EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II GENE IN COMMERCIAL STRAINS OF BROILER CHICKENS ADMINISTERED AQUEOUS EXTRACT OF GINGER (*Zingiber officinale*)

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

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ANIMAL PRODUCTION

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DECLARATION

I hereby declare that this thesis titled **"Expression of major histocompatibility complex Class II gene in commercial strains of broiler chickens administered aqueous extract of ginger (*Zingiber officinale*)"** is a collection of my original research work and that it has not been presented for any other qualification anywhere. Information from other sources (published or unpublished) has been duly acknowledged.

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SIGNATURE/DATE

CERTIFICATION

This is to certify that this thesis titled **"Expression of major histocompatibility complex Class II gene in commercial strains of broiler chickens administered aqueous extract of ginger (*Zingiber officinale*)"** carried out by OKOLO, Gabriel Papa (MTech/SAAT/2019/9398) meets the regulations governing the award of the degree of Masters of Technology (MTech) of the Federal University of Technology, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

I dedicate this work to God Almighty the author and finisher of our faith. Also, to my family, friends and loved ones both at home and in the diaspora.

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Benjamin's, Lawrence, Simon, Arome, Gabriel and Isreals) I love you all. Idris, M.O., I cannot say how grateful I am for having you in my life. Keep being you and doing yourself.

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ABSTRACT

Due to regulation of the usage of synthetic antibiotics as a result of the development of resistance by micro-organisms, the growth performance, haematology and expression of Major Histocompatibility Complex (MHC) Class II gene in broiler chickens administered varying levels of aqueous ginger extract was investigated. Two hundred and seventy day-old Arbor Acres Plus, Cobb 500 and Ross 308 chicks were used in the experiment. The birds were randomly divided into three groups of 90 birds along strains and designated as T1, T2, and T3. They were further divided into 3 treatments of 30 birds with 3 replicates of 10 chicks each. The control was administered 0.2g of Oxytetracycline$^{®}$ in 2 litres of water following the recommendation of the manufacturer; 4 and 6 % aqueous ginger extract was administered via drinking water to birds in treatments 2 and 3 respectively. A single phase feeding was done with the diet consisting 2,948.05 kcal/kg ME; 22.34 % CP fed to the birds for 568 days. Growth performance parameters were evaluated during the experiment. Liver samples fixed in RNA Later solution and blood samples from the vein of the birds were collected at the end of the experiment and used for the gene expression and haematology study. Results for growth performance showed that the Cobb 500 performed significantly ($P<0.05$) better (1662 g) than Ross 308 (1503 g) and Arbor Acres Plus birds (1501 g) in average final body weight gain. For the effect of varying ginger extracts on growth performance, birds administered 6 % ginger extract performed significantly ($P<0.05$) better than those given 4 % ginger extract and the control (0 %). Haematology showed a significant ($P<0.05$) strain effect on haemoglobin concentration, packed cell volume, and red blood cell count of the birds; Arbor Acres Plus birds had better values of the parameters (18.00 g/dl, 38.33 %, 2.52 x 10^{6}/mm^{3}) than the other strains. The level of inclusion of the aqueous ginger extract also significantly ($P<0.05$) influenced the red blood cell count and white blood cell count of the birds with those administered 6 % aqueous ginger extract having better red blood cell count (2.70) while those administered 4 % aqueous ginger extract had better white blood cell count (108.83). Arbor Acres Plus, Cobb 500 and Ross 308 birds administered aqueous ginger extract expressed more of the gene compared to those on the control. For the breed effect, expression of the gene was better in Ross 308 strain (1.07 fold change), Arbor Acres Plus (0.76 fold change), while Cobb 500 had the least (0.62 fold change). For the effect of varying ginger extract levels on the expression of the gene, it was observed to have been upregulated most in birds administered 6 % ginger extract (1.97 fold change), 4% ginger extract (1.26 fold change), while the control (0 %) had the least value (0.00 fold change). It was concluded that the effect of the aqueous ginger extract is breed and level of administration dependent and it is therefore recommended that could be used up to 6 % in the drinking water of the three broiler chicken strains as it led to upregulation of the gene and also influenced growth performance and haematology positively.

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CHAPTER ONE

1.0   **INTRODUCTION**

1.1   Background to the Study

Poultry species including turkey, goose, fowl, guinea fowl and duck are domesticated birds that are raised primarily for egg or meat production (Oluyemi and Roberts, 2007). They are very popular and vital sources of animal protein that are consumed all over the globe without any religious and or cultural barriers. To satisfy consumers' demand, chickens have been genetically improved to increase their body weight gain, feed efficiency, growth rate, and breast muscle weight (Wang *et al*., 2012). These processes of selection have led to the production of modern commercial broiler strains with increased growth rate, breast meat yield, better feed conversion rate, and concomitantly higher body fat compared with unselected lines (Baéza and Bihan- Duval, 2013).

Feed additives are nutritive and non-nutritive compounds that are added to poultry ration to improve feed efficiency and weight gain (Abouelfetouh and Moussa, 2012; Al-Mashhadani, 2014; Fadlalla *et al*., 2010; Mohamed *et al*., 2018). Antibiotics were the most common feed additives used in time past; however, the use of synthetic antibiotics is being regulated worldwide because of the development of resistant micro-organisms and their negative effect on animal and human health (Joseph *et al*., 2015; Yahya *et al*., 2014). In recent times, herbs and spices have gained useful applications in broiler production. This could be attributed to their inherent antimicrobial, growth-promoting and fat-reducing properties. Ginger is one of the spices reported as a natural growth enhancer and it contains several compounds such as shogaols, gingerdione, gingerol, phenolic, and gingerdiol (Zhao *et al*., 2011). It has been reported that some of these

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essential phytochemicals in ginger improved weight gain and impart pharmacological benefits on broiler chickens' health (Ali *et al*., 2008).

The major histocompatibility complex (MHC) region is a conserved region of all vertebrates. It contains genes that are highly polymorphic in different species and also generates quantifiable soluble products categorized as Class I, II and III genetic markers including erythrocyte antigens, polymorphic plasma, and erythrocytes proteins (Hull, 1970). The discovery of this locus via the study of the compatibility of tissue transfer birthed the name of this locus. The MHC Class I molecules span the membrane of almost every cell in an organism, whereas, the Class II molecules are restricted to cells of the immune system (macrophages and lymphocytes). Several genes encode these molecules in chickens which are all clustered in the same region on chromosome 16. Each gene has an unusually large number of alleles. The purpose of MHC molecules is to bind and display pathogen-derived peptide fragments on the cell surface for recognition by the proper T cells. The results are almost always harmful to the pathogen: virus-infected cells are killed; macrophages are activated to kill bacteria living inside of them; and B cells are activated to produce antibodies that destroy or neutralise extracellular pathogens. Thus, there is strong selective pressure in favour of any pathogen that has mutated in such a way that it escapes presentation by an MHC molecule (Gallegos *et al*., 2016).

Most inorganic growth-promoting substances have failed in achieving growth in livestock without adverse effects on the chickens' health because of the MHC reaction as a result of the animals developing immune responses to the foreign agent. The mechanism for the detrimental effect of these inorganic antibiotics on immune memory and memory protection is incompletely understood. The use of organic substances like

ginger is hence necessitated in promoting growth and enhancing the expression of the MHC gene to encourage immune memory development and support long-lasting protection from reinfection.

1.2   Statement of the Research Problem

Broiler chickens are highly susceptible to infectious diseases usually caused by pathogenic agents as well as non-infectious diseases which are major setbacks in poultry production. This situation could be implicated in the promotion of synthetic antibiotics use in poultry production. The use of synthetic antibiotics is however being regulated due to their public health implications (Hosseinzadeh *et al.*, 2014). Genetic resistance to several poultry pathogens has been unequivocally linked to the major histocompatibility B complex Kellye (2006). However, few MHC associated research have been conducted in commercial strains of broiler chickens. While there are several reports on aqueous ginger usage in poultry production and their effect on haematological indices, the nature of the expression of immune boosting genes such as the MHC Class II has not been thoroughly investigated for different strains of broiler chickens. This study will therefore investigate the growth performance, haematology and expression of the MHC Class II gene of three different commercial strains of broiler chickens following the administration of varying levels of aqueous ginger extracts in drinking water.

1.3   Justification of the Study

The phytochemical compounds in ginger have effects on the health of broiler chickens and also enhance the rate of body weight gain. The antimicrobial properties of ginger suggest its usefulness as a possible replacement for synthetic antibiotics for the prevention or treatment of bacterial infections. Expression of immune-regulating genes can be influenced by the administration of natural immune-boosting substances such as

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ginger which does not pose any residual consequences compared to synthetic antibiotics whose residual effects are linked with drug resistance in humans. Similarly, the study of the MHC Class II gene will help to determine the resistance or susceptibility of chickens to auto-immune diseases through the activity of immune stimulating substances such as ginger extract. Studies of MHC Class II gene expression and function will help elucidate their role in immune responses against pathogens. This knowledge may in turn contribute to the development of natural methods of enhancing immunity.

1.4   Aim and Objectives of the Study

This research is aimed at investigating the effect of varying concentrations of aqueous ginger extract intakes on the growth performance, haematology, and expression of the MHC Class II gene in commercial strains of broiler chickens. The objectives of the study are to:

i.   evaluate the effect of administering varying concentrations of aqueous ginger extract on the growth performance of three commercial strains of broiler chickens.

ii.   determine the effect of administering varying concentrations of aqueous ginger extract on the haematological indices of the three commercial strains of broilers chickens;

iii.   evaluate the effect of administering varying concentrations of aqueous ginger extract on the expression of the MHC Class II gene within and between the three commercial strains of broiler chickens.

