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# EFFECTIVENESS OF PHYTOBIOTIC OILS DIETARY ADDITION AS ANTIOXIDANT ON BROILER GROWTH PERFORMANCE, GUT HEALTH, AND IMMUNE RESPONSE TO ALLEVIATE COLD STRESS

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**ABSTRACT**

This work aims to reveal the influence of Phytobiotic dietary addition as an antioxidant on broiler growth and health status, to alleviate cold stress. 600 (Ross-308) one-day-old broiler chicks were distributed into six treatments, and four replicates were as follows: T1- control: 0.0% (basal diet), T2- BHT (butyl-hydroxy-toluene)150 mg/kg basal diet, Phytobiotic oils: T3- Betony oil (0.6 ml/kg diet). T4- Peppermint oil (0.6 ml/kg diet), T5- Basil oil (0.6 ml/kg diet), T6- 0.6 ml/kg diet combination of 0.2 ml/kg diet of each Phytobiotic oil. The dietary results additive of (Betony, Peppermint, and Basil) oils and their combination had significantly higher body weight, body weight gain (BWG), feasibility, goblet cells No., villus height, villi and crypt width of the small intestine, duodenum pH and digestive enzymes (amylase, lipase, and trypsin) concentrations, high count of *Lactobacillus* bacterial, serum total protein, high density lipoprotein (HDL), antioxidant enzymes activities in serum superoxide dismutase, glutathione peroxidase, Catalase and total antioxidant capacity. However, mortality%, bacterial counts of *E.Coli* in the small intestine, glucose, total cholesterol, low density lipoprotein (LDL), triglycerides, and heat shock proteins (40 and 70), corticosterone hormone concentration, H/L ratio, and Malondialdehyde were significantly lower compared to the BHT-T2 and the control T1. In turn, all treatments of oil additives had achieved superiority in all characteristics.



## INTRODUCTION

Phytobiotic or phytogenic feed additives are bioactive compounds of plant origin that protect against bacteria, viruses, fungi, and oxidative damage caused by ultraviolet radiation (Kikusato 2021). Phyto additives such as dry plants, essential oils, and plant extracts can be used in broiler nutrition to increase performance, nutrient digestibility, intestinal health, and overall health in the broiler (Saracila *et al.,* 2020).

Poultry production faces continuous challenges in developing welfare management practices to optimize chicken efficiency while maintaining food safety. Traditionally, antimicrobials have been broadly used for improving poultry productivity and hygiene; Ricke *et al.,* (2020) warned that the risk of pathogens developing cross-resistance to antibiotics has risen in the gradual removal of antibiotics for therapeutic and prophylactic uses of animal food. Essential oils of plants have an aromatic compound that enhances attention in studies for their ability as antioxidants, anti-carcinogens, and anti-inflammatories (Alizadeh *et al*., 2013).

Ghazaghi *et al*., (2014) record the positive role on animal health, appetite stimulation, productivity, and improvement of enzyme action and secretion correlated to diet digestion and absorption for example, trypsin, amylase, and jejunum chime.

Betony (*Stachys kurdica*) belongs to the *Lamiaceae* family (Özal *et al*., 2019), it encloses various alkaloids, glycosides, tannins, saponins, and volatile oil as active ingredients, it is a rather real herbal healing in case of bronchitis, diarrhea, and nervousness, outstanding of glycoside component, that is supposed to lower blood pressure. It relaxes the whole body and gives quiet, arouses the digestive system, purifies the blood system, and even provides protection, good health, and fortune, and it is used as an anti-stressor (Vogl *et al.,* 2013). Peppermint (*Mentha piperita*) is an aromatic, practically fully constant, infrequently annual, herb, widely spread, and can be found in many environments (Brickell, 2011). Mint essential oil and menthol are extensively used as flavorings and antiseptics. The activity of the mint rests on the abundant volatile oil, which has been found to have hydrocarbon and more oxygenated compounds (Chopra *et al*., 1956). Shalayel *et al*. (2017) pointed out that peppermint oil contains menthol and thymol compounds as well as flavonoids and carotenoids that develop the immune organs and systems of birds. Basil oil (*Ocimum basilicum* Linn.) is a natural antioxidant including methyl chavicol (estragole), linalool, eugenol, and methyl cinnamate as main pharmacological constituents (Ahmed *et al.,* 2019).

