

## Supplementation of hen diets with dried or freshly harvested *Talinum triangulare*: the effect on egg production and egg lipid profile

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### ABSTRACT

This study investigated the comparative effects of administration of dried and fresh *Talinum triangulare* (Tt) plant supplements on the egg production and egg quality of laying hens. Two hundred and ten pullets, purchased at the age of 18 weeks, were randomly assigned to seven study groups: A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>; B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>; and C. Aqueous extracts derived from a dried sample of Tt were administered at 62.5, 250, and 1000 mg/L doses for treatment of Group A (A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub>); or from freshly harvested samples of Tt at 62.5, 250, and 1000 mg/L dose for treatment of Group B (B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>). Hens in Group C (CC) served as an un-supplemented experimental control group. Laying rate and egg lipid profile were determined at two-month intervals following standard procedures. Supplementation with Tt significantly enhanced egg production, with extended higher laying rates in groups A and B. A significant (P<0.05) reduction in low-density lipoprotein (LDL) concentration and an increase in the high density lipoprotein (HDL) concentration of eggs were observed in the supplemented groups (A and B). Dietary supplementation with dried samples of Tt at doses of 250 and 1000 mg/L increased the number of eggs laid, and resulted in the production of eggs with lower LDL and higher HDL cholesterol. Further research is needed to elucidate the exact mechanisms behind the hypolipidemic principle and egg production enhancement.

**Key words:** hens; dietary supplementation; *Talinum triangulare*; egg production; egg cholesterol

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## Introduction

Eggs are an almost perfect, complete protein food of high quality. They are a functional food that offers great advantages, in view of their excellent nutrient composition, affordability in terms of price, and wide acceptability (REHAULT-GODBERT et al., 2019). The issue of nutrition in many of the world's poorer countries could be ameliorated through controlled interventions that foster chicken and egg production. However, the current economic recession and consequences of COVID-19 on agriculture and the global economy have radically affected egg production. Many small-scale producers have gone out of business and production levels of large-scale farmers have decreased significantly. This negative impact on the agribusiness is due to the high costs and unavailability of feed ingredients and feed supplements on the market (WORLD POULTRY, 2013). Therefore, the possibility of producing eggs at prices which make them more accessible to a moderately poor population is attracting researchers in this field (HAFEZ and ATTIA, 2020).

Moreover, the issue of saturated fat and cholesterol content in egg and egg-derived foods has become critical due to the high level of awareness created by nutrition experts (MIRANDA et al., 2015). As a result of this growing health concern among product consumers, a decline in egg consumption in some Western nations, such as the USA and Spain, has been reported (GUERRERO-LEGARRETA and HUI, 2010). The possibility of manipulating the fatty acid composition in eggs by diet was recognized as far back as 1934 by Cruickshank (SIM, 1998). This concept of functional eggs has contributed immensely to re-establishing the important role of eggs as a healthy and safe food item in human history (RAJASEKARAN and KALAIVANI, 2013). Egg consumption has been on the increase in Nigeria (HEISE et al., 2015), despite the negative publicity with regards to cholesterol. This is especially true in urban areas, as per capita income increases, and as consumers become increasingly aware of the rich nutritional value of eggs.

*Talinum triangulare* is a perennial leguminous vegetable that is rich in nutrients and phytochemical

components, including omega-3 fatty acid (EZEKWE et al., 2006). It occurs naturally in bushes, parks, on roadsides, and could even be planted within and around homes and gardens. In general, the intake of vegetables has been associated with a relatively lower occurrence of major chronic diseases, such as hypertension, coronary heart disease, stroke, and chronic inflammatory bowel disease, and have thus been recommended for their prevention and management (HUNG et al., 2004; BOEING et al., 2012; NAHAK et al., 2014; WALLACE et al., 2020; NYANCHOKA et al., 2022). *Talinum triangulare* is available and accessible to most small-scale farmers who represent 90% of the total poultry production in Nigeria (SONAIYA and SWAN, 2004; HEISE et al., 2015). The possibility of supply of Tt in dried form to city dwellers presents great opportunities for its utilization. There are no reports in the available literature on the effects of supplementation of layer hen feed with Tt on egg production or the nutrient content of eggs. This study investigated the effects of dietary supplementation with graded doses of dried or freshly harvested Tt extracts on the laying rate and lipid profile of eggs laid by treated hens.

