



Research Article

Determination of Weight and Biochemical Changes in Layers Experimentally Infected with *Escherichia coli*

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ABSTRACT

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This study determined the effect of experimental exposure of layer chickens to *Escherichia coli* (*E. coli*) on weight, egg production and some plasma biochemical parameters. A total of 20 laying chickens (20 weeks old) were acquired and assigned, at random, into infected and control groups, of 10 layers each. Birds in the infected group were challenged with 0.5 ml of bacterial aliquot containing 10^9 colony forming units (CFU) of the bacteria, administered intra-tracheally. Five birds, from each group were selected at random and their live body weights were determined on days 0, 2, 4, 6, 14, 21, 28, 35 and 42. Also, egg production was monitored, and the weekly percentage egg production of birds was calculated. Two (2) ml of blood were collected from each bird in both groups and used for plasma biochemical analyses. Finding from the research showed that *E. coli* infection caused reduction in feed and water consumption by day 3 post infection (pi); with subsequent reduction in body weight and egg production. The mean plasma aspartate aminotransferase (AST) activity in the infected group progressively increased from day 6 pi to reach a significantly higher ($p < 0.05$) value (66.4 ± 0.93 IU/L) on day 28 pi while the mean plasma alanine aminotransferase (ALT) activity progressively increased in the infected group from day 6 pi to attain the highest significant ($p < 0.05$) value (74.4 ± 2.11 IU/L) on day 21 pi. The mean plasma total protein concentration in the infected group decreased from day 4 pi and reached the lowest significant ($p < 0.05$) value (5.12 ± 0.28 mg/dl) on day 35 pi. In conclusion, the drop in egg production, loss of body weight, increase hepatic transaminases and decrease plasma protein may be attributed to anorexia or possible impairment of intestinal, renal reabsorption of nutrients from the infection.

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INTRODUCTION

Poultry production is a big industry in Nigeria and is an important component of the livestock sub-sector that provides employment, income, animal protein for urban and rural dwellers as well as manure for crop production (Obi *et al.*, 2008). Avian colibacillosis is considered as one of the major bacterial diseases that afflicts the poultry industry worldwide, and along with salmonellosis, is also one of the most common avian diseases communicable to humans (Singh *et al.*, 2011). Despite the recognition of the disease for over a century, avian colibacillosis remained majorly endemic with consequent huge economic losses (Olsen *et al.*, 2016b).

Colibacillosis in laying hens is characterized by acute mortality without prior clinical signs of disease and without a significant effect on egg production or quality. Normally, colibacillosis is a secondary infection that appears after a situation of immunosuppression caused by another bacterial or viral infection; although Asif *et al.* (2021) proposed that it may act as a primary pathogen as well in some cases. The most common route of infection is via the respiratory tract frequently followed by septicemia (Fang *et al.*, 2021). Most of the outbreaks of colibacillosis occur around the period of peak egg production, which is believed to be an important stress factor contributing to the disease but continues as the flock ages. Cumulative mortality for the disease is usually between 5 and 10% during a single outbreak (Asif *et al.*, 2021). However, the proportion of hens that suffer from the disease often reaches more than 50% (Mudenda *et al.*, 2023).

In view of the high mortality due to egg peritonitis Syndrome, the disease results in great economic losses at the farm level and likely also for the poultry farming from loss of body weight and reduce egg production (Olsen *et al.*, 2016a). However, published data on the percentage quantification of mentioned losses are not available. Therefore, in the present

work, the effect of experimental *E. coli* infection on body weight and egg production in layer chickens was investigated. Also, the search for laboratory diagnosis of colibacillosis in layers requires establishing specific biochemical changes, consequent to the disease, which necessitated this study.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was sought for from the Ethical Committee on Animal Use and Care of Ahmadu Bello University, Zaria (ABUECAUC), with Approval number: ABUECAUC/2017/026

Experimental Animals, Design and Source of bacterial organism

A total of twenty (20) laying (20 weeks old) chickens that were specifically raised for research purposes and vaccinated against endemic vaccinal diseases in the area, except *E. coli* infections, were purchased from a reputable farm in Kaduna State, Nigeria. The birds were housed and managed intensively in the poultry research pens of the Department of Veterinary Pathology, Ahmadu Bello, University Zaria, Kaduna State, Nigeria. Prior to arrival of the birds, the pens were thoroughly washed with detergent and sprayed with formalin at a concentration of 4 ml/litre of water. Throughout the experiment, the birds were fed standard commercial layer mash (Hybrid Feeds[®]) and water was provided to the birds *ad libitum*. The *E. coli* used in this experiment, APEC serotype O1K1, was obtained from the bacteria bank of the National Veterinary Research Institute, Vom, Plateau State, Nigeria.