Cold stress is an important problem facing broiler rearing in winter, as it has a negative influence on growth performance, health, and most disease-resistant immune system capacity (Qureshi *et al.,* 2018), by increasing the levels of corticosterone in the blood (Olfati *et al.,* 2018); therefore, the use of Phytobiotic oils may dissolve this problem.

The study was designed to evaluate synthetic and natural antioxidant sources as anti-stressors for the productive, antioxidant, gut well-being, and immune performance of cold-stressed broilers.

## MATERIALS AND METHODS

### Ethical approve

This study was approved by the Poultry farm in Grdarasha, College of Agricultural Engineering Science, Erbil/ Iraq, with the reference number UM.VET.2024.129 dated 01.11.2024.

### Design of experiment

This work was conducted in a private poultry project hall in Erbil/Iraq. 600-day-old broilers (Ross-308) were kept in floor pens of matching size (1.5×2 m), and reared at 32, 27, and 23°C during the 1$^{st}$, 2$^{nd,}$ and 3$^{rd}$ weeks, respectively. Cold stress was applied during the 4$^{th}$ and 5$^{th}$ weeks for the resting 17°C afternoon and 15°C at night throughout the day (24 hours) in winter (10/November to 15/December 2024). The birds were randomly distributed in **6**treatments, and **4**replicates were as T1- Control: without adding (basal diet), T2- BHT 150 mg/kg basal diet (butyl-hydroxy-toluene: synthetic antioxidant), Phytobiotic oils: T3- Betony oil (0.6 ml/kg diet). T4- Peppermint oil (0.6 ml/ kg diet), T5- Basil oil (0.6 ml/ kg diet), T6- 0.6 ml/ kg diet combination of 0.2 ml/ kg diet of each Betony, Peppermint, and Basil oils. The average initial body weight was 43.1g, birds were provided water and feed *ad libitum,* Feed in pellet form and freshwater were provided *ad libitum* the feed was prepared by a special feed company diets respectively as suggested in NRC (1994) Table (1), and lighting (22 h light: 2 h dark) was applied throughout the experimental period. The vaccination agenda consisted of Newcastle at 7 and 21 days and IB at 14 days of rearing, as mentioned in the Ross-308 guide (Ross-308 AP, 2018).

Table (1): Composition and nutritional levels of experimental starter and grower diets

| Ingredients | Starter (%) (1-20 d) | Grower (%) (21-35 d) |
|---|---|---|
| Corn | 42.71 | 50.350 |
| Soya bean meal 44% | 39 | 31.510 |
| Wheat | 11 | 11 |
| Fish meal 56% | 2 | 1.600 |
| Toxin binder | 0.150 | 0.150 |
| Mono Di calcium phosphate 21% | 1.200 | 1.200 |
| Soybean oil | 1.500 | 2.100 |
| DL-Methionine | 0.200 | 0.200 |
| L-Lysine | 0.140 | 0.140 |
| salt | 0.300 | 0.300 |
| Enzyme | 0.050 | 0.050 |
| Calcium Ca % | 1.700 | 1.350 |
| Anticoccidial | 0.050 | 0.050 |
| Total | 100 | 100 |
| Calculated chemical analyses | | |
| Metabolic energy kcal/ kg | 2875 | 3004 |
| Crud protein % | 22.93 | 20.30 |
| Fat % | 3.837 | 4.591 |
| fiber | 4.024 | 3.687 |
| Avail. P for poultry | 0.377 | 0.359 |
| Calcium Ca % | 1.099 | 0.919 |
| Total phosphorus P% | 0.664 | 0.623 |
| Salt | 0.349 | 0.341 |
| Arginine | 1.030 | 1.263 |
| Lysine | 1.200 | 1.120 |
| Methionine+ Cystine | 0.880 | 0.777 |
| Methionine | 0.500 | 0.496 |
| Threonine | 0.750 | 0.719 |
| Tryptophan | 0.220 | 0.238 |

**Body performance determination**

Five birds from each replicate were randomly chosen for 35 days, and body weight (BW), body weight gain (BWG), mortality, feed intake (FI), feed conversion ratio (FCR), and feasibility were determined (Abdul-Majeed and Al-Krad, 2023; Qader and Tayeb, 2024).

