P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2022.135

Effects of Hexane Extract of Garlic on Hematological, Biochemical and Histological Parameters in F_1 Crossbred Chicks Non-Infected and Infected with *Salmonella typhimurium*

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Article History: 21-429 Received: 26-Oct-21 Revised: 15-Nov-21 Accepted: 18-Nov-21

ABSTRACT

An experiment was undertaken to evaluate the effects of garlic extract on hematological, biochemical, and histopathological profiles in F1 crossbred chicks (White Leghorn cops × Nigerian Heavy Ecotype chickens) non-infected and infected with *Salmonella typhimurium*. This experiment was carried out to contribute to the genetic improvement of local Nigerian chickens while controlling salmonellosis and combating antibiotic resistance. Two hundred and forty (240) chicks at two weeks old were randomly divided into six treatments: T1 (No Salmonella and no garlic), T2 (garlic only), T3 (Ciprofloxacin only), T4 (Salmonella + Ciprofloxacin), T5 (Salmonella + garlic) and T6 (Salmonella without garlic or ciprofloxacin) and reared for six days. On day seven, birds were sacrificed, and blood samples were collected for hematology and biochemistry analysis, while liver and small intestines were also collected for histopathology. Data obtained were subjected to analysis of variance (ANOVA), and were means were found to be significant, Duncan's New Multiple Range Test was used to separate the means at a 5% probability level. The results revealed that white blood cell counts, neutrophils percentage, liver enzymes, total protein, and the weight of organs increased significantly on T6. In contrast, packed cell volume, red blood cell counts, and hemoglobin decreased significantly. Liver and small intestines damage were also observed. However, feeding birds with garlic counteracted the effect of ST on those parameters. It can be concluded that hexane extract of garlic can alleviate the harmful effect of ST in birds' systems and, thus, used in Poultry Farms against antibiotic resistance.

Key words: *Allium sativum*, Birds, Blood parameters, Histopathology and *S. typhimurium.*

INTRODUCTION

Animal Breeders in Nigeria have over the past years researched on improving the performance and disease resistance of the indigenous native chickens. The target is to make available safe and cheap animal protein to a greater proportion of the citizens, thus achieving the Sustainable Development Goals (SDGs) 1 and 2 (Morton et al. 2017). The Nigeria native chickens are preferred by the population for their good source of protein, the flavour of their meat and eggs compared to exotic chickens. They are also characterised by their capacities to hatch their eggs, hardy and resistant to diseases (Iyasere et al. 2019; Abou Zeid et al. 2020). However, the productivity rate of these local chickens remains very low due to inadequate nutrition, poor disease management and unimproved genetic potential in terms of their body weight (Munisi et al. 2015). To increase the productivity (body weight and eggs laying rate) of the Nigeria indigenous heavy ecotype chickens, they can be crossed with an improved breed of chicken such as the White Leghorn which are known for their higher egg and meat production. This productivity can only be maximized if poultry related diseases such as salmonellosis are also controlled.

Salmonella is a gram-negative bacterium that colonizes the gastrointestinal tract of chicken (Lee et al. 2020). The Centres for Disease Control and Prevention has reported that, Salmonella is one of the bacteria that causes foodborne disease (Froebel et al. 2020). Several *Salmonella serovars* have been identified by scientists and,

Cite This Article as: Tchoupou-Tchoupou EC, Ndofor-Foleng HM, Nwenya JM, Okenyi NJ, Ikeh Nnana E, Ngwu NR, Nwakpu PE, Ossei J and Onyimoniy EA, 2022. Effects of hexane extract of garlic on hematological, biochemical and histological parameters in F_1 crossbred chicks non-infected and infected with *Salmonella typhimurium*. International Journal of Veterinary Science 11(4): 435-442. https://doi.org/10.47278/journal.ijvs/2022.135

among them *Salmonella enterica* serovar typhimurium (Wang et al. 2020). Birds can be infected with *Salmonella enterica* serovar typhimurium (*S. typhimurium*) after ingesting contaminated feed or water. Salmonellosis symptoms in poultry farm includes diarrhea, ruffled feathers, wings drooping, reluctant to move and egg laying disorder (Bandyopadhyay 2017). It has been reported that, *S. typhimurium* is a zoonosis transmitted to humans through contaminated chicken eggs and meat. It causes considerable mortality and morbidity in animals and humans (EFSA BIOHAZ et al. 2019). Antibiotics such as ciprofloxacin have been used during the past century to fight against this bacterium. However, the misuse of these products has resulted in the emergence of a resistant microbial population (Aldeyab et al. 2020). For this reason, it is essential to control salmonellosis by increasing the natural resistance of chickens, thereby reducing the socioeconomic damages worldwide in the one hand and fight against antibiotic resistance on the other hand. Several alternatives, such as probiotics, prebiotics, phytobiotics and feed additive have been used worldwide to combat the spread of salmonella infection (Rebollada-Merino et al. 2020; Sugiharto et al. 2020).

Garlic (*Allium sativum* L.) plant of the Alliaceae family has long been used as a seasoning in kitchen, as well as an ethno-medical plant to prevent and treat infectious diseases. The medical characteristics of garlic depends on many compounds including diallyl thiosulfonate (allicin), diallyl sulfide, S-allyl-cysteine sulfoxide (alliin), amino acids and vitamins (Kodera et al. 2017; Mansingh et al. 2018; Shang et al. 2019). Used in humans, animals and plants, garlic and its formulations have various biological properties such as antiviral, antimicrobial, antibacterial, anti-inflammatory and antioxidant (Rouf et al. 2020; Chitra 2020). Due to such properties, garlic can therefore be used in poultry farms as a potential alternative to antibiotics in order to improve bird health and production performance.

The current work focused on evaluating the effects of hexane extract of garlic on haematological, biochemical and histopathological profile in F_1 crossbred chicks (White Leghorn cocks × Nigerian Heavy Ecotype hens) non-infected and infected with *S. typhimurium*.

