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Expression of major histocompatibility complex class II gene in commercial strains of broiler chickens administered varying levels of aqueous ginger extract

***[1,2]Okolo, G. P., [2]Egena, S. S. A., [2]Otu, B. O. and [3]Sikiru, A. B.**

[1]Department of Agricultural Technology, Kogi State Polytechnic, P.M.B 1101, Lokoja, Kogi State, Nigeria.
[2]Department of Animal Production, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria.
[3]Department of Animal Science, Federal University of Agriculture, Zuru, Kebbi State, Nigeria.
Corresponding author: acheneje.egena@futminna.edu.ng

Abstract

Due to regulation on the usage of synthetic antibiotics as a result of the development of resistance by microorganisms, the expression of Major Histocompatibility Complex (MHC) class II gene in three commercial strains of broiler chickens administered varying levels of ginger extract was investigated. Ninety each of Arbor acres Plus, Cobb 500 and Ross 308 were used in the experiment. The birds were randomly divided into three treatment groups, on breed basis, of 30 birds, designated as T1, T2, and T3. Each treatment per breed were further divided into 3 replicates of 10 chicks. The control was administered a leveled table spoonful of Oxytetracycline® in 2L of water as recommended by the manufacturer while 4% and 6% of aqueous ginger extract was given to treatment 2, and 3 birds, respectively via drinking water. A single phase diet (22.34% CP; 2948.05 ME kcal/kg) was fed to the birds for 8 weeks. Liver samples were collected at the end of the experiment, fixed in RNA Later, and used for the expression study. Results showed significant (P<0.05) effect of treatment on the expression of MHC complex class II gene of the birds. Arbor acres Plus, Cobb 500 and Ross 308 birds administered ginger extract expressed more of the gene compared to the control. For the breed effect, expression of MHC class II gene was better in Ross 308 strain (1.07 fold change) followed by 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 MHC class II gene, it was observed to have been upregulated most in birds administered 4% ginger extract (1.26 FC), 6% ginger extract (1.19 FC), while the control (0%) had the least value (0.00 FC). In conclusion, aqueous ginger extract could be used up to 6% in the drinking water of the three broiler chickens strains as it led to upregulation of the MHC class II gene expression which could help in conferring immunity to the birds against exogenous microbial infection.

Keywords: MHC complex II gene, upregulation, Oxytetracycline®, immunity, ginger extract.

Expression du gène du complexe majeur d'histocompatibilité de classe II dans des souches commerciales de poulets de chair administrés à des niveaux variables d'extrait aqueux de gingembre

Résumé

En raison de la réglementation sur l'utilisation d'antibiotiques synthétiques à la suite du développement d'une résistance par des micro-organismes, l'expression du gène de classe II du complexe majeur d'histocompatibilité (CMH) dans trois souches commerciales de poulets à griller administrées à des niveaux variables d'extrait de gingembre a été étudiée. Quatre-vingt-dix chacun des acres Arbor Plus, Cobb 500 et Ross 308 ont été utilisés dans l'expérience. Les oiseaux ont été divisés au hasard en trois groupes de traitement, sur la base de la race, de 30 oiseaux, désignés par T1, T2 et T3. Chaque traitement par race a ensuite été divisé en 3 répétitions de 10 poussins. Le témoin a reçu une cuillerée de table rase d'Oxytetracycline® dans 2L d'eau comme recommandé par le fabricant tandis que 4% et 6% d'extrait aqueux de gingembre ont été administrés aux oiseaux de traitement 2 et 3, respectivement via l'eau potable. Un régime à une seule phase (22,34 % de PB ; 2 948,05 ME kcal/kg) a été administré aux oiseaux pendant