CHAPTER TWO

2.0　　　　　　　　LITERATURE REVIEW

2.1　　Ginger and its Components

Ginger (*Zingiber officinale*) is a flowering plant whose rhizome and root are widely used as a spice and folk medicine. It is an herbaceous perennial which grows annual pseudo stems (false stems made of the rolled bases of leaves) about one metre tall and bearing narrow leaf blades.

According to Zick *et al*. (2008), the characteristic fragrance and flavour of ginger result from the volatile oils and oleoresin, a non-volatile pungent component that makes up about 1% to 3% of the weight of fresh ginger. Sesquiterpene hydrocarbons and phenolic compounds, such as gingerol and shogaol, as well as lipophilic rhizome extracts, were the main terpene components that were identified in ginger. Gingerols can be converted to shogaols, zingerone, and paradol (Govindarajan and Connell, 1983; Hasan *et al*., 2012), with gingerol as the major pungent compound (An *et al*., 2016). Zingerone is produced from gingerols during drying, having lower pungency and a spicy-sweet aroma. Shogaols are more pungent and have higher antioxidant activity but are not found in raw ginger; it is formed from gingerols during heating, storage or via acidity (An *et al*., 2016). Fresh ginger also contains an enzyme, zingibain, which is a cysteine protease and has similar properties to rennet.

Raw ginger is composed of 79% water, 18 % carbohydrates, 2% protein, and 1 % fat. About 100g g of raw ginger supplies 333 Kilojoules (80 Kilocalories) of food energy and contains moderate amounts of vitamin B6 (12 % of the Daily Value, DV), and the dietary minerals magnesium (12 % DV) and manganese (11 % DV) but otherwise, is

low in nutrient content. When used as a spice powder in a common serving amount of one US tablespoon, ground dried ginger (9 % water) provides negligible content of essential nutrients, with the exception of manganese (Condé, 2014).

Few researches have been conducted on the toxic effects of using ginger as a supplement in poultry feed. However, it was reported by Herawati (2010) reported that broiler chickens fed diets containing 0.5, 1.0 and 1.5 % red ginger showed oedema, necrosis and inflammation in the muscles. All phytobiotics have toxic characteristics and the intensity of their toxicity is determined by the dose and duration of the feeding period (Herawati, 2010). Feeding such substances at higher doses causes symptoms of congestion, oedema, inflammation and necrosis (Ganiswarna, 1995).

2.2   Immunomodulatory Effect of Ginger in Chickens

The surface of the gastrointestinal tract in chickens respond very quickly to alterations in nutrient intake (Dou *et al*., 2002). The histology of the intestinal villi and epithelial cells on the apical surface is commonly affected by dietary feed components (Yamauchi *et al*., 2006). It has been suggested that longer villi absorb a greater amount of available nutrients due to an increase in surface area (Caspary, 1992). Greater villi height and more mitosis in the gut indicate that the function of intestinal villi is stimulated as a result of enhanced absorption (Langhout *et al*., 1999; Yasar and Forbes, 1999). Incharoen and Yamauchi (2009) showed that the villus height, surface area, cell area and cell mitosis in the intestinal segment had higher values in ginger-fed laying hens compared to the control birds. Kausar *et al*. (1999) reported that a carminative mixture containing ginger at the dose rate of 4 ml/l of drinking water increased the mean litre in primary and secondary responses against Newcastle disease, suggesting an immunomodulating effect of ginger. Sudrashan *et al*. (2010) reported that essential oil

isolated from ginger resulted in a significant reduction in the bacterial counts of *Staphylococcus*, *Escherichia coli* and *Salmonella spp* when applied as a decontaminating agent in the ratio of 1:150, 1:250 and 1:500 in chicken meat. Zhao *et al*. (2011) found that ginger reduced the oxidation of stored feed which may be partially responsible for improved laying performance and for the serum and egg yolk antioxidant contents.

2.3. The Chicken's MHC Class II Gene Location on Chromosomes

Chicken MHC-B and MHC-Y regions are located on the micro-chromosome 16, which is found on the same side of the nucleolar organizing region (Miller and Taylor, 2016). MHC-B and MHC-Y are inherited independently because they are separated by a guanine cytosine (GC) rich region (Miller *et al*., 2004). Until now, 46 genes within a sequence covering 241,833bp in MHCB have been identified (Shiina *et al*., 2007), including three highly polymorphic gene groups: BF, BL, and BG, which encode Class I, II, and Class IV glycoproteins on cell surfaces, respectively (Lamont *et al*., 1987). The encoded proteins play important roles in rapid allograft rejection, immune responses and in determining the susceptibility/resistance to pathogen infections (Kaufman, 2018).

The mammalian MHC is polygenic, consisting of numerous genes, pseudogenes, and repetitive paralogous regions located on different chromosomes. The mammalian MHC is also polymorphic, with multiple alleles of each gene within a population (Murphy and Weaver, 2016; Wieczorek *et al*., 2017). Thus, the mammalian MHC is large and complex, with distantly related genes that provide extensive diversity to its antigen-presenting glycoproteins. In contrast, the chicken MHC is small and simple, yet it contains the essential counterparts of genes present in the mammalian MHC. For this

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reason, the chicken MHC is considered a minimal essential set of genes, despite the differences from mammalian MHCs in organization and structure (Kaufman *et al*., 1999). Upon discovery, the chicken MHC was classified as the B locus or B blood group, coding for agglutination factors present on the surface of chicken red blood cells (Briles *et al*., 1950).

Subsequently, these genes were associated with skin graft rejection and therefore, histocompatibility (Schierman and Nordskog, 1961). Ultimately, it was discovered that the chicken MHC B locus is located on chicken micro chromosome 16 and contains the B-F/B-L and the B-G regions corresponding to MHC Class I, II, III, and IV (B-G) genes (Briles *et al*., 1993). In addition to the B locus, a separate group of non-classical MHC Class I and II genes were identified and named Rfp-Y, which are also on micro chromosome 16 but, are genetically distant and unlinked to the B locus (Delany *et al*., 2009).

2.4   The Roles of MHC Class II Gene

Having MHC Class II molecules present is essential for the overall immune function. Because the MHC Class II gene is loaded with extracellular proteins, it is mainly concerned with the presentation of extracellular pathogens (for example, bacteria) that might be infecting a wound or the blood. Class II molecules interact mainly with immune cells, like the T-cell (CD4+). The peptide present regulates how T-cells respond to infection (Owen *et al*., 2009). Stable peptide binding is essential to preventing the detachment and degradation of a peptide, which could occur without secure attachment to the MHC molecule. This would prevent T-cell recognition of the antigen, T-cell recruitment and a proper immune response (Owen *et al*., 2009). The triggered appropriate immune response may include localized inflammation and

swelling due to the recruitment of phagocytes or may lead to a full-force antibody immune response due to activation of B cells.

2.5   Expression Pattern of Major Histocompatibility Complex Class II Gene

The MHC Class II molecules are constitutively expressed in professional, immune antigen-presenting cells, but may also be induced in other cells by interferon γ (Ting and Trowsdale, 2002). They are expressed on the epithelial cells in the thymus and the antigen presenting cells (APCs) in the periphery. MHC Class II expression is closely regulated in APCs by Class II, major histocompatibility complex, transactivator (CIITA), which is the MHC Class II transactivator. CIITA is solely expressed on professional APCs; however, non-professional APCs can also regulate CIITA activity and MHC II expression. Interferon γ (IFN γ) also triggers the expression of CIITA and is responsible for converting monocytes which are MHC Class II negative cells into functional APCs that express MHC Class II on their surfaces (Roche and Furuta, 2015). MHC Class II is also expressed in group 3 innate lymphoid cells.

The alcoholic ginger extract decreased the MHC II expression on lipopolysaccharide (LPS) activated macrophages. The functional consequence of the inhibitory effect of ginger extract on macrophages was seen in the form of a decrease in T-cell proliferation in the primary mixed lymphocyte reaction (MLR). This is further supported by decreased production of IL-2 and IFNγ in the primary MLR containing ginger extract-treated macrophages as APCs (Tripathi *et al*., 2008).

Expression of MHC Class II gene from different bat species, pigs, mice or chickens are also reported to confer susceptibility to infection. Notably, the infection of mice with bat influenza A virus resulted in robust virus replication in the upper respiratory tract, whereas mice deficient for MHC Class II gene were resistant (Karakus *et al.*, 2019). Class II MHC molecules are highly expressed on the surface of epithelial cells in both the lung and intestine, although the functional consequences of this expression are not fully understood (Wosen *et al.*, 2018). There are studies that implicate a particular viral protein in the downregulation of MHC Class II gene or decreased CD4+ T-cell activation, but the host targets have not yet been identified. For example, the ebola encoded protein VP35 decreases MHC Class II gene surface expression when encoded in the genome of the Herpes Virus Simplex (Jin *et al.*, 2010). Furthermore, dendritic cells infected with this construct are less able to activate CD4+ T-cells (Jin *et al.*, 2010). The levels of MHC Class I molecule expression differ among chicken lines and the expression correlates with Marek's disease susceptibility (Dalgaard *et al.*, 2003). For example, resistant B21 haplotype chickens have significantly lower MHC expression than the susceptible B19 haplotype birds. However, variation in response to Mareks disease virus by sublines that are identical at the B locus illustrates the significance of non-MHC gene effects on Marek's disease response (Bacon, 1987)

2.6   Major Histocompatibility Complex (MHC) and its Mode of Presenting Antigens

The MHC controls how the immune system detects and responds to specific antigens. Antigen specificity of T-cell recognition is controlled by MHC molecules with different antigen presentations between MHC Class I and Class II molecules (Stoakes, 2018). The two MHC Classes function similarly and this function involves the delivery of short peptides to the cell surface for recognition by CD8+ and CD4+ T-cells, respectively.

