

## EFFECT OF CLOVE (FEED AND WATER GRADE) ON LAYERS PERFORMANCE AND EGG QUALITY

### ABSTRACT

An experiment was conducted for 8 weeks to determine the effect of clove (feed and water grade) on laying performance, egg quality and chemical composition. Two hundred and ten (210) point of lay (18 weeks old) of Isa brown breed were randomly allotted after weigh balance into seven treatments of five replicates. Seven Dietary treatments (Diet 1,2,3,4,5,6,7) were established for control, 0.5, 1.0g/kg in feed, 0.5, 1.0g/liter of water, 0.25g/kg and 0.25g/litre, 0.5g/kg and 0.5g/litre of clove powder in feed and water respectively. Egg samples were stored at 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> week of the experiments and analyzed at the end of the experiment to have 4, 2 and 0 week(s) of storage. Proximate composition and lipid profile of eggs was chemically and statistically analyzed a completely randomized experimental design of a factorial arrangement. The results showed that the inclusion of clove (*Syzygium aromaticum*) increased performance of the layer birds in term of egg production at 0.5g (clove in their water) and 0.5g+0.5g (clove powder in their feed and water) respectively. The result also indicated that internal egg qualities were best at the combination of feed and water (0.5g+0.5g). The interaction effect of storage time and mode of administration of clove on the proximate composition of eggs laid by the birds was best at 0 week and 4 weeks storage duration. The storage time on lipid profile of eggs laid by birds fed with clove was observed to be best at 4 weeks due to the reduction of cholesterol. In conclusion, the addition of clove in feed and water at 0.5g+0.5g can be best recommended due to the increase in laying performance, egg quality. Also, the proximate composition and lipid profile of eggs stored was best at this level of inclusion.

### INTRODUCTION

Eggs are among the few foods that are used throughout the world regardless of religion and ethnic groups (Stadelman and Cotterilli, 2001). Egg is an affordable nutrient rich food that contains highly digestible proteins, minerals, lipids and vitamins which are essential in human diet. Eggs are one of the most complete foods yet, the industry is been put under severe pressure largely due to the controversial reports regarding its cholesterol content (Basmacioglu et al., 2003). Cardiovascular diseases are more related to the fatty acid profile of eggs rather than the cholesterol content itself (Simopolus, 2000). Fatty acids are the compound most susceptible to oxidation (Fennema, 2000).

Antioxidants are added to layers diet to minimize lipid oxidation and to enhance the consumer satisfaction of eggs (Galobart et al., 2001). Antioxidants can be categorized into two main classes; synthetic and natural antioxidants, and are frequently used in the food industry to prevent oxidation of lipids. Natural antioxidant such as garlic has been found to improve laying performance and egg quality in laying hens (Mulugeta and Olobatoke, 2011). Clove possesses strong antioxidant activity which is comparable to the activities of the natural antioxidant (Dorman, 2000). Clove has the highest capacity to give off hydrogen and reduce lipid peroxidation. The antioxidant activity of clove bud extract and its major aroma components, eugenol and eugenol acetate were comparable to that of natural antioxidant  $\alpha$ -tocopherol (Lee and Shibamoto, 2001). Clove also possesses antifungal, antiviral, antimicrobial, antidiabetic, anti-inflammatory and antithrombotic properties. Clove also showed a significant inhibitory effect against hydroxyl radicals and act as iron chelator (Gulcin et al., 2004).

**Comment [R1]:** The title suggests that 2 types of additive were used not to methods of administration, rephrase, please

**Comment [R2]:** Some formattinh mistakes, such as capital letters of words in the middle of the sentence or the lack of space between number (value) of trait and its unit

**Comment [R3]:** Only by numbers

**Comment [R4]:** What does it mean? Point of lay?

**Comment [R5]:** Isa Brown is not breed but commercial laying-type hybrid hen

**Comment [R6]:** This statement may be justified by newer reference, as well as not book

**Comment [R7]:** The order of authors' names is important in quotation

Egg lipids are highly concentrated in yolk and are made up of lipoprotein, triacylglycerol and cholesterol. The lipid content in fresh egg yolk as observed by Niesel (1998) is approximately 30%. Egg composition is relatively consistent in terms of total protein, total lipid, phosphorus, essential amino acids, iron and phospholipids. Some other components such as fatty acid composition, vitamins, cholesterol content, carotenoids, mineral contents and antioxidants are influenced by the diets of the hen and are more variable (Hargis *et al.*, 1991). Egg quality and its healthy nutrient composition are determined by the hen's age, environment, genetic and feed quality especially manipulated with added nutrients (Bertechini, 2003). The quality of eggs is affected by both internal and external factors. Egg quality is determined by the egg shape, strength of the shell, quality of albumen and yolk index. External and internal quality of eggs depends mainly on breed and duration of storage.

**Comment [R8]:** This statement may be justified by more valuable reference

As egg is laid, deterioration starts due to loss of moisture, CO<sub>2</sub> and entrance of bacteria via the egg shell pores. Shin *et al* (2012) reported that refrigeration preserves egg for a long period of time. However, considering the economic challenges in power supply there is need to establish an alternative means of preservation. The effects of natural antioxidant have been found to prevent deterioration and improve quality of stored eggs. (Cherian *et al.*, 1996). The general aim of the study was to determine the effect of clove (feed and water grade) on laying performance, egg quality and chemical composition.

