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(Research Article)

Telferia occidentalis and *Ocimum gratissimum* Leaf meal additives on lipids and antioxidants composition of laying chicken eggs

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Abstract

In this study, *Telferia occidentalis* and *Ocimum gratissimum* meals were used as additives in the diets of laying chickens to assess the lipids and antioxidant composition of the eggs. A total of 420 Isa-brown point-of-lay pullets were randomly allocated in a completely randomized design to seven dietary treatments (T1 - T7). T1 served as the control diet with no inclusion of test ingredients; pumpkin leaf meal (PLM) and scent leaf meal (SLM). In other treatments or diets PLM and SLM were included per 100kg feed as follows; T2 250g PLM, T3 250g SLM, T4 500g PLM, T5 500g SLM, T6 250gPLM+250gSLM, and T7 500gPLM+500g SLM. Data were collected on egg production in three phases (cycles) of egg laying. Phase one was marked as egg production period during which the birds were between 18 and 28 weeks old, phase two spanned from week 28 to 38 weeks old, while phase three covered the laying periods from 38 to 48 weeks old. At the end of each laying cycle, two eggs were taken per replicate of each treatment for laboratory analyses to determine the presence and composition of dietary lipids and antioxidants in the raw egg. The dietary lipids and antioxidant parameters examined were cholesterol, triglycerides, total phenol, flavonoid and carotenoid. Results showed that, use of pumpkin leaf (PLM) and scent-leaf leaf meals at 250gPLM+250gSLM/100kg feed (T6) significantly ($P>0.05$) decreased egg cholesterol and triglyceride at first and third laying cycles. The phenol and flavonoid were not significantly ($P>0.05$) increased by the supplementation of laying chicken diets with PLM and SLM across various compositions applied in all the cycles suggesting that the herbs did not have negative effect on the egg phenol and flavonoid compositions. The laying chickens that received 500gPLM+500gSLM (T7) had significantly highest carotenoid value. Carotenoid is an important pigment that gives egg yolk its brilliant yellow colour and also enhances albumen quality. It can be concluded from the above that the use of *Telferia occidentalis* and *Ocimum gratissimum* meals in layer egg production as was done in this study improved the quality of eggs by lowering the lipids and increasing carotenoid constituents of the eggs, and by this, the health and wellbeing of the egg consumers can be enhanced.

Keywords: *Telferia occidentalis*; *Ocimum gratissimum*; Additives; Antioxidants; Eggs

1. Introduction

The supply and consumption of animal protein in the developing countries is low, thus the shortage of animal protein for human nutrition is one of the serious problems in Nigeria. Food and Agriculture Organization (FAO, 2013) recommended minimum of 53.8g per capita daily protein intake for an adult Nigerian, while Oloyede (2005) stated that 35g of the recommended protein requirement for an adult Nigerian should come from animal source. The commonest sources of animal protein especially in the meals of growing children in Nigeria are chickens and eggs. Hence, the problem of low protein intakes of Nigerians can be solved by increasing the productions of meat and eggs using poultry species that have fast growth rate and excellent meat and egg quality.

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One of the challenges in commercial layer production is how to keep a healthy flock and enhance maximum production of quality meat and eggs. Thus, farmers often resolve to the use of antibiotics and other drugs to maintain the health of their birds and increase growth and egg production. However, these frequently come with consequences of cost implications and drug residues in poultry products (Jawad *et al.*, 2014). Abbas *et al.* (2012) reported the use of herbs and plant products as alternatives to drugs and boosters in keeping a disease-free poultry farm. Also, Onu (2012) observed increasing interest in the anti-oxidant ability and benefits of phytochemicals in vegetables and other tropical herbs as feed ingredients in poultry. However, one of the common vegetables which are very rich in phytochemicals and antioxidant substances is *Telferia occidentalis* (fluted pumpkin leaf).

