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RESEARCH ARTICLE

Green Poultry Production: Efficacy of Commercial Herb Additives as Improving Bird Performance, Immune Responses and Intestinal Microbiota in Domesticated Quails

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Abstract

Poultry meat will persist as the real and indispensable haven to provide people's needs for animal protein, and researchers are doing their best to make it safer, free of any harmful constituents that affect human health. The green economy, including the poultry sector, could be achieved by replacing chemical therapy with plant and organic medication. The purpose of the present study was to assess the effect of the adoption of commercial herb product as additives in domesticated quails. In the current study, a total of 140 Japanese quails were divided into 4 groups: 3 groups (b–d) were additively supplemented with Coxan (herb product), Clazo-Fort (chemical anti-coccidial product), and Coxan alternately with Clazo-Fort, respectively. While group "a" act as a control, non-additive group. The assessment of different parameters in the quails after the additive supplementation revealed that the quails supplemented with the Coxan product had an increase in body weight gain in most period durations and was significant at 7–14 days of age with an increase in the index of bursa of fabricius. The HI titer against NDV vaccines and CD4 T cells showed an elevation in their levels in the Coxan group. In addition, the intestinal microbiota revealed that there was a significant increase in lactobacillus counts in the three additive-supplemented groups, with higher significance in the Coxan group ($p<0.0001$) with the normal histological structure of the intestine. Overall, these results confirm the acceptable improvement in quail performance and the related parameters by using herb product containing essential oils (oregano and garlic oils) as additive supplements, which may make them a probable alternative for chemical compounds in healthy quail farms.

Keywords:

Quails, *Coturnix coturnix japonica*, Herbal essential oils, Immunity, Body performance

Introduction

Quail production is considered a branch of the modern poultry industry, like an animal production system. As a result of the increased demand for animal proteins in Egypt due to human population growth, quail breeding must receive more attention in order to increase and prosper its productivity as a source of meat protein production [1,2]. Also, it is encouraged to raise quail as a substitute for chickens due to the spread of contagious infectious diseases in chickens and the subsequent big economic losses in the industry [1]. When compared to other poultry species, quail are relatively small in size, which lowers the cost of feed. Furthermore, they have a brief life cycle, lay a lot of eggs, and have high resistance to several diseases. In addition to their significance for breeding, quail has become important for scientific research as experimental animals in studies concerning pathology, physiology, nutrition, embryology, genetics, and toxicology [3,4].

Antibiotics have been commonly used as a feed additive in the poultry ration as a growth promoter and frequently for prophylaxis against diseases in birds [5]. Moreover, the main method of coccidiosis control is the addition of anti-coccidial to feed or water [6,7]. The continuous feeding of antibiotics at sub-therapeutic levels caused an imbalance of gut flora, antibiotic residue, and the development of drug-resistant bacteria, which raised serious concerns for the public's health [8,9]. Because of this, there is a concentrated effort to find antibiotic alternatives, and various replacements have been researched globally [10,11]. Consequently, it is increasingly imperative to identify effective alternatives for managing infectious diseases and limiting the propagation of resistant bacteria. Moreover, it is imperious to preserve the utility of antibiotics for the future, particularly for urgent human needs. In European countries, the prophylactic use of antibiotics and anticoccidial chemicals that are used as growth promoters and feed additives in animal production has been strictly limited since 2006, and a full ban has been proposed to be effective in 2021 (Council Directive of 2011/50/EU of the European Council) [12,13].

Medicinal plants and their products, either extracts or essential oils, are prospective alternatives that may be used in poultry production [14]. Several of these bioactive compounds can impact the health and production of poultry, as well as have a variety of anti-inflammatory, antioxidant, immunomodulatory, anti-bacterial, and anti-coccidiosis properties and can modify the efficiency of the digestive system [15].

The oregano (*Origanum vulgare subsp. hirtum*) and garlic (*Allium sativum*) are aromatic-medicinal plants and are considered prospective sources of bioactive compounds that could be employed as a natural performance-enhancing antioxidant with strong anticoccidial capabilities [16-20]. Oregano and its main bioactive compounds have a significant influence on intestinal microbiota and the function of intestinal cells [21-23]. Also, it can be used as a functional element in the veterinary field as well as the food and feed industries [24,25]. The garlic contains primary components called Organosulfurs, which are thought to be important players in anti-inflammatory and antioxidant pathways and have strong anticoccidial activity that protects host tissue from parasite-caused damage [26]. Additionally, garlic is rich with aromatic oils that improve digestion and the respiratory system [27].

A more complete understanding of the effect of these alternative additives on the body's performance will give us the chance to develop new therapies to prevent and control diseases in poultry species. Also, it will allow comprehension of how these alternative materials differ from current additives such as anti-coccidiosis [28].

The use of herbal remedies has been proposed because of their natural stimulation of the immune system, enhanced growth performance, and anticoccidial effects. Consistently, the purpose of the present study was to evaluate the effect of one commercial herbal product on domesticated quail body weight, immune responses, intestinal morphology, and microbiota and compare it to one of the chemical products used in the field as a preventive (prophylactic additive) and control of coccidiosis.

Materials and Methods

Commercial products of herbal and chemotherapy

The herb product is Coxan, obtained from the company of Phytotherapic Solutions, S.L., Spain, and it contains oregano oil (8%) and garlic oil (2.5%). The other chemotherapy product is Clazo-Fort from the company 2M Group, which contains diclazuril at 2%. In this study, the phyto and chemotherapy products were used as additives in drinking water with a dose of 1 ml/liter as recommended by the manufacture's structure and 0.5 ml/10 liters at a concentration of 1 ppm according to El-Banna *et al*. [29], respectively.

Design of experiment

A total of 145 one-day-old healthy Japanese quails (*Coturnix coturnix japonica*) were bought from a commercial hatchery. Five birds were euthanized for blood collection. The birds (n = 140) were allocated to the experimental units and reared in a battery system under strict hygienic conditions. The quails were fed a standard commercial diet that was formulated without anticoccidial or antibiotic growth promoters. Feeding and watering were given ad libitum. At 7 days of age, the chicks of quail were randomly divided into four groups, with 35 birds per group. The first group was a control group (G-a: control). Commercial phytotherapy and chemotherapy products were added to the drinking water of the second and third bird groups (G-b: Coxan and G-c: Clazo-Fort), respectively. In the fourth group, Coxan and Clazo-Fort products were added alternately every 12 hours per day (G-d: Coxan and Clazo-Fort). Routinely, the quails were vaccinated against Newcastle disease virus at 6 and 13 days of age using MEVAC^{TM} ND HB1 and MEVAC^{TM} ND Elite (live attenuated vaccines) via eye dropping, respectively. The experimental trial was conducted strictly in compliance with the Institutional Animal Care and Use Committee at Zagazig University in Egypt's guidelines for using animals in experiments (approval No.: ZU-IACUC/2/F/75/2021).

