Archiva Zootechnica 25:1,50-59, 2022
DOI: 10.2478/azibna-2022-0004

Dietary clove leaf meal supplementation: influence on egg qualities and reproductive morphometry of domestic laying birds

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ABSTRACT

This study investigated the effects of dietary *Syzygium aromaticum* supplementation on egg qualities, egg cholesterol and reproductive morphometry of laying hens. Three experimental diets were formulated containing 0.00%, 0.25% and 0.50% *Syzygium aromaticum* leaf meal (SLM). One hundred and fifty (20 weeks old) Isa-Brown birds were randomly assigned to three-treatment group which comprised 50 birds replicated five times with 10 birds each. Six eggs were collected from each replicate three days to the end of the 1st cycle of laying (4 weeks), 2nd and 3rd cycles and properly labelled for egg qualities assessment. At the end of the 12 weeks feeding trial, 45 birds (3 birds per replicate, that is, 15 birds per treatment) were slaughtered and dissected to evaluate their reproductive parameters. The result showed positive dietary effect (P<0.05) on shell thickness, yolk height, yolk index and egg mass. Cholesterol level of whole eggs, egg yolk and albumen were significantly lowered. Weight of entire reproductive tract and ovary as well as length of infundibulum, magnum, isthmus, uterus and vagina were significantly increased by dietary SLM. Conclusively, clove supplementation at 0.25% and 0.50% could be adopted for improved egg shell thickness and low-cholesterol eggs in poultry production

Keywords: Clove, Layers, Egg, morphometry, cholesterol.

Introduction

The ban on the use of anti-microbial growth promoter has triggered the use of plant-based additives in livestock production. Thus, the use of feeds as well as additives that are safe and of high nutritive qualities are crucial for optimum performance of livestock (Oladeji et al., 2019). Hence, much recognition is gained by phytogenic feed additives in the poultry industry as it does not only contribute to the nutrient requirements of the birds but also act as perfect substitute for premixes, antibiotics and antioxidants (Suganya et al., 2016). Lack of known residual effects of the phytogenic feed additives on human health alongside with its availability at little or no cost has enhanced its adoption in recent times (Abdelli et al, 2021). Clove (*Syzygium aromaticum*) is an evergreen, aromatic plant which is available all year round with varying harvest seasons in different countries (Yun, 2018). It contains numerous bio-active compounds such as eugenol (72–90%), eugenol acetate, β-caryophyllene, flavonoids and triterpenoids (Jimoh et al. 2017) and vitamins such as vitamins A, K, B6, B1 and C (Dorman and Dean, 2000).

Chicken egg obtained from *Gallus domesticus* is commonly consumed by numerous households worldwide. In 2009, the total metric tons of egg produced worldwide from a total laying flock of 6.4 billion hens was estimated to be 62.1 million. Whole egg especially its yolk possesses valuable amount of high-quality protein and choline (Howe et al., 2004), vitamins and other bioactive components such as lecithins and carotenoid (Andersen, 2015) and are recommended as part of a healthy diet in the 2015–2020 US dietary guidelines (USDA and HHS, 2015). Numerous studies have reported clove as an effective growth promoter in poultry especially broiler production. Boyraz and Ozcan, 2006 reported that clove extract in poultry diet at 400mg/kg was a good substitute for antibiotics as it enhanced the performance of the birds. Also, diets supplemented with 0.80% clove powder and 0.80% clove flower oil combination alongside drinking water treated with 0.40% aqueous clove extract was reported by Salman and Ibrahim (2012) to improve the performance of broiler chickens exposed to heat stress. Based on the numerous studies that has affirmed the potency of clove to broiler chickens' performance and paucity of information in relation to domestic laying hen, investigating the effects of clove on domestic laying hens would be a great contribution to knowledge. Therefore, this study focuses on the influence of dietary clove (*Syzygium aromaticum*) on reproductive morphometry and egg qualities of domestic laying birds.

Materials and methods

This experiment was conducted at the poultry section of the Teaching and Research Farm, Federal University of Technology, Akure. Dry *S. aromaticum* leaf was sourced from the local markets in SouthWestern, Nigeria. It was ground with the aid of hammer mill to about 100µm to produce *S. aromaticum* leaf meal (SLM). The antioxidant activity and phytochemical composition of SLM is presented in Table 1.