Telferia ocidentalis leaves are popularly consumed in many homes in Nigeria as a result of the various medicinal potentials ascribed to the plant. *Telfairia occidentalis* (Fluted pumpkin) commonly known as ugwu is a creeping vegetable that spreads across the ground with lobed leaves and thrives best in soils rich in organic matter. *Telfairia occidentalis* leaves play important role in human and livestock nutrition as it is believed to be source of protein, oils, fats, minerals and vitamins. Some investigations have revealed that the leaf of this plant is very rich in phytochemicals and antioxidant substances such as phenols and ascorbic acid. Many phenolics such as flavonoids have antioxidant capacities that are much stronger than those of vitamin C and E. Apart from the antioxident properties of this plant (*Telfairia occidentalis*) and its leaves, it is used locally in the treatments of some ailments such as leukemia, convulsion and anemia in some locallities. As a result, there has been increased consumption of its water extract, amongst anaemic and pregnant humans with claims that it increases the blood volume and potency. Another herb, which has recently showed medicinal importance, is *Ocimum gratissimum* (scent leaf).

Scent leaf (*Ocimum gratissimum*), a perennial plant crop which is available in Nigeria throughout the year with its high yield in raining season, is a fully developed flowering plant with root, stem and leaves systems. It is a widely used local plant in the tropics of Africa and Asia for therapeutic purposes and others. It prefers moist and fertile soil during growth, but will tolerate drought after flowering. Nweze and Ekwe (2012) concluded that *Ocimum gratissimum* leaf extract can be used to improve growth performance, stabilize the blood components and reduce the gut and blood micro-organisms for finishing broilers. Utilization of scent leaf in livestock nutrition has not been widely and scientifically exploited. The successful use of scent leaf and pumpkin leaf in laying pullets may help increase the knowledge for the search of natural herbs that would replace the use of synthetic antibiotics and multivitamins and reduce cost of egg production. According to Salihu *et al.* (2020), scent leaf can be used as an alternative to common artificial growth promoters like antibiotics in poultry feed. Ijeh *et al.* (2004) noted that scent leaf is rich in alkaloids, tannins, phytates, flavonoids, oligosaccharides, terpenoids, thymol and saponin. Therefore, its use as an additive in poultry feed will offer a beneficial effect on birds' immunity and growth. For instance, Nte *et al.* (2017) observed that broilers fed aqueous extract of scent leaf had better growth rate at starter phase while Anugom and Ofongo (2019) reported a higher body weight, feed intake and feed conversion ratio in broilers fed aqueous extract of scent leaf at finisher phase, than the control group. Nevertheless, there are limited reports on the use of both pumpkin leaf and scent leaf meals in laying chicken nutrition. Thus, the findings of this study would be useful to poultry farmers, as detailed information on how nutrient content of *Telferia occidentalis* and *Ocimum grattisimum*, as dietary additives affected the external and internal characteristics of the eggs of Isa-brown layers.

2. Material and methods

2.1. Experimental design and management of birds

A total of four hundred and twenty (420) Isa-brown point-of-lay pullets were used for this study. The birds were randomly allocated to seven (7) dietary treatment groups with a total of sixty (60) birds per treatment. Each treatment was replicated three (3) times with 20 birds per replicate. This experiment was laid out in a completely randomized design. The birds were raised on a deep litter house and fed commercial layer mash with or without supplemented pumpkin and scent leaf meals throughout the experimental period.

Routine vaccination against Newcastle disease was strictly adhered to during the study. This was combined with adequate bio-security measures to prevent outbreak of infectious diseases. Other necessary routine management practices including de-worming and delousing were done. The birds had *ad libitum* access to feed and water throughout the period of the experiment.

2.2. Experimental feed/ ingredients

These included pumpkin leaf meal, scent leaf meal and commercial layer mash.

2.3. Collection and Processing of Pumpkin leaf meal and Scent leaf meal

The test ingredients which are the pumpkin leaf (*Telferia ocidentails*) and scent leaf (*Ocimum gratissimum*) were obtained from Ega market in Idah, Kogi State, Nigeria. The leaves were destalked, and immediately air-dried on a clean concrete floor in a well-ventilated room. It was left to dry for about fourteen days until moisture content becomes stabilized at 12%. The air-dried leaves were milled using harmer milling machine of 0.02 mesh size. The milled *Telfera occidentalis* leaf meal (PLM - Pumpkin Leaf Meal) and *Ocimum gratissimum* leaf meal (SLM - Scent Leaf Meal) were collected in jute bags separately and tightly tied to prevent the attack of insects and pests. They were kept in a clean ventilated room prior to the feed formulation to ensure that their qualities are maintained. Samples were taken from the bagged pumpkin leaf meal and scent leaf meal for proximate analysis, vitamin and mineral determination. Thereafter the pumpkin leaf meal and scent leaf meal were incorporated into commercial layers feed at different levels of inclusion.

