Evaluation of the Growth Performance And Expression of Interferon Gamma (IFN-γ) Gene in Purebred And Exotic-Local Crossbred Chicks Under the Influence of Garlic

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Abstract **-** A total of 240 chicks (120 Nigerian heavy ecotype local chicken and 120 White Leghorn x local crossbred chicken) were used for this study, which was aimed at evaluating the performance and expression of interferon gamma (IFN-γ) gene in purebred and exotic-local crossbred chicks under the influence of garlic. The experimental design used was a 2x3 factorial (2 genotypes x 3 treatments: control, ciprofloxacin and garlic) in a completely randomized design. At 2 months and 4 months, 2gram of the tissues samples of the small intestinal was cut and immersed into RNA Later for expression of interferon gamma (IFN-γ) gene. cDNA were obtained using Solis Firescript cDNA synthesis kit according to manufacturer's protocol. The interaction effect between the genotypes and treatment on body weight and body weight gains of F_1 offspring showed that highly ($P<0.01$) significant mean values of body weight were reported at day old, at 2 months and 4 months. Weight gains were significantly ($P<0.01$) different at 2 and 4 months of age. This shows that garlic helped in promoting growth features in the local x exotic crossbred genotype. At 2 months of age, the mRNA expression of interferon gamma (IFN-γ) gene in the small intestine for the crossbred genotype administered garlic were upregulated compared to the control group while the mRNA expressions of the ciprofloxacin group were down regulated compared to the control group. It was therefore revealed that the effect of garlic on the expression of interferon gamma (IFN-γ) gene was short-termed.

Index Terms **-** Crossbred, Garlic extract, Heavy ecotype, Interferon gamma gene, Purebred

INTRODUCTION

Poultry industry has seen an unparalleled growth during last three decades and is now recognized as one of the fastest growing component of agriculture sector. This has happened due to increased consumption of eggs and meat with their easy availability, relatively inexpensive cost and rich in all essential nutrients which can meet the deficiencies of critical dietary minerals, vitamins and amino acids (Hafez and Attia, 2020).

Globally, indigenous chickens are highly valued for their unique characteristics including robustness, disease resistance and adaptability to local climate conditions. In Nigeria, the Nigerian Heavy Ecotype chicken stands out as one of the indigenous poultry breeds. This breed has demonstrated exceptional resilience in harsh tropical environments and under less-than-ideal nutritional conditions, showcasing remarkable resistance to specific diseases. The genetic advantages inherent in the Nigerian Heavy Ecotype chicken present a valuable foundation for breeding programs aimed at producing breeds tailored to local conditions. While exotic poultry breeds are recognized for their superior growth traits, they often struggle to adapt to the local environments. To enhance economic traits like body weight in local chickens, one strategy is to harness the genetic diversity available through crossbreeding, particularly between exotic and indigenous stocks.

Drug resistance associated with use of antibiotics in poultry production has been a big push to find alternative treatment methods for common poultry ailments. The alternatives to antibiotics are needed to maintain the gut health, integrity and performance by controlling pathogens and increased nutrient digestion and absorption. Prominent alternatives in poultry production include phytogenics, organic acids, probiotics, prebiotics, synbiotics, herbal drugs, vitamins, minerals and plant extracts (Abd El-Hack *et al.*, 2022). Garlic (*Allium sativum*) is one of such phytogenics, which can be used as substitute for antibiotics (Hossain *et al*., 2023). A number of studies have shown that garlic-active components have beneficial effects in livestock and poultry production, having antimicrobial, hypocholesterolemic, antioxidant effect and growth-promoting properties (Tchoupou-Tchoupou *et al*., 2022; Bar *et al*., 2022; Baysa *et al*., 2023). It has been observed that garlic supplementation has growth-enhancing activities on broiler chickens (Rusl *et al.*, 2022). The growth-enhancing effect of garlic in animals could be attributed in part to the distinct phytochemical content that results in improved gut activities, immunity and health status (Rafeeq *et al*., 2022).