## Materials and methods

**Ethic statement.** The study complied with the University of Nigeria standard ethical/humane practices for the use of animals in research. The Experimental Animal Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, approved the animal experimental protocol in compliance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC).

**Plant collection and extraction.** *Talinum triangulare* (waterleaf) plants were planted in a small backyard garden near the students' practice poultry house belonging to the Department of Veterinary Animal Health and Production, University of Nigeria, Nsukka. A plant taxonomist at the herbarium that belongs to the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, identified the test plant (Tt). In this study, Tt was presented in two forms:

The dried-pulverised form, suitable for use in urban areas and cities where fresh vegetables are relatively expensive and scarce;

The freshly harvested or wet form, readily available to small-scale farmers mostly located in rural areas with an abundance of land for vegetable cultivation.

The extracts from the dried and fresh samples of Tt that were used for treatment of groups A and B hens were prepared following the method of ARONU et al. (2018), and were administered in the pullets' drinking water, from January to December 2019.

*Experimental Animals and Study design.* Two hundred and ten ready-to-lay Isa-brown pullets were purchased from Kosy Veterinary Consult in Enugu, Nigeria. Records from the source of the birds showed that the following standard prescribed vaccinations had been administered to the birds: Newcastle Disease (intra ocular, LaSota and Komarov), Gomboro and Fowl Pox. Amprolium hydrochloride anti-coccidial drug was administered at the preventive dose of 1g/5litres of drinking water for 10 days to prevent coccidiosis at intervals in the experimental flock. The experimental birds were housed on deep litter in the poultry houses of the Experimental Poultry Housing Unit of the Department of Animal Health and Production, University of Nigeria, Nsukka. The birds were acclimatized for one month. The study started at the 22<sup>nd</sup> week (five and half months) of the pullets' life and lasted for 11 months (i.e. 16 months of age). Rations were isocaloric and isonitrogenous, except for Tt supplementation in the treated groups (A and B).

The pullets were randomly assigned to three experimental groups (A, B, and C). Extracts obtained from dried and pulverized samples of Tt were administered at doses of 62.5, 250, and 1000 mg/L to experimental group A (A), labelled A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub> respectively. Extracts from freshly harvested Tt were administered at doses of 62.5, 250, and 1000 mg/L to experimental group B, also labelled B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> respectively. These doses were chosen on the basis of their effects reported earlier on the health, serum lipid profile and productivity of birds (ARONU et al., 2018;

2019; 2020). Pullets in the experimental group C (CC) served as the un-supplemented control. Each of the experimental groups (A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>; B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>; and CC) was further separated into three replicates. Each of the replicates was placed in a separate well ventilated 5cm deep litter pen which housed 10 pullets. The pens were lit with 12 h of day light and for an additional two hours into the night, and were cleaned every 3 months. The eggs were collected twice daily, at 8 am and 1pm respectively.

Replicates in each group were given the same group-specific treatments. Feed and water were administered *ad libitum*. Top Feed® (from Premier Feed Mills Co Ltd, Nigeria), was used throughout the study. All the birds received pullet rearing ration up to 18 weeks of age. The pullet rearing ration had 14% crude protein content, 8% fat, 15% crude fibre, 0.9% calcium, 0.35% available phosphorus and a metabolizable energy content of 2,600 Kcal/kg. A pre-layer diet was introduced at the 18<sup>th</sup> week and was continued until the laying percentage had reached five percent. The pre-layer diet composed of 16.5% crude protein, 5.5% fat, 13% crude fibre, 2% calcium, 0.38% available phosphorus, and a metabolizable energy content of 2,750 Kcal/kg. Then, the hen feed was gradually changed to layer diet. The layer diet was composed of 16.5% crude protein, 5% fat, 10% crude fibre, 3.5% calcium, 0.4% available phosphorus, and 2,890 Kcal/kg of metabolizable energy. The number of eggs laid per experimental group was recorded daily for 11 months, and egg lipid profile was assessed every two months (Month 0, 2, 4, 6 and 8) for a period of 8 months.

