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Immunosorbent Assay (ELISA) using crushed *Melissa officinalis* and *Laurus nobilis* leaves for broiler chickens

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Abstract

The importance of herbal medicine compounds lies in their effectiveness as an antioxidant activity, thus protecting cells and maintaining their vital functions. The study was conducted in broiler chicken fields of College of Veterinary Medicine, Fallujah University, from September 9, 2025 to November 14, 2025, with the aim of investigating the effect of *Melissa* and *Laurus* leaf powder, in addition to a synthetic vitamin E, on some immune indicators of Newcastle and infectious bursa disease viruses. Four hundred and fifty sexless pullets of the Ross 308 strain were used, randomly distributed into 18 pens. The study comprised six treatments, each with an average of 75 chicks, grouped into three replicates. The treatments were designed as follows: T₁- negative control; T₂- positive control (vitamin E at 3 g /kg); T₃ and T₄ of *Melissa officinalis* leaf powder (3 and 6 g /kg) respectively; T₅ and T₆ of *Laurus* leaf (3 and 6 g/kg) respectively. In the 3rd week, the negative control group (T₁) exhibited the highest antibody titer (1.06 ± 0.30^a), followed by T₃ (*Melissa officinalis* group; 3 g/kg at 0.80 ± 0.23^{ab} , and T₆; *Laurus* at 6 g/kg (0.99 ± 0.17^a), with no significant differences among groups. A higher (6 g/kg) dose of T₄; *Melissa officinalis* at 0.39 ± 0.10^{bc} and T₅; *Laurus nobilis* (3 g/kg) at 0.14 ± 0.05^c resulted in significantly lower titers compared to the control group. Vitamin E (T₂: 0.57 ± 0.27^{bc}) showed an average immune response. By the 5th week, the negative control (T₁) again showed highest level (1.71 ± 0.33^a), followed by vitamin E (T₂: 1.47 ± 0.36^{ab}), *Melissa officinalis* at 3 g/kg (T₃: 1.44 ± 0.39^{ab}), and *laurel* at 6 g/kg (T₆: 1.40 ± 0.57^{ab}), all without significant differences, highlighting their role in supporting long-term immune responses. In contrast, the higher dose of *Melissa officinalis* (T₄: 0.83 ± 0.18^c) and *laurel* at 3 g/kg (T₅: 0.99 ± 0.55^{bc}). The differences between the coefficients were statistically significant at $p \leq 0.05$ level as evidenced by the LSD values and statistical character differences.

Keywords: Broiler chicken, Immunosorbent assay, ELISA, *Melissa officinalis* leaves, *Laurus nobilis* leaves, vitamin E

Introduction

Poultry production is one of the largest animal sectors and has a critical role in supplying the world with high-quality protein for humans [1]. This is achieved by producing healthy birds with well-developed immunity that support optimal growth and production efficiency [2]. One of the major challenges to successful broiler chicken production over the last several years has been oxidative stress due to an increase in free radicals which can lead to impaired immunity [3], reduced growth and high disease incidence; researchers have been investigating the role of both natural and synthetic antioxidants in improving the health and performance of immune systems [4]. A recent trend with regards to antioxidants has been the use of medicinal herbs as they contain phenolic compounds, terpenoids and other active ingredients that can help improve immunity and reduce oxidative damage [5].

Melissa (*Melissa officinalis*) is an aromatic herb from the mint family that has historically been used for its sedative and antimicrobial properties [6]. It is composed of a variety of active substances including rosmarinic acid, caffeic acid, flavonoids, and volatile oils such as citral and linalool, which have all been shown to have free radical-scavenging and anti-inflammatory effects [7]. Recent research has indicated that adding *Melissa* extract or powder to poultry diets is associated with increased humoral and cellular immunity, increased antibody production [8],

