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Research Article

Haematological, Serum Biochemical and Immunological Changes Associated with the Inclusion of Garlic (*Allium sativum* L.) Meal in the Diet of Commercial Layer Chickens

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ABSTRACT: This work examined the impact of garlic as feed-inclusion on the hematology, serum proteins and enzymes, as well as, immunity of layer chickens. Day-old Isa Brown chicks (500) were separated into four groups, A, B, C and D (with each group having three replicates of 42 or 43 each), placed in rearing and given garlic meal in feed at a rate of 0.125%, 0.25%, 0.5% and 0%, respectively. Chickens (n=10, for each group) were randomly selected and bled at 6, 13, 23, 42, 50 and 56 weeks of age for the assessment of haematological parameters, serum proteins and enzymes using standard procedures. ELISA methods were used to assess Newcastle disease (ND) and infectious bursal disease (IBD), vaccinal immune response and interferon gamma (IFN-ɣ) concentrations. Hemagglutination inhibition test was also used to determine ND vaccinal immune response. Mean values were compared using Dunnett's test ($p<0.05$). PCV was significantly higher in Group D at 23 (29.7±0.78%) and 42 weeks-old (34.5±0.17%) while at 50 week-old, it was significantly higher in C (32.5±0.5%) compared to control Group D (22.1±0.41%). Heterophilia in D at 13 week-old and lymphocytosis in B and C at 23 and 42 weeks-old resulted in leucocytosis. Total protein values were lowest in D, albumin concentrations were significantly higher in garlic groups at 42 and 56 weeks-old and globulin concentrations were lowest in D. AST level was significantly higher in B at 42 week-old and in A and C at 56 week-old without corresponding increase in CK. Newcastle disease and IBD antibody titers were significantly lower in D only at 6 week-old. Increase in IFN-ɣ concentration post-challenge were 22 and 40 pg/ml in A and B, respectively, while it decreased by 51 pg/ml in D. Thus, garlic feed inclusion resulted in lymphocytosis, increased level of serum proteins and enhanced cellular immunity in commercial layers. However, with prolonged use, it could cause hepatocellular damage.

Keywords: Chicken, Garlic, Haematology, Immunology, Serum Biochemistry

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INTRODUCTION

The inclusion of growth promoters into poultry feed as additives is as old as commercial poultry production and it is a common practice all over the world. These growth promoters are usually antibiotics, based on the fact that pathogenic microorganisms abound in the environment and easily contaminate feed, water, clothing and equipment in farm premises. These microorganisms in small quantities cause subclinical infections which although might not result in disease, would cause sub-optimal performance i.e. reduced growth rate and egg production in poultry. Thus, inclusion of sub-therapeutic doses of antibiotics in poultry feed was introduced over half a century ago with resultant improvement in growth (1, 2).

However, the use of antibiotics as growth promoters has recently faced intense criticism for public health reasons. Over the years, antibiotic resistant strains of pathogens have evolved, thereby challenging effective therapy in both poultry and humans. Studies have shown that the use of antibiotic growth promoters have resulted in poultry flocks and products being contaminated with antibiotic resistant pathogens such as *Campylobacter, Enterrococcus,* and *Salmonella species,* as well as, *Escherichia coli* (3, 4). The European Union banned the use of most antibiotics as growth promoters in farm animals in order to preserve their effectiveness in humans, in 1999 (5). While the United States is yet to adopt this broad policy, a ban was placed on the use of Enrofloxacin for therapeutic uses in food animals in 2004 by the U.S. Food and Drug Administration due to its contribution to fluoroquinolone resistance in human pathogens (4). Antibiotic residues have been reported in poultry meat and eggs made available for human consumption in Nigeria (6, 7, 8), thereby making consumers to be passive users of these antibiotics with attendant risk of ineffectiveness when indicated.

These unpleasant effects of the use of antibiotic growth promoter in poultry production and the consequent ban on their use have caused producers to search for alternatives (9). The use of non-conventional growth promoters in poultry feed has been found to improve nutrient digestibility, control pathogenic micro-organisms, facilitate favourable intestinal microbial balance and enhance absorption of calorigenic nutrients across the gut wall through increased absorptive capacity (10, 11). One of such non-conventional growth promoters is garlic (12).

Garlic (*Allium sativum*), is a well known spice and herbal medicine for the prevention and treatment of a variety of diseases (13). It is a member of the onion family *Alliceae* and has been shown to exhibit antimicrobial, antioxidant, and anti-hypertensive properties (14, 15). Allicin (diallyl-thiosulfinate) is the major organosulfur compound in garlic which is considered to be biologically active (16). As a natural feed additive, garlic has been reported to improve broiler growth and feed conversion ratio, and caused decreased mortality (17, 18, 19).

