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Research Article

Aframomum Chrysanthum Seed Meal Modulated Cytokine Gene Expression and Improved Sensory Attributes of Meat without Altering Feed Efficiency in Meat-Type Chickens

Philip O. Akporhuarho[1], Ufuoma Godstime Sorhue[1*], Adimabua Mike Moemeka[2], Onyinye Stella Onwumere-Idolor[3], Jonathan Ujomu[1], Emmanuel Abadah[1], Jennfer Aaron[1]

[1]Department of Animal Science, Faculty of Agriculture, Delta State University, Abraka, PMB 1 Abraka Nigeria; [2]Department of Animal Production, Faculty of Agriculture, Dennis Osadebey University, Asaba, Nigeria; [3]Department of Animal Production, Delta State University of Science and Technology Ozoro.

Abstract | This study was conducted to examine the growth performance, sensory attributes, and expression patterns of the interleukin-10 and interleukin-1β genes in the spleen of chickens subjected to Aframomum chrysanthum seed meal (ACSM) as a replacement for synthetic antibiotic in broiler production. A total of one hundred and forty-four day-old Aboracre strains of broilers were randomly allotted into four dietary treatments. Treatments one (control) had no ACSM in the diet but was given routine antibiotics medication for broilers. Treatments two (T2), three (T3), and four (T4) had ACSM at 1%, 1.5%, and 2% inclusion levels. The experimental diet was fed for 8weeks; Daily feed intake and weekly body weight were collected, panelist were trained for sensory evaluation of meat, while spleen samples were collected after the feeding trial for gene expression analysis. The feed intake data showed no significant changes ($P>0.05$);likewise the final weight, feed conversion ratio and feed efficiency, while weight gain was significantly different ($P<0.05$) with values of 1986.74 ± 13.54 (T2) to 2202.59 ± 53.34 (T1). Sensory attributes varied significantly ($P<0.05$) and improved with increased inclusion of Aframomum chrysanthum seed meal (ACSM). There were also no significant differences ($P>0.05$) in expression patterns of interleukin 10 (1L-10) and interleukin 1β (1L-1β) in the spleen of chickens fed ACSM. However, the expression of IL-10 increased with increased inclusion of the test ingredients while IL-1β decreased with increased inclusion of the test ingredients. The study concludes that ACSM has the phytochemical potentials of regulating inflammation and immune response in broiler chickens without affecting feed efficiency and also improving sensory attributes of meat.

Keywords | Inflammation, Immune response, Photochemical, Meat quality, Medicinal plants

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***Correspondence** | Ufuoma Godstime Sorhue, Department of Animal Science, Faculty of Agriculture, Delta State University, Abraka, PMB 1 Abraka Nigeria; **Email:** gtsorhue@yahoo.com
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INTRODUCTION

Natural growth promoters can be incorporated into broiler chickens diets without any adverse effect on their performance (Onainor *et al*., 2023). The augmentation of endogenous digestive enzyme production, activation of immunological responses, as well as anti-microbial, and antioxidant activities are only a few of the advantageous qualities of bioactive plant elements in animal nutrition (Al-Amin *et al*., 2006).

Table 1: Analyzed composition of test ingredient.

	Moisture %	Crude protein %	Crude fat %	Crude fiber %	Total Ash %	NFE %
Test ingredient	13.52±0.00	6.86±0.06	5.56±0.13	0.89±0.03	2.65±0.01	70.58±0.21

NFE: Nitrogen Free Extract (Carbohydrate CHO).

Aframomum chrysanthum J. M. Lock belongs to the genus Aframomum. Aframomum has over seventy species, including chrysanthum; it is among the Zingiberaceae, which is also known as the ginger family (Doherty *et al.* 2010). In Nigeria, the seed is commonly called Banga spice or Ataiko, and extracts from Aframomum chrysanthum seeds are useful anti-diarrhoea supplements (Nwachoko *et al*., 2016). The presence of phenols in the seeds of Aframomum chrysanthum indicates that this species may play a role as a germicide since phenols are globally used as antiseptics and standards for the juxtaposition of bactericides (Okwu, 2001). Very few works on Aframomum chrysanthum exist, unlike Aframomum melegueta which is a more popular member of the Aframomum class.

The loss of interest in antibiotic usage has aroused interest in medicinal plants that are suitable for improving performance due to their phytochemical constituents that are capable of replacing synthethic antibiotics (Ndukwe *et al.* 2020). Studies have demonstrated how phytochemical-containing plants may boost the levels of certain cytokines in broilers to improve their immune systems (Budai *et al*., 2013; Altincik *et al*., 2014; Sorhue *et al*., 2021).

