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Full Length Research

Evaluation of egg quality traits in Lohmann Brown layers treated with clomiphene citrate

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ABSTRACT: The study's objective was to assess how Clomiphene Citrate (CC) treatment affected several aspects of egg quality in Lohmann Brown (LB) layer characteristics. The study was Phase I (Non-mating Phase) research, which deals with the properties of eggs. In this experiment, CC was given orally to 18 LB hens for six weeks (stimulatory stage) and then withheld for another six weeks (withdrawal stage) using a repeated measures completely randomised design. The Statistical Package for the Social Sciences (SPSS) version 16.0 and GraphPad InStat software were used to analyse the data. When compared to the equivalent characteristics after CC administration, the egg weight, width and length, yolk and albumen volumes and shell weight were significantly different (P<0.05). Therefore, it is advised to give CC orally at 15 mg/kg to LB chickens in order to improve the egg quality indices.

Keywords: Clomiphene citrate, egg quality, non-mating phase, stimulatory stage, withdrawal stage.

INTRODUCTION

One of the cheapest, most affordable and acceptable animal products is the poultry egg because it is highly nutritious and serves important roles in many food products due to its functional properties (Silversides and Scott, 2001). According to the Population Reference Bureau (PRB, 2013), Nigeria has a population of 174 million people, but over 70% of the population lives on less than a dollar per day (Watts, 2006). The increase, however, is not proportional to the growth in poultry production due to various drawbacks such as infectious diseases, management problems, hatchery problems, among others (Kalita *et al*., 2013). Poultry production thus offers an excellent means through which the animal protein needs of the populace can be met (Boland *et al*., 2013; Oleforuh-okoleh *et al*., 2016) because they have short generational and incubation intervals, high prolificacy, fast growth rate and are easy to raise (Oluyemi and Roberts, 2000; Kabir and Olufemi, 2013).

Poultry meat and egg production have been reported to show an increasing trend with the introduction of improved breeds/ strains (Haleem, 2009). The Lohmann Brown is a hybrid chicken layer selectively bred from the New Hampshires and other brown layer strains. They start to lay when they are about 18 weeks of age and can lay up to 300 brown eggs in a year, at one egg per hen per day (Feltwell, 2011). They are a very inquisitive strain of chicken that lay their eggs daily – normally at dawn or dusk. Most Lohmann Brown hens have a caramel/ brown shade of feathers in a pattern around their necks, and white feathers at the tips of their tail feathers (Feltwell, 2011; Riggs *et al*., 2011). An egg is a biological structure intended by nature for reproduction. The yolk present in the egg protects and provides nourishment for the developing embryo and serves as the principal source of food during the first few days of the chick's life (Tabeekh, 2011).

Several factors that affect egg quality include: egg size (Butcher and Miles, 2003), age of birds (Coutts and Wilson, 1990), genetics and diseases (Jones, 2006), and stress (Igbokwe, 2018). The efficiency of reproduction of hens has been reported to change with age, which is related to the internal egg composition or ratio; larger egg weight, poor shell quality, increased early and late embryo mortality (Leeson and Summers, 2005), albumen quality deterioration (Tona *et al*., 2004) and an increase in yolk cholesterol content (Dikmen and Sahan, 2007).

Egg quality is a factor which contributes to better profitability in terms of fertile and table eggs (Hanusová *et al*., 2015). Quantitative traits of economic importance that show continuous variability include egg morphometric variables such as weight, size, albumen and yolk content (Islam and Dutta, 2010). It is a fact that the shell, albumen and yolk of an egg are in direct proportion to its weight, and these vary significantly among breeds and strains of poultry species (Jones *et al*., 2010; Momoh *et al*., 2010). It is also reported that as hens age, the weight of their eggs increases (Roque and Soares, 1994), shell thickness decreases (Peebles *et al*., 2000), and the proportion of yolk increases at the expense of albumen and eggshell (Suarez *et al*., 1997). Genetic and environmental factors influence egg quality; therefore, hens laying substandard eggs should be culled, even though improving their management can help in improving the quality of the eggs (Melesse *et al*., 2013). Eggs that have unnatural shape or poor shell quality, too, are not desired as such eggs usually have poor hatchability, and if the chicks hatch out, they rarely survive or grow well (Ori, 2011).