Grouping and inoculation of birds with *E. coli*

The birds were kept for 4 weeks to acclimatize to the new environment and other handling conditions, after which they were divided at random into two groups (infected and control) of 10 layers each. The control birds were housed in a pen located far away from the pen in which birds of the infected group were

housed. The bacteria from a previously prepared slant were reactivated by sub-culturing on Eosin and methylene blue (EMB). The resulting colonies were then examined for their characteristic features, colour, morphology and tested for gram stain reaction. On the day of infection (Day 0), bacterial inoculum was prepared using McFarland standards, which were prepared by adding barium chloride to sulphuric acid to obtain a barium precipitate of different turbidity standards. These were used to estimate the number of bacteria present in a liquid suspension (Gadzama *et al.*, 2019). In this test, the turbidity of suspension of bacteria was compared with turbidity of the appropriate standard. Each of the birds in the infected group was then challenged by inoculating each of the birds in the infected group with 0.5 ml of bacterial aliquot containing 10^9 colony forming units (CFU) of the bacteria intra-tracheally (Antao *et al.*, 2008). After inoculation, the bacteria were recovered from infected birds by following conventional culture, isolation, and identification of bacteria by standard procedures as documented in Cowan and Steel (Barrow and Feltham, 2000) and Cheesbrough (2006).

Clinical Observations, Evaluation of Mean Body Weight, and Egg Production

Starting from day 0 of infection and throughout the experimental period, birds in the infected group were closely monitored for clinical signs of colibacillosis and recorded accordingly. Five birds, each, from the infected and control groups were selected at random and their live body weights were determined on days 0, 2, 4, 6, 14, 21, 28, 35 and 42 using a digital weighing balance. Also, egg production was monitored and the weekly percentage egg production of birds in the infected and control groups was calculated.

***E. coli* Recovery**

Escherichia coli organisms were recovered from the blood of the infected birds showing clinical signs of colibacillosis as indicated by presence of colonies with green metallic sheen on the EMB culture (Plate I). To further confirm the bacterium, Gram's staining was carried out and the organism was a Gram-negative rod which was catalase positive, produced acid and gas on triple sugar iron agar. It was indole positive, citrate and urease negative, methyl red positive, Voges Proskauer negative and was motile.



Plate I: Photograph of *E. coli* culture on EMB showing characteristic green metallic sheen recovered from blood of bird in the infected group.

Collection of Blood Sample for Plasma Biochemical Analyses

Beginning from day 0 and, subsequently, on days 2, 4, 6, 14, 21, 28, 35 and 42 post-infection, Blood samples (2 ml) were collected from the brachial vein of each of 5 birds selected at random from each group and dispensed into heparinized vacutainer tube. The blood was centrifuged in the laboratory at 2200 g for 10 minutes. The plasma was then dispensed into vials and stored at -20 °C until used for determination of activities of AST, ALT, and total plasma protein.

Determination of Liver Enzymes

AST and ALT activities were determined by the enzymatic method using reagent kits (Agape Diagnostic laboratory, Switzerland) in accordance with the manufacturer instructions. While total plasma protein was estimated using hand refractometric method according to Sirois (1995).

Statistical Analysis

All the data obtained were subjected to statistical analysis including the calculation of the means and standard error of the means. Data between groups were evaluated using student t-test and values of $P < 0.05$ were considered significant using Graph Pad prism version 5.00 for windows, Graph pad Software, San Diego California USA.

RESULTS AND DISCUSSION

The clinical signs observed in the *E. coli*-infected layers in this study, namely, reduced feed and water consumption, decreased egg production, watery yellowish faeces, weakness, and loss of weight by this organism

were the classical findings in colibacillosis (Barnes *et al.*, 2008). The gross lesions observed in the *E. coli*-infected layers such as the congested lungs, congested serosal blood vessels of the oviduct, congested ovarian follicles with yellowish fibrinous materials and hyperaemic intestine were also part of the reported postmortem findings in birds with colibacillosis (Tonu *et al.*, 2011; Srinivasan *et al.*, 2013). The reduced feed intake and loss of body weight observed in this study may be part of the acute inflammatory response immediately after *E. coli* contacts host tissues and partly because of acute phase of endotoxemia (Mireles *et al.*, 2005).