MATERIALS AND METHODS

Location of the Study Area

This research work was carried out in the Poultry Unit of the Department of Animal Science Teaching and Research Farm (DASTRF), University of Nigeria, Nsukka (UNN). The farm is situated within the equatorial rainforest belt of the tropics and falls specifically within the derived savannah vegetation zone. Nsukka is positioned on latitude 5°22' North, longitude of 07°24'East and altitude of 447°25m above sea level; with two main seasons, dry season (November to March) and rainy season (April to October) (Ndofor-Foleng et al. 2015). The study was carried out after having consulted the institutional guideline on the use of animals for scientific research and obtaining ethical clearance.

Experimental

Preparation of *Allium sativum* Hexane Extract

Fresh pinkish garlic (*Allium sativum*) was purchased from Orie Orba Market, Nigeria and the hexane extract of garlic prepared according to Tchoupou-Tchoupou et al. (2021) was used.

Experimental Animals/Breeding Protocol

The base population for the exotic chickens (White Leghorn) were acquired from the local market (Orie Orba Market, Nigeria) and quarantined before being introduced into the experimental unit. The population of the local birds (female) were the heavy ecotype chickens which had undergone 7^{th} generation of index selection at the DASTRF, UNN.

The White Leghorn males were crossed with the heavy ecotype female using Artificial Insemination. Eggs were collected and hatched to have a population of 240 F_1 chicks. On hatching, the chicks were weighed and distributed according to treatments (Fig. 1): T1 (Negative Control: No Salmonella and no extract), T2 (Extract only), T3 (Ciprofloxacin only), T4 (Salmonella + Ciprofloxacin), T5 (Salmonella + Extract) and T6 (Positive Control: Salmonella without extract or ciprofloxacin). Each treatment had 40 birds, replicated 4 times with 10 birds per replicate. The birds were raised on conventional deep litter. Pens and all routine management practices were carried out.

A standard strain of *S. typhimurium* was received from the National Veterinary Research Institute in Vom Jos. The Chicks received a challenge of 10^8 organisms/ml NaCl solution of *S. typhimurium* by oral gavage (0.5mL) at two weeks of age and the faecal swab samples were used to determine the presence or absence of salmonella infection. The samples were cultured in tetrathionate broth (TTB) then, incubated for 18h at 37°C. The growth of TTB broth was streaked on Brilliant Green Agar (BGA) and MacConkey agar. These culture plates were incubated for 48h at 37°C. The plates were inspected for typical colonies of Salmonella. On MacConkey agar, the colour of the colonies is pale why on BGA the colour is pink (Dar et al. 2018).

The garlic hexane extract (800mg/kg) was diluted in drinking water and administered within a period of 5 days after infection to T2 and T5 while ciprofloxacin (antibiotic) used as a standard product was administered to T3 and T4. Throughout the experiment, birds were equally observed daily for clinical signs and mortality.

Hematological and Biochemical Parameters

After five days of treatment, blood samples were collected from three birds per replicate to determine the hematological indices such as white blood cell counts (WBC), packed cell volume (PCV), red blood cell counts (RBC), hemoglobin (HB), neutrophile, lymphocyte, monocyte, basophile and eosinophile. The blood was collected in sample bottles containing ethylene diamine tetra acetic acid as anticoagulant. Blood samples were also collected for serum biochemistry analysis such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total protein (TP).

Histopathology

At the end of the experiment, liver and small intestine (ileum) were collected and fixed in 10% neutral buffered formalin for 48 hours before being dehydrated in 4 grades (70, 80, 90 and 100%) of ethyl alcohol. The tissues samples were then prepared according to the standard procedure

described by Luna (1968). The slides were mounted in coverslips with distyrene, plasticizer and xylene, and examined with a Motic™ compound light microscope using x4, x10 and x40 objective lenses. The photomicrographs were taken using a Motic™ 5.0 megapixels microscope camera at x160 and x200 magnification.

Statistical Analysis

Data generated on the haematological and biochemical parameters were subjected to analysis of variance (ANOVA) using IBM statistic version 20 (IBM Corp, Armonk, NY, USA). Significantly different means were compared by using Duncan's New Multiple Range Test procedure accepted at 5% probability test.

RESULTS

The result of the effects of feeding garlic hexane extract to uninfected and infected birds with *S. typhimurium* on hematological parameters is presented in Table 1. White blood cells (WBC), PCV, lymphocytes, RBC and HB were highly significant affected by treatment with the p-value of ($P<0.0001$). Moreover, neutrophil and lymphocyte were also affected by treatments, while monocyte, basophil and eosinophil were not.

The average values of WBC on T4 (13.83×10^3/L), T5 (13.32×10^3/L) and T6 (13.60×10^3/L) were significantly ($P<0.05$) higher than the values of 8.98×10^3/L, 8.93×10^3/L and 8.95×10^3/L recorded for birds in the control groups T1, T2 and T3 respectively. However, the average values of RBC and HB counts were significantly decrease ($P<0.05$) in the birds infested with *S. typhimurium* on T4, T5 and T6 compared to groups T1, T2 and T3 non-infested. The highest value was obtained on T1, while the lowest was recorded in positive control. Packed Cell Volume was significant increase ($P<0.05$) in the non-infected groups compared to the infected groups. The average values in birds fed with garlic significantly increased from 26.35 to 31.25% when compared to the positive control (21.75%). The lymphocytes count showed the highest values in the non-infected groups compared to the infected groups. However, the neutrophils in the infected birds had higher values than the values in the non-infected birds.

The result of serum biochemistry analysis presented in Table 2 showed that birds in the positive control group had higher AST, ALT, ALP and TP than groups T1, T2, T3, T4 and T5. Nevertheless, the concentrations of these parameters were reduced in groups fed garlic (T2 and T5) and ciprofloxacin (T3 and T4).

The weights of the liver and small intestine (Table 3) were significantly ($P<0.05$) affected by treatments. The value recorded on T6 was higher compared to the values obtained in other treatments. Feeding bird with garlic or ciprofloxacin reduce the weight of the organs.

Table 1: Least square mean±SEM of hematologycal parameters.

