



Effects of Ginger and Garlic Extract on Performance and Interleukin (IL-12) Gene Expression of Broiler Chickens Experimentally Challenged with *Salmonella typhimurium*

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ABSTRACT

Background: *Salmonella typhimurium* is an avian pathogen that can cause severe intestinal disease, especially in younger chickens and adverse economic losses in the poultry industry. It affects the major organs of the birds; Newly hatched/younger birds are more affected by salmonellosis than older birds due to an underdeveloped organ. As a result of this problem, solutions are sought by researchers to reduce the problems of microorganisms' resistance to antibiotics by replacing them with some phytogetic growth promoters that are more accessible, less expensive, have a low risk of toxicity, low health hazard on the consumers and are environmentally friendly. This experiment was conducted during the late rainy period of September and October 2023; to evaluate the effects of ginger and garlic extracts as a substitute to ciprofloxacin on the growth performance and the immune modulation of interleukin (IL-12) gene expression in broiler chickens, experimentally challenged with *Salmonella typhimurium* infection.

Methods: A total of 240 day-old-chicks were assigned to five treatments in a completely randomized design, with the following dosage levels: T1 (Negative Control, no ciprofloxacin, Ginger and garlic) T2 (positive control - ciprofloxacin without Ginger nor garlic); T3 contained 10 ml of ginger extract; T4 contained 10 ml of garlic extract; T5 contained 10 ml of both ginger and garlic extracts. The birds were orally challenged with 10 ml/liter of water containing *Salmonella typhimurium* infection before commencing the various treatments already enunciated above. Three weeks post-infection, sample organs were harvested and analyzed for IL-12 mRNA expressions.

Result: Results obtained showed that IL-12 was upregulated in the liver in all the treatments when compared to the control treatment, whereas only (T5), showed an upregulated IL-12 mRNA expression in the caecum. The results obtained from the growth parameters showed that ginger and garlic extract had no detrimental effect on the weekly growth performance parameters. Nevertheless, birds fed with a mixture of 10 ml of ginger and garlic extracts performed best in terms of body weight gain followed by the control treatment that contained ciprofloxacin; while T2 performed the least when compared to other treatment groups.

Key words: Bacteria, Cytokines, Garlic, Ginger, Interleukin (IL-12), *Salmonella typhimurium*.

INTRODUCTION

Salmonella is a rod-shaped, gram-negative facultative anaerobe that belongs to the family *Enterobacteriaceae* (Vargas *et al.*, 2022). Eng *et al.* (2015) had the pathogenic capability of all strains of *Salmonella* as they have such ability to invade, replicate and survive in their host cells, resulting in potentially fatal diseases. Salmonellosis is characterized by rapid laborious breathing, shivering, lack of coordination, lethargy, fluffed-up feathers, droopiness, diarrhea and convulsions (Okoro *et al.*, 2016). Salmonellosis can make birds vomit food, water and drool saliva in severe cases. Eng *et al.* (2015) reported that *Salmonella typhimurium* may infect blood vessels and gain access to the circulatory system, causing lesions in a wide range of tissues all throughout the body (sepsis). More than 20 million humans and animals are infected with *Salmonella* each year. It decreases animal productivity and causes 150,000 human and animal fatalities each year all around the World (Vargas *et al.*, 2022).

In conventional management, *Salmonella* is prevented and regulated in feed (or water) through the administration

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of broad-spectrum or synthetic antibiotics such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, fluoroquinolones, cephalosporins, ciprofloxacin, etc (Shu-Kee *et al.*, 2015). However, Igwe *et al.* (2024) noted the promotion of antimicrobial resistance (AMR) in the use