Apart from management systems and other improved husbandry techniques, the reproductive performance of other livestock has been improved using fertility drugs like CC (Clomid®) to enhance semen and egg production (Adebambo, 2005). CC is a non-steroidal anti-estrogen which also possesses weak estrogen properties (McGinnis and Wallace, 1973). CC significantly increased early egg production in some pullets when fed for approximately 8 weeks prior to the beginning of egg production (WHO, 2015). Allanah and Bratte (2015) reported that oral administration of 15 mg/kg CC body weight improves fertility in cocks. However, high dietary levels (1000 mg/kg) administered for one week produced a severe reduction in egg production and a heavy moult in hens (McGinnis and Wallace, 1973). CC is reported to be absorbed in the gastrointestinal tract, metabolised in the liver and generally well tolerated in the body (Ara and Asmatullah, 2011). This work seeks to compare egg quality parameters during and after oral administration of Clomiphene Citrate (CC) in Lohmann Brown hens in the non-mating phase.

MATERIALS AND METHODS

The Experiment was carried out at the Poultry Unit of the Department of Animal Science (GPS Coordinates: N11.97643º, E008.42995º), located within the Faculty of Agriculture Teaching and Research Farm, Bayero University, Kano.

Sample size determination

The resource equation of Mead *et al*. (2012) was used in determining the sample size. The equation is as follows:

$$N-1 = T + E$$

Where: N = number of experimental units, T = number of degrees of freedom (number of test groups – 1), and E = number that estimates the error of the remainder.

Mead *et al*. (2012) proposed that E should be between 10 and 20. The sample size was calculated as follows:

$$18 - 1 = (2 - 1) + E$$

$$N - 1 = T + E$$

$$E = 18 - 2 = 16$$

Therefore, the sample size used was 18 because it gives an estimate of error (E) that is in the range of 10 to 20.

Experimental design and treatment

The study was laid out in a repeated measures design with two treatments (Clomiphene Citrate stimulation and Clomiphene Citrate withdrawal). Clomiphene Citrate (CC) (Global Napi Pharmaceuticals, Sanofi Aventis, France) was administered to eighteen (18) Lohmann Brown hens at 15 mg/kg body weight per *os* for six weeks (stimulatory stage) and withdrawn for another six weeks (withdrawal stage). Therefore, the two stages of the experiment lasted for a total period of twelve weeks.

Acquisition and management of experimental birds

A total of 36 Lohmann Brown layers weighing between 1.2 and 2.0 kg, aged 37 weeks, were used for the study. The birds were purchased at point of lay from the Sovet International Limited and housed in battery cages. Layer mash was purchased from the Sovet International Limited and fed to the hens in the morning around 8 am and afternoon around 2 pm with a commercial layer ration (Super Layer®) containing 16.0 % crude protein, 5.0 % fat, 6.0 % fibre, 3.5 % calcium, 0.4 % phosphorus and 2600 kcal/kg metabolizable energy. Clean water was served *ad libitum*. The birds were given Anupco Vitalyte Extra® Multivitamin anti-stressor (Anglian Nutrition Products Company, UK) through their drinking water at 0.5 g per litter during the stabilisation period of two weeks, to reduce transportation stress.

Egg quality parameters

Egg quality parameters during the stimulatory and withdrawal stages of the experiment were recorded as

