Impact of dietary Anise seeds powder and oil (*Pimpinella anisum*) as natural additive on Immune response and some blood parameters of broilers chickens

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Abstract

This experiment, which was carried out in the Sako poultry project in Erbil, Iraq, from January 9 to December 10, 2021, sought to ascertain the effects of using anise seed and oils as natural additives in broiler feed on the immune feed response and several blood parameters of broilers. We randomly allocated 180 broiler chicks into four groups. T1: control; T2: a standard diet with 2 g of anise powder per kg of food; T3: a standard diet with 1 ml of anise oil per kg of diet; T4: a regular diet with 1 g of anise powder and 0.5 ml of anise oil; and Results demonstrate that adding anise seed powder and oil as a natural additive to ration boosts broiler immunity and WBC count while having no effect on broiler RBC, PCV, or Hb.

Keywords: Anise, natural additive, Immune, broilers chickens.

Introduction

At the present time, there are many different feed additions for broiler rations, such as aromatic herbs and the essential oils or extracts that go along with them, to enhance performance (1. Additionally, adding herbs to a broiler's diet could improve their health, immune system, and digestion by boosting endogenous enzymes and improving their immune response .Additionally, customers view these plants and their constituent parts as natural and secure. (2).

According to WHO, 80 percent of people worldwide rely on conventional medicine. for their main healthcare requirements, and the majority of this therapy uses plant extracts or their active ingredients (3). Consumers believe that those plants and the parts they make up are natural and secure. These ingredients have long been used as flavorings in both human and animal feed.

A member of the Apiaceae family, anise (Pimpinella anisum L.) is a fragrant plant (4 ,5). It can manifest in a variety of hot climates around the world, including southern Europe and Southeast Asia (6). Anise formed active compounds with powerful pharmacological effects as a result of the high temperature (7). Coumarins, polyacetylenes, polyenes, phenolic acids, essential oils, eugenol, anisaldehyde, trans-anethole, and estragole are just a few of the bioactive compounds that can be found in anise seeds (6). As a result, anise is thought to contain qualities that support growth as well as those that are antioxidant, antibacterial, and immune-stimulant .Anise is also known to have powerful natural anti-inflammatory, anti-tumor, anti-platelet, anti-cancer, and anti-cancer impacts (8).

The successful addition of anise to the meal has boosted the broiler hens' development efficiency, feed utilization, immunity, antibacterial resistance, and antioxidant status (9).

Numerous studies, evaluation the use of anise seed or oil in poultry nutrition as growth promoters. These studies demonstrate that anise seed or oil greatly enhances growth rate , excess weight, and feed conversion ratio. (10) .

An annual herb called anise (Piminella anisum L.) is indigenous to many hot climates around the world, including Iran, India, Turkey, and Egypt. In addition to its potential direct antiviral effects (11), anise has been used as a medical plant as a digestive stimulant, antiparasitic, antibacterial, antifungal, and antipyretic (12). Only one study has revealed any impact of anise seeds on broiler performance, and there have been relatively few investigations on the effects of anise or other essential oils on the performance of poultry (13) The impact of those seeds on blood parameters, the immune system, or carcass quality, however, has not been examined.

So, this study was aimed to evaluate the effects of use anise seed and oils in broiler as natural additive on Immune feed response and some blood parameters of broilers (Ross 308) compared with control treatment.

Materials and Methods

This study examined the effects of using anise seed and oils as a natural additive in broiler

feed on immune feed response and a few blood markers in broilers. Between January 9 and December 10, 2021, it was carried out at the Sako poultry project unit in Gurato, Mergasor, and Erbil.

Experimental design

180 day-old, unsexed broiler chicks (Ross 308) were randomly assigned to four treatments, each of which was then split into three replicates of 15 chicks.

- T1: control (standard diet).
- T2 : standard diet + 2 g anise powder/ kg diet.
- T3: standard diet + 1 ml anise oil / kg diet.
- T4: standard diet + 1 g anise powder + 0.5 ml anise oil

Each treatment's chicks were kept in full environmental control houses, and all of the experimental feeds were created to satisfy (18) recommendations for the nutritional needs of broiler chicks (Table 1) Water and food were freely available.

Anti-infective precautions were taken against the most prevalent infectious disorders. As shown in Table 2, various Newcastle disease vaccines were used to immunize the chicks against the disease.

