

Data generated were subjected to one-way analysis of variance (ANOVA) using SPSS 15.0 for Windows. Variant means were further separated using the least significant difference method. Significance was accepted at P < 0.05.

3. Results

There were no significant variations (P > 0.05) in the PCV, HBC, RBC counts, and TWBC counts of the three groups of pullets, though at all times of assay the values recorded for pullets in group PA were higher than those of pullets in groups PB and PC (Table 2). No significant variations (P > 0.05) were recorded for serum levels of TChol among the groups all throughout the study. The serum HDL-C levels of pullets in groups PA and PB were significantly higher (P < 0.05) than those of pullets in group PC at months 4 and 6 of the study (Table 3). The serum LDL-C levels of pullets in groups PA and PB, however, were significantly lower (P < 0.05) than those of pullets in group PC at months 2, 4, and 6 of the experiment (Table 3). The serum TAG and VLDL-C levels in the three groups did not significantly vary (P > 0.05) throughout the study, though both parameters increased with time in all the groups (Table 4).

Results of the serum enzyme assays showed that there were no significant variations (P > 0.05) in the serum ALT activity among the three groups of pullets, but the serum AST activity of pullets in groups PA and PB was significantly lower (P < 0.05) than that of pullets in group PC at months 4 and 6 of assay (Table 5).

Table 2. Mean packed cell volume (%), haemoglobin concentration (g/dL), red blood cell
counts (10 ⁶ / μ L), and total white blood cell count (×10 ³ / μ L) ±SEM of pullets given aqueous
extract of Talinum triangulare (TT).

C	Age of pullets at sample collection						
Group	Month 2	Month 4	Month 6				
PCV (%) ± standard error of mean							
PA (dried TT)	26.83 ± 0.44	28.83 ± 1.09	29.43 ± 1.11				
PB (fresh harvested TT)	26.50 ± 0.29	27.50 ± 0.76	28.27 ± 0.87				
PC (untreated control)	25.63 ± 0.98	27.83 ± 1.30	28.02 ± 0.99				
No significant variations acro	ss the groups, P > 0	.05					
Hemoglobin concentration (g	/dL) ± standard err	or of mean					
PA (dried TT)	10.29 ± 0.21	10.21 ± 0.88	10.18 ± 0.72				
PB (freshly harvested TT)	8.65 ± 0.50	8.97 ± 0.22	9.56 ± 0.48				
PC (untreated control)	9.48 ± 0.63	8.79 ± 0.68	9.43 ± 0.72				
No significant variations acro	ss the groups, P > 0	.05					
RBC counts (10 ⁶ /µL) ± standa	rd error of mean						
PA (dried TT)	21.33 ± 1.96	18.78 ± 1.63	19.02 ± 1.83				
PB (freshly harvested TT)	19.50 ± 2.72	18.85 ± 0.92	18.78 ± 1.43				
PC (untreated control)	19.60 ± 0.79	19.07 ± 1.50	18.97 ± 1.48				
No significant variations across the groups, P > 0.05							
Total white blood cell count \pm SEM (×10 ³ /µL) of mean							
PA (dried TT)	21.33 ± 1.96	18.78 ± 1.63	19.02 ± 1.83				
PB (freshly harvested TT)	19.50 ± 2.72	18.85 ± 0.92	18.78 ± 1.43				
PC (untreated control)	19.60 ± 0.79	19.07 ± 1.50	18.97 ± 1.48				
No significant variations across the groups, P > 0.05							

Serum total protein and globulin levels did not significantly vary (P > 0.05) across the groups throughout the study, but the serum albumin levels of groups PA and PB were significantly higher (P > 0.05) than those of pullets in group PC at month 4 of the experiment (Table 6).

4. Discussion

The hematological parameters of pullets in this study showed no adverse health effects of treatment with *T. triangulare* as revealed by the health indices of the experimental birds. The lack of significant variation in the PCV, HBC, and RBC counts of pullets given *T. triangulare* extracts in the present study is not in agreement with the findings of Ezekwe et al. [23], who reported that methanolic extracts of *T. triangulare* significantly enhanced PCV and RBC counts of rats. The findings in the present study, however, are in agreement with reports by Aronu et al. [9] in layers given *T. triangulare* supplements.