8 semaines. Des échantillons de foie ont été prélevés à la fin de l'expérience, fixés ultérieurement dans l'ARN et utilisés pour l'étude d'expression. Les résultats ont montré un effet significatif (P<0,05) du traitement sur l'expression du gène du complexe CMH de classe II des oiseaux. Les oiseaux Arbor acres Plus, Cobb 500 et Ross 308 ayant reçu de l'extrait de gingembre ont exprimé plus de gène que le témoin. Pour l'effet de race, l'expression du gène CMH de classe II était meilleure dans la souche Ross 308 (changement de 1,07 fois) suivie par Arbor acres plus (changement de 0,76 fois) tandis que Cobb 500 avait le moins (changement de 0,62 fois). Pour l'effet de la variation des niveaux d'extrait de gingembre sur l'expression du gène du CMH de classe II, il a été observé qu'il avait été régulé positivement chez les oiseaux ayant reçu 4 % d'extrait de gingembre (1,26 FC), 6 % d'extrait de gingembre (1,19 FC), tandis que le contrôle (0%) avait la valeur la plus faible (0,00 FC). En conclusion, l'extrait aqueux de gingembre pourrait être utilisé jusqu'à 6% dans l'eau potable des trois souches de poulets à griller car il a conduit à une régulation positive de l'expression du gène MHC de classe II qui pourrait aider à conférer une immunité aux oiseaux contre les infections microbiennes exogènes.

Mots-clés : Gène du complexe II du CMH, régulation positive, Oxytétracycline®, immunité, extrait de gingembre.

Introduction

One of the key problems facing the production of poultry is microbial infections like *Eimeria spp* (Willis *et al.,* 2010a; Willis *et al.,* 2011) and bacterial infections caused by Clostridia, Salmonella and *E. coli* (Willis *et al.,* 2010b; Ohimain and Ofongo, 2012). Generally, different microbes have been linked with many poultry diseases, including Colibacillosis, Salmonelosis, Poultry Cholera, Clostridiosis, Crysipelas, Pasteurellosis, Mycobacteriosis, and Spirochetosis (Potter, 1998; Ohimain and Ofongo, 2013). Synthetic antibiotics are the most common feed additives used for the prevention and treatment of these microbes and recent trends are discouraging the use of synthetic antibiotics due to the development of resistant microorganisms, and their effects on human health (Yahya *et al*., 2014; Joseph *et al*., 2015; Afolami and Onifade, 2018; Ruvalcaba-Gomez, 2022). Antibiotic resistant Salmonella has been isolated for instance, in most endemic parts of the world (Afolami and Onifade, 2018). As a result, antibiotics are not so effective anymore and this has encouraged research into the development of innovative alternatives that can avert and deal with Salmonellosis (Gut *et al.,* 2018) and other diseases.

In view of the above, herbs and spices have gained useful applications in broiler chickens production for the prevention and control of microbes. This is attributed to their inherent antimicrobial, growth-promoting, and fat reducing properties. Ginger is one of the spices reported for its natural growth enhancing ability as it contains several compounds: shogaols, gingerdione, gingerol, phenolic, and gingerdiol (Zhao *et al.,* 2011). Some of the essential phytochemicals in ginger are reported to improve weight gain, and impact pharmacological benefits on broiler chickens health (Ali *et al.,* 2008). Reports abound about the antimicrobial, anti-inflammatory, immunomodulatory, antioxidant effects, and free radical scavenging activity of ginger (Suciyati *et al.,* 2021). This is achieved via an innate immune defense system which recognizes and destroys bacteria upon their infesting the host cell. The activities of the bacteria produced substances such as reactive oxygen species, nitric oxide, proteolytic enzymes, and lysozyme; by-products whose effects are countered by ginger, which hunts for superoxide and hydroxyl radicals and also generates oxygen-containing free radicals. This immunomodulatory activity of ginger relieves the

intestinal mucosal structure from being injured (Mao *et al.,* 2019; Zhang *et al.,* 2022).