IL1β and IL10 are signature members of the cytokine family because of their obvious roles as chief pro- and anti-inflammatory cytokines. Inflammation that is caused by cytokines after recruiting other cells against infections is a principal factor that affects the growth performance of chickens (Sorhue *et al*., 2021). *Escherichia coli* and Eimeria tenella-infected birds had reduced IL10 and increased IL1β at the zenith of infection (Yu *et al*., 2021). In like manner, the body weight of birds infected with Eimeria maxima was reportedly reduced at peak periods of infection (Schneiders *et al*., 2019). This implies that reduced IL10 gene expression in chickens is caused by an insufficient amount of the required phytochemicals or treatments to fight infections.

Phytochemical compounds present in medicinal plants have also been reported to reduce tenderness, increase flavor, and juiciness of meat (Odoemelam *et al*., 2013; Edeoga *et al*., 2006). Hence, plant materials that could improve the organoleptic qualities of meat, improve body weight, and bring the bird's health status to equilibrium without causing retained chemical residues occasioned by antibiotic usage are a veritable tool for the poultry industry. This study was therefore designed to test the effectiveness of ACSM on feed efficiency, sensory attributes, and expression patterns of two signature members of the cytokine family in meat-type chickens.

MATERIALS AND METHODS

Source and processing of test ingredient (Aframomumchrysanthum seed meal)

The Aframomum chrysanthum used for the experiment was procured from commercial food spices market in Oghara, Delta State, Nigeria. The Aframomum chrysanthum seeds were blended into powder to form Aframomum chrysanthum seeds meal (ACSM).

Experimental diet

Four experimental diets were formulated to contain different levels of ACSM at 0% (control), 1%, 1.5%, and 2%, respectively. The birds were fed with formulated broiler starter mash mixed with different dietary levels of ACSM from 0 to 4 weeks and a finisher diet from 5 to 8 weeks. Tables 1 and 2, shows the proximate composition of the test ingredient and the experimental diet.

Table 2: Analyzed composition of experimental diet.

Starter diet%	T1	T2	T3	T4
Moisture	11.31±0.24	12.08±0.13	14.12±0.02	12.58±0.12
Crude protein	16.47±0.7	17.14±0.19	17.88±0.03	18.81±0.05
Crude fat	1.68±0.01	1.28±0.02	1.10±0.01	0.93±0.03
Crude fibre	6.85±0.01	8.58±0.03	8.83±0.03	9.51±0.03
Total ash	5.05±0.06	5.36±0.03	5.73±0.03	6.77±0.04
NFE	58.64±0.12	55.57±0.06	52.36±0.01	51.86±0.62
Finisher diet%				
Moisture	11.37±0.07	12.89±0.29	10.60±0.24	13.03±0.08
Crude protein	17.15±0.06	17.66±0.06	17.25±0.08	19.13±0.02
Crude fat	4.39±0.03	4.20±0.02	4.18±0.02	4.16±0.02
Crude fibre	11.51±0.02	11.65±0.03	10.85±0.03	11.10±0.11
Total ash	6.81±0.02	7.01±0.03	7.91±0.04	8.78±0.04
NFE	48.78±0.14	46.60±0.50	49.24±0.10	43.82±0.07

NFE: Nitrogen Free Extract (Carbohydrate CHO).

Experimental birds and their management

One hundred and forty-four (144) day-old broiler chicks were sourced for this research. The birds were housed in pens using the deep litter system. Before the arrival of the birds, the poultry house was thoroughly washed, disinfected, and air-dried for a week. The surroundings of the poultry house were cleared of grasses and disposed of harmful substances. A Foot dip containing disinfectant solution was placed at the poultry house. Feeders and drinkers were washed thoroughly and disinfected. The heating of the brooding house was done using electric bulbs, a charcoal stove, and kerosene lanterns at different times during the brooding phase. During this phase, the floor of the brooding house was covered with newspapers and then, later, changed to wood shavings. The Poultry house was kept dry during the experiment by replacing wet litters with dried ones. On arrival, birds were given antibiotics (keproceryl) for control birds only and anti-stress (glucose) for all treatments. Feed and water were supplied ad libitum throughout the experiment. Standard management and health practices were followed, and all the necessary vaccinations were timely administered.

Experimental design

One hundred and forty-four birds were randomly allotted to four dietary treatments of 12 birds per treatment group, with 3 replicates, making a total of 36 birds per treatment. Treatment 1 (0% ACSM) served as the control, followed by Treatment 2 (1% ACSM), Treatment 3 (1.5% ACSM), and Treatment 4 (2% ACSM).