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and improved digestion due to its antibacterial and antiviral capabilities ^[9]. Bay leaves (*Laurus nobilis*) are simple plants that have a lot of taste due to their essential oils and phenol-based chemicals like eugenol, cineole, and tannins ^[10]. Since bay leaves contain many essential oils, they can provide there is an antioxidant and antimicrobial benefit by improving both the absorption of nutrients and digestion leading to improved bird health and growth ^[11]. Research has shown that including bay leaves in broiler diets has improved body weight while increasing the birds' ability to fight off disease, reduced oxidative stress, and increased the activity of antioxidant enzymes such as catalase and glutathione peroxidase ^[12]. While synthetic antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and synthetic tocopherols continue in use today to reduce the impacts of free radicals in the diets of poultry, they are facing increasing scrutiny due to concerns over the safety of consuming these products by the human population and their accumulation within poultry tissues ^[13]. The rise in market prices combined with an increasing preference by global consumers to rely more on "natural products" instead of synthetic substances has led researchers to explore using plant-based alternatives ^[14]. Based on scientific research, medicinal plants appear to be a viable source for natural antioxidants with medicinal plants generally deemed safe and effective when applied at proper concentrations and processed through proper techniques such as drying and grinding ^[15]. The Enzyme-Linked Immunosorbent Assay (ELISA), a vital biological tool for evaluating the immune response in veterinary science, is one of the most common methods used today ^[16]. This technique detects and measures antibody levels in the blood serum of animals by providing an estimation of the level of immune activity in the animal that was exposed to either a pathogen or a vaccine ^[17]. Furthermore, researchers have used the ELISA test extensively to study the effects of feed additives on the immune system of chickens ^[18]. In addition to providing information on the effect of adding plant extracts to chicken feed, previous studies have shown that the ELISA test will allow researchers to quantify the increase in IgG and IgM levels as the result of feeding chicken with plant extracts compared to a control group ^[19]. Prior research suggests that utilizing medicinal plants as natural sources of antioxidants has enhanced the immune response of avians to the common vaccines of Newcastle and Gumboro ^[20]. Other studies have further concluded that using dried leaves for powder is more stable and easier to blend with leeches than using liquid extracts ^[21]. However, there is currently a lack of substantive direct comparisons between natural medicinal plant-based sources and synthetic supporters, indicating a need for greater systematic investigations that examine performance, oxidative stress, and immune response ^[22]. This study was conducted to determine the effect of adding *Melissa* leaf and laurel leaf powder to chicken feed, and vitamin E (synthetic antioxidant), on the immune response, utilizing the ELISA method for measuring antibody levels in blood serum and monitoring the productive performance and overall health parameters. This study aims at providing scientific data on the effectiveness of plant-based

alternatives to support the health of chickens through the use of natural antioxidants and immune stimulating properties and represents a step towards reducing chemical dependence on industrial material and providing sustainable, healthy production of animal protein.

Materials and Methods
Experiment design and bird management

The field experiment was conducted on the grounds of the University of Fallujah's College of Veterinary Medicine between September 9, 2025, and November 14, 2025. During the experiment, broiler chickens were provided with appropriate environmental conditions such as lighting, ventilation, temperature and periodic feed fortification according to the standard program developed for broiler production. A total of 450 Day Old Ross 308 broiler chicks were randomly assigned to six treatments using a completely randomized design (CRD) with a replicable unit size of ten chicks. Each treatment contained a total of three replicates that had a repeatable unit size measuring 180 cm long x 250 cm wide. Each treatment contained an evaluation of one of six different additives used at levels of 3 g/kg; 6 g/kg; designated as T₁ - negative control (Standard feed without additives); T₂ - positive control (Antioxidant vitamin E @ 3 g/kg of feed); T₃ - *Melissa officinalis* leaf powder @ 3 g/kg of feed; T₄ - *Melissa officinalis* leaf powder @ 6 g/kg of feed; T₅ - *Laurus nobilis* @ 3 g/kg of feed; T₆ - *Laurus nobilis* @ 6 g/kg of feed. Plant additives were created by shade-drying the leaves prior to grinding in an electric grinder into a fine powder.