2.4. Experimental diets

Seven dietary treatments were prepared using the commercial layer mash with the test ingredients (pumpkin and scent leaves) added as additives.

Treatment 1, control diet with no inclusion of test ingredients (PLM and SLM); Treatment 2, 250gPLM/100kgfeed; Treatment 3, 50gSLM/100kgfeed; Treatment 4, 500gPLM/100kgfeed; Treatment 5, 500gSLM/100kgfeed; Treatment 6, 250gPLM+250gSLM/100kgfeed,Treatment 7; 500g PLM+500gSLM/100kg feed.

2.5. Data collection

Data collection was done in three phases. Phase one was marked as egg production period during which the birds were between 18 and 28 weeks old, phase two spanned from week 28 to 38 weeks old, while phase three covered the laying periods from 38 to 48 weeks old.

2.6. Dietary lipids and antioxidants in raw eggs

At the end of each laying cycle, two egg were taken per replicate of each treatment for laboratory analyses to determine the presence and composition of dietary lipids and antioxidants in the raw eggs. The parameters examined were cholesterol, triglycerides, phenol, flavonoid and carotenoid. They were determined according to the procedures described by Amaya (2004) and Patel *et al.* (2010).

2.7. Statistical analysis

The data collected for lipids and antioxidants were subjected to one way analysis of variance (ANOVA) using general linear model incorporated in SPSS version 16 (SPSS, 2011). Significant means were separated using Duncan multiple range test, incorporated in the same software at 5% level of significance.

3. Results and discussion

Tables 1, 2 and 3 show the lipid profile and antioxidant of eggs in layers fed diets containing different levels of dried pumpkin leaf and scent leaf meals; T_1(Control), T_2(250gPLM), T_3(250g SLM), T_4(500g PLM), T_5(500g SLM), T_6 (250g PLM + 250g SLM) and T_7 (500g PLM + 500g SLM) at first, second and third laying cycles.

Table 1 Lipid profile and antioxidants in eggs of laying birds fed diets containing different levels of dried pumpkin leaf and scent leaf meals at first laying cycle (18-28 weeks)

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	T_7	SEM±
Triglyceride (mg/dl)	705.30^b	463.20^f	796.50^a	536.80^c	498.20^d	403.50^g	524.10^d	1.15
Cholesterol (mg/dl)	204.30^c	148.80^e	245.90^b	268.90^a	245.70^b	138.60^f	189.40^d	1.14
Phenol (%)	0.16	0.14	0.16	0.18	0.16	0.12	0.12^{NS}	1.16
Flavonoid (%)	1.13	1.28	1.23	1.26	1.14	1.21	1.22^{NS}	1.13
Carotenoid (%)	136.45^f	194.51^d	198.73^c	188.69^e	210.61^b	201.34^c	216.34^a	1.15

Means in the same row with different superscripts are significantly different ($p<0.05$): Note: PLM = Pumpkin leaf meal, SLM= Scent leaf meal, NS= Non-significant, T_1: (Control), T_2: (250g PLM/ 100Kg feed), T_3: (250g SLM/100kg feed), T_4: (500g PLM/100kg feed), T_5:(500g SLM/ 100kg feed), T_6: (250g PLM and 250g SLM mixture/ 100kg feed), T_7: (500g PLM and 500g SLM mixture/ 100kg feed), SEM±: Standard error of mean

Table 2 Lipid profile and antioxidants in eggs of laying birds fed diets containing different levels of dried pumpkin leaf and scent leaf meals at second laying cycle (28-38 weeks)