	TREATMENTS					
PARAMETERS	T1	T2	T3	T4	T5	T6
PCV (%)	33.25±1.37^{a}	31.25±1.10^{a}	32.00±.0.81^{a}	26.25±1.31^{b}	26.35±0.92^{b}	21.75± 0.5^{c}
RBC $*10^6$/L	2.13±0.04^{a}	2.08±0.06^{a}	1.98±0.03^{a}	1.46±0.06^{b}	1.57±0.10^{b}	1.57±0.04^{b}
WBC $*10^3$/L	8.98±0.39^{b}	8.93±0.28^{b}	8.95±0.20^{b}	13.83±0.10^{a}	13.32±0.99^{a}	13.60±0.55^{a}
HB (g/dl)	10.32±0.14^{a}	10.15±0.29^{a}	10.17±0.21^{a}	7.10±0.27^{b}	7.27±0.47^{b}	6.77±0.22^{b}
Neutrophil (%)	15.75±2.17^{c}	21.00±1.91^{bc}	22.25±3.01^{abc}	36.00±7.25^{ab}	40.250±10.61^{a}	34.25±2.83^{ab}
Lymphocyte (%)	80.50±3.01^{a}	76.75±1.37^{a}	76.00±2.82^{a}	62.00±7.29^{ab}	49.500±10.97^{b}	63.500±3.42^{at}
Monocyte (%)	2.00±1.08	1.50±0.28	1.25±0.47	1.00±0.40	1.07 ±0.41	1.50±0.64^{ns}
Basophile (%)	1.00±0.70	0.50±0.28	0.50±0.28	0.50±0.28	0.37±0.23	0.50±0.28 ^{ns}
Eosinophil (%)	0.75±0.47	0.25±0.25	0.00 ±0.00	0.50±0.50	0.07±0.07	0.25±0.25 ^{ns}

Data shown are Mean±SE. ^{a,b,c} -Row means with different superscripts are either significant at $P<0.05$ or highly significant at $P<0.01$. T1=control; T2=Garlic only; T3=Cipro only; T4=Salmonella+Cipro; T5=Salmonella+Garlic; T6=Salmonella only; WBC=White Blood Cell; PCV=Packed cell volume; RBC=Red Blood Cell; HB=Hemoglobin concentration

Table 2: Least square means±SEM of some serum biochemical parameters in different treatments

	TREATMENTS					
PARAMETERS	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
AST (U/L)	209.30±17.64^{ab}	208.95±18.67^{ab}	181.15±7.66^{b}	187.55±7.70^{ab}	209.02±9.93^{ab}	222.95±2.77^{a}
ALT (U/L)	16.58±1.90^{b}	15.40±2.77^{b}	11.87±1.67^{b}	29.50±5.91^{a}	16.31±1.59^{b}	30.19±7.10^{a}
ALP (IU/L)	4860±640^{b}	4309±723^{b}	4635±423^{b}	7650±490^{a}	5291±863^{b}	8794±910^{a}
TP (g/dL)	3.02±0.13^{ab}	3.10±0.40^{ab}	3.10±0.17^{ab}	3.20±0.15^{ab}	2.90±0.58^{b}	3.65±0.13^{a}

Data shown are Mean±SE. ^{a,b,c} -Row means with different superscripts are either significant at $P<0.05$ or highly significant at $P<0.01$. T1=control; T2=Garlic only; T3=Cipro only; T4=Salmonella+Cipro; T5=Salmonella+Garlic; T6=Salmonella only.

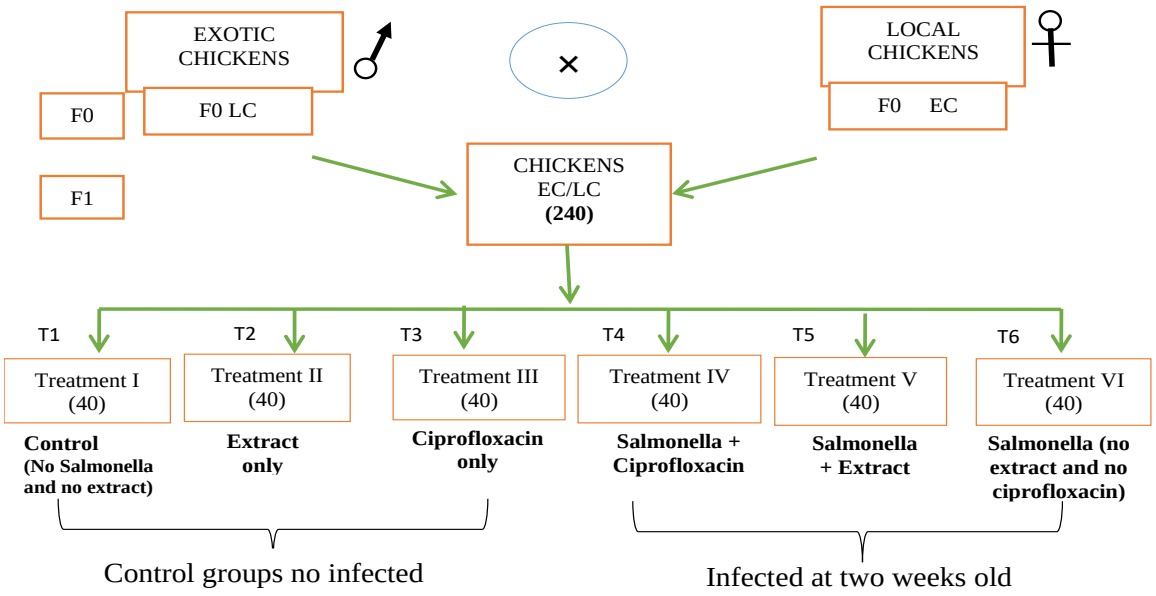

Fig. 1: Treatment protocol.