Chicks rearing
The chicks were raised with a rosso mattress of sawdust, with a range of thickness between 3 and 5 cm. The chicks received circular feeders (feeding trays) for the first week, then circular feeders suspended to a level equal to the bird's chest. This helped to encourage the chicks to eat and to waste less food. The chicks received water freely for the first week using inverted plastic containers of water, and then were provided suspended containers for water in the second week after which the chicks received three different diets, starter feed for the first eleven days, grower feed for the next eleven days, and finisher feed for the last eleven days (the finisher would be given to them until thirty-five days old).

Composition of the diet: Dale ^[23] described how broiler chickens can be fed according to specific nutrient standards and have equal protein and energy levels among diets. Each diet contained an equal amount of each additive to achieve reasonable uniformity in the distribution of each additive throughout the entire diet.

Vaccination schedule and preventive program: All birds were vaccinated with basic vaccines to ensure consistent assessment of the immune response via ELISA testing in all groups. The preventive program was implemented as mentioned in Table (1).

Table 1: Shows the preventive program used during the period of raising broilers

Age/ day	Prophylactic treatment	Administration's Mode	Manufacturer, country
1	Oil vaccine (ND + IB)	injected S/C neck	
1-3	Giving the antibiotic Enrosol	drinking water	Baytril®, Bayer, Germen
7	Newcastle vaccine, first strain (Lasotta)		Creative Diagnostics, USA
14	Infectious Bursal Disease Virus (IBDV) vaccine is a medium-peak dose		
18	Newcastle vaccine, second strain (Lasota)		
22-26	Antibiotic tylosin		Baytril®, Bayer, Germen
Note: Vitamin AD3E was given after each vaccine			

Blood sampling and Immunoassay using ELISA kit

During the 3rd and 5th wk of the bird's life, blood samples were collected from the wing vein of 6 randomly chosen birds from each treatment group ($N = 72$). Blood was then collected in special tubes, clotting was allowed to occur, and then centrifuged at 3000 rpm for 15 minutes. The resulting serum samples were frozen in Eppendorf tubes at -20 degrees C until use in ELISA methods. Antibody levels of the Newcastle and IBD vaccines were measured using commercially available kits and following the manufacturer's instructions. To perform the assays, 100 μ l of serum was added to a predetermined number of wells and was then incubated at 37 degrees C for 1 hour before they were washed three times with wash solution. After washing, compound was added and incubated for 30 minutes, substrate (TMB) was then added to each well and allowed to stand for 15 minutes, then stop solution was added. Absorbance was read at 450 nm using a microplate reader and antibody levels were calculated using optical density values based on the manufacturer's equation for each assay.

Statistical analysis

The quantitative ELISA data were evaluated using a one-way analysis of variance (ANOVA) [24], and if the ANOVA indicated a significant difference using the least-squares difference test (LSD), the group average means were compared [25]. All birds were classified into a positive or

negative classification based on the ELISA cut-off value, and a chi-square test was used to determine the distribution of positive and negative birds in each group as classified, and the level of significance was set at $p \leq 0.05$.

Results

Results indicated that various factors influenced NDV & IBV antibody levels from plant extracts (*Melissa officinalis* & *Laurus nobilis*) based on the ratio of the extract's dose to an animal's body weight. In the 3rd week, the greatest antibody level (1.06 ± 0.30^a) was found in T₁ (control) followed by T₃ (3 g/kg of *Melissa officinalis*; 0.80 ± 0.23^{ab}) and T₆ (6g/kg of *Laurus nobilis*; 0.99 ± 0.17^a) with no difference. *Melissa officinalis* (6 g/kg; T₄; 0.39 ± 0.10^{bc}) & *Laurus nobilis* (3 g/kg; T₅; 0.14 ± 0.05^c) produced significantly lower antibody levels than T₁ while vitamin E (T₂; 0.57 ± 0.27^{bc}) produced an intermediate response. In the 5th week, all groups had higher antibody levels compared to the 3rd week; T₁ (1.71 ± 0.33^a) had the highest, followed by T₂ (1.47 ± 0.36^{ab}), T₃ (1.44 ± 0.39^{ab}), and T₆ (1.40 ± 0.57^{ab}), with no significant difference among those groups. T₄ (0.83 ± 0.18^c) & T₅ (0.99 ± 0.55^{bc}) had much lower antibody levels than T₁. All differences between groups were statistically significant at $p \leq 0.05$ according to their LSD values being significant and/or by the difference in their statistic characteristics.