Major histocompatibility complex (MHC) class II molecules are critical in the initiation of antigen-specific immune response. The MHC region is a conserved region of all vertebrates. It contains genes that are highly polymorphic in different species and generates quantifiable soluble products categorized as class I, II, and III genetic markers including erythrocyte antigens, polymorphic plasma, and erythrocytes proteins (Flajnik *et al*., 1999). It is located on chromosome 16 with 6 exons. The MHC organization differs both among and within bird orders. In the chicken (*Gallus gallus*) of the order Galliformes, the arrangement is simple against what is obtainable in many birds of the order Passeriformes where it is more complex and with a larger number of MHC class I and II genes; Chicken MHC genes are found at two independent loci: classical MHC-B, and non-classical MHC-Y (Karlsson and Westerdahl, 2013). The discovery of this locus via the study of the compatibility of tissue transfer, birthed the name of this locus. The molecules of class II MHC are essentially expressed in professional, immune antigen-presenting cells, but may also be induced on other cells by interferon γ (Ting and Trowsdale, 2002). These genes are expressed on the epithelial cells in the thymus and on antigen-presenting cells in the periphery. It has a lot of biological functions ranging from peptide antigen assembly, immune response, antigen processing and presentation of exogenous peptide antigens, and positive regulation of T cell activation (Ensembl.org, 2022). The MHC gene controls how the immune system detects and respond to specific antigens. Antigen specificity of T-cell recognition is controlled by MHC molecules with different antigen presentation between MHC class I and class II molecules. 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+ of 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).

Researches linking the expression of the MHC complex class II gene in chickens when dosed with aqueous ginger extract is somewhat scarce. It is imperative to evaluate this effect as it may be a way of helping to boost the immune response of birds to sundry diseases. The aim of the study therefore, was to evaluate the expression of the MHC complex class II gene in three commercial strains of broiler chickens administered varying levels of aqueous ginger extract.

Materials and Methods

Experimental site

The research work was carried out at the poultry unit of the Teaching and Research Farm of the Department of Animal Production, Federal University of Technology, Gidan Kwanu campus, Minna, Niger State, Nigeria. Gidan Kwanu lies between latitude 9°32' and 9°42' N, and longitude 6°30' and 6°40' E. The day light temperature fluctuates between 24°C at the middle of the wet season, and 35°C at the pinnacle of the dry season; the annual rainfall is between 1200-1300mm (Ojimaduka *et al.*, 2020).

Experimental diet and animal management

Fresh ginger rhizomes were sourced, thoroughly washed with water, peeled, and cut into chips. The chips were then ground with a warring blender (Polyester electric blender, model PV-BL999B, China) into mash. The concentrated ginger juice obtained from the mash using an extractor was stored in a bottle and refrigerated at 4°C until the time of usage (Joseph *et al.,* 2015). The chicken's diet was formulated to contain metabolizable energy of 2948.05Kcal/kg with a protein level of 22.34% CP. It was compounded

from maize, groundnut cake, fish meal, wheat offal, limestone, bone meal, palm oil, common salt, lysine, methionine, premix, and toxin binder. The single phase diet was fed to the birds for the whole experiment. Two hundred and seventy (270) day old commercial broilers chicks (90 each of Arbor acres Plus, Cobb 500 and Ross 308) were used for the experiment. The birds were arranged in such a way that they could be analyzed using both the completely randomized and randomized complete block designs. The chicks in each group were divided into 3 replicates of 10 chicks. Treatment 1 birds were administered a table spoonful of Oxytetracycline® in 2 litres of water (control). Birds in T2 and T3 were administered 4% and 6% aqueous ginger extract, respectively. After finishing this, fresh water was then provided to the chickens *ad libitum*. They were administered preventive vaccination using attenuated live vaccines of Gumboro and Lasota on days 7, 14, 21, and 28, respectively.