MHC Class I molecules present antigens that are intracellular or endogenous, whilst MHC Class II molecules present antigens that are extracellular or exogenous. The MHC Class I complex at the surface of the cell disconnects over time, leading to internalization into the endosome and entrance into the MHC Class II pathway (Stoakes, 2018). The cross presentation also occurs where MHC Class I molecules present extracellular antigens to CD8+ T-cells. Degradation through autophagy can cause endogenous antigens to be presented by MHC Class II molecules. Many viruses have evolved proteins that prevent antigen presentation by MHC molecules through the degradation or mislocalization of MHC molecules. Cross presentation is particularly important for responding to viruses that do not readily infect antigen-presenting cells (Stoakes, 2018).

2.7 Major Histocompatibility Complex (MHC) Associated Disease Resistance

Disease resistance is a multigenic trait governed by a diverse repertoire of immunological factors. Phenotypic variation in disease resistance is associated with intrinsic polymorphism of immune intermediary proteins such as MHC molecules, T-cell receptors, immunoglobulins, and secreted cytokines and antibodies (Hawken *et al*., 1998). The association between MHC polymorphism and resistance or susceptibility differences to several pathogens has long been recognized and is the best characterized example of genetic-mediated disease resistance Lamont (1998). The MHC mediates interactions between various cellular components of the immune system, and as such, has a profound effect on the immune response to specific antigens. Genetic enhancement of natural immunity may be a plausible mechanism for increasing resistance to whole classes of pathogenic organisms, thus, protecting birds in production under a wide variety of environmental conditions.

It has been found that mortality following challenge with a virulent Marek's disease virus strain was lower in high-antibody producing chickens expressing select MHC erythrocyte antigens (Pinard *et al*., 1993). Leitner *et al*. (1990, 1992) reported that lines selected for rapid early antibody response to *E. coli* were more resistant to *E. coli* challenge. Differences in the frequency of MHC Class IV restriction fragment length polymorphisms have also been found in meat-type chicken lines divergently selected for early antibody response to *E. coli* vaccination (Uni *et al*., 1993). More recent studies have shown that MHC associated gene regions Tap 2 and B-F are associated with *E. coli* antibody production (Cahaner *et al.,* 1997).

The mechanism behind the association between MHC and autoimmune disease has not been fully defined but is potentially reflecting a breakdown in tolerance to self-antigens in abnormal MHC Class II molecule antigen presentation. Specific MHC Class II alleles are, therefore, likely determinants of autoantigen targeting, resulting in disease association (Stoakes, 2018). Studies have shown an association between MHC haplotypes and increased levels of resistance to Northern fowl mite infestations. This resistance has been associated with skin inflammation that limits access to and feeding of mites on the host's blood (Owen *et al*., 2008).

MHC-linked genetic resistance to Infectious Laryngotracheitis Virus (ILTV) has been studied since the 1990s. Homozygous B2 haplotype birds were found to show higher resistance to ILTV infections than heterozygous B15/B21, and B2/B15 haplotype chickens. B2 birds were observed to be capable of mounting an immune response with a smaller infectious dose than other tested chicken lines (Poulsen *et al*., 1998). In a recent study (Dunn, 2020), MHC congenic chicken lines bearing homozygous B2, B5, B12,

B13, B19, and B21 haplotypes were challenged with ILTV to evaluate disease incidence. B2 and B5 haplotypes demonstrated greater resistance than the others by showing less severe clinical signs and reduced viral load. Two different lines (lines 6 and 7) containing the B2 haplotype, but a different genetic background, were compared; line 6 demonstrated greater resistance than line 7 implying the role of non-MHC genes in genetic resistance to this virus.

When studying MHC-linked genetic resistance to Infectious Bursal Disease Virus (IBDV) using B haplotype homozygote chickens, it was noticed that the antibody response to IBDV is MHC II-restricted and T-cell-dependent (Juul-Madsen *et al*., 2006). In a study to test the protection efficacy of a vectorized fowl pox–IBDV vaccine, chicken lines bearing the B haplotypes (B2, B12, and B15) were vaccinated and challenged with a virulent IBDV strain and their bursals assessed. It was discovered that B15 does not confer any protection against IBDV, whereas, B2 and B12 conferred protection to vaccinated and infected birds (Butter *et al*., 2013).

2.8   The Major Histocompatibility Complex (MHC) and Tissue Allorecognition

Allorecognition is the ability of an organism to distinguish between its tissues and those of another organism within the same species. This has important implications for transplantation of organs and tissues. A risk of organ transplantation is the alloresponse, where the histoincompatible antigen is recognized, producing an adaptive immune response via the employment of allospecific T-cells. This can lead to rejection of the transplanted tissue. The MHC is involved in the direct mechanism of allorecognition where T-cells recognize determinants on the donor MHC molecule-peptide complex displayed at the cell surface. This is because the MHC molecules display an antigenic determinant called an epitope that is either self or non-self, with antigens from the

transplanted cells recognized as non-self. To prevent an alloresponse in non-tolerant recipients, immunosuppressive drugs are provided but these are known to also cause long-term adverse effects increasing the understanding of the role of MHC in tissue (Stoakes, 2018).

2.9   Housekeeping   Gene: Glyceraldehyde-3-Phosphate   Dehydrogenase (GAPDH)

Quantitative gene expression data are often normalized to the expression levels of controls or what is popularly called "housekeeping" genes. An inherent assumption in the use of housekeeping genes is that expression of the genes remains constant in the cells or tissues under investigation. Although exceptions to this assumption are well documented, housekeeping genes are of value in fully characterized systems. GAPDH is one of the most commonly used housekeeping genes in comparisons of gene expression data (Robert *et al*., 2005). GAPDH is an enzyme of about 37kDa that catalyses the sixth step of glycolysis and thus, serves to break down glucose for energy and carbon molecules. In addition to this long established metabolic function, GAPDH has recently been implicated in several non-metabolic processes including transcription activation and initiation of apoptosis (Tarze *et al*., 2007) endoplasmic reticulum to Golgi vesicle shuttling, and fast axonal, or axoplasmic transport. Mostly because the GAPDH gene is stably and constitutively expressed at high levels in most tissues and cells, it is considered a housekeeping gene. For this reason, it is commonly used by biological researchers as a loading control for western blot and as a control for qPCR. However, researchers have reported different regulations of GAPDH under specific conditions (Patel *et al*., 2016); the transcription factor MZF-1 for example, has been shown to regulate the GAPDH gene (Zheng *et al.,* 2003).

CHAPTER THREE

3.0   MATERIALS AND METHODS

3.1   Study Area

The research was carried out at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Production, School of Agriculture and Agricultural Technology, Federal University of Technology, Gidan Kwano Campus, Minna, Niger State, Nigeria. Gidan Kwano is between latitude 9°32' and 9°42' N, and longitude 6°30' and 6°40' E. The daylight temperature of the area fluctuates between 24°C in the middle of the wet season, and 35°C at the peak of the dry season where the annual rainfall is between 1200 and 1300 mm (Ojimaduka *et al.*, 2020).

3.2   Formulation of Experimental Diet

The experimental diet was formulated to provide a metabolizable energy of 2,948.05 kcal/kg and a protein content of 22.34 % crude protein (Table 3.1.). The diet was fed single phased.

3.3   Preparation of Ginger Extract

Fresh ginger rhizomes were purchased from Kure Ultra-modern market, thoroughly washed with water to remove dirt, peeled, and cut into chips. The ginger chips were then ground with a warring blender (Polyster electric blender of model PV-BL999B, Nigeria) into a mash. The concentrated ginger juice was then collected from the mash using an extractor. The squeezed juice was stored in a bottle and refrigerated at 4°C until the time it was used (Joseph *et al.*, 2015).

Table 3.1: Composition of experimental diet

Ingredients	(%)
Maize	59.90
Groundnut cake	33.00
Fish meal	3.00
Wheat offal	4.00
Limestone powder	1.00
Bone meal	2.00
Palm oil	1.00
Salt	0.25
Lysine	0.25
Methionine	0.25
Toxin binder	0.10
Premix	0.25
Total (kg)	**100.00**
Calculated analysis	
Crude protein (%)	22.34
Crude fibre (%)	3.70
Ether extract (%)	5.50
Ash (%)	3.04
Calcium (%)	1.31
Available Phosphorus (%)	1.00
Lysine	0.53
Methionine	0.62
ME (kcal/kg)	2,948.05

Feed of 25kg contained; Vitamin. A 2,500,000 IU: Vitamin. D-500,000IU, Vitamin. E – 5.000IU, vitamin. k- 562.5mg; Thiamine – 42.5mg; Riboflavin – 1,250mg; Pyridoxine – 687.5mg; Niacin- 6,875mg; vitamin B12- 3.75mg; Pantothenic acid – 1,875mg; Folic acid – 1,875mg; Biotin – 12.5mg; Manganese – 20g; Zinc – 12.5g; Copper – 1.25g; Iodine 0.38g; Selenium – 50mg and Cobalt – 50mg

3.4   Experimental Design

A total of 270 day old broiler chicks of three strains (Arbor Acres Plus, Cobb 500 and Ross 308) were used for this experiment. The randomized complete block design was used for the experiment with the strain and level of administration as the blocks. The chicks in each group were divided into 3 replicates of 10 chicks per treatment. The control had 0.2 g of Oxytetracycline® in 2 litres of water administered to them. The 4 % aqueous ginger extract was obtained by measuring 192ml of water and mixed with 8ml of ginger extract while the 6 % aqueous ginger extract was obtained by measuring 188ml of water added to 12ml of ginger extract concentration, which was then administered to the experimental birds.

3.5   Management of the Experimental Birds

Before the arrival of the birds, the experimental house was washed and fully disinfected. The birds were housed on deep litter for 56 days, using wood shavings as the litter material. The chicks on arrival were weighed individually and distributed randomly to the various experimental groups. The experimental birds were administered antistress (vitalite) through drinking water, also preventive vaccination in the form of attenuated live vaccines of Gumboro were administered on days 7 and 21 and while Lasota vaccine was administered on days 14 and 28 of the experiment.