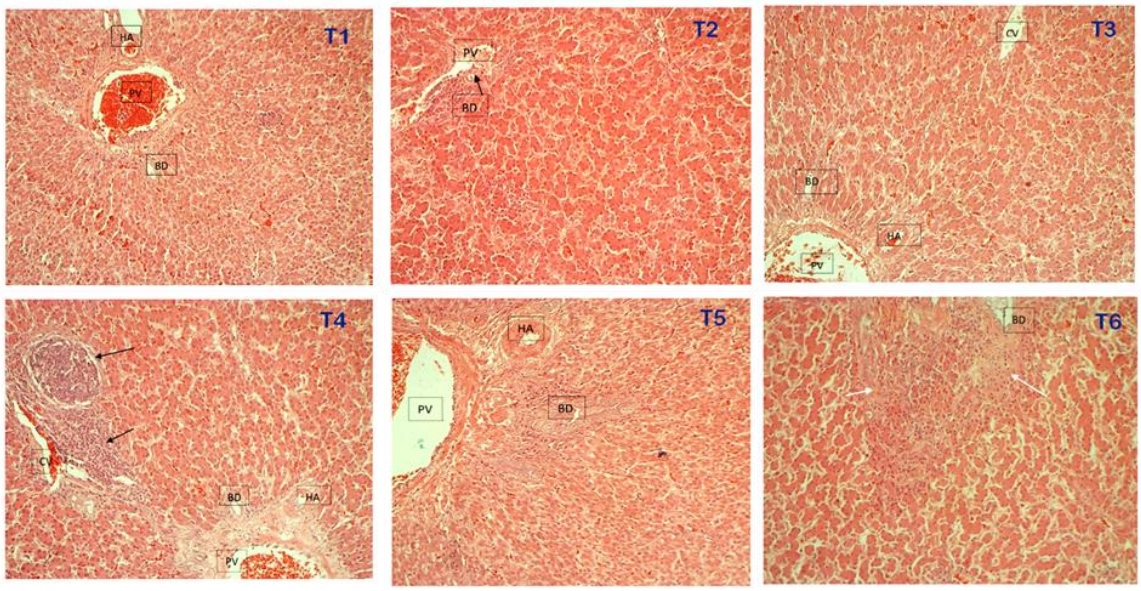

Fig. 2: Photomicrograph of liver sections from experimental birds of treatments 1, 2, 3, 4, 5 and 6, H and E ×400: Hepatic artery (HA), Portal vein (PV), Bile ductile (BD), Centrilobular venule (CV); (T1, T2 and T3): normal histology; (T4): aggregates of lymphoid cells (arrow); (T5): normal hepatic histomorphology; (T6): multifocal areas of necrosis (arrow) and multifocal aggregates of lymphoid cells.

Table 3: Organ's weight

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Liver	10.23±0.42^{ab}	8.74±0.99^{b}	9.81±0.56^{ab}	10.19±0.41^{ab}	8.97±0.76^{ab}	10.88±0.48^{a}
Small Intestine	22.47±1.37^{b}	21.13±2.22^{b}	28.45±1.81^{a}	21.01±0.97^{b}	23.75±1.10^{b}	28.52±0.78^{a}

Data shown are Mean±SE. ^{a,b,c} -Row means with different superscripts are either significant at P<0.05 or highly significant at P<0.01. T1=control; T2=Garlic only; T3=Cipro only; T4=Salmonella+Cipro; T5=Salmonella+Garlic; T6=Salmonella only.

Photomicrographs of liver and ileum sections from experimental birds on T1, T2, T3, T4, T5 and T6 are shown in Fig. 2 and 3 respectively. Fig. 2 shows normal hepatocytes with normal hepatic sinusoids containing RBCs on T1, T2, T3 and T5. The sections on T4 presented random multifocal variably sized and mostly well circumscribed aggregates of lymphoid cells (arrow). These lymphoid aggregates were mostly organized adjacent to the centrilobular vein or the portal tracts. On T6, it was observed multifocal areas of necrosis (arrow), multifocal aggregates of lymphoid cells and dissociated hepatocytes. Fig. 3 revealed normal intestinal villi (white arrow) and crypts (black arrow) lined by normal enterocytes with normal lamina propria of the mucosa on T1, T2 and T3. The sections on T4 showed a relatively normal intestinal histomorphology. However, a few areas presented intense

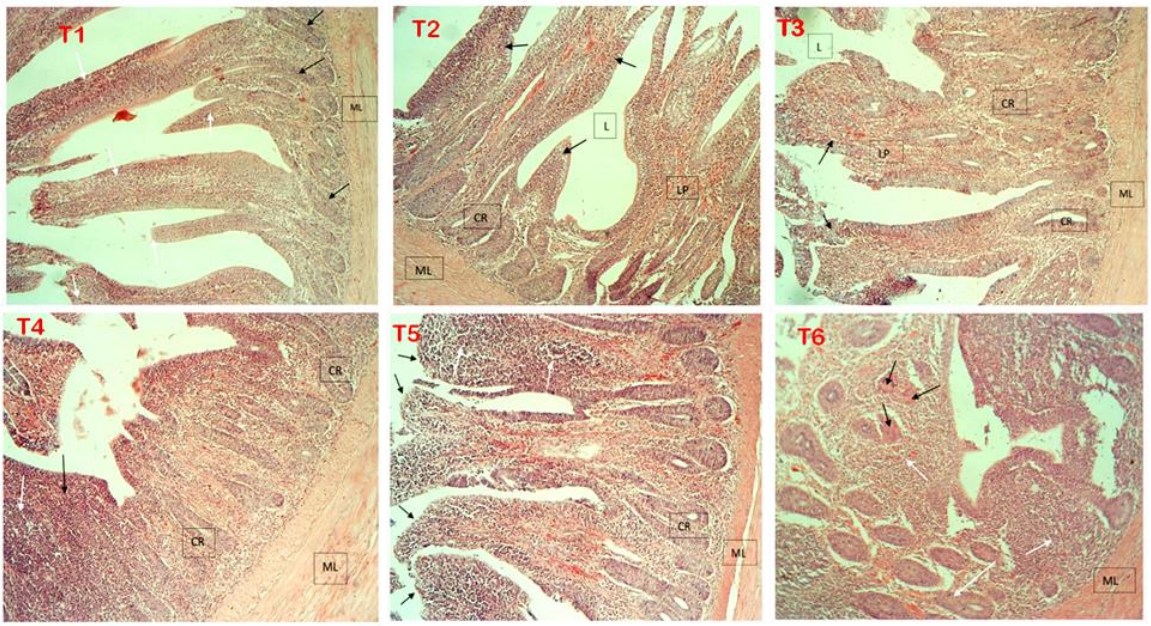

Fig. 3: Photomicrograph of small intestine sections from experimental birds of treatments 1, 2, 3, 4, 5 and 6, H and E ×400: Muscularis layer (ML), Lumen (L), Lamina propria of mucosa (LP), Crypts (CR); (T1): Normal intestinal villi (white arrow) and crypts (black arrow); (T2 and T3): normal enterocytes (arrow); (T4): cellular infiltration of the lamina propria (black arrow) with formation of lymphoid nodules (white arrow); (T5): necrosis and sloughing of the enterocytes at the villus tips (black arrow) and leucocytic infiltration of the lamina propria (white arrow); (T6): necrosis of the enterocytes at the villus tips with marked leucocytic infiltration of the lamina propria (white arrow) and necrosis of the crypt lining cells.

cellular infiltration of the lamina propria of the mucosa (black arrow) with formation of variably sized and often well circumscribed lymphoid nodules (white arrow). On T5, necrosis and sloughing of the enterocytes at the villus tips (black arrow) with mild to moderate leucocytic infiltration of the lamina propria were observed (white arrow). The sections observed on T6 revealed marked necrosis of the enterocytes at the villus tips with marked leucocytic infiltration of the lamina propria (white arrow) as well as necrosis of the crypt lining cells (black arrow).