The reduction in LDL-C induced by treatment with *T. triangulare* is important considering that this is the component of the lipid profile considered to increase

the risk of cardiovascular diseases and atherosclerosis, for which elevated levels of LDL-C are the chief cause [24]. Also incriminated in the risk of cardiovascular diseases and atherosclerosis are elevated TAG [25] and VLDL levels [26]. The TChol and TAG values were not significantly different with *T. triangulare* treatment, but the final outcome reveals higher values for the untreated group than for the treated. Most important here is the fact that the HDL-C increased significantly with treatment. HDL is said to inhibit oxidation, inflammation, activation of the endothelium, coagulation, and platelet aggregation [27]. Risk of atherosclerosis is therefore said to be inversely correlated with HDL-C levels; for these reasons and more, HDL is called the "good" cholesterol [28].

Conversely, increased LDL-C in the blood is associated with increased risk of cardiovascular disease [29]. New approaches to lowering LDL-C levels and increasing HDL-C levels are attractive avenues for the development of novel classes of antiatherogenic drugs [29]. The ability of *T. triangulare* extract to both reduce LDL-C and increase HDL-C in pullets in this study suggests that it may be a

Table 3. Mean total cholesterol (TChol) (mg/dL), high-density lipoprotein cholesterol (HDL-C) (mg/
dL), and low-density lipoprotein cholesterol (mg/dL) ± SEM of pullets given aqueous extract of <i>Talinum</i>
triangulare.

Group	Age of pullets at sample collection					
	Month 2	Month 4	Month 6			
TChol ± SEM (mg/dL)						
PA (dried TT)	128.94 ± 4.71	127.41 ± 5.33	123.27 ± 9.38			
PB (freshly harvested TT)	115.78 ± 1.78	118.33 ± 29.96	116.37 ± 12.04			
PC (untreated control)	136.47 ± 7.64	111.30 ± 22.62	119.37 ± 8.86			
No significant variations acro	ss the groups, $P > 0.05$					
HDL-C ± SEM (mg/dL)						
PA (dried TT)	78.85 ± 10.97	75.89 ± 6.19^{a}	78.11 ± 8.37^{a}			
PB (freshly harvested TT)	70.11 ± 3.44	81.69 ± 15.42^{a}	78.21 ± 10.11^{a}			
PC (untreated control)	72.53 ± 2.81	44.87 ± 8.56 $^{\rm b}$	51.82 ± 6.23^{b}			
^{a, b} Different superscripts in a column indicate significant differences between the groups, P < 0.05						
LDL-C ± SEM (mg/dL)						
PA (dried TT)	50.10 ± 7.45	51.82 ± 3.98	$45.16\pm5.09^{\rm a}$			
PB (freshly harvested TT)	50.32 ± 6.05	46.61 ± 5.36	$41.03\pm6.36^{\rm a}$			
PC (untreated control)	63.94 ± 5.39	66.42 ± 18.58	67.55 ± 12.82^{b}			
No significant variations across the groups, P > 0.05						

Table 4. Mean triacylglycerol (TAG) (mg/dL) and very low-density lipoprotein (VLDL) (mg/dL) ± SEM of pullets given aqueous extract of *Talinum triangulare*.

Group	Age of pullets at sample collection				
	Month 2	Month 4	Month 6		
TAG ± SEM (mg/dL)					
PA (dried TT)	102.13 ± 11.22	791.39 ± 53.26	802.11 ± 46.11		
PB (freshly harvested TT)	108.04 ± 5.26	824.94 ± 86.64	789.23 ± 46.27		
PC (untreated control)	99.54 ± 3.57	792.87 ± 71.29	810.23 ± 60.11		
No significant variations across the groups, P > 0.05					
VLDL ± SEM (mg/dL)					
PA (dried TT)	20. 43 ± 11.22	158.39 ± 53.26	160.42 ± 46.11		
PB (freshly harvested TT)	21.61 ± 5.26	164.99 ± 86.64	157.85 ± 46.27		
PC (untreated control)	19.91 ± 3.57	158.57 ± 71.29	162.05 ± 60.11		
No significant variations across the groups, P > 0.05					

good low-cost candidate for use as an antiatherogenic drug. Further research may be geared towards identifying, isolating, and establishing the mode of operation of the active agents responsible for the antiatherogenic properties of *T. triangulare*.