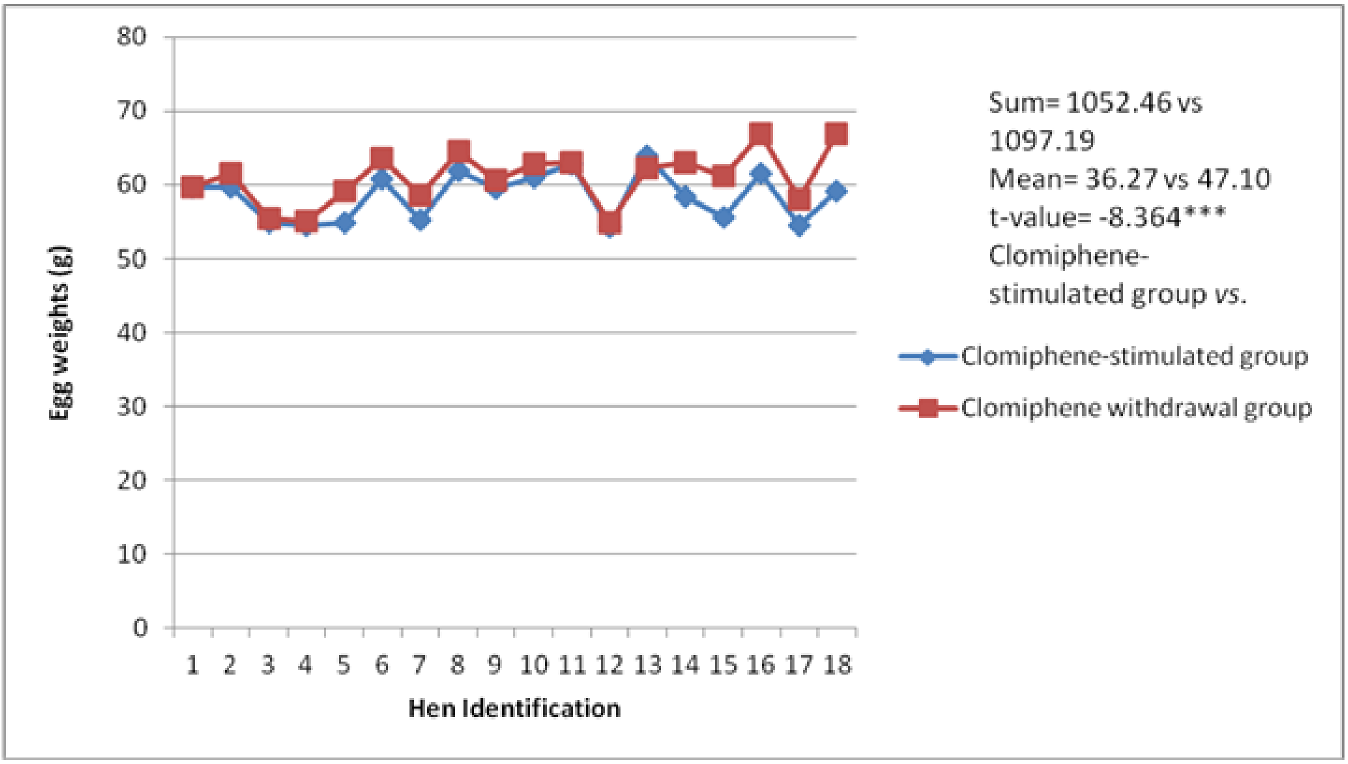

Figure 1. Egg weight of lohmann brown eggs during and after oral clomiphene citrate administration.

follows: Egg length and egg width were measured with a Vernier calliper along the longitudinal and equatorial axis accordingly. A hole was made in each egg to expel the albumen into a measuring cylinder, after which the egg shell was gently broken, and the yolk was carefully poured into another measuring cylinder. Yolk and albumen volumes of each egg were measured from the measuring cylinders in millimeters (ml) (Oyebanji and Atoki, 2018).

Egg shells were dried at room temperature for 24 hours after cleaning the remaining albumen inside the shell with a tissue. Shell weight (with membrane) was measured using a laboratory scale by placing the dried shell on the scale and recording its weight as described by Anderson *et al*. (2004).

Shell thickness (with membrane) was measured at the sharp poles, blunt poles and equatorial parts of each egg with a Vernier calliper. Shell thickness was obtained from the average values of these three parts (Oyebanji and Atoki, 2018).

Statistical analysis

The data obtained was analysed with a paired samples t-test in the Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA), to compare egg quality parameters during and after Clomiphene Citrate administration in the Lohmann Brown hens.

RESULTS

Egg quality parameters

Changes in egg weight during and after administering Clomiphene Citrate (CC) to the Lohmann Brown hens are presented in Figure 1. The mean egg weight (47.10 g) after the CC administration was significantly (t-value = -8.364; $P<0.001$) higher than the mean egg weight (36.27 g) during the administration period. Changes in egg width of the Lohmann Brown eggs during and after oral CC administration are presented in Figure 2. The mean egg width (33.23 mm) after CC treatment was significantly (t-value = -7.76; $p<0.001$) higher than the corresponding mean value (25.47 mm) during the time of CC administration. Egg length was significantly (t-value = -6.43; $P<0.001$) higher at 44.27 mm after the treatment period compared to the corresponding value of 35.26 mm recorded for the CC treatment period (Figure 3). Changes in the mean shell thickness of the Lohmann Brown eggs after the CC withdrawal period (0.31 mm) were (t-value = -1.6; $p>0.05$) lower than during the period of CC administration at 0.33 mm (Figure 4), even though differences were not significant. The mean shell weight (6.18 g) of eggs collected after the withdrawal period was significantly (t-value = -11.52; $P<0.001$) higher than the mean shell weight (4.44 g) of eggs collected during the administration period (Figure 5). The mean yolk volume of