Organoleptic quality assessment

At the end the of eight-week feeding trial, six birds were randomly selected from each treatment (i.e., two from each replicate), fasted for 16 hours, and slaughtered by severing the jugular vein and letting it bleed for 5 minutes. The birds were later scalded at 65 °C in steaming water for 30 seconds before de-feathering. Thereafter, the carcasses were eviscerated, and the thighs were collected for organoleptic quality assessments. They were deboned, and the muscles were carefully cut into 10 pieces of about 2 g each, with absolute care taken to remove the tendons. Each piece of the sample was dipped into a super saturated (50%) brine solution for about 5 seconds and put into a double-layered polythene bag with a label on each for identification. The samples were subjected to boiling at about 100 °C for 20 minutes. They were thereafter assigned to 10 panelists for tasting. A nine-point hedonic questionnaire was constructed to solicit responses regarding the tenderness, flavour, texture, and general acceptability of the meat. Prior to the commencement of the assessment, a short training was organized for the panelists concerning the tenderness, flavor, and texture of meat.

Each panelist was given 10 samples to taste prior to finishing the pertinent questionnaire. Clean water at 20 °C was available for rinsing of the mouth in between samples throughout the tasting sessions. The survey comprised a 9-point hedonic scale, and the panelists responded to how they liked or disliked the sample. Table 3 show the scale used to categorize the panelist responses.

The samples were rated using the 9-point hedonic scale, with 9 as the maximum score and 1 as the least score (AMSA, 1995).

Data collection

Data were collected on daily feed intake (DFI) and weekly body weight for eight weeks and were used to calculate weight gain (WG), feed conversion ratio (FCR), and feed efficiency (FE) using standard methods.

Sample collection, RNA concentration, reverse transcription and real-time qPCR

Four birds from each of the treatment groups were slaughtered at the end of the feeding trial (8th week) to examine the mRNA expression patterns of genes regulating immune responses (IL-10 and IL-1β). Spleen samples were collected from experimental birds and stored in eppendorf tubes with the aid of RNALater solution in a freezer prior to RNA extraction. RNA was extracted according to the protocol contained in the 100-preps Geneaid™ Tissue Total RNA Mini Kit with DNase. The concentration and purity of the isolated RNA were assessed by a spectrophotometer (Nanodrop), followed by reverse transcription. Reverse transcription and real-time PCR were done according to Sorhue *et al.* (2022). RNA extraction through to Real-time PCR was done at the African Biosciences molecular Laboratory in Ibadan, Nigeria. Table 4 shows details of the primers used for this study.

Table 3: Nine-point hedonic scale for panelist responses.

Scale	1	2	3	4	5	6	7	8	9
Response	Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Table 4: Primer sequence for real time PCR.

S/N	Genes	F/R	Primer sequence for chicken	NCBI accession number	% GC
1	IL1B	F	5'GTCAACATCGCCACCTACAA3'	NM_204524.1	50
		R	5'CGGTACATACGAGATGGAAACC3'		50
2	IL10	F	5'AGCTGAGGGTGAAGTTTGAG3'	NM_001004414.2	48
		R	5'AACTCATCCAGCAGTTCAGAG3'		50
3	GAPDH	F	5'CCTCTCTGGCAAAGTCCAAG3'	NM_204305.1	45
		R	5'CATCTGCCCATTTGATGTTG3'		55

IL1B: Interleukin1beta; IL10: Interleukin 10; F: Forward, R: Reverse; GC: Guanine-Cytosine content (Sorhue *et al.*, 2021).

Table 5: Performance indices of broiler chickens fed ACSM.

		T2	T3	T4
Initial BWT	12.41±0.73	12.26±0.23	12.00±0.11	12.16±0.35
Total feed intake	1355.74±5.78	1353.70± 5.78	1369.26±11.55	1349.95± 23.09
Final body weight	2215.00±41.24	1999.00±70.14	2199.00±107.4	2115.00±58.14
Total weight gain	2202.59±53.34^{ac}	1986.74±13.54^{b}	2187±50.23^{ac}	2102.84±11.06^{a}
FCR	0.62±0.01	0.68±0.01	0.63±0.02	0.64±0.02
Feed efficiency	1.62±0.12	1.47±0.06	1.60±0.12	1.56±0.13

FCR: Feed Conversion ratio, Means carrying different superscript are significantly different (P < 0.05).

Table 6: Organoleptic qualities of broilers fed different levels of ACSM.

	T1	T2	T3	T4
Flavor	5.50±0.56^{a}	6.67±0.422^{b}	7.17±0.543^{c}	7.00±0.577^{c}
Juiciness	4.83±0.60^{a}	5.17±0.307^{b}	6.83±0.833^{c}	6.33±0.615^{d}
Tenderness	5.67±0.333^{a}	5.83±0.601^{a}	6.50±0.428^{b}	6.17±0. 477^{c}
General acceptability	8.00±0.365^{a}	7.83±0.307^{a}	8.33±0.211^{b}	8.50±0.500^{b}

Means carrying different superscript are significantly different (P<0.05).