3.6.   Proximate Analysis

Proximate analysis of aqueous ginger extract was carried out according to the method outlined by AOAC (2000), at the Animal Production Laboratory of the Federal University of Technology, Minna, Niger State.

3.7   Data Collection

Data collected during the experiment were on growth performance, some haematological parameters and expression of MHC Class II gene.

3.7.1 Growth parameters of the different strains of broiler chickens administered various concentrations of aqueous ginger extracts

3.7.1.1   *Average initial body weight (AIBW)*

This is the average live weight of the chicks taken per replicate before the commencement of the feeding trial. It was determined thus:

$$\text{AIBWG (g)} = \frac{\text{Weight of the total chicks in a replicate (g)}}{\text{Total number of chicks in a replicate}}$$

3.7.1.2   *Average feed intake (AFI)*

This is the difference between the amount of feed offered to the bird and the feed left over after 24 hours divided by the number of birds. This parameter was determined thus;

$$\text{AFI (g)} = \frac{\text{Amount of feed offered to the animal (g)} - \text{feed left over after 24 hours (g)}}{\text{Total number of birds}}$$

3.7.1.3   *Average body weight gain (ABWG)*

This is the difference between the final body weight and the initial body weight, divided by the number of birds, as described by Owen *et al*. (2013).

$$\text{ABWG (g)} = \frac{\text{Final weight} - \text{Initial weight (g)}}{\text{Total number of birds}}$$

3.7.1.4   *Feed conversion ratio (FCR)*

The feed conversion ratio (FCR) was obtained by dividing the total feed intake by the total body weight gain of birds per replicate, using the formula below (Mohapatra *et al*. 2014).

$$FCR = \frac{\text{Total feed intake (g)}}{\text{Total weight gain}}$$

3.7.2   Haematological evaluation

At the end of the eight weeks, nine birds per treatment were randomly selected making a total of twenty sevens birds for blood sample collection. Blood was collected from the wing vein of the birds into tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) for haematological determination. The blood collected was analysed using a Cell-Dyn 3500 Haematology system (Abbott Laboratories, Abbott Park, IL, USA) to measure total and differential White Blood Cell count (WBC) and other blood parameters including Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell count (RBC), and Mean Corpuscular Haemoglobin (MCH).

3.7.3   Expression of major histocompatibility complex class II gene

At the end of the eight weeks of the experiment, three birds were randomly selected from each of the three treatment groups, and a total of nine tissue samples from the broiler chickens were collected from the liver of the birds, stored in plain bottles and completely submerged in RNAlater solution and kept refrigerated until when required for use. The guanidinium thiocyanatephenol-chloroform method was used to extract genomic RNA from the liver tissues as described by Chomczynski and Sacchi (1987). The forward and reverse primers used in the study are:

MHC2F 5…CTCGAGGTCATGATCAGCAA…3 (forward)
MHC2R 3…TGTAAACGTCTCCCCTTTGG…5 (reverse)

The extracted RNA was converted to cDNA using the FIREScript RT cDNA synthesis kit. The process involved using 1 ul of reverse transcriptase, 2 ul of 10 x reaction buffer, 0.5 ul RNase inhibitor (Ribogrip), 0.5 ul of primers with a 5 uM concentration and 10 ul of the RNA sample (at 50 ng/µl). Nuclease free water was used to balance the reaction volume to 20 ul. The thermocycling conditions were as follows; annealing at 25° C for 10 minutes, reverse transcription at 45° C for 30 minutes, and enzyme inactivation at 85° C for 5 minutes. The synthesized cDNA was amplified using the My IQ single colour real time cycler. The qPCRmix used was Solis Biodyne 5x HOT FirePol qPCR supermix plus. The reaction was done in 25 µl reactions consisting of 4 µl of the 5x HOT Firepol qPCR Mix, 0.4 µl each of the forward and reverse primers and specific probe which had a concentration of 250 nM, 18.2 µl of nuclease free water and 2 µl cDNA template 100 ng. The cycling conditions were as follows; an initial activation at 95° C for 12 minutes, followed by denaturation at 95° C for 15 seconds, then annealing at 53 and 55°C for 20 seconds (for MHC 2 and GAPDH), and a final elongation at 72° C for 20 seconds.

3.8   Data Analysis

Data obtained from the evaluation of the effect of breed and level of administration of aqueous ginger extract on the growth performance, haematology and the expression of MHC Class II gene were analyzed using the model;

$Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$

Where Y_{ijk} is the observed response, μ is the overall mean, α_i is the effect of the ith strain of broilers chicken, β_j is the effect of the jth level of administration of the aqueous ginger extract, and e_{ijk} is the random error.

The effect of administering varying concentration levels of aqueous ginger extract on the expression of MHC Class II gene in Arbor Acres Plus, Ross 308 and Cobb 500 broiler chickens were evaluated using the model;

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

Where Y_{ij} is the observed response, μ is the overall mean, αi is the effect of the ith broilers chicken strain, and e_{ij} is the random error.

Data were analyzed using SPSS statistical software version 20.0. Where significant differences were observed between the means (P<0.05), least significant difference was used to separate them.

<center>**CHAPTER FOUR**</center>

4.0 **RESULTS AND DISCUSSION**

4.1 **Results**

4.1.1 Proximate composition of the test ingredient (aqueous ginger extract)

The proximate composition of the experimental test ingredient (aqueous ginger extract) is shown in Table 4.1. The result on proximate composition of aqueous ginger extract revealed content of the parameters measured; the moisture content was (91.63%), crude fibre (3.14%), ash (0.02%) and nitrogen free extract (5.21%).

4.1.2 Breed effect on the growth performance of broiler chickens administered varying levels of aqueous extract of ginger

The breed effect on the growth performance of broiler chickens administered aqueous extract of ginger is presented in Figure 4.1. There were significant ($P<0.05$) differences in the average initial body weight (AIBW), average feed intake (AFI), average final body weight (AFBW) and average body weight gain (ABWG) of the birds used. Average initial body weight ranged from 35.51 g (Cobb 500) to 44.26 g (Ross 308). Average final body weight was highest in the Cobb 500 strain (1,662 g), followed by the Ross 308 (1,503 g) while the Arbor Acres Plus had the least AFBW (1501 g). The average body weight gain (ABWG), was highest in the Cobb 500 strain (1627 g) while both the Ross 308 and Arbor Acres Plus had the same value (1460 g). Average feed intake was observed to be higher for the Cobb 500 (2,485 g) followed by the Ross 308 (2,317 g) while the least was observed among the Arbor Acres Plus strains of broiler chickens (2,275 g). However, there was no significant ($P>0.05$) difference in the results obtained for the feed conversion ratio.

<center>22</center>

Table 4.1: Proximate composition of aqueous ginger extract

Parameters	%
Moisture content	91.63
Crude fibre	3.14
Ash	0.02
Nitrogen free extract	5.21

	AIBW (g) *	AFBW (g) *	ABWG (g) *	ATFI (g) *	AFRC (%)
■ ARBOR ACERS	41.49	1501	1460	2275	1.56
■ COBB 500	35.51	1556	1627	2485	1.54
■ Ross 308	43.26	1503	1460	2317	1.59
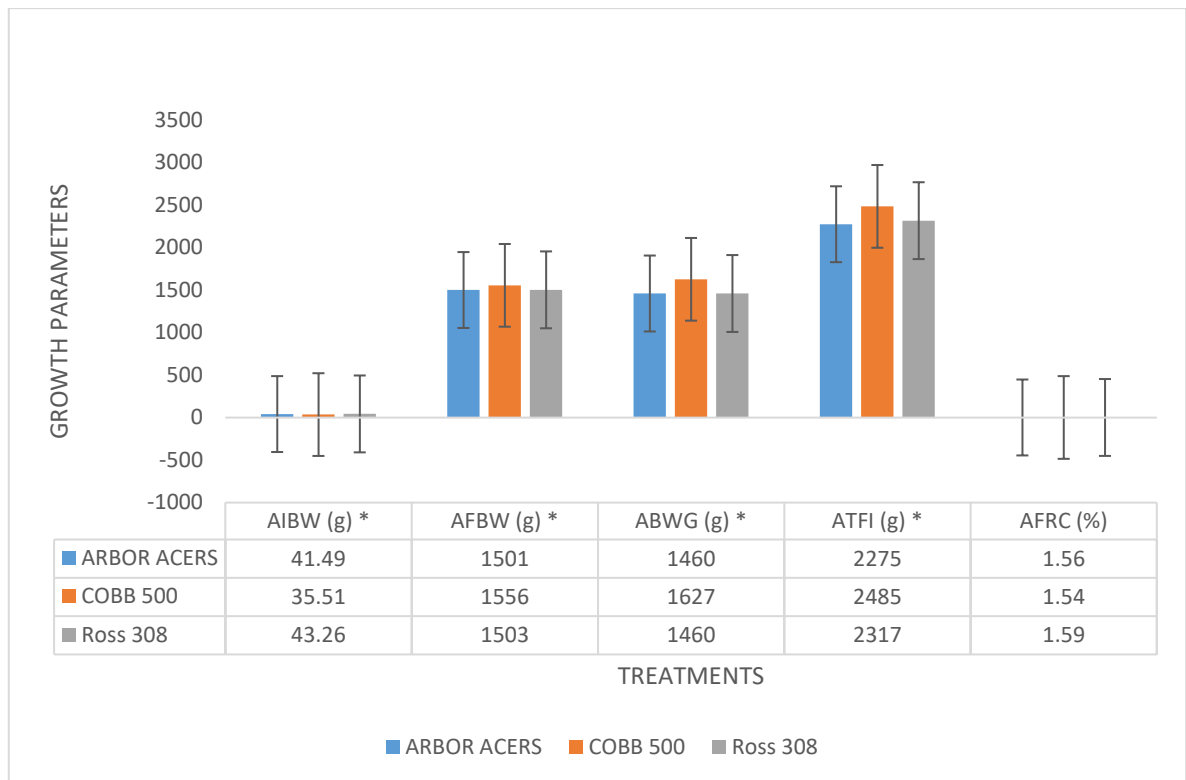
Figure 4.1: Effect of breed on the growth performance of broiler chickens administered aqueous extract of ginger

4.1.3 Treatments effect on the performance of broiler chickens administered varying levels of aqueous extract of ginger

The treatment effect of administering different levels of aqueous ginger extract on the performance of broiler chickens is shown in Figure 4.2. There were no significant (P>0.05) differences in all the parameters measured for growth performance. The average initial body weight (AIBW) ranged between 40.18 g (0 % extract) to 40.00 g (6 % ginger extract). Also, broiler chickens administered 6 % ginger extract were numerically better in average final body weight (1,635 g), average body weight gain (1595 g), and average feed conversion ratio (1.52). Broiler chickens administered 4 % ginger recorded the highest average feed intake (2,883) while those administered 6 % ginger extract recorded the lowest average feed intake (2,300 g) when compared to their feed conversion ratio.