*Parameters investigated.* The hens' egg production parameter: The laying rate (LR) was computed as the percentage of the total number of eggs over the total number of days by the number of hens, i.e., egg laying rate (%) = [(total number of eggs) / (total number of days × number of hens)] × 100.

*Egg Collection for Lipid Profile Assay and Preparation for Assay.* Three eggs were randomly selected from each replicate group on the day of oviposition with a total of nine eggs per experimental group. The selected eggs were used the next day for laboratory investigations on egg lipid parameters.

Each egg was cleaned and carefully cracked from one side. The albumin was allowed to flow out leaving the yolk in the shell. A sterile 2 ml syringe (without a needle) was used to puncture the yolk and 1 ml of its contents was aspirated and emptied into a clean labelled test tube. 1 ml of deionized water was added to the yolk in the test tube to make a 1:1 dilution. The contents of the test tube were made into a uniform mixture by shaking and rolling. From this mixture, a sample was collected for analysis of the egg for determination of total cholesterol, HDL cholesterol and triacylglycerol and the result obtained was multiplied by two.

**Egg Lipid Profile:** The following procedures were employed in the evaluation of individual egg lipid profile parameters: Total cholesterol (TCh) levels in the eggs were determined by the enzymatic colorimetric test (CHOD-PAP) method (ALLAIN et al., 1974), while the triacylglycerol levels in the eggs were evaluated by the glycerol-phosphate oxidase method (BUCCOLO and DAVID, 1973). The levels of high-density lipoprotein (HDL) cholesterol in the eggs were determined by the dextran sulphate-Mg (II) precipitation method (ALBERS et al., 1978), while the levels of low density lipoproteins (LDL) cholesterol were calculated using Friedewald's formula ( $LDL-C = Total\ cholesterol - HDL-C - Triglyceride/5$ )

(FRIEDEWALD et al., 1972). The levels of very low-density lipoprotein (VLDL) in the eggs were calculated as one-fifth of the triacylglycerol (WARNICK et al., 1990).

**Statistical analyses:** Data generated from the study were subjected to one-way analysis of variance, and variant means were separated post-hoc using the least significant difference method. Version 16.0 of the Statistical Package for Social Sciences (SPSS) was used for the analyses. Significance was set at  $P < 0.05$ .

## Results

**Egg production.** Dietary supplementation with both dried and freshly harvested forms of aqueous extract of Tt at all three doses delivered significantly ( $P < 0.05$ ) increased egg laying in most of the months during the period of study (Table 1). Hens in groups A2 and A3, which were given 250 and 1000 mg/L of dried Tt, respectively, had the highest egg production most of the time, when compared to the other groups (Table 1). Laying rates improved significantly ( $P < 0.05$ ) after month five (5) and up to the end of laying in the treated groups, especially in the group treated with 1000 mg/L dried Tt (Table 1).

Table 1. Mean Laying Rate  $\pm$  SEM (%) in different groups during the trial.