Three quails from each group were randomly selected and euthanized at 7, 14, 18, 21, and 22 days of age, and five quails at 28 days of age for the blood collection and serological examination, as well as tissue sample collection of intestinal segments such as the cecai for pathological and histopathological examination (at 18 and 22 days), additionally weighting lymphoid organs (on day 28). The use of Coxan and Clazo-Fort as additives in drinking water was assessed based on the following criteria:

Performance parameters

Birds were weighed on days zero, 7, 14, 21, and 28 to detect the average live body weight (ABW) and calculate the average body weight gain (BWG) according to Brody [30].

Immune organs weights

On day 28, five birds were selected from each group and euthanized. The birds were dissected and the weights of the bursa, thymus, and spleen were recorded. The values were adjusted for BW according to Sedaghat and Karimi Torshizi [31].

Humoral immune response

The immune status of quails was evaluated under the supplementation of the products by detecting the antibody titers against NDV in the separated serum that was collected weekly using the hemagglutination inhibition (HI) test. The HI test was performed using 1% washed chicken RBCs and 4 hemagglutination (HA) units from the LaSota vaccine strain (Pestikal® LASOTA SPF) according to OIE [32]. Two-fold dilutions were applied with sera of half initially diluted, and the titer of HI was expressed as $\log_2$.

Measurement of CD4 and CD8 T-cell molecules

The Avian Cluster of Differentiation 4 and 8 (CD4, CD8) ELISA Kit (SunLong Biotech Co., LTD) was used to determine the concentrations of CD4 and CD8 molecules in the separated serum based on the manufacturer's instructions.

Intestinal microbiota enumeration

At 28 days of age (the end of the experiment), the digesta and the mucosal scraping of the ileum and caecum of five deceased quails from each group were collected into sterile tubes under aseptic conditions. Briefly, ten-fold serial dilutions of one gram of the homogenized content were carried out using 96-well standard plates for microdilutions. According to Herigstad *et al*. [33], 10 µL of each diluted sample was plated onto the following media using the drop plate technique: MacConkey agar (HiMedia Laboratories Pvt. Ltd., India) for coliform bacteria and De Man-Rogosa-Sharpe agar (MRS, HiMedia Laboratories Pvt. Ltd., India) for lactobacillus count. Lactobacilli were cultivated at 37°C in 3% CO2 for 48–72 h. Coliform bacteria were incubated at 37°C for 24–48 h. The results were stated as $\log_{10}$ colony-forming units (CFU) per gram of ilio-caecal content.

Histopathology examination

During the dissection of the euthanized birds on days 18 and 22, intestinal segments (cecai) were collected from the different groups and fixed in 10% neutral buffered formalin. Followed by regular tissue processing in various alcohol grades, xylene changes, and lastly, embedding in melted paraffin wax. Then five-micrometer sections were cut and stained with hematoxylin and eosin (H&E) [34]. The stained tissue slices were examined under a light microscope, and photomicrographs were taken.

Statistical analysis

The data were represented as the mean ± Standard Error of Mean (SEM). One-way ANOVA measurements, followed by Tukey's multiple comparisons test were used to examine group differences. The statistical significance was defined as $P< 0.05$ using SPSS version 21.0 (SPSS, IBM Corp., Armonk, NY) and GraphPad Prism 9.0.2 (GraphPad Software, Inc.).

Results

Body weight gain (BWG)

Generally, the body weight gain of quails with the additive Coxan (G-b) was improved when compared with other additives and non-additive groups (Figure 1). At 7–14 days of age, the Coxan group's BWG was significantly higher than the other groups.

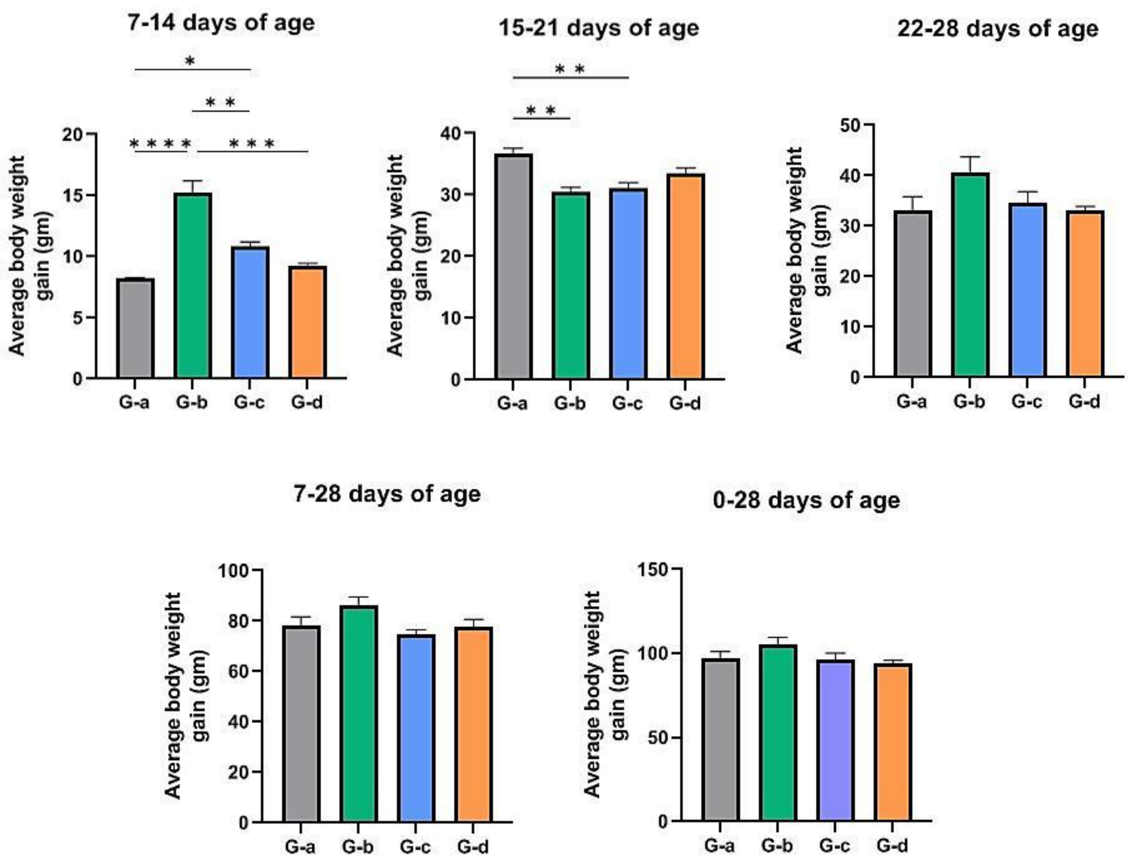

Figure 1. Average body weight gain among quails groups either additives or non-additive at different durations of experimental period. The figures display the mean ± standard error of the mean (bar), with significant value p<0.05.

Weight of Immune organs

The effects of different additives on the weights of immune organs (bursa of fabricius, thymus, and spleen) are given in Table 1. In this study, the bursa index increased in the Coxan and Clazo-Fort groups, with the highest in the Coxan group. In contrast, Coxan, alternately with Clazo-Fort, had a negative impact. The three additive groups did not improve the weight of the spleen and thymus compared with the control group.