**pH and histomorphology of the small intestine**

At age 35 days, three birds were euthanized by cervical displacement. Intestinal duodenum, jejunum, and ileum sections were desiccated, pellucid, and fixed in paraffin oil. Serial sections were cut at 5 µm and set on glass slides. For all tests, segments were deparaffinized in xylene and dehydrated in a graded alcohol series. Sections were observed by a light microscope mentioned by (Mohammed and Al-Taee, 2024; Sadeeq and Bsski, 2024) to estimate goblet cell numbers, villus height and width, and crypt depth.

**Duodenum enzyme determination**

The frozen intestine samples were thawed in 4°C water to determine enzyme activity after homogenizing the duodenal mucosa in cold water (0.1g/ml) and centrifuging for 5 minutes at 1000 rpm and 4°C. Enzymes were determined according to the procedure's description: amylase, Trypsin, and Lipase were measured using a Randox reagent kit produced in Northern Ireland.

**Duodenum pH and microbial determination**

After the abdominal cavity was opened from two birds in each treatment replicate, the duodenum was collected in flasks to read pH. Duodenum contents were attentively kept in disinfected Petri dishes at -20°C until examined. Collected 1 gram of each homogenized sample and put it into 10 ml sterile saline solution for dilution. Then all samples were stretched on an agar plate medium, using MRS for *lactobacillus*, and McConkey for *E. Coli* (Hassan *et al.,* 2011).

**H/L ratio**

Blood smears were stained with Wright-Giemsa to determine the heterophils and lymphocytes, and calculate the ratio.

**Blood biochemical determinations**

Blood samples were collected in a heparinized test tube from the jugular vein, then centrifuged at 3000 rpm for 15 minutes to separate plasma samples, which were kept in Eppendorf tubes and frozen at 20-ºC until tests (Al-Najmawi and Al-Zubaidy, 2024). Glucose, total protein, HDL, LDL, total cholesterol, and triglyceride parameters were analysed using a spectrophotometer, and corticosterone concentrations were measured by ELISA, the kits from Buyer's Guide from Life Science Biocompare. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) enzyme activities were measured in serum (Ahmed and Abdul-Rahman, 2023), kits from Randox Laboratories Ltd, UK. Heat shock proteins 40 and 70 were examined by a kit produced by Cusabio Biotech Co. (Mustafa, 2019). The OD concentrations were determined by ELISA by Randox Laboratories Ltd, UK. Also, antibody titter response against NDV and IB (Mustafa, and Mohammed, 2020; Mohammad and Al-Mahmood, 2023), and immunoglobulin (IgA and IgG) were measured by the ELISA Kits from Bio Compare.

**Statistical analysis**

All data were analyzed by the SAS Institute program (SAS, 2014) using CRD (Completely Randomized Design) at 0.01 and 0.05 depending on the statistical model.

## RESULTS AND DISCUSSION

Table 2 displays that Betony, Peppermint, and Basil oils, or their combination, added to broilers' diet had significantly ($P \leq 0.01$) higher BW, BWG, and Feasibility (economic profit $/kg) compared to the T2 (BHT) and the control T1. While all treatments of oil additives and BHT had significantly (P≤0.05) less feed intake, mortality was significantly (P≤0.01) lower in oil treatments and BHT compared with the control. In most characteristics, the treatments of oil additives and their combinations had superior performance. Phytobiotic oil is used in broiler diets for its beneficial effects on growth, digestion, microflora, immune strength, and oxidant activity. Studies testified to the influence of these feed additives, choosing the proper protection types, techniques, and physicochemical properties to control the location and timing of the release of lively compounds (Abdelli *et al.,* 2021).