Increase of triacylglycerol by over 600% at 4 months of age, corresponding to the age when pullets attain physiological puberty, was reported. The elevation in triacylglycerol levels in the blood of pullets at puberty may be a prerequisite for fat mobilization and subsequent production and concentration of the same in oocytes, which culminates in a very high level of TAG (of over 2000 mg/dL in most cases) in pullet eggs. This observation reveals a critical point of high TAG demand in pullet

Group	Age of pullets at sample collection						
	Month 2	Month 4	Month 6				
ALT ± SEM (IU/L)							
PA (dried TT)	13.40 ± 1.22	9.25 ± 2.20	12.73 ± 1.86				
PB (freshly harvested TT)	13.32 ± 1.36	13.93 ± 1.27	12.82 ± 1.97				
PC (untreated control)	13.40 ± 0.70	12.01 ± 0.26	12.78 ± 1.09				
No significant variations acro	oss the groups, P > 0.0	05					
AST ± SEM (IU/L)							
PA (dried TT)	92.63 ± 4.58	68.87 ± 2.24^{a}	72.36 ± 2.08^{a}				
PB (freshly harvested TT)	94.30 ± 5.71	73.36 ± 1.34^{ab}	80.63 ± 3.02^{ab}				
PC (untreated control)	89.59 ± 2.04	$78.43 \pm 2.07^{\rm b}$	84.21 ± 2.49^{b}				

Table 5. Mean serum alanine aminotransferase (ALT) (IU/L) and serum aspartate aminotransferase (AST) ± SEM (IU/L) of pullets given aqueous extract of *Talinum triangulare*.

Table 6. Mean serum total proteins (IU/L), albumin (g/dL), and globulin (g/dL) \pm SEM of pullets given aqueous extract of *Talinum triangulare*.

Group	Age of pullets at sample collection					
	Month 2	Month 4	Month 6			
Total proteins ± SEM (g/dL)						
PA (dried TT)	4.05 ± 0.12	4.75 ± 0.20	4.92 ± 0.34			
PB (freshly harvested TT)	3.80 ± 0.20	4.91 ± 0.62	4.89 ± 0.28			
PC (untreated control)	4.14 ± 0.17	4.66 ± 0.20	4.82 ± 0.25			
No significant variations across	s the groups, P > 0.05					
Albumin ± SEM (g/dL)						
PA (dried TT)	1.37 ± 0.10	1.84 ± 0.11 a	1.80 ± 0.12			
PB (freshly harvested TT)	1.21 ± 0.05	1.78 ± 0.28 ^a	1.81 ± 0.18			
PC (untreated control)	1.39 ± 0.06	1.44 ± 0.22 ^b	1.76 ± 0.16			
^{a, b} Different superscripts in a column indicate significant differences between the groups, P < 0.05						
Serum globulin ± SEM (g/dL)						
PA (dried TT)	2.68 ± 0.21	2.92 ± 0.10	3.12 ± 0.17			
PB (freshly harvested TT)	2.59 ± 0.17	3.13 ± 0.35	3.08 ± 0.21			
PC (untreated control)	2.75 ± 0.16	3.22 ± 0.26	3.06 ± 0.18			
No significant variations across the groups, P > 0.05						

nutrition at the age of puberty. Thus, an adjustment in the nutrient requirements of pullets at the rearing stage to accommodate this finding may have a significant impact on subsequent egg laying. Sato et al. [30] observed that high plasma levels of lipids in laying hens reflect the significant demand for yolk lipids by growing oocytes. Triacylglycerols play an important role in metabolism as energy sources and in lipid transport [31]. In this study ALT and AST were within normal ranges for pullets throughout the study; they were therefore not adversely affected by treatment with *T. triangulare*. This is an indication that the treatment had no harmful effect on liver cells and did not negatively interfere with normal physiological levels at the dose and forms that the *T. triangulare* extracts were administered. The reduction in AST observed for pullets points to some degree of liver membrane stability conferred on the liver hepatocytes with administration of *T. triangulare* [32,33].