**Comment [R9]:** This sentence is too complex

**Comment [R10]:** Actually, in this paper Torrico et al (2012) reported...

**Comment [R11]:** The same as in case of the title

## MATERIALS AND METHODS

### Experimental site

The experiment was carried out at the Poultry Unit of Teaching and Research Farm, Faculty of Agricultural sciences, LadokeAkintola University of Technology, Oyo state, Nigeria which lies on longitude 4°10' East at Greenwich meridian and latitude 8°10' North at equator. The altitude is between 300 and 600m above sea level while the mean temperature and annual rainfall are 27°C and 1247mm respectively (Ayinla and Odetoje, 2015).

### Procurement of test ingredient

Dried clove buds was purchased from OjaJagun market in Ogbomosho, Oyo state. The dried clove buds was ground into a powdered form and stored for usage.

### Experimental animals and management

Two hundred and ten (210) point of lay (18 weeks old) of Isa Brown breed were purchased from a reputable farm. The birds was weighed prior to the commencement of the experiment and distributed randomly into seven (7) treatments. Thirty birds were assigned to each treatment which was replicated ten times at three (3) birds per replicate. The birds were managed in a 2-tier battery cage. Before the arrival of birds the cage and pen area was cleaned and disinfected. All necessary vaccination and medication was administered. Feeding (Metabolizable Energy (ME): 2700Kcal/kg; Crude Protein (CP): 18%), drinking, drugs, vaccination, egg collection was properly monitored. The experiment lasted for 8(weeks).

**Comment [R12]:** The bioactive substances content in clove buds was analyzed? It is impossible to standardized the plant material as well as some chemical components may have negative influence on birds' organism

**Comment [R13]:** It is not necessary to write numbers by words

### Experimental layout

Diet 1: Control (0% clove in feed and water).

Diet 2: 0.5g of clove/1kg of feed.

Diet 3: 1.0g of clove/1kg of feed.

Diet 4: 0.5g of clove/1litre of water.

Diet 5: 1.0g of clove/1litre of water.

Diet 6: 0.25g of clove/1kg of feed and 0.25g of clove/litre of water.

Diet 7: 0.5g of clove/1kg of feed and 0.5g of clove/litre of water.

**Comment [R14]:** Some grammar mistakes. Probably the native speaker revision is needed

**Comment [R15]:** How the experiment factor was given to feed/water? Before feed mixture preparation? The feed form was mesh or pelleted? The additive was administered every day or periodically? How the water with experimental factor was applied?

## Data collection

### Feed intake

The experimental diets offered to the birds were weighed and leftovers were collected and weighed. The difference between the quantity offered and the leftover was calculated to get the daily feed intake as follows:

$$\text{Feed intake (gram/bird/day)} = \frac{\text{Quantity supplied} - \text{Leftover}}{\text{Number of birds} \times \text{Number of days}}$$

**Comment [R16]:** These formulas are very basic and well known. It is not necessary to present them

### Weight gain

The weight of birds was taken and recorded weekly using a weighing scale. The average weight of birds was calculated among each replicates and treatments.

**Comment [R17]:** Balance paramters

### Feed conversion ratio

Feed conversion ratio is calculated thus;  $\frac{\text{Feed intake}}{\text{Weight gain}}$

**Hen-day production (HDEP):** This was calculated as

$$\text{HDEP} = \frac{\text{Total number of eggs produced during the week}}{\text{Total number of birds} \times \text{No of days}} \times 100$$

### Mortality

Mortality was also recorded.

### Egg quality analysis

Egg was analyzed based on;

#### Internal egg quality analysis

$$\text{Yolk index} = \frac{\text{Yolk height}}{\text{Yolk width}}$$

Albumen height using spherometer.

Haugh unit =  $100 \log(H + 1.57 - 1.7W^{0.37})$ ; H= Albumen height W= weight of the egg.

Albumen weight = Egg weight - (yolk weight + shell weight + membrane weight).

Yolk colour using yolk fan.

$$\text{Albumen percentage} = \frac{\text{Albumen weight}}{\text{Egg weight}} \times 100$$

$$\text{Yolk percentage} = \frac{\text{Yolk weight}}{\text{Egg weight}} \times 100$$

**Comment [R18]:** The traits of eggs should be presented from external to internal, it is impossible to determine yolk index if egg is not broken. So, from non-destructive to destructive traits, or from external to internal ones

**Comment [R19]:** How many eggs were analyzed? Per group? In total?

**Comment [R20]:** How it was measured? Any laboratory equipment was used?

**Comment [R21]:** What kind of equipment it was?

**Comment [R22]:** Brand of producer

**Comment [R23]:** Again obvious formulas, rather information that proportion were calculated would be better

#### External egg quality analysis

$$\text{Egg shape index} = \frac{\text{Egg width}}{\text{Egg height}}$$

$$\text{Egg shell thickness} = \frac{\text{measurement of the middle} + \text{bottom} + \text{upper Part of the egg}}{3}$$

**Comment [R24]:** Any equipment?

Egg weight using weighing scale

Egg storage

Egg was stored at 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup> weeks and analyzed at the end of the experiment to have 4, 2 and 0 week(s) of storage respectively.

**Comment [R25]:** Accuracy, producer of balance

**Comment [R26]:** How many eggs were stored? The microclimate conditions of the storage place should be added, as well as information where eggs were placed, in eggboxes, eggtrays etc. made of plastic, carton etc.