DISCUSSION

The main objective of this study was to evaluated the effects of garlic on F_1 chicks (White Leghorn cocks × Nigerian Heavy Ecotype hens) uninfected and infected with *S. typhimurium*. It is well known that haematological profile (Table 1) is an essential indicator of the physiological or pathophysiological status of the animals' well-being (Onasanya et al. 2015). The present findings revealed that, the range of WBC in the infected groups was higher than the normal ranged from 8.85-10.22×10^3/L for bird reported by Abdi-Hachesoo et al. (2011). Moreover, it was also observed the increase in the number of neutrophils in those groups. This may probably be because of the present of *S. typhimurium* in the bird systems, which is responsible for infection and inflammation (Khan and Chousalkar 2020). It has been reported that, during the early stage of an infection a large number of neutrophils migrate from the peripheral blood to the site of inflammation where the microorganism is eliminated by both phagocytic and non-phagocytic mechanisms (Mortaz et al. 2018). The observed increase in the number of neutrophils in the treatments fed garlic (T2 and T5) compared to the negative control could prove the capacity of garlic to stimulate these leukocytes, which are the most dominant and efficient cells during both inflammation and phagocytosis (Rosales 2018). These results may be attributed to the diallyl sulfide, a sulfur compound found in garlic with the potential to increase immunity by stimulating several cells including macrophages, neutrophils and lymphocytes (Subramanian et al. 2020). In agreement with the present results, Ademola et al. (2004) stated that birds fed garlic powder have an increase value of neutrophils count than their counterparts without garlic inclusion. This observation against immuno-stimulatory effects of garlic also corroborates with the report of Zeryehun et al. (2017).

The significant decrease ($P<0.05$) of the RBC and HB counts in groups challenged with Salmonella indicates that, the bacteria negatively affect the erythrocyte precursor hormone (erythropoietin) responsible for the synthesis of RBC. Zivot et al. (2018) reported that, the production of the RBC is stimulated by erythropoietin (EPO), which is a humoral cytokine produced in the kidney and released into the systemic circulation with the primary role to regulate oxygen transport to organs. However, the infection of this organ can decrease the quantity of RBC into the bloodstream leading to the dyserythropoietic diseases including leukaemia and anaemia. Consequently, inefficient erythropoiesis results in the increase in quantity of immature RBC precursors and leukocytes in the general circulation (Moras et al. 2017). The reduction of the oxygen carrying capacity of these cells therefore affect the well-being of birds. Feeding bird with garlic without *S. typhimurium* infection did not show any negative effect. This could mean that the consumption of garlic for 6 days did not cause anaemia and hypoxia to birds. This could be attributed to the capacity of garlic to stimulate EPO trough erythropoiesis and thus preventing tissue hypoxia (Brines and Cerami 2012). In contrast, Al-Jowari (2017) reported

that, the administration of garlic powder at 1% and 5% compared to the control group in male rabbits for 21 days cause a significant (P<0.05) increase in the RBCs count. This difference may be due to the garlic preparation, the duration of the experiment or/and the species variation.

One of the tests giving more information about anaemia and the rate of dehydration is PCV. The normal PCV for chickens ranged from 22-43% as reported by Santoso et al. (2015). The authors observed on this study that, the percentage of PCV on T6 was lower than the ranges earlier reported. This may be due to the harmful effect of *S. typhimurium* inducing liver and kidney inflammation, folic acid and serum iron deficiency (Shubham et al. 2020; El-Lattief et al. 2020). In addition, Chandra et al. (2014) stated that, Salmonella can also destroy the haematopoietic precursors in the organs such as liver. In the same vein, Dar et al. (2018) also reported that RBC, PCV and HB decreased significantly (P<0.05) in bird system after the *S. typhimurium* infection. However, feeding bird with garlic did not cause any common conditions. This is because allicin, the main phytochemical in garlic has the potential to inhibit the growth of *S. typhimurium* and maintain normal the level of RBC in the organism (Salehi et al. 2019). In addition, Vitamin C or ascorbic acid an efficient water-soluble antioxidant found in garlic can also improves the absorption of iron by capturing non-heme iron and storing it in a form that can be absorbed by the organism. Iron plays a key role in the production of hemoglobin, which is responsible for oxygen transportation (He et al. 2018; Timoshnikov et al. 2020). This result agrees with the findings of Zeryehun et al. (2017) who noted that the administration of garlic extract at 1% significantly (P<0.01) improved the PCV value of White Leghorn chicken.

The values recorded for lymphocytes count were within the normal ranged from 24-84% for chickens as reported by Santoso et al. (2015). The non-significant values obtained in the infected groups could be attributed to the natural resistance of the local chicken used as parent stock.