Table (2) Influence of Phytobiotic dietary addition as an antioxidant on broiler body performance at 35 days.

| Traits | T 1 | T 2 | T 3 | T 4 | T 5 | T 6 | SE |
|---|---|---|---|---|---|---|---|
| FI (g) | 3818.5 a | 3706.4 b | 3618.7 b | 3601.4 b | 3557.1 b | 3658.0 b | 173 |
| BW(g) | 2217.5 d | 2295.0 c | 2393.3 ab | 2385.0ab | 2335.6 b | 2455.0 a | 117 |
| BWG (g) | 2174.4 d | 2251. 9c | 2350.2ab | 2341.9 ab | 2292.5 b | 2411.9 a | 94 |
| FCR | 1.722 a | 1.615 b | 1.512 bc | 1.510 bc | 1.523 bc | 1.490 c | 0.10 |
| Mortality % | 2.67 a | 1.50 b | 0.75 cd | 1.00 c | 1.50 b | 0.50 d | 0.08 |
| Feasibility ($/kg) | 0.60 c | 0.74 b | 0.91 a | 0.89 a | 0.84 a | 0.92 a | 0.13 |

T1: control, T2: BHT- butylhydroxytoluene (150 mg/ kg diet), T3: Betony oil (0.6 ml/kg diet), T4: Peppermint oil (0.6 ml/kg diet), T5: Basil oil (0.6 ml/kg diet), T6: Combination of each (Betony, Peppermint, and Basil) oils (0.2 ml/kg diet). The same superscripts within rows mean insignificant: [a,b,c,d.] Means within rows with unlike superscripts differ significantly at (P≤0.01), (P≤0.05).

Betony included flavonoids, phenylethanoid glycosides, iridoids, quinic acid derivatives, aliphatic alcohol glycosides, oligosaccharides, and antimicrobial activity towards Gram-positive bacteria (Napolitano *et al.,* 2022). Linanyl acetate, α-terpineol, germacrene D, β-myrcene, α-pinene, linalool, caryophyllene oxide, β-caryophyllene, and spathulenol were detected as the major components of the essential oils of Stachys, and the most abundant components were linoleic, oleic, and palmitic acid (Kiliç *et al.,* 2017). Due to their valuable antioxidant, neuroprotective, and hypoglycemic properties (Bahadori *et al.,* 2020). Peppermint oil can be considered a growth promoter due to its high digestibility. So peppermint oil contains menthol and thymol compounds as well as flavonoids and carotenoids (Shalayel *et al*., 2017), Basil contains natural antioxidants such as linalool, eugenol, methyl chavicol, and methyl cinnamate considered pharmacological constituents (Ahmed *et al.,* 2019), these compounds may increase appetite stimulants through their effect on the appetite center in the brain via the olfactory axis and pharyngeal to stimulate birds to eat feed (Nguyen *et al.,* 2023), caused an improvement in broiler BW, BWG, FCR developments might be qualified to the antimicrobial and antioxidant activities of the EO used, which can improve gut health and stimulate digestive enzymes, results of improving the digestibility of dietary nutrients for growth (Du *et al.,* 2016) during cold stress condition. Vlaicu *et al.* (2023) see the EOs additions in broiler diets as an antibiotic replacement to improve growth, FCR, and reduce feed costs. The development of digestive enzyme secretion (Table 4) as amylase, lipase, trypsin, *lactobacillus* (microflora) counts, villi height and length of the small intestine (Table 3), the antioxidant status (Table 5) and immune response (Table 6) this advantage to maximize the availability nutrients in poultry feed by raising the permeability of intestinal mucosa lead to the high absorption of nutrients, which led to removal of cold stress and to improve the public health of broilers, so effect on the proliferation of satellite cells (muscle cells) that is reflected the precursor of myogenic cells, which affects increasing of growth and development of skeletal musculature (Hussein *et al.,* 2021; Beghoul *et al.,* 2022).

The dietary addition that betony, peppermint, and basil, or their combination oils, as the BHT (T2) had significantly (P≤0.01) higher goblet cell numbers, villus height, villi width and crypt width in all parts of broiler small intestine (duodenum, jejunum and ileum) compared with the control T0, except T2 in jejunum crept depth had insignificant with the control as clarified in (Table 3). In turn, oil additive treatments had achieved superiority in small intestine histology compared with BHT and the control. This development is seen in BW, BWG, and FCR.

The benefit of antioxidants originates in Phytobiotic, as the protection of intestinal villi occurs through the cellular activity of antioxidants, which enhances nutrient absorption in the villi. Additionally, the active compounds in Phytobiotic oils act as factors that increase digestibility, balancing the intestinal microbial ecosystem, and encourage secretion of endogenous enzymes, resulting in enhancing growth in poultry (Emami *et al*., 2012, and Ghazanfari *et al*., 2024).