Statistical analysis

The data collected were analyzed using one-way analysis of variance (ANOVA) in a completely randomized design with the aid of Prism 7.0 software (Graphpad Software Inc., San Diego, California), and significantly different means were compared using Turkey's multiple comparisons test.

RESULTS AND DISCUSSION

Performance indices of broiler chickens fed ACSM

The performance indices of broiler chickens after 8 weeks of experimentation are presented in Table 5. The initial body weight of birds across treatments was statistically not significant (P>0.05). Significant differences (P < 0.05) among treatments were only recorded for total weight gain, with T1 gaining more weight while T2 gaining the least weight; this trend accompanied FCR and feed conversion ratio, but the later were not significantly different (P > 0.05). The non-significant differences (P > 0.05) reported in this study are not knew, as phytochemical-containing plant parts have been reported to improve performance in birds (Ndukwe *et al*., 2020); though the antibiotic-based treatments performed better in terms of weight gain, FCR, and FE, the values were not significant except for weight gain. The report from our study runs contrary to records from Onainor *et al*. (2022) using scent leaf meal which reported that birds fed 1.5% scent leaf meal, performed better than control birds; these authors also reported significant differences (P < 0.05) in final body weight, feed intake, and FCR. However, the significant differences (P < 0.05) in total weight gain in this study align with Onainor *et al*. (2023). Medicinal plants were also shown to have no significant effect on carcass characteristics as a replacement for antibiotics at inclusion levels of 0.5% to 2% in broiler chicken diets (Onainor *et al*., 2023). The results from this study infer that ACSM contains the requisite phytochemicals necessary to improve gut health and enhance feed utilization because plant materials have properties that could potentially improve feed utilization and the performance of birds (Ertas *et al*., 2005). Aframomum melegueta seed meal obtained from species of the Aframomum genus was reported to have no significant effect on feed consumption, growth, meat sensory attributes, or FCR in domestic rabbits (Olatunji *et al*., 2018).

Organoleptic qualities of broilers fed different levels of ACSM

The result of organoleptic qualities of broilers fed different levels of *Aframomumchrysanthum* seed meal is presented in Table 6. The result shows that treatment varied significantly (P<0.05) in all sensory attributes evaluated. The best performance for flavor, juiciness and tenderness was recorded for treatment three (1.5%) ACSM, while control diet with 0% ACSM had the least. This is an indication that the test ingredient improved the sensory attributes of broiler meat at all levels of inclusion. These reports are in line with reports from Odoemelam *et al*. (2013) after feeding scent leaf-based-diets to broiler chickens. This study aligns with Adomeh and Eguaoje's (2019) finding that the garlic and ginger mixture improved organoleptic qualities, with the best performance recorded for 1.5% inclusion levels. The increased tenderness with an increase in the test ingredient is an indication that the phytochemicals present are sufficient to the extent of releasing juicy contents from the meat of birds, leading to low pH and water loss. The improvement in flavor of treatments receiving ACSM is an indication that the test ingredient had a positive effect on this parameter as perceived by the panelists. Edeoga *et al.* (2006) reported that ingredients with sufficient alkaloids and flavonoids are expected to improve meat flour, which confirms the presence of these vital compounds in the test ingredients. The results showed an increase in meat juiciness with increase in ACSM levels. The least value of (5.50 ± 0.563) quality of juiciness was recorded in broilers without ACSM, while the highest (7.17 ± 0.543) quality of juiciness was recorded in broilers treated with 1.0% ACSM. However, this study contradicts the report by Nisar *et al.*, (2020) and Mehdipour *et al.* (2013) that the inclusion of phytochemicals had no significant effect (P > 0.05) on broiler and quail meat quality at the varying levels.

Relative expression of interleukin 10 and interleukin 1β genes in spleen of broiler chickens

The result of interleukin 10 gene expression in the spleen of broilers fed Aframomum chrysanthum seed meal is presented in Figure 1. The result showed that the test ingredient affected the expression of the target gene. IL10 gene expression was higher in birds fed 2% ACSM; however, the control group was higher than birds fed 1% and 1.5% ACSM, respectively. The fold change indicates a ratio of 1.82, 1.39, and 0.81 folds for T2, T3 and T4 as compared to the control group. There were no significant differences (P > 0.05) in the expression pattern of the gene. The result of interleukin-1β (IL1β) gene expression in the spleen of broilers fed ACSM is presented in Figure 2. The result showed that the test ingredient affected the expression of the target gene. IL1β gene expression was higher in birds fed 1% ACSM; however, the control group was higher compared to birds fed 1.5% and 2% ACSM, respectively. The fold change for IL1β indicates a ratio of 1.16, 1.20, and 4.0 folds for T2, T3, and T4 as compared to the control group. There were also no significant differences (P > 0.05) in the expression pattern of the gene.