### Chemical analysis

#### Proximate analysis

The proximate analysis of the eggs was carried out according to the method of A.O.A.C (2005).

#### Lipid profile

Lipid profile of egg samples was determined as published by Richmond (1973) to estimate the total cholesterol, low density lipoprotein, high density lipoprotein, triacylglyceride.

**Comment [R27]:** What kind of parameters were evaluated?

### Statistical analysis

Data collected were analyzed within a completely randomized design in a factorial arrangement (SAS,2000).Significant differences among treatment were separated by Duncan's multiple range test.

**Comment [R28]:** Anova? Manova? What kind of test was used to verify the normal distribution?

## RESULTS AND DISCUSSION

### RESULTS

#### Performance of laying birds fed with clove

Table 1 shows the performance of layers fed with different inclusion levels of clove. There were significant ( $p<0.05$ ) differences among all parameters except water intake and body weight gain. The inclusion level of (0.5g+0.5g) in feed + water has the best performance in all parameters except for feed conversion ratio.

**Comment [R29]:** Rather "had"

#### Inclusion levels of Clove on Physico-Chemical Properties of eggs

Table 2 shows the effect of different inclusion level of clove on egg quality. There were significant ( $p<0.05$ ) differences among the internal egg parameters such as albumen height, haugh unit, yolk index, yolk color, yolk and albumen. The result also shows significant differences ( $p<0.05$ ) among the external parameters such as egg weight, egg shell thickness except for egg shape index, membrane % and shell. The Chemical properties showed that control gave the highest ( $P<0.05$ ) in crude protein, moisture contents and crude fiber (Table 3). The inclusion of clove at 1.0g in either water or feed gave a superior value for Ash, Crude protein and calorific values with 2.55, 36.79 and 1516.82 respectively. Inclusion of clove at 1.0 g in diet and water also improved ( $P<0.05$ ) the lipid profile with a lowest values gotten in LDL (65.46mg/di) highest value in HDL (4.98 mg/di) and Lipid peroxidation (18.36).

**Comment [R30]:** Information that significant differences were found does not contribute anything important. Much more important would be the statement which groups were better or worse than others

#### Mode of Administration of Clove On Physico-Chemical Properties of eggs

Table 3 showed the effect of mode of administration of clove on physico-chemical properties of egg. There were significant differences ( $p<0.05$ ) among the treatments except egg shape index, yolk index yolk (%) percentage and albumen (%) percentage. Birds on feed containing clove had the highest ( $p<0.05$ ) egg weight (48.44), haugh unit (50.48) and shell (%) percentage (10.11) respectively followed those on water containing clove had the highest ( $p<0.05$ ) egg shell thickness (0.31) and albumen height (5.17) while those fed on feed + water containing clove had the highest ( $p<0.05$ ) egg shell thickness (0.31), albumen height (5.39) and yolk colour (4.42). Moreover, birds on control containing no clove had the lowest ( $p<0.05$ ) egg weight (44.87).Eggs from feed+water grade inclusion of clove improved ( $P<0.05$ ) the crude protein and calorific values as compared to other mode of administration and control. Water grade had superior ( $P<0.05$ ) for ash while the highest value for CHO was found in feed grade.Generally, all modes of administration of clove improved ( $P<0.05$ ) the lipid profile of eggs as compared with the control.

**Comment [R31]:** It is the surname of the scientist who published the formula, so from capital letter

#### Storage time on Physico-Chemical Properties of eggs from laying birds fed with Clove

All parameters declined with the storage time for both external and internal quality assessment of egg from layers fed with antioxidants (Table 4). Day 0 recorded highest values ( $P<0.05$ ) for egg weight, shell thickness, albumen height, albumen %, yolk colour and shell % while the least was found at 4<sup>th</sup> week of storage. Similar trend was followed with the

proximate composition but reverse was the case in lipid profile as the quality was improved as the storage time increased.

### Interaction Effect of Method of Administration and Different Inclusion Levels of Clove on Physical and Chemical Properties of Eggs

Table 5 shows the effect of different inclusion levels of clove on physical quality of eggs. There were significant differences in eggshell thickness, albumen height, Haugh unit ( $p < 0.05$ ).

Table 6 shows the effect of method of administration and different inclusion levels of clove on Chemical composition of eggs. Crude protein was positively improved at the 'feed+water' and 'water' method of administration at both inclusion levels as shown in table 4. Crude lipid was increased at 0.5g/kg + 0.5g/kg clove inclusion in feed and water. Energy (calorific) was best at all methods of administration and different inclusion levels compared to control. Cholesterol decreased at the 'water' method of administration at both inclusion levels and at 0.5g/kg +0.5g/l inclusion level in feed and water respectively.

Table 1: Performance of layers fed with different inclusion levels of clove.