However, in order to adopt the use of garlic as a feed additive in poultry production, it is important to investigate its effect on the systems of chickens. This will further elucidate its growth promoting effect and reveal the possibilities of toxicity, if any. This study was therefore designed to investigate the effect of garlic feed inclusion on the haematology, serum proteins and enzyme levels, as well as, the immunological response of commercial chicken layers.

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MATERIALS and METHODS

Experimental Chickens, Rearing and Sampling

This study was carried out with the institutional approval of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/App/2015/065).

Five hundred, one day-old, Isa Brown pullets were placed in the brooder house in 4 separate groups A, B, C and D (with each group having three replicates of 42 or 43 each), randomly selected. They were fed chick mash as appropriate from day-old till 7 weeks of age, growers mash till 19 weeks of age and layers mash from then till end of egg production. Garlic meal (Patent No. NG/P/2012/285) was included in feed of chickens in Groups A, B and C at a ratio of 0.125%, 0.25% and 0.5%, respectively, while Group D had no garlic meal inclusion. Vaccinations to protect the chickens against prevalent diseases were as follows; Marek's disease vaccine, HVT strain, was administered at day-old; Newcastle disease vaccine, LaSota strain at 2 week-old, Komarov at 8 week-old and Komarov oil emulsion at 15 and 40 weeks-old, while infectious bursal disease vaccines were administered at 14 and 23 days-old. A minimum of 10 chickens per group were randomly selected and bled via jugular venipuncture (5 ml) at 6, 13, 23, 42, 50 and 56 weeks of age; with 2 ml of blood into EDTA containing bottles for the assessment of haematological parameters and 3 ml into plain bottles for serum protein and enzyme assays.

Haematology

Packed cell volume (PCV) of each blood sample was determined immediately after collection using the microhematocrit method. Total leucocyte count was determined using the Coulter counter method. Twenty-five microliter of whole blood from each blood sample was diluted in 5 ml of avian diluting fluid (sodium citrate 3.8 g, neutral formalin 0.2 ml, brilliant cresyl blue 0.5 g and 100 ml of distilled water). Leucocytes were counted using the light microscope while differential leucocytes counts were determined as described by Campbell (20) i.e. thin smear preparation of blood samples were Giemsa stained and cells were counted with the light microscope at x100 objective lens using oil emulsion.

Serum Enzymes and Proteins Assay

Sera were harvested and levels of aspartate aminotransferase, creatine kinase, total protein and albumin from the different chicken groups were determined by spectrophotometry using Fortress diagnostics^{R} kits (Fortress Diagnostics Limited, Antrim, BT41 1QS, UK).

Humoral Immune Response

Newcastle disease vaccinal immune response: Antibody response to Newcastle disease vaccinations was assayed at 6, 13, 23, 42 and 51 weeks of age by both enzyme linked immunosorbent assay and hemagglutination inhibition test.

Hemagglutination inhibition test was conducted as described by OIE (21). Briefly, 50 µl of 4HA units Newcastle disease inactivated virus (antigen) was dispensed into each well of U-bottom microtiter plates using two rows per serum sample. Fifty µl of serum sample was then added to the first well of two rows of wells. Two-fold serial dilution was carried out from the first row to the last well (picking 50µl and transferring into the next well) and 50µl of 0.5% washed chicken RBC was dispensed into each well. The plate was incubated for 30 minutes at room temperature after which HI titers were read visually as the last well showing haemagglutination inhibition.

Enzyme linked immunosorbent assay (ELISA) was conducted using a kit manufactured by Affinitech Incorporation, USA. Reagents were warmed to room temperature, mixed by swirling and inverting and appropriately diluted with deionized water. The negative, positive and test samples were shaken and diluted in a ratio of 2 µl to 800 µl sample diluents. One hundred µl of negative and positive controls were then dispensed to duplicate wells each. One hundred µl of diluted test serum was dispensed per well. This was allowed to stand for 30 minutes at room (27°C) before the plates was emptied and washed by dispensing 300 µl wash solution per well. Washing was done three times. One hundred µl of conjugate was dispensed per well and allowed to stand for 30 minutes at room temperature. The plate was washed three times and one hundred µl of substrate was immediately dispensed per well and allowed to stand for 30 minutes at room temperature. One hundred µl stop solution was immediately dispensed per well. The plate was then read using dual wavelength microplate reader blanked on air, and a 405nm primary filter was used while a 630nm was used as reference filter.