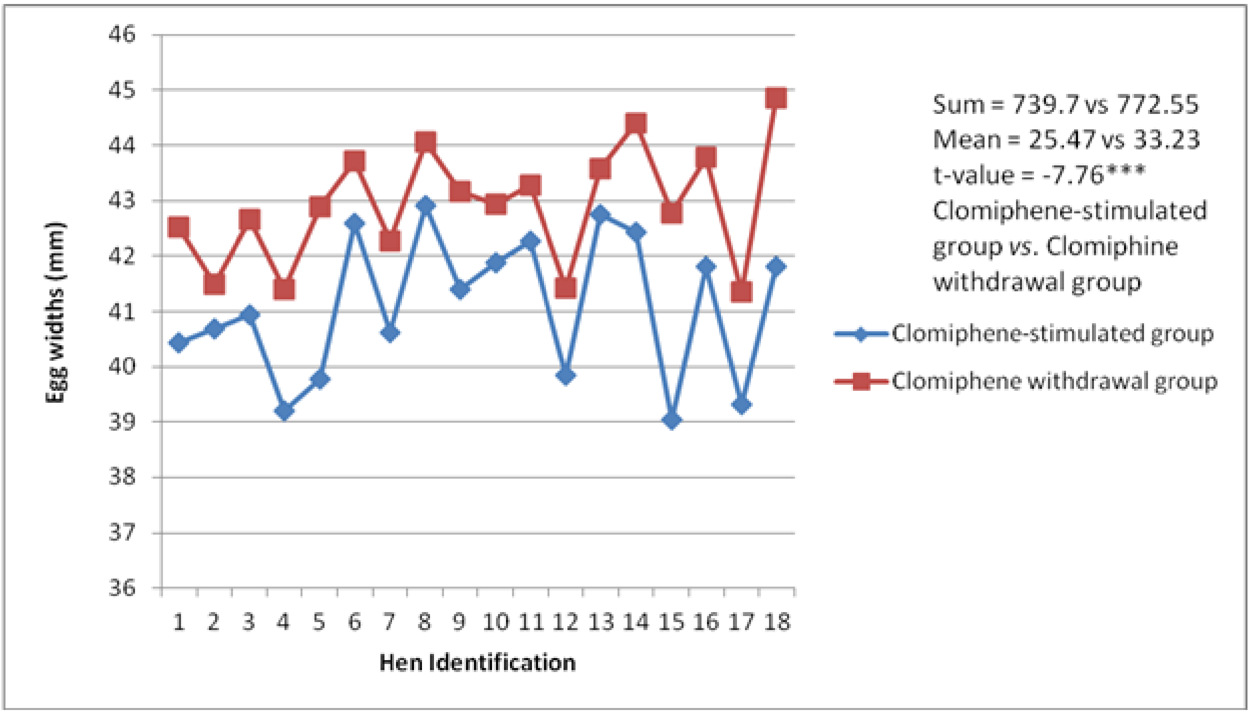

Figure 2. Egg width of Lohmann Brown eggs during and after oral clomiphene citrate administration.