Nevertheless, it is important to acknowledge that nutrition plays a pivotal role in modulating gene expression. Advances in molecular genetics have enabled the investigation of how nutrition, including substances like garlic (*Allium sativum*), influences animal growth and immune responses. This emerging field, known as nutrigenomics, explores the intricate interplay between nutrition and genes. Despite its potential, there remains a lack of comprehensive research on the impact of garlic on gene expression in Nigerian Heavy Ecotype chickens, and their crossbred counterparts. The aim of the study was to evaluate the growth performance and expression of interferon gamma (IFN-γ) gene in purebred and exotic-local crossbred chicks under the influence of garlic. The specific objectives of the study were to: (i) evaluate the effects of *Allium sativum* on growth performance of two genotypes of chickens, (ii) evaluate the effects of *Allium sativum* on haematological indices of the two genotypes of chickens and (iii) evaluate the effects of *Allium sativum* on the expression of

interferon gamma (IFN-γ) gene in the small intestine of the two genotypes of chickens.

MATERIALS AND METHODS

Experimental Design and Management of the Animals

The research employed an experimental design that followed a 2x3 factorial arrangement (2 genotypes and 3 treatment groups) within a completely randomized design carried out at the Research Farm, Animal Science Department, University of Nigeria, Nsukka, Nigeria. The two genotypes under investigation were the local Nigerian Heavy Ecotype and a crossbreed of the exotic White Leghorn with the local Nigerian Heavy Ecotype. The study had three distinct treatments: a Control group (provided with water and no garlic extract, a group of birds administered with ciprofloxacin, and a group administered with *Allium sativum* extract. A total of 240 F_1 chicks were involved in the study, with 120 chicks resulting from the two different crosses each: Local x Local and Exotic x Local. These chicks were randomly allocated into three groups, with each group containing 40 chicks per treatment. Within each treatment group, there were four replicates, each comprising 10 chicks. The birds were fed *ad-libitum* with Chikun commercial feed. The data collected were measurements of body weights and feed intake from the day of hatching until the birds reached 6 months of age. Additionally, weight gain and feed conversion ratio (FCR) were calculated at 2, 4 and 6 months of age. The evaluation of hematological indices was conducted at the same points. Furthermore, the study involved the analysis of the expression of interferon gamma (IFN-γ) gene in the small intestine using the quantitative polymerase chain reaction (qPCR) method at 2 and 4 months of age.

Gene Expression Analysis

At the 2nd and 4th month of the experimental period 3 birds per replicate were sacrificed and 2 gram of the tissues samples of the small intestinal 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 Interferon gamma (IFN-γ) gene of interest. cDNA were obtained using Solis Firescript cDNA synthesis kit according to manufacturer's protocol.

The primers used for IFN-γ gene amplification were:

Forward: 5'- AAAGCCGCACATCAAACACA -3'
Reverse: 3'- GCCATCAGGAAGGTTGTTTTTC -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 were 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 IFN-γ gene mRNA.

Statistical Analysis

The experimental design used was a 2x3 factorial (2 genotypes x 3 treatments: control, ciprofloxacin and garlic) in a completely randomized design using statistical the model below;

$Y_{ijk} = u + A_i + B_j + (AB)_{ij} + e_{ijk}$
Y_{ijk} is individual observation,
u is population mean,
Ai is effect of Local and Crossbred,
Bj is effect of treatment (Control, Ciprofloxacin and Garlic),
$(AB)_{ij}$ is interaction effect of genotype and treatment
e_{ijk} is the experimental error.

Data on growth performance and haematological parameters were subjected to analysis of variance and where means were found to be significant, Duncan's New Multiple Range test was used to separate them.

RESULTS

The effect of genotype on the expression of interferon gamma (IFN-γ) gene showed significant differences (P<0.01) among the genotypes at 2 and 4 months of age (Figure 1). Specifically, at 2 months of age, it was observed that the expression of IFN-γ gene were significantly upregulated in the crossbred in comparison to the local genotype. Similar trend ensued at 4 months of age, as local genotype expressed a lower mRNA levels compared to the crossbred.

The treatment effect on the expression of interferon gamma (IFN-γ) gene showed significant variations (P<0.01) among the treatment at 2 months of age (Figure 2); whereas at 4 months of age, there was a non-significant (P>0.05) variation among the treatment groups.