Table 1. Effect of additives on weight of the lymphoid organs of Japanese quails

Groups	**Weight of lymphoid organ (g/100 g of BW)***		
	Bursa index	Thymus index	Spleen index
G-a; control, non-additive group	0.097±0.019	0.539±0.133	0.375±0.185
G-b; Coxan group	0.144±0.133	0.469±0.090	0.071±0.006
G-c; Clazo-Fort group	0.106±0.207	0.450±0.061	0.101±0.026
G-d; Coxan with Clazo-Fort	0.081±0.192	0.356±0.049	0.0896±0.019

*****The organ index is expressed as lymphoid organ weight (g) per BW (g).

Humoral immune response specific to Newcastle disease virus vaccines

Figure 2 shows the mean antibody titers detected by HI for NDV in different groups of quails, and the maternal-derived antibody (MDA) was 6.33±0.33 log $_2$. The mean HI antibody titers in all the additive groups were elevated, comparable with the non-additive group (G-a) at 21 days of age, and were 6±1.73, 7±1.53, and 7±0.58 log $_2$ in the Coxan, Clazo-Fort, and Coxan with Clazo-Fort groups, respectively, while in the control group, it was 4.66±1.20 log $_2$.

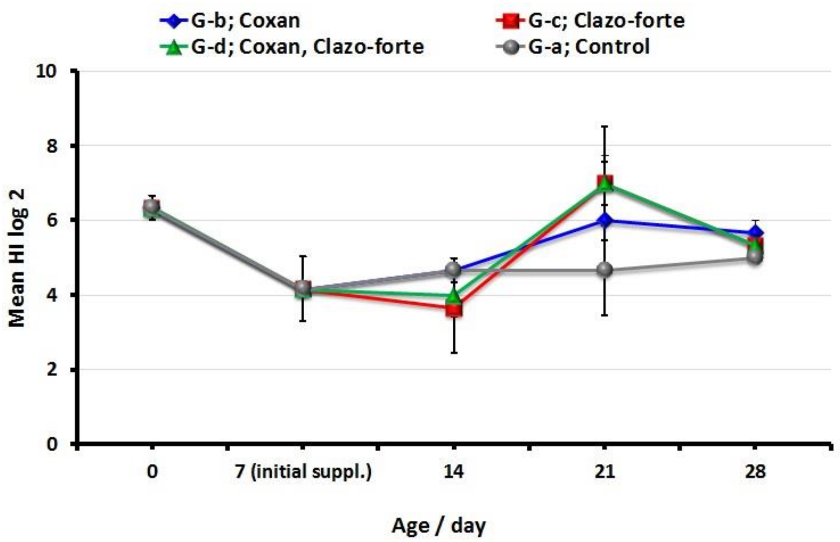

Figure 2. Mean hemagglutination inhibition (HI) titers against Newcastle disease virus vaccines among additives and non-additive quail groups (mean HI titer ±SEM)

CD4 and CD8 T-cells molecules

Figure 3 illustrates the CD4 and CD8 T-cell molecule values determined using ELISA. The high elevation of CD4 value was detected in the Coxan group (G-b) at 14 days of age (1762±87.5 pg/ml). At 28 days of age, the CD4 was significantly increased in the Coxan (G-b) and Clazo-Fort (G-c) groups with a value of 350±28.87 and 516±30.05 pg/ml, respectively, comparable to the non-additive (G-a) group. The Coxan with Clazo-Fort group (G-d) showed a significant decrease when compared with the non-additive and Coxan groups on day 21 and with the Clazo-Fort group at the age of 28 days. As for the CD8 value, it was the highest (25.83±19.27 ng/ml) in the Coxan group at 21 days of age. At 28 days of age, its value was 13.08±11.49 and 13.83±11.49 ng/ml in the Coxan and Clazo-Fort groups, respectively with no significant difference when compared with the value in the other groups.

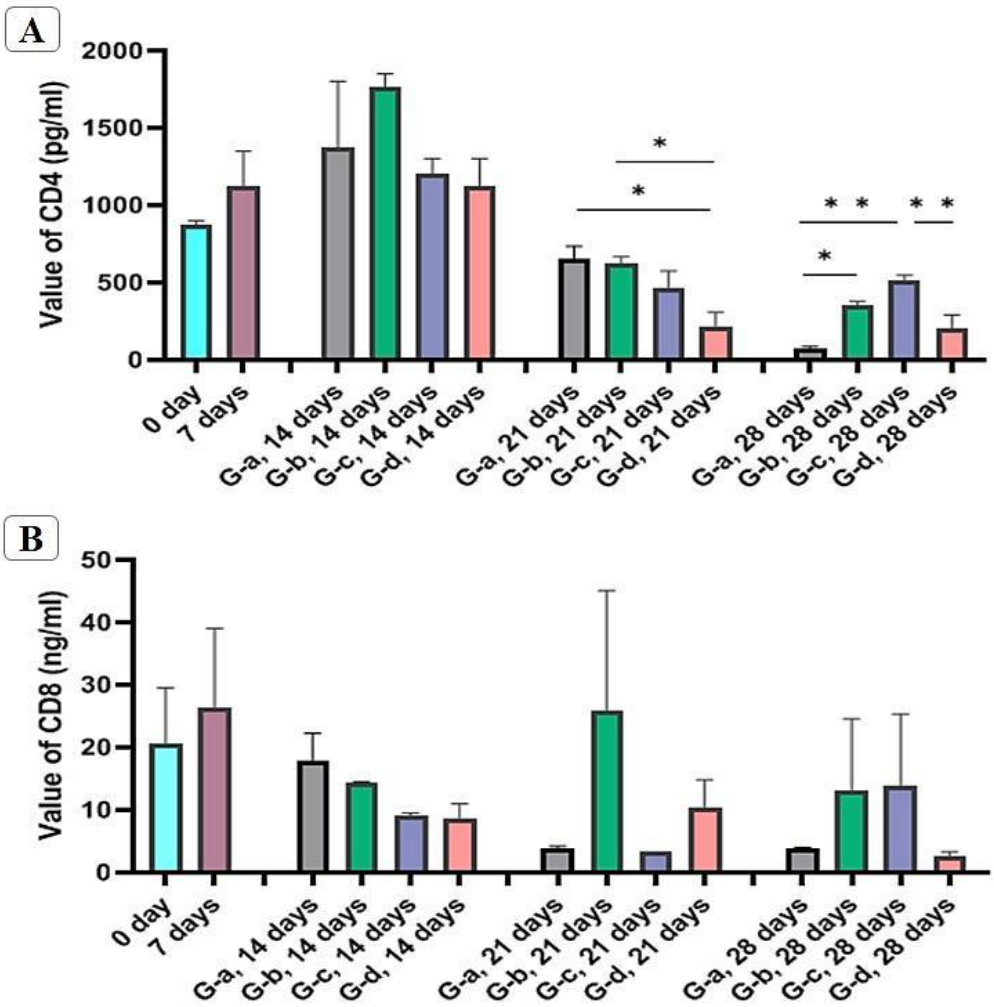

Figure 3. The value of CD4 (A), and CD8 (B) T-cell molecule in the collected serum from non-additive and additive quail groups using ELISA

Intestinal microbiota and microscopic examination

The coliform and Lactobacillus populations of the quail's ileo-cecal at 28 days of age are presented in Figure 4. There were non-significant variations in the population of coliform bacteria in the non-additive group (G-a) and the additive groups of Coxan (G-b) and Clazo-Fort (G-c). In the Coxan with Clazo-Fort group (G-d), there was a significant elevation in the coliform populations (8.50±0.499 log_{10} CFU/g, *p*=0.0357) compared to the non-additive group. A significant (P<0.05) increase in the Lactobacillus population was detected in the three additive groups when compared with the quails in the non-additives group, and the higher Lactobacillus counting was in the Coxan group (9.48±0.209 log_{10} CFU/g, p < 0.0001).