Table (3) Influence of Phytobiotic dietary addition as an antioxidant on small intestine histomorphology of broiler at age 35 days.

| Traits | | T 1 | T 2 | T 3 | T 4 | T 5 | T 6 | SE |
|---|---|---|---|---|---|---|---|---|
| Duodenum | NGC[1] | 9.03 c | 10.42 b | 14.10 a | 14.35 a | 13.21 ab | 13.95 a | 1.35 |
| | VH (µm) | 882.7 d | 1040.3 c | 1301.4 ab | 1305.0 ab | 1283.1 b | 1345.3 a | 130 |
| | VW (µm) | 123.6 c | 175.4 b | 217.9 a | 220.0 a | 211.3 a | 225.3 a | 6.8 |
| | CW(µm) | 90.0 c | 102.5 b | 132.0 a | 127.1 a | 124.3 a | 135.5 a | 5.0 |
| Jejunum | NGC[1] | 9.85 d | 11.05 c | 16.70 a | 15.90 ab | 15.11 b | 16.45 a | 1.13 |
| | VH (µm) | 929.3 d | 1230.3 c | 1493.4 b | 1455.0 ab | 1407.8 b | 1513.1 a | 141 |
| | VW (µm) | 121.3 c | 170.1 b | 217.3 a | 205.3 a | 199.5 a | 213.6 a | 5.7 |
| | CW(µm) | 87.0 c | 89.4 c | 110.3 a | 112.4 a | 104.8 b | 116.8 a | 4.3 |
| Ileum | NGC[1] | 8.15 d | 9.55 c | 12.60 ab | 11.91 b | 11.77 b | 13.29 a | 1.24 |
| | VH (µm) | 863.6 d | 1170.1 c | 1281.5 a | 1245.8ab | 1233.6 ab | 1275.5 a | 141 |
| | VW (µm) | 120.3 c | 168.1 b | 205.7 a | 200.3 a | 192.0 a | 203.2 a | 6.2 |
| | CW(µm) | 89.5 d | 101.3 c | 117.6 ab | 115.4 ab | 110.8 b | 119.0 a | 4.7 |

[1]NGC: number of goblet cells per mm of villus length. VH: villus height, VW: villi width, CW: crypt width. $^{a-d}$ Means within rows with different superscripts differ significantly at (P≤ 0.01).

The results in table (4) explain significant (P≤0.01) high improvement in the duodenum pH and digestive enzymes (amylase, lipase, and trypsin) concentrations in the oils additive treatment compared with the BHT and the control treatment. BHT in turn better in all characteristics compared with the control. Also, the same table reveals small intestine gut health, the results show significantly ($P \leq 0.01$) higher *Lactobacillus* bacterial counts (microflora), however ($P \leq 0.01$) lower bacterial counts of *E.Coli* in the small intestine in the oils and BHT treatments compared with the control. The results of all characteristics show the fulfillment of oil treatments compared with the BHT and the control. Adding essential oils to the diet increases the intestine goblet cell numbers that have responsibility for the intestinal mucin production, the main component of the mucus layer that lines the chicken intestine, it defends the host against luminal microflora, prevents gastrointestinal pathology and partakes in nutrients digestion and absorption (Ghazanfari *et al.,* 2024). Agostini *et al.* (2012) conclude that adding essential oils to broiler diet increased *Lactobacilli* counts, the positive choice of active compounds by lactic acid bacteria.

Table (4) Influence of Phytobiotic dietary addition as an antioxidant on duodenum pH, digestive enzymes concentration (ppm), and small intestine bacterial counts (cfu×log $10^{-3}$) in broiler at age 35 days.

| Traits | | T 1 | T 2 | T 3 | T 4 | T 5 | T 6 | SE |
|---|---|---|---|---|---|---|---|---|
| duodenum parameters | pH | 4.83 c | 5.20 b | 6.73 a | 6.58 a | 6.51 a | 6.57 a | 0.33 |
| | Amylase (ppm) | 2.23 c | 2.89 b | 3.68 a | 3.71 a | 3.70 a | 3.79 a | 0.26 |
| | Lipase (ppm) | 9.83 d | 11.25 c | 13.06 a | 11.88 b | 13.02 a | 12.93 a | 0.38 |
| | Trypsin (ppm) | 22.78 d | 25.67 c | 29.01 a | 28.55 ab | 27.68 b | 29.65 a | 1.25 |
| bacterial counts | *Lactobacillus* (cfu × log $10^{-3}$) | 5.04 c | 6.32 b | 8.55 a | 8.73 a | 8.95 a | 8.90 a | 0.41 |
| | *E. Coli* (cfu × log $10^{-3}$) | 9.75 a | 5.61 b | 2.31 c | 2.11 c | 2.05 c | 2.10 c | 0.26 |