At 2 months of age, the mRNA expression of interferon gamma (IFN-γ) gene for the garlic group were upregulated compared to the control group while the mRNA expressions of the ciprofloxacin group were down regulated compared to the control group.

The interaction effect between genotype and treatment on the mRNA expression of interferon gamma (IFN-γ) gene showed no significant variations (P<0.01) among the interaction groups at 2 months of age (Figure 3); whereas at 4 months of age (Figure 4), there were significant (P>0.05). At 4 months of age, the $2^{-\Delta\Delta CT}$ means manifested the highest value of 1.00 in crossbred fed control, which is different from other interaction groups. This highest value was followed by crossbred fed ciprofloxacin (0.72), crossbred fed garlic (0.54) and local fed garlic (0.38). The least significant values among the interactions were recorded in local fed control and local fed ciprofloxacin (0.01 and 0.03).

DISCUSSION

Interferon gamma (IFN-γ) is a type of interferon, a protein that is released by cells in response to a virus or other foreign invader (Chen *et al.*, 2022). It is a critical element of the immune system, and its primary function is to activate macrophages, which are a special type of white blood cell that can ingest and destroy bacteria and other foreign elements. IFN-γ also helps to regulate other immune cells, such as natural killer cells, T cells, and B cells. It also helps to activate the production of cytokines, which are molecules that can affect the body's inflammatory response. In addition, IFN-γ has been shown to have anti-viral and anti-tumor activities and