According to the manufacturer's instruction, test results were considered valid when the difference between the optical densities of the positive and negative controls was 0.7 or greater.

The readings of the samples were calculated as shown below:

$$Sp = \frac{\text{Abs.test samples - Ave. Abs Negative}}{\text{Ave. Abs positive - Ave Abs. Negative}}$$

Sp : Sample to positive ratio

Sp × (100) = ELISA Unit(EU). Positive control value was set at 100EU.

Infectious bursal disease vaccinal immune response: Infectious bursal disease virus antibody production to vaccination was assayed and monitored using the ELISA technique as described above.

Cellular Immunity

Ten layer chickens were separated from each group at 50 week-old and bled for serum via jugular venipuncture. Interferon gamma (IFN-ɣ) level was assayed as described by Lambrecht et al. (22) using a commercial ELISA kit (NOVATEINBIO Incorporation, USA) based on standard sandwich technology. The chickens were challenged the following day with Newcastle disease vaccine, R_2B strain, at 60 doses per bird and bled for serum at 4 days post-challenge.

One hundred µl of each serum sample was dispensed into each well of the microtiter plate already pre-coated with capture antibody. IFN-gamma standards provided were also dispensed into wells. Fifty µl of the conjugate (IFN-gamma: Horse-radish peroxidase) was added to all the wells, which was mixed properly and covered with adhesive before incubating for one hour at 37°C. The plate was washed manually five times and blot-dried using absorbent paper. Fifty µl each of chromogenic substrate A

(Streptavidin – Horse radish peroxidase) and B (TMB developing reagent) as provided by the manufacturer were added and incubated for 10 minutes at 20°C after which stop solution was added to stop the reaction. The Optical Density (OD) of reaction solutions were read at 450nm using ELISA reader (Optic Ivymen® system 2100-c). Concentrations of IFN-gamma in serum samples were derived from the standard curve generated from the IFN-gamma standards provided.

Statistical Analysis

Mean values for each parameter per group were calculated and comparison was made between groups for statistical significance differences using Analysis of Variance (ANOVA) and Dunnett's test (Graph Pad Prism 5) at p<0.01 and 0.05.

RESULTS

Haematology

Mean values obtained for PCV (%) ranged from 24.14±1.06 (A) to 26.6±0.98 (B) at 6 week-old and from 27.83±0.8 (A) to 28.5±0.58 (C) at 13 week-old, with no significant difference (Figure 1). At 23 week-old, PCV ranged from 27.0±0.52 (A) to 29.7±0.78 (D) with Group D having significantly higher value (p<0.05) than Group A. At 42 week-old, PCV ranged from 26.4±0.22 (B) to 34.5±0.17 (D) which was significantly higher (p<0.05) than in other groups. However, at 50 week-old, values ranged from 21.03±0.3 (A) to 32.5±0.5 (C) which was significantly higher (p<0.05) than values in other groups. Also, at 56 week-old, PCV ranged from 23.0±0.49 (B) to 24.8±0.44 (A) with no significant difference between the groups.

Mean hemoglobin concentration (gm%) followed a similar trend as PCV ranging from 7.91±0.35 (A) to 8.72±0.33 (B), from 9.14±0.26 (A) to 9.41±0.16 (C), from 8.86±0.56 (A) to 9.83±0.75 (D), from 8.58±0.05 (B) to 11.38±0.05 (D), from 6.86±0.1 (A) to 10.33±0.38 (C) and from 7.53±0.17 (B) to 8.14±0.15 (A) at 6, 13, 23, 42, 50 and 56 weeks old, respectively.

At 13 week-old, mean total leucocyte count (x10^9/L) was highest (p<0.05) in Group D (12.2±0.57) with significant reduction up till 56 week-old (Figure 2). Group B had highest (p<0.05) values at 23 (9.26±0.73) and 42 (11.66±0.78) weeks-old. While Group D had the highest total leucocyte counts (p<0.05, 0.01) at 13 week-old, it had the lowest counts at 42, 50 and 56 weeks of age.

With regards to Lymphocyte counts (x 10^9/L), Group B had the highest (p<0.05) values at 23 (6.82±0.53) and 42 (8.44±0.53 – p<0.01) weeks of age (Figure 3), while Group D had the highest (p<0.05) value at 13 week-old (7.27±0.4) and the lowest (p<0.05) at 42 week-old (4.91±0.43). Highest heterophil count (10^9/L) at 13 week-old was in Group D (4.77±0.23 – p<0.01) and at 23 (2.85±0.15), 50 (2.51±0.26) and 56 (2.49±0.22) weeks-old in Group C (Figure 4), while the lowest counts at 13 (2.45±0.27), 23 (1.88±0.1) and 42 (1.70±0.18) weeks of age were in Group A (p<0.01). Throughout the study period, Platelet count was highest in Group D, the difference being significant at 42 (p<0.05) and 50 (p<0.01) weeks of age (Figure 5).

