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Expression Analysis of Interleukin-13 Gene in the Small Intestine of Three Genotypes of chickens Administered Conventional Antibiotics and Garlic

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ABSTRACT

This study aimed at evaluating the interleukin-13 gene in small intestine of three genotypes of chickens reared in the derived savannah. The study adopted a 3 x 3 factorial (3 genotypes and 3 treatments) in a completely randomized design. The genotypes that were used for the study were exotic genotype (White Leghorn), local (Nigerian Heavy Ecotype) chicken and crossbred (cross between exotic and local chicken). The treatments were: Control (water + No extract), Ciprofloxacin (water + Ciprofloxacin) and Garlic (water + *Allium sativum* extract at a dose of 800 mg/ml). A total of 360 F_1 chicks (120 chicks from each genotype were randomly distributed into 3 groups (40 chicks per treatment) having 4 replicates (10 chicks in each). At 2, 4, and 6 months of age, the small intestines were collected and stored in RNA later for expression of interleukin-13 using the qPCR method expression studies. The effect of genotype x treatment interaction on the expression of interleukin-13 gene in the small intestinal tissues showed significant variations ($P<0.05$) at 2, 4 and 6 months of age. The local and crossbred birds fed garlic showed a greater upregulation of the interleukin-13 gene compared to the rest of the group. The results suggested chicken administered garlic had a higher expression of interleukin-13 gene in the small intestine at 2 and 4 months compared with chickens on the control diet and those given ciprofloxacin. The effect of garlic on gene expression however reduced at 6 months of age.

Key words: Crossbreeding, Expression Analysis, Genotype Chicken Interleukin-13 and Nutrigenomic

INTRODUCTION

Overpopulation is one of the major causes of the world's problems. Developing countries are the most hit by population increase as it leads to poverty and malnutrition. Over population also leads to enormous strain on the planet's resources and possess a wide range of complex challenges for nutritionist physiologist and most of all animal breeders who have to work extra hard to fight malnutrition by increasing animal protein intake (Fulla 2022).

The African continent is home to diverse population of livestock breeds adapted to harsh environmental conditions. Animal productivity is less than optimal because it is faced by problems like lack of adequate nutrition, inbreeding and lack of proper management (Iyasere et al. 2019). The consequence is decrease in livestock productivity which is unable to match demand and population growth and has led to a low level of animal protein intake (Attia et al. 2022). To solve this problem, breeders have resorted to looking at the local breeds that are adapted to the harsh environment. Example of such livestock is the Nigerian heavy chicken ecotype chicken.

Nigerian heavy ecotype chicken (Fulani) is one of the indigenous breeds of poultry found in Nigeria. It is well adapted to the harsh tropical environment and poor nutritional setups with excellent resistance against certain diseases (Ndofor-Foleng et al. 2015). They have poor growth and reproductive performance when compared to the exotic. Exotic chickens are known for their large body sizes and high productivity. High production trait of the exotic chickens can be used to improve the Nigerian heavy ecotype chicken. The genetic potentials of local chicken and exotic chicken breeds could therefore be harnessed crossbreeding to improve the heavy ecotype

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chicken species in the Nigeria, and thus generate a new genetic base that can be used to develop a breed (Iyasere et al. 2019).

However, animal breeders have introduced several methods to increase productive performance of indigenous chickens by crossing with the exotic birds. In spite of the advantages of crossbreeding, lack of good health, lack of feed all-round the year and inadequate feed ingredients and feed additives has continual to be a major constraint to the survival of crossbred chickens compounds (Tchoupou-Tchoupou et al. 2021). A major feed additive that has been extensively used in poultry feed is antibiotics.

The use of antibiotics for any purpose in the husbandry of livestock includes not only the treatment or prophylaxis of infection but also the use of sub-therapeutic doses in animal feed to promote growth and improve feed efficiency in contemporary intensive animal farming. Incidentally, the use of antibiotics in animal feed has shown several side effects such as resistance towards the drug and evidence of resistant strains that become zoonotic (Tchoupou-Tchoupou et al. 2021) which could end up in human food chain if the withdrawal period is not maintained. This concern has led international organizations (WHO, FAO and OIE 2010) to adopt concerted plans to optimize antibiotic use and promote research for alternative solutions. This recommendation has led to an increased interest in natural medicinal products given that more than 80% of drug substances are either directly derived from natural products or developed from natural compounds (Tchoupou-Tchoupou et al. 2021).