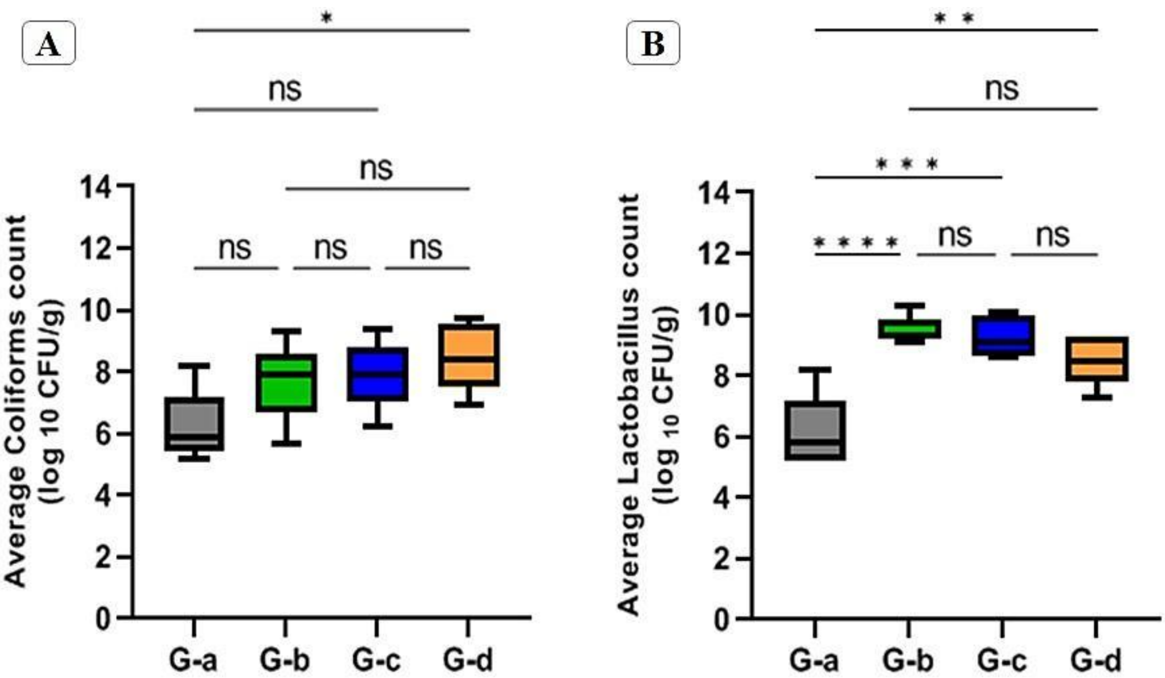

Figure 4. Average Coliforms (A), and Lactobacillus (B) bacterial counting in the non-additive and additive groups at 28 days of age. The Data represent mean values (mean ± SEM) (bar). The figures display the mean ± standard error of the mean (bar), with significant value <0.05.

Microscopic examination of cecal tissue sections of all additive groups (G-b to G-c) revealed the absence of any histopathological changes in the microscopic normal structure of the intestine, as shown in Figure 5.

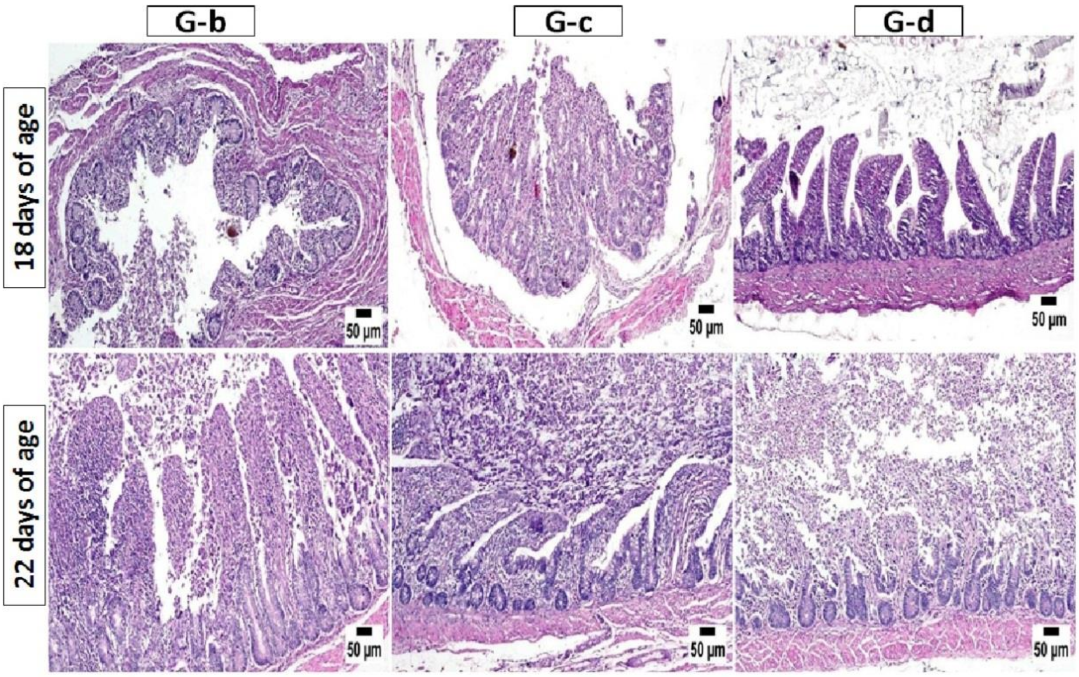

Figure 5. Photomicrographs of cecum, H&E stained showing normal histological structure of cecal wall in the additives groups.

Discussion

Antibiotics have been extensively employed in animal production since the 1940s [35]. The risks of using antibiotics in livestock operations, such as the development of antibiotic resistance in humans and animals as well as environmental pollution, have led poultry producers to invest in alternative feed additives in an effort to raise animal performance to the level of its genetic potential over time [36]. Prior studies have revealed the positive influences of herbal supplements on production performance and carcass quality [37,38]. Essential oils (EOs) are significant aromatic components of herbs that have antimicrobial, antifungal, antiparasitic, and antiviral properties. They are used as natural alternatives to antibiotic growth promoters (AGPs) in poultry. In addition, EOs have other advantageous benefits, including enhancing the secretion of digestive enzymes and triggering the immune system [39]. In response to the World Health Organization's directives on strict limitations in using antibiotics as growth promoters and feed additives and in consistency with the commitment of the Egyptian government to the replacement of natural products that have minimal side effects on poultry, humans, and the environment. This article was designated to investigate the effectiveness of the use of a commercial herb product containing essential oils such as oregano and garlic oils on the quail's performance and related parameters. This investigation was carried out on domesticated Japanese quails, which are considered a respectable source of animal proteins with a lower breeding cost compared to other poultry species.