Data collection

At the end of the experiment, a total of twenty seven (27) tissue samples were collected from the liver of the birds, completely submerged in RNA Later and stored in Eppendorf tubes until the time of use. The guanidinium thiocyanate-phenol-chloroform method was used to extract total RNA from the liver as described by Chomczynski and Sacchi (1987). The extracted RNA was converted to cDNA using the FIREScript RT cDNA Synthesis Kit. The process of conversion was done according to the manufacturers protocol.

The forward and reverse primers used in the study were designed at the African Biosciences Laboratory, Ibadan, Oyo State and are:

MHC2F……CTCGAGGTCATGATCAGCAA (forward)

MHC2R……TGTAAACGTCTCCCCTTTGG (reverse)

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 25µl reactions consisting of 4µl of the 5x HOT Firepol qPCR Mix, 0.4µl each of the forward and reverse primers and a specific probe which had a concentration of 250nM, 18.2µl of nuclease free water and 2µl cDNA template (100ng). The cycling conditions were 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.

Data analysis

The data generated from the study were analyzed using SPSS software version 20.00 (IBM, USA). Figures were generated using MS Excel.

RESULTS

Figure 1 shows the results of the expression of MHC complex class II gene among the Arbor acres Plus strain of broiler chickens administered varying levels of aqueous ginger extract. The results showed significant ($P<0.05$) differences in the expression of the MHC complex class II gene between birds on the control, and those on the aqueous ginger extract. The gene was upregulated in Arbor acres plus birds administered aqueous ginger extract while those

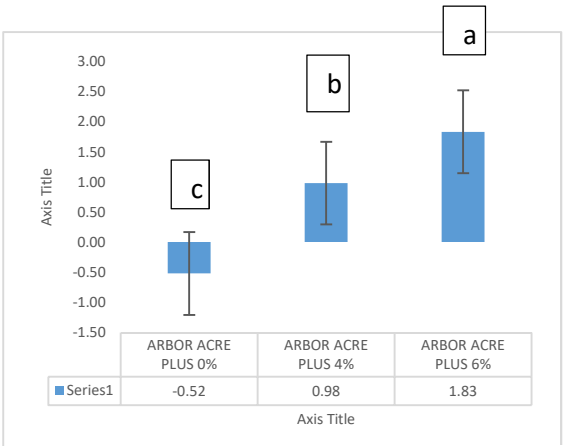

Figure 1: MHC complex class II gene expression pattern (*Arbor acres* plus)

Figure 2 shows the results of the expression of MHC complex class II gene among the Ross 308 strain of broiler chickens administered varying levels of aqueous ginger extract. The expression of the gene was significantly (P<0.05) affected by the different aqueous ginger extract administration. The gene was better upregulated in birds administered the ginger extract compared to those on the antibiotic regime.

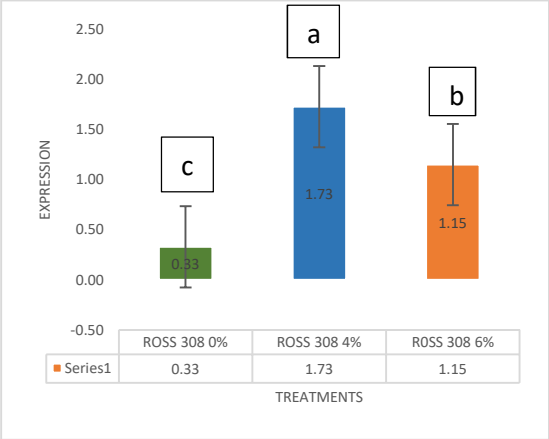

Figure 2: MHC complex class II gene expression pattern (Ross 308)

Figure 3 shows the results of the expression of MHC complex class II gene among the Cobb 500 strain of broiler chickens administered varying levels of aqueous ginger extract. Significant (P<0.05) differences was observed in the expression of the MHC complex class II gene among the Cobb 500 birds. The gene was upregulated greatly in the Cobb 500 birds administered the different levels of aqueous ginger extract when compared to birds administered the control.