Parameters	FEED			WATER		FEED + WATER	
	Control	0.5g	1.0g	0.5g	1.0g	0.25g+0.25g	0.5g+0.5g
Initial	1.33 <sup>c</sup>	1.32 <sup>c</sup>	1.3 <sup>c</sup>	1.38 <sup>b</sup>	1.27 <sup>d</sup>	1.25 <sup>d</sup>	1.41 <sup>a</sup>
Final	1.33 <sup>b</sup>	1.36 <sup>a</sup>	1.31 <sup>b</sup>	1.38 <sup>a</sup>	1.27 <sup>c</sup>	1.26 <sup>c</sup>	1.42 <sup>a</sup>
BWG	0	0.04	0.01	0	0	0.01	0.01
FI(Kg/bird/day)	0.081 <sup>ab</sup>	0.065 <sup>d</sup>	0.076 <sup>c</sup>	0.083 <sup>a</sup>	0.08 <sup>ab</sup>	0.076 <sup>c</sup>	0.085 <sup>a</sup>
MORTALITY	0	0	0	0	0	0	0
%PROD	77.74 <sup>c</sup>	69.88 <sup>e</sup>	71.19 <sup>d</sup>	80.24 <sup>b</sup>	76.9 <sup>c</sup>	72.74 <sup>d</sup>	87.26 <sup>a</sup>
No of egg	11.66 <sup>c</sup>	10.48 <sup>e</sup>	10.68 <sup>d</sup>	12.04 <sup>b</sup>	11.54 <sup>c</sup>	10.91 <sup>d</sup>	13.09 <sup>a</sup>
HDEP	0.78 <sup>c</sup>	0.71 <sup>d</sup>	0.71 <sup>d</sup>	0.81 <sup>b</sup>	0.69 <sup>d</sup>	0.74 <sup>d</sup>	0.87 <sup>a</sup>
FCR	1.28 <sup>c</sup>	1.41 <sup>a</sup>	1.23 <sup>d</sup>	1.27 <sup>c</sup>	1.31 <sup>b</sup>	1.29 <sup>c</sup>	1.28 <sup>c</sup>
WI(L/bird/day)	0.033	0.033	0.033	0.034	0.034	0.034	0.035

<sup>abc</sup> - Means along the same row with different superscripts are significantly different ( $P < 0.05$ ).

BWG- Body weight gain, % PROD - % Production, HDEP- Hen day egg production, FCR- Feed conversion ratio, WI- Water intake.

Table 2: Effect of inclusion levels of Clove on Physico-Chemical properties of Eggs

Parameters	Control	0.5g	1.0g	SEM
Physical Properties				
Egg weight	48.44 <sup>a</sup>	45.11 <sup>b</sup>	45.31 <sup>b</sup>	2.34
Egg shape index	0.78	0.76	0.77	0.23
Egg shell thickness	0.28	0.30	0.30	0.21
Albumen height	4.99	4.50	5.03	1.02
Haugh unit	46.77 <sup>b</sup>	50.43 <sup>a</sup>	47.49 <sup>b</sup>	3.21
Yolk index	0.25	0.21	0.26	0.21
Yolk colour	3.78	3.84	3.83	1.02
Yolk %	27.76 <sup>a</sup>	28.16 <sup>a</sup>	26.39 <sup>b</sup>	2.34

Comment [R32]: The numbers after decimal point should be unified

Comment [R33]: The liveability amounted at 100%? If so this information may be given in results section, and here the row with mortality may be deleted

Comment [R34]: Number of eggs per hen? Per group?

Comment [R35]: Units of all traits must be added

Comment [R36]: There is no information in M&M section that water intake was registered. If so how it was conducted?

Comment [R37]: Units must be added to all traits

Comment [R38]: What with results of last two groups? (0.25g+0.25g and 0.5g+0.5g)

Comment [R39]: It is in fresh eggs?

Comment [R40]: What kind of test was used to compare groups in case of yolk colour?

Albumen %	65.21 <sup>a</sup>	61.32 <sup>b</sup>	62.75 <sup>b</sup>	3.24
Shell %	9.52	10.06	10.00	1.45
Membrane %	0.60	0.52	0.56	0.23
<b>Proximate</b>				
Crude Lipid	22.49 <sup>a</sup>	20.49 <sup>b</sup>	20.78 <sup>b</sup>	0.42
Ash	2.24 <sup>b</sup>	2.40 <sup>a</sup>	2.55 <sup>a</sup>	0.74
Moisture	30.12 <sup>a</sup>	26.84 <sup>b</sup>	27.43 <sup>b</sup>	0.53
Crude protein	34.58 <sup>b</sup>	36.11 <sup>a</sup>	36.79 <sup>a</sup>	0.53
CF	1.64 <sup>a</sup>	1.47 <sup>a</sup>	1.47 <sup>b</sup>	0.20
CHO	13.48 <sup>b</sup>	18.74 <sup>a</sup>	15.75 <sup>b</sup>	0.65
Calorific	1392.05 <sup>b</sup>	1494.32 <sup>a</sup>	1516.82 <sup>a</sup>	10.18
<b>Lipid profile</b>				
CHOLESTROL(mg/di)	276.560	274.300	249.15	5.02
HDL(mg/di)	1.99 <sup>c</sup>	2.03 <sup>b</sup>	4.98 <sup>a</sup>	0.23
LDL(mg/di)	88.86 <sup>a</sup>	68.64 <sup>b</sup>	65.46 <sup>b</sup>	2.43
TRIGLYCERIDE	318.31 <sup>b</sup>	384.19 <sup>a</sup>	311.96 <sup>b</sup>	3.43
Lipid peroxidation	17.24 <sup>a</sup>	16.35 <sup>b</sup>	18.36 <sup>a</sup>	1.21