of these antibiotics as growth promoters in animal feed and therapeutics in veterinary medicine. *Salmonella* spp resistance towards these antibiotics is generally referred to as multi-drug resistant (MDR). The transmission of MDR *Salmonella* strains from animals to humans via the ingestion of food or water contaminated with the animals' feces, direct contact or the consumption of infected food animals poses a high risk of zoonotic disease. Consequently, the AMR problem may cause hundreds of millions of human deaths along with severe financial crisis and severe damage in livestock production by 2050 (Eng *et al.*, 2015). Bacteria counteract the actions of antibiotics by four well-known mechanisms namely, enzyme modification, alteration in target binding sites, efflux activity and decreased permeability of bacterial membrane (Bassetti *et al.*, 2013). This expression of resistance towards antibiotics by bacteria could either be intrinsic or acquired. Intrinsic resistance is due to inherent properties within the bacteria chromosome such as mutations in genes and chromosomally inducible enzyme production Davies (2007) whereas acquired resistance could be due to the transmission of resistance genes from the environment and/or horizontal transfer from other bacteria (Jarriyawattanachaiikul and Chokesajjawatee, 2016). Ciprofloxacin belongs to the group of drugs called fluoroquinolones (Emmerson and Jones, 2003). In general, it is effective against Gram-negative organisms and some mycobacteria. The molecular formula of Ciprofloxacin is $C_{17}H_{18}FN_3O_3$ having a molar mass of 331.4g/mol. Cytokines are important protein secreted by cells that plays an essential role in the immune and inflammatory responses of humans and animals against diseases (Charles *et al.*, 2001). Cytokines determine the type of immune responses and the effect or process generated to mediate resistance of the attacking disease. Cytokines such as interleukin and interferon-gamma are important components of the immune system. In addition to connecting to both adaptive and innate aspects of the immune system. These molecules alert the immune system by providing initial warning signs of potential threats.

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product that enables it to produce end products, proteins, or non-coding RNA and ultimately affect a phenotype as the final effect. Interleukin (IL)-12 and IL-10 are immune-regulatory cytokines with an antagonistic effect on the T-helper (Th)1/Th2 cytokine balance and they provide a functional link between innate resistance and the adaptive immune response (Haritova and Stanilova, 2012).

The essence of this study is to determine the effect of ginger and garlic on the ability of broiler chicken to combat attenuated *Salmonella typhimurium* bacteria and to ascertain how Ginger and Garlic can help boost the secretion of cytokines, its effect on the liver and caeca of the broiler chickens and how the genes were expressed towards the treatment given to the experimental birds.

MATERIALS AND METHODS

Experimental site

The research took place at the Teaching and Research farm of the Department of Animal Science, Faculty of Agriculture and Natural Resources, Ebonyi State University, CAS campus, Abakaliki, Nigeria. The research work took place between September and October 2023 during the peak of the late rainy period.

Experimental materials

An isolate of *Salmonella typhimurium* was sourced from National Veterinary Research Institute (NVRI) Vom, Jos, Nigeria which was replicated by re-culturing *Salmonella typhimurium* before administering it to the birds. After one week of acclimatization, the birds were orally challenged with 10ml of salmonella typhimurium per liter of water. Ciprofloxacin was sourced from a pharmacy while fresh rhizomes and bulbs of ginger and garlic respectively were sourced from the local market at the International Market, Abakaliki, Ebonyi state, Nigeria. The rhizomes and bulb were dried, peeled with a laboratory knife and ground with a hammer mill to get the desired texture.

Experimental animals and management

A total of 240 Day-old chicks were purchased from Agrico-International Technology Trade (Agrited). The birds were weighed on arrival to get their initial weight. The birds were offered clean water and fed *ad libitum* throughout the experiment. After one week of acclimatization, birds were orally challenged with *Salmonella typhimurium*. The treatment was administered through the drinking water of the birds at 10 g/1000 ml of treatment/quantity of water for treatments 2, 3 and 4. While the control group which is Ciprofloxacin, followed the manufacturer's prescription of 2.5ml per liter of water. The experiment was made up of five treatments with negative and positive control inclusive. The various treatment groups and their dosage levels include the two control groups, (without ciprofloxacin, ginger or garlic) treatment (2.5 ml Ciprofloxacin/1000 ml water), without Garlic or ginger as the positive and negative control group; the Ginger group or GG (10 g/ liter of water for hexane ginger extract), the Garlic group or GLG (10 g/liter of garlic water for hexane garlic extract) and the Garlic plus Ginger group GLGG (10 g/liter of water for hexane garlic and ginger extract) with three replicates per treatment. All the birds in each treatment were orally challenged with 10 ml/liter water of the salmonella after one week of acclimatization and about three weeks of post-infection; sample tissues (liver and caecum) were collected for IL-12 expression analysis. Each treatment had a total of 48 birds comprising of three replicates each of 16 birds per replicate in a completely randomized design (CRD).