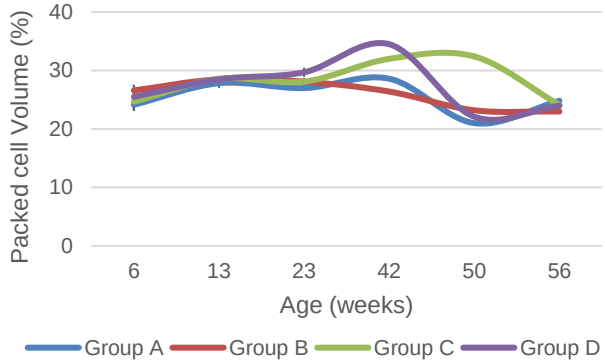

Figure 1. Packed cell volume of layer chickens on graded doses of garlic feed inclusion

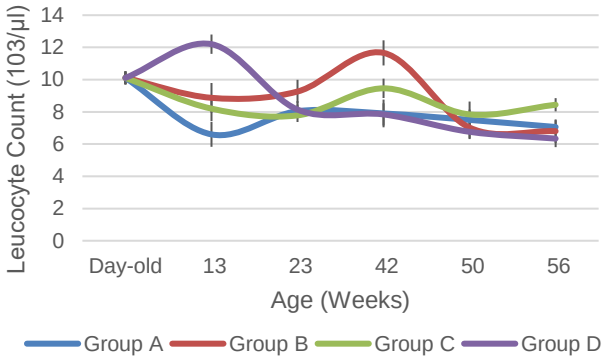

Figure 2. Total leucocyte counts in layer chickens on graded doses of garlic feed Inclusion

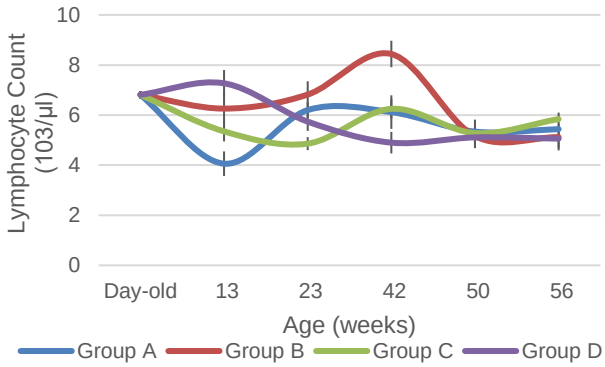

Figure 3. Lymphocyte counts in layer chickens on graded doses of garlic feed inclusion

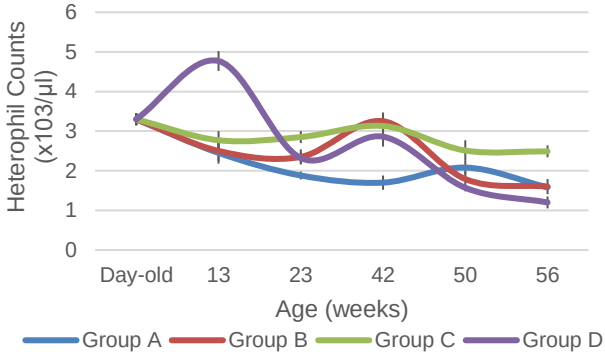

Figure 4. Heterophil counts in layer chickens on graded doses of garlic feed inclusion

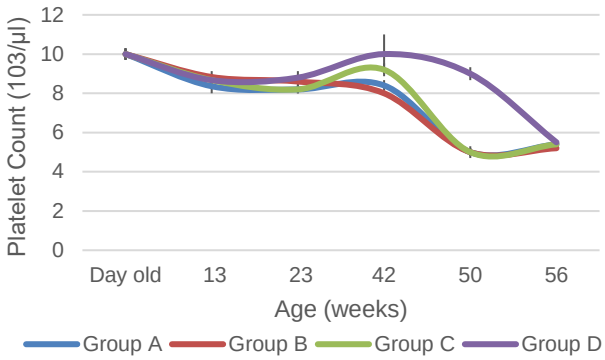

Figure 5. Platelet counts in layer chickens on graded doses of garlic feed inclusion