Serum biochemistry profiling is a major tool used in the diagnosis and treatment of livestock diseases through the investigation of many parameters influencing serum bio-chemical indices (Onasanya et al. 2015). Aspartate aminotransaminase (AST), ALT, ALP and TP are often used to detect liver damage and dysfunction. This is because during pathological infection, ALT and AST are released by the liver in the blood to reflect the status of the animal and, TP to express the parenchymal damages (Kalra et al. 2021). The increased quantity of ALP in the blood, is mostly due to liver diseases, bile duct obstruction, gallbladder diseases, or bone disorders (Babu et al. 2020). The normal ranges of the concentration of liver enzymes and total protein are: 70-220U/L (AST), 568-8831U/L (ALP) (Odunitan-Wayas et al. 2018) and 2.8-3.32g/dL (TP) (Tóthová et al. 2019). In the present study, AST and TP on T6 were out of the normal ranges earlier reported. Alkaline phosphatase was within the normal ranges for all the treatment. The significantly (P<0.05) decreased results of the liver enzymes and TP in chicks fed garlic could be due to the protective effect of the biochemical components it contains such as saccharides and phenolic compounds (Mhyson 2017). The plant extract may also be non-toxic to the liver. These results agree to the findings of Omer et al. (2019) who also made similar observation on the capacity of garlic to improve bird health.

In this study, the increase weights of the liver and small intestine recorded in the positive control may be a result of the competition of the bacteria with the microbiota for nutrients in order to overcomes colonization resistance and disseminate in the liver (Gart et al. 2016). *Salmonella typhimurium* is able to alter the nutritional environment by metabolizing several compounds, such as ethanolamine produces in the intestine, as a source of carbon and nitrogen. The used of ethanolamine is to produced necessary energy to enhance its growth (Bäumler and Sperandio 2016). Feeding bird with garlic or ciprofloxacin reduce the weight of the organs and thus the mortality rate (result not shown). The findings of this study compare favorably with the results of Adibmoradi et al. (2006) who reported that garlic is able to activate the absorptive process as an antibiotic alternative in chicken. The beneficial effect of garlic observed is probably due to the potency of the bioactive compounds it contains, which are responsible for the antibacterial activity of the garlic hexane extract (El-Saber Batiha et al. 2020; Tchoupou-Tchoupou et al. 2021).

According to the previous results, *S. typhimurium* is responsible to several injuries in the liver (Fig. 2) and ileum (Fig. 3) tissues, even though no gross lesions were observed. The harmful effects of the bacteria recorded on T6 had also been reported by Muna et al. (2016) during *S. typhimurium* outbreak in Sudan. These authors stated that natural infestation of chicks with Salmonella damaged organs such as liver, intestine, kidney and heart after post-mortem examinations. In addition, Kumari et al. (2013) also stated a desquamation of mucosal epithelium, villi and lumen necrosis, infiltration of granulated and mononuclear leukocytes, pathologic hyperplasia, and focal fibroblastic tumors when broiler chickens were infected with *S. gallinarum*. These observations testify the severity of the infiltration of Salmonella in the bird's system. However, feeding bird with garlic significantly reduced the tissues lesions. It could be supposed that the mechanisms by which garlic alleviated the hepatocytes lesions in chicks infected with *S. typhimurium* may be attributed to its antioxidant compounds including allicin. Allicin reacts with thiol-possessing enzymes to inhibit nitric oxide, hydroxyl and superoxide radical accumulation responsible to induce oxidative stress and tissues damages (Salehi et al. 2019). This study also showed that garlic did not causes abnormalities in the bird intestine. It can then be supposed that garlic can be used to balance the microbiota and overcome dysbiosis in order to enhanced diversity of commensal microbes in the gastrointestinal tract.

Conclusion

This study has emphasized on the effects of garlic in improving the health of F_1 chicks (White Leghorn cocks × Nigerian Heavy Ecotype hens) uninfected and infected with *S. typhimurium*. It was observed that garlic could be used in poultry industry as an alternative to antibiotics (such as ciprofloxacin) to combat salmonellosis and fight antibiotic resistance. The administration of this probiotic prevents the destruction of the liver parenchyma and cell dysfunction. Garlic also has immuno-stimulatory effects of neutrophils whose role is known in the destruction of the

pathogen in a cell/organism. Histopathological analysis showed that garlic hexane extract has a hepatoprotective power. This demonstrates that garlic hexane extract can be used in poultry farms to fight against microbial resistance induce by antibiotics and then prevent humans from zoonosis.

Acknowledgements

We thank all the technical and research staffs of the Departments of Animal Science and Veterinary Medicine, Faculty of Agriculture, UNN who contributed to the data collection.

Funding

I would like to thank the German Academic Exchange Service (DAAD) for the generous financial support. It is because of you that I have been able to do this work. I am extremely grateful.

REFERENCES

Abdi-Hachesoo B, Talebi A and Asri-Rezaei S, 2011. Comparative study on blood profiles of indigenous and Ross-308 broiler breeders. Global Veterinaria 7: 238-241.

Abou Zeid MAM, Nasef SA, Ali GIE and Hegazy AM, 2020. A field study on biochemical changes associated with salmonella infection in ducklings. Journal of World's Poultry Research 10: 250-262. https://doi.org/10.36380/JWPR.2020.31

Ademola SG, Farinu GO, Obe AA and Babatunde GM, 2004. Growth, haematological and biochemical studies on garlic and ginger fed broiler chickens. Moor Journal of Agricultural Research 5: 122-128. https://doi.org/10.4314/mjar.v5i2.31809

Adibmoradi M, Navidshad B, Seifdavati J and Royan M, 2006. Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. The Journal of Poultry Science 43: 378-383. https://doi.org/10.2141/jpsa.43.378

Aldeyab M, López-Lozano JM and Gould IM, 2020. Global antibiotics use and resistance. In Global Pharmaceutical Policy, Palgrave Macmillan, Singapore, pp: 331-344. https://doi.org/10.1007/978-981-15-2724-1_13

Al-Jowari SAK, 2017. Effect of garlic powder (*Allium sativum*) on blood constituents in male rabbits. Al-Nahrain Journal of Science 17: 132-137. https://doi.org/10.22401/JNUS.17.3.18

Babu NA, Masthan KMK, Krupaa RJ and Hariharan R, 2020. Alkaline Phosphatase and its clinical importance-A Review. European Journal of Molecular and Clinical Medicine 7: 1409-1413.