There were no significant differences (P > 0.05) in the expression patterns of the genes studied. Expression of cytokine genes in the spleen of broiler chickens using real-time polymerase chain reaction (RT-PCR) has been reported over twenty years ago (Fujiki *et al.*, 2000). The results of the present study show evidence of immune gene modulation by the test ingredients, as varying levels impacted gene expression of IL-10 and IL-1β. Increased inclusion of the test ingredient reduced the expression of IL-1β, while simultaneously increasing IL-10 expression. Sun *et al.* (2019) reported that a high IL10 level inhibits the production of IL1β. This is an indication that the test ingredients contain sufficient phytogenic substances capable of sustaining immune functions in broilers, which is in line with a study that found *Escherichia coli* serotypes-challenged birds had reduced IL10 as IL1β increased (Elnagar *et al.*, 2021). The report from this study is similar to earlier studies reported by Sorhue *et al.* (2021) using Ocimum gratissimum leaf meal in two chicken strains. According to FAO (2014), enzymes and antibiotics are mostly used as feed additives for the purpose of reducing microbial load in the gut, leading to an abundance of nutrients for birds. Brisbin *et al.* (2008) further stated that this systematic use of antibiotics and enzymes helps to reduce inflammation by reducing the effects of IL-1β and increase the expression of IL-10. This statement implies that the test ingredient used in this study followed the systemic action of antibiotics in the spleen of the experimental birds, as evident in the relative expression patterns of IL-10 and IL-1β.

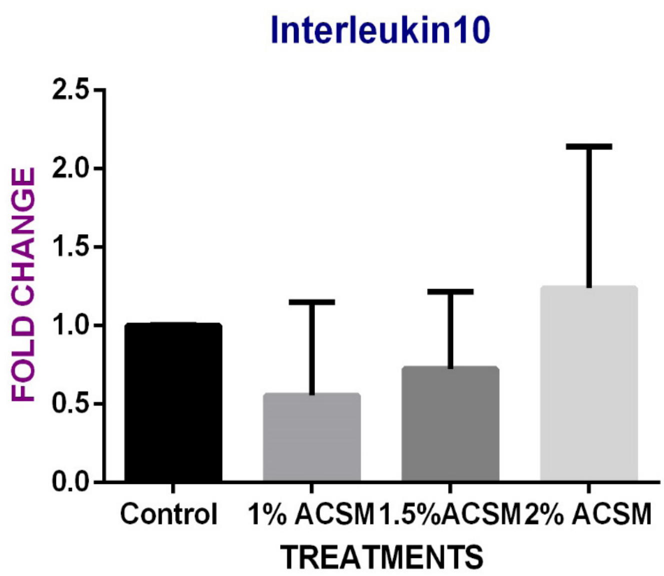

Figure 1: Relative expression of interleukin 10 gene in spleen of broiler chicken fed varying levels of Aframomum Chrysathum seed meal.

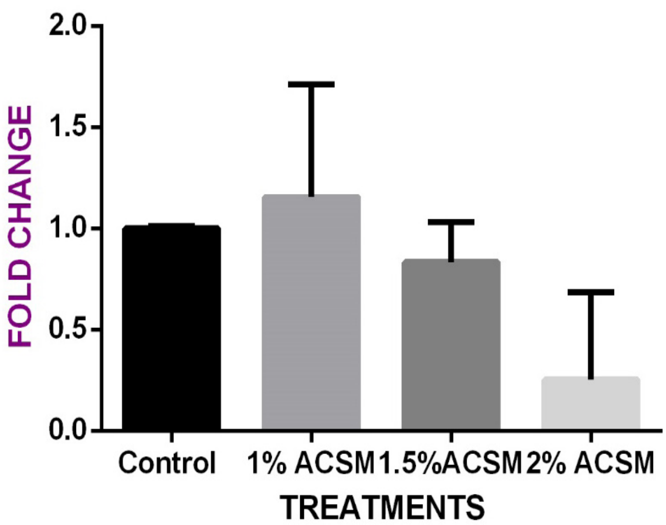

Figure 2: Relative expression of interleukin-1b gene in spleen of broiler chicken fed varying levels of Aframomum Chrysathum seed meal.