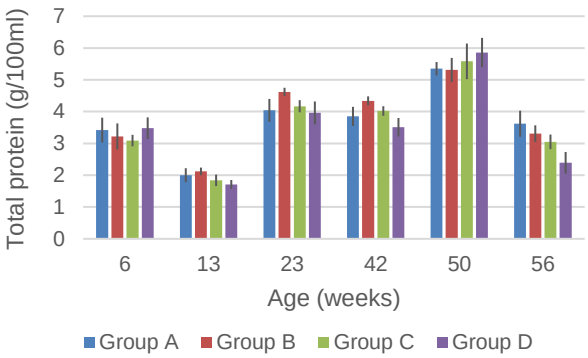

Figure 6. Total protein levels in layer chickens on graded doses of garlic feed inclusion

Serum Proteins and Enzymes

Total protein levels (g/dL) in serum ranged from 3.09±0.18 (C) to 3.48±0.34 (D) at 6 week-old, 1.71±0.14 (D) to 2.12±0.12 (B) at 13 week-old and 3.96±0.36 (D) to 4.62±0.13 (B) at 23 week-old with no significant difference between the groups (Figure 6). At 42 week-old, the range was 3.51±0.29 (D) to 4.34±0.14 (B) being significantly higher ($p<0.05$) than those of other groups, while at 50 week-old, values ranged from 5.31±0.38 (B) to 5.86±0.46 (D) with no significant difference between the groups. However, at 56 week-old, total protein level ranged from 2.39±0.34 in Group D to 3.62±0.41 in Group A with significant difference ($p<0.05$). Albumin values (g/dL) ranged from 0.71±0.09 (B) to 0.84±0.09 (D) at 6 week-old, from 0.48±0.03 (B) to 0.74±0.10 (D) at 13 week-old and from 1.52±0.13 (A) to 1.65±0.07 (C) at 23 week-old with no significant difference between the groups (Figure 7). At 42 week-old, albumin level in groups A (1.36±0.06) and B (1.40±0.05) were significantly higher($p<0.01$) than those of groups C (1.21±0.03) and D (1.14±0.05), while at 50 week-old there was no significant difference between the groups with values ranging from 1.11±0.19 (C) to 1.38±0.08 (B). However, at 56 week-old, values in groups A (1.19±0.11), B (1.12±0.04) and C (1.03±0.01) were significantly higher ($p<0.01$) than that of Group D (0.59±0.14). Globulin levels were not significantly different between the groups at 6, 42, 50 and 56 weeks of age (Figure 8). However, at 13 and 23 weeks of age, levels were significantly higher ($p<0.05$) in Groups B (1.61±0.12) and C (3.46±0.25), respectively, than in other groups. Also, Group D had the lowest values at 13, 23, 42 and 56 weeks of age.

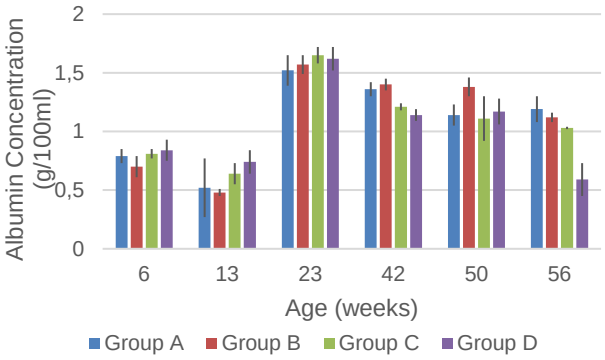

Figure 7. Albumin concentrations in layer chickens on graded doses of garlic feed inclusion

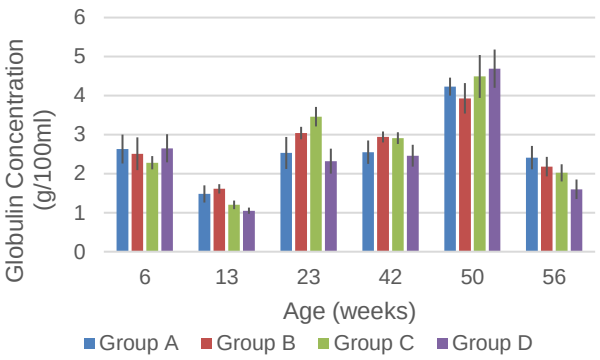

Figure 8. Globulin concentrations in layer chickens on graded doses of garlic feed inclusion