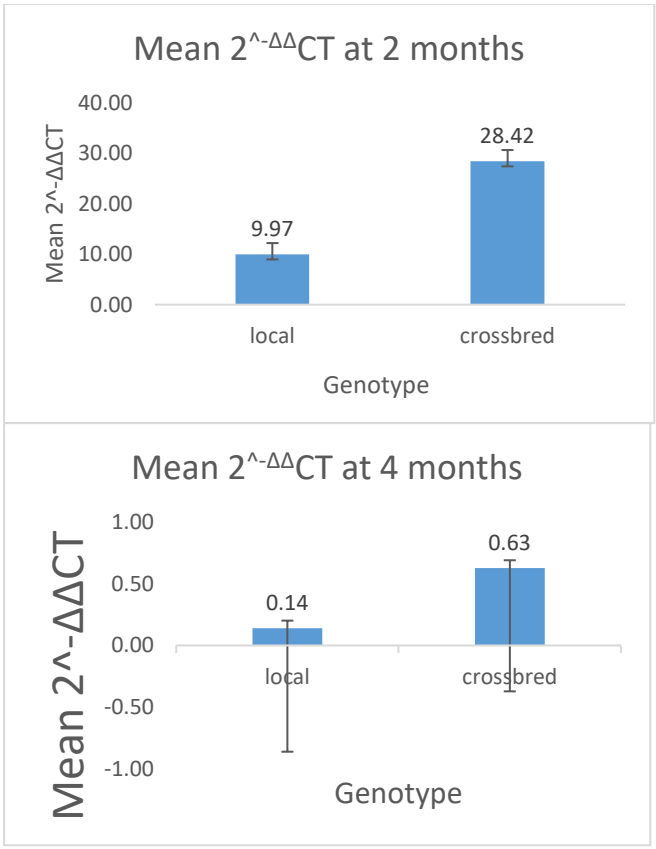

Figure 1: Effect of genotype on expression of IFN-γ at 2 and 4 months of age

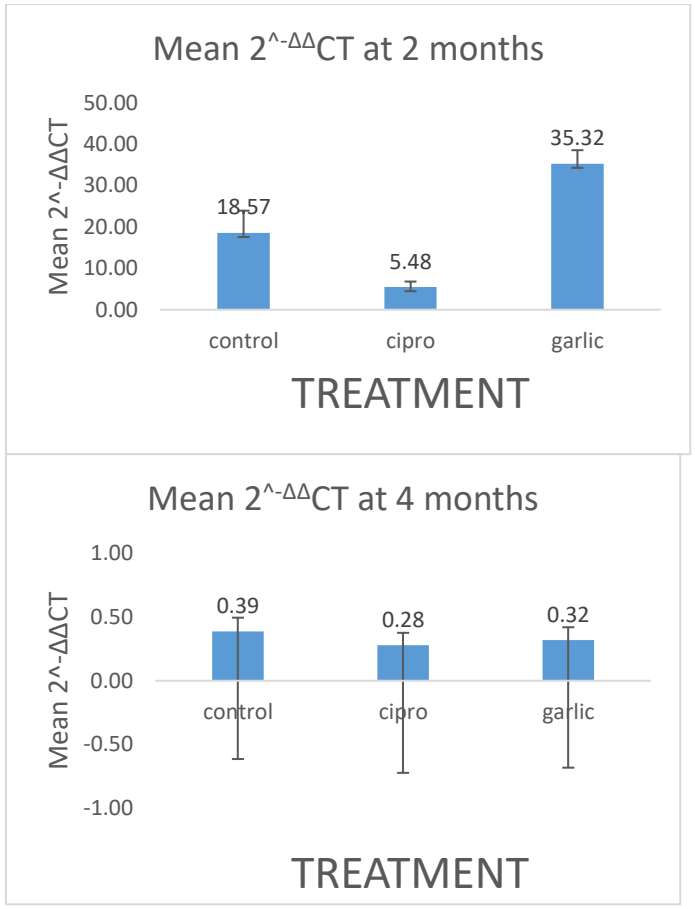

Figure 2: effect of treatment on expression of IFN-γ at 2 and 4 months of age

also helps to regulate the production of other interferons, such as interferon alpha and beta, which are important in the body's fight against viruses (Kak *et al.*, 2018; Castro *et al.*, 2018; Ottum *et al.*, 2015).

The results of this study suggest that the expression of interferon gamma (IFN-γ) gene is affected by genotype. Specifically, the expression of the gene was significantly higher in the crossbred in comparison to the local genotype at both 2 and 4 months of age. This indicates that the genetic make-up of the genotype can influence the expression of this gene, which could have implications for the overall health of the animal (Tallentire *et al.*, 2016; Malgw *et al.*, 2022). The expression of the IFN-γ gene has been linked to the immune response of an animal, and higher expression of the gene has been associated with an improved immune response (Antoni and Dhabhar, 2019; McRae *et al.*, 2015). Therefore, the results of this study suggest that the crossbred may have better immunity due to its higher expression of the gene.

The results of this study also have significant implications for our understanding of the biological effects of garlic and cipro on the expression of interferon gamma (IFN-γ) gene. The upregulation of the gene in the garlic group compared to the control group suggests that garlic has a positive effect on the expression of the gene, which may be beneficial in terms of immunomodulation and immune function regulation (Elmowalid *et al.*, 2019; Mohamed *et al.*, 2016). On the other hand, the down regulation of the gene in the ciprofloxacin group compared to the control group suggests that ciprofloxacin may have a negative effect on the expression of the gene, which could potentially lead to immune system dysregulation (Dolowschiak *et al.*, 2016).

The result on the interaction of genotype and treatment suggests that the mRNA expression of the IFN-γ gene varies significantly depending on the genotype of the animal and the treatment administered. This could be due to genetic differences between the two genotypes (Tallentire *et al.*, 2016; Malgw *et al.*, 2022). The result also suggests that the administration of ciprofloxacin and garlic may have an effect on the expression of the IFN-γ gene at 4 months of age. In particular, the crossbred fed ciprofloxacin and crossbred fed garlic groups had higher expression levels than the local fed ciprofloxacin and local fed garlic groups. This suggests that the crossbred may be more responsive to the treatments than the local genotype.