However, while antibiotic usage may reduce IL-1β expression and prevent inflammation, which could eventually reduce body weight loss and stunted growth during infections in broiler chickens, its usage has led to a high incidence of necrotic enteritis and dysbacteriosis following the development of resistance from its prolonged usage (Huyghebaert *et al*., 2011). Although antibiotics help to reduce cases of pathogenic bacteria (Ravindrer, 2016), treatment of infections, prevention, promoting growth, and improving production are the main causes of antibiotic usage in broiler production (Castanin, 2007; Mattew *et al*., 2009). Following reports by the last four quoted authors, the result of the expression of pro- and anti-inflammatory cytokines IL-10 and IL-1β infers that the test ingredient could also assume the functions of antibiotics, having reduced pro-inflammatory cytokines expression while simultaneously increasing anti-inflammatory cytokine in chicken spleen. It is known that bacteria use enzyme modification, efflux activity, and decreased permeability of their membranes to counter the actions of antibiotics (Basselti *et al*., 2013). Natural sources such as ACSM contain phytogenic substances such as phenol, flavonoid, and tannins that are efficient without creating room for bacteria to develop resistance to their usage. If natural sources such as ACSM are not effectively used to replace antibiotics, the public health challenge posed by antibiotic use will increase. The findings from this study support the suggestion that antibiotic alternatives ought to have antibodies and gut microbiome-modulating effects (Teirlynck *et al*., 2009). Nofrarias *et al*. (2006) have earlier reported that phytogenic feed additives control inflammatory processes in the intestinal mucosa, which aligns with the results of this study carried out in the spleen. Since cytokines (IL-10 and IL-1β), are said to coordinate, propagate, and inhibit immune responses, by virtue of the evolution of the immune system (Fujiki *et al*., 2000), plant materials containing phytochemicals capable of modulating immune gene expression can be established as a veritable feed additive in broiler production. Kogut-Rothwell *et al*. (2005) reported that chicken IL-1β expression is increased in response to bacterial, viral, and parasitic challenges. Yu *et al*. (2021) also reported that IL10 gene expression was downregulated as Eimeria tenella infection approached its peak period (6-8 days post-infection); this incidence could be averted by introducing anticoccidial, antibiotic, medicinal plant, or phytobiotic treatments. This implies that medicinal plant parts such as ACSM are expected to reduce expression of IL1β gene since they possess anti-bacterial, anti-viral, and anti-parasitic properties. Earlier reports also showed that intraepithelial lymphocytes removed from the jejunum of Eimeria maxima-infected chickens contained high levels of IL-1β mRNA and less of IL-10 (Hong *et al*., 2006). Since feed conversion ratio and body weight gain are affected by inflammation that may arise from the expression of IL-1β, the inclusion of ACSM could be an alternative to antibiotics (Dinarello, 2011). Excessive inflammation and immune response are directly correlated with poor growth rates in birds, and the non–significant differences obtained in this study prove that there were no detrimental effects of feeding ACSM as a replacement for antibiotics, and this was also the case for feed conversion ratio and feed efficiency.

CONCLUSIONS AND RECOMMENDATIONS

This study concludes that ACSM is a good feed additive for improving the sensory attributes of meat without having a detrimental effect on growth performance or immune responses in chickens. However, inclusion at 1.5% level is recommended for optimum performance.

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NOVELTY STATEMENT

This paper's novelty rest on the fact that most studies conducted using medicinal plants in broiler chicken are centred on growth performance, blood indices or organoleptice qualities alone, without considering its effect on immune functions. On the other, studies that have based their objectives on immune responses haven't linked the results with performance traits. This study has established a linkage with all the aforementioned characteristics using Aframomum crysanthum seed meal.

AUTHOR'S CONTRIBUTION

Conception-UGS
Execution-UGS, AMM, EA, OSO, AJ, UJ
Data curation-UGS, POA, OSO
Analysis- UGS, OSO
Writing original draft-EA, AJ, UJ
Editing and revising-UGS, POA
Supervision-POA, UGS

Experimental location and ethical approval

The experiment was conducted at the poultry research farm of Delta State University Abraka. The experiment was approved by the Departmental Board of study, with approval reference number PGD – 18012023.

REFERENCES

Adomeh EE, Eguaoje AS (2019). Performance and organoleptic qualities of broiler chickens fed and raised with varying levels of ginger and garlic mixture. Nigerian J. Anim. Sci., 21(2): 310-318.

Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M (2006). Anti-diabetic and hypolipidaemic properties of ginger (Zingiberofficinale) in streptozotocin-induced diabetic rats. Braz. J. Nutr., 96: 660-666. https://doi.org/10.1079/BJN20061849

Altincik A, S€onme F, Yenisey C, Duman S, Can A, Akev N, Kirdar S, Sezak M (2014). Effects of Aloe vera leaf gel extract on rat peritonitis model. Indian J. Pharmacol., 46: 322–327. https://doi.org/10.4103/0253-7613.132184

American Meat Science Association (1995). Research guidelines for cookery, sensory evaluation and instrumental measurements for fresh meat. Natl. Livest. Meat Board, Chicago, IL, USA

Bassetti M, Merelli M, Temperoni C, Astilean A (2013). New antibiotics for bad bugs: Where are we? Ann. Clin. Microbiol. Antimicrob., 12: 22. https://doi.org/10.1186/1476-0711-12-22

Brisbin JT, Gong J, Sharif S (2008). Interactions between commensal bacteria and the gut-associated immune system of the chicken. Anim. Health Res. Rev., 9: 101–110. https://doi.org/10.1017/S146625230800145X

Budai MM, Varga A, Milesz ST, Ozser J, Benko S (2013). Aloe veradownregulates LPS-induced inflammatory cytokine production and expression of NLRP3 inflammasome in human macrophages. Mol. Immunol., 56: 471–479. https://doi.org/10.1016/j.molimm.2013.05.005

Castanon JIR (2007). History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci., 86: 2466-2471. https://doi.org/10.3382/ps.2007-00249

Dinarello CA (2011). Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood, 117(14): 3720-3732. https://doi.org/10.1182/blood-2010-07-273417

Doherty VF, Olaniran OO, Kanife UC (2010). Antimicrobial activities of Aframomummelegueta (alligator pepper). Int. J. Biol., 2(2): 126-131. https://doi.org/10.5539/ijb.v2n2p126

Edeoga HO, Omosun GIC, Uche IC (2006). Chemical composition of *HyptisSuavaolens* and *Ocimumgratissimum* hybrids from Nigeria. Afr. J. Biotechnol., 5: 892-895.

Elnagar R, Elkenany R, Younis G (2021). Interleukin gene expression in broiler chickens infected by different *Escherichia coli* serotypes. Vet. World, 14(10): 2727-2734. https://doi.org/10.14202/vetworld.2021.2727-2734

Ertas ON, Guler T, Ciftci M, Dalkilic B, Simsek G (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. Int. J. Poult. Sci., 4(11): 879-884. https://doi.org/10.3923/ijps.2005.879.884

Food and Agriculture Organization (2014). Feed supplements and additives Poultry feed availability and nutrition in developing countries. Website https://www.fao.org/3/al704e/al704e.pdf accessed online (20th August 2023).

Fujiki K, Shin D, Nakao M, Yano T (2000). Molecular cloning and expression analysis of carp (Cyprinus carpio) interleukin-1_, high affinity immunoglobulin E Fc receptor _ subunit and serum amyloid A. Fish Shellfish. Immunology., 10: 229–42. https://doi.org/10.1006/fsim.1999.0253

Hong YH, Lillehoj HS, Lee SH, Dalloul RA, Lillehoj EP (2006). Analysis of chicken cytokine and chemokine gene expression following Eimeria acervulina and Eimeria tenella infections.. Vet. Immunol. and Immunopathol., 114 (3-4): 209-223. https://doi.org/10.1016/j.vetimm.2006.07.007

Huyghebaert G, Ducatelle R, Van-Immerseel F (2011). An update on alternatives to antimicrobial growth promoters for broilers. Vet. J., 187:182–188.

Kogut MH, Rothwell L, Kaiser P (2005). IFN-gamma priming of chicken heterophils upregulates the expression of proinflammatory and Th1 cytokine mRNA following receptor-mediated phagocytosis of Salmonella entericaserovarenteritidis. J. Interf. Cyt. Res., 25(2): 73-81. https://doi.org/10.1089/jir.2005.25.73

Lovkova MY, Buzuk GN, Sokolova SM, Kliment'eva NI (2001). Chemical features of medicinal plants (Review). Appl. Biochem. Microbiol., 37: 229–237. https://doi.org/10.1023/A:1010254131166

Mathew AG, Liamthong S, Lin J (2009). Evidence of intestinal transfer between *Escherichia coli* and *Salmonella typhi*. Food Biol., 6(8): 959-964. https://doi.org/10.1089/fpd.2009.0263

Mehdipour P, Afsharmanesh M, Sami M (2013). Effects of dietary synbiotic and cinnamon (*Cinnamomumverum*) supplementation on growth performance and meat quality in Japanese quail. Livest. Sci., 154(1-3): 152-15. https://doi.org/10.1016/j.livsci.2013.03.014

Ndukwe GI, Tetam JG, Clark PD (2020). Phytochemical screening and *in vitro* antioxidant assessment of *Cassia alata* (Linn) leaf extracts. Chem. Search J., 11(2): 64–72.