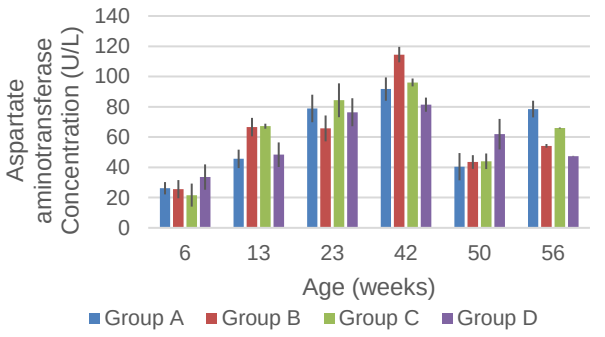

Figure 9. Aspartate aminotransferase concentrations in layer chickens on graded doses of garlic feed inclusion

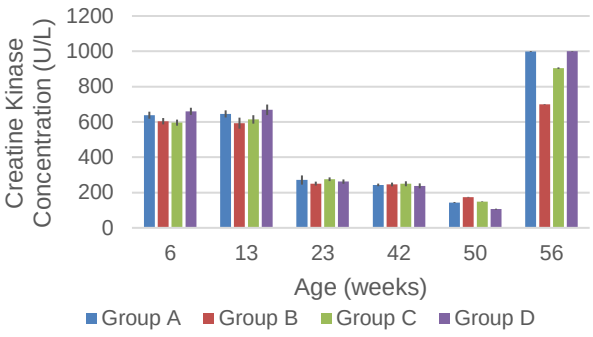

Figure 10. Creatine kinase concentrations in layer chickens on graded doses of garlic feed inclusion

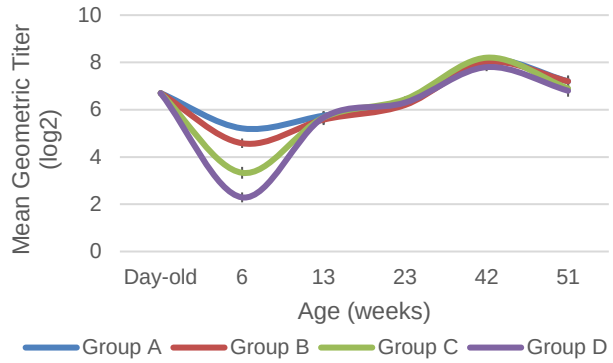

Figure 11. Newcastle disease hemagglutination inhibition antibody titers (MGT) in layer chickens on graded doses of garlic feed inclusion

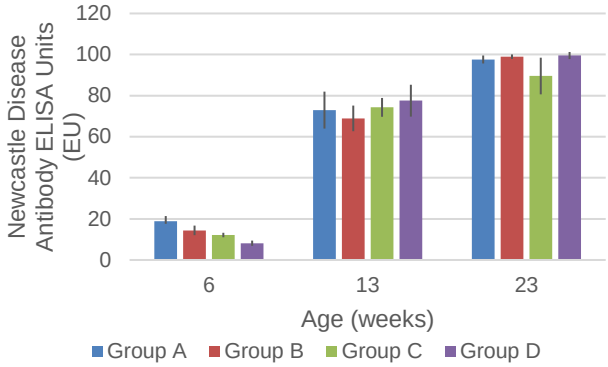

Figure 12. Mean Newcastle disease antibody (ELISA) titers in layer chickens on graded doses of garlic feed inclusion

Aspartate aminotransferase levels (µ/L) ranged from 21.64±7.61 (C) to 33.56±8.37 (D) at 6 week-old, from 45.58±6.1 (A) to 67.17±1.65 (C) at 13 week-old, from 65.70±8.55 (B) to 84.30±11.18 (C) at 23 week-old and from 40.40±9.01 (A) to 61.90±10.09 (D) with no significant difference between the groups (Figure 9). However at 42 week-old, AST levels ranged from 81.40±4.67 (D) to 114.50±5.1 (B) which was significantly higher ($p<0.01$) than the levels in other groups, while at 56 week-old, values ranged between 47.40±0.16 (D) to 78.50±5.52 (A) with values in groups A and C being significantly higher ($p<0.01$) than those of groups B and D. At the sampling times when significantly, increased AST levels were observed in some garlic groups, creatinine kinase (CK) concentration ranged from 237.80±13.88 (D) to 249.60±14.09 (C) with no significant difference at 42 week-old (Figure 10) and ranged from 699.20±1.76 (B) to 1000.10±1.63 (D) at 56 week-old, with levels in groups B and C being significantly reduced ($p<0.01$).