Natural medicinal products originating from herbs and spices have been used as feed additives for farm animals (Singh and Gaikwad 2020). It has been reported that, the use of phytobiotics such as *Allium sativum* (garlic) extract could be a major asset in the fight against bacterial infections. In recent years, garlic has been found to be effective in its application as antibacterial (Tchoupou-Tchoupou et al. 2021), antiviral, antifungal, antimicrobial, antioxidant, insecticidal, antiprotozoal and antitumor. Moreover, it boosts the immune system and it reduces the level of cholesterol and triglyceride in the serum of broilers thus helping in improving their lipid profile (Sheoran et al. 2017).

More recently, it has been possible to study the impact of diet on the organism at the molecular level (nutrigenomic). Nutrigenomics is a science that explores the relationship between nutrition and gene expression and how it affects health (Felisbino et al. 2022). It provides an understanding of how diets help the immune response of animals by up regulate or down regulating the expression of genes in chickens. This also depends on polymorphism or variability of the various genotypes (local, exotic and crossbred) to the dietary components or feed additives (garlic). Garlic contains allicin, diallyl disulfide and diallyl trisulfide. These compounds have been found to modulate the expression of genes involved in cell growth, apoptosis, and inflammation (Tchoupou-Tchoupou et al. 2021). One of the genes that could be studied in relation to garlic is interleukin-13 (IL-13).

IL-13 is a Protein Coding gene made by a type of T lymphocyte. It reduces inflammation by blocking production of cytokines by macrophages. It has important immunomodulatory activities and exert influence on a wide variety of immune cells, such as B cells, eosinophils, basophils, monocytes, fibroblasts, endothelial cells, airway epithelial cells, smooth muscle cells, and keratinocytes (Abdellatif et al. 2020**)**. Therefore, this study aimed at evaluating the expression analysis of interleukin-13 gene in the small intestine of three genotype chickens reared in the derived savannah.

MATERIALS AND METHODS

The Animal and Management Conditions

Three genotypes: heavy ecotype (HE), exotic chicken and crossbred (HE X EC) were used. One hundred and twenty (120) day old chicks selected from each genotype were brooded and reared at the University of Nigeria, Nsukka animal Research Farm. They were allotted to 3 treatments - (control, ciprofloxacin, garlic), each treatment had 40 birds replicated 4 times with 10 chicks per replicate. Ciprofloxacin was administered as a commercial antibiotic according to manufacturer's recommendations) and the Garlic group was administered garlic at a dose of 800 mg/ml**)**. Administration of the treatment lasted for 6 months. Birds were fed *ad-libitum* with the same commercial Chikun chicks feed and layer mash of Hybrid Feeds Limited for F_1 off springs. Clean drinking water was also provided. Birds were raised in the same conditions of humidity, ventilation, and temperature. Appropriate prophylactic medications and vaccinations against common diseases were given to ensure the optimal health of the birds. The study was carried out after having consulted the institutional guideline on the use of animals for scientific research and obtaining ethical clearance.

Gene Expression Analysis

At the 2nd, 4th, and 6th month of the experimental period, 3 birds per replicate were sacrificed and the tissues samples of the small intestinal were collected and weighed. Two grams of the sample was cut and immersed into 1ml of RNA Later (QIAGEN Ltd., West Sussex, United Kingdom) contain in a 1.5 ml Eppendorf tube. These samples were kept under normal temperature overnight to allow RNA later to penetrate inside the tissues and prevent RNA degradation. The samples were then stored at -20°C to retain the integrity of the RNA in the tissues. Extraction of mRNA was done, and specific primers were used to target the region that had the interlukin-13 gene of interest. cDNA was obtained using Solis Firescript cDNA synthesis kit according to manufacturer's protocol.