Table 1. Antioxidant activity and phytochemical composition *of Syzygium aromaticum* leaf meal

Parameters	*Syzygium aromaticum* leaf meal
Saponin (mg/g)	19.17±0.18
Flavoniod (mg/g)	0.04±0.00
Phenol (mg/g)	4.24±0.58
Terpeniod (mg/g)	52.39±0.03
DPPH (%)	80.55±0.07

One hundred and fifty Isa brown pullets purchased at 20 weeks of age was used for the study which lasted for 12 weeks. The birds were weighed and randomly assigned to three experimental diets: diet 1 (control) has 0% supplementation, diet 2 contains 0.25% SLM and diet 3 contains 0.50% SLM which was formulated according to (NRC, 1994) nutritional recommendations (Table 2).

Table 2. Composition of layer's experimental diet

Ingredients	% Composition
Maize	50.00
Rice bran	8.00
Palm kernel Cake	2.60
Groundnut Cake	15.00
Soybean Meal	15.00
Fishmeal	5.00
Limestone	1.50
Premix	0.30
Di-Calcium Phosphate	1.00
Phosphorus	1.00
Methionine	0.20
Lysine	0.10
Salt	0.30
Total	100
Calculated Nutrients	
Metabolizable Energy (Kcal/kg)	2783.50
Crude Protein (%)	20.68
Fibre (%)	3.88
Calcium (%)	1.20
Phosphorus (%)	0.66
Methionine (%)	0.51
Lysine (%)	1.11

Fifty birds were randomly distributed into treatment groups of five replicates per treatment and ten birds were assigned to each replicate. The birds were served the formulated layers' mash in limited amount (110g/hen) and fresh clean water was provided regularly.

Six (6) eggs were collected from each replicate three (3) days to the end of the 1st cycle of laying (that is, 4 weeks/ month) and properly labelled using permanent marker. This was repeated for the 2nd and 3rd cycle and their external and internal qualities were assessed. Egg weight was obtained with the aid of sensitive scale while egg height and width were measured using Vernier Caliper. Egg shape index was calculated as a ratio of egg diameter to egg length (Allen and Young, 1980). Thereafter the eggs were broken and the two egg membranes were pulled off the shells immediately and the shells peeled were air-dried at room temperature for 24 hours and weighed to obtain egg shell weight using sensitive scale and the egg shell thickness was determined with a micrometer screw gauge. The yolk and albumen were carefully separated and weighed to determine yolk and albumen weight. The vernier caliper was used to measure albumen height, albumen length, yolk weight and height and yolk index was obtained by dividing the height of the yolk by the average of its diameter while the Haugh Units (HU) was calculated from the height of the albumin and the weight of the egg as: Haugh's unit = 100 x log (Albumen height + 7.57 - 1.7 x Egg weight0.37). Likewise, some of the eggs were prepared according to the procedures described by Elkin et al. (1999). The eggs were first hard-cooked, allowed to cool, after which the weight of the boiled egg was noted. The egg shell was peeled off and also weighed followed by the careful removal of the egg white (albumen). The yolks were separated, weighed and crushed. One gramme (1g) of the sample of each yolk was homogenized with 15ml of chloroform-methanol 2:1 (v/v), thoroughly mixed and filtered. Egg homogenate filtrates were designated as egg yolk samples. The total cholesterol of the egg yolk samples was determined using the RANDOX® cholesterol assay kit. The kit contained cholesterol assay reagent and standard cholesterol solution were used for the calibration curve.

At the end of 12 weeks trial, forty-five (45) birds, that is, 3 birds per replicate were slaughtered and dissected. The various parts of the reproductive organs such as the ovary, infundibulum, magnum, isthmus, uterus, vagina and the entire reproductive tract were weighed and measured accordingly (Aro, 2019).

All data collected were subjected to completely randomized design procedures of SAS (2008, version 9.2). The means were compared using Duncan Multiple Range Test of the same software where the analysis of variance indicated significant treatment effect at 5% level of significance.

RESULTS AND DISCUSSION

The effect of the dietary *Syzygium aromaticum* leaf meal on external egg qualities parameters is illustrated in Table 3.

