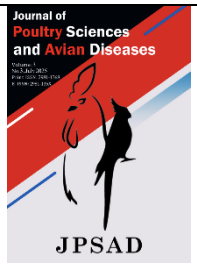
Journal of Poultry Sciences and Avian Diseases

Journal homepage: www.jpsad.com



Effects of Encapsulated Mixtures of Plant Essential Oils and Organic Acids as an Alternative to Antibiotic Growth Promoters on Humoral Immune Response and Expression of Interleukin-4 and Interferon-gamma Genes in Broilers

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A r t i c l e I n f o

Article type:
Original Research

How to cite this article:
Khezri, A., Shafabakhsh, H., Alizadeh, A., Mohammadabadi, M., & Shakeri, M. (2025). Effects of Encapsulated Mixtures of Plant Essential Oils and Organic Acids as an Alternative to Antibiotic Growth Promoters on Humoral Immune Response and Expression of Interleukin-4 and Interferon-gamma Genes in Broilers. *Journal of Poultry Sciences and Avian Diseases, 3*(3), 12-19.
http://dx.doi.org/10.61838/kman.jpsad.3.3.3

A B S T R A C T

The poultry industry has achieved remarkable advancements in growth rates over the decades, primarily through antibiotic growth promoters (AGPs). Despite the benefits of AGPs, their use has sparked serious concerns regarding the rise of antibiotic-resistant bacteria, potential residues in animal products, and their broader implications for human health. These challenges have prompted increased regulatory scrutiny and a shift toward alternative strategies, such as nutrition, for maintaining poultry health and productivity. Therefore, this study evaluated the effect of an encapsulated mixture of essential oils and organic acids as an alternative to AGPs on humoral immunity, lymphoid organs, interleukin-4, and Interferon-gamma gene expression. A total number of 270 one-day-old male Ross-308 were assigned to three groups: control diet, control diet plus flavophospholipol (600 mg/kg), or control diet plus an encapsulated mixture of essential oils and organic acids (KaroGut™; 1L/1000L in drinking water). Humoral immunity was assessed via antibody titers against sheep red blood cells (SRBC) and Newcastle Disease Virus (NDV). The weight of Fabricius and the spleen's bursa and the expression of IL-4 and IFN-γ in the jejunum were measured. Results showed that birds supplemented with essential oils and organic acids increased anti-SRBC and NDV antibody titers *vs* control (p<0.05). The relative weights of the bursa of Fabricius and the spleen as key lymphoid organs tended to be higher for essential oils and organic acids. IL-4 and IFN-γ expression in the jejunum were significantly higher in birds supplemented with essential oils and organic acids *vs* control (p<0.05). These findings suggest that an encapsulated mixture of essential oils and organic acids could enhance immunity and related key gene expression, offering a viable alternative to AGPs in broilers.

Keywords: *essential oils, organic acids, cytokine genes expression, immunity, broiler.*

Article history:
Received 04 April 2025
Revised 23 May 2025
Accepted 29 May 2025
Published online 01 July 2025

1 Introduction

Chicken products such as meat play a vital socio-economic role for communities in low-income countries across Africa and Asia (1)due to their short generation interval and adaptability to diverse agro-ecological conditions(2, 3). This widespread popularity is largely attributable to their valuable traits, including adaptation to different environmental conditions and efficiently utilizing lower-quality feed resources (4, 5). The industry has witnessed remarkable advancements in growth performance and feed efficiency for years, primarily due to the effects of antibiotic growth promoters (AGPs) in broiler diets (6). However, besides the positive effects of AGPs on growth performance, long-term use of AGPs has raised significant concerns regarding the development of antibiotic-resistant bacteria, potential residues in animal products, and their impact on human health (7, 8). The growing concern prompted several countries to implement restrictions or bans on the use of AGPs in broiler diets, while others continue to permit their use without limitations. Further research is essential to address this challenge in the poultry industry. A practical approach involves implementing nutritional strategies, such as adding essential oils and organic acids to broiler diets.

Essential oils, derived from plant parts like leaves, flowers, and seeds, are volatile concentrates rich in terpenes, terpenoids, and phenolics, known to modulate immune responses and cytokine expression, including Iinterleukin-4 (IL-4) and Interferon-gamma (IFN-γ), in broiler chickens (9). IL-4 is critical in developing humoral immunity by stimulating B cell activation and antibody production (10). Interleukins regulate interactions between lymphocytes and other leukocytes, enabling rapid responses to poultry infections (11). IFN-γ produces a mature protein consisting of 145 amino acids(12)(a component of the Th1 immune response) and is a critical cytokine involved in the immune system's regulations, including activating macrophages and enhancing antigen presentation.