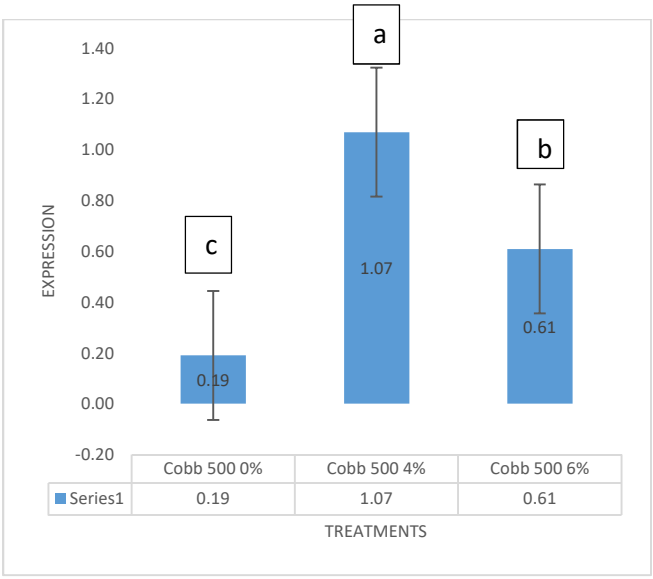

Figure 3: MHC complex class II gene expression pattern (Cobb 500)

Figure 4 shows the results of the expression of MHC complex class II gene comparing the Arbor acres Plus, Ross 308 and Cobb 500 strains of broiler chickens administered varying levels of aqueous ginger extract. There were significant (P<0.05) differences observed in the expression of the MHC complex class II gene between the different strains of broiler chickens. The gene was upregulated in all the strains of broiler chickens; the Ross 308 strains of broiler chickens had higher value while Cobb 500 birds recorded the lowest value.

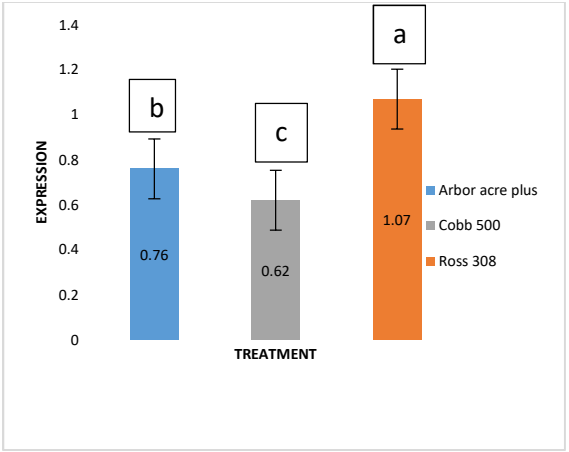

Figure 4. Effect of breed on the expression of MHC complex class II gene

The effect of different levels of ginger extracts on the expression of MHC complex class II gene in the different strains of broiler chickens is presented in Figure 5. The level of administration of the aqueous ginger extract significantly ($P<0.05$) influenced the expression of the gene in the birds. The gene was observed to be upregulated with increasing level of administration with birds on 6% aqueous ginger extract having the most upregulated level followed by those on the 4% aqueous ginger extract.

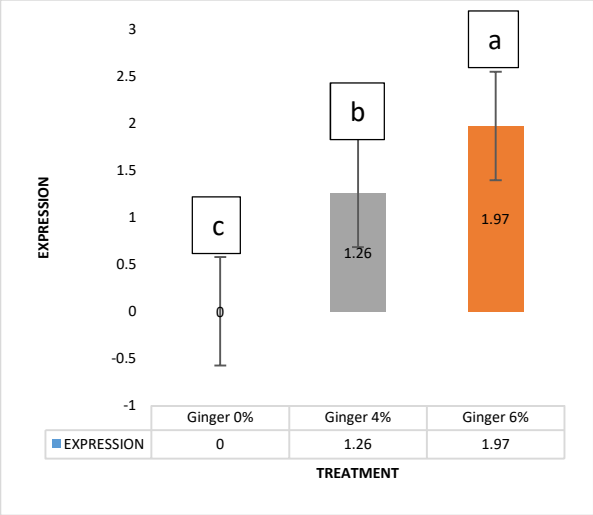

Figure 5. Expression of MHC complex class II gene due to levels of aqueous ginger extract administration