	Monthly mean laying rate (%), with standard error in brackets										
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11
Dried Tt. 62.5 mg/L	50.67 <sup>a</sup> (4.37)	73.33 <sup>ab</sup> (2.55)	86.33 <sup>a</sup> (3.09)	86.20 <sup>a</sup> (5.91)	76.43 <sup>a</sup> (6.12)	76.57 <sup>b</sup> (4.73)	84.20 <sup>a</sup> (3.94)	80.80 <sup>a</sup> (3.52)	68.97 <sup>a</sup> (7.36)	61.27 <sup>ab</sup> (6.10)	63.20 <sup>b</sup> (2.12)
Dried Tt. 250 mg/L	49.67 <sup>a</sup> (3.85)	69.33 <sup>b</sup> (3.86)	71.37 <sup>b</sup> (2.96)	75.23 <sup>ab</sup> (5.34)	86.57 <sup>a</sup> (5.42)	71.43 <sup>b</sup> (5.40)	71.47 <sup>b</sup> (4.96)	62.20 <sup>b</sup> (4.27)	73.27 <sup>a</sup> (7.46)	72.80 <sup>a</sup> (6.90)	71.43 <sup>a</sup> (4.66)
Dried Tt. 1000 mg/L	34.33 <sup>b</sup> (5.22)	77.00 <sup>a</sup> (3.26)	79.67 <sup>a</sup> (3.41)	73.70 <sup>ab</sup> (5.65)	84.50 <sup>a</sup> (5.08)	85.27 <sup>a</sup> (3.81)	84.53 <sup>a</sup> (4.12)	70.77 <sup>b</sup> (3.89)	72.30 <sup>a</sup> (7.82)	73.17 <sup>a</sup> (6.28)	72.13 <sup>a</sup> (3.34)
Fresh Tt. 62.5 mg/L	47.67 <sup>a</sup> (4.14)	80.23 <sup>a</sup> (1.86)	82.97 <sup>a</sup> (1.97)	83.03 <sup>ab</sup> (3.66)	85.67 <sup>a</sup> (4.09)	76.23 <sup>b</sup> (3.71)	81.63 <sup>a</sup> (4.14)	64.13 <sup>b</sup> (2.48)	59.10 <sup>ab</sup> (5.57)	57.57 <sup>ab</sup> (5.85)	41.83 <sup>c</sup> (1.32)

Table 1. Mean Laying Rate ± SEM (%) in different groups during the trial. (continued)

	Monthly mean laying rate (%), with standard error in brackets										
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10	Month 11
Fresh Tt. 250 mg/L	35.57 <sup>b</sup> (3.67)	70.57 <sup>b</sup> (1.64)	86.90 <sup>a</sup> (2.16)	80.10 <sup>ab</sup> (4.36)	84.07 <sup>a</sup> (4.50)	75.40 <sup>b</sup> (3.12)	76.03 <sup>ab</sup> (3.99)	62.37 <sup>b</sup> (2.36)	56.00 <sup>ab</sup> (5.49)	57.17 <sup>ab</sup> (6.09)	47.47 <sup>c</sup> (2.09)
Fresh Tt. 1000 mg/L	42.73 <sup>ab</sup> (4.40)	77.53 <sup>a</sup> (1.59)	84.10 <sup>a</sup> (2.10)	79.83 <sup>ab</sup> (4.50)	73.97 <sup>a</sup> (3.33)	76.50 <sup>b</sup> (3.00)	75.60 <sup>ab</sup> (3.32)	65.33 <sup>b</sup> (3.52)	53.10 <sup>b</sup> (5.72)	58.80 <sup>ab</sup> (5.98)	58.30 <sup>b</sup> (2.90)
Unsupple- mented Control	33.40 <sup>b</sup> (3.97)	68.47 <sup>b</sup> (2.87)	79.37 <sup>a</sup> (2.24)	70.00 <sup>b</sup> (3.61)	62.97 <sup>b</sup> (3.11)	59.33 <sup>c</sup> (3.58)	62.9 <sup>b</sup> (2.72)	49.83 <sup>c</sup> (2.51)	36.77 <sup>c</sup> (4.11)	50.00 <sup>b</sup> (5.63)	35.70 <sup>c</sup> (1.71)

<sup>a, b, c</sup> Different superscripts in a column indicate significant differences between the groups, P<0.05

*Lipid Profile.* Supplementation with dried Tt at the dose of 1000 mg/L of drinking water significantly (P<0.05) lowered the mean TCh

content of eggs produced by group A pullets at month two and group B at month four (Table 2).

