

Effect of supplementation *Origanum Majorana* powder to Pigeon diets on antioxidant status, performance and blood Hematology

Abstract

This study aimed to evaluate the effect of adding *Origanum majorana* (OM) powder to domestic pigeon diets on growth performance; blood hematology; blood biochemical parameters; blood inflammatory and oxidative markers; carcass characteristics. Fifty-four unsexed pigeon squabs (average body weight 321g and 30 days old) were randomly divided into three groups. The first group was fed the grower basal diet without adding OM powder, while it was added at the levels of 0.5 and 1% into the basal diets of the 2nd and 3rd groups, respectively. Obtained result show that, there are significant increase in globulin level, and glutathione peroxidase enzyme, While heterophils, heterophil to lymphocyte ratio, malondialdehyde level was significantly ($P<0.05$) reduced. Moreover, blood examination showed positive responses to OM powder as red blood cells, hemoglobin, hematocrit, mean corpuscular volume, white blood cells, and lymphocytes. These results indicated that adding OM powder to the pigeon diet may improve their immunity, increase their antioxidant status, and correct some hematological disorders.

Keywords: *Origanum majorana* powder, performance, antioxidant, malondialdehyde hematology

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Introduction

Compared to other animal outputs, poultry production is distinguished by reduced costs, greater feed conversion, and fewer associated environmental and health issues.^{1,2} It was crucial for researchers to look into new feed additives and incorporate them into chicken diets in order to improve the health, growth performance, and immune response of poultry. The use of herbal plants and their extracts as growth promoters and immunological modulators in place of antibiotics, which have negative effects on poultry, is seen to be a promising additive in chicken production.^{3,4}

Origanum majorana (OM), also known as sweet marjoram, is a creeping, aromatic, medicinal herb plant⁵ that belongs to the *Lamiaceae* family and is widely used in North Africa and Western Asia.⁶ Because of its high content of phenolic compounds, flavonoids, and essential oils⁷ containing borneol, terpinene, pinene, sabinene, and terpineol, *Origanum majorana*⁸ has antioxidant, antibacterial, antifungal, antiseptic, analgesic, immune modulator, and metabolism inducing properties.⁹ Additionally, OM extract may guard against hyperlipidemia¹⁰ and liver and kidney damage.⁶

When broilers were fed diets supplemented with prebiotics, probiotics, or herbal blends (*Origanum majorana*, *Carum carvi*, and *Foeniculum vulgare*) as antibiotic alternatives, the herbal blend group outperformed the others.¹¹ Additionally, when Saleh et al.¹²

supplemented the diets of laying hens with OM and another medicinal herbal plant, they observed an improvement in the hens' productive performance, including the feed conversion ratio and the quality and quantity of their eggs.

Domestic pigeons are frequently sold in Egypt due to its extremely marketable and delectable flesh, which suggests that it has a good nutritional value, easy maintenance and raising, and quick weight gain.¹³ To our knowledge, there haven't been many research done to assess the OM powder's impacts on the pigeon diet's nutritional, behavioral, antioxidant, and immunomodulatory components.

In this study, we hypothesize that adding OM powder to pigeon diets may modulate their growth performance, immune response, and antioxidant status. Thus, the effect of adding OM powder to the pigeon diet on growth performance, feeding and drinking behavior, blood hematology, blood biochemical parameters, antioxidant and inflammatory markers was investigated in this study.

Material and methods

The experiment was carried out in the research animal house belonging to the Faculty of Veterinary Medicine, Assiut University, Egypt. Ethical bird management and treatment were followed according to the guides by the Animal Care Committee of Assiut University, Egypt. 6102017Yahya

Origanum majorana powder

Origanum majorana (OM) powder was obtained from a commercial source (Organic, Natural Oil Factory, Assiut, Egypt), organized, and analyzed in accordance with the AOAC¹⁴ strategies in the Animal Nutrition and Clinical Nutrition Lab., Faculty of Veterinary Medicine, New Valley University, Egypt. Herein, 95.5% dry matter (DM, AOAC official method 930.15), 14% crude protein (CP, AOAC official method 984.13), 3.3% ether extract (EE, AOAC official method 920.39), 17.5% crude fiber (CF, AOAC method 978.10), and 10.3% ash (AOAC official method 942.05) made up the chemical composition. Metabolizable energy (ME) (2712 Kcal/Kg diet) was calculated using NRC (15's chemical composition). Furthermore, the active principles of OM powder were examined in the Chemistry Lab at Assiut University's Faculty of Science in Assiut.¹⁵ The active principles included thymol (4.2%), terpineol contents (alpha-terpinene 6.8%, gamma-terpinene 5.5%), alpha-terpinolene 3.6%, alpha-phellandrene 2.07% and alpha-terpinolene 1.5%, (1-Pyrrolyl) phenol 1.7%, caryophyllene 1.5%, carene 1.1%, fluoro-5-(1-hydroxy-2-(methylamino)ethyl) phenol 0.2%, aminopropyl phenol 0.09%, 5-methyl phenol 0.14%, and cathine 0.02%. Previous studies^{11,12} were used to determine the amount of OM powder to include in the diets.