Bandyopadhyay S, 2017. Systemic Clinical and Metabolic Diseases. Pet Bird Diseases and Care 167-252. https://doi.org/10.1007/978-981-10-3674-3_3

Bäumler AJ and Sperandio V, 2016. Interactions between the microbiota and pathogenic bacteria in the gut. Nature 535: 85-93. https://doi.org/10.1038/nature18849

Brines M and Cerami A, 2012. The receptor that tames the innate immune response. Molecular Medicine 18: 486-96. https://doi.org/10.2119/molmed.2011.00414

Chandra H, Chandra S, Kaushik R, Bhat N and Shrivastava V, 2014. Hemophagocytosis on Bone Marrow Aspirate Cytology: Single Center Experience in North Himalayan Region of India. Annals of Medical and Health Sciences Research 4: 692-696. https://doi.org/10.4103/2141-9248.141515

Chitra P, 2020. Study the effect of dietary supplementation of garlic (*Allium sativum*) tulsi (*Ocimum sanctum*) leaf powder on growth performance of broilers. The Pharma Innovation Journal 9: 70-72.

Dar MA, Ahmed R, Urwat U, Ahmad SM, Dar PA, Kushoo ZA, Dar TA, Muntaz PT, Bhat SA, Amin U, Shabir N, Bhat HF, Shah RA, Gnai NA and Heidari M, 2018. Expression kinetics of natural resistance associated macrophage protein (NRAMP) genes in *Salmonella typhimurium* infected chicken. BioMed Central Veterinary Research 14: 180. https://doi.org/10.1186/s12917-018-1510-4

El-Lattief AA, Marouf S, El-Bialy A and El-Jakee J, 2020. Development of a duplex real-time PCR for differentiation of Salmonella Typhimurium and monophasic serovars. Journal of World's Poultry Research 10: 299-325. https://doi.org/10.36380/jwpr.2020.36

El-Saber Batiha G, Beshbishy A, GL Wasef, Elewa Y, Al-Sagan A, El-Hack A, Taha A, Abd-Elhakim M and Devkota H, 2020. Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A review. Nutrients 12: 872. https://doi.org/10.3390/nu12030872

Froebel LK, Froebel LE and Duong T, 2020. Refined Functional Carbohydrates reduce adhesion of Salmonella and Campylobacter to poultry epithelial cells in vitro. Poultry Science 99: 7027-7034. https://doi.org/10.1016/j.psj.2020.09.031

Gart EV, Suchodolski JS, Welsh JRTH, Alaniz RC, Randel RD and Lawhon SD, 2016. *Salmonella typhimurium* and multidirectional communication in the gut. Frontiers in Microbiology 7: 1827. https://doi.org/10.3389/fmicb.2016.01827

He H, Qiao Y, Zhang Z, Wu Z, Liu D, Liao Z, Yin D and He M, 2018. Dual action of vitamin C in iron supplement therapeutics for iron deficiency anaemia: prevention of liver damage induced by iron overload. Food and function 9: 5390-5401. https://doi.org/10.1039/C7FO02057K

Iyasere OS, Ajayi OD, Alade SO and Akinbode VO, 2019. Behaviour, physiology and body mass of Nigerian indigenous hens during brooding. Agricultura Tropica et Subtropica 52: 43-47. https://doi.org/10.2478/ats-2019-0005

Kalra A, Yetiskul E, Wehrle CJ and Tuma F, 2021. Physiology, Liver. [Updated 2021 May 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK535438/

Khan S and Chousalkar KK, 2020. *Salmonella typhimurium* infection disrupts but continuous feeding of Bacillus based probiotic restores gut microbiota in infected hens. Journal of Animal Science and Biotechnology 11: 29. https://doi.org/10.1186/s40104-020-0433-7

Kodera Y, Ushijima M, Amano H, Suzuki J and Matsutomo T, 2017. Chemical and biological properties of S-1-propenyl-L-cysteine in aged garlic extract. Molecules 22: 570. https://doi.org/10.3390/molecules22040570

EFSA BIOHAZ P, Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid Chemaly M, De Cesare A, and Davies R, 2019. Salmonella control in poultry flocks and its public health impact. EFSA Journal 17: e05596. https://doi.org/10.2903/j.efsa.2019.5596

Kumari D, Mishra SK and Lather D, 2013. Pathomicrobial studies on *Salmonella gallinarum* infection in broiler chickens. Veterinary World 6: 725. https://doi.org/10.14202/vetworld.2013.725-729

Lee M, Hosseindoust A, Oh S, Ko H, Cho E, Sa S, Kim Y, Choi J and Kim J, 2020. Impact of an anti-*Salmonella Typhimurium* Bacteriophage on intestinal microbiota and immunity status of laying hens. Journal of Animal Physiology and Animal Nutrition. https://doi.org/10.1111/jpn.13424

Luna LG, 1968. Manual Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Ed, McGraw Hill Book Co., New York, USA, pp: 58.

Mansingh DP, Dalpati N, Sali VK and Vasanthi AHR, 2018. Alliin the precursor of allicin in garlic extract mitigates proliferation of gastric adenocarcinoma cells by modulating apoptosis. Pharmacognozy Magazine 14: S84–S91. https://doi.org/10.4103/pm.pm_342_17

Mhyson AS, 2017. The effect of garlic and neomycin supplementation in diet on productive and some blood parameters of experimentally infected broiler chickens with *Salmonella typhimurium*. Al-Qadisiyah Journal of Veterinary Medicine Sciences 16: 16-23. https://doi.org/10.29079/vol16iss1art411

Moras M, Lefevre SD and Ostuni MA, 2017. From Erythroblasts to Mature Red Blood Cells: Organelle Clearance in Mammals. Frontiers in Physiology 8: 1076. https://doi.org/10.3389/fphys.2017.01076

Mortaz E, Alipoor SD, Adcock IM, Mumby S and Koenderman L, 2018. Update on Neutrophil Function in Severe Inflammation. Frontiers in Immunology 9: 2171. https://doi.org/10.3389/fimmu.2018.02171

Morton S, Pencheon D and Squires N, 2017. Sustainable Development Goals (SDGs), and their implementation: A national global framework for health, development and equity needs a systems approach at every level. British Medical Bulletin 124: 1-10. https://doi.org/10.1093/bmb/ldx031

Muna EA, Salih MH, Zakia AM, Halima MO, Abeer AM, Ameera MM, Ali HO and Idris SB, 2016. Pathology of Broiler Chicks Naturally Infected with *Salmonella enteritidis* (*S. enteritidis*) and *Salmonella typhimurium* (*S. typhimurium*) During an Outbreak in Sudan. Journal of Scientific Research and Reports 1-8. https://doi.org/10.9734/JSRR/2016/23431