Table 2: Feed additives on the humoral immune response against Newcastle (ND) and infectious bursal disease (IBV) at 3 and 5 weeks of age in broiler chickens

Group	Week 3	Week 5
T ₁ negative control	1.0 ± 0.30^a	1.71 ± 0.33^a
T ₂ positive control Vit E 3g/kg	0.57 ± 0.27^{bc}	1.47 ± 0.36^{ab}
T ₃ <i>Melissa</i> 3 g/kg	0.80 ± 0.23^{ab}	1.44 ± 0.39^{ab}
T ₄ <i>Melissa</i> 6 g/kg	0.39 ± 0.10^{bc}	0.83 ± 0.18^c
T ₅ <i>Laurus</i> 3 g/kg	0.14 ± 0.05^c	0.99 ± 0.55^{bc}
T ₆ <i>Laurus</i> 6 g/kg	0.99 ± 0.17^a	1.40 ± 0.57^{ab}
LSD (0.05)	0.42	0.51
*Values with different letters (a, b, c) indicate significant differences at $p \leq 0.05$ between groups. * S/P Ratio (Mean $\pm$ SE)		

Statistical analysis using a chi-squared test identified that there were statistically significant differences ($p < 0.05$) in the distribution of positive birds versus negative birds across treatment groups through week 3, continuing through week 5, with the *Melissa officinalis* groups tending to have numerically the highest percentage of positive birds and *Laurus nobilis* (most clearly at the 6 g/kg dose) groups tending to have the lowest percentage of positive birds. This indicates that these birds had a relatively weak immune

response in their early development compared to their counterparts receiving one of the other treatments. Development of the acquired immune response was detected by the significantly increased percentage of positive birds in the two *Melissa officinalis* and vitamin E treatment groups with age. Furthermore, even though the *Laurus nobilis* groups' results improved after week 3, these remained below those for any of the other treatment groups as shown in table 3.

Table 3: *Melissa officinalis*, *Laurus nobilis* and Vitamins E Supplementation, on the Immune responses of Birds

Group	3 rd weeks		5 th weeks	
	Positive (≥ 0.5)	Negative (< 0.5)	Positive (≥ 0.5)	Negative (< 0.5)
T ₁	4	2	6	0
T ₂	3	3	5	1
T ₃	4	2	5	1
T ₄	2	4	3	3
T ₅	1	5	4	2
T ₆	5	1	5	1
Significant $p < 0.05$ Chi square	0.019, $\chi^2 = 9.87$		0.01, $\chi^2 = 8.64$	

Discussion

According to Table (2), the data revealed that statistically significant differences existed in the humoral immunity response when comparing *Melissa officinalis* (lemon balm) and *Laurus nobilis* (laurel) as well as vitamin E during the third and fifth week of treatment. There was an apparent effect of the different types of food addition (*Melissa officinalis* versus *Laurus nobilis*) and amounts used (3 g/kg versus 1 g/kg), along with age (young vs old), on the relative effectiveness of generating an immune response against Newcastle disease virus (NDV) vaccines and infectious bursal virus (IBV) vaccines [26]. When examining the immune response during the third week of the study *Melissa officinalis* treatment (3 g/kg feed) had significantly higher levels of antibody response when compared to the control [27]. *Melissa officinalis* has been shown to possess active phenolic components, for example, flavonoids, rosmarinic acid, and volatile oils. These active phenolic compounds may have an important role in decreasing oxidative stress in addition to stimulating B lymphocyte activation during early growth; therefore, supporting the work of [28]. When we look at the *Laurus nobilis* coefficients, we see that at the high dose (6 g/kg) there is a relative decrease in the antibody concentrations over the third week of life of the chicks. This may be due to the effect of high levels of some of the active components, namely the volatile oils that have strong biological activity. The high levels of these volatile oils may induce a temporary physiological stress response in young birds and disrupt their oxidative balance, subsequently decreasing their initial immune responses [29].