The primers used for IL-13 gene amplification were:
Forward: 5'- CTGCCCTTGCTCTCCTCTGT-3'
Reverse: 3'- CCTGCACTCCTCTGTTGAGCTT-5'

Quantitative PCR Analysis/Gene Expression: The BIOER 96-well line K series thermal cycler using the Solis Eva-green qPCR (no ROX) master mix according to the manufacturer's protocol was used. β-actin gene was used as a housekeeping gene. The $2^{-\Delta\Delta CT}$ method by Livak and Schmittgen (2001) was used to obtain the relative quantification of IL-13 gene mRNA. The relative quantification of IL-13 gene was calculated using the

Comparative C_T Method ($2^{-\Delta\Delta C}{}_T$ Method) described by Livak and Schmittgen (2001), where $\Delta\Delta C_T$ corresponded to the difference between the C_T measured for the mRNA level of each tissue and the C_T measured for the mRNA level of the housekeeping gene,

$\Delta C_T = C_T$ (target gene) - mean C_T (β-actin).

Where ΔΔCT is the gap between the threshold cycle measured for each tissue's mRNA level and the threshold cycle measured for the reference gene's mRNA level.

Statistical Analysis

The experimental design used was a 3x3 factorial (3genotype x 3treatment groups) in a completely randomized design according to the model:

$Y_{ijk} = u + A_i + B_j + (AB)_{ij} + e_{ijk}$

Y_{ijk} is individual observation.

u is population mean

Ai is effect of Local, Exotic and Crossbred

Bj is effect of treatment

$\mathbf{(AB)_{ij}}$ is interaction effect of breed

$\mathbf{e_{ijk}}$ is the experimental error

Data collected were subjected to analysis of variance and where means were found to be significant; Duncan's New Multiple Range test was used to separate the means.

RESULTS

The effect of the different treatments on the expression of interleukin 13 gene in the small intestinal tissues of the chickens is presented in Fig. 1. There were highly significant ($P<0.01$) differences between treatments at 2, 4, and 6 months. Birds (2-months old)**,** on garlic showed the highest expression value of interleukin 13 genes in the small intestinal tissues whereas birds on the control and Ciprofloxacin groups were similar ($P>0.05$). At **4** months of age, the mean expression values of interleukin-13 gene for control and Ciprofloxacin treated birds were significantly ($P<0.05$) lower compared to the birds fed garlic extracts. However, at 6 months of age**,** the trend was different with highly significant ($P<0.01$) difference obtained among the treatments with the of interleukin 13 gene being upregulated in birds