Nisar HI, Sharif MI, Rahman MAI, Rehman SI, Kamboh AAII Saeed M. (2020). Effects of dietary supplementations of Synbiotics on growth performance, carcass characteristics and nutrient digestibility of broiler chicken. Braz. J. Poult. Sci., 23(2): 1-9. https://doi.org/10.1590/1806-9061-2020-1388

Nofrarias M, Manzanilla EG, Pujols J, Gibert X, Majo N, Gasa J. (2006). Effects of spray-dried porcine plasma and plant extracts on intestinal morphology and on leukocyte cell subsets on weaned pigs. J. Anim. Sci., 84: 2735–2742. https://doi.org/10.2527/jas.2005-414

Nwachoko N, Essien EB, Ayalogu EO (2016). Antidiarrhoea activities of aqueous extract of Aframomumchrysanthum seed in albino rats. Int. J. Orig. Resour., 2(3): 172-177.

Odoemelam VU, Nwaogu KO, Ukachukwu SN, Esonu BO, Okoli IC, Etuk EB, Etuk IF, Aladi NO, Ogbuewu IP. (2013). Growth response, carcass quality and organoleptic assessment of broiler chicken fed *Ocimumgratissimum* L. supplemented diets. Int. J. Agric. Rural Dev., 16(2): 1521-1528.

Okwu DE (2001). Improving nutritive value of cassava tapioca meal with local spices. J. Nutr. Funct. Med. Foods, 3: 43–51. https://doi.org/10.1300/J133v03n04_05

Olatunji OI, Olaniyan OS, Olayiwola OB, Olatunbosun OS, Odunsi AA (2018). Evaluation of growth performance, carcass quality and organoleptic indices of weaner rabbits fed two dietary protein levels with or without alligator pepper (*Aframomum melegueta*). Niger. J. Anim. Sci., 20(3): 304-312.

Onainor ER, Sorhue, UG, Moemeka AM, Omeje IS, Bratte L, Efienokwu J (2022). Harnessing phytobiotic qualities of scent leaf (ocimum gratissimum) meal as substitute for in-feed antibiotics in broiler production: implication on economic traits and immune responses. International Congress on Domestic Animal Breeding, Genetics and Husbandry - (ICABGEH-22) Samsun, Turkey, October

03 - 05: 134

Onainor ER, Sorhue UG, Bratte L, Omeje IS, Moemeka AM, Uguru J. (2023). Blood and carcass characteristics of two chicken strains subjected to *Ocimum graticimum* leaf based diet as substitute for synthetic antibiotics. Anim. Rev., 10(1): 12–20. https://doi.org/10.18488/92.v10i1.3401

Ravindran M (2016). Poultry feed availability and nutrition in developing countries: Feed supplements and additives. Available at http://www.fao.org/3/a-al704e.pdf (accessed 20th August 2023).

Schneiders GH, Foutz JC, Milfort MC, Ghareeb AFA, Sorhue UG, Richter JN, Fuller AL, Williams SM, Rekaya R, Aggrey SE (2019). Ontogeny of intestinal permeability in chickens infected with eimeria maxima: implications for intestinal health. J. Adv. Parasitol., 6(3): 41-50.

Sorhue UG, Emenim RO, Moemeka AM, Peterson IP (2021). Dietary inclusion of scent leaf meal (*Ocimum gratissimum*) affects immune genes expression in chicken spleen at 28 and 56days. Anim. Rev., 8(1): 10-19.

Sorhue UG, Imide IO, Moemeka AM, Omeje SI, Udeh I, Awlolowo F, Idama L (2022). Effect of Unconventional Feeds on Production Cost, Growth Performance and Expression of quantitative Genes in Growing Pigs. J. Indonesia Trop. Anim. Agric., 47(4):301-317. https://doi.org/10.14710/jitaa.47.4.301-317

Sun Y, Ma J, Li D, Li P, Zhou X, Li Y, He Z, Qin L, Liang L, Luo X (2019). Interleukin-10 inhibits interleukin-1β production and inflammasome activation of microglia in epileptic seizures. J. Neuroinflam., 16: 66. https://doi.org/10.1186/s12974-019-1452-1

Teirlynck E, Bjerrum L, Eeckhaut V, Huyghebaert G, Pasmans F, Haesebrouck F (2009). The cereal type in feed influences gut wall morphology and intestinal immune cell infiltration in broiler chickens. Brazilian J. Nutr., 102: 1453–1461. https://doi.org/10.1017/S0007114509990407

William P, Losa R (2001). The use of essential oils and their compounds in poultry nutrition. World's Poult., 17: 14-15.

Yu H, Zou W, Mi C, Wang Q, Dai G, Zhang T, Zhang G, Xie K, Wang J, Shiy H (2021). Research note: Expression of T cell-related cytokines in chicken cecal and spleen tissues following *Eimeria tenella* infection *in vivo*. Poult. Sci., 100(7): 1-7. https://doi.org/10.1016/j.psj.2021.101161