The mean body weights of birds in the infected and control groups were as shown in Figure 1. The daily mean weight of *E. coli*-infected group of layers showed a significant decrease ($P < 0.05$) that was progressive starting from day 4 pi value (1576 ± 44.56 g) to a lowest value (1480 ± 48.89 g) on day 6 pi followed by a progressive but non-significant ($P > 0.05$) increase in the infected layers, beginning from day 28 and continued until termination of the experiment. On the contrary, an increase in mean body weight was observed in the control group, all through the study. The loss of body weight observed in the infected group of layers may be because of combined effects of reduced feed intake and the diarrhoea due to enteritis, which could have impaired digestion as well as intestinal absorption of the nutrients needed for normal body functions and reproduction in layers (Abdu, 2014).

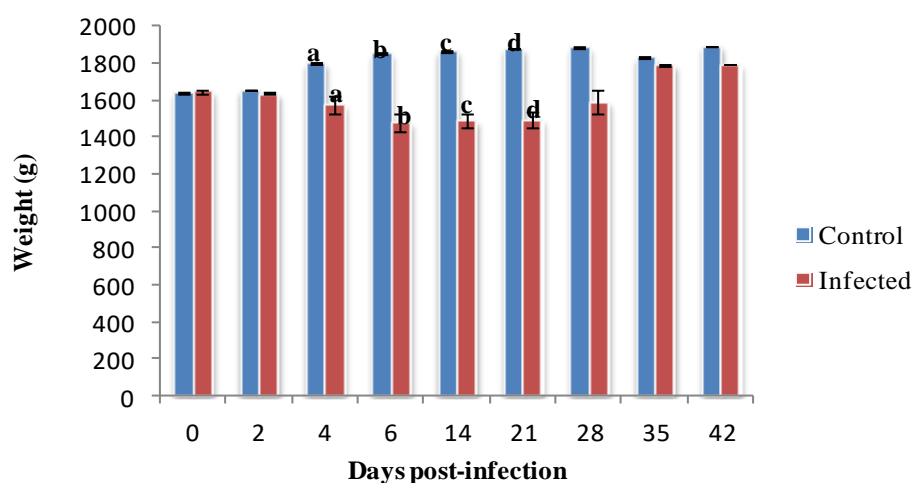


Figure 1: Mean (\pm SEM) daily weight in *E. coli*-infected and control layers. Values with the same superscript alphabets are significantly different with $p < 0.05$

Weekly percentage egg production

The percentage egg production in the infected and control groups are shown in Figure 2. A significant ($P < 0.05$) progressive decrease in the mean weekly percentage egg production was observed in the *E. coli*-infected layers starting from week 1 to reach the lowest value of $40 \pm 4.47\%$ in week 3. Following this, the percentage egg production remained consistently low with minor fluctuations until termination of the experiment at week 6. The decrease in egg production may be due to possible decreased progesterone levels, which has been reported to be responsible for

preovulatory Luteinizing hormone (LH) surges and the induction of ovulation (Johnson, 2014). The decrease in egg production may also be due to the hampered uptake of gut calcium (Franco-Jimenez and Beck, 2007) or the decreased production of cholecalciferol-1-hydroxylase, which has a momentous role in calcium homeostasis (Gadzama *et al.*, 2019). Although the activity of cholecalciferol-1-hydroxylase was not investigated in this study, decreased production of this enzyme could negatively affect calcium metabolism with consequent effect on egg production.