Table 2. Mean Total Cholesterol Concentration of eggs ± SEM (mg/dl) from different groups during the trial.

Groups	Mean egg total cholesterol (mg/dl), with standard error in bracket				
	Month 0	Month 2	Month 4	Month 6	Month 8
A <sub>1</sub> : Dried Tt 62.5 mg/L	2024.11 (80.27)	1942.95 <sup>abc</sup> (65.65)	2024.21 <sup>a</sup> (32.49)	2007.23 (83.13)	2010.05 (23.00)
A <sub>2</sub> : Dried Tt 250 mg/L	2063.02 (74.08)	1892.57 <sup>ab</sup> (17.70)	1999.25 <sup>a</sup> (33.08)	2028.19 (62.54)	2032.11 (28.79)
A <sub>3</sub> : Dried Tt 1000 mg/L	2034.46 (66.83)	1860.37 <sup>a</sup> (17.01)	2060.71 <sup>a</sup> (30.55)	2011.92 (21.72)	2001.83 (41.39)
B <sub>1</sub> : Fresh Tt 62.5 mg/L	2072.11 (60.56)	2007.00 <sup>b</sup> (51.88)	2080.45 <sup>a</sup> (41.99)	2116.35 (59.02)	2024.62 (44.22)
B <sub>2</sub> : Fresh Tt (250 mg/L)	2053.14 (54.68)	1951.71 <sup>abc</sup> (28.95)	2070.76 <sup>a</sup> (19.25)	1972.54 (42.75)	2064.01 (20.47)
B <sub>3</sub> : Fresh Tt 1000 mg/L	2062.40 (59.83)	2031.36 <sup>c</sup> (30.74)	1976.54 <sup>b</sup> (20.12)	2024.57 (61.64)	1953.33 (31.98)
CC: Unsupplemented Control	2048.61 (69.87)	2031.84 <sup>c</sup> (35.62)	2074.48 <sup>a</sup> (6.62)	2091.06 (52.79)	2073.07 (80.48)

<sup>a, b, c</sup> Different superscripts in a column indicate significant differences between the groups, P<0.05.

However, no significant variations ( $P>0.05$ ) were recorded in the mean triacylglycerol levels of the eggs from the different groups across the experimental period (Table 3). Significantly ( $P<0.05$ ) higher values for HDL- C were recorded for eggs collected from the supplemented groups (A and B), especially those given 250 mg/L and 1000 mg/L of both the dried and fresh Tt, from month four of laying up to the end of the study (Table 4). Eggs from the treated groups had significantly ( $P<0.05$ ) lower levels of LDL-C from the fourth

month of laying until the end of the experiment (Table 5). At the end of the study the difference in LDL cholesterol concentrations between the unsupplemented group (CC) and B<sub>3</sub> (1000 mg /L freshly harvested Tt sample) was 39 % in favour of B<sub>3</sub>, and this was statistically significant ( $P<0.05$ ). The effects of both dried and fresh Tt on the LDL-C was dose dependent: the higher the concentration of Tt extract administered to hens, the greater the lowering effect on LDL-C, and vice versa.

Table 3. Mean Triacylglycerol Concentration of eggs  $\pm$  SEM (mg/dl) from different groups during the trial.