4.1.4 Breed effect on the haematological indices of broiler chickens administered varying levels of aqueous extract of ginger

The effect of breed on haematological parameters of the different strains of broiler chickens administered varying levels of aqueous ginger extract is presented in Figure 4.3. There were significant (P<0.05) differences in the values obtained for haemoglobin concentration, packed cell volume and red blood cell count of the broiler strains used. The Arbor Acres Plus strain had the highest value for haemoglobin concentration (18.00 g/dl), while Cobb 500 chickens recorded the lowest value (15.17 g/dl). Packed cell volume ranged from 29.50 % (Cobb 500) to 38.33 % in the Arbor Acres Plus. The red blood cell count was lowest in Cobb 500 (2.0 X 10^6/mm^3). The white blood cells count, basophil, eosinophil and mean corpuscular haemoglobin showed no significant (P>0.05) differences.

	AIBW (g)	AFBW (g)	ABWG (g)	ATFI (g)	AFRC (%)
Ginger 0%	40.18	1510	1470	2300	1.56
Ginger 4%	40.08	1521	1481	2883	1.61
Ginger 6%	40	1635	1595	2395	1.52
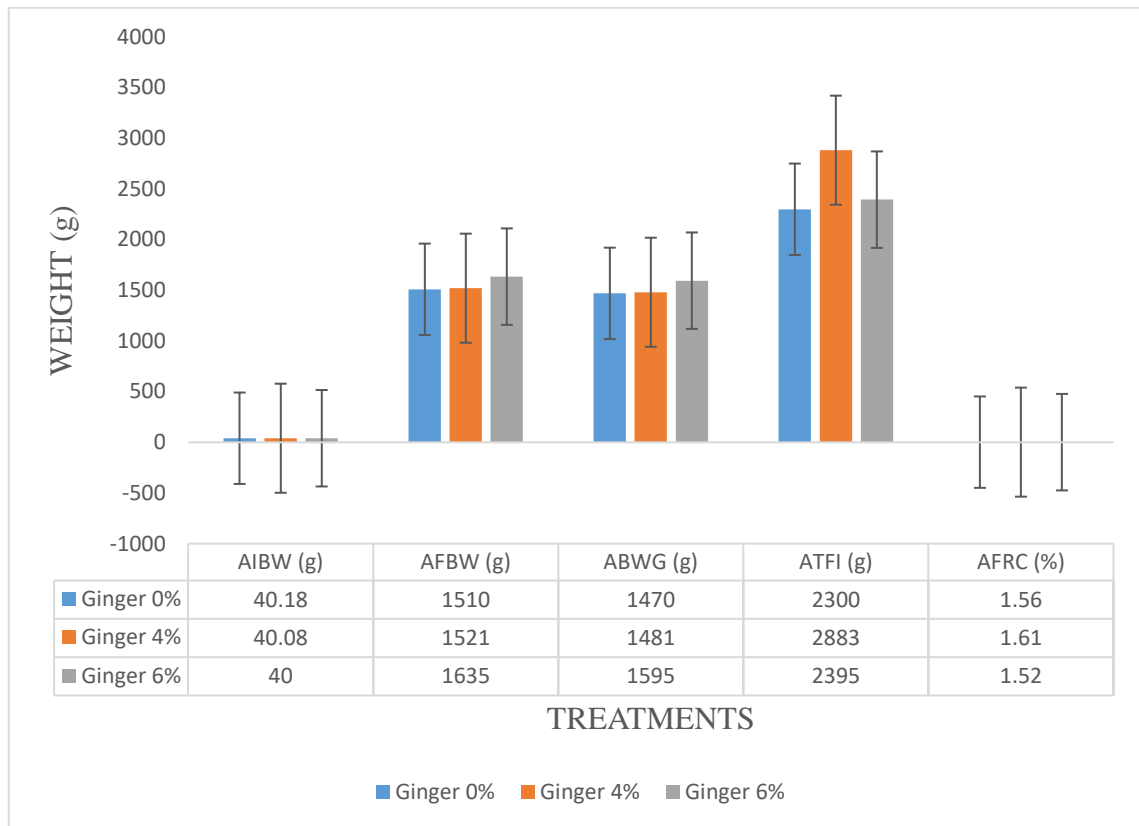
Figure 4.2: Treatments effect on the performance of broiler chickens administered varying levels of aqueous extract of ginger

	HB (g/dl) *	PCV (%) *	RBC (X 106/mm 3) *	WBC (µl)	BASOPH IL (µm)	EOSINO PHIL (cells/m cL	MHC (pg)
Arbor Acer +	18	38.33	2.52	77.17	0.33	0.67	24.17
Cobb 500	15.17	29.5	2	87.33	0.23	0.48	19.33
Ross 308	16.67	33.5	2.72	94.67	0.35	0.67	23.17

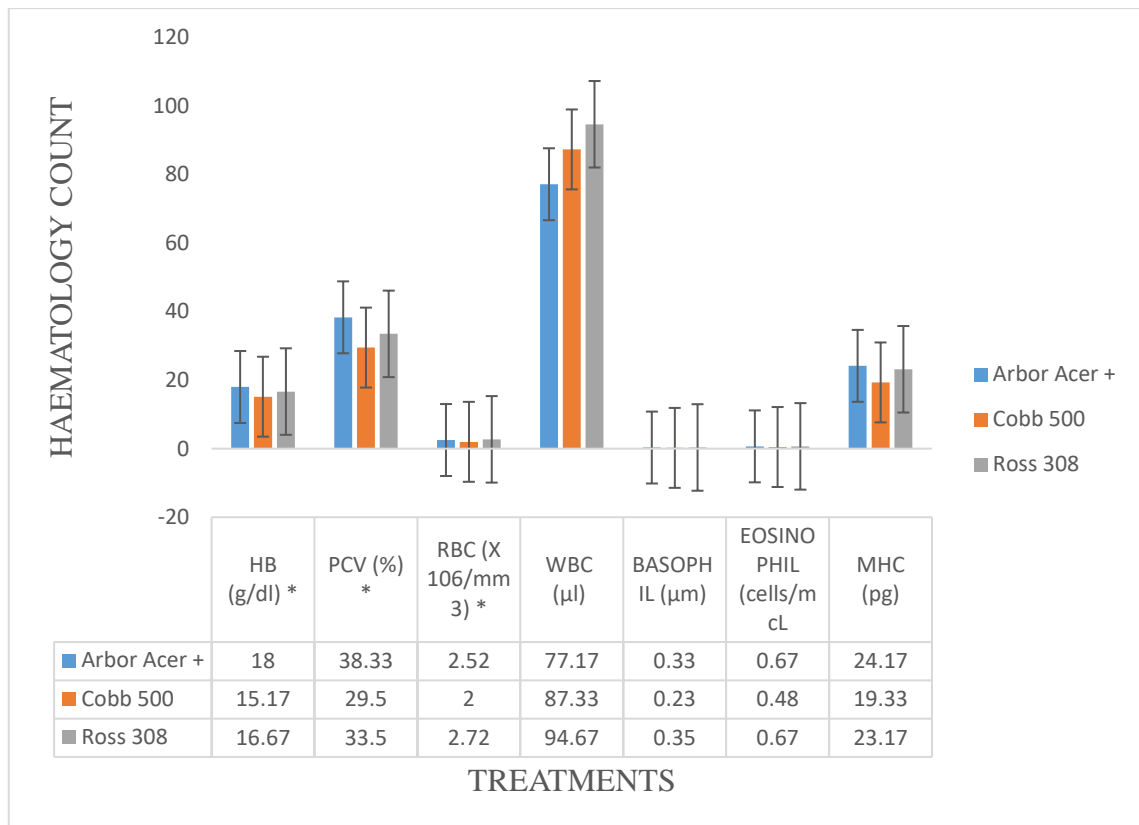**Figure 4.3: Breed effect on the haematological indices of broiler chickens administered varying levels of aqueous extract of ginger**

4.1.5 Treatment effect on the haematological indices of broiler chickens administered varying levels of aqueous extract of ginger

Figure 4.4 shows the results of the effect of different levels of aqueous ginger extract administration on the haematological parameters of the broiler chickens. There were significant (P<0.05) differences in the values obtained for the red blood and white blood cell count of the birds. Red blood cell count was lowest in birds administered 0 % aqueous extract of ginger ($2.12 \times 10^6/mm^3$) and highest in those administered 6 % of the extract ($2.70 \times 10^6/mm^3$) even though it is statistically similar to those administered 4 % of the aqueous ginger extract. For white blood cells count, the highest value was recorded in birds administered 4 % aqueous ginger extract ($103.83 \times 10^3/\mu l$) while the lowest was recorded in birds administered 0 % aqueous ginger extract ($73.33 \times 10^3/\mu l$). The results showed no significant (P>0.05) differences in the values obtained for haemoglobin concentration, packed cell volume, basophil, eosinophil and mean corpuscular haemoglobin of the birds used for the experiment.