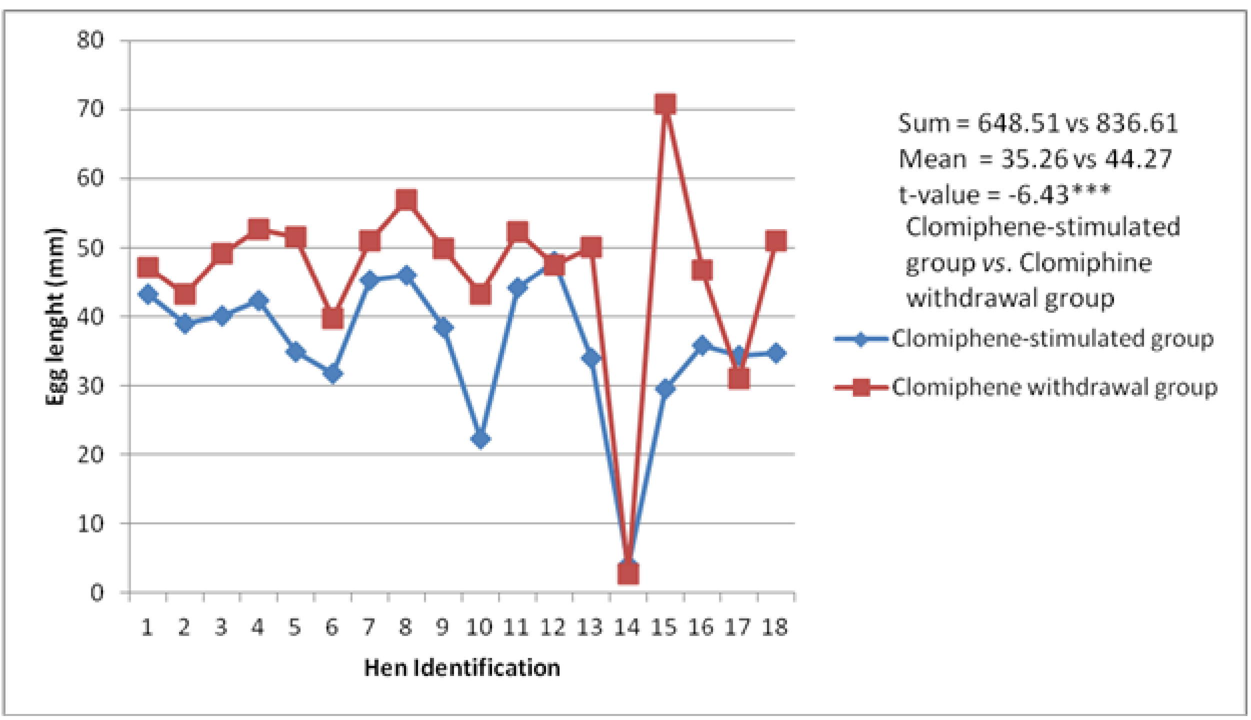

Figure 3. Egg length of Lohmann brown eggs during and after oral clomiphene citrate administration.

the eggs was significantly (t-value = -8.434; P<0.001) more after the withdrawal stage (12.27 ml) than at the stimulatory stage (9.42 ml) (Figure 6). Similarly, albumen volume was significantly (t-value = -8.40; P<0.001) higher in eggs collected after the withdrawal (29.48 ml) than during the stimulatory (22.64 ml) stages (Figure 7).

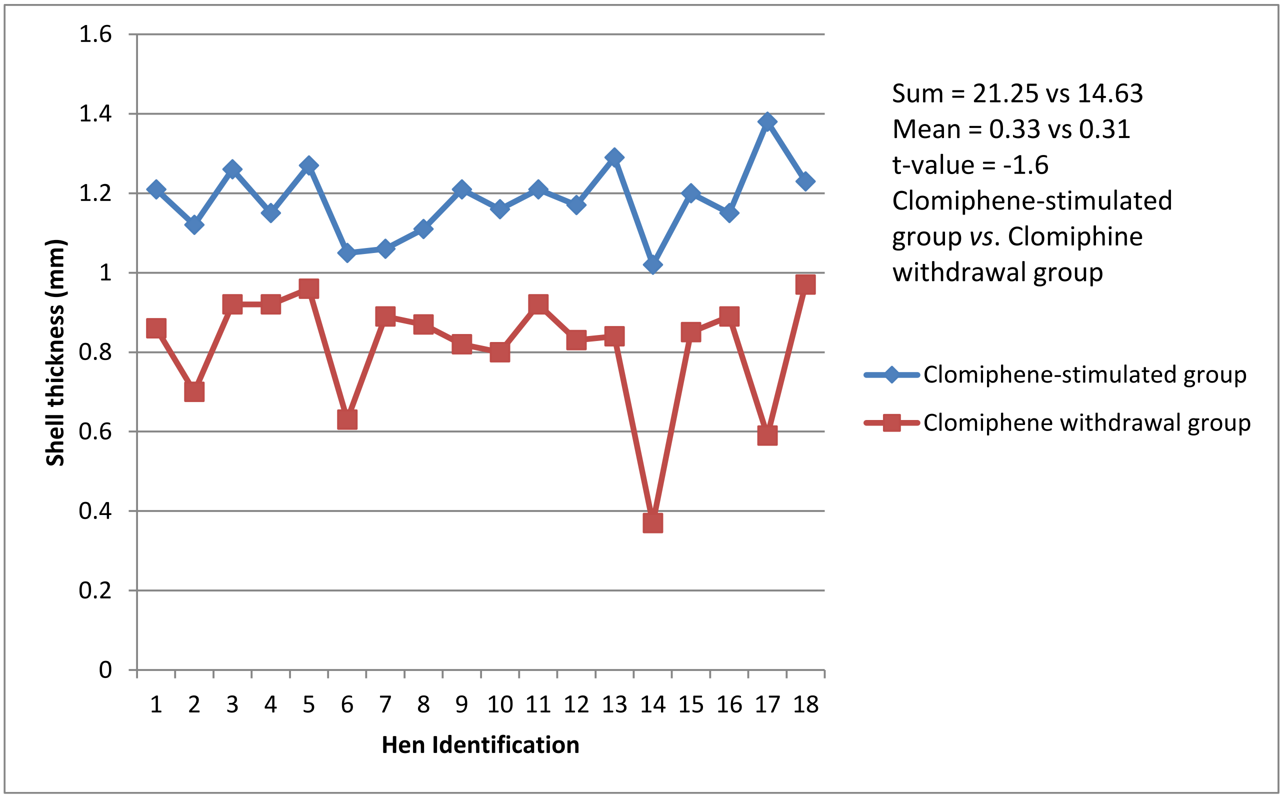

Figure 4. Shell thickness of Lohmann Brown Eggs during and after oral clomiphene citrate administration.