Groups	Mean egg Triacylglycerol (mg/dl), with standard error in bracket				
	Month 0	Month 2	Month 4	Month 6	Month 8
A <sub>1</sub> : Dried Tt 62.5 mg/L	2005.23 (42.18)	2053.96 (32.12)	2122.85 (160.93)	1892.21 (161.35)	2201.21 (132.31)
A <sub>2</sub> : Dried Tt 250 mg/L	2037.89 (33.33)	2033.56 (40.25)	2009.69 (169.12)	1952.38 (102.61)	2139.64 (219.10)
A <sub>3</sub> : Dried Tt 1000 mg/L	2028.69 (41.28)	1991.53 (56.15)	2106.87 (142.01)	1933.33 (56.18)	2180.85 (177.92)
B <sub>1</sub> : Fresh Tt 62.5 mg/L	2030.66 (27.81)	2058.86 (34.74)	2069.23 (73.75)	2004.33 (270.12)	2270.97 (123.51)
B <sub>2</sub> : Fresh Tt (250 mg/L)	1998.62 (39.64)	2015.17 (48.74)	2106.49 (60.90)	2005.19 (216.13)	2153.21 (214.78)
B <sub>3</sub> : Fresh Tt 1000 mg/L	2026.41 (52.11)	2000.89 (36.35)	2017.68 (133.84)	2144.59 (140.87)	2211.88 (284.70)
CC: Unsupplemented Control	2032.01 (38.06)	2025.03 (21.09)	2034.93 (47.04)	2033.33 (130.91)	2102.79 (188.51)

No significant variations across the groups,  $P>0.05$

Table 4. Mean high-density lipoprotein (HDL) cholesterol content of eggs  $\pm$  SEM (mg/dl) from different groups during the trial.

Groups	Means of serum HDL cholesterol (mg/dl), with standard error in brackets				
	Month 0	Month 2	Month 4	Month 6	Month 8
A <sub>1</sub> : Dried Tt 62.5 mg/L	1349.36 (30.64)	1307.45 (12.54)	1490.44 <sup>ab</sup> (47.75)	1486.31 (15.89)	1534.33 <sup>ab</sup> (50.95)
A <sub>2</sub> : Dried Tt 250 mg/L	1325.93 (52.14)	1263.11 (42.55)	1522.38 <sup>a</sup> (30.11)	1556.93 (75.00)	1580.15 <sup>ab</sup> (49.69)
A <sub>3</sub> : Dried Tt 1000 mg/L	1333.63 (42.89)	1285.12 (14.23)	1557.30 <sup>a</sup> (41.88)	1537.64 (7.46)	1633.20 <sup>a</sup> (65.52)

Table 4. Mean high-density lipoprotein (HDL) cholesterol content of eggs  $\pm$  SEM (mg/dl) from different groups during the trial. (continued)

Groups	Means of serum HDL cholesterol (mg/dl), with standard error in brackets				
	Month 0	Month 2	Month 4	Month 6	Month 8
B <sub>1</sub> : Fresh Tt 62.5 mg/L	1359.84 (31.36)	1291.91 (23.26)	1464.81 <sup>ab</sup> (59.80)	1548.15 (102.20)	1502.21 <sup>ab</sup> (53.94)
B <sub>2</sub> : Fresh Tt (250 mg/L)	1328.96 (56.19)	1277.67 (65.80)	1582.98 <sup>a</sup> (34.53)	1576.91 (52.52)	1556.34 <sup>ab</sup> (34.79)
B <sub>3</sub> : Fresh Tt 1000 mg/L	1368.91 (40.21)	1291.26 (15.81)	1572.98 <sup>a</sup> (34.53)	1596.90 (31.15)	1589.63 <sup>ab</sup> (49.99)
CC: Unsupplemented Control	1353.60 (36.83)	1302.59 (61.40)	1436.95 <sup>b</sup> (23.80)	1462.88 (10.17)	1475.15 <sup>b</sup> (11.34)

<sup>a, b, c</sup> Different superscripts in a column indicate significant differences between the groups,  $P < 0.05$

Table 5. Mean low-density lipoprotein (LDL) cholesterol content of eggs  $\pm$  SEM (mg/dl) from different groups during the trial.