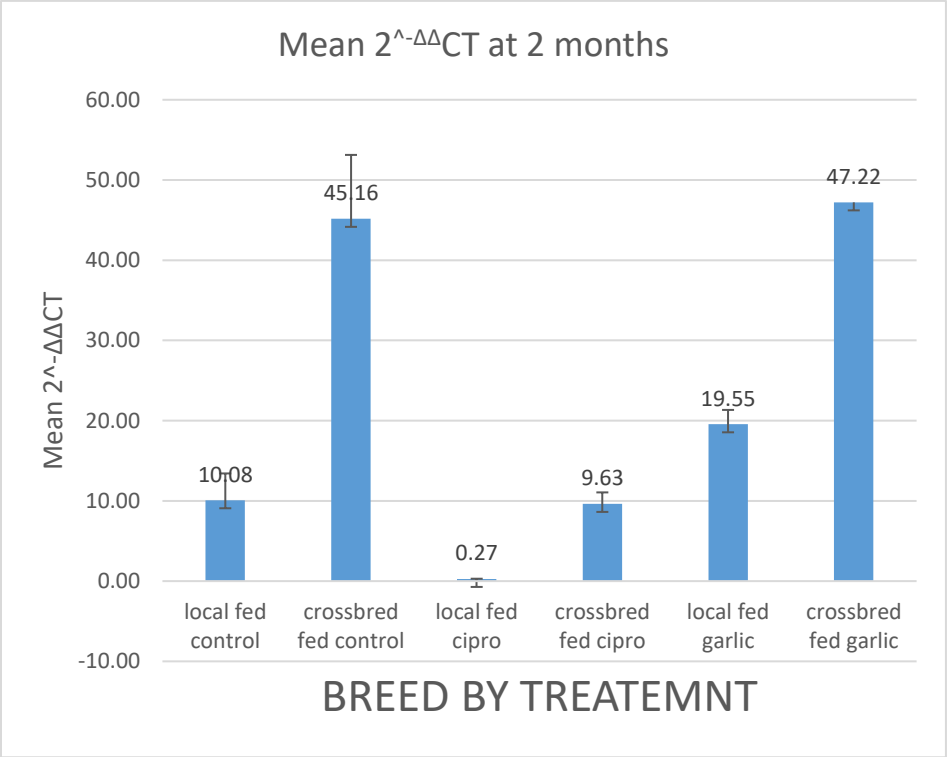

Figure 3: Interaction effect of genotype and treatment on expression of (IFN-γ) at 2 months of age