Vitamin E supplementation was associated with improved antibody production compared to the controls, further supporting vitamin E's known antioxidant function in preserving the normal structure of immune cells while decreasing oxidative injury to cells during the early stages of development [30]. By the 5th week of this study there was clear evidence of a change in the overall immune response in the form of increased antibody production in the majority of treatments indicating maturation of the immune system and accumulation of the effects of the addition of food additives [31]. Antibody levels were similar with *Melissa officinalis* at both dosages (3 and 6 g/kg) and did not differ significantly from the controls which is indicative of a balanced effect for this herb which provided antioxidant and calming effects to the immune system without overstimulation of the immune system. These results support Miraj and Kiani, [32] findings regarding the immunomodulatory effects of *Melissa* would vary based on the amount used and the age of the subject.

According to previous studies Vitamin E produced the most antibodies in week five (1.47); therefore, it must enhance the function of lymphocytes, thereby aiding in the maturation of the adaptive immune response [30]. These findings support previous research indicating that antioxidants (vitamins) produce a lasting and significant effect, whereas herbal products are influenced by their dosage and their metabolic relationship with other therapeutic agents [33].

With regard to *Laurus nobilis*, there was a positive trend throughout weeks three and five in the quantity of antibody levels; however, they were lower than those of the other treatments. This indicates that *Laurus nobilis* may provide a more gradual and less effective means of enhancing humoral immunity although it has been shown to be beneficial for antimicrobial and enhance digestive function. This finding is also consistent with the findings of Shehata *et al.* [34] and

Badawy *et al.* [26], who report that given the high activity of the essential oils found in *Laurus nobilis*, large doses may have a negative impact on the immune system.

Research indicates that herbal medicines can modulate the immune system depending on the amount consumed, therefore when too little of the medicine is consumed, this can cause partially inhibited antibodies from being produced, due to plant compounds affecting lymphocyte signalling or being part of an oxidative balance within lymphocytes [35]. Furthermore, at both time points, there is a relative similarity in immune responses between the two doses of *Melissa officinalis*, suggesting that the immunomodulatory effects of *Melissa officinalis* are not linear with respect to dose, which is consistent with the findings of Al-Gharawi *et al.* [36].

According to the results of the chi-square test presented in Table (3), both the 3rd week and the 5th week revealed statistically significant differences between the distributions of positive and negative birds and their corresponding coefficients with respect to the third and fifth weeks. The percentage of birds that tested positive for treatments utilizing *Melissa* increased during the 3rd week; therefore, these treatments were found to be effective in providing protection at an earlier stage [27]. When comparing the 5th week, the highest percentage of birds that tested positive were found in both the *Melissa* and vitamin E treatment groups, which would suggest that collective immunity improved and that immune response became more uniform with age [37].