In addition to plant essential oils, organic acids have emerged as promising candidates for replacing AGPs in broiler diets (13). Short-chain organic acids, including formic, propionic, and butyric acids, are recognized for suppressing pathogens and enhancing intestinal health in poultry (14). These acids lower the gastrointestinal pH, inhibit pathogenic bacteria growth, and promote beneficial microbiota proliferation (15). Among the three sections of the intestine, the jejunum is the primary site for IL-4 and IFN-γ expression due to its central role in nutrient absorption, microbial interactions, and immune regulation within the gut-associated lymphoid tissue (GALT) of broilers. Furthermore, the jejunum hosts a dense population of immune cells, including T-lymphocytes and antigen-presenting cells, critical for initiating and modulating immune responses to dietary additives such as essential oils and organic acids (16). These compounds influence gut microbiota and epithelial integrity, particularly in the jejunum, affecting cytokine production and systemic immunity. Encapsulation techniques involving protective coatings for essential oils and organic acids ensure gradual release throughout the gastrointestinal tract, enhancing efficacy and minimizing nutrient loss during digestion.

Previous studies have demonstrated the positive effects of organic acids and essential oil compounds when supplemented separately on broiler performance and gut health (17, 18). However, no available studies have examined alterations in gene expression of IL-4 and IFN-γ as immunity-related genes in Ross-308 broilers supplemented with encapsulated mixtures of plant essential oils and organic acids. Therefore, this study aimed to investigate the effects of encapsulated mixtures of plant essential oils and organic acids on humoral immune responses and the expression of IL-4 and IFN-γ in the jejunum of Ross-308 broilers. By elucidating their immunomodulatory and growth-promoting mechanisms, this research seeks to establish these alternatives as viable and sustainable replacements for AGPs in commercial poultry production.

2 Method

2.1 Experimental Design

The study used 270 one-day-old male broiler chickens (Ross-308) with an initial average body weight of 43±2g. On arrival, all chickens were weighed individually and randomly assigned to 18 equally sized pens. Average body weight was recorded weekly starting from day zero (on arrival). Feed and water were provided *ad libitum* throughout the trial. The temperature was maintained at 32°C during the first three days, then gradually reduced to 23°C on day 10, which was kept constant thereafter. Relative humidity was maintained at an average of 60±5%. Light was provided 24 hours/day for the first three days, followed by 23 hours/day for the rest of the study. All broiler chickens were vaccinated against Newcastle Disease Virus (NDV) on days 7 and 18. The experiment included three dietary

treatments: control diet (without additive), control diet+AGPs (flavophospholipol antibiotic at 600 mg/kg), and control diet+essential oil and organic acids (KaroGut™ at 1L/1000 L in drinking water). Each dietary treatment consisted of 6 replicates and 15 birds/replicate. The feed was provided as a starter (1–14 days), grower (15–21 days), and finisher (22–42 days) (Table 1).

Table 1. Ingredients and composition of the control diets (% of DM)

Item	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-42 days)
Ingredients, % of DM			
Corn grain, ground	55.6	59.6	66.6
Soybean Meal	37.9	35.0	29.0
Soybean Oil	2.3	2.0	1.5
Calcium Carbonate	0.99	0.72	0.59
Di-calcium Phosphate	1.79	1.45	1.28
Salt	0.25	0.25	0.25
DL-Methionine	0.25	0.2	0.15
L-Lysine Hydrochloride	0.4	0.15	0.2
Threonine	0.2	0.06	0.04
Vitamin Premix[1]	0.25	0.25	0.25
Mineral Premix[2]			
Chemical composition, % unless stated			
Metabolizable Energy (kcal/kg)	3000	3050	3100
Crude protein (CP)	21.70	20.60	18.5
Lysine	1.45	1.21	1.13
Methionine + Cystine	0.74	0.69	0.57
Threonine	0.91	0.75	0.67
Calcium	1.01	0.95	0.91
Phosphorus	0.42	0.38	0.36