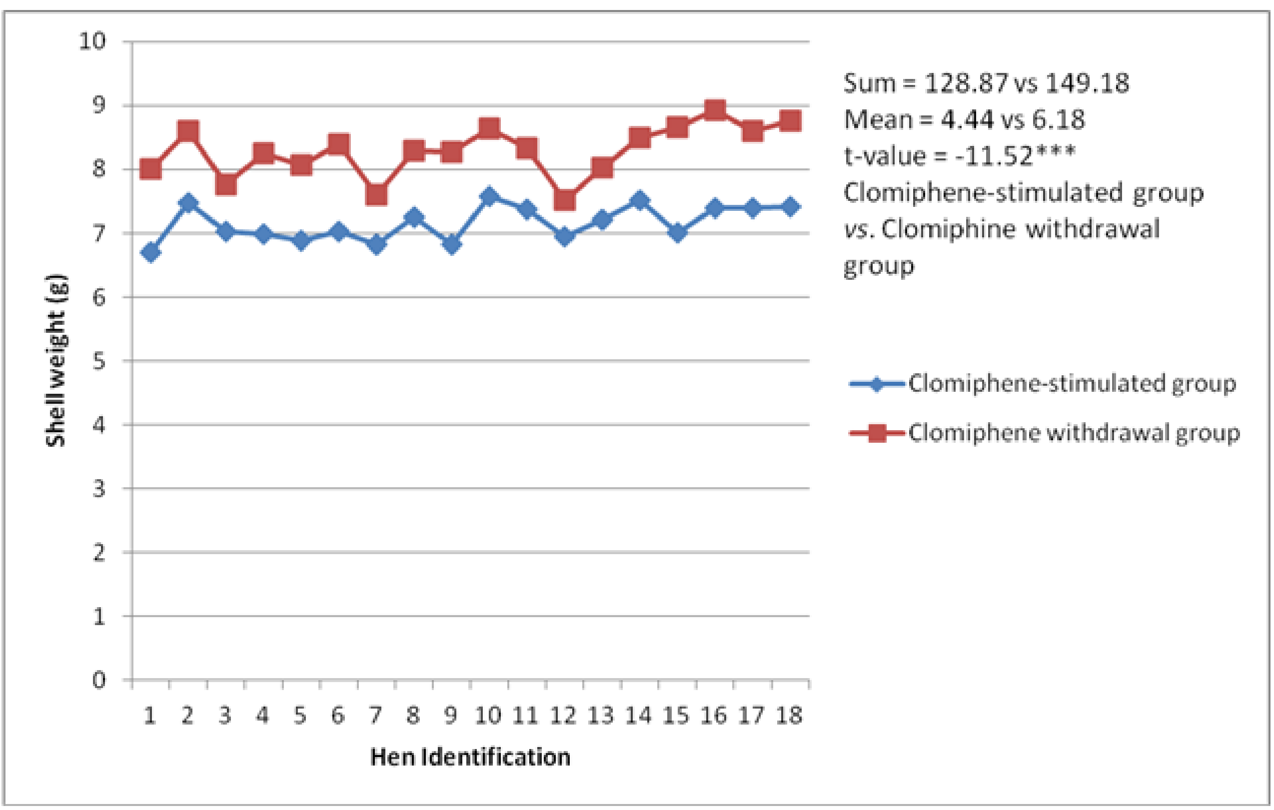

Figure 5. Shell weight of Lohmann Brown eggs during and after oral clomiphene citrate administration.