4.1.6 Expression of MHC Class II gene in Arbor Acres Plus, Ross 308 and Cobb 500 strains of broiler chickens administered varying levels of aqueous ginger extract

Figure 4.5 presents the result of the expression of MHC Class II gene in Arbor Acres Plus broiler chickens as influenced by the levels of the treatment. The results showed that there were significant (P<0.05) differences in the expression of the gene as a result of the effect of administration of aqueous ginger extract. The MHC Class II gene was most upregulated in birds administered 6 % aqueous ginger extract (1.83 fold change), followed by those administered 4 % (0.98 fold change). However, there was a downward regulation of the MHC Class II gene observed in Arbor Acres Plus birds administered 0 % aqueous ginger extract (-0.52 fold change).

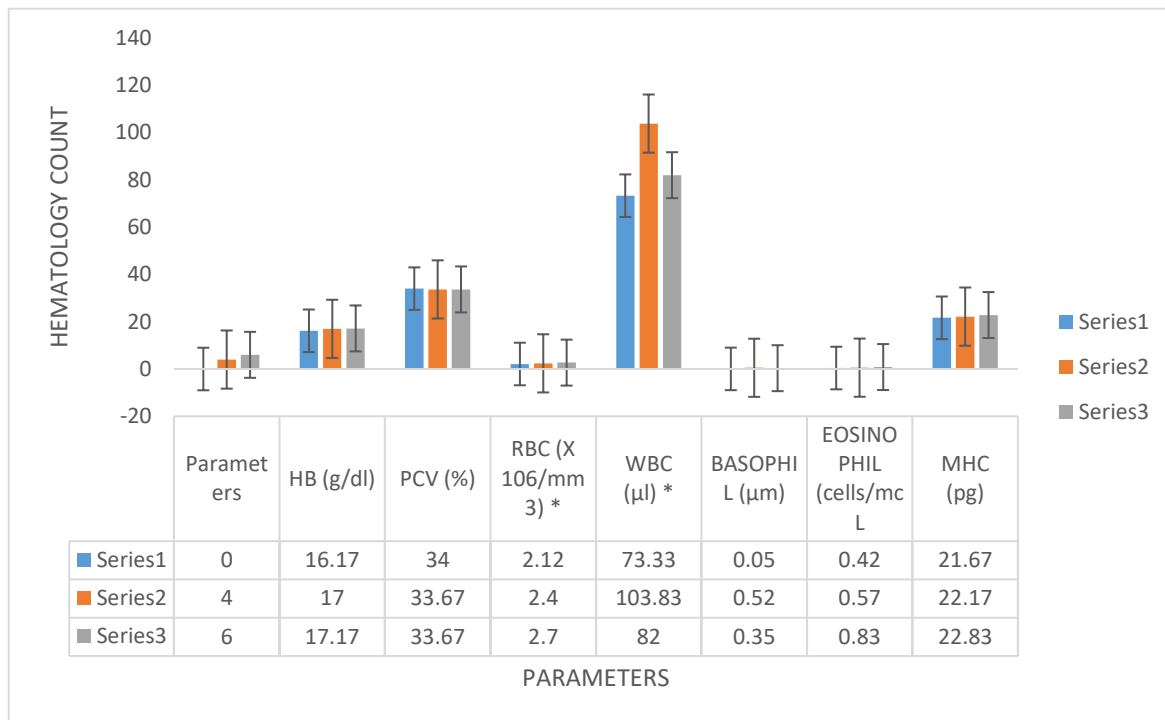|	Parameters	HB (g/dl)	PCV (%)	RBC (X 106/mm 3) *	WBC (μl) *	BASOPHI L (μm)	EOSINO PHIL (cells/mc L	MHC (pg)
Series1	0	16.17	34	2.12	73.33	0.05	0.42	21.67
Series2	4	17	33.67	2.4	103.83	0.52	0.57	22.17
Series3	6	17.17	33.67	2.7	82	0.35	0.83	22.83

Figure 4.4: Treatments effect on haematological indices of broiler chickens administered varying levels of aqueous extract of ginger

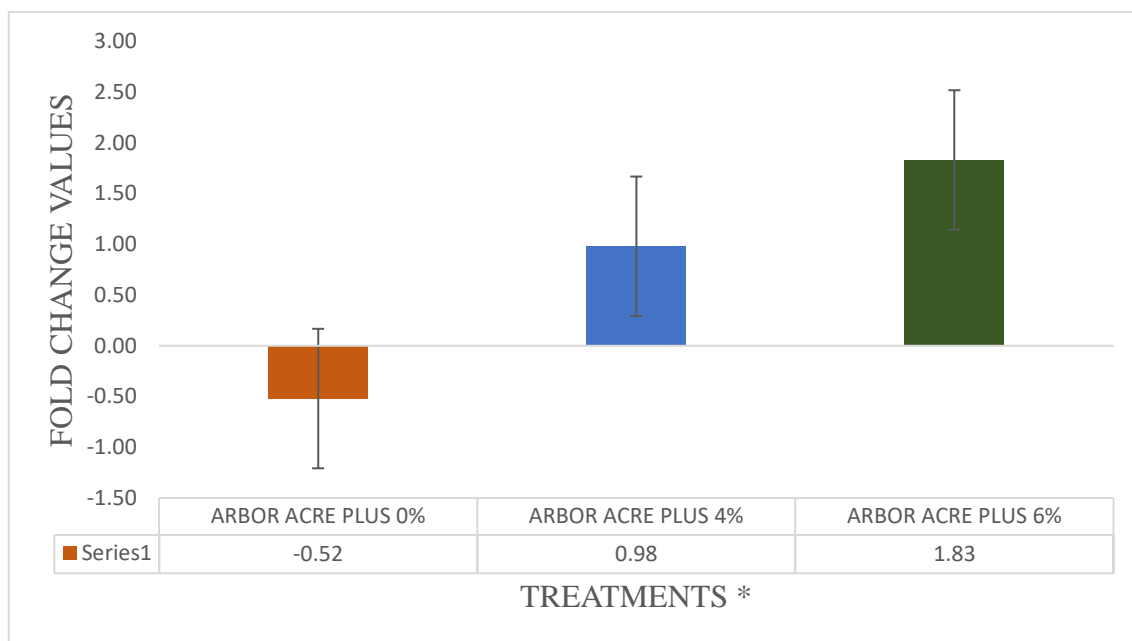|	ARBOR ACRE PLUS 0%	ARBOR ACRE PLUS 4%	ARBOR ACRE PLUS 6%
■ Series1	-0.52	0.98	1.83

TREATMENTS *

Figure 4.5: Expression of MHC Class II gene in Arbor Acres Plus broiler chickens administered varying levels of aqueous ginger extract

The expression of the MHC Class II gene in the Ross 308 strain of broiler chickens administered varying levels of aqueous extract of ginger is depicted in Figure 4.6. The results of the expression pattern showed that there were significant ($P<0.05$) differences in Ross 308 strains of broiler chickens administered aqueous extract of ginger. There was higher upregulation of the MHC Class II gene in broiler chickens administered 4 % aqueous ginger extract (1.73 fold change), followed by those groups administered 6 % (1.15 fold change), while birds administered 0 % recorded the lowest (0.33 fold change).

The results of the expression of the MHC Class II gene in the Cobb 500 strain of broiler chickens administered varying levels of aqueous ginger extracts is presented in Figure 4. 7. The results showed significant ($P<0.05$) differences in the expression values obtained for the Cobb 500 strain of broiler birds; the expression of the MHC Class II gene was highly upregulated in broiler chickens administered 4 % aqueous ginger extract in drinking water (1.07 fold change), and lowest in birds administered 0 % of the aqueous extract (0.19 fold change).

	ROSS 308 0%	ROSS 308 4%	R0SS 308 6%
■ Series1	0.33	1.73	1.15

TREATMENTS*
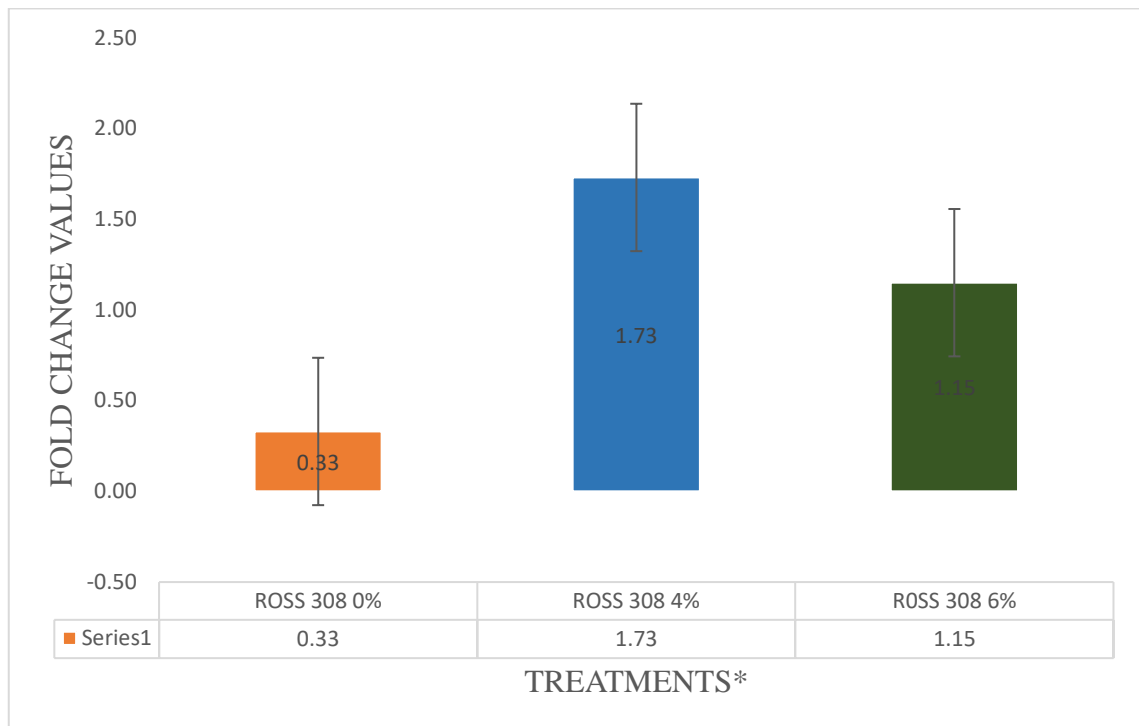
Figure 4.6: Expression of MHC Class II gene in Ross 308 broiler chickens administered varying levels of aqueous ginger extract