[a – d] Means within rows with different superscripts differ significantly at ($P \leq 0.01$).

Table (5) confirms the dietary addition of Phytobiotic oils in T3, T4, and T5 or their combination in T6 shows a significant ($P \leq 0.01$) increase in serum total protein, HDL, antioxidant enzymes activities in serum SOD, GPx, CAT and TAC in all treatments of oil additives and BHT compared with the control T1. Otherwise, glucose, TCH, LDL, TG, and stress indicators: HSP 40, HSP 70, corticosterone hormone concentration, and H/L ratio significantly ($P \leq 0.01$), and MDA had a significant ($P \leq 0.05$) decrease in oil treatments compared to BHT and the control.

Cells commonly generate reactive oxygen species (ROS) and reactive nitrogen species (RNS), which interrupt normal oxidant and antioxidant cellular homeostasis, leading to oxidative stress (Alfadda and Sallam, 2012). The oxygen having compounds can be approximately categorized by their oxygen-containing capability, and potent non-radicals (Lushchak, 2014; Oyinloye *et al.,* 2015). ROS accumulated levels are more reactive and potent than normal oxygen and nitrogen, thus causing deleterious effects on the living system. Despite all negativities associated with accumulated cellular ROS, several studies have shown that, at low or moderate levels of unknown concentration, ROS perform important beneficial roles, including acting as subordinate messengers in signal transfer, which enhances immune resistance in antibacterial infections in the phagosome (Alfadda and Sallam, 2012). Furthermore, oil additives, including fatty acids and volatile oils, which help reduce heat shock proteins (HSP) 40 and 70, Phytobiotic oils can be rather useful in treating and managing various HSPs, which in turn leads to lower corticosterone and MDA in plasma concentrations and H /L ratio.

Conversely, GPx, SOD, and CAT concentrations increased in plasma in all treatments of oil and BHT compared with the T1, remarkably oil additives resulted in higher improvement compared with BHT (synthetic antioxidant) and the control T1. H / L ratios correlate positively with corticosterone levels in blood and stress. Stress factors such as food, water insufficiency, temperature excesses, constant light, and exposure to novel social conditions increase the number of heterophils and decrease the number of lymphocytes, leading to an increased H/L ratio in blood (Kraft *et al.,* 2019). The results in Table (5) prove that adding Phytobiotic oils to the broiler diet or their mix led to a lower H/L ratio in the blood, an indicator of improving mood, health status, and welfare of broilers.

Table (5): Influence of Phytobiotic dietary addition as an antioxidant on some biochemical parameters, antioxidant enzymes activities, stress indicators in broiler serum at age 35 days.