The results for liver enzyme activity obtained in this study agree with the finding of Ezekwe et al. [23] that the methanolic extract of *T. triangulare* had no significant effect on the level of ALT and ALP. The finding in this present study also concurs with the reports of Liang et al. [34], who investigated the antioxidant and hepatoprotective properties of polysaccharides from *Talinum triangulare* and showed that they reduced the levels of AST, ALT, and MDA in CCL₄-injured mice and restored activities of defense antioxidant substances (SOD and GSH).

The results obtained in this study for serum proteins are an indication of the wellbeing of the studied pullets and of the high nutritional status of the *T. triangulare* extract. Protein is involved in enzyme, hormone, and antibody mechanisms, as well as osmotic balance. It also serves as a reserve source of nutrition for the body's tissues and muscles. Similarly, increased albumin levels observed with treatment are an indication of enhanced metabolic balance and confirm the protein-rich diet. The facts presented above support the proposed use of leaf meals and leaf protein concentrates as nonconventional feed ingredients. Leaf meals such as *T. triangulare* extract may serve as good substitutes, in part, to some other expensive conventional

References

- Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS et al. Solutions for a cultivated planet. Nature 2011; 478: 337-342.
- Fry JP, Love DC, MacDonald GK, West PC, Engstromm PM et al. Environmental health impacts of feeding crops to farmed fish. Environment International 2016; 91: 201-214.
- Tacon AG, Hasan MR, Metian M. Demand and Supply of Feed Ingredients for Farmed Fish and Crustaceans. Rome, Italy: FAO; 2011.
- Hassan MR, Hecht T, De Silva SS, Tacon AGJ. Study and Analysis of Feeds and Fertilizers for Sustainable Aquaculture Development. UNFAO Fisheries Technical Paper No. 497. Rome, Italy: FAO; 2007.
- Institute of Medicine (editor). Genes, Behavior and the Social Environment: Moving Beyond the Nature/Nurture Debate. Washington, DC, USA: National Academies Press; 2006.
- Izar MC, Tegani DM, Kasmas SH, Fonseca FA. Phytosterols and phytosterolemia: gene – diet interactions. Genes & Nutrition 2011; 6 (1): 17-26.
- Okitoi LO, Ondwasy HO, Siamba DN, Nkurumah D. Traditional herbal preparations for indigenous poultry health management in Western Kenya. Livestock Research for Rural Development 2007; 19: (5): 19072.

protein sources like fish meal, soybean, and groundnut oil meals as well as other animal sources of protein in feed formulations.

The relatively higher values for hematological parameters in the group given dried T. triangulare highlighted the fact that the dried sample is more potent in achieving a boost in hematological parameters than the fresh sample. This may be due to the fact that dried samples present the components of the plant in a more concentrated form with the evaporation of most of the water contained in the plant. Aja et al. [8] also showed that dried samples of T. triangulare had higher levels of bioactive substances than the fresh sample. They reasoned that this is possibly because the bioactive compounds are not volatile compounds and have higher dried weights. The proposed use of T. triangulare as a blood tonic in children and women was also substantiated in this study as treatment was found to lead to relatively higher values for the erythrocyte parameters.

In conclusion, this study showed the potency of *T. triangulare* to reduce LDL and to enhance HDL cholesterol levels in growing pullets. Positive effects on lipid profiles may target the production of heart-friendly eggs, which is the desire of researchers who seek to meet consumer demands.

- Aja PM, Okaka ANC, Onu PN, Ibiam UA, Uraku AJ. Phytochemical composition of *Talinum triangulare* (waterleaf) leaves. Pakistan Journal of Nutrition 2010; 9 (6): 527-530.
- Aronu CJ, Okoye JOA, Ihedioha JI, Marire BN, Anika SM. The effects of aqueous extracts of dried-pulverized or the fresh form of *Talinum triangulare* on the haematology and serum biochemistry of layers. Veterinarski Arhiv 2018; 88 (5): 687-707.
- Fasuyi AO. Nutritional potentials of some tropical vegetable leaf meals: chemical characterization and functional properties. African Journal of Biotechnology 2006; 65 (1): 49-53.
- Anaga AO, Onuoha A, Igboeli OO, Ezeja M. Antihyperglycemic effects of the methanol leaf extract of *Diaphananthe bidens* in normoglycemic and streptozotocin-induced hyperglycaemic rats. Asian Pacific Journal of Tropical Biomedicine 2012; 5 (3): 192-196.
- Ihedioha JI, Idika IK, Ogamba GN, Akam CJA. Changes in the haematological values of avian blood samples stored at varying temperatures for a period of up to 72 hours. Comparative Clinical Pathology 2008; 17 (2): 73-79. doi: 10.1007/s00580-008-0723-5
- Thrall MA, Weiser MG. Laboratory Procedures for Veterinary Technicians. Fourth Edition. Saint Louis, MO, USA: Mosby-Year Book, Inc.; 2002.