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	T_7	SEM±
Triglyceride (mg/dl)	400.00^f	425.26^e	835.10^c	466.70^d	459.60^d	859.60^b	919.30^a	1.25
Cholesterol (mg/dl)	111.50^d	164.30^c	278.00^a	133.50^d	174.60^c	220.60^b	286.10^a	2.14
Phenol (%)	0.15	0.14	0.16	0.16	0.12	0.12	0.14^{NS}	0.89
Flavonol (%)	1.15	1.19	1.24	1.24	1.18	1.25	1.26^{NS}	1.54
Carotenoid (%)	190.45^c	195.53^c	196.14^c	192.59^c	204.63^b	206.37^{ab}	211.48^a	1.26

Means in the same row with different superscripts are significantly different ($p<0.05$) Note: PLM = Pumpkin leaf meal, SLM= Scent leaf meal, NS= Non-significant, T_1: (Control), T_2: (250g PLM/ 100Kg feed), T_3: (250g SLM/100kg feed), T_4: (500g PLM/100kg feed), T_5:(500g SLM/ 100kg feed), T_6: (250g PLM and 250g SLM mixture/ 100kg feed), T_7: (500g PLM and 500g SLM mixture/ 100kg feed), SEM±: Standard error of mean

Table 3 Lipid profile and antioxidants in eggs of laying birds fed diets containing different levels of dried pumpkin leaf and scent leaf meals at third laying cycle (38-48 weeks)

Parameters	T_1	T_2	T_3	T_4	T_5	T_6	T_7	SEM±
Triglyceride (mg/dl)	599.50^b	393.70^f	677.00^a	456.30^c	423.50^e	343.00^g	445.50^d	1.54
Cholesterol (mg/dl)	173.70^c	126.50^e	209.00^b	228.60^a	208.80^b	117.80^f	161.00^d	1.54
Phenol (%)	0.14	0.18	0.15	0.15	0.16	0.14	0.16	1.54
Flavonoid (%)	1.22	1.17	1.14	1.21	1.25	1.24	1.28	1.54
Carotenoid (%)	196.83^e	214.41^a	200.86^d	192.73^f	191.18^f	208.36^c	221.10^b	1.54

Means in the same row with different superscripts are significantly different ($p<0.05$)Note: PLM = Pumpkin leaf meal, SLM= Scent leaf meal, NS= Non-significant, T_1: (Control), T_2: (250g PLM/ 100Kg feed), T_3: (250g SLM/100kg feed), T_4: (500g PLM/100kg feed), T_5:(500g SLM/ 100kg feed), T_6: (250g PLM and 250g SLM mixture/ 100kg feed), T_7: (500g PLM and 500g SLM mixture/ 100kg feed), SEM±: Standard error of mean

At first laying cycle, the results show that triglyceride, cholesterol and carotenoid contents of the eggs were significantly ($p<0.05$) influenced by the different dietary treatments given to the laying chickens. However, the phenol and flavonoid were not significantly ($p>0.05$) affected (Table 1). The eggs in laying birds fed diet T_3 had significantly ($p<0.05$) highest value of triglyceride (796.50 mg/dl) while the value was least in T_6 (403.50 mg/dl). The triglyceride content of the eggs in the laying birds fed diets T_7 and T_5 were comparable. The cholesterol content was more significantly ($p<0.05$) influenced in eggs in the laying birds placed on T_4 (268.90 mg/dl) and 138.60 mg/dl in T_6, the least. The eggs in the layers placed on T_3 and T_5 had comparable values of cholesterol. The carotenoid ranged from T_1 (136.45 %) to T_7 (216.34 %).

At the second laying cycle (Table 2), the lipid profile parameters were significantly (p<0.05) affected by the dietary treatments but, phenol and flavonoid were not. Triglyceride was significantly (P<0.05) highest (919.30 mg/dl) in Diet 7 and least in 1 (400.00 mg/dl). The cholesterol contents of eggs in the laying birds placed on diet 7 (286.10 mg/dl) was also significantly (P<0.05) highest while eggs of laying birds on diet 1 (111.50 mg/dl) was least followed by diet 6 (220.60mg/dl). Further, the eggs of the laying birds fed diet 7 had significantly (p<0.05) highest value of carotenoid (211.48 %) while the control diet 1 was least, 190.45 %.