Birds, diets, and experimental design

A total number of 54 unsexed pigeon squabs (30 days old, average body weight; 321g) obtained from a local commercial source were randomly divided into three groups (n=18), with three replicates of 6 birds per replicate. The first group was fed the grower basal diet without supplementing it with OM powder, whereas the second and third groups' basal diets were supplemented with it at 0.5 and 1%, respectively. The diet was in the form of mash. Table 1 shows the ingredients and chemical composition of the grower basal diet. The temperature was adjusted to meet the needs of the birds (18-23 °C). There was both natural and mechanical ventilation. There was free access to both water and feed.

Table 1 Ingredient and chemical composition of the grower basal diet of pigeon squabs.

| Item | Control diet* |
|-------------------------------|---------------|
| Ingredient, % DM | |
| Yellow corn | 75.94 |
| Soybean meal | 19.72 |
| Supplement | 4.34 |
| Chemical composition | |
| CP, % DM | 16.0 |
| CF, % DM | 2.88 |
| EE, % DM | 2.97 |
| Available Ph, % DM | 0.40 |
| Ca, % DM | 1.20 |
| Lysine, % DM | 0.80 |
| Methionine, % DM | 0.30 |
| ME, Kcal/Kg diet ¹ | 2988 |

*Grower basal diet was fed to the three groups of pigeons with supplementation of Marjoram powder at the level of 0.5% and 1% to the 2nd and 3rd groups of pigeons. ¹ME: metabolizable energy was calculated using ME of the ingredients according to NRC (1994).

Growth performance parameters

Each bird's body weight was recorded at the start of the experiment. Following that, pigeon squabs' individual body weight, cumulative

body weight, and feed intake were all recorded weekly. The feed conversion ratio (FCR) was calculated using the body weight gains and feed consumption. European production efficiency index (EPI) and relative growth rate (RGR) were calculated according to^{16, 17}, respectively.

Carcass characteristics and blood examination

The experiment lasted 45 days. At the experimental end, three birds from each group were randomly selected, weighed, and euthanized by slaughtering. During slaughtering, blood samples were taken from the *cervical vein*. Heparinized and non-heparinized tubes (Vacutainer, Becton Dickinson, USA) were used to store the blood.¹⁸

Blood hematology

The tubes containing heparin were utilized for assessing the number of red blood cells (RBCs), white blood cells (WBCs), concentration of hemoglobin (Hb) in the blood, average size of red blood cells (MCV), concentration of hemoglobin in each red blood cell (MCH), volume of packed red blood cells (HCT), and the breakdown of different types of white blood cells. The ratio of heterophils to lymphocytes was calculated. RBCs and WBCs were counted with a hemocytometer, and blood smears were stained with the Wright-Giemsa stain.

Blood biochemical parameters

The blood samples in the remaining tubes were spun (for 15 min, at 3000 rpm, 4 °C) and stored at -20 °C until additional examination. The levels of complete proteins, albumin, globulin, overall cholesterol, urea, and creatinine were measured utilizing commercially available kits (Egyptian Company for Biotechnology, Cairo, Egypt).

Serum inflammatory and oxidative markers

TNF- α and interleukin 6 (IL6) were measured for inflammation using an ELISA Kit for chicken (Egyptian Company for Biotechnology, Cairo, Egypt). The levels of malondialdehyde (MDA) and glutathione peroxidase (GPx) as oxidative markers were determined using commercial colorimetric kits (Egyptian Company for Biotechnology, Cairo, Egypt) and a spectrophotometer (Unico UV 2000; Spectra Lab Scientific Inc., VA, USA).

Statistical analysis

Statistical analysis of the effect of OM on growth parameters, behavior, carcass characteristics, blood hematology, blood parameters, and inflammatory and oxidative markers in pigeons was performed using the Statistical Package for Social Science (SPSS 26.0). The Duncan's Multiple Range Test was used for treatment means comparison, in significance level at 5%.