Combining both quantitative methods (ANOVA and LSD) and qualitative methods (Chi-square) gives you a better understanding of the immune system's function. For example, not only does it show that average immunosuppressive low-level (low) IgG antibody titers were increased but also more birds had a functional protective immune response [38]. Based on the biological data, both types of immune-modulating effects are based on the dose; therefore, it appears that the immune-modulating effect of herbs is dose related, *i.e.*, herbs used at an optimal dose (*Melissa* at 3 g/kg and *Laurus* at 6 g/kg) have immunostimulating and supportive effects [26]. The study supports existing literature that suggests a high dose of some plants can lower the effect of the immune system by altering lymphocyte signaling pathways or having an effect on intracellular oxidative balance [39] as seen in partial inhibition of antibody production when given inappropriate dosages (*Melissa* 6 g/kg and *Laurus* 3 g/kg). The results of this study provide confirmation of the beneficial effects that long-term, consistent, and strategic use of herbal plants (in particular, *Melissa officinalis*) and vitamin E in poultry nutrition have on the humoral immune response to NDV and IBV vaccines. Yet all of these factors are necessarily influenced by specific parameters such as the kind of plant used, the quantity of the ingredient used, the age of the birds [40], and there is therefore a need for additional research to determine the most appropriate dosage of these ingredients to maximise their immunoanabolic effects while minimising any possible adverse consequences for use of these products. This is particularly relevant given increasing interest in using naturally occurring bioactive materials as immunostimulants and as a means of decreasing reliance on antibiotic treatment [41, 42].

Conclusion

The study results showed that adding *Melissa officinalis* to broiler diets, particularly at a low dose with feed, is a promising nutritional option for enhancing the immune response against ND and IBV, while maintaining an

appropriate balance between efficacy and safety. Vitamin E also showed a moderate supportive effect on immunity, especially in advanced stages of life, without significant superiority over the control group. The results showed that the immune effect of both *Melissa officinalis* and *Laurus nobilis* was clearly dose-dependent; a low dose of laurel inhibited the immune response, while a higher dose improved this response. In contrast, a higher dose of *Melissa officinalis* showed a persistent inhibitory effect on antibody production. These results suggest that active plant compounds may modulate inflammatory pathways and immune cell signaling, potentially leading to immunosuppressive effects at high or inappropriate doses. This underscores the importance of determining the optimal dose to achieve the best immune response and avoid potential adverse effects.

Conflict of Interest

Not available.

Financial Support

Not available.