In this study, the live body weight gain (BWG) trended to increase in the most time intervals during the experiment in the quail group supplemented with Coxan, with significant elevation at 7–14 days of age. El-Sayed *et al.* [40] detected that the feed additive supplementation of oregano essential oil and garlic powder succeeded in increasing the average body weight gain of quails. Also, Fotea *et al.* [41] showed that the highest body weight gain was obtained in broiler chicks fed diets supplemented with oregano essential oil. Moreover, the use of garlic powder or extract as feed additives in growing broiler chickens improved body weight gain [42,43].

These results can be attributed to the fact that OEs have positive effects on nutrient digestibility [44,45]. Oregano oil has the ability to increase digestive enzyme production and consequently improve the utilization of digestive products [44]. In addition, garlic oil has sulphur compounds, minerals, enzymes, amino acids, and alkaloids such as allicin. Allicin benefits recovering and developing the functional structure of the intestinal epithelium layer and results in helping the digestive system via improved absorption of valuable nutrients [46].

There were outcomes to previous studies contrary to these results as Cross *et al*. [47] stated that dietary inclusion of oregano essential oil in broilers diets could not affect the parameters of growth performance and Demir *et al*. [48] determined that the addition of garlic powder and thyme to the diet of broiler didn't affect growth and food conversion rate during the experiment.

Regarding the effect of the additive supplementation on the weight of immune organs, it revealed that the Coxan and Clazo-Fort swingly increased the index of the bursa, which was highest in the Coxan. While the three supplemented groups, either herbal or chemical, decreased the thymus and spleen indices compared to the control group. Abdou and Rashed [49] revealed an increase in the relative weight of the lymphoid organs as the bursa and thymus when Japanese quails were given garlic powder in the diet; however, there was no significant difference in spleen values. As a result of the stimulating influence of herbal essential oils on the generation of endogenous digestive enzymes, an enhancement of digestibility may be supported by a decrease in the weights of the organs [50].

Resistance to various illnesses is greatly influenced by the state of the host immune system. The use of essential oils could enhance the cellular, humoral, and phagocyte system immune responses in poultry, in addition to the immune-stimulating properties of the polyphenol fractions of thymol and carvacrol, and consequently improve the ability of the defense system to deal with infectious organisms [51]. In these results, the mean HI titer of the humoral immune response against the ND vaccine in Japanese quails was improved in the three additive groups by using herbal or chemical products or a combination of them at 21 days of age after duplication of the vaccination dose on day 13. This means that the two products stimulate the immune response. However, several researchers concluded that using herbal additives or essential oils had no beneficial influence on the humoral immune response of poultry [52-55]. On the other hand, El-Latif *et al*. [56] and Gholamrezaie *et al*. [57] reported positive impacts of herbal additive supplementation and improved immunity.

The presence of some components in essential oils may bind to receptors of immunoglobulin G and stimulate the immune response [58,59]. Additionally, certain bioactive plant chemicals control the expression of numerous genes involved in the immune response [60].

T lymphocytes play a critical role in stimulating the immune system in reaction to stressors and specific diseases [61]. These cells are clustered into differentiation $CD4^+$ and $CD8^+$. The $CD4^+$ T cells produce co-stimulatory molecules and cytokines that support other immune cells, such as innate immunity cells and β cells. In contrast, $CD8^+$ T cells are mostly killers that produce cytotoxic molecules like perforin that can eliminate host-infected cells [62].

The CD4 value was markedly elevated in the Coxan group (G-b) at 14 and 28 days of age. Also, a significant increase was detected in the Clazo-Fort (G-c) group at 28 days of age when compared with the control (G-a) group, while the Coxan with Clazo-Fort group (G-d) showed a significant decrease, and this may be attributed to the use of both the plant and chemical products interchangeably, which were added 12 hours per each, not 24 hours as in the other groups. There were no significant differences in the CD8 value among additives and non-additive groups, but the value trended towards an increase in the Coxan group (G-b) at 21 days of age and in the Coxan (G-b) and Clazo-Fort (G-c) groups at 28 days of age.

The gastrointestinal tract's microbes are essential for maintaining the health and productivity of poultry [63,64]. In birds, the gut microbiota diversity varies by region; the cecum has the highest microbial diversity and is a significant location of fermentation [65]. Also, the distal portion of the small intestine, or ileum, has a vital role in digestion, absorption, and mucosal immunity, and it is rich in microbiota [66]. The gut microbiota performs a variety of roles in the body, including enhancing food utilization, preventing pathogen colonization, enhancing growth, and metabolizing mycotoxins in feed [67,68]. In this investigation, there were non-significant variations in the population of coliform bacteria in the ileocecal part of the intestine among the Coxan, Clazo-Fort, and control groups. This means that the herbal and chemical additives used under the circumstances of this experiment maintain the limiting numbers of pathogenic bacteria as coliforms. In contrast, when using the Coxan alternately with the Clazo-Fort, there was a significant increase in the coliform populations. On the contrary, El-Sayed *et al*. [40] detected that the supplementation of oregano oil and garlic powder as feed additives in the quails was effective on the pathogenic population, which decreased the total microbial and pathogen counts in the intestine.

On the other hand, there was a significant increase in the Lactobacillus population in the three additive groups, and the detection of the higher counts was in the Coxan group. This result is consistent with those reported by Wenk [69] and Apajalahti *et al*. [70], who found that the antimicrobial activity of essential oil stimulates the growth of beneficial microorganisms.

Generally, the ratio of lactobacilli to coliforms was increased, and this result is consolidated with that reported by Castillo *et al*. [71], who mentioned that the herbs have the ability to modulate the composition of the microbial population by their prebiotic and antimicrobial activities. Subsequently, the microbiota can minimize the colonization of pathogenic bacteria (competition exclusion) by attaching to the enterocyte epithelial walls and forming a protective layer [72,73].

The detection of the normal histology structure of the large intestine (cecai) in the three trials of additive supplements indicates the safe doses of Coxan and Clazo-Fort used for this experiment. In which some herbs may cause toxicity or inflammation when they are administered in large quantities [74].

In the current study, adding the herb product containing oregano and garlic oils to the quails without any combination with the chemical product seems to be adequate in achieving a favorable result in increasing the body weight gain, elevating the humoral and cellular immune response, and increasing the lactobacillus populations in the ileocecal part of the intestine while maintaining its normal histological structure. All the results mentioned above achieved by the herb product are similar to or higher than those achieved by using the chemical product or even in the attempt to combine the herbal and chemical products, and this is considered a good parameter to obtain better productivity in quails, a good immunization response to vaccines such as the NDV vaccine, and an alternative to antibiotics in quail production.

Conflict of Interest

No potential conflict of interest was reported by the author(s).

References

[1] Arafat, N. and Abbas, I. (2018): Coccidia of Japanese quail: from identification, prevalence, infection, and immunization. J parasitol, 104(1): 23-30.