| Traits | | T 1 | T 2 | T 3 | T 4 | T 5 | T 6 | SE |
|---|---|---|---|---|---|---|---|---|
| Glucose (mg/dl) | | 154.5 a | 139.0 a | 99.7 b | 105.2 b | 102.9 b | 98.1 b | 5.47 |
| Total protein (g/dl) | | 2.89 b | 3.07 b | 4.02 a | 3.89 a | 3.95 a | 4.13 a | 0.27 |
| Lipid profile (mg/dl) | HDL | 24.31 d | 31.00 c | 41.50 ab | 40.19 b | 43.8 a | 41.35 ab | 2.16 |
| | LDL | 129.19 a | 106.3 b | 67.8 d | 73.01 c | 67.60 d | 67.75 d | 3.08 |
| | TCH | 153.5 a | 137.3 b | 109.3 c | 113.2 c | 111.4 c | 109.1 c | 4.28 |
| | TG | 277.5 a | 243.0 b | 187.2 c | 190.0 c | 189.4 c | 185.6 c | 5.07 |
| Anti-oxidant enzyme activities (U/mL)[1-4] | SOD[1] | 113.7 c | 128.5 b | 139.1 a | 136.4 a | 138.3 a | 140.3 a | 4.25 |
| | GPx[2] | 119.4 d | 142.7 c | 168.9 a | 165.1 ab | 163.4 b | 170.0 a | 6.28 |
| | CAT[3] | 6.75 c | 11.38 b | 15.37 a | 15.12 a | 14.79 a | 15.20 a | 0.61 |
| | TAC[4] | 17.28 d | 23.09 c | 28.25 a | 26.98 ab | 26.33 b | 29.05 a | 1.93 |
| | MDA (nmol/ml) | 9.05 a | 6.75 b | 6.08 b | 6.42 b | 6.33 b | 6.13 b | 0.19 |
| Stress indicators | HSP 40 (ng/ml) | 136.1 a | 119.2 a | 64.3 c | 75.3 bc | 82.8 b | 67.5 c | 2.17 |
| | HSP 70 (ng/ml) | 215.6 a | 193.1 a | 117.4 b | 120.0 b | 123.8 b | 119.5 b | 4.18 |
| | Cs (ng/ml) | 18.5 a | 16.72 a | 11.6 b | 12.5 b | 12.9 b | 12.04 b | 0.53 |
| | H / L ratio | 0.469 a | 0.351 b | 0.193 d | 0.208 c | 0.215 c | 0.204 cd | 0.094 |

HDL: High density lipoprotein (mg/dl), LDL: low density lipoprotein (mg/dl), TCH: Total cholesterol (mg/dl), TG: Triglycerides (mg/dl). SOD activity: super oxide dismutase (U/mL), GPx: glutathione peroxidase(U/mL), CAT: Catalase (U/mL), TAC: total antioxidant capacity (U/mL), MAD: Malondialdehyde (nmol/ml), HSP: Heat Sock Protein (ng/ml), Cs: Corticosterone (ng/ml). [a,b,c,d] Means within rows with different superscripts are different at ($P \leq 0.01$) and ($P \leq 0.05$).

Table (6) points out the dietary addition of betony, peppermint, and basil, or their combination oils, the BHT-T2 had significantly ($P \leq 0.01$) higher on broilers antibodies titer against ND and IBV by ELISA, the concentration of Immunoglobulins (IgA and IgG) in blood serum had significantly ($P \leq 0.01$) higher increased in the all-addition treatments compared with the control T0. In all characteristics, the dietary oil additives in T3, T4, T5, and T6 improved compared with the BHT (synthetic antioxidant) in T1.

The Phytobiotic oils may develop cells' immunity, increase the struggling ability of macrophage cells, and improve the action of T and B cells, which are responsible for the immune state (Mustafa and Shokry, 2024). The improvement in the immune status of birds that received Phytobiotic oils may be due to phytochemical compounds such as flavonoids and phenolic, which have strong antimicrobial and antioxidant properties led to scavenging and redox possessing, also for their ability to neutralize and quench free radicals and pathogenic microbes attacking birds' digestive and immune systems (Oblakova *et al.,* 2022).

The immune system plays a significant role in conserving health and averting diseases. A healthy immune system assists broilers in fighting infections, prevents diseases, and reduces their development. In the future, constructing a balance between the growth rate and the activity of the immune system creates more demand for peak poultry production (Galarneau *et al.,* 2020).

Table (6): Influence of Phytobiotic dietary addition as an antioxidant on broiler immune status at age 35 days.

| Traits | | T 1 | T 2 | T 3 | T 4 | T 5 | T 6 | SE |
|---|---|---|---|---|---|---|---|---|
| Antibodies titter (ng/ml) | ND | 2349 d | 3178 c | 4900 ab | 4882 ab | 4718 b | 5025 a | 136.0 |
| | IBV | 1368 d | 2170 c | 3142 a | 3049 ab | 2855 b | 3015 ab | 86.3 |
| Immuno-globulins (mg/ml) | IgA | 1.19 c | 1.46 b | 2.40 a | 2.26 a | 2.01 ab | 2.35 a | 0.153 |
| | IgG | 8.56 c | 10.92 b | 14.03 a | 13.93 a | 13.85 a | 14.20 a | 0.349 |

ND: Newcastle disease, IB: Infectious Bronchitis Viral disease. [a,b,c,d] Means within rows with different superscripts are significant at (P≤ 0.01).