Group	Means of serum LDL cholesterol (mg/dl), with standard error in brackets				
	Month 0	Month 2	Month 4	Month 6	Month 8
A <sub>1</sub> : Dried Tt 62.5 mg/L	736.36 (56.84)	635.51 (70.84)	533.77 <sup>ab</sup> (72.71)	520.92 <sup>ab</sup> (97.35)	475.69 <sup>ab</sup> (39.92)
A <sub>2</sub> : Dried Tt 250 mg/L	687.19 (43.11)	629.46 (45.23)	476.88 <sup>a</sup> (23.03)	471.25 <sup>ab</sup> (72.30)	451.95 <sup>ab</sup> (66.12)
A <sub>3</sub> : Dried Tt 1000 mg/L	704.22 (28.59)	575.26 (31.08)	483.41 <sup>a</sup> (35.35)	424.28 <sup>a</sup> (20.44)	368.63 <sup>a</sup> (47.30)
B <sub>1</sub> : Fresh Tt 62.5 mg/L	731.33 (46.21)	715.05 (45.14)	615.64 <sup>b</sup> (19.13)	368.63 <sup>a</sup> (43.30)	522.41 <sup>ab</sup> (92.69)
B <sub>2</sub> : Fresh Tt (250 mg/L)	698.62 (50.36)	674.03 (57.59)	488.59 <sup>a</sup> (27.27)	395.63 <sup>a</sup> (39.80)	507.67 <sup>ab</sup> (19.31)
B <sub>3</sub> : Fresh Tt 1000 mg/L	756.11 (40.25)	704.10 (18.39)	503.56 <sup>ab</sup> (15.27)	427.67 <sup>a</sup> (31.03)	363.69 <sup>a</sup> (79.61)
CC: Unsupplemented Control	742.33 (30.87)	729.25 (96.31)	637.53 <sup>b</sup> (26.60)	627.18 <sup>b</sup> (45.08)	597.92 <sup>b</sup> (76.27)

<sup>a, b, c</sup> Different superscripts in a column indicate significant differences between the groups,  $P < 0.05$

## Discussion

Supplementation with Tt, in both types (dried or freshly harvested) and three concentrations (62.5, or 250, or 1000 mg/ L of drinking water), administered to treated groups (A and B) in this study significantly enhanced egg production, with prolonged higher laying rates. It is thought that the higher and prolonged laying rates recorded in the Tt treated hens in this study are a result of the nutrient (especially micronutrients) and bioactive phytochemical contents of Tt which were reported earlier in the literature (AJA et al., 2010; AGUNBIADE et al., 2015; IKEWUCHI et al., 2016; BIOLTIF, 2020). Nutritional modulation and influences on egg laying rate, duration and egg quality have been extensively reported in literature (HIEP and SWICK, 2017; WANG et al., 2017; BRYDEN et al., 2021; KOWALSKA et al., 2021). This research outcome corroborates with IRVINE (1956) proposition that waterleaf plant stimulates egg lay. The fact that a good laying rate was sustained by the dried sample groups (A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub>) until the end of the feeding trial is of great importance to the egg production industry, because it opens prospects for poultry farmers in areas where Tt is not cultivated and fresh Tt is not available, to get dried samples for use and application in enhancing egg production. This suggests that supplementation with Tt may be useful to laying birds in their second year of lay; for a higher laying rate and longer duration of laying that would translate to higher income from the sale of eggs.

Treatment with Tt also reduced the level of “bad” cholesterol (LDL-C) in the egg, whereas, the level of the “good” cholesterol (HDL-C) was enhanced. It is thought that the positive effects of Tt treatment on egg levels of LDL-C may be related to the documented beneficial effects of dietary fibre on serum levels of LDL-C, which have been attributed to such factors as the ability of some dietary fibres to bind bile acids and cholesterol during intraluminal formation of micelles that leads to an overall reduction of the cholesterol content of liver cells, upregulation of LDL-C receptors, and thus increased clearance of LDL cholesterol (ANDERSON and TIETYEN-CLARK, 1986; BROWN et al., 1999). It