[2] Ramadan, M.; Elmadawy, R. and Tolba, I. (2021): Coccidiosis in Japanese quails (*Coturnix coturnix japonica*) in Kalioubia governorate: prevalence and treatment trials. BVMJ, 40(2): 131-136.

[3] Berto, B.P.; Cardozo, S.V.; Teixeira Filho, W.L.; Ferreira, A.M.R. and Lopes, C.W.G. (2008): Aflatoxin effect on the oocysts morphometry and contribution on the morphology of Eimeria bateri Bhatia, Pandey and Pande, 1965 from the japanese quail Coturnix japonica in Brazil. Rev. Bras. DE Parasitol. Vet., 17: 235-238.

[4] Jatoi, A.S.; Sahota, A.W.; Akram, M.; Javed, K.; Jaspal, M.H.; Hussain, J.; Mirani, A.H. and Mehmood, S. (2013): Effect of different body weight categories on the productive performance of four close-bred flocks of Japanese quails (*Coturnix coturnix japonica*). Small, 180(225): 200-250.

[5] Sun, X.; McElroy, A.; Webb, K.E.; Sefton, Jr.A.E. and Novak, C. (2005): Broiler performance and intestinal alterations

when fed drug-free diets. Poult Sci, 84(8): 1294–1302.

[6] Chapman, H.D. (1984): Drug resistance in avian coccidia. Vet Parasitol, 15(1): 11-27.

[7] Peek, H.W. and Landman, W.J.M. (2003): Resistance to anticoccidial drugs of Dutch avian *Eimeria* spp. field isolates originating from 1996, 1999 and 2001. Avian Pathol, 32(4): 391-401.

[8] Jensen, B.B. (1998): The impact of feed additives on the microbial ecology of the gut in young pigs. J Anim Feed Sci, 7: 45- 64.

[9] Andremont, A. (2000): Consequences of antibiotic therapy to the intestinal ecosystem. Ann Fr AnesthReanim, 19(5): 395–402.

[10] Mehrabi, Z.; Firouzbakhsh, F. and Jafarpour, A. (2012): Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (Oncorhynchus mykiss) fingerlings. J Anim Physiol Anim Nutr, 96(3): 474-481.

[11] Berrama, Z.; Temim, S.; Souames, S. and Ainbaziz, H. (2017): Growth performance, carcass and viscera yields, blood constituents and thyroid hormone concentrations of chronic heat stressed broilers fed diets supplemented with cumin seeds (*Cuminum cyminumL*.). Kafkas Üniv Vet Fakül Derg, 23(5): 735-742.

[12] Sultan, A.; Ullah, T.; Khan, S. and Khan, R.U. (2012): Effect of organic acid supplementation on the performance and ileal microflora of broiler during finishing period. Pakistan J Zool, 47(3):635–639.

[13] Muthamilselvan, T.; Kuo, T.F.; Wu, Y.C. and Yang, W.C. (2016): Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. Evidence-based complementary and alternative medicine: eCAM, Volume 2016, Article ID 2657981, 19 pages.

[14] Wati, T.; Ghosh, T.K.; Syed, B. and Haldar, S. (2015): Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens. Anim Nutr, 1(3): 213-219.

[15] Rafeeq, M.; Rashid, N.; Tariq, M.M.; Shahzad, I.; Sheikh, M.Z.M.; Shafee, M.; Mehmood, K.; Bilal, R.M. and Asmat, T. (2021): Effect of herbal extract on the growth performance, serum biochemical composition, ileal histo-morphology and immune response of broiler chickens. Pak J Zool, 53: 1793.

[16] Stanaćev, V.; Glamočić, D.; Milošević, N.; Puvača, N.; Stanaćev, V. and Plavša, N. (2011): Effect of garlic (Allium sativum L.) in fattening chicks nutrition. Afr J Agric Res, 6(4): 943-948.

[17] Tsinas, A.; Giannenas, I.; Voidarou, C.; Tzora, A. and Skoufos, J. (2011): Effects of an oregano based dietary supplement on performance of broiler chickens experimentally infected with *Eimeria acervulina* and *Eimeria maxima*. Poult Sci, 48(3): 194-200.

[18] Bozkurt, M.; Giannenas, I.; Küçükyilmaz, K.; Christaki, E. and Florou-Paneri, P. (2013): An update on approaches to controlling coccidia in poultry using botanical extracts. Br Poult Sci, 54(6): 713-727.

[19] Pourali, M.; Kermanshahi, H.; Golian, A.; Razmi, G.R. and Soukhtanloo, M. (2014): Antioxidant and anticoccidial effects of garlic powder and sulfur amino acids on Eimeria-infected and uninfected broiler chickens. Iran J Vet Res, 15(3): 227-232.

[20] Sheoran, N.; Kumar, R.; Kumar, A.; Batra, K.; Sihag, S.; Maan, S. and Maan, N.S. (2017): Nutrigenomic evaluation of garlic (*Allium sativum*) and holy basil (*Ocimum sanctum*) leaf powder supplementation on growth performance and immune characteristics in broilers. Vet world, 10(1): 121.

[21] Paraskeuas, V.; Fegeros, K.; Palamidi, I.; Hunger, C. and Mountzouris, K.C. (2017): Growth performance, nutrient digestibility, antioxidant capacity, blood biochemical biomarkers and cytokines expression in broiler chickens fed different phytogenic levels. Anim Nutr, 3(2): 114-120.

[22] Tzora, A.; Giannenas, I.; Karamoutsios, A.; Papaioannou, N.; Papanastasiou, D.; Bonos, E. Skoufos, S.; Bartzanas, T. and Skoufos, I. (2017): Effects of oregano, attapulgite, benzoic acid and their blend on chicken performance, intestinal microbiology and intestinal morphology. Poult Sci, 54(3): 218-227.

[23] Giannenas, I.; Bonos, E.; Christaki, E. and Florou-Paneri, P. (2018): Oregano: a feed additive with functional properties. In Holban, A.M. and Grumezescu, A.M. editors. Therapeutic Foods, Handbook of Food Engineering, (Vol 8). London, UK: Elsevier Academic Press.

[24] Bozkurt, M.; Ege, G.; Aysul, N.; Akşit, H.; Tüzün, A.E.; Küçükyılmaz, K.; Borum, A.E.; Uygun, M.; Akşit, D.; Aypak, S.; Şimşek, E.; Seyrek, K.; Koçer, B.; Bintaş, E. and Orojpour, A. (2016): Effect of anticoccidial monensin with oregano essential oil on broilers experimentally challenged with mixed Eimeria spp. Poult Sci, 95(8): 1858-1868.

[25] Giannenas, I.; Sidiropoulou, E.; Bonos, E.; Christaki, E. and Florou-Paneri, P. (2019): The history of herbs, medicinal and aromatic plants, and their extracts: past, current situation and future perspectives. In Florou-Paneri, P.; Christaki, E. and Giannenas, I. editors. Feed Additives: Aromatic Plants and Herbs in Animal Nutrition and Health. London, UK: Elsevier.