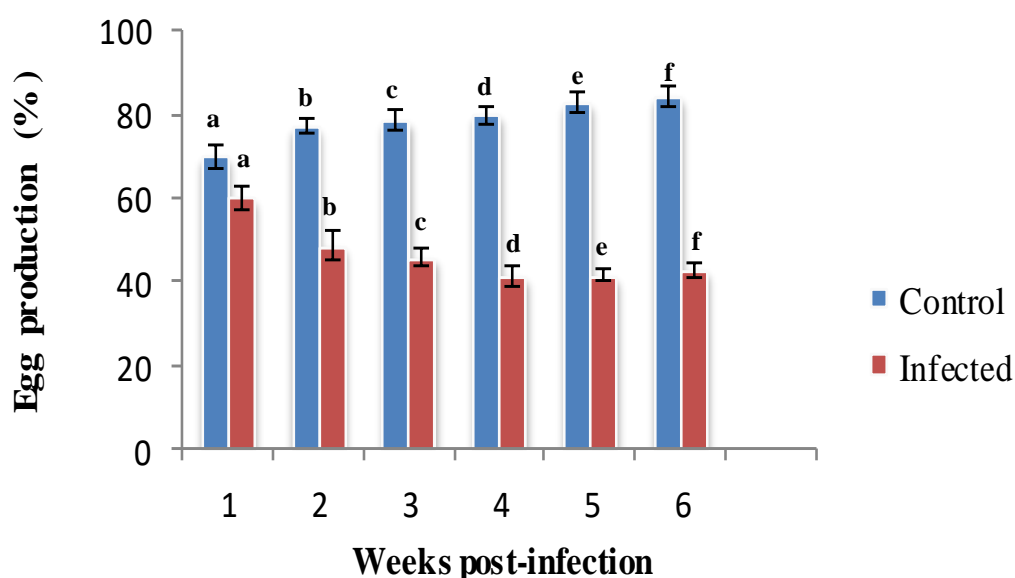


Figure 2: Mean \pm SEM of weekly percentage egg production in *E. coli* infected and control layers. Values with the same superscript alphabets are significantly different with $p < 0.05$

The mean AST and ALT activity in the *E. coli*-infected and control layers are presented in Figure 3 and 4, respectively. The mean plasma AST and ALT activity in the infected group progressively increased from day 6 pi (59.2 ± 1.20 IU/L and 70.2 ± 1.2 IU/L, respectively) to a significant ($p < 0.05$) value (66.4 ± 0.93 IU/L and 74.4 ± 2.11 IU/L, respectively) on day 21 and 28 pi, respectively, when compared with those in the control group. The mean plasma values of both

AST and ALT in the infected group remained higher than that in the control up to day 42 when this experiment was terminated. However, the mean plasma total protein concentration in the infected group progressively decreased starting from day 4 pi (6.04 ± 0.32 mg/dl) to a lowest value (5.12 ± 0.28 mg/dl) on day 35 pi that differed significantly ($p < 0.05$) from that in the control group. The low levels of plasma total protein concentration were maintained in the infected

group up to day 42 pi when the experiment was terminated Figure 5. The increase in the mean plasma AST activity is indicative of cellular injury to hepatocytes and cardiac myocytes, while the elevated plasma ALT activity was most probably due to presence of the hepatic lesions observed in the *E. coli*-infected layers in the present study. Similar increases in plasma ALT and AST activities in *E. coli*-infected chickens were earlier reported by Chiroma *et al.* (2018) and Gadzama *et al.* (2019). The observed significant decrease in

the mean total plasma protein concentration in the *E. coli*-infected group in this study may partly be attributed to impaired synthesis of these proteins due to the *E. coli* infection-induced hepatic damage (Oladele *et al.*, 2005). Malnutrition resulting from anorexia (Petersen *et al.*, 2001) and impaired digestion and malabsorption of nutrients from the intestinal tract could have played a part in the observed decrease in total plasma protein (Mudenda *et al.*, 2020).

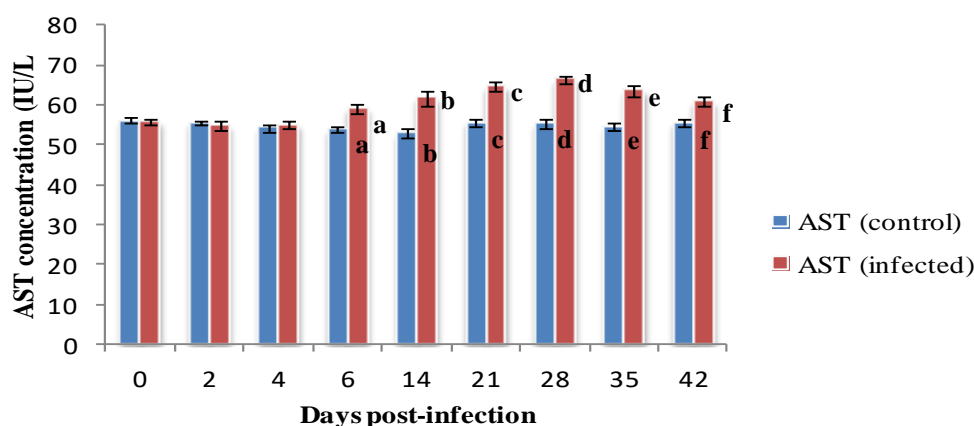


Figure 3: Mean (\pm SEM) aspartate aminotransferase activity in *E. coli*-infected and control groups of layers. Values with the same superscript alphabets are significantly different with $p < 0.05$