Humoral Immune Response

Hemagglutination inhibition test for Newcastle disease (ND) antibody showed mean geometric titer (GMT) of maternal antibody at day-old to be 6.7±0.11 (log_2) as presented in Figure 11. At 6 week-old, titers ranged from 2.29±0.09 (D) to 5.21±0.21 (A), with significant difference ($p<0.01$). At 13, 23, 42 and 51 weeks of age, MGT values ranged from 5.58±0.23 (B) to 5.75±0.17 (A), from 6.2±0.09 to 6.44±0.12 (C), from 7.8±0.17 (D) to 8.2±0.09 (C) and from 6.8±0.25 (D) to 7.2±0.25 (B), respectively, with no significant difference between the groups. Also, ELISA Units (EU) for ND antibody ranged from 8.16±1.27 (D) to 18.86±2.52 (A) with significant difference ($p<0.01$) at 6 week-old (Figure 12), while the range was 68.92±6.24 (B) to 77.54±7.76 (D) at 13 week-old and 89.55±8.92 (C) to 99.53±1.72 (D) at 23 week-old with no significant difference between the groups. The EU values for IBD antibody titers at 6 week-old ranged from 96.84±11.32 (D) to 125.13±6.31 (A) with significant difference ($p<0.05$) and from 93.52±8.81 (D) to 105.18±6.62 (C) at 13 week-old and 57.44±7.97 (C) to 73.69±9.06 (B) at 23 week-old, with no significant difference (Figure 13).

Cellular Immune Response

Interferon-gamma (IFN-gamma) concentration (pg/ml) in serum pre-challenge were 40, 35, 55 and 93 in groups A, B, C and D, respectively (Figure 14). Post-challenge, the values were 62, 75, 55 and 42 in groups A, B, C and D, respectively, with groups A and B showing increases of 22 and 40, Group C showed no increase while Group D showed a decrease of 51.

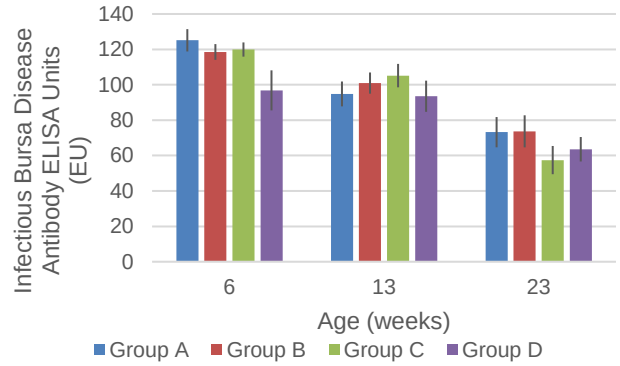

Figure 13. Infectious bursal disease antibody (ELISA) titers in layer chickens on graded doses of garlic feed inclusion

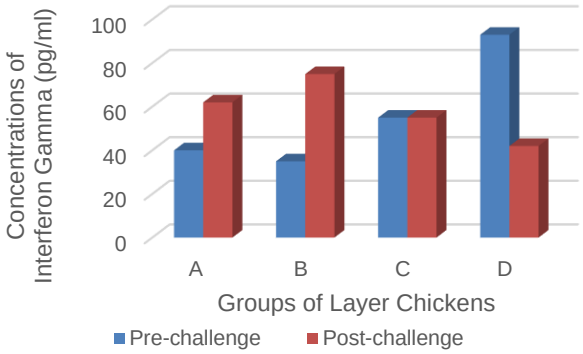

Figure 14. Interferon gamma (IFN-ɣ) concentrations in layer chickens on graded doses of garlic feed inclusion