[26] Dkhil, M.A.; Abdel-Baki, A.S.; Wunderlich, F.; Sies, H. and Al-Quraishy, S. (2011): Anticoccidial and anti-inflammatory activity of garlic in murine *Eimeria papillata* infections. Vet Parasitol, 175(1-2): 66-72.

[27] Abou-Elkhair, R.; Gaafar, K.M.; Elbahy, N.M.; Helal, M.A.; Mahboub, H.D. and Sameh, G. (2014): Bioactive effect of dietary supplementation with essential oils blend of oregano, thyme and garlic oils on performance of broilers infected with *Eimeria* species. Glob Vet, 13(6): 977-985.

[28] Lu, H.; Adedokun, S.A.; Adeola, L. and Ajuwon, K.M. (2014): Anti-inflammatory effects of non-antibiotic alternatives in coccidia challenged broiler chickens. Poult Sci, 51(1): 14-21.

[29] El-Banna, H.A.; El-Bahy, M.M.; El-Zorba, H.Y. and El-Hady, M. (2005): Anticoccidial efficacy of drinking water soluble diclazuril on experimental and field coccidiosis in broiler chickens. J Vet Med Series A, 52(6): 287-291.

[30] Brody, S., (1945): Bioenergetics and growth with special reference to the efficiency complex in domestic animals. Reinhold publication Corp.; New York, Ny.

[31] Sedaghat, A. and Torshizi, M. K. (2017): Immune responses, intestinal microbiota, performance and blood characteristics of Japanese quail fed on diets containing camphor. animal, 11(12): 2139-2146.

[32] OIE, 2012. Terrestrial Manual. Chapter 2. 3. 14. Newcastle disease.

[33] Herigstad, B.; Hamilton, M. and Heersink, J. (2001): How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods, 44(2): 121-129.

[34] Bancroft, J.D. and Gamble, M. (2008): Theory and practice of histological techniques. Elsevier health sciences.

[35] Dibner, J.J. and Richards, J.D. (2005): Antibiotic Growth Promoters in Agriculture: History and Mode of Action. Poult Sci, 84(4): 634-643.

[36] Sacakli, P.; Çınar, Ö.Ö.; Ceylan, A.; Ramay, M.S.; Harijaona, J.A.; Bayraktaroglu, A.G.; Shastak, Y. and Calik, A. (2023): Performance and gut health status of broilers fed diets

supplemented with two graded levels of a monoglyceride blend. Poult Sci, 102(2): 102359.

[37] Tekeli, A.; Celik, L.; Kutlu, H.R. and Gorgulu, M. (2006): Effect of dietary supplemental plant extracts on performance, carcass characteristics, digestive system development, intestinal microflora and some blood parameters of broiler chicks. In Proceedings of 12th European Poultry Conference, pp: 10-14.

[38] Tekeli, A.; Kutlu, H.R.; Celik, L.; Var, I.; Yurdakul, E. and Avcy, A. (2008): The use of Propolis as an alternative to antibiotics growth promoters in broiler diets. Proceedings of 23rd World's Poultry Congress, June 30-July 4, Brisbance, Australia, pp: 482.

[39] Krishan, G. and Narang, A. (2014): Use of essential oils in poultry nutrition: A new approach. J Adv Vet Anim Res, 1(4): 156-162.

[40] El-sayed, Z.S.; El-Garhy, O.H.M.; Abdella, M.M. and El-Sayaad, G.A. (2017): Effect of Adding Antibiotic, Oregano Essential Oil and Garlic Powder on Growth Performance, Carcass Traits and Intestinal Pathogenic Microrganisms of Quails. Egypt J Anim Prod, 54(1): 55-66.

[41] Fotea, L.; Costăchescu, E.; Hoha G. and Leonte, D. (2008): The effect of oregano essential oil (*Origanum vulgar L.*) on broiler performance. LucrăriŞtiinţifice, 53: 253-256.

[42] Lewis, M.R.; Rose, S.P.; Mackenzie, A.M. and Tucker, L.A. (2003): Effects of dietary inclusion of plant extracts on the growth performance of male broiler chickens. Br Poult Sci, 44(S1): 43-44.

[43] Raeesi, M.; Hoseini-Aliabad, S.A.; Roofchaee, A.; Shahneh, A.Z. and Pirali, S. (2010): Effect of periodically use of garlic (*Allium sativum*) powder on performance and carcass characteristics in broiler chickens. Int J Anim Vet Sci, 4(8): 683-689.

[44] Hernandez, F.; Madrid, J.; Garcia, V.; Orengo, J. and Megias, M.D. (2004): Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poult Sci, 83(2): 169-174.

[45] Jamroz, D.; Wiliczkiewicz, A.; Wertelecki, T.; Orda, J. and Skorupińska, J. (2005): Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. Br Poult Sci, 46(4): 485-493.

[46] Adibmoradi, M.; Navidshad, B.; Seifdavati, J. and Royan, M. (2006): Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. Poult Sci, 43(4): 378-383.

[47] Cross, D.E.; McDevitt, R.M.; Hillman, K. and Acamovic, T. (2007): The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Br Poult Sci, 48(4): 496-506.

[48] Demir, E.; Sarica, Ş.; Özcan, M.A. and Sui Mez, M. (2003): The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. Br Poult Sci, 44(S1): 44-45.

[49] Abdou, A. and Rashed, G.G. (2015): Effect of black seed (*Nigella sativa*) and garlic (*Allium sativum*) feed supplements on productive performance and some physiological and immunological responses of japanese quail. Egyptian Journal of Nutrition and Feeds, 18(1): 129-141.

[50] Bampidis, V.A.; Christodoulou, V.; Florou-Paneri, P.; Christaki, E.; Chatzopoulou, P. S.; Tsiligianni, T. and Spais, A.B. (2005): Effect of dietary dried oregano leaves on growth performance, carcass characteristics and serum cholesterol of female early maturing turkeys. Br Poult Sci, 46(5): 595-601.

[51] Pérez-Rosés, R.; Risco, E.; Vila, R.; Peñalver, P. and Cañigueral, S. (2015): Effect of some essential oils on phagocytosis and complement system activity. J Agric Food Chem, 63(5): 1496–1504.

[52] Jafari, R.; Ghorbanpoor, M. and Hoshmand, D.S. (2009): Study on immunomodulatory activity of dietary garlic in chickens vaccinated against avian influenza virus (subtype H9N2). Int J Poult Sci, 8(4): 401-403.

[53] Pourali, M.; Mirghelenj, S.A. and Kermanshahi, H. (2010): Effects of garlic powder on productive performance and immune response of broiler chickens challenged with Newcastle Disease Virus. Glob Vet, 4(6): 616-621.

[54] Özek, K.; Wellmann, K.T.; Ertekin, B. and Tarım, B. (2011): Effects of dietary herbal essential oil mixture and organic acid preparation on laying traits, gastrointestinal tract characteristics, blood parameters and immune response of laying hens in a hot summer season. J Anim Feed Sci, 20(4): 575-586.