Results and discussion

Growth performance parameters

Table 2 show that, there are non-significant effect of *Origanum majorana* powder's using in pigeon squab diet on growth performance parameters (body weight, weight gain, feed intake, feed conversion, production index, or relative growth rate) this data agreed with previously obtained data of Khattab et al.¹⁹

Hematological parameters

The impact of the powder on blood hematology is presented in Table 3. With OM powder, red blood cells [RBCs], hemoglobin [Hb],

hematocrit [HCT], mean corpuscular volume [MCV], white blood cells [WBCs], and lymphocyte percentage all showed elevated values [$p < 0.05$]. In contrast, the percentage of heterophils and the ratio of heterophils to lymphocytes were significantly lower [$p < 0.01$].

Origanum majorana powder greatly boosted both red blood cell (with 1% OM) and white blood cell counts, potentially due to thymol (active ingredient in OM), which enhances the immune response.²⁰ Meanwhile, the beneficial effect of OM on hemoglobin levels may be attributed to its higher iron content, which is a vital nutrient for the production of hemoglobin.²¹

Blood biochemical parameters, serum inflammatory markers, and oxidative markers

Table 4 illustrated that, OM supplements are available. The powder had no effect on serum total protein, albumin, creatine, or urea levels. In contrast, globulin was higher [$p < 0.05$], and the albumin-to-globulin ratio was lower [$p < 0.05$] in the treated groups. This data agreed with finding of Shawky et al.²² Moreover, hypocholesterolemia data of OM was agree with¹² and may be due to carvacrol and thymol present in OM could reduce cholesterol levels by inhibiting hepatic 3-hydroxy-3-methyl-glutaril198 CoA reductase.²³

Table 2 The effect of incorporating *Origanum majorana* powder into the diet of pigeons on growth performance parameters

| Item | Groups* | | | SEM | P-value |
|---------------|---------|------|------|------|---------|
| | Control | 0.5M | 1M | | |
| Initial BW, g | 322 | 321 | 324 | 10.3 | 0.98 |
| Final BW, g | 424 | 438 | 439 | 13.8 | 0.68 |
| BWG, g | 102 | 115 | 117 | 10.5 | 0.57 |
| Total FI, g | 1178 | 1207 | 1237 | 41 | 0.62 |
| FCR, g/g | 4.7 | 5.8 | 5.2 | 0.87 | 0.73 |
| EPI | 12.8 | 12.5 | 12.2 | 1.97 | 0.98 |
| RGR | 17.5 | 19.2 | 19.1 | 2.34 | 0.85 |

Means within the same row with different superscripts differ significantly ($P < 0.05$). *Groups: Pigeons fed only basal diet - control group, 0.5M - pigeons fed with addition of marjoram powder at the level of 0.5%, 1.0M - pigeons fed with addition of Marjoram powder at the level of 1%, BW, bodyweight; BWG, bodyweight gain; FCR, feed conversion ratio; FI, feed intake; EPI; RGR; relative growth rate; European production index.

Table 3 The effect of adding *Origanum majorana* powder to pigeon diet on blood hematology

| Item | Groups* | | | SEM | P-value |
|------------------------------------|--------------------|--------------------|--------------------|-------|---------|
| | Control | 0.5M | 1M | | |
| RBCs ($\times 10^6/\text{mm}^3$) | 4.27 ^b | 4.43 ^b | 5.37 ^a | 0.145 | < 0.01 |
| Hb (g/dl) | 11.70 ^c | 12.30 ^b | 14.83 ^a | 0.135 | < 0.01 |
| HCT (%) | 38.90 ^c | 40.00 ^b | 48.70 ^a | 0.149 | < 0.01 |
| MCV (fl) | 89.15 ^c | 92.10 ^b | 96.55 ^a | 0.648 | < 0.01 |
| MCH (pg) | 27.63 | 27.76 | 27.96 | 0.652 | 0.93 |
| WBCs ($\times 10^3/\text{mm}^3$) | 38.33 ^b | 43.33 ^a | 44.00 ^a | 0.981 | 0.01 |
| Monocyte% | 7.00 | 7.00 | 8.00 | 0.577 | 0.42 |
| Heterophil% | 42.33 ^a | 27.33 ^b | 27.33 ^b | 2.769 | 0.01 |
| Lymphocyte% | 45.00 ^c | 73.00 ^a | 65.66 ^b | 1.981 | < 0.01 |
| H/L ratio | 0.95 ^a | 0.385 ^b | 0.42 ^b | 0.049 | < 0.01 |

Means within the same row with different superscripts differ significantly ($P < 0.05$). *Groups: Pigeons fed only basal diet - control group, 0.5M - pigeons fed with addition of Marjoram powder at the level of 0.5%, 1.0M - pigeons fed with addition of Marjoram powder at the level of 1%; H/L ratio, heterophil to lymphocyte ratio; WBCs, white blood cells; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; HCT, hematocrit value; Hb, blood hemoglobin; RBCs, red blood cells count.