Sample collection

After 3 weeks of treatment, three birds per replicate were slaughtered through cervical dislocation; liver and cecum tissue samples were dissected and submerged in RNA

later solution in 0.5 ml Eperdorff tubes. This was done to store the samples at 20°C and retain the RNA integrity for isolation.

Data collection

Growth performance using parameters such as

a. Body weight gain

Initial weights of the birds were obtained by weighing the chicks on arrival by use of the Digital weighing scale of 100 kg capacity. Their daily weight gains were obtained by:

$$\text{Average daily weight gain} = \frac{\text{Total body weight gain}}{\text{Number of experimental days}}$$

b. Feed efficiency: This was obtained by:

$$\text{Feed efficiency} = \frac{\text{Average daily feed intake}}{\text{Average daily weight gain}}$$

Gene (*Interleukin* (IL-12)) expression Analysis.

The liver and caecum of three chicks per treatment were harvested and analyzed in the laboratory. The gene expression was analyzed to determine if the interleukin IL-12 gene was upregulated or downregulated because of the treatment. The mRNA expression of interleukin (IL-12) of the broiler birds was determined by the quantitative real-time PCR (polymerase chain reaction) run and was carried out on the BIOER96 - well line K series thermal cycler using the Solis Eva-green qPCR (noROX) master mix according to the manufacture's protocol.

Data analysis

Data from growth parameters was subjected to a one-way Analysis of Variance (ANOVA) using a computer-aided statistical software package SPSS 23.0 and Duncan's new multiple range test (Duncan, 1955) was used to separate significantly different means at $P < 0.05$.

RESULTS AND DISCUSSION

From Fig 1, it can be seen that when ciprofloxacin was administered to the liver of infected broiler birds, the IL-12 gene was neither upregulated nor down-regulated; while upregulation of IL-12 gene was observed among other treatment groups of ginger, garlic and a combination of both materials when administered to the birds as seen in Fig 2. It can also be observed that the treatment effects were not significant in all treatment groups at $p < 0.05$ on the liver sample. This implies that the birds were able to resist the bacteria (*Salmonella typhimurium*).

From Fig 3. It can also be observed that there was downregulation of IL-12 expression as observed in the -ddCt as seen in Fig 4 of the Infected + Ginger treatment group and the infected + Garlic treatment group. The infected + ginger + garlic treatment group showed an up-regulation of IL-12 in the caecum of infected birds in Fig 4. The treatment effect of the experiment was not significant at T1, T3 and T4 but was significant at T2 at $p < 0.05$. Results from the present study showed that Ginger and Garlic up-regulated IL-12 in the liver and boosted the immunity of the salmonella-infected birds as stated by (Eckmann and



Fig 1: A bar chart showing the effect of ginger and garlic on IL-12 expression on the liver.

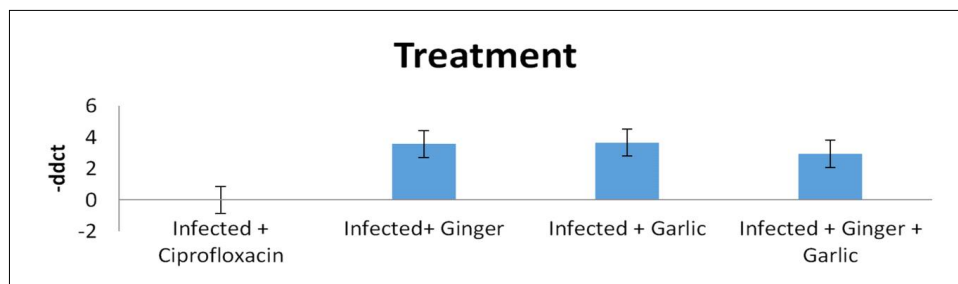


Fig 2: A bar chart showing the effect of ginger and garlic on IL-12 expression on the liver with standard error.