[1] For each kilogram of the feed: 12,000 International Units of Vitamin A (Retinol), 500 International Units of Vitamin D3 (Cholecalciferol), 80,000 International Units of Vitamin E, 3 mg of Vitamin B1 (Thiamine), 7.5 mg of Vitamin B2 (Riboflavin), 51 mg of Vitamin B3 (Niacin), 4.5 mg of Vitamin B6 (Pyridoxine), 0.02 mg of Vitamin B12 (Cyanocobalamin), 2.55 mg of Vitamin K3, 1.5 mg of Vitamin B9 (Folic Acid), 13.5 mg of Vitamin B5 (Pantothenic Acid), 0.2 mg of Vitamin B7 (Biotin), and 250 mg of Choline Chloride.

[2] For each kilogram of the feed: 120 mg of Manganese, 40 mg of Iron, 16 mg of Copper, 1 mg of Iodine, and 0.01 mg of Zinc

KaroGut™ (produced by Aral Fanavar Karo Co., Iran) is a coated blend of plant essential oils and organic acids (thymol, carvacrol, eugenol) and organic acids (formic, malic, butyric, benzoic, acetic) designed to regulate digestive pH and inhibit the growth of pathogenic microorganisms. KaroGut™ employs a microencapsulation technique involving a lipid-based coating to protect essential oils and organic acids from degradation in the acidic gastric environment. This coating enhances compound stability by preventing volatilization and oxidation, ensuring a gradual release throughout the small intestine, particularly in the jejunum, to maximize antimicrobial and immunomodulatory effects.

2.2 Immune Response Analysis

Humoral immunity was assessed by challenging broilers with sheep red blood cells (SRBC). On days 21 and 35, two birds/replicates were randomly (marked for identification) received an intramuscular injection of 1mL 0.5% SRBC suspension to stimulate antibody production. This procedure aimed to assess antibody production specific to SRBC. The remaining chickens in each replicate were maintained under identical conditions. To confirm the stability of the immune response, the same birds received a second injection after 7 days (on days 28 and 35, respectively). Two birds/replicates were randomly selected to collect blood samples on days 28

and 42. Blood samples (2 mL) were drawn from the brachial vein 7 days after each injection (on days 35 and 42). Blood was centrifuged at 3000×g for 10 minutes at 4°C to collect serum. The obtained serum was stored at -80°C for subsequent antibody titer analyses. Following the final blood collection on day 42, the birds were humanely euthanized via cervical dislocation. Total IgG and anti-SRBC antibody levels were determined using hemagglutination assays in a U-bottomed 96-well microplate. The IgM titer was calculated by subtracting the IgG titer from the total antibody titer (19). Additionally, the humoral immune response to NDV was assessed. NDV antibody titers were measured using a hemagglutination-inhibition test (19).

2.3 Interleukin-4 and Interferon-gamma Gene Expression

On days 21 and 42, total RNA was extracted from the jejunum using the Denazist RNA Purification Kit, following the manufacturer's instructions (Denazist Co., Iran). The quality and quantity of the extracted RNA were assessed using electrophoresis on a 2% agarose gel and NanoDrop®ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The extracted RNA was then reverse-transcribed into complementary DNA (cDNA) using the Parstous cDNA Synthesis Kit, according to the manufacturer's protocol (Parstous Co., Iran). The resulting cDNA was stored at -80°C until further analysis. Real-time PCR with SYBR Green measured IL-4 and IFN-γ gene expression in the jejunum. Each PCR reaction contained 6.25 ng of cDNA, adjusted to a final concentration of 0.25 ng/µL in a 25 µL reaction volume with SYBR Green. GAPDH was used as an endogenous control to normalize gene expression levels. The PCR protocol included 40 cycles, followed by melting curve analysis, which was conducted by gradually heating the reaction mixtures from 55°C to 95°C at a rate of 0.2°C per second while recording the SYBR Green fluorescence signal. Fluorescence data were collected during the final stage, combining SYBR Green with DNA amplification. The difference between the cycle threshold (CT) values of the IL-4 and IFN-γ genes and the reference gene (GAPDH) was calculated as ΔCT, where higher ΔCT values indicate lower gene expression. The relative expression levels of IL-4 mRNA were determined using the 2^−ΔΔCT method. The primers are listed in Table 2.