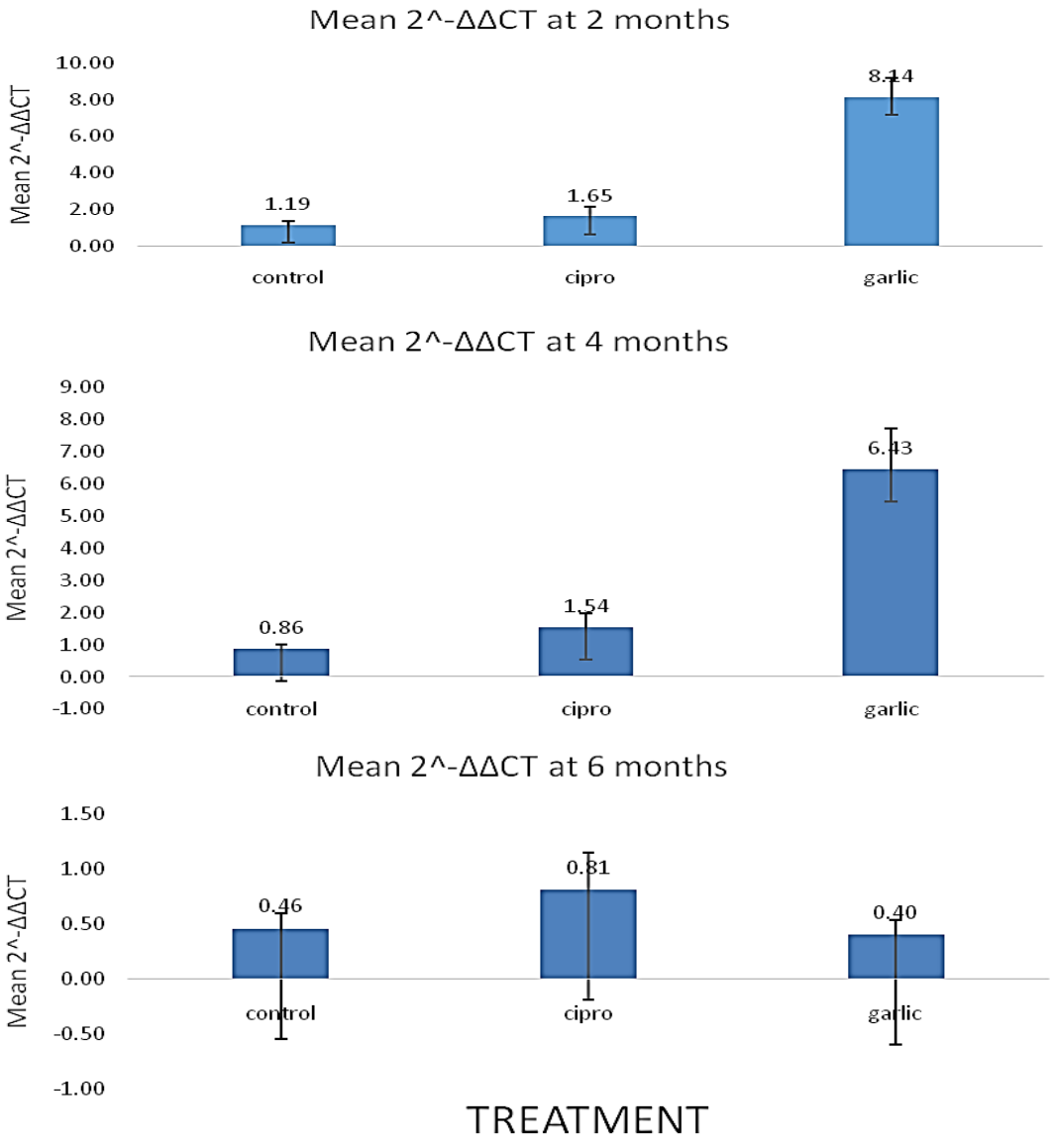

Fig. 1: Effect of treatment on the expression of interleukin-13 gene in the small intestine.

administered with ciprofloxacin while the interleukin-13 gene in birds fed garlic at 6 months age was down regulated.

At 2 and 4 months of age (Fig. 2), there was no significant (P>0.05) genotype effect on the expression of interleukin 13 gene in the small intestine of the chickens. On the other hand, at 6 months, expression of interleukin - 13 gene in the small intestine of the crossbred genotypes was significantly (P<0.05) up regulated compared with the local and exotic genotype.

The exotic birds showed the least expression value of the interleukin 13 gene. The interaction effect of genotype and treatment on the expression of interleukin-13 gene in the small intestinal tissues (Fig. 3) showed significant variations (P<0.05) at 2, 4 and 6 months of age. At 2 and 4 months, the interleukin-13 gene of the small intestine of the local genotype fed garlic and crossbred genotype fed garlic showed a greater upregulation. Lower values were obtained in exotic genotype fed garlic. The lowest mean value was recorded among the birds on the control group and those administered Ciprofloxacin.

DISCUSSION

The main objective of this study was to evaluate the expression level of interleukin-13 gene in the small intestine of three genotype chickens. It is a fact that gene expression is important because a specific protein can be produced only when its gene is turned on. The primary function of interleukins is to modulate growth, differentiation, and activation during inflammatory and immune responses. Interleukins can elicit many reactions in cells and tissues by binding to high-affinity receptors in cell surfaces (Justiz Vaillant and Qurie 2022).