**Comment [R41]:** How membranes were separated from shells?

**Comment [R42]:** All abbreviations must be explained when 1<sup>st</sup> time appear and also in legend under the table. These parameters are not mentioned in M&M section

UNDER PEER REVIEW

Table 3 Effect of Mode of Administration of Clove on Physico-Chemical properties of Eggs

Parameters	Control	Feeds	Water	Feed+Water	SEM
<b>Physical Properties</b>					
Egg weight	44.87 <sup>c</sup>	48.44 <sup>a</sup>	45.79 <sup>b</sup>	45.78 <sup>b</sup>	0.55
Egg shape index	0.78	0.78	0.76	0.78	1.08
Egg shell thickness	0.28 <sup>b</sup>	0.30 <sup>a</sup>	0.31 <sup>a</sup>	0.31 <sup>a</sup>	0.01
Albumen height	4.99 <sup>b</sup>	4.37 <sup>b</sup>	5.17 <sup>a</sup>	5.39 <sup>a</sup>	0.14
Haugh unit	46.77 <sup>b</sup>	50.48 <sup>a</sup>	47.45 <sup>b</sup>	46.46 <sup>b</sup>	0.80
Yolk index	0.25	0.22	0.26	0.28	0.01
Yolk colour	3.78 <sup>b</sup>	3.67 <sup>b</sup>	3.95 <sup>b</sup>	4.42 <sup>a</sup>	0.16
Yolk %	27.76	27.54	27.01	27.72	0.50
Albumen %	64.21	61.49	62.58	62.76	0.67
Shell %	9.52 <sup>b</sup>	10.11 <sup>a</sup>	9.95 <sup>b</sup>	9.94 <sup>b</sup>	0.20
Membrane %	0.60 <sup>a</sup>	0.55 <sup>b</sup>	0.53 <sup>b</sup>	0.55 <sup>b</sup>	0.04
<b>Proximate composition</b>					
Crude Lipid	25.48 <sup>a</sup>	19.51 <sup>b</sup>	21.18 <sup>ab</sup>	21.20 <sup>ab</sup>	2.32
Ash	2.24 <sup>b</sup>	2.21 <sup>b</sup>	2.91 <sup>a</sup>	1.78 <sup>c</sup>	1.02
Moisture	30.12 <sup>a</sup>	25.37 <sup>c</sup>	28.50 <sup>b</sup>	27.54 <sup>b</sup>	2.12
Crude protein	32.60 <sup>c</sup>	35.11 <sup>b</sup>	35.11 <sup>b</sup>	36.59 <sup>a</sup>	3.23
CF	1.64 <sup>a</sup>	1.66 <sup>a</sup>	1.55 <sup>b</sup>	1.42 <sup>c</sup>	1.07
CHO	13.49 <sup>c</sup>	21.28 <sup>a</sup>	13.09 <sup>c</sup>	17.36 <sup>b</sup>	1.32
Calorific	1392.05 <sup>c</sup>	1526.13 <sup>a</sup>	1454.45 <sup>b</sup>	1536.12 <sup>a</sup>	7.43
<b>Lipid profile</b>					
CHOLESTROL(mg/di)	401.57 <sup>a</sup>	306.38 <sup>b</sup>	276.56 <sup>c</sup>	210.14 <sup>d</sup>	7.28
HDL(mg/di)	4.98 <sup>a</sup>	2.52 <sup>c</sup>	3.90 <sup>b</sup>	1.161 <sup>d</sup>	1.98
LDL(mg/di)	82.42 <sup>a</sup>	68.64 <sup>b</sup>	66.52 <sup>b</sup>	67.54 <sup>b</sup>	3.23
TRIGLYCERIDE	318.31 <sup>c</sup>	396.45 <sup>a</sup>	318.09 <sup>c</sup>	329.70 <sup>b</sup>	3.32
Lipid peroxidation	17.24 <sup>b</sup>	14.19 <sup>c</sup>	23.66 <sup>a</sup>	21.26 <sup>a</sup>	2.46

**Comment [R43]:** According to M&M section 7 different groups were compared, so the same schema should be kept here.

**Comment [R44]:** Again units must be added to each trait

Table 4: Effect of storage time on Physico-Chemical properties of Eggs from layers feed with clove