Table 2. Primary sequences used in the experiment

Primer	Sequence 5'–3'	Access No.	Size (bp)	Temp (°C)
GAPDH Fw	5′-CCTAGGATACACAGAGGACCAGGTT- 3′	NM_204305	128	60
GAPDH Rev	5′-GGTGGAGGAATGGCTGTCA- 3′			
IL-4 Fw	5′-AGCACTGCCACAAGAACCTG- 3′	NM_001007079	145	59
IL-4 Rev	5′-CCTGCTGCCGTGGGACAT-3′			
IFN-γ Fw	5′-GCTCCCGATGAACGACTTGA-3′	NM_205149	112	61
IFN-γ Rev	5′-TGTAAGATGCTGAAGAGTTCATTCG-3′			

2.4 Statistical Analysis

The data were analyzed by ANOVA using the General Linear Model procedure of SAS software (SAS Institute, 2011) as a randomized complete design, and differences in treatment means were evaluated using Tukey's multiple range tests. All statements of significance were based on $p<0.05$.

3 Results

Birds receiving essential oil and organic acids showed significantly higher total antibody titers at 28 and 42 days compared to the control ($p=0.02$ and $p=0.03$, respectively), while the antibiotic group showed intermediate values, indicating an enhanced humoral immune response (Table 3). There were no significant changes among diets or days on bursa Fabricius or spleen weight (Table 4).

On day 42, birds supplemented with essential oil and organic acid upregulated IL-4 (1.57-fold, $p=0.03$) and IFN-γ (1.61-fold, $p=0.04$) expression compared to the control,

surpassing the antibiotic group (1.32-fold for IL-4 and 1.44-fold for IFN-γ). As a Th2 cytokine, IL-4 supports B-cell maturation and antibody production, particularly IgG, which corroborates the elevated IgG titers observed in the essential oil and organic acid group (Table 5).

Table 3. Impact of dietary treatments on immune response parameters (log_2) of broilers at d 28 and 42.

	Total Antibody		IgG	
Dietary treatments*	day 28	day 42	day 28	day 42
Control diet	5.8±0.6^{b}	6.2±0.7^{a}	2.9±0.4	3.1±0.5^{b}
Antibiotic diet	6.5±0.7^{ab}	7.0±0.8^{ab}	3.4±0.5	3.7±0.6^{ab}
KaroGut™ diet	7.1±0.7^{a}	7.6±0.7^{a}	3.8±0.6	4.1±0.6^{a}
p-value	0.02	0.03	0.05	0.04
	IgM		Antibody Response to NDV	
	day 28	day 42	day 28	day 42
Control diet	2.9±0.4	3.1±0.4	4.9±0.6^{b}	5.3±0.7^{b}
Antibiotic diet	3.1±0.5	3.3±0.5	5.6±0.7^{ab}	6.1±0.8^{ab}
KaroGut™ diet	3.3±0.5	3.5±0.5	6.2±0.7^{b}	6.8±0.7^{a}
p-value	0.08	0.06	0.04	0.03

*Control (Control Diet), Antibiotic diet (600 mg/kg), KaroGut™ diet (1 L/1000 L). All values are presented as Mean±SEM. Values within the same row that have different superscripts are significantly different ($p<0.05$).

Table 4. Impact of dietary treatments on the relative weight of Bursa fabricus and spleen (% of body weight) of broilers at 21 and 42 days

	Bursa Fabricius (g)		Spleen (g)	
Dietary treatments*	day 21	day 42	day 21	day 42
Control diet	0.18±0.03	0.12±0.03	0.11±0.03	0.12±0.04
Antibiotic diet	0.21±0.04	0.15±0.05	0.12±0.03	0.15±0.06
KaroGut™ diet	0.24±0.05	0.17±0.06	0.15±0.04	0.16±0.06
p-value	0.06	0.05	0.07	0.08

*Control (Control Diet), Antibiotic diet (600 mg/kg), KaroGut™ diet (1 L/1000 L). All values are presented as Mean±SEM. Values within the same row that have different superscripts are significantly different ($p<0.05$).