Table 4 The effect of adding *Origanum majorana* powder to pigeon diet on blood biochemical parameters as well as serum inflammatory and oxidative markers

| Item | Groups* | | | SEM | P-value |
|---------------------|-------------------|-------------------|-------------------|------|---------|
| | Control | 0.5M | 1M | | |
| Total protein, g/dl | 5.05 | 5.37 | 5.30 | 0.16 | 0.37 |
| Albumin, g/dl | 3.40 | 3.23 | 3.25 | 0.08 | 0.34 |
| Globulin, g/dl | 1.65 ^b | 2.13 ^a | 2.05 ^a | 0.11 | 0.04 |
| A/G ratio | 2.06 ^a | 1.53 ^b | 1.59 ^b | 0.08 | 0.01 |
| Cholesterol, mg/dl | 194 ^a | 159 ^b | 148 ^b | 9.46 | 0.03 |
| Urea, mg/dl | 37.1 | 34.1 | 37.7 | 2.07 | 0.47 |
| Creatinine, mg/ dl | 0.39 | 0.48 | 0.43 | 0.02 | 0.18 |
| Interleukin 6 ng/l | 273 | 284 | 279 | 23.2 | 0.95 |
| TNF α Pg/ml | 222 ^b | 230 ^a | 236 ^a | 6.90 | 0.09 |
| MDA nmol/ml | 8.95 ^a | 6.17 ^b | 4.50 ^c | 0.32 | < 0.01 |
| GPx mu/ml | 38.5 ^c | 102 ^b | 124 ^a | 5.57 | < 0.01 |

Means within the same row with different superscripts differ significantly ($P < 0.05$). * Groups: Pigeons fed only basal diet - control group, 0.5M - pigeons fed with addition of Marjoram powder at the level of 0.5%, 1.0M - pigeons fed with addition of marjoram powder at the level of 1%; GPx, glutathione peroxidase; MDA, malondialdehyde; TNF α , tumor necrosis factor α ; A/G ratio, albumin/globulin ratio.

Finally, Interleukin 6 remained unaffected, while tumor necrosis factor appeared to be [$p = 0.09$] elevated by adding OM powder. Furthermore, serum oxidative markers, specifically malondialdehyde and glutathione peroxidase enzyme, decreased and increased significantly, respectively. Substances that stimulate leukocytosis may induce cytokine secretion from these cells, including interleukin 6 and TNF.²⁴ Thus, the tendency for TNF to increase may be a compensatory response to leukocytosis. In contrast to our findings, Arranz et al.²⁵ proposed that the essential oil derived from OM has anti-inflammatory properties because it contains terpineol and sabinene hydrate, both of which have a negative impact on cytokine production.

In the present investigation, OM powder significantly reduced the MDA level and enhanced the glutathione peroxidase level. The relationship between the abundance of phenolics and flavonoids in OM (such as carnosol, carnosic acid, and hydroxycinnamic acid) and its antioxidant activity has been studied [6]. Consequently, OM can have a crucial function in upholding the regular physiology, production, health, and welfare of animals.

Conclusion

Supplementation of OM powder into the grower diet for pigeons increased the body weight, serum globulin level, lymphocyte and WBCs count. These findings indicate that OM powder could potentially boost bird immune system. The elevated levels of Hb, HCT, and MCV suggest its potential to promote blood cell production. The reduction in H/L ratio, MDA, and increase in GPx levels suggest that OM powder may possess antioxidant properties. To summarize, incorporating OM powder into pigeon diet could be crucial in reducing stress, addressing certain blood-related issues, and maintaining overall bird health and well-being. However, further research is still needed to fully understand its effects.

Conflicts of interest

The authors declared that there are no conflicts of interest.