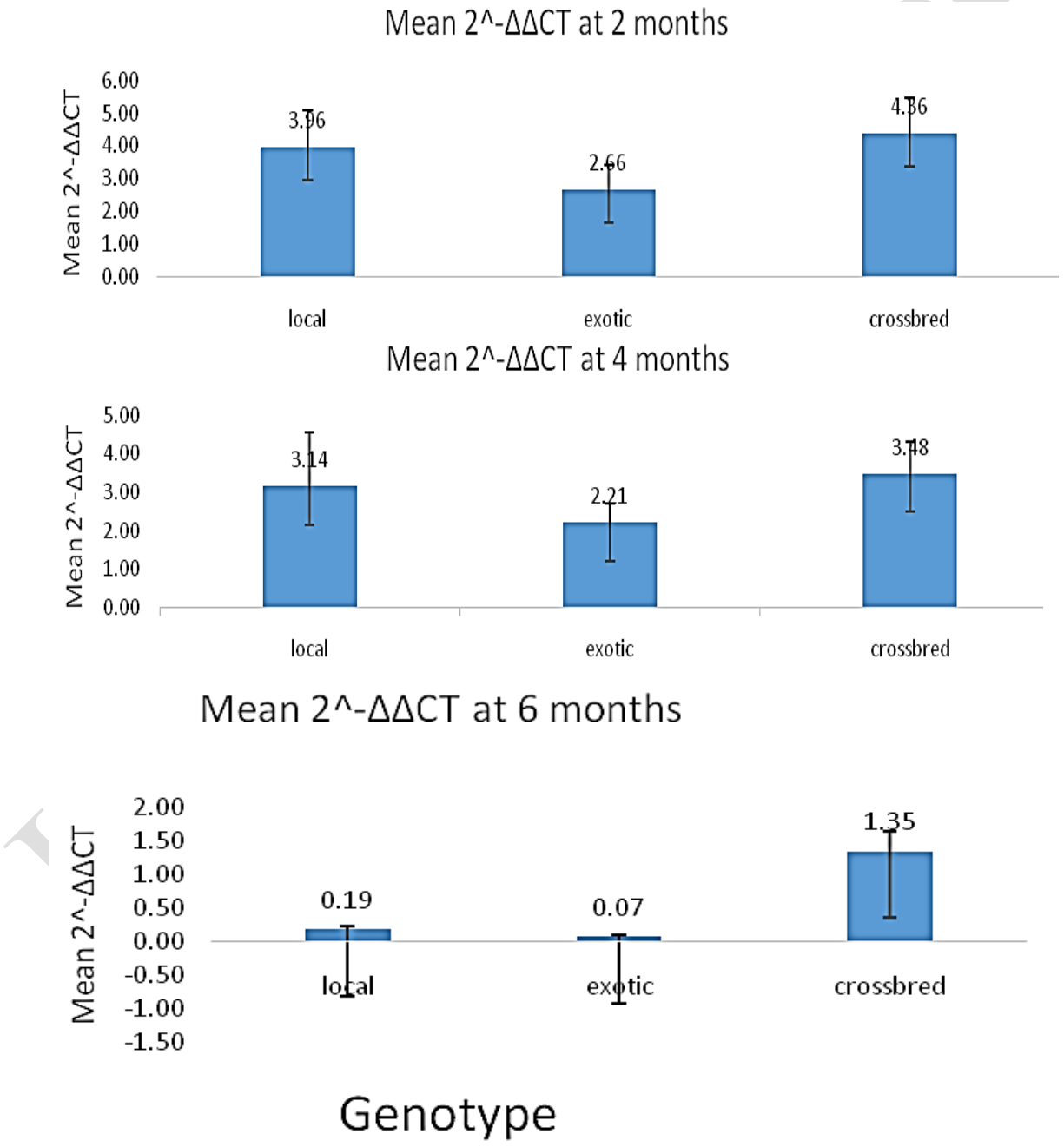

Fig. 2: Effect of genotype on the expression of interleukin-13 gene in the small intestine.