Kagnoff, 2001) while in the caecum of the birds in T4 which had the combination of both ginger and garlic showed a positive expression of IL-12. This could be a function of the antibacterial properties of ginger and garlic. This implies that IL-12 mRNA peak expression was found in the liver followed by the caecum. This agrees with findings from (Dorhoi *et al.*, 2006) who reported that both Interleukin 12 and Interferon gamma promote Th1 immune responses that play a vital role in stimulating protective immunological responses against invading pathogens. The present study is not consistent with (Dorhoi *et al.*, 2006) findings which indicated that Interleukin-12 mRNA peak expression was found in the caecum followed by the liver and spleen at 3

dpi. Results from the present study are correlated with findings from (Haghighi, *et al.*, 2008) that Garlic extracts have been found to suppress pro-inflammatory cytokines like IL-2 and elevate interleukin (IL) -10 and IL-12 in monocytes. Similarly, (Haghighi *et al.*, 2008) results showed an increase in interleukin (IL) 12 in response to *Salmonella typhimurium* that was maintained at control levels by probiotic treatment. The result from the present study agrees with the findings from Szigeti *et al.* (2008) that Interleukin IL-12, IL-18 and Interferon-gamma showed an upregulated mRNA expression in the caecum, liver and spleen compared to the uninfected group of age-matched though the present study doesn't agree with his statistical

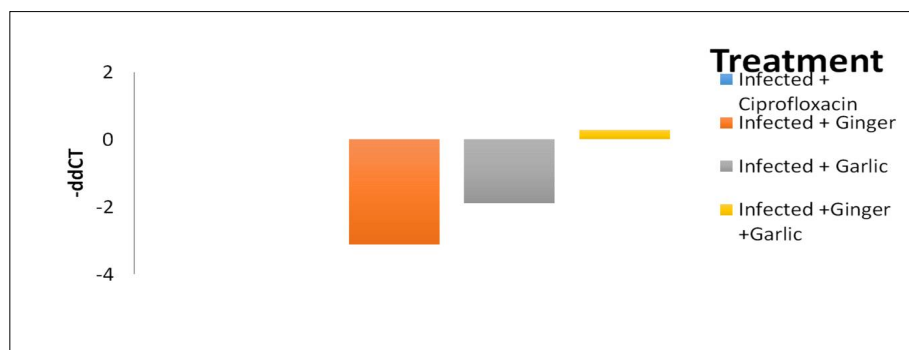


Fig 3: A bar chart showing the effects of ginger and garlic extract on IL-12 expression on the caecum sample.

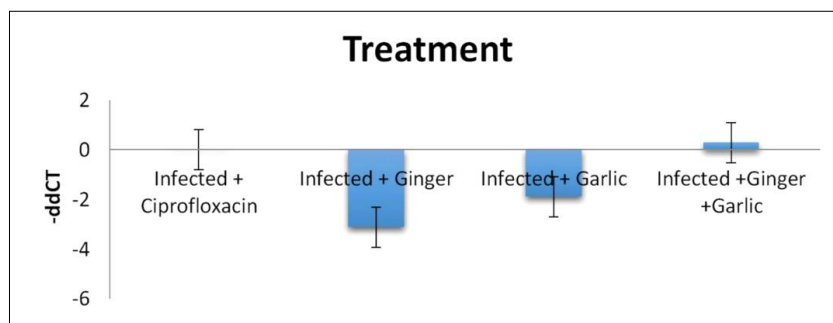


Fig 4: A bar chart showing the effects of ginger and garlic extract on IL-12 expression on caecum sample with standard error.

Table 1: Effects of ginger and garlic on body weight gain.