Table 5. Impact of dietary treatments on relative gene expression ($2^{-}\Delta\Delta CT$) of IL-4 and IFN-γ in Jejunum Tissue of broilers at 21 and 42 days

	IL-4		IFN-γ	
Dietary treatments*	day 21	day 42	day 21	day 42
Control diet	1.00±0.20	1.00±0.22^{b}	1.00±0.26	1.00±0.24^{a}
Antibiotic diet	1.25±0.28	1.32±0.25^{ab}	1.38±0.31	1.44±0.30^{ab}
KaroGut™ diet	1.51±0.39	1.57±0.36^{a}	1.42±0.35	1.61±0.32^{b}
p-value	0.05	0.03	0.07	0.04

* Control (Control Diet), Antibiotic diet (600 mg/kg), KaroGut™ diet (1 L/1000 L). All values are presented as Mean±SEM. Values within the same row that have different superscripts are significantly different ($p<0.05$).

4 Discussion

Birds that received essential oil and organic acids showed significantly higher total antibody titers at 28 and 42 days than the control. In contrast, the antibiotic group showed intermediate values, indicating an enhanced humoral immune response. On day 42, the IgG levels followed a similar trend, whereas essential oil and organic acid had the highest IgG titers, reflecting their potential to promote long-term immunity. While differences in IgM levels among treatments were less pronounced, essential oil and organic acid still resulted in a modest numerical improvement. Additionally, antibody titers against NDV were notably higher in the essential oil and organic acid group than in the control group, suggesting improved vaccine efficacy with this supplement. The superior immune response in essential oil and organic acid groups may be attributed to its

constituents' antimicrobial and immunomodulatory properties. Compounds like thymol and carvacrol effectively reduce harmful bacteria populations, including *Escherichia coli* and *Clostridium perfringens*, in the broiler gut (20), reducing inflammation and allowing greater resource allocation to the immune system. Organic acids such as formic and butyric acid could lower intestinal pH, fostering a favorable microbial environment that supports immune development. Our results align with studies (6, 21) showing flavophospholipol effectively controls gut pathogens but may suppress microbial diversity, potentially limiting immune priming compared to essential oil and organic acid. For example, a study reported that broilers supplemented with essential oils exhibited higher SRBC antibody titers than AGPs, attributing this to improved gut health and immune stimulation (21).

The current results showed that the relative weights of the bursa of Fabricius and spleen, key lymphoid organs in poultry, were not significantly influenced by the treatments but exhibited a higher tendency for birds supplemented with essential oil and organic acid than the control. The increase in lymphoid organ weights suggests enhanced immune organ development, likely linked to improved humoral immunity and cytokine expression. The bursa of Fabricius, critical for B-cell maturation, benefits from a healthier gut environment, as essential oils and organic acids reduce pathogen loads and inflammation (22). Similarly, the spleen, a secondary lymphoid organ, supports systemic immunity, and its increased size may reflect greater lymphocyte proliferation driven by IL-4 and IFN-γ (23). The antibiotic group's results suggest that while flavophospholipol supports immune function via pathogen control, it lacks the broader stimulatory effects of essential oil and organic acid constituents. These findings are consistent with a study that reported that bursa and spleen weights increased when birds were supplemented with organic acids and essential oils (24)but differ from a study where AGPs had minimal impact on lymphoid organ size (25).

IFN-γ, a Th1 cytokine, enhances macrophage activity and cellular immunity, suggesting a balanced Th1/Th2 response induced by essential oil and organic acid. The upregulation of IL-4 and IFN-γ likely reflects the essential oil compounds' ability to modulate GALT. Citral and terpinene, for instance, possess anti-inflammatory properties that may reduce oxidative stress in the jejunum, facilitating cytokine production (26). Organic acids, by stabilizing gut microbiota, may further enhance GALT activity, as evidenced by studies showing increased IFN-γ expression with butyric acid supplementation (27). While showing moderate increases in cytokine expression, the antibiotic group did not match essential oil and organic acid, possibly due to its narrower antimicrobial spectrum and lack of direct immunomodulatory effects. These findings are consistent with a study that reported elevated IL-4 and IFN-γ expression in broilers fed phytogenic additives as AGP alternatives (28).

While the current study offers evidence supporting the effectiveness of encapsulated essential oils and organic acids in enhancing growth rates and immunity, a larger sample size could improve data accuracy and statistical reliability, as specific trends were identified in the current dataset (p=0.05–0.08). Furthermore, initiating challenges on birds from day 28 may not fully account for the long-term immunomodulatory effects that extend beyond the finisher phase. A longer observation period could provide deeper insights into sustained immune responses and overall health outcomes. Additionally, variability in the composition of essential oils impacted by factors such as plant source, extraction method, and batch consistency may impact reproducibility. Considering that the differences mentioned above may lead to variations in efficacy, careful standardization is required to ensure consistent outcomes in broiler diets.