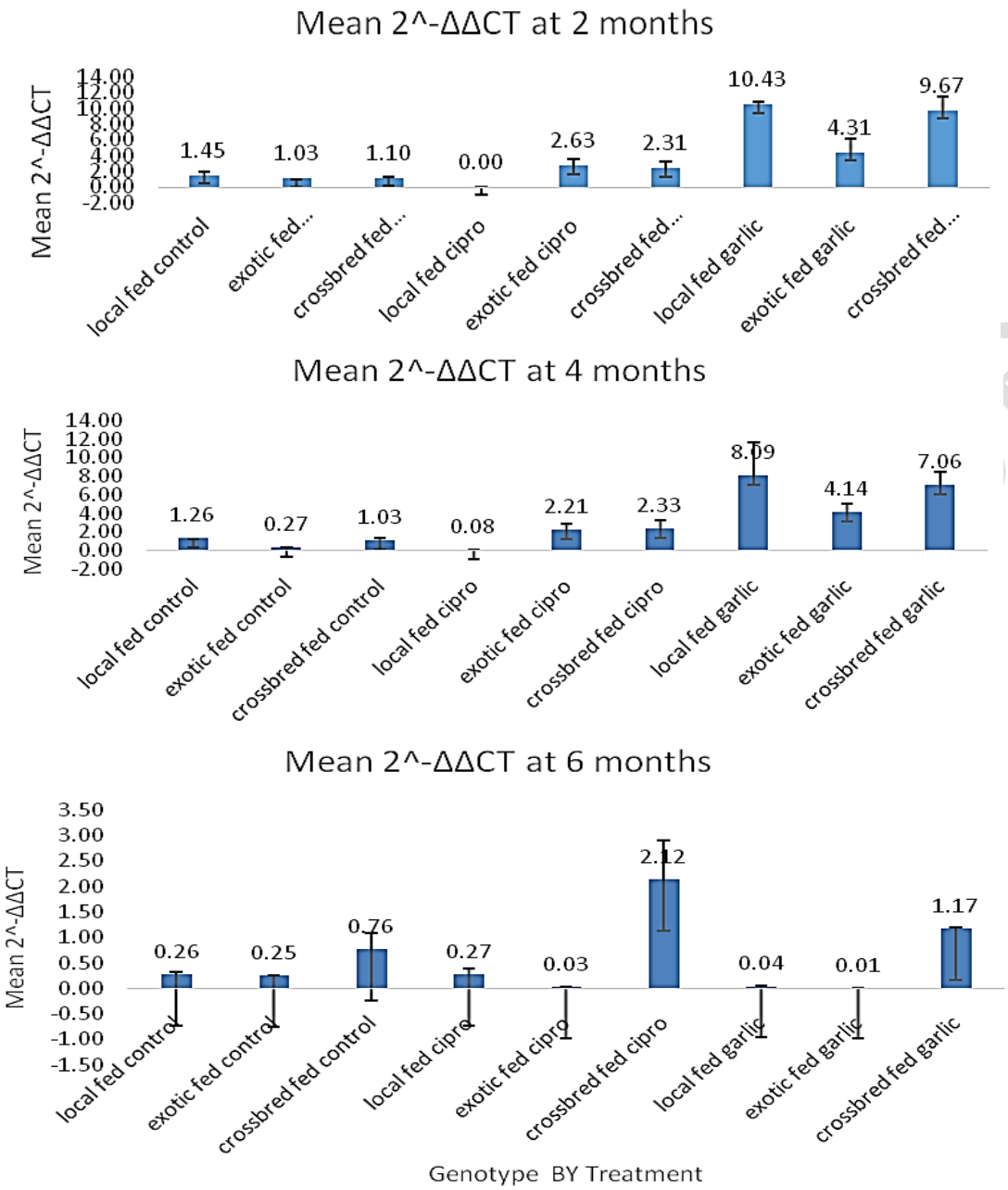

Fig. 3: Interaction effect of genotype and treatment on the expression of interleukin 13 gene in the small intestinal tissues.

The present findings revealed that at 2 and 4 months, the IL 13 genes in birds fed with garlic was upregulated while at an older age (6 months), there was down regulation of these genes in the small intestine. Down regulation of gene is a process by which a cell decreases the quantity of a cellular component such as RNA or protein, in response to an external stimulus, the complementary process that involves increases of such components is called upregulation (Goel et al. 2021).

Therefore, an increase in the exposure of these animals at an older age (6 months) to garlic led to the down regulations of the interleukin-13 gene in the birds. This suggests that the effects of garlic treatment on IL-13 expression might have been short-lived and might not have been sustained over time. Abdellatif et al. (2020) opined that the short-lived effect of garlic diminishes over time because garlic has its binding site in the intestine of birds. As the animal gets older and is being fed continuously with garlic, the site gets saturated and as such the anti-inflammatory and immunostimulatory properties may be reduced leading to a decrease in the expression of IL-13.

Considering the effect of the genotype in this study, it was observed that, at 6 months of age, the expression of interleukin-13 gene in the small intestine of crossbred genotypes was significantly upregulated compared to the expression levels observed in the local and exotic genotypes. This suggests that crossbred birds may have a higher level of immunity against certain diseases or pathogens (Sugiharto et al. 2018). The upregulation of interleukin-13 in the crossbred birds could be the result of the combination of genetic material from both the local

and exotic genotypes, which has provided them with an increased level of immunity (Bailey et al. 2020). On the other hand, the exotic birds showed the least expression levels, suggesting that these birds reared in Nigeria, may be more susceptible to certain diseases or pathogens.