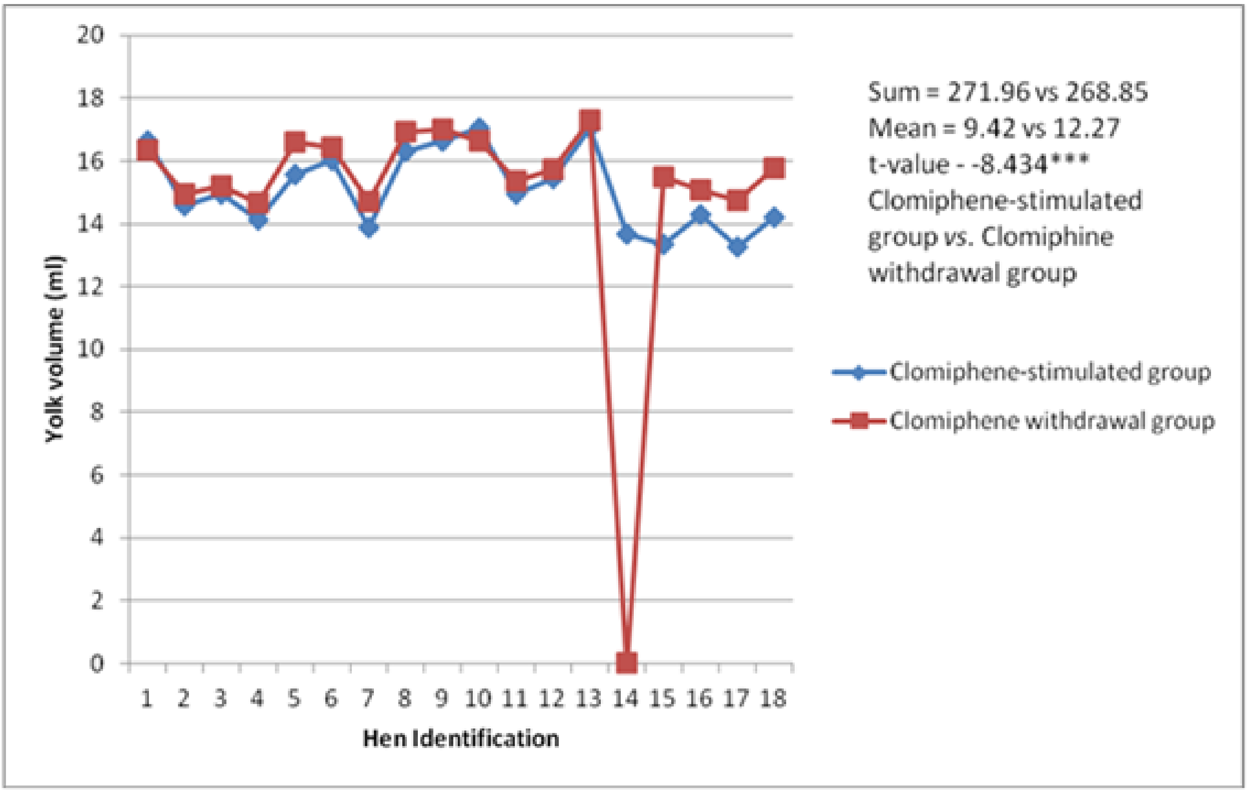

Figure 6. Yolk volume of Lohmann Brown eggs during and after oral clomiphene citrate administration.