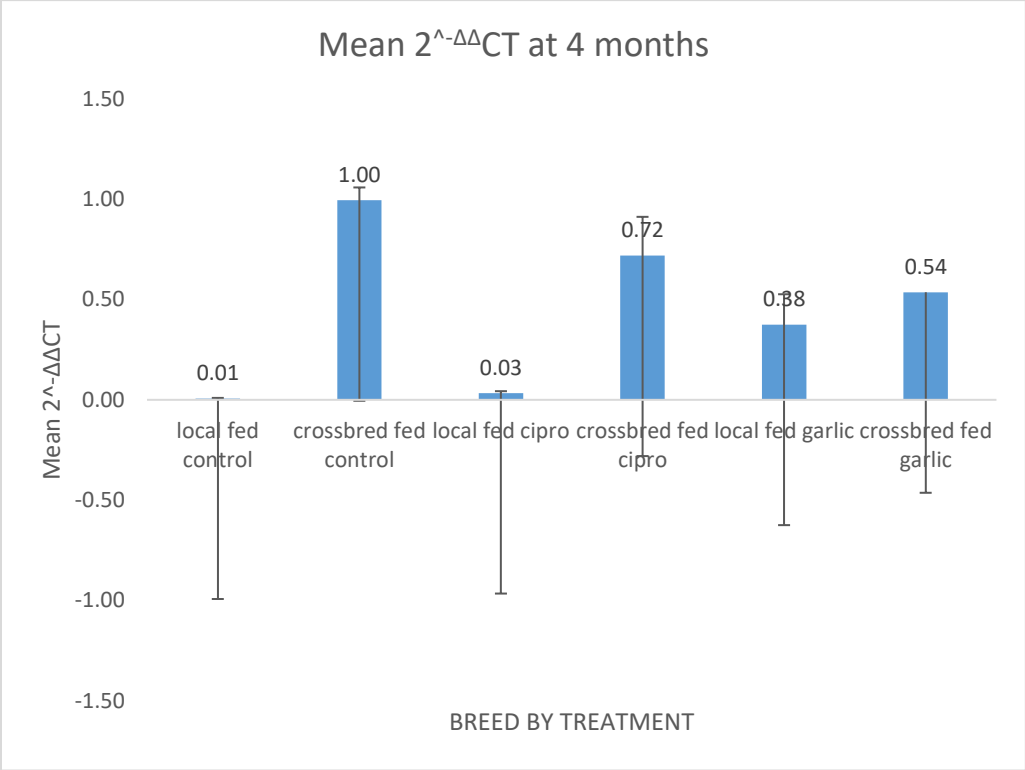

Figure 4: Interaction effect of genotype and treatment on expression of (IFN-γ) at 4 months of age

The results of this study revealed also that the use of garlic extract as alternative to antibiotics was essential for growth development, improvement of haematological indices and expression of interferon gamma (IFN-**γ**) gene in chickens. It was observed that garlic helped in promoting growth features in the local x exotic crossbred genotype used in the study, and did not have adverse effects on the blood parameters of the birds. Garlic, with its antibacterial effects, also improved the feed conversion ratio of the birds especially the crossbred genotype, hence it was a good alternative to antibiotics.

The crossbred genotype at 2 months of age, had an upregulated expression of interferon gamma (IFN-**γ**) gene in the small intestine more than the local counterpart. The implication was that the immunity or succeptibility of birds to diseases can be influenced by genotype due to the level of expression of genes responsible for immunity. Also, hybrid vigour might have contributed to a better acquired immune responses in the crossbred than the local genotype.

It was equally observed from this study that nutrition could have a significant impact on the expresion of interferon gamma (IFN-γ) gene in small intestinal tissues. This is because the garlic extract showed a positive effect on the expression of interferon gamma (IFN-γ) gene at 2 months of age which was an indication of a beneficial inflammatory response in the body. This could be attributed to the potentials of garlic to promote the health of the intestinal tissues.

The results of the study however, suggested that age affected the level of expression of interferon gamma (IFN-γ) gene, as it was not sustained over time. There were no significant variations ($P<0.01$) among the genotypes observed at 4 months of age, which implies that the effects of diet and genotype on the expression of interferon gamma (IFN-γ) gene may be short-termed.

Statements and Declarations:

Statement of Animal Rights: The study was carried out after having consulted the institutional guidelines on the use of animals for scientific research and obtaining ethical clearance.

Conflict of Interest: The authors declare that there was no conflict of interest.

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Author Contributions: Nwenya JM: Principal Researcher. Ndofor-Foleng HM: Project Supervisor. Ikeh NE: Designed the Research. Okenyi NJ: Data Collection and Statistical Analysis. Tchoupou-Tchoupou EC: Data Collection and Statistical Analysis. Nwose RN: Assisted in the Collection of Samples and Laboratory Work.

REFERENCES

[1] Abd El-Hack, M. E., El-Saadony, M. T., Salem, H. M., El-Tahan, A. M., Soliman, M. M., Youssef, G. B. & Swelum, A. A. (2022). Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. *Poultry science*, 101696.

[2] Antoni, M. H., and Dhabhar, F. S. (2019). The impact of psychosocial stress and stress management on immune responses in patients with cancer. *Cancer*, *125*(9), 1417-1431.

[3] Bar, M., Binduga, U. E., & Szychowski, K. A. (2022). Methods of isolation of active substances from garlic (Allium sativum L.) and its impact on the composition and biological properties of garlic extracts. *Antioxidants*, *11*(7), 1345.

[4] Baysal, G., Olcay, H. S., & Günneç, Ç. (2023). Encapsulation and antibacterial studies of goji berry and garlic extract in the biodegradable chitosan. *Journal of Bioactive and Compatible Polymers*, 08839115231157097.

[5] Castro F, Cardoso AP, Gonçalves RM, Serre K and Oliveira MJ. (2018). Interferon-Gamma at the Crossroads of Tumor Immune Surveillance or Evasion. *Front. Immunol*. 9: 847. doi: 10.3389/fimmu.2018.00847

[6] Chen, J. K., Hsiao, C., Yang, P. Y., Periyasamy, T., and Wang, C. Y. (2022). Characterization of agapornis fischeri interferon gamma and its activity against beak and feather disease virus. *Virus Research*, *308*, 198647.