Garlic contains a number of compounds that are known to have an effect on gene expression. Allicin is one of the most studied compounds and is responsible for the pungent smell of garlic. It has been found to have anti-inflammatory, anti-bacterial, and antioxidant properties (Mandal et al. 2019). Studies have also shown that allicin is capable of modulating gene expression, in particular, down regulating the expression of pro-inflammatory genes (Horev-Azaria et al. 2009; Salehi et al. 2019). S-allylcysteine is another compound found in garlic that has been found to induce the expression of genes involved in apoptosis, and is able to modulate the expression of genes involved in inflammation, as well as genes involved in the metabolism of cholesterol, fatty acids, and glucose (Ansary et al. 2020).

The expression of IL-13 gene in small intestinal tissues plays an important role in maintaining the homeostasis of the intestinal microbiota. The change in gene expression levels in specific host cells in response to the identification of a pathogen is the most important host response (Liu et al. 2001). Therefore, any significant changes in the expression of IL-13 in these tissues can have serious implications for the health and well-being of the host animal (McKenzie et al. 2017).

Considering the treatment x genotype effect, it could be notated that garlic has been shown to have anti-inflammatory and anti-microbial properties, which may explain the upregulation of interleukin-13 gene expression in the Nigerian heavy ecotype genotypes fed garlic and the crossbred genotype fed garlic (Elbaz et al. 2021; Semple et al. 2022). Also it may suggest that these crossbred genotypes may have a more robust immune system, which would explain why they were able to respond better to the garlic treatment. The high sulphur content in garlic gives it antibiotic properties and thus helps in keeping the digestive system clean by flushing out toxins. It also builds immunity against common cold and prevents heart ailments by clearing up blocked arteries.

On the other hand, the lowest mean value was recorded among local genotypes fed Ciprofloxacin. This may be due to the fact that Ciprofloxacin is an antibiotic and has been shown to have a wide range of anti-microbial effects. Antibiotics are known to interfere with the normal functioning of the immune system and can lead to a decrease in immune response (Maggini et al. 2018). This could explain why the Local genotypes fed Ciprofloxacin had the lowest mean value for interleukin-13 gene expression. The result of this study goes a long way to buttress the importance of nutrigenomics in animals. Therefore, nutrigenomics is based on the idea that the nutrition provided to animals can affect the way their genes are expressed (Malgwi et al. 2022). Through this research, it is possible to identify the best nutritional strategies for animal health and performance. In addition, nutrigenomics can be useful for predicting the response to dietary changes and for identifying potential health risks.

Conclusion

In conclusion, this study highlighted the nutritional advantages of using garlic and Ciprofloxacin in the gene expression of interleukin 13 gene in three genotypes (exotic, local and crossbred) chickens. The garlic extract had a positive effect on the expression of interleukin 13 gene in the small intestinal tissues at two and four months of age. This could be an indication of a beneficial inflammatory response in the body, which could indicate the potential for garlic to promote the health of small intestinal tissue.

It can also be concluded from this study that nutrition and genotype can have a significant impact on the expression of interleukin-13 gene in small intestinal tissues. This suggests that diet and genotype play a role in the regulation of interleukin-13 gene expression, and that different diets and genotypes can lead to different levels of expression. Furthermore, the results suggest that the expression of the gene is not sustained over time, as there were no significant differences observed at four and six months of age. This implies that the effects of diet and genotype on the expression of interleukin-13 gene may be short-term.

The expression of interleukin-13 gene in the small intestine of crossbred genotypes was upregulated at six months of age compared to the expression in the local and exotic birds. This implies that the genotype of the bird affects the expression of the gene, which can in turn influence the bird's immunity and susceptibility to diseases. Additionally, this indicates that crossbred birds may have acquired better immune responses than local and exotic birds at six months of age.

Conflict of Interest: The authors declare that there have no conflicts of interest.

Authors Contribution

Okenyi NJ: Principal researcher. Ndofor-Foleng HM: Project supervisor. Onyimonyi AE: Designed the research. Tchoupou-Tchoupou EC: data collection and statistical analysis. Ikeh NE: data collection and statistical analysis. Nwenya JM, assisted in the field work. Amaefule BC: assisted in the field work and collection of blood samples. Uberu CPN: Participated in data collection. Nwosu IC: Assisted in the laboratory work.

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