Munisi WG, Katule AM and Mbaga SH, 2015. Comparative growth and liability performance of exotic, indigenous chickens and their crosses in Tanzania. Livestock Research for Rural Development 27: 66. http://www.lrrd.org/lrrd27/4/muni27066.htm

Ndofor-Foleng HM, Oleforuh-Okoleh V, Musongong GA, Ohageni J and Duru UE, 2015. Evaluation of growth and reproductive traits of Nigerian local chicken and exotic chicken. Indian Journal of Animal Research 49: 155-160. https://doi.org/10.5958/0976-0555.2015.00046.1

Odunitan-Wayas F, Kolanisi U and Chimonyo M, 2018. Haematological and serum biochemical responses of Ovambo chickens fed provitamin a biofortified maize. Brazilian Journal of Poultry Science 20: 425-434. https://doi.org/10.1590/1806-9061-2016-0444

Omer HAA, Ahmed SM, Abdel-Magid SS, El-Mallah GMH, Bakr AA and Fattah MMA, 2019. Nutritional impact of inclusion of garlic (*Allium sativum*) and/or onion (*Allium cepa* L.) powder in laying hens' diets on their performance, egg quality and some blood constituents. Bulletin of the National Research Centre 43: 23. https://doi.org/10.1186/s42269-019-0061-6

Onasanya GO, Oke FO, Sanni TM and Muhammad AI, 2015. Parameters influencing haematological, serum and bio-chemical references in livestock animals under different management systems. Open Journal of Veterinary Medicine 5: 181-189. https://doi: 10.4236/ojvm.2015.58025

Rebollada-Merino A, Ugarte-Ruiz M, Hernández M, Miguela-Villoldo P, Abad D, Rodríguez-Lázaro D, Lucía de Juan, Domínguez L and Rodríguez-Bertos A, 2020. Reduction of *Salmonella typhimurium* caecal colonisation and improvement of intestinal health in broilers supplemented with fermented defatted 'alperujo', an olive oil by-product. Animals 10: 1931. https://doi.org/10.3390/ani10101931

Rosales C, 2018. Neutrophil: A cell with many roles in inflammation or several cell types? Frontiers in physiology 9: 113. https://doi.org/10.3389/fphys.2018.00113

Rouf R, Uddin SJ, Sarker DK, Islam MT, Ali ES, Shilpi JA, Nahar L, Tiralongo E and Sarker SD, 2020. Anti-viral potential of garlic (*Allium sativum*) and it's organosulfur compounds: A systematic update of pre-clinical and clinical data. Trends in Food Science and Technology 104: 219-234. https://doi.org/10.1016/j.tifs.2020.08.006

Salehi B, Zucca P, Orhan IE, Azzini E, Adetunji CO, Mohammed SA, Banerjee SK, Sharopov F, Rigano D, Sharifi-Rad J, Armstrong L, Martorell M, Sureda A, Martins N, Selamoğlu Z and Ahmad Z, 2019. Allicin and health: A comprehensive review. Trends in Food Science and Technology 86: 502-516. https://doi.org/10.1016/j.tifs.2019.03.003

Santoso U, Fenita Y and Kususiyah K, 2015. Effect of fermented *Sauropus androgynus* leaves on blood lipid fraction and haematological profile in broiler chickens. Journal of Indonesian Tropical Animal Agriculture 40: 199-207. https://doi.org/10.14710/jitaa.40.4.199-207

Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, Mavumengwana V and Li HB, 2019. Bioactive Compounds and Biological Functions of Garlic (*Allium sativum* L.). Foods (Basel, Switzerland) 8: 246. https://doi.org/10.3390/foods8070246

Shubham K, Anukiruthika T, Dutta S, Kashyap AV, Moses JA and Anandharamakrishnan C, 2020. Iron deficiency anemia: A comprehensive review on iron absorption, bioavailability and emerging food fortification approaches. Trends in Food Science and Technology 99: 58-75. https://doi.org/10.1016/j.tifs.2020.02.021

Subramanian MS, Nandagopal MSG, Amin Nordin S, Thilakavathy K and Joseph N, 2020. prevailing knowledge on the bioavailability and biological activities of sulphur compounds from Alliums: A Potential Drug Candidate. Molecules 25: 4111. https://doi.org/10.3390/molecules25184111

Sugiharto S, Widiastuti E, Isroli I, Wahyuni HI and Yudiarti T, 2020. Effect of a fermented mixture of papaya leaf and seed meal on production traits and intestinal ecology of the indonesian indigenous crossbred chickens. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 68: 707-718. https://doi.org/10.11118/actaun202068040707

Tchoupou-Tchoupou EC, Osseyi EG, Ndofor-Foleng HM and Onyimonyi EA, 2021. In vitro antibacterial activity of *Allium sativum* (garlic) extract against *Salmonella typhimurium*. European journal of pharmaceutical and medical research 8: 172-178.

Timoshnikov VA, Kobzeva TV, Polyakov NE and Kontoghiorghes GJ, 2020. Redox Interactions of Vitamin C and Iron: Inhibition of the pro-oxidant activity by deferiprone. International Journal of Molecular Sciences 21: 3967. https://doi.org/10.3390/ijms21113967

Tóthová C, Sesztáková E, Bielik B and Nagy O, 2019. Changes of total protein and protein fractions in broiler chickens during the fattening period. Veterinary World 12: 598-604. https://doi.org/10.14202/vetworld.2019.598-604

Wang M, Qazi IH, Wang L, Zhou G and Han H, 2020. Salmonella Virulence and Immune Escape. Microorganisms 8: 407. https://doi.org/10.3390/microorganisms8030407

Zeryehun T, Asrat M, Amha N and Urge M, 2017. Effects of supplementation of different levels of garlic (*Allium Sativum*) on selected blood profile and immunity of White Leghorn chicken. Biotechnology in animal husbandry 33: 333–348. https://doi.org/10.2298/BAH1703333Z

Zivot A, Lipton JM, Narla A and Blanc L, 2018. Erythropoiesis: insights into pathophysiology and treatments in 2017. Molecular medicine (Cambridge, Mass.) 24: 11. https://doi.org/10.1186/s10020-018-0011-z