5 Conclusions

In conclusion, essential oil and organic acid enhance humoral immunity and cytokine expression in birds more effectively than flavophospholipol, offering a promising alternative to AGPs. These findings underscore the role of nutrigenomics in poultry, demonstrating how dietary interventions can modulate gene expression and immune function to improve health and performance. Although this study provides valuable information, further research is needed to investigate several more critical areas, including the specific mechanisms and pathways involved, the long-term effects on growth, and the influence of various environmental and dietary conditions to further understand the effects of encapsulated plant essential oils and organic acids as alternatives to antibiotic growth promoters in broilers.

Acknowledgements

The authors thank our laboratory staff and farm personnel for contributing to this study.

Conflict of Interest

The authors declare no conflict of interest related to this study.

Author Contributions

Amin Khezri: conceptualizing, methodology, supervision, editing/reviewing/revising. Hamidreza Shafabakhsh: investigator, data collection, and first draft writer. Ali Alizadeh and Mohammadreza Mohammadabadi: analysis and revisions. Majid Shakeri: guideline, editing/reviewing/revising. All authors have read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this published article, and its supplementary information is available from the corresponding author upon reasonable request.

Ethical Considerations

This study was conducted following the ethical guidelines for animal research. All experimental procedures were approved by the Animal Care and Use Committee of Bahonar University according to the guidelines of the Iranian Council of Animal Care (1995). Animal welfare was strictly maintained, and all efforts were made to minimize discomfort and stress to the broilers used in this study

Funding

The author received no grants or funding while preparing the contents of this work.

References

1. Mohamadinejad F, Mohammadabadi M, Roudbari Z, Eskandarynasab Siahkouhi S, Babenko O, Klopenko N, et al. Analysis of liver transcriptome data to identify the genes affecting lipid metabolism during the embryonic and hatching periods in ROSS breeder broilers. Journal of Livestock Science and Technologies. 2024;12(2):61-7. [DOI]
2. Padhi MK. Importance of Indigenous Breeds of Chicken for Rural Economy and Their Improvements for Higher Production Performance. Scientifica (Cairo). 2016;2016:2604685. [PMID: 27144053] [PMCID: PMC4838803] [DOI]
3. Moodi D, Khezri A, Naserian AA, Ghazizadeh-Ahsaee M, Dayani O, Bahrampour V. Predicting blood beta-hydroxybutyric acid in dairy cow herds through machine learning-based feature selection: On-farm data basis. Journal of Livestock Science and Technologies. 2025;13(1):67-78. [DOI]
4. Amirteymoori E, Amin K, Omid D, Mohammadreza M, Sepideh K, Amir M, et al. Effects of linseed processing method (ground versus extruded) and dietary crude protein content on performance, digestibility, ruminal fermentation pattern, and rumen protozoa population in growing lambs. Italian Journal of Animal Science. 2021;20(1):1506-17. [DOI]
5. Khezri A, Dayani O, Tahmasbi R. Effect of increasing levels of wasted date palm on digestion, rumen fermentation and microbial protein synthesis in sheep. Journal of Animal Physiology and Animal Nutrition. 2017;101(1):53-60. [PMID: 27039841] [DOI]
6. Niewold TA. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult Sci. 2007;86(4):605-9. [PMID: 17369528] [DOI]
7. Hao H, Cheng G, Iqbal Z, Ai X, Hussain HI, Huang L, et al. Benefits and risks of antimicrobial use in food-producing animals. Frontiers in Microbiology. 2014;Volume 5 - 2014. [DOI]
8. Saeedi S, Dayani O, Tahmasbi R, Khezri A. Effect of supplementation of calf starter with fennel powder on performance, weaning age and fermentation characteristics in Holstein dairy calves. J Anim Physiol Anim Nutr (Berl). 2017;101(1):81-7. [PMID: 28063208] [DOI]
9. Wang J, Su S, Pender C, Murugesan R, Syed B, Kim WK. Effect of a Phytogenic Feed Additive on Growth Performance, Nutrient Digestion, and Immune Response in Broiler-Fed Diets with Two Different Levels of Crude Protein. Animals. 2021;11(3):775. [PMID: 33799557] [PMCID: PMC7999929] [DOI]
10. Marcano VC, Susta L, Diel DG, Cardenas-Garcia S, Miller PJ, Afonso CL, et al. Evaluation of chickens infected with a recombinant virulent NDV clone expressing chicken IL4. Microb Pathog. 2021;159:105116. [PMID: 34339794] [DOI]
11. Chaudhari AA, Kim WH, Lillehoj HS. Interleukin-4 (IL-4) may regulate alternative activation of macrophage-like cells in chickens: A sequential study using novel and specific neutralizing monoclonal antibodies against chicken IL-4. Veterinary Immunology and Immunopathology. 2018;205:72-82. [PMID: 30459004] [DOI]
12. Digby MR, Lowenthal JW. Cloning and expression of the chicken interferon-γ gene. Journal of Interferon & Cytokine Research. 1995;15(11):939-45. [PMID: 8590305] [DOI]
13. Chukwudi P, Umeugokwe PI, Ikeh NE, Amaefule BC. The effects of organic acids on broiler chicken nutrition: A review. Animal Research and One Health. 2025;3(1):43-53. [DOI]
14. Dibner J, Buttin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research. 2002;11(4):453-63. [DOI]
15. Ricke S. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Science. 2003;82(4):632-9. [PMID: 12710485] [DOI]
16. Bar-Shira E, Friedman A. Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. Developmental & Comparative Immunology. 2006;30(10):930-41. [PMID: 16430960] [DOI]
17. Waghmare S, Gupta M, Bahiram K, Korde J, Bhat R, Datar Y. Effects of organic acid blends on the growth performance, intestinal morphology, microbiota, and serum lipid parameters of broiler chickens. Poultry Science. 2025;104(1):104546. [PMID: 39566172] [PMCID: PMC11617460] [DOI]
18. Iqbal H, Rahman A, Khanum S, Arshad M, Badar I, Asif A. Effect of essential oil and organic acid on performance, gut health, bacterial count and serological parameters in broiler. Brazilian Journal of Poultry Science. 2021;23(03):eRBCA-2021-1443. [DOI]
19. Salehimanesh M. The Metaphors Existed in The Examples of Qur'an and Bedouin culture. Journal of Religion and