Figure 4.7: Expression of MHC Class II gene in Cobb 500 broiler chickens administered varying levels of aqueous ginger extract

4.1.7 Breed effect on the expression of MHC Class II gene in broiler chickens administered varying levels of aqueous extract of ginger

Figure 4.8 shows the results of the effect of breed on the expression of the MHC Class II gene in the different strains of broiler chickens administered varying levels of aqueous ginger extract. The results showed significant ($P<0.05$) differences in the expression values obtained for the different strains of broiler chickens used for the experiment. Ross 308 strain of broiler chickens had the highest expression value for the MHC Class II gene (1.07 fold change), followed by the Arbor Acres Plus (0.76 fold change), while it was least in the Cobb 500 strain (0.62 fold change).
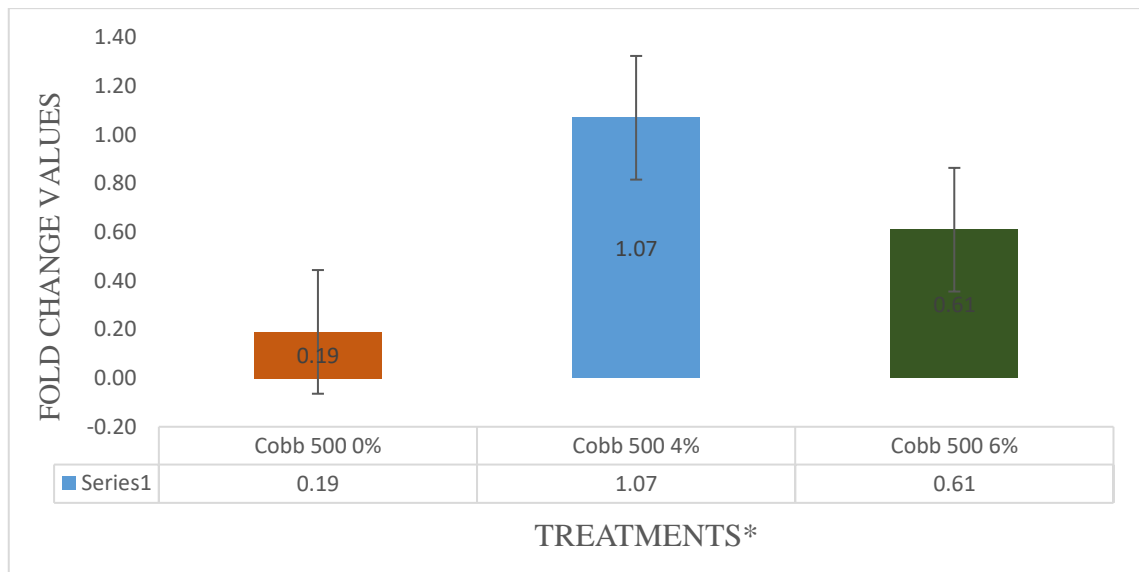
4.1.8 Treatment effect on the expression of MHC Class II gene in broiler chickens administered varying levels of aqueous extract of ginger

Figure 4.9 shows the results of the effect of administering varying levels of aqueous ginger extract on the expression of MHC Class II gene in the different strains of broiler chickens. The results showed that the gene was significantly ($P<0.05$) upregulated in the birds; birds administered 6% aqueous ginger extract expressed the gene better than those administered 4 % and 0 % (the control).

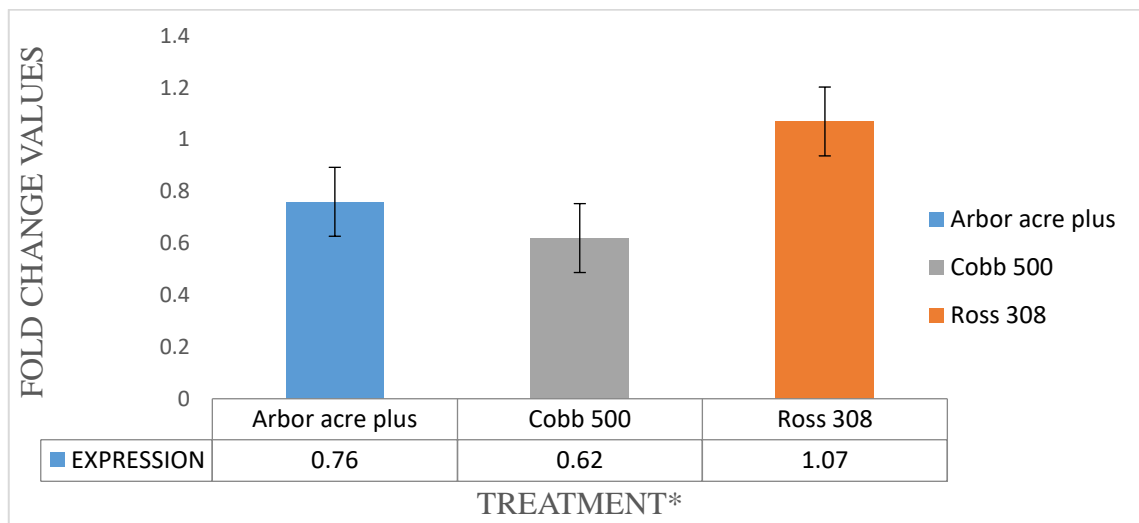|	Arbor acre plus	Cobb 500	Ross 308
◼ EXPRESSION	0.76	0.62	1.07

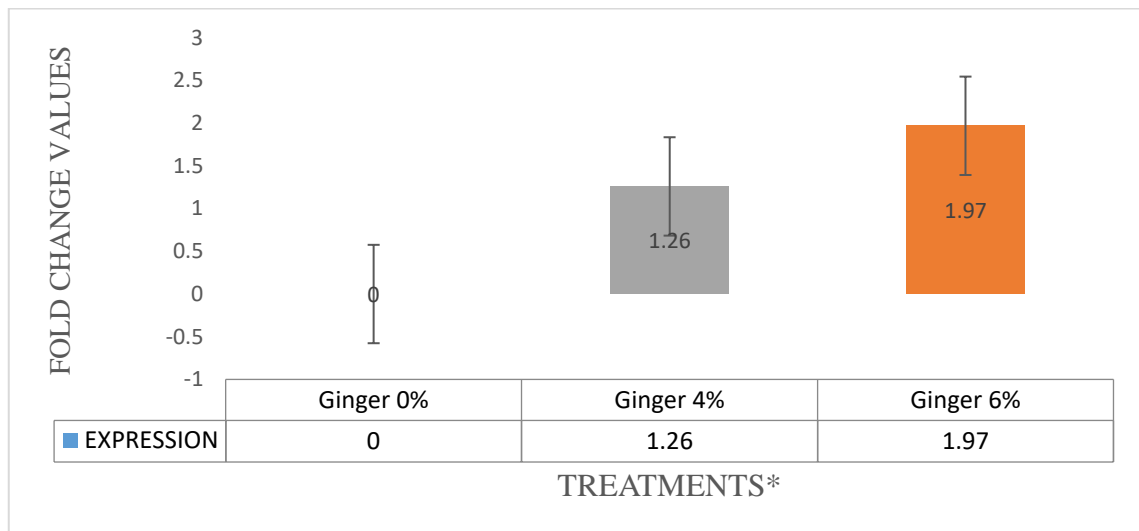**Figure 4.8: Effect of breed on the expression of MHC Class II gene of broiler chickens administered varying levels of aqueous extract of ginger**

Figure 4.9: Effect of administering varying levels of aqueous extract of ginger on the expression of MHC Class II gene of broiler chickens

4.2   Discussion

4.2.1   Proximate composition of the test ingredient

The moisture content of the aqueous ginger extract is slightly lower than that reported by Sa'aci *et al.* (2018) who reported 93.34 % moisture content and 7.0 % ash and by Adebiyi *et al*. (2014) who reported a difference in the moisture content of this present study could probably be attributed to the method of processing or specie of ginger used.

4.2.2   Breed effect on the growth performance of broiler chickens administered varying levels of aqueous extract of ginger

The Cobb 500 strain despite its low initial body weight appeared to have been more adaptable to the treatment in recording the highest final body weight gain compared to the other two strains used for the experiment. This may be a result of their ability to easily digest and convert feed to body weight than the other strains. The superiority of the weight gain of the Cobb 500 strain over the other strains affirms earlier reports (Abdullah *et al*., 2010; Fernandes *et al*., 2013); who reported that the Cobb 500 strain of broiler chickens gained higher body weight than other strains of broiler chickens. Also, the result of feed intake and feed conversion ratio agrees with past reports that the strain of the chicken and different stage of growth affects the degree of nutrient digestibility, efficiency of feed conversion and feed intake (Al-Marzooqi *et al*., 2019; Ghanem, 2014; Udeh *et al*., 2015).