Weeks (g)	T1	T2	T3	T4	T5	SEM	P-value
1	60.44	52.50	50.25	60.02	70.99	±156.06	0.84 ^{ns}
2	480	479	473	456	480.33	±5.68	0.58 ^{ns}
3	779.40	739.38	700	725.76	718.18	±6.94	0.22 ^{ns}
4	1370.30	1257.3	1250.33	1172.33	1237.33	±32.66	0.50 ^{ns}
5	2393.5	2093	2075	1966	2153	±17.63	0.19 ^{ns}
6	2600.4	2400.5	2760	2533	2526	±78.81	0.42 ^{ns}
7	2596.20 ^b	2696.66 ^a	2733.33 ^a	2266.67 ^a	2696.66 ^a	±78.38	0.04 [*]
8	2876.5	2816.6	2750	2766.67	2833.33	±144.33	0.99 ^{ns}

Note: There is a significant difference at P-value< 0.05.

a,b,c = Means on the same row with the same superscripts are not significantly different (P-value>0.05). SEM Standard Error of means.

Table 2: Feed efficiency at various weeks for broiler birds.

Weeks	T1	T2	T3	T4	T5	SEM
1	3.8 ^a	3.8 ^a	3.62 ^a	3.77 ^a	3.70 ^a	0.19
2	1.06 ^b	1.06 ^b	1.07 ^b	1.14 ^b	1.14 ^b	0.12
3	1.12 ^c	1.12 ^c	1.12 ^c	1.11 ^c	1.09 ^c	0.03
4	1.02 ^a	1.02 ^a	1.00 ^a	1.05 ^a	1.02 ^a	0.02
5	0.62 ^c	0.62 ^c	0.65 ^c	0.67 ^c	0.66 ^c	0.03
6	0.57 ^c	0.57 ^c	0.55 ^c	0.55 ^c	0.58 ^c	0.91
7	0.59 ^a	0.59 ^a	0.61 ^b	0.70 ^b	0.62 ^c	0.02
8	0.58 ^b	0.58 ^b	0.55 ^b	0.57 ^b	0.61 ^b	0.55

a,b,c = Means on the same row with the same superscripts are not significantly.

Different (P-value > 0.05). T1= (control), T2= (Cipro) T3 = (10 ml/liter ginger extract), T4= (10 ml/liter garlic extract), T5= (5 ml ginger extract + 5 ml/liter garlic extract). Note: There is a significant difference at P-value< 0.05.

decision as results obtained has no statistically significant difference compared to Szabo *et al.* (2013) results.

Effects of ginger and garlic extract on growth

Performance parameters

In this study we accessed the following growth parameters, body weight gain and feed conversion ratio.

From the results obtained, combination of ginger and garlic showed higher performance in body weight gain and weight change and gave the best performance in feed efficiency as shown in Table 2. The inclusion of garlic and ginger had a strong effect on the growth performance traits monitored. However, there was no significant difference ($p>0.05$) in weeks 1-6 and 8. The highest performance of the combination of ginger and garlic (916 g) in body weight change Compared to the control group (473.34 g). Table 1 is a result of the inclusion of ginger and garlic on the body weight gain. This may be a result of the good health status caused by the inclusion of ginger and garlic extract which has useful pharmacological substances such as allicin and zingiberene which are fat soluble (Igwe *et al.*, 2024). The properties of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) which are antimicrobial, immune-stimulant, reducer of fat and cholesterol properties (Jarriyawattanachai *et al.*, 2016; Okoro *et al.*, 2016) could also be a major contributory factor. Tekeli *et al.* (2006) also reported that garlic improves the live weight and feed conversion ratio (Table 2) of broiler chicken and increases the intestinal lactic acid bacteria. Similarly, Cosby *et al.*, (2015) stated in their report that adding sun-dried garlic powder and garlic oil does not affect growth performance. However, Ciprofloxacin also performed greatly on the feed efficiency and body weight gain which may be a result of its characteristics as an antibiotic. The lowest performance observed from the ginger group (T2) is consistent with Bandna Chand (2013) research where he stated that ginger had weaker antibacterial and may be used along with garlic as an antibacterial agent. However, he recommended that further studies should be conducted to determine if better antibacterial activity is achieved by combining the two spices.

CONCLUSION

In summary, it can be concluded that both garlic and ginger combination extracts have a strong positive impact on the immune modulation of IL-12 genes and on the growth performance of broiler chicks experimentally challenged with *Salmonella typhimurium*.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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