Parameters	weeks			SEM
	0	2	4	
<b>Physical Properties</b>				
Egg weight	48.55 <sup>a</sup>	46.43 <sup>b</sup>	42.60 <sup>c</sup>	0.36
Egg shape index	0.76	2.59	0.78	0.71
Egg shell thickness	0.51 <sup>a</sup>	0.21 <sup>b</sup>	0.30 <sup>b</sup>	0.12
Albumen height	7.52 <sup>a</sup>	3.62 <sup>b</sup>	3.78 <sup>b</sup>	0.09
Haugh unit	13.10 <sup>c</sup>	61.84 <sup>b</sup>	65.43 <sup>a</sup>	0.52
Yolk index	0.37 <sup>a</sup>	0.21 <sup>b</sup>	0.17 <sup>c</sup>	0.00
Yolk colour	5.45 <sup>a</sup>	5.43 <sup>a</sup>	1.05 <sup>b</sup>	0.10
Yolk %	23.71 <sup>b</sup>	28.88 <sup>a</sup>	29.26 <sup>a</sup>	0.33
Albumen %	32.03 <sup>a</sup>	28.37 <sup>b</sup>	25.79 <sup>c</sup>	0.33
Shell %	66.05 <sup>a</sup>	61.01 <sup>b</sup>	60.60 <sup>b</sup>	0.44
Membrane %	9.66 <sup>b</sup>	9.57 <sup>b</sup>	10.55 <sup>a</sup>	0.13
<b>Proximate</b>				
Crude Lipid				
Ash	2.77 <sup>a</sup>	2.48 <sup>b</sup>	1.61	1.21
Moisture	27.04	27.62	28.03	3.23
Crude protein	40.00 <sup>a</sup>	33.80 <sup>b</sup>	34.77 <sup>c</sup>	4.23
CF	1.49 <sup>b</sup>	1.53 <sup>b</sup>	1.66 <sup>a</sup>	1.32
CHO	10.94 <sup>c</sup>	16.59 <sup>b</sup>	22.58 <sup>a</sup>	3.23
Calorific	1519.85 <sup>a</sup>	1424.60 <sup>b</sup>	1523.60 <sup>a</sup>	4.23
<b>Lipid profile</b>				
CHOLESTROL(mg/di)	284.90	284.31	223.75	4.67
HDL(mg/di)	3.92	3.72	3.90	0.23
LDL(mg/di)	73.58	86.62	54.49	2.13
TRIGLYCERIDE	361.14	414.52	262.96	3.45
Lipid peroxidation	14.02	11.44	26.56	2.01

**Comment [R45]:** It is time of eggs collection or weeks of storage? It seems that it is senseless to show the obvious impact of time on the general quality of eggs. It would be much more informative and valuable to present the changes in the quality of eggs from particular groups.

**Comment [R46]:** Units, abbreviations (see comments above).

**Comment [R47]:** What kind of unit it is? miligrams per what?

Table 5: Interaction effect of mode of administration and inclusion level of clove on egg quality parameters.

Parameters	Control	Feed		Water		Feed +Water		SEM
		0.5	1.0	0.5	1.0	0.25+0.25	0.5+0.5	
Egg wt(g)	48.44 <sup>a</sup>	44.67 <sup>d</sup>	45.06 <sup>cd</sup>	46.02 <sup>b</sup>	45.55 <sup>d</sup>	45.00 <sup>cd</sup>	46.56 <sup>bc</sup>	0.55
Egg shape index	0.78	0.78	0.78	0.77	0.75	0.78	0.78	1.08
STH(mm)	0.28	0.30	0.30	0.32	0.30	0.30	0.32	0.01
Albumen height(mm)	4.99 <sup>bc</sup>	4.06 <sup>d</sup>	4.67 <sup>c</sup>	4.94 <sup>bc</sup>	5.39 <sup>a</sup>	5.22 <sup>ab</sup>	5.56 <sup>a</sup>	0.14
Haugh unit	46.77 <sup>b</sup>	55.24 <sup>b</sup>	45.71 <sup>b</sup>	45.62 <sup>b</sup>	49.27 <sup>a</sup>	45.56 <sup>ab</sup>	47.35 <sup>ab</sup>	0.80
Yolk index	0.25 <sup>bc</sup>	0.20 <sup>d</sup>	0.23 <sup>cd</sup>	0.22 <sup>cd</sup>	0.29 <sup>a</sup>	0.27 <sup>ab</sup>	0.29 <sup>a</sup>	0.01
Yolk colour	3.78 <sup>c</sup>	3.67 <sup>c</sup>	3.67 <sup>c</sup>	4.0 <sup>bc</sup>	3.89 <sup>bc</sup>	4.28 <sup>ab</sup>	4.56 <sup>a</sup>	0.16
Yolk %	27.76 <sup>ab</sup>	29.02 <sup>a</sup>	26.06 <sup>b</sup>	27.30 <sup>b</sup>	26.72 <sup>b</sup>	26.64 <sup>c</sup>	28.79 <sup>a</sup>	0.50
Albumen %	64.21 <sup>ab</sup>	60.35 <sup>d</sup>	62.62 <sup>bc</sup>	62.28 <sup>bcd</sup>	62.88 <sup>ab</sup>	64.80 <sup>a</sup>	60.72 <sup>cd</sup>	0.67
Shell %	9.52	10.15	10.06	9.96	9.94	9.89	9.98	0.20
Membrane%	0.60	0.51	0.58	0.53	0.53	0.63	0.47	0.04

<sup>abc</sup> - Means along the same row with different superscripts are significantly different (P<0.05).

Egg wt – Egg weight (mm), STH (mm) - Shell Thickness

**Comment [R48]:** According to statistical model described in M&M section only this form of results presentation is correct but probably it is possible to combine tables 3, 4 and 5?