Table 3. Effect of dietary SLM on external egg qualities of laying hens

Parameters	Diet 1(0.00%)	Diet 2 (0.25%)	Diet 3 (0.50%)	P-Value
Egg wt (g)	48.11±0.84	50.94±0.59	52.00±0.76	0.09
Egg shell length (mm)	3.84±0.06	4.04±0.04	4.10±0.05	0.13
Egg shell width (mm)	2.71±0.09	2.81±0.04	2.89±0.05	0.18
Shell wt (g)	4.70±0.18	4.78±0.07	4.97±0.08	0.13
Shell mem (g)	0.98±0.05	1.01±0.03	1.08±0.03	0.13
Shell thickness (mm)	0.28±0.00^{b}	0.29±0.01^{b}	0.34±0.03^{a}	0.01
Esindex	69.58±0.62	70.59±2.30	70.67±0.77	0.54

$^{a, b, ab}$ = Means on the same row but with different superscripts are significant at P<0.05

Egg wt- Egg weight, Shell wt- Shell weight, Shell mem- Shell membrane, Esindex -Egg shell index, P-Val -P-Value

Addition of dietary SLM to laying birds diets resulted in a significant (P<0.05) increase in egg shell thickness which agrees with the findings of Nasiroleslami and Torki (2010) who reported that herbal supplementation of laying birds' diets improved egg shell thickness. The increased egg shell thickness can be attributed to the mineral constituents especially calcium (623mg/100g) present in clove(https://fitaudit.com/food/193582/minerals).

The improved shell thickness thereby reduced the number of cracked eggs collected. Laying birds fed with 0.5% dietary SLM had higher value for egg shell thickness when compared with the laying hen fed control diet. In contrast, egg weight, egg shell length, shell width, shell weight, shell membrane and egg shell index were not statistically (P>0.05) influenced by dietary SLM supplemented in the birds' diet which aligns with the findings of Damaziak et al. (2017 and Ayed et al. (2018). However, there was slight numerical increase in the mean values of non-significant eggs parameters previously mentioned, collected from treatment 2 and 3 groups which indicated that clove is rich in minerals and other food/feed enhancing phytochemicals.

Egg yolk height, yolk index and egg mass were significantly increased (P<0.05) by the dietary SLM fed to the laying hens as shown in Table 4. Eggs collected from birds fed with 0.25% and 0.50% dietary SLM had higher values for yolk height, yolk index and egg mass compared to the control. The yolk index of eggs collected from treatment group 2 and 3 revealed that the standing-up quality of the yolk was improved by eugenol present in clove (Asrat et al., 2018).

Table 4. Effect of dietary SLM on internal egg qualities of laying hens

Parameters	Diet 1 (0.00%)	Diet 2 (0.25%)	Diet 3 (0.50%)	P-Value
Albhgt (mm)	0.71±0.07	0.71±0.01	0.73±0.01	0.02
Albwt(g)	12.12±0.63	13.96±0.48	14.26±0.46	0.25
Alblength (mm)	6.62±0.15	6.70±0.08	6.65±0.17	0.55
Yolkhgt (mm)	1.41±0.05^{b}	1.77±0.01^{a}	1.77±0.02^{a}	0.00
Yolkwt (g)	12.28±0.36	12.49±0.13	12.81±0.18	0.24
Yolklength (mm)	2.81±0.08	2.93±0.03	2.88±0.04	0.38
Yolkindex	0.50±0.02^{b}	0.61±0.01^{a}	0.62±0.01^{a}	0.01
Eggmass (g)	30.62±1.42^{b}	35.93±0.55^{a}	35.95±0.72^{a}	0.01
Haughunit	99.99±0.01	99.97±0.02	100.05±0.04	0.15

$^{a, b, ab}$ = Means on the same row but with different superscripts are significant at P<0.05.
Albhght – Albumen height, Albwt- Albumen weight, Alblength- Albumen length, Yolkhgt- Yolkheight, Yolkwt- Yolk weight.

No significant differences (P>0.05) were observed on the albumen weight, albumen height, albumen length, yolk weight, yolk length and haugh unit of eggs collected in the three treatment groups although the data presented in Table 3 revealed numerical increase in the values of albumen weight for eggs collected in treatment. The improvement in albumen weight agrees with the findings of Ayed et al. (2018) and Reham et al. (2018) as the bio-active compounds of herbal plants enhance albumen secretion in laying hen through the protection of the magnum and uterus (Nadia et al., 2008). Hence, the increase in the numerical values of albumen weight resulted into the positive influence on egg mass. The haugh unit were non-significant but were in the range of good-quality eggs.