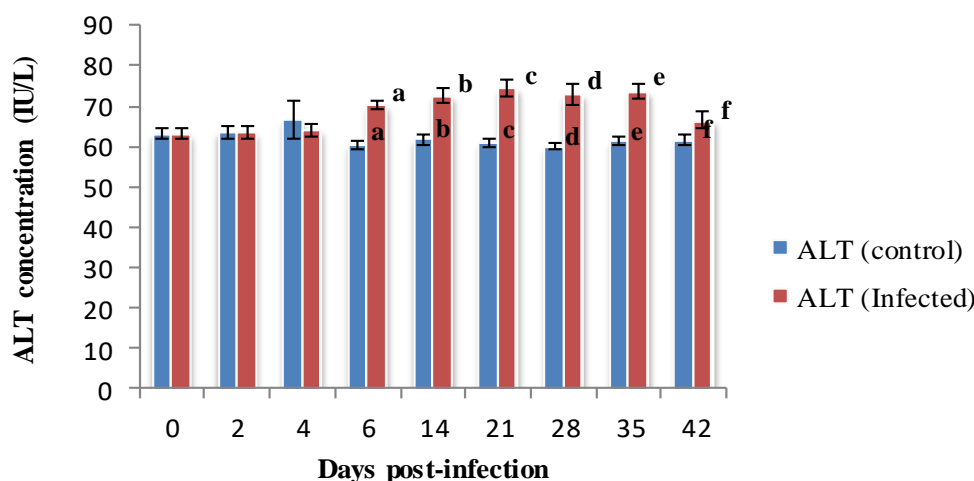


Figure 4: Mean (\pm SEM) alanine aminotransferase activity of *E. coli*-infected and control groups in layers. Values with the same superscript alphabets are significantly different with $p < 0.05$.

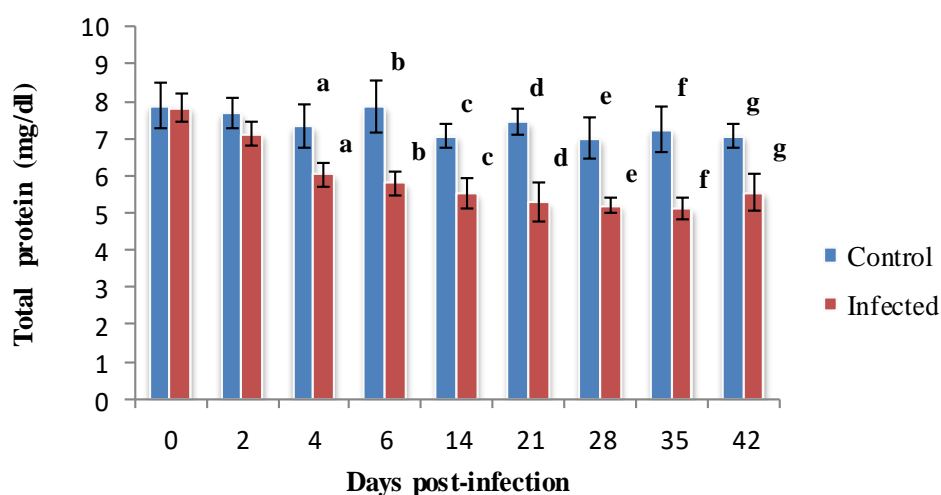


Figure 5: Mean (\pm SEM) total protein concentrations of *E. coli*-infected and control groups in layers. Values with the same superscript alphabets are significantly different with $p < 0.05$

CONCLUSION

Finding from this study observed that, experimental infection with *Escherichia coli* in layers caused the full-blown disease, colibacillosis, with its characteristic clinical manifestations (reduced feed and water consumption, decreased egg production, watery yellowish faeces, weakness) evident by day 3 post-infection. Also, since *Escherichia coli* in layers caused an increase in mean plasma aspartate aminotransferase and alanine aminotransferase activities that differed significantly ($P < 0.05$) relative to that of the control; it is recommended that biosecurity measures should be well instituted in poultry farms to minimize the associated losses due to colibacillosis. In addition, more studies should be carried out to determine the effect of *E. coli* infection on hepatic synthesis and plasma levels of vitellogenins, which bind calcium and plays vital role during eggshell formation in chickens.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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