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References

1. Dagher N, Diab-El-Harake M, Kharroubi S. Poultry production and its effects on food security in the Middle Eastern and North African region. *J Appl Poult Res*. 2021;30:100110.
2. Puvaca N, Tufarelli V, Giannenas I. Essential oils in broiler chicken production, immunity and meat quality: Review of *Thymus vulgaris*, *Origanum vulgare*, and *Rosmarinus officinalis*. *Agriculture*. 2022;12(6):874.
3. Seidavi A, Tavakoli M, Slozhenkina M, et al. The use of some plant-derived products as effective alternatives to antibiotic growth promoters in organic poultry production: A review. *Environ Sci Pollut Res Int*. 2021;28(35):47856–47868.
4. Sakr SA, El-Emam HA, Naiel MAE, et al. The impact of paulownia leaves extract enriched diets on performance, blood biochemical, antioxidant, immunological indices and related gene expression of broilers. *Front Vet Sci*. 2022;9:859.
5. Cala A, Salcedo JR, Torres A, et al. Study on the phytotoxic potential of the seasoning herb marjoram [*Origanum majorana* L.] leaves. *Molecules*. 2021;26(11):3356.
6. Yen LT, Park J. The complete chloroplast genome sequence of *Origanum majorana* L. *Mitochondrial DNA B Resour*. 2021;6(3):1224–1225.
7. Kordali S, Kabaagac G, Sen I, et al. Phytotoxic effects of three *Origanum* species extracts and essential oil on seed germinations and seedling growths of four weed species. *Agronomy*. 2022;12(10):2581.
8. Banchio E, Bogino PC, Zygadlo J, et al. Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanum majorana* L. *Biochem Syst Ecol*. 2008;36:766–771.
9. Bina F, Rahimi R. Sweet marjoram: A review of ethnopharmacology, phytochemistry, and biological activities. *J Evid.-Based Complement Altern Med*. 2017;22(1):175–185.
10. El-Ghany A, Nanees Y. Effect of marjoram leaves on injured liver in experimental rats. *Rep Opin*. 2010;2(12):181–191.
11. Ahmed LA, Ramadan RS, Mohamed RA. Biochemical and histopathological studies on the water extracts of marjoram and chicory herbs and their mixture in obese rats. *Pak J Nutr*. 2009;8:1581–1587.
12. Saleh AA, Hamed S, Hassan AM, et al. Productive performance, ovarian follicular development, lipid peroxidation, antioxidative status, and egg quality in laying hens fed diets supplemented with salvia officinalis and *Origanum majorana* powder levels. *Animals*. 2021;11(12):3513.
13. Salem HM, Yehia N, Al-Otaibi S, et al. The prevalence and intensity of external parasites in domestic pigeons [*Columba livia domestica*] in Egypt with special reference to the role of deltamethrin as insecticidal agent. *Saudi J Biol Sci*. 2022;29(3):1825–1831.
14. AOAC. *Official Methods of Analysis*. Association of Official Analytical Chemist, 2012; 19th ed. AOAC: Washington, DC, USA.
15. NRC. National Research Council. *Nutrient Requirements of Poultry*. 1994; National Academies Press: Cambridge, MA, USA.
16. Marcu A, Vacaru-Opri I, Dumitrescu G, et al. The influence of genetics on economic efficiency of broiler chickens growth. *Anim Sci Biotechnol*. 2013;46:339–346.
17. Fattah AFA, Roushdy ESM, Tukur HA, et al. Comparing the effect of different management and rearing systems on pigeon squab welfare and performance after the loss of one or both parents. *Animals*. 2019;9(4):165.
18. George D, Mallery P. *IBM SPSS Statistics 26 Step by Step: A Simple Guide and Reference*. 2019, Routledge: New York, NY, USA.

19. Khattab MA, Roshdy AR, Ali AM. Effect of some medicinal plants on broiler performance. *Sinai J Appl Sci.* 2018;7(2):131–140.
20. Shad AA, Bakht J, Shah HU, et al. Antioxidant activity and nutritional assessment of under-utilized medicinal plants. *Pak J Pharm Sci.* 2016;29(6):2039–2045.
21. ALGarni EH, Hafez DA. Effect of extracts of some herbs on fertility of male diabetic rats. *J Am Sci.* 2015;11:165–175.
22. Shawky SM, Orabi SH, Dawod A. Effect of marjoram supplementation on growth performance and some immunological indices in broilers. *Int J Vet Sci.* 2020;9(2):297–300.
23. Shad AA, Bakht J, Shah HU, et al. Antioxidant activity and nutritional assessment of under-utilized medicinal plants. *Pak J Pharm Sci.* 2016;29:2039–2045.
24. Sevimli A, Bulbul T, Bulbul A, et al. Chicken amyloid arthropathy: Serum amyloid A, interleukin-1, interleukin-6, tumour necrosis factor and nitric oxide profile in acute phase [12th hour]. *Pol J Vet Sci.* 2013;16(2):241–247.
25. Arranz E, Jaime L, de Las Hazas ML, et al. Supercritical fluid extraction as an alternative process to obtain essential oils with anti-inflammatory properties from marjoram and sweet basil. *Ind Crops Prod.* 2015;67:121–129.