References

1. Nkukwana TT. Global poultry production: Current impact and future outlook on the South African poultry industry. *South African J Anim Sci*. 2018;48(5):869-884.
2. Korver DR. Current challenges in poultry nutrition, health, and welfare. *Animal*. 2023;17:100755.
3. Lauridsen C. From oxidative stress to inflammation: redox balance and immune system. *Poultry Sci*. 2019;98(10):4240-4246.
4. Halliwell B. Understanding mechanisms of antioxidant action in health and disease. *Nature Reviews Mol Cell Biol*. 2024;25(1):13-33.
5. Dar RA, Shah Nawaz M, Ahanger MA, Majid IU. Exploring the diverse bioactive compounds from medicinal plants: a review. *J Phytopharm*. 2023;12(3):189-195. doi: 10.1111/jpn.14107.
6. Awlqadr FH, Altemimi AB, Qadir SA, Mohammed OA, Saeed MN, Hesarinejad MA, *et al*. Bioactive Compounds, Medicinal Benefits, and Contemporary Extraction Methods for Lemon Balm (*Melissa officinalis*). *Food Sci Nutr*. 2025;13(9):e70864.
7. Petrisor G, Motelica L, Craciun LN, Oprea OC, Ficai D, Ficai A. *Melissa officinalis*: Composition, pharmacological effects and derived release systems—A review. *Int J Mol Sci*. 2022;23(7):3591.
8. Phillips CJ, Hosseintabar-Ghasemabad B, Gorlov IF, Slozhenkina MI, Mosolov AA, Seidavi A. Immunomodulatory effects of natural feed additives for meat chickens. *Life*. 2023;13(6):1287.
9. Alem WT. Effect of herbal extracts in animal nutrition as feed additives. *Heliyon*. 2024;10(3):e24836.
10. Paparella A, Nawade B, Shaltiel-Harpaz L, Ibdah M. A review of the botany, volatile composition, biochemical and molecular aspects, and traditional uses of *Laurus nobilis*. *Plants*. 2022;11(9):1209.
11. Yadav S, Jha R. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *J Animal Sci Biotechnol*. 2019;10(1):2.
12. Li Y, Wang K, Li C. Oxidative stress in poultry and the therapeutic role of herbal medicine in intestinal health. *Antioxidants*. 2024;13(11):1375.
13. Fellenberg MA, Speisky H. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. *World's Poultry Sci J*. 2006;62(1):53-70.
14. de Castro Carvalho G, Carneiro JDDS, Rocha RAR, Pereira E. Plant-based trends: consumer's perception and factors that influence the purchase of plant-based products. *British Food J*. 2025. [Epub ahead of print].
15. Nafiu MO, Hamid AA, Muritala HF, Adeyemi SB. Preparation, standardization, and quality control of medicinal plants in Africa. In: Kuete V, editor. *Medicinal spices and vegetables from Africa*. London: Academic Press; 2017. p. 171-204.
16. Hosseini S, Vázquez-Villegas P, Rito-Palomares M, Martínez-Chapa SO. Enzyme-linked immunosorbent assay (ELISA): from A to Z. Singapore: Springer Nature; 2018. p. 67-115.
17. Hao X, Zhang F, Yang Y, Shang S. The evaluation of cellular immunity to avian viral diseases: methods, applications, and challenges. *Frontiers Microbiol*. 2021;12:794514.
18. Kiczorowska B, Samolińska W, Al-Yasiry ARM, Kiczorowski P, Winiarska-Mieczan A. The natural feed additives as immunostimulants in monogastric animal nutrition—a review. *Annals Animal Sci*. 2017;17(3):605-625.
19. Liu Y, Che TM, Song M, Lee JJ, Almeida JAS, Bravo D, *et al*. Dietary plant extracts improve immune responses and growth efficiency of pigs experimentally infected with porcine reproductive and respiratory syndrome virus. *J Animal Sci*. 2013;91(12):5668-5679.
20. Raza A, Muhammad F, Bashir S, Anwar MI, Awais MM, Akhtar M, *et al*. Antiviral and immune boosting activities of different medicinal plants against Newcastle disease virus in poultry. *World's Poultry Sci J*. 2015;71(3):523-532.
21. Shah MD, Maran BAV, Tan JK, Yong YS, Fui CF, Shaleh SRM, *et al*. The anti-leech potential of the solvent extract of Bornean neem leaves and ultra-high performance liquid chromatography-high-resolution mass spectrometry profiling. *J King Saud Univ-Sci*. 2021;33(6):101541.
22. Chaachouay N. Synergy, additive effects, and antagonism of drugs with plant bioactive compounds. *Drugs and Drug Candidates*. 2025;4(1):4-28.
23. Dale N. National Research Council Nutrient Requirements of Poultry - Ninth Revised Edition (1994). *J Applied Poultry Res*. 1994;3(1):101. doi: 10.1093/japr/3.1.101.
24. Nasrizal AA, Lowell JJ, Idris J, Nazaruddin AT, Bolong N. The Application of Statistical ANOVA, LSD and RSM to Agro-Based Filter Design Optimization. In: *International Conference on Water Resources*. Singapore: Springer Nature; 2021. p. 199-209.
25. Liechti ME. Modern clinical research on LSD. *Neuropsychopharmacology*. 2017;42(11):2114-2127.
26. Badawy M, Attia AI, Reda F, Sherasiya A, Swelum AA, El-Mekkawy MM. Effects of dietary supplementation with *Laurus nobilis* extract on growth performance, carcass features, blood lipid profile, immunity, antioxidative status, digestive enzymes, and gut microbial load in growing New Zealand white rabbits. *Trop Animal Health Prod*. 2025;57(4):1-12.
27. Travel A, Petit A, Barat P, Collin A, Bourrier-Clairat C, Pertusa M, *et al*. Methodologies to assess the bioactivity of an herbal extract on immunity, health, welfare and production performance in the chicken: The case of