the Contemporary World. 2016;3(1):39-52. [PMID: 26847817] [DOI]
20. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology. 2004;94(3):223-53. [PMID: 15246235] [DOI]
21. El-Sayed Y, Khalil W, Fayez N, Mohamed Abdel-Fattah AF. Enhancing effect of oregano essential oil and Bacillus subtilis on broiler immune function, intestinal morphology and growth performance. BMC Veterinary Research. 2024;20(1):112. [PMID: 38519928] [PMCID: PMC10958958] [DOI]
22. Ducatelle R, Goossens E, Eeckhaut V, Van Immerseel F. Poultry gut health and beyond. Animal Nutrition. 2023;13:240-8. [PMID: 37484993] [PMCID: PMC10362088] [DOI]
23. Lee Y, Lee R, Kim J, Han YH, Hunter C, Park J. Comparative analysis of changes in immune cell in the chicken spleen across different ages using flow cytometry. BMC Veterinary Research. 2024;20(1):429. [PMID: 39334332] [PMCID: PMC11438354] [DOI]
24. Dong Y, Gao X, Qiao C, Han M, Miao Z, Liu C. Effects of mixed organic acids and essential oils in drinking water on growth performance, intestinal digestive capacity, and immune status in broiler chickens. Animals. 2024;14(15):2160. [PMID: 39123686] [PMCID: PMC11311025] [DOI]
25. Gadde U, Kim WH, Oh ST, Lillehoj HS. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Animal Health Research Reviews. 2017;18(1):26-45. [PMID: 28485263] [DOI]
26. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils–a review. Food and Chemical Toxicology. 2008;46(2):446-75. [PMID: 17996351] [DOI]
27. Zhou ZY, Packialakshmi B, Makkar SK, Dridi S, Rath NC. Effect of butyrate on immune response of a chicken macrophage cell line. Veterinary Immunology and Immunopathology. 2014;162(1-2):24-32. [PMID: 25265870] [PMCID: PMC7112829] [DOI]
28. Lillehoj H, Liu Y, Calsamiglia S, Fernandez-Miyakawa ME, Chi F, Cravens RL. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Veterinary Research. 2018;49:1-18. [PMID: 30060764] [PMCID: PMC6066919] [DOI]