- Higgins T, Bentler E, Doumas BT. Haemoglobin, iron and bilirubin. In: Burtis CA (editor). Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 6th ed. Amsterdam, the Netherlands: Elsevier; 2003. pp. 509-526.
- Campbell JY. Inspecting the mechanism: an analytical approach to the stochastic growth model. Journal of Monetary Economics 1994; 33 (3): 463-506.
- Allian CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total cholesterol. Clinical Chemistry 1974; 20 (4): 470-475.
- Buccolo G, David H. Quantitative determination of serum triglycerides by the use of enzyme. Clinical Chemistry 1973; 19: 476.
- Albers JJ, Warnick GR, Cheung MC. Quantification of highdensity lipoproteins. Lipids 1978; 31: 926-932.
- Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. Clinical Chemistry 1990; 36 (1): 15-19.
- Reitman S, Frankel S. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 1957; 28: 5.
- Lubran MM. The measurement of total serum proteins by the biuret method. Annals of Clinical & Laboratory Science 1978; 8 (2): 106-110.
- 22. Doumas BT, Peters T Jr. Serum and urine albumin: a progress report on their measurement and clinical significance. Clinica Chimica Acta 1997; 258: 3-20.
- Ezekwe CI, Uzomba C, Ugwu Okechukwu PC. The effect of methanol extract of *Talinum triangulare* (water leaf) on the haematology and some liver parameters of experimental rats. Global Journal of Biochemistry and Biotechnology 2013; 8 (2): 5-60.
- Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH et al. Triglyceride and vascular disease: a scientific statement from the American Heart Association. Circulation 2011; 123: 2292-2333.

- Fowkes FGR, Low GBO, Housley E. Cross linked fibrin degradation products, risk of coronary heart disease and progression of peripheral arterial disease. Lancet 1993; 342: 84-86.
- 26. Oslon RE. Discovery of the lipoproteins, their role in fat transport and their significance as risk factors. In: Olson RE (editor). Evolution of Ideas about the Nutritional Value of Dietary Fat. Rockville, MD, USA: American Society for Nutritional Sciences; 1998. pp. 439-443.
- 27. Linsel-Nitschke P, Tall AR. HDL as a target in the treatment of atherosclerotic cardiovascular disease. Nature Reviews Drug Discovery 2005; 4: 193-205.
- Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA 2009; 302: 1993-2000.
- Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001; 104: 503-516.
- 30. Sato K, Fukao K, Seki Y, Akiba Y. Expression of the chicken peroxisome proliferator-activated receptor-gamma gene is influenced by aging, nutrition and agonist administration. Poultry Science 2004; 83: 1342-1347.
- 31. Anderson CJ, Blesso CN, Park YK, Barona J, Pham T et al. Carbohydrate restriction favourably affects HDL metabolism in men and women with metabolic syndrome. Addition of egg yolk further increases HDL particles. FASEB Journal 2012; 26 (Suppl. 6): 254.
- Thapa BR, Walia A. Liver function tests and their interpretation. Indian Journal of Pediatrics 2007; 74 (7): 666-671.
- Ronald J, Grisanti DC. How to Quickly and Easily Understand Your Blood Tests without a Medical Degree. Greer, SC, USA: Sequoia Educational Systems; 2004.
- Liang D, Zhou Q, Gong W, Wang Y, Nie Z et al. Studies on the antioxidant and hepatoprotective activities of polysaccharides from *Talinum triangulare*. Journals of Ethnopharmacology 2011; 136: 316-321.