- Melissa officinalis* L. extract. *Frontiers Vet Sci.* 2021;8:759456.
28. Abdelhakeam MA, Zounouny AI, Ali SM, El-Shafei MA, Tantawi AA. Influence of *Melissa officinalis* supplementation on growth performance, blood parameters, biochemical indices, antioxidant status, and rumen fluid in growing Ossimi male sheep. *Egyptian J Nutr Feeds.* 2025;28(1):29-42.
 29. Ali NAL, Al-Shuhaib MBS. Highly effective dietary inclusion of laurel (*Laurus nobilis*) leaves on productive traits of broiler chickens. *Acta Scientiarum Animal Sci.* 2021;43:e52198.
 30. Lee GY, Han SN. The role of vitamin E in immunity. *Nutrients.* 2018;10(11):1614.
 31. Ayres VE. The Effects of Feed Additives and the Use of a Novel Wood Boiler Heat Exchanger on Litter Quality, Broiler Performance, and Immune Status [Thesis]. Morgantown: West Virginia University; 2022.
 32. Miraj S, Rafieian-Kopaei SA, Kiani S. *Melissa officinalis* L: A Review study with an antioxidant prospective. *J Evidence-based Compl Alternative Med.* 2017;22(3):385-394.
 33. Ashour EA, Aldhalimi AK, Ismail IE, Kamal M, Elolimy AA, Swelum AA, *et al.* The effect of using Echinacea extract as an immune system stimulant and antioxidant on blood indicators, growth efficiency, and carcass characteristics in broiler chickens to produce a healthy product. *Poultry Sci.* 2025;104(1):104392.
 34. Shehata AI, Shahin SA, Taha SA, Elmaghraby AM, Alhoshy M, Soliman AA, *et al.* Essential Oil of Bay Laurel (*Laurus nobilis*) Enhances Growth and Immunity in Cold-Stressed Nile Tilapia (*Oreochromis niloticus*). *J Animal Physiology Animal Nutr.* 2025. [Epub ahead of print].
 35. Draginic N, Andjic M, Jeremic J, Zivkovic V, Kocovic A, Tomovic M, *et al.* Anti-inflammatory and Antioxidant Effects of *Melissa officinalis* Extracts: A Comparative Study. *Iran J Pharm Res.* 2022;21(1):e126561. doi: 10.5812/ijpr-126561.
 36. Al-Gharawi JK, Al-Yasiry A, Qader GK, Kiczorowska B, Samolinska W, Al Salman NTS, *et al.* Effect of using different methods of adding *Melissa officinalis* L. on some physiological and immunological traits of broiler. *J Al-Muthanna Agricultural Sci.* 2023;10(1):1-15.
 37. Johnson ML. The effect of maternal antioxidant nutrient supplementation and age on chick post-hatch innate immune function [Thesis]. Edmonton: University of Alberta; 2013.
 38. Gonzalez Herrero ME, Kuehn C. A qualitative mathematical model of the immune response under the effect of stress. *Chaos.* 2021;31(6):061104. doi: 10.1063/5.0055784.
 39. Emerald M. Medicinal plants: Therapeutic potential, safety, and toxicity. In: *Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays.* Cham: Springer International Publishing; 2024. p. 1327-1397.
 40. Salhi DAAM, AL-Rubaie HM, AL-Amery AM. Effect of Artemisia herba-alba and Urtica dioica herbs extracts in the biological of Eimeria tenella parasite in broiler chicks. *Iraqi J Vet Med.* 2015;39(1):1-4.
 41. Tiwari R, Latheef SK, Ahmed I, Iqbal HM, Bule MH, Dhama K, *et al.* Herbal immunomodulators-a remedial panacea for designing and developing effective drugs and medicines: current scenario and future prospects. *Current Drug Metabolism.* 2018;19(3):264-301.

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