Table 5 showed the cholesterol level in the egg yolk, whole egg and egg albumen of laying hen fed with dietary SLM.

Table 5. Effects of dietary SLM on the cholesterol contents of eggs of laying hen

Variables	Diet 1 (0.00%)	Diet 2 (2.50%)	Diet 3(5.00%)	P-Value
Yolk	15.00±0.42^{a}	11.99±0.06^{b}	12.73±0.41^{b}	0.0019
Wegg	229.74±1.94^{a}	171.48±4.67^{b}	183.27±6.14^{b}	0.0002
Albumen	214.47±2.15^{a}	159.23±4.68^{b}	170.26±6.04^{b}	0.0003

$^{a, b, ab}$ = Means on the same row but with different superscripts are significant at P<0.05

Significant differences (P<0.05) were observed among the three treatment groups which followed similar trend. Laying hens in the control group had higher significant (P<0.05) value for cholesterol content in the yolk, whole egg and albumen. The cholesterol level of eggs evaluated for birds fed with diet 2 & 3 were similar. The cholesterol contents in the egg yolk, whole egg and albumen of laying hen fed with 0.25% dietary SLM were numerically lower than other treatment groups which indicated that clove possess hypocholesterolemic property. This study affirms the report of Balasasirekha and Lakshmi (2012) who stated that plants that contain eugenol have hypolipidemic activity.

The reproductive tract morphometry of the laying chickens fed varying levels of dietary SLM presented in Table 6.

Table 6. Reproductive tract morphometry of laying hen fed different levels of dietary SLM

Parameters	Diet I (0.00%)	Diet 2 (0.25%)	Diet 3 (0.50%)	P-Value
Wt of ent. tract (g)	91.60±1.41^{b}	94.53±0.35^{a}	92.03 ±0.72^{ab}	0.03
Wt of ovary (g)	34.80±0.02^{b}	40.63±0.01^{a}	40.33±0.02^{a}	0.03
Len of Infund (cm)	5.77±0.15^{c}	6.47±0.58^{b}	8.70±0.08^{a}	0.01
Len of Magnum (cm)	32.23±0.03^{b}	34.80±0.01^{ab}	35.10±0.10^{a}	0.04
Len of Isthmus (cm)	9.57±0.54	9.37±1.41	9.53±1.24	0.35
Len of Uterus (cm)	7.17±1.69	7.10±0.80	7.70±0.30	0.10
Len of Vagina (cm)	5.10±0.74	5.20±0.40	5.70±0.96	0.70

$^{a, b, ab}$ = Means on the same row but with different superscripts are significant at P<0.05
Wt- weight, ent. -entire, Infund- Infundibulum, Len- Length

Hens fed 0.25 and 0.50% SLM had higher significant (P<0.05) weights for both entire tract and the ovary when compared with the values recorded on the control diet. The increment in ovarian weight can be attributed to synergetic activity of follicles stimulating hormone and luteinizing hormone triggered by dietary SLM in the bird's diet that enhanced the level of oestrogen secretion which resulted into improvement of the weight of the entire reproductive tract. However, this finding disagreed with the findings of Akinwande et al. (2019) and Okukpe et al. (2018) who reported a decrease in follicle stimulating hormone secretion in male wistar rats and buck respectively fed clove extract. Infundibulum and magnum length were significantly higher (p<0.05) among hen fed 0.25 and 0.50% SLM. The length of the infundibulum still fell within the normal range as reported by Rahman (2013). Also, the increased length of the magnum could be responsible for the higher albumen weight recorded in Table 3.

CONCLUSION

The results of the present study revealed that dietary SLM improved egg qualities of economic importance. Egg shell thickness was enhanced at 0.25 and 0.50% supplementation rate due to the mineral constituent of clove thereby leading to a reduction in the number of cracked eggs. However, the cholesterol content of eggs was better reduced at 0.25% dietary supplementation level. Therefore, the usage of SLM at 0.25% level could be encouraged in hens' nutrition.

ACKNOWLEDGEMENTS

All authors are immensely grateful to the Technologist of Animal Nutrition Laboratory and Head of Teaching and Research Farm, Federal University of Technology for providing the necessary support for the successful conduct of the research.

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