Table 6: Effect of Method of Administration and Different Inclusion Levels of Clove on Proximate Composition of Egg

Parameters	Feed			Water		Feed+ Water		SEM
	Control	0.5g/kg	1.0g/kg	0.5g/l	1.0g/l	0.25g/kg+0.25g/l	0.5g/kg+0.5g/l	
<b>Proximate</b>								
Crude lipid	21.49 <sup>a</sup>	19.67 <sup>b</sup>	19.35 <sup>b</sup>	23.27 <sup>a</sup>	19.10 <sup>b</sup>	18.53 <sup>c</sup>	23.90 <sup>a</sup>	0.73
Ash	2.24 <sup>b</sup>	2.48 <sup>b</sup>	1.94 <sup>c</sup>	3.02 <sup>a</sup>	2.80 <sup>a</sup>	1.56 <sup>c</sup>	2.00 <sup>b</sup>	0.13
Moisture	30.12 <sup>a</sup>	26.65 <sup>b</sup>	24.10 <sup>c</sup>	26.93 <sup>b</sup>	30.07 <sup>a</sup>	26.96 <sup>b</sup>	28.13 <sup>b</sup>	0.92
Crude protein	34.57 <sup>b</sup>	34.57 <sup>b</sup>	35.66 <sup>b</sup>	36.33 <sup>a</sup>	36.86 <sup>a</sup>	37.43 <sup>a</sup>	37.87 <sup>a</sup>	0.21
Crude fiber	1.64 <sup>b</sup>	1.58 <sup>b</sup>	1.74 <sup>a</sup>	1.47 <sup>c</sup>	1.64 <sup>b</sup>	1.36 <sup>c</sup>	1.49 <sup>c</sup>	0.04
Cholesterol	13.48 <sup>b</sup>	20.70 <sup>a</sup>	21.85 <sup>a</sup>	13.38 <sup>b</sup>	12.80 <sup>c</sup>	22.12 <sup>a</sup>	12.61 <sup>c</sup>	1.13
Calorific	1392.05 <sup>c</sup>	1499.64 <sup>b</sup>	1552.64 <sup>a</sup>	1454.90 <sup>b</sup>	1454.02 <sup>b</sup>	1595.93 <sup>a</sup>	1476.31 <sup>b</sup>	17.64
<b>Lipid profile</b>								
CHOL	276.56	326.07	286.69	265.82	271.49	231.01	189.26	3.21
HDL	4.98	2.10	0.83	1.88	4.04	2.10	1.11	0.36
LDL	68.64	87.62	77.22	89.25	43.79	59.71	75.37	3.32
TAG	318.31	427.61	365.28	436.90	199.28	288.07	371.33	3.32
LIPID	17.24	11.42	16.96	14.07	19.17	23.56	18.96	2.54
<b>PEROXIDATION</b>								

<sup>a,b,c</sup> shows that there are significant differences between means having different superscripts within the row.

## DISCUSSION

The inclusion of clove in the feed and drinking water of layers have effect on the Final body weight, body weight gain, daily feed intake, feed conversion ratio, % Production, number of eggs produced and egg production rate. The influence of clove on the body of these birds could be as a result of its antimicrobial and anti-protozoal properties which according to Kale *et al.*, (2003); Bishnuet *al.*, (2009), helped to reduce the microbial load and thus improved the feed consumption and feed efficiency of the birds. Hanafy and Hatam, (1991) corroborated the claim the improvement in weight gain of the birds using clove in their feed and water may probably be due to the fact that clove extract inhibits growth of intestinal bacteria such as *S. aureus* and *E. coli*.

This result was also in line with the report of Siddig and Abdelati, (2001) in their work on broiler fed rations containing turmeric and cloves showing higher weight gain. Canogullariet *al.*, (2009) found that 1% garlic supplementation gave heavier weight of hen compared to the 2% dose. There was no mortality among the treated birds throughout the duration of experiment an indication that clove in the feed and drinking water of the birds were not deleterious to the health of the laying hens. Although birds fed rations supplemented with clove powder utilized their feed more efficiently as those fed ration without addition of the clove and improved their % production even better than control. These results agreed with results of Kaya *et al.*, (2013), Gandomaniet *al.*, (2014), Bozkurt *et al.*, (2012), who observed improvement of egg production of laying hens fed diets supplemented with clove.

Effects of different inclusion level of clove on egg quality (Table 2) shows that the egg weight was highest in control group and those birds placed on treatments shows depressed value in term of egg weight. The addition of clove powder supplementation to feed and water had a significant effect. These results are in contrast with Gandomaniet *al.*, (2014), who introduced that the clove buds added into hens feed had significant effect on egg weight. Li *et al.*, (2010) also reported that there is a significant correlation between daily feed intake on egg production and egg weight; they reported that a reduction in feed intake decreased egg weight. Egg shell thickness were depressed in all treatment groups and only 0.5g in water and 0.5g+0.5g in feed + water that shows better shell thickness. The significance decreased in egg shell thickness may be due to an inadequate or improper absorption of calcium due to supplementation of clove. This disagrees with the findings of Lawal, (1992) who reported that supplementation of cassava root meal in the diets of hens was not impaired with the calcium metabolism. Shell thickness is a function of the calcium and phosphorus levels in layers ration. It is also an indication of the specific gravity (relative density of eggs) since both are positively correlated Oluyemi and Roberts, (1979). The results indicate that the addition of clove powder into complete feed mixtures and drinking water for laying hens had a negative impact on albumen height. Haugh unit score, the key indicator of interior egg quality, similarity was observed in all dietary treatment. This result is corroborate with the findings of Radwanet *al.*, (2008) who reported that non- significance influenced by diets supplemented with thyme powder.