may also be as a result of the effects of Tt treatment (as a source of dietary fibre) on hepatic fatty acid synthesis and intestinal motility (SCHNEEMAN and GALLAHER, 1985; SCHNEEMAN, 1987; NISHINA and FREEDLAND, 1990; BROWN et al, 1999). Supplementation with the dry sample gave a better result with regards to the LDL-C, with higher doses giving much better results. This research outcome agrees with that of EZEKWE et al. (2006) who reported that waterleaf reduces the risk of CVD by reducing TChol, LDL-C, and increasing HDL-C in humans and animals. SANDA (2015) also attested to the same finding. BROWN et al. (1999) and ANDARWULAN et al. (2015) reasoned that the presence of dietary fibre in Tt may be responsible for lowering cholesterol levels. High Density Lipoprotein and their lipid and protein parts (apolipoprotein type A) inhibit inflammation, endothelial activation, coagulation and platelet aggregation, and consequently oxidation, and thus are inversely associated with the risk of atherosclerotic CVD (EREN et al., 2012). The ability of Tt to reduce LDL-C and increase HDL-C levels in eggs produced by treated hens is an added advantage for the eggs so produced, could alleviate the fears of health-conscious individuals, and encourage, among all ages, the consumption of eggs.

## Conclusions

Administration of extracts from both dried and freshly harvested Tt significantly improved laying rates, reduced the total cholesterol and LDL cholesterol content, and enhanced the HDL cholesterol content of the eggs. The positive effects of the supplementation were recorded mainly at doses of 250 mg/L and 1000 mg/L.

The authors recommend dietary supplementation with *T. triangulare* for the enhancement of egg production and production of functionally healthier eggs. The possibility of having *T. triangulare* packaged in its dried form and supplied to urban dwellers all through the year is a promising emerging business for jobless and rural women in developing nations. Further studies to elucidate the hypolipidemic principles in *T. triangulare* is proposed by the authors.

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**ARONU, C. J., J. O. A. OKOYE, J. I. IHEDIOHA, S. M. ANIKA: Prehrana kokoši obogaćena sušenom ili svježe ubranom biljkom *Talinum triangulare*: učinak na proizvodnju i lipidni profil jaja. Vet. arhiv 93, 317-328 2023.**

#### SAŽETAK

U ovom su radu istraženi komparativni učinci dodatka prehrani sušene i svježe biljke *Talinum triangulare* (Tt) na proizvodnju i kvalitetu jaja kokoši nesilica. Ukupno je 210 kokoši u dobi od 18 tjedana nasumično podijeljeno u sedam pokusnih skupina: A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> te C. Vodeni ekstrakti dobiveni od sušene biljke *Talinum triangulare* primijenjeni su u dozama od 62,5, 250 i 1000 mg/L u skupini A (A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub>), dok su ekstrakti dobiveni od svježe ubrane biljke Tt u istim dozama primijenjeni u skupini B (B<sub>1</sub>, B<sub>2</sub>, i B<sub>3</sub>). Kokoši u skupini C (CC) poslužile su kao kontrolna skupina i nisu dobile dodatak prehrani. Stopa nesivosti i lipidni profil jaja određeni su u dvomjesečnim intervalima prema standardnim postupcima. Dodatak ekstrakta Tt prehrani kokoši znakovito je povećao proizvodnju jaja uz povećanu stopu nesenja u skupinama A i B. Osim toga znakovito je smanjena ( $P < 0,05$ ) koncentracija lipoproteina niske gustoće (LDL), a povećana koncentracija lipoproteina visoke gustoće (HDL) u skupinama koje su dobivale dodatak prehrani (A i B). Prehrana s dodatkom ekstrakta dobivenog od sušene biljke Tt u dozama od 250 i 1000 mg/L povećala je broj snesenih jaja i rezultirala proizvodnjom jaja s nižim LDL-om i višim HDL-om. Potrebna su daljnja istraživanja kako bi se razjasnili točni mehanizmi koji se nalaze u podlozi ovog hipolipidemijskog principa i povećanja proizvodnje jaja.

**Ključne riječi:** kokoši; dodatak prehrani; *Talinum triangulare*; proizvodnja jaja; kolesterol

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