[55] Bozkurt, M.; Aysul, N.; Küçükyilmaz, K.; Aypak, S.; Ege, G.; Çatli, A.; Akşit, H.; Çöven, F.; Seyrek, K. and Çınar, M. (2014): Efficacy of in-feed preparations of an anticoccidial, multienzyme, prebiotic, probiotic, and herbal essential oil mixture in healthy and Eimeria spp.-infected broilers. Poult Sci, 93(2): 389-399

[56] El-Latif, A.S.A.; Saleh, N.S.; Allam, T.S. and Ghazy, E.W. (2013): The effects of rosemary (*Rosemarinus officinalis*) and garlic (*Allium sativum*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. Br Poult Sci, 2(2): 16-24.

[57] Gholamrezaie, S.L.; Mohammadi, M.; Jalali, S.J.; Abolghasemi, S. and Roostaie-Ali-Mehr, M. (2013): Extract and leaf powder effect of Artemisia annua on performance, cellular and humoral immunity in broilers. Iranian J Vet Res, 14(1): 15-20.

[58] Nimmerjahn, F. and Ravetch, J.V. (2010): Antibody-mediated modulation of immune responses. Immunolog Rev, 236(1): 265–275.

[59] Ahmed, S.T.; Hossain, M.E.; Kim, G.M.; Hwang, J.A.; Ji, H. and Yang, C.J. (2013): Effects of resveratrol and essential oils on growth performance immunity, digestibility and fecal microbial shedding in challenged piglets. Asian– Australas. J Anim Sci, 26(5): 683–690.

[60] Liu, Y.; Song, M.; Che, T.M.; Lee, J.J.; Bravo, D.; Maddox, C.W. and Pettigrew, J.E. (2014): Dietary plant extracts modulate gene expression profiles in ileal mucosa of weaned pigs after an *Escherichia coli* infection. J Anim Sci, 92(5): 2050-2062.

[61] Yuniwarti, E.Y.W.; Asmara, W.; Artama, W.T. and Tabbu, C.R. (2012): The effect of virgin coconut oil on lymphocyte and CD4 in chicken vaccinated against Avian Influenza virus. J Indones Trop Anim Agric, 37(1): 64–69.

[62] Kang, I. (2013): Analysis of T cells using flow cytometry. J Rheum Dis, 20(2): 83–86.

[63] Broom, L.J. (2019): Host-microbe interactions and gut health in poultry: focus on innate responses. Microorganisms, 7(5): 139.

[64] Rychlik, I. (2020): Composition and function of chicken gut microbiota. Animals (Basel), 10(1): 103.

[65] Oakley, B.B.; Lillehoj, H.S.; Kogut, M.H.; Kim, W.K.; Maurer, J.J.; Pedroso, A.; Lee. M.D.; Collett, S.R.; Johnson, T.J. and Cox, N.A. (2014): The chicken gastrointestinal microbiome. FEMS Microbiol. Lett., 360(2): 100–112.

[66] Shini, S. and Bryden, W.L. (2021): Probiotics and gut health: linking gut homeostasis and poultry productivity. Anim Prod Sci, 62(12): 1090-1112.

[67] Barnes, E.M.; Mead, G.C.; Barnuml, D.A. and Harry, E.G. (1972): The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br Poult Sci, 13(3): 311–326.

[68] Slizewska, K. and Piotrowska, M. (2014): Reduction of ochratoxin A in chicken

feed using probiotic. Ann Agric Environ Med, 21(4): 676–680.
[69] Wenk, C. (2000): Recent advances in animal feed additives such as metabolic modifiers, antimicrobial agents, probiotics, enzymes and highly available minerals. Asian-australas J Anim Sci, 13(1):86–95.
[70] Apajalahti, J.; Kettunen, A. and Graham, H. (2004): Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult Sci J, 60(2):223–232.
[71] Castillo, M.; Martín-Orúe, S.M.; Roca, M.; Manzanilla, E.G.; Badiola, I.; Perez, J. F. and Gasa, J. (2006): The response of gastrointestinal microbiota to avilamycin, butyrate, and plant extracts in early-weaned pigs. J Anim Sci, 84(10): 2725-2734.
[72] Jeurissen, S.H.; Lewis, F.; van der Klis, J.D.; Mroz, Z.; Rebel, J.M. and Ter Huurne, A.A. (2002): Parameters and techniques to determine intestinal health of poultry as constituted by immunity, integrity, and functionality. Current issues in intestinal microbiology, 3(1): 1-14.
[73] Yegani, M. and Korver, D.R. (2008): Factors affecting intestinal health in poultry. Poult Sci, 87(10): 2052-2063.
[74] Corrier, D.E. and DeLoach, J.R. (1990): Evaluation of cell-mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. Poult Sci, 69(3): 403-408.

الملخص العربي

إنتاج الدواجن الخضراء: فعالية الإضافات العشبية التجارية في تحسين أداء الطيور والاستجابات المناعية والميكروبات المعوية في طيور السمان المستأنسة

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ستظل لحوم الدواجن هي الملاذ الحقيقي الذي لا غنى عنه لتوفير إحتياجات الإنسان من البروتين الحيواني، ويبذل الباحثون قصارى جهدهم لجعلها أكثر أماناً وخالية من أي مسببات ضارة تؤثرعلى صحة الإنسان. ويمكن تحقيق الإقتصاد الأخضر، بما في ذلك قطاع الدواجن، من خلال إستبدال العلاج الكيميائي بالأدوية النباتية والعضوية. كان الغرض من هذه الدراسة هو تقييم تأثير منتجات الأعشاب التجارية كإضافات في طائر السمان المستأنس. حيث تم تقسيم عدد 140 طائر سمان ياباني إلى 4 مجموعات: 3 مجموعات (ب-د) تم إضافة كوكسان (منتج عشبي)، وكلازوفورت (منتج كيميائي مضاد للكوكسيديا)، وكوكسان بالتناوب مع كلازوفورت، على التوالي. بينما تعمل المجموعة "أ" كمجموعة تحكم غير مضافة. أظهر تقييم العوامل المختلفة في طيور السمان بعد المكملات المضافة أن الطيور المضاف اليها منتج الكوكسان (العشبي) شهدت زيادة كبيرة في وزن الجسم المكتسب في معظم فترات التجربه، مع زيادة ملحوظة عند عمر 7-14 يومًا الى جانب زيادة في الوزن النسبى لجراب الفابريشيوس. أظهر عيار نشاط التلازن الدموي لكرات الدم الحمراء ضد لقاحات النيوكاسل وخلايا CD4 T ارتفاعًا في مستوياتها في مجموعة الكوكسان. بالإضافة إلى ذلك، كشفت الكائنات الحية الدقيقة المعوية عن وجود زيادة كبيرة في أعداد العصيات اللبنية في المجموعات الثلاث المضاف إليها المكملات، مع أهمية أعلى في مجموعة كوكسان ($P < 0.0001$) مع التركيب النسيجي الطبيعي للأمعاء. بشكل عام، تؤكد هذه النتائج التحسن المقبول في أداء طائر السمان والمؤشرات المرتبطة به باستخدام المنتج العشبي الذى يحتوي على الزيوت العطرية (زيت الأوريجانو والثوم) كمكملات إضافية، مما قد يجعلها بديلاً محتملاً للمركبات الكيميائية في مزارع السمان.