In egg yolk index, similarity was observed but it was improved in 1.0g in water, (0.25g+0.25g) in feed + water and (0.5g+0.5g) in feed + water respectively. This disagree with Nasiroleslamiet *al.*, (2010), who adding essential oils of fennel (*Foeniculum vulgare*) and ginger (*Zingiberofficinale*) to diets did not effect on egg index and yolk index. In egg yolk colour, similarity was also observed but it increased in (0.25+0.25 feed and water) and (0.5+0.5 feed and water) supplemented with clove powder. Shades of yellow colours on the

**Comment [R49]:** These bacteria are generally harmful but not only in intestines

**Comment [R50]:** Weight of what? Body weight gain?

**Comment [R51]:** What is the connection between this paper and the cited one?

**Comment [R52]:** In this paper thyme, sage and mentha were used

**Comment [R53]:** In this article additive composed of 6 essential oils was used as follow: oregano oil (*Origanum sp.*), laurel leaf oil (*Laurusnobilis*), sage leaf oil (*Salvia triloba*), myrtle leaf oil (*Myrtuscommunis*), fennel seed oil (*Foeniculum vulgare*), and citrus peel oil (*Citrus sp.*). There were no cloves

**Comment [R54]:** there is no specific gravity in the results

**Comment [R55]:** maybe it would be better to use numbers of groups?

colour scale Hoffman La Roche were in the normal range for the add clove powder. Gandomaniet *et al.*, (2014) observed after the addition of clove bud no significant difference to the yolk colour. (Yang *et al.*, 2003) increased the intensity in yolk colour of addition 2% concentration of green tea. Canogullariet *et al.*, (2009) and Aziza *et al.*, (2013) recorded in the experimental group with the addition of saffron in a dose of 20 mg/kg significantly different in yolk colour.

Comment [R56]: ????

Comment [R57]: The yolks were very bright in all groups and increased in factorial groups

Comment [R58]: ???

Interaction effect of storage time and mode of administration of clove on the proximate composition of eggs laid by the birds was also significance on the ether extract, ash, moisture, crude protein, crude fibre, cholesterol and calorific. Ether extract is favorably influenced by the clove supplementation and duration of storage. There is a significant correlation between 1.0g in feed and 0.5g in water at 0 week. Similar trends also observed at 4weeks. The possible reason behind this might be the higher moisture retention by clove. This finds contradict with the findings of Goswami *et al.*, (2013) on effect of natural preservatives on sensory and microbiological properties of quail egg reported no significant differences for the mean protein, fat and ash contents in between the treatments throughout the storage period. However, protein and cholesterol content decreased with minor values during storage in all treatments due to lipid and protein oxidation.

Comment [R59]: The results presentation doesn't show interaction between supplementation and time of storage

The incorporation of clove affected considerably total egg cholesterol, high density lipoprotein, Low Density Lipoprotein (LDL) and Triacylglyceride (TAG). Therefore, during the 0 week and 2 weeks the total cholesterol in eggs increased again. Canogullariet *et al.*, (2009), Khan *et al.*, (2007), Yalçinet *et al.*, (2006) and Chowdhury *et al.*, (2002) reported that clove supplementation powder significantly decreased the egg cholesterol content. Azeke and Ekpo, (2009), incorporated garlic and tea powder with different doses into laying hen feeds. They observed a significant reduction in total cholesterol of egg yolk, low density lipoprotein (LDL), high density lipoprotein (HDL) and total triglycerides which is also observed in this study. Though weeks of egg storage laid by laying birds supplemented clove into their feed and water especially at 4weeks storage were affected favourably. Azeke and Ekpo, (2009) explained further that anticholesterolemic agents found in clove can be responsible for reducing the cholesterol content of egg yolk. Many researches proved clinically the cholesterol-lowering effect of clove in blood plasma. Indeed, Warshafsky *et al.*, (1993) also finds that the results of meta-analysis of the controlled trials of garlic showed a significant reduction in total cholesterol levels.

Comment [R60]: Based on other references own results should be justified

Comment [R61]: Typically used abbreviation is TG

Comment [R62]: It is not showed anywhere. Also it would be justified if the cholesterol level would be verified after supplementation. Not after storage

## CONCLUSION AND RECOMMENDATIONS

### Conclusion

Addition of clove (*Syzygium aromaticum*) powder slightly increased performance of the layer birds in term of egg production at 0.5g (clove in their water) and 0.5g+ 0.5g (clove powder in their feed and water) levels respectively. The results also indicated that internal egg qualities were best at the combination of feed and water (0.5g+ 0.5g).

The interaction effect of storage time and mode of administration of clove on the proximate composition of eggs laid by the birds was best at 0 week and 4 weeks storage duration.

Finally, storage time on lipid profile of eggs laid by birds fed with clove was observed to be best at 4 weeks because it is reduced egg cholesterol.

Comment [R63]: And here, at the end of article, it absolutely confusing. What kind of plant derived additive was used? Cloves (*Syzygium aromaticum*) or cloves of garlic (*Allium sativum*)???? Probably these inconsistencies, of which there are many throughout the work, are due to incorrect translation from English

### Recommendations

Clove inclusion in feed and water at 0.5g+0.5g respectively can be recommended to laying birds to improve their laying performance, egg quality and chemical composition.

Comment [R64]: It is impossible to conclude it from presented results especially as there are no statistically significant differences in the table

Further investigations need to be carried out to increase the level of clove powder above stated level in neither water nor feed of laying birds, may be it will increased the egg production above 87% as obtained in this study.

**Comment [R65]:** ???

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**Comment [R66]:** Generally, cited references are relatively old, there is a lot of newer references linked to the topic

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**Comment [R71]:** The original title must be kept

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