At third laying cycle (Table 3), the results show that triglyceride content of the eggs was significantly (p<0.05) highest in eggs of the laying birds placed on diet 3 (677.00), followed in significantly (P<0.05) decreasing order by 599.50, 456.30, 445.50, 393.70 and 343.00 mg/dl obtained in eggs of the laying birds fed diets 1, 4, 7, 5, 2 and 6 respectively. The cholesterol contents of the eggs in the experimental layers were significantly (p<0.05) influenced with the values 228.60>209.00≥208.80>173.70≥161.00>126.50>117.80 mg/dl (in that order) in eggs of the birds maintained on diets 4, 3, 5, 1, 7, 2 and 6 respectively. The phenol and flavonoid contents of the eggs were not significantly (P>0.05) affected by the experimental diets. The carotenoid contents of the eggs were significantly (p<0.05) increased by the diets and the highest value was obtained in eggs of the laying birds fed diet 7 (221.10 %) followed by the values from diets 2 (214.41), 6 (208.36), 3 (200.86), 1 (196.83), 4 (192.73) and 5 (191.18 %) as shown in Table 3.

4. Discussion

Lipid composition of egg is paramount as it directly affects metabolism. In this study, use of pumpkin leaf (PLM) and scent leaf meals at 250g PLM+250g SLM composite mix/100kg feed (T_6) resulted in the decrease of egg triglyceride and cholesterol at first and third laying cycles. These findings agree with those of another study showing that herbal extracts and flax seed reduced egg and sera blood cholesterol of laying hens (Hosseini *et al. 2023*). Hosseini *et al.* (2023), also reported that layers fed herb-supplemented diets had a lower value of yolk triglycerides and total cholesterol than the control. The positive impacts of PLM and SLM can be due to the inhibitory effect of phenolic compound 3-hydroxy-3-methylglutaryl coenzyme A, which is one of the most important components of cholesterol synthesis (Oh *et al.,* 2017). However, the reduced cholesterol and triglycerides recorded in the eggs of laying chickens fed control diet at second laying cycle may suggest that the metabolism of lipid in laying chickens depends on age of the birds and stage of egg production. In addition, in practice, laying chickens often attain peak of egg production about the age they were in the second laying cycle in this study, and this implies that at about this age, birds might require higher nutritional intake for both maintenance and egg formation. Nevertheless, this study submitted that supplementation of laying chicken diets with PLM and SLM at 250gPLM+250g SLM composite mix gave better egg lipid composition than the rest of the treatments. However, more investigations are necessary to get insights into the underlying mechanisms modifying egg lipid content by these dietary herbs.

The phenol and flavonoid were not significantly influenced by the supplementation of laying chicken diets with PLM and SLM across various compositions applied. This suggests that the herbs might not have negative effect on the egg phenol and flavonoid compositions. The non-significant values of phenol and flavonoid in the eggs of laying chickens across all the treatments is expected because phenol and flavonoid are more abundant in fruits than in vegetables (Vinson *et al*., 2001) hence, the supplementation of the laying chicken diets with PLM and SLM did not exert significant influence on the eggs. However, the laying chickens that received 500g PLM+500g SLM composite mix had significantly highest carotenoid value. Carotenoid is an important pigment that gives egg yolk its brilliant yellow colour and also enhances albumen quality (Schaeffer *et al.,* 1988). This suggests that the eggs of laying chickens fed supplemental PLM and SLM may be better in terms of quality than the control because the quality of yolk and albumen greatly determine egg quality.

5. Conclusion

In this study, use of pumpkin leaf (PLM) and scent-leaf leaf meals at 250g PLM+250g SLM composite mix/100kg feed (T_6) resulted in the decrease of egg triglyceride and cholesterol at first and third laying cycles. Lipid composition of egg is paramount as it directly affects metabolism. The phenol and flavonoid were not significantly influenced by the supplementation of laying chicken diets with PLM and SLM across various compositions applied, suggesting that the herbs might not have had negative effect on the egg phenol and flavonoid compositions. However, the laying chickens that received 500g PLM+500g SLM composite mix had significantly highest carotenoid percent. Carotenoid is an important pigment that gives egg yolk its brilliant yellow colour and also enhances albumen quality (Schaeffer *et al.,* 1988). From the above, it can be concluded that the eggs of laying chickens fed supplemental PLM and SLM may be better because the quality of yolk, albumen, cholesterol and triglyceride greatly determine egg quality.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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