DISCUSSION

This study investigated the effects of garlic inclusion in the feed of commercial chicken layers on their hematological parameters, serum proteins and enzymes, as well as, their humoral and cellular immunity. Values for PCV recorded were similar to those reported by Igwe et al. (23) for control birds. The significantly higher PCVs recorded for no-garlic group D at 23 and 42 weeks of age than garlic groups is believed to be due to hemodilution in the garlic groups as it was observed that water intake in these groups was higher, although it was not subjected to empirical studies. Also, a study by Aji et al. (24) reported increased water intake in broilers on garlic feed, although with no statistically significant difference when compared with the control group. However, the significantly higher PCV recorded at 50 week-old in Group C with the highest level of garlic (0.5%) is believed to be due to lice infestation (*Menacanthus stramineus*) observed in the groups at this time which was with the exception of layers in Group C. This louse is known to gnaw the skin at the base of feathers and feed on blood (5) causing anemia in addition to restlessness which prevents the chickens from feeding as appropriate. This finding indicates a probable antiparasitic activity of garlic against poultry ectoparasites as earlier reported by Shohreh et al. (26) in chicken layers and by Militz et al. (27) in aquaculture management. Expectedly, results for hemoglobin concentration followed the same pattern as PCV. Increased total leucocyte counts observed in garlic groups from 23 to 56 weeks of age which was particularly significant in Group B at 23 and 42 weeks of age was due to increased lymphocyte counts. Increased leucocyte count was reported in garlic-fed broilers by Fadlalla et al. (28), and in garlic treatment of *Trypanosoma brucei brucei* infection in rabbits (29). Earlier, Ghazanfari et al. (30) reported that garlic enhanced cellular immunity in mice. Also, improved NK cell and T cell functions, phagocytosis and cytokine release had been reported by Nantz et al. (31), by garlic. The leukocytosis recorded in Group D at 13 week-old was due to heterophilia, an indication of inflammation, most likely due to bacterial infections, in this group which was absent in the garlic groups, with Group A particularly having the least counts at 13, 23 and 42 weeks of age. As had been reported by earlier workers, garlic consumption results in reduced platelet count in animals and humans (32; 33). Thus, the observation of reduced platelet counts in garlic groups in this study is not unusual.

Total protein and albumin values were generally higher in garlic groups than the control Group D with Group A showing highest values at most of the sampling times. Also, Group D generally showed lowest globulin levels. According to Doneley (34), the liver is the site of synthesis of proteins including albumin and macroglobulin's, thus, the higher levels recorded in garlic groups could indicate enhanced hepatic function. Liver enzyme AST was only significantly higher in garlic group B at 42 week-old and in garlic groups A and C at 56 week-old with no corresponding increase in CK levels. This might be due to some degree of damage to hepatocytes by the consistent and prolonged inclusion of garlic in feed of the chickens. This finding corroborates the report of Rana et al. (35) that garlic in high doses (1.0-0.5 g/kg body weight/day) showed significant deterioration of liver function in Wistar rats while lower doses did not exhibit deleterious effects.

Antibody response to ND vaccinations was significantly different between the groups only at 6 week-old by both the HI test and ELISA with control Group D having the lowest values. Also, antibody response to IBD vaccination differed significantly only at 6 week-old with Group D also having the least response. It is worthy of note that there was a natural outbreak of IBD at 4 week-old in this flock involving all the groups of pullets with groups B and C being more severely affected. Infectious bursal disease is an immunosuppressive disease affecting the hosts' ability to respond to vaccinations via antibody production (36). It is however remarkable that even though garlic groups B and C were more severely affected, they were able to mount higher titers of both ND and IBD antibodies than control group D, two weeks after the IBD outbreak.

Assay of IFN-ɣ following antigen stimulation in these groups of chickens is a logical means of assessing cellular immune response. It is a multifunctional protein initially believed to interfere with viral replication (37) but now known to regulate several aspects of the immune response. It stimulates bactericidal activity of phagocytes and stimulates antigen presentation through class I and class II major histocompatibility complex (MHC) molecules amongst others (38). IFN-ɣ is secreted by CD4+ T-helper lymphocytes, CD8+ cytotoxic lymphocytes (39; 40), B lymphocytes, NKT cells and antigen presenting cells-APCs (41; 42; 43). According to Frucht et al. (44) and Sen (45), IFN-ɣ secreted by NK cells and probably APCs could be important in early host defence against infection whereas secretion from T lymphocytes is essential in adaptive immune response. The increased IFN-ɣ concentrations recorded for groups A and B post-challenge which was higher in Group B signifies the ability of these groups to mount cellular response in the face of challenge by pathogenic organisms. Group C with the highest dose of garlic meal (0.5%) used in this study could not demonstrate this ability. On the contrary, control Group D had the highest IFN-ɣ concentration pre-experimental challenge, an indication of probable pre-existing natural challenge to the chickens (which could not be detected in the garlic groups)' and showed a decrease post-challenge. It is probable that the immune system of the control chickens had been overwhelmed by constant sub-clinical infections from the environment and thus could not adequately respond to the experimental challenge.

The immunostimulatory activity of garlic as reviewed by Arreola et al. (46) has been verified in this study by the lymphocytosis, enhanced humoral response to ND and IBD vaccinations in the face of immunosuppression and increased serum concentrations of IFN-ɣ post-challenge exhibited by the garlic groups A and B. The evidence of hepatocellular damage demonstrated in this study by significantly high serum AST levels in garlic groups from 42 week-old calls for caution in the inclusion of garlic in feed for chickens on a daily basis in spite of the numerous health advantages.

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