[7] Dolowschiak, T., Mueller, A. A., Pisan, L. J., Feigelman, R., Felmy, B., Sellin, M. E., Namineni, S., Nguyen, B. D., Wotzka, S.Y., Heikenwalder, M., von Mering, C., Mueller, C., and Hardt, W. D. (2016). IFN-γ hinders recovery from mucosal inflammation during antibiotic therapy for

Salmonella gut infection. *Cell host & microbe*, *20*(2), 238-249.

[8] Elmowalid, G. A., Abd El-Hamid, M. I., Abd El-Wahab, A. M., Atta, M., Abd El-Naser, G., and Attia, A. M. (2019). Garlic and ginger extracts modulated broiler chicks innate immune responses and enhanced multidrug resistant Escherichia coli O78 clearance. *Comparative immunology, microbiology and infectious diseases*, *66*, 101334.

[9] Hafez, H. M., & Attia, Y. A. (2020). Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. *Frontiers in veterinary science*, *7*, 516.

[10] Hossain, M. S., Shahiduzzaman, M., Rahim, M. A., Paul, M., Sarkar, R., Chaity, F. S., ... & Islam, S. (2023). Bioactive properties and organosulfur compounds profiling of newly developed garlic varieties of Bangladesh. *Food Chemistry: X*, *17*, 100577.

[11] Kak, G., Raza, M. and Tiwari, B.K. (2018). Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. *BioMol Concepts*; 9: 64–79.

[12] Malgwi, I. H., Halas, V., Grünvald, P., Schiavon, S., and Jócsák, I. (2022). Genes Related to Fat Metabolism in Pigs and Intramuscular Fat Content of Pork: A Focus on Nutrigenetics and Nutrigenomics. *Animals*, *12*(2), 150.

[13] McRae, K. M., Stear, M. J., Good, B., and Keane, O. M. (2015). The host immune response to gastrointestinal nematode infection in sheep. *Parasite immunology*, *37*(12), 605-613.

[14] Mohamed, E. H., Baiomy, A. A. A., Ibrahim, Z. S., & Soliman, M. M. (2016). Modulatory effects of levamisole and garlic oil on the immune response of Wistar rats: Biochemical, immunohistochemical, molecular and immunological study. *Molecular medicine reports*, *14*(3), 2755-2763.

[15] Ottum, P. A., Arellano, G., Reyes, L. I., Iruretagoyena, M., and Naves, R. (2015). Opposing roles of interferon-gamma on cells of the central nervous system in autoimmune neuroinflammation. *Frontiers in immunology*, *6*, 539.

[16] Rafeeq, M., Bilal, R. M., Alagawany, M., Batool, F., Yameen, K., Farag, M. R., ... & El-Shall, N. A. (2022). The use of some herbal plants as effective alternatives to antibiotic growth enhancers in poultry nutrition. *World's Poultry Science Journal*, *78*(4), 1067-1085.

[17] Rusli, R. K., Sadarman, S., Hidayat, C., Sholikin, M. M., Hilmi, M., Yuniza, A., ... & Irawan, A. (2022). A meta-analysis to evaluate the effects of garlic supplementation on performance and blood lipids profile of broiler chickens. *Livestock Science*, *263*, 105022.

[18] Tallentire, C. W., Leinonen, I., and Kyriazakis, I. (2016). Breeding for efficiency in the broiler chicken: a review. *Agronomy for Sustainable Development*, *36*(4), 1-16.

[19] Tchoupou-Tchoupou, E. C., Ndofor-Foleng, H. M., Nwenya, J. M., Okenyi, N. J., Ikeh Nnana, E., Ngwu, N. R., ... & Onyimoniy, E. A. (2022). Effects of hexane extract of garlic on hematological, biochemical and histological parameters in F1 crossbred chicks non-infected and infected with Salmonella typhimurium. *International Journal of Veterinary Science*, *11*(4), 435-442.

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