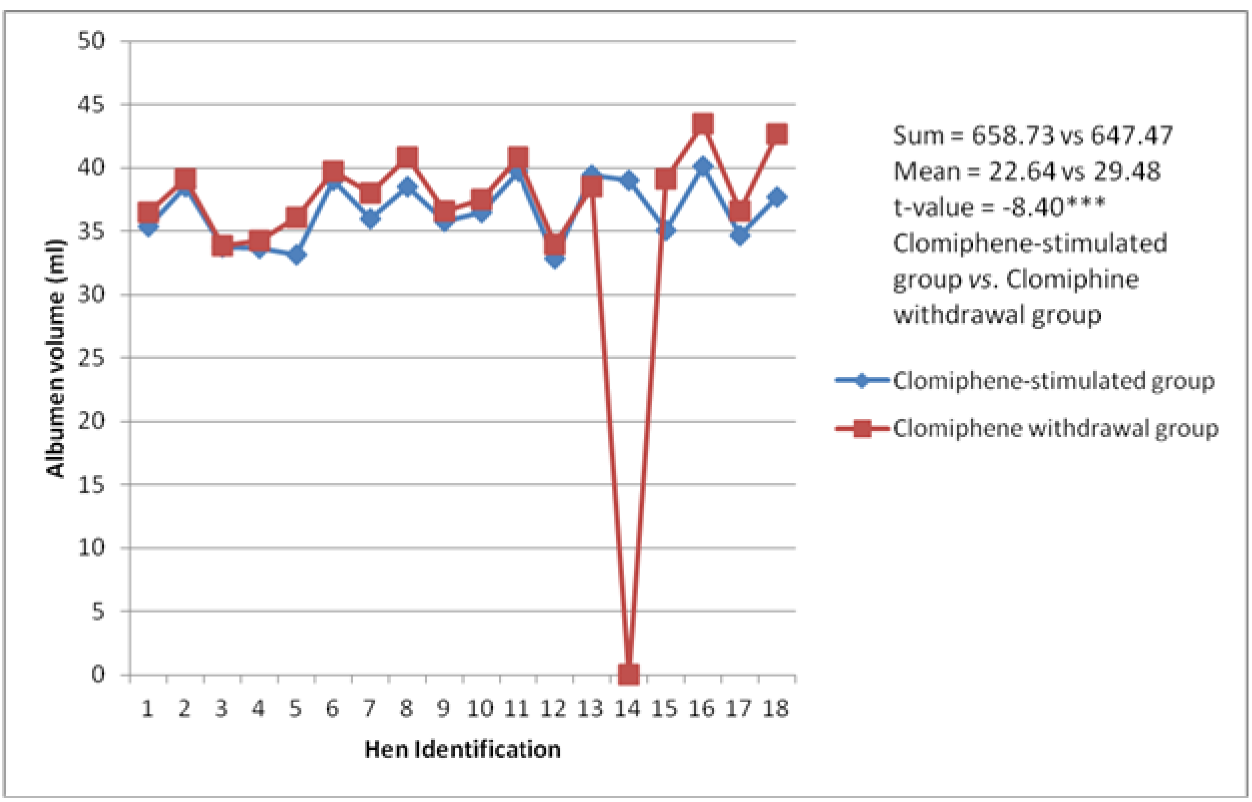

Figure 7. Albumen volume of Lohmann Brown eggs during and after oral clomiphene citrate administration.

DISCUSSION

External egg quality parameters

The result for the egg weights did not agree with the findings of McGinnis and Wallace (1973), who reported that Clomiphene Citrate (CC) did not affect egg weight of Single Comb White Leghorn Layers. The differences could be attributable to the fact that in one trial, they used 19-week-old pullets kept in floor pens and administered CC at 0 and 120 mg/kg of feed. In their other trial, they used 15 to 18-month-old cage layers and administered CC at 0, 250, 500 and 1000 mg/kg of feed for 7 days with no light restriction and observed non-significant increases in egg weight. The current study administered CC at 15 mg/kg orally in drinking water and not through the feed; light was not provided for 24 hours but between 6 and 10 hours daily, and the Lohmann Brown layers used were 36 weeks old. The weight of the eggs correlated with the age of the hens, as older hens are reported to lay heavier eggs than younger ones (Padhi *et al*., 2013), considering that older hens were used in the current study and administered CC for six weeks before withdrawal for another six weeks. High dietary levels of CC have been reported to cause inhibition of gonadotropin secretion (McGinnis and Wallace, 1973), but the level administered did not inhibit gonadotropic secretion in the hens. Zhang *et al*. (2005) reported that the weight of an egg influences the weight of its components as well. This is in line with the present result, whereby all the other components of egg quality were lower in hens administered CC compared to those on withdrawal, except for shell thickness, which appears higher in those administered CC at the stimulation stage, even though differences did not reach significant levels. Shell weight in the hens after CC administration was higher compared to the hens at stimulation in the current study. No observed differences were found on the relative shell content of eggs (Williams, 2015), while egg shell percentage and shell thickness (mm) in another study were observed to be significantly affected, with the highest records obtained in some local chickens administered 1 mg per kg body weight (Hanafy, 2011) of CC. In the current study, however, shell thickness was not affected. It has been estimated to be necessary for an egg to have at least a 50% chance to withstand normal handling conditions without breakage, but it should at least have an egg shell thickness of 0.33 mm (Stadelman, 1995). This work entails that CC had an effect on shell weight in the study; hens were administered at 15 mg/kg body weight which could be as a result of the fact that those that laid heavier eggs recorded higher weights and higher internal content weights compared to those with smaller eggs having low internal content weights. This is because heavier eggs were laid at the withdrawal stage than at the stimulatory stage, even though the same strain of Lohmann Brown was used.

Internal egg quality parameters

Wagner and Williams (2007) reported that tamoxifen had no effect on albumen content of eggs. Biswas *et al.* (2010) also did not observe any differences in yolk and albumen weight when they administered Tamoxifen at 1, 0.5 and 2.5 mg per kg body weight, contrary to the 15 mg per kg body weight used in this study. Tamoxifen is shown to have comparable efficacy to clomiphene citrate, which is why it was used to compare the results of the current study. Oyebanji and Atoki (2018) reported lower levels of internal egg quality parameters in Japanese quails administered 1.5 mg/ kg body weight of Clomiphene citrate, a much lower value than the 15 mg/ kg body weight used in the current study. Clomiphene citrate has been reported to possess gonadotropin-stimulating effects in rats and mice when administered at low levels and to possess gonadotropin-inhibiting effects at high levels (Holtkamp *et al*.,1966). There were not many studies on clomiphene citrate in laying hens on egg quality parameters, thereby limiting comparisons with the current results obtained,

Conclusion

The current study has demonstrated that CC administration at 15 mg/kg influenced egg quality parameters in Lohmann Brown hens, leading to an increase in the external and internal contents of the eggs observed. It is therefore recommended based on the findings that CC should be given to laying LB hens during the Non-mating Phase at a dose of 15 mg/kg per os in order to improve the number of eggs laid and internal contents of the eggs i.e egg quality. However, further reading should look into administering CC to the control, stimulatory and withdrawal groups to observe the effects on the egg quality parameters.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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