Discussion

The upward regulation of MHC complex class II gene in all the broiler strains administered aqueous ginger extract when compared to those in the control is an indication of possibly better immunity boosting by the aqueous ginger extract at the MHC gene locus of the broiler chickens. This immune status conferred by the aqueous ginger extract would prevent the outbreak of common bacterial diseases in the flock and in turn reduce loss for farmers. This is in line with the findings of Nidaullah *et al.* (2010) who reported that aqueous ginger extract enhanced the immune performance of broilers against common bacterial diseases. The downward regulation of MHC complex class II gene in Arbor acres plus birds (-0.52) may be because, the antibiotic rather than boost the immunity on this class of birds, affected it negatively leading to depressed expression of the gene either because the birds developed resistance to the dose administered, or as a result of treatment failure. This can have negative impact on the immune status of the birds as the birds might come down with diseases during production. Generally, it was observed that birds administered with antibiotic had lower levels of the MHC complex class II gene compared to those administered with the aqueous ginger extract. This is a plus for the usage of aqueous ginger extract as a non synthetic immune booster in broiler chickens production. It may well be that, some level of resistance to the antibiotic occurred in the birds on this treatment which somewhat affected the activation of the gene under study. Yahya *et al*. (2014), and Joseph *et al.* (2015) had both reported on the need to regulate the use of synthetic antibiotics because of the development of resistance in microorganisms and their effects on both animal and human health.

With regard to the breed effect after administration of the aqueous ginger extract, the upward regulation 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 genetics and how they were able to utilize the ginger extract considering other environmental variables. This result is in line with the findings of Dalgaard (2003) who reported that, levels of MHC molecule expression differ among different chicken lines and the expression correlates with Mark's disease susceptibility.

With regard to the effect of administration of varying levels of the aqueous ginger extract, the expression of the MHC class II gene was in the order 6% > 4% > 0%. This could mean that, increasing the level of aqueous ginger extract will

also boost the immunity of birds. Beside this, the result also pointed to the better utilization of aqueous ginger extract than the Oxytetracycline® by the birds used in the study. This is good news to health conscious farmers who have been advocating for the use of alternative, non-synthetic and less harmful additives and or ingredients in the rearing of poultry and other livestock. Farmers will also benefit from reduced cost of rearing birds to market weight. The observed increase in the expression of MHC complex class II gene with increasing usage of aqueous ginger extract in the broiler chickens is however, not in consonance with the findings of Tripathi *et al*. (2008) who showed that alcoholic ginger extract decrease the MHC complex class II gene expression on Lipopolysaccharide (LPS) activated macrophages. The difference could be due to the differences in the medium of extraction, and could indicate that the aqueous extract is better than the alcoholically extracted ginger extract for broiler chickens with regards to the expression of MHC gene.

Conclusion

Ginger is a medicinal plant that has been reported to have immune stimulating properties on livestock. Results of the expression of MHC class II genes differs among chicken strains used in the study, and the level of administration of the extract. The pattern of expression of the MHC class II gene can be used to predict the possible immune response of the broiler strains. The use of aqueous ginger extracts up to 6% upregulated the gene in the different strains of broiler chickens. On the other hand, the synthetic antibiotics (Oxytetracycline®) produced varied effect on the expression of the MHC class II gene; while it led to some levels of upregulation in Ross 308 and Cobb 500 birds, it led to down-regulation in Arbor acres plus strain. This therefore, suggests that, aqueous ginger extract possess immune stimulating properties and can be used to provide protection to broiler chickens against disease causing micro-organisms.

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