4.2.3   Treatment effect on the performance of broiler chickens administered varying aqueous extracts of ginger

The aqueous ginger extract positively affected the growth performance of the different strains of broiler chickens. This may be as a result of the immunomodulatory effect of

ginger on the digestive juices, microflora and nutrient assimilation in the digestive tract. This assertion aligns with the reports of George *et al.* (2013) who found that shogaols, gingerdione, gingerol, phenol and gingerdiol (all active compounds in ginger) stimulate the intake of feed, and positively enhance feed conversion ratio resulting in increased weight gain of broiler chickens. Arkan *et al.* (2012) and Talukder *et al.* (2017) also reported that broiler chickens fed with supplemented feed ration of 2 and 6% aqueous ginger extract recorded an increase in weight. However, the results of the present research negate the findings of Wafaa *et al.* (2012) that aqueous ginger extract did not influence weight gain, feed intake and feed conversion ratio of broiler chickens. The different performance of the strains of broiler chickens used for the study appears to be as a result of genotype differences.

4.2.4 Breed effect on haematological parameters of broiler chickens administered varying levels of aqueous extract of ginger

Haematological parameters help in determining the level of inflammation, anaemia, infections, blood clotting disorders, haemophilia, and many more. The haematological values in this research showed that the three strains of broilers were in good health. The values obtained for haemoglobin concentration, packed cell volume, red blood and white blood cell count, basophil, eosinophil and mean corpuscular haemoglobin in the birds were similar to those reported by Jain (1993). The values recorded for haemoglobin concentration in the three strains of broilers were however, lower than the value reported by Wikivet (2012). For packed cell volume, the values recorded for Arbor Acres Plus and Ross 308 strains were higher than that reported by Onyishi *et al.* (2017) who reported a packed cell volume of 29.92 % for broiler chickens; the values recorded for the Cobb 500 strain is, however, within the range of 25-45% reported for broiler chickens by Al-Nedawi (2018). The values obtained for red blood cell count in

Arbor Acres Plus and Ross 308 strains were also within the normal range of general reference interval (2.5-3.9 $\times 10^2$/L) reported by Harrison and Lightfoot (2005) while that of Cobb 500 was lesser. The differences in the white blood cell count of the Ross 308 and Cobb 500 compared to that of the Arbor Acres Plus could be due to the differences in strain and weight of the birds. Adeyemo et al. (2018) reported weight, age, sex, diet type, strain and climate as factors that could cause variation in the haematological indices of chickens.

4.2.5 Treatment effect on haematological parameters of different strains of broiler chickens administered varying levels of aqueous extract of ginger

Haemoglobin, packed cell volume and mean corpuscular haemoglobin are the main parameters for measuring circulatory erythrocytes and are very important in diagnosing anaemia (Peters et al., 2011). They also serve as useful parameters of the bone marrow's ability to generate red blood cells in mammals (Chineke et al., 2006). The number of erythrocytes in chickens impacts their general conditions (Mitruka et al., 1977). The increase in packed cell volume, haemoglobin concentration and red blood cell count of the broiler chickens administered varying levels of the ginger extract is an indication that the oxygen-carrying capacity of the birds' blood was enhanced as a result of administering aqueous ginger extract to the birds. The increase in white blood cell count however, negates the reports of Ademola et al. (2009) who observed that ginger administered to chickens at a concentration of 1.0 % led to a significant decrease in the total number of white blood cell count.

4.2.6 Expression of MHC Class II gene in Arbor Acres Plus, Ross 308 and Cobb 500 broiler chickens administered varying levels of aqueous extract of ginger

The MHC Class II gene was upregulated in birds administered 4 and 6 % aqueous ginger extract compared with those administered the control. The upward regulation is an indication of better immunity provided by the aqueous ginger extract at the MHC Class II gene locus of the broiler chickens. This immune status must have minimized the activities of microorganisms and prevented the outbreak of common bacterial diseases in the flock. This is in line with the findings of Nidaullah *et al.* (2010) that, aqueous ginger extract enhances the immune performance of broiler chickens against common bacterial diseases; the authors also observed that aqueous extract of ginger mixed with water acted as an immune stimulant against coccidiosis. The downward regulation of the MHC Class II gene in the control birds may be because, the antibiotic administered to the birds rather than boost their immunity, affected it negatively. It may well be that some level of resistance to the antibiotic occurred in the control birds which somewhat affected the activation of the gene under study. Yahya *et al*. (2014), and Joseph *et al.* (2015) both reported on the need to regulate the use of synthetic antibiotics because of the development of resistance by micro-organisms and their effect on both animal and human health. This is a boost for the usage of aqueous ginger extract as a non-synthetic immune booster in broiler chickens' production.

4.2.7   Breed effect on the expression of MHC gene of different strains of broiler chickens administered varying levels of aqueous ginger extract

The upregulation of the MHC complex Class II gene was in the order of Ross 308 > Arbor Acres Plus > Cobb 500. The differences observed in the ability of the birds to

express the gene could be due to the effect of their genetic makeup, how they were able to utilize the ginger extract considering other environmental variables and/or due to how the different strains of birds responded to the treatment at the MHC gene locus. This result is in line with the findings of Dalgaard *et al*. (2003) who reported that MHC molecule expression differs among chicken lines and that the expression is positively correlated with Marek's disease susceptibility. Despite the better performance of the Cobb 500 strain, they were more susceptible to diseases than the other strains of broiler chickens judging from the haematology and expression results. However, better growth performance and haematological indices of the different strains of broiler chickens used for this research could be linked to how they upregulated the MHC Class II gene which is an indication of better immunity conferred on birds by the test ingredients ginger and the ability of the birds to individually utilize this ingredient in other to resist infection and gain good weight.

4.2.8 Treatment effect on the expression of MHC class II gene of different strains of broiler chickens administered aqueous extract of ginger

With regard to the effect of the administration of varying levels of the aqueous ginger extract on the expression of the MHC Class II gene, it was highly upregulated in birds administered 6 % ginger extract than the other levels of 0 % and 4 %, respectively. This is an indication that the administration of ginger extracts up to 6 % is suitable for better expression of the gene in broiler chickens. Besides this, the result of growth performance and haematology also points to the better utilization of aqueous ginger extract than the synthetic Oxytetracycline® used in the study. A protein digesting enzyme (zingibain) found in ginger is believed to improve digestion as well as kill parasites and their eggs. Furthermore, the properties of ginger tend to enhance antibacterial and anti-inflammatory factors (Mohammed and Yusuf, 2011). Tekeli *et al*.

(2011) stated that due to the active ingredients in these herbs, there is the formation of more stable intestinal flora and improved feed conversion efficiency as a consequence of better digestion. This is good news to health-conscious consumers who have all these while advocating for the use of alternative organic and less harmful additives or ingredients in the rearing of poultry and other livestock. Farmers are also likely to benefit from the reduced cost of producing birds to market weight when using aqueous ginger extract than antibiotics. The processing method used in the production of the aqueous ginger extract is also easily available to farmers even in rural areas. This finding is however in disagreement with the reports of Tripathi *et al*. (2008) that alcoholic ginger extract decreases the expression of the MHC Class II gene in Lipopolysaccharide activated macrophages.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Based on the findings of this work, the following conclusions were drawn: Administration of varying concentrations of aqueous ginger extract up to 6 % in the drinking water of the three strains of broiler chickens improved their average feed intake, average body weight, feed conversion ratio and average final body weight gain. The Cobb 500 strain performed significantly ($P<0.05$) better than the Arbor Acres Plus and the Ross 308.

Haemoglobin concentration, packed cell and red blood cell counts of the birds were significantly ($P>0.05$) affected by the administration of varying concentrations of aqueous ginger extract up to 6 % in the birds. The level of administration also significantly ($P<0.05$) affected the red and white blood cell counts of the birds.

Results of the expression of MHC Class II genes show that it was significantly ($P<0.05$) upregulated in the three broiler strains as a result of the administration of the aqueous ginger extract compared to those on the control. Ross 308 strain of broiler chickens expressed ($P<0.05$) the MHC Class II gene better than the Arbor Acres Plus and Cobb 500 strains.

5.2 Recommendations

Based on the above conclusion, the following are recommended:

i.   That aqueous ginger extract could be administered in the drinking water of the three strains of broiler chickens up to 6 % without any adverse effect on

the growth performance, haematology, expression of the MHC Class II gene and health of the birds.

ii.   The Cobb 500 and Ross 308 strains of broiler chickens should be administered 4 % aqueous ginger extract in drinking water for better expression of the MHC Class II gene, while 6 % should be administered to Arbor Acres Plus chickens to help confer immunity on the chickens.

iii.   Further research could be carried out to examine the effect of varying concentrations of aqueous ginger extract on the regulation of the expression of MHC Class I and III genes in commercial strains of broiler chickens.

5.3   Contributions to Knowledge

The inclusion of varying concentrations of ginger extract in the drinking water of the three different strains of broiler chickens significantly ($P<0.05$) enhanced growth performance and led to the upregulation of the MHC Class II gene in the birds compared to those on the control (antibiotic regime). There was a 1.07 (Ross 308), 0.76 (Arbor Acres Plus) and 0.62 (Cobb 500) fold change observed in the expression of genes over what is observed in birds on antibiotics (control).

In this study it is worth noting that the effect of varying levels of aqueous ginger extract on the expression of genes, was discovered to have been upregulated only in broiler chickens administered the aqueous ginger extract; birds on 6% ginger extract treatment recorded a 1.97 fold change, those on 4% ginger extract treatment had a 1.26 fold change, whereas the control which had no ginger extract (0%) had no (0.00) fold change.

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