## CONCLUSIONS

The effect of dynamic properties of Phytobiotic oils in betony, peppermint, and basil as antioxidants for scavenging free radicals to improve body performance in broilers exposed to cold stress, also improving BWG and FCR, furthermore lowering mortality, which reflects on the feasibility of this trial project. Likewise, the improvement of small intestine morphology, particularly the duodenum, enhanced the secretion of duodenum digestive lactobacillus and enzymes. Volatile oils and values of nutrient in the oil's dietary addition enhanced birds' appetite which increases the immune response to viral diseases, decreased the efforts of cold stress may lower HSP expression and impairment in intestinal mucosa, which could effectively scavenge oxygen free radicals, induce body oxidation decline system imbalance, lessen oxygen free radical damage to cells in mucosal tissues, and increase the absorbency of intestinal mucosa, thus efficiently preserving the structure and function of the intestinal barrier. Finally, the use of 0.6 ml oil/kg diet of each betony, peppermint, and basil or their combination (0.2 ml/kg of each one) in this study plays an important role in cold stress, especially betony oil and the oil combination group achieved more improvement during the cold condition.

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**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

## تأثير إضافة الزيوت النباتية الحيوية إلى العلف كمضاد للأكسدة على الأداء الانتاجي لدجاج فروج اللحم وصحة الأمعاء والاستجابة المناعية للتخفيف من الإجهاد البارد

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**الخلاصة**

هدفت هذه الدراسة إلى البحث عن تأثير إضافة الزيوت النباتية كمضاد للأكسدة على نمو دجاج فروج اللحم وحالته الصحية، للتخفيف من الإجهاد البارد. تم توزيع ستمائة (روس–308) من افراخ دجاج اللاحم بعمر يوم واحد عشوائيًا على ست معاملات وأربع مكررات على النحو التالي: T1– السيطرة: 0.0٪ (العلف الأساسي)، T2– BHT (بيوتيل هيدروكسي تولوين) 150 ملغم/ كغم علف، الزيوت النباتية،: T3 زيت البطنج (0.6 مل/ كغم علف)،) T4 زيت النعناع (0.6 مل/ كغم علف)، T5 زيت الريحان (0.6 مل/ كغم علف)، T6 خليط 0.2 مل/ كغم لكل من الزيوت (البطنج والنعناع والريحان). أظهرت النتائج الغذائية للإضافة الزيوت (البطنج، النعناع والريحان) أو خليطهما ارتفاعًا معنويًا في وزن الجسم، الزيادة الوزنية، والجدوى الاقتصادي، وزيادة في أعداد ال خلايا الكأسية، وارتفاع الزغابات، وعرض الزغابات في الأمعاء الدقيقة (الاثني عشر، الصائم واللفائفي)، ودرجة حموضة في الاثني عشر وتركيزالإنزيمات الهضمية (الأميليز، اللابيز والتربسين)، وارتفاع عدد بكتيريا اللاكتوباسيلوس (البكتريا اللبنية)، وارتفاع مصل الدم من (البروتين الكلي، البروتين الدهني عالية الكثافة HDL، وأنشطة الإنزيمات المضادة للأكسدة SOD، والجلوتاثيون بيروكسيديز، والكاتاليز، (TAC، في حين نسبة الهلاكات، عدد بكتيريا القولون في الأمعاء الدقيقة، الكلوكوز، الكوليسترول الكلي، البروتين الدهني واطئة الكثافة LDL، الدهون الثلاثية، وبروتينات الصدمة الحرارية HSP 40 و70 HSP، تركيز هرمون الكورتيكوستيرون، ونسبة H/L، و المالونداياالديهايد (MDA) انخفض معنويا في معاملات اضافة الزيوت النباتية مقارنة بيوتايل هيدروكسي تولوينBHT–T2ومعاملة السيطرةT1. حققت جميع معاملات إضافات الزيت تفوقًا في جميع الصفات المدروسة.

الكلمات المفتاحية: مضادات الأكسدة، فروج اللحم، الإجهاد البارد، الأداء الإنتاجي، الزيوت النباتية.

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