Table 1. Analyses of the basal diet's content and chemicals

Components	beginning a diet	Growing diet	completer diet
Protein percentage	22.50	20.00	18.00
Energy (Kcal/kg)	3069	3185	3250
Lipid%	4.450	5.610	5.950
Lysine %	1.330	1.210	1.210
Methionine %	0.640	0.560	0.500
Calcium%	0.980	0.950	0.900

P %	0.620	0.580	0.540

Table 2. Vaccination schedule for broiler chicks during the trial.

Age (days)	Vaccine	Route of vaccination
1-3	N.D+I.B	ED
10-12	I.B.D	ED/DW
16-17	H.P.S	Sub cut.1/2 cc
22	I.B.D	D/W
28	N.D	D/W

Estimated blood parameter

At 42 days old, five birds from each experimental treatment had five ml of blood drawn. Campbell performed erythrocyte (RBC) and leucocyte counts (WBC) in accordance with accepted avian standards (1984). Wright-Giemsa staining and a PCV test were performed afterward, and Blood H:L ratios were examined, according to (14).

Immune response

At 42 days old, five chickens from each experimental treatment were butchered for this reason. To determine the antibody titers against the diseases Newcastle disease (ND) and infectious bursal disease (IBD), the blood was drawn, stored in the refrigerator for three to four hours, and the serum was collected in plastic vials and frozen at -20° C for subsequent analysis.

Statistical analysis

The General Linear Models technique in the SPSS software was used to statistically analyses the data (15). The Duncan's multiple range test was used to distinguish between treatments with statistically significant changes at a 5% probability level.

Results and Discussion

The production and wellbeing of finfish species are improved by medicinal herbs in a number of ways (16,17). One of these herbal remedies is anise, which has potential immunological, antibacterial, and antioxidant effects (6).

Anise successfully altered antibiotic usage in the poultry business (9). Aquatic animals' blood biochemical and hematological changes serve as a useful indicator of their physiological state and level of fitness (18). Additionally, the effects of nutritional feed components on the health and fitness of fish are known, and they may be assessed based on the bio-signatures values (19). The precise constant values of the hematological and blood biochemical values change according on the species, sizes, feeding methods, and environmental factors (20). Fish with abnormal results have a dynamic metabolic function, which might result in better or worse values (21,22).

A- Effect of anis seed and its oils on RBCs, PCV and Hb level in broilers.

This study in Table (3) cleared that there were no significant differences of the effect of anis seed and its oils on RBCs, PCV and Hb level in broilers. This study illustrated that the higher level of RBCs observed in the group 2 that treated with standard diet with 2 g anise powder/ Kg diet followed by its level in the group 4 that treated with standard diet with 1 g anise powder and 0.5 ml anise oil as its level reached to 2.11 X 10^6 , while, the lower level of RBCs observed in control group as its level reached to 2.10 X 10^6 and in the group 3 that treated with standard diet with 1 ml anise oil/Kg diet.

While, the level of PCV % not show any significant variations ($P > 0.05$) among different treatment means where the high level of PCV % observed in group 2 that treated with treated with standard diet with 2 g anise powder/ Kg diet followed by its level followed by its level in group 3 that treated with that treated with standard diet with 1 ml anise oil/Kg diet and the lower PCV % observed in the group 4 that treated with standard diet with 1 g anise powder and 0.5 ml anise oil as its level reached to 31.83 % and the lower level of PCV observed in control group as its level was 31.33.

While, the results of Hb level the results cleared that, there was no any significant differences ($P > 0.05$) among different treatment groups where the high level of Hb observed in group in group 2 that treated with standard diet with 2g anise powder /Kg diet as its level reached to 10.69 mg/dl followed by group 3 that treated that treated with that treated with standard diet with 1 ml anise oil/Kg diet as its level reached to 10.66. While the lower level observed in control group as its level reached to 10.44 mg/dl and the group 4 that treated with 4 that treated with standard diet with 1 g anise powder and 0.5 ml anise oil as its level reached to 10.61 mg/dl.

These findings were in agreement with those discovered that, with the exception of the hematocrit, hemoglobin, RBCs, and WBCs, the feeding dietary anise has normal values for hematological and biochemical blood indicators. The findings show that chickens are in good health and that anise has no negative effects on metabolism or general wellbeing. Additionally,

increased hematocrit, hemoglobin, and RBC levels correlate with better metabolic performance, an abundance of nutrients, and a lack of anemia-related symptoms.(23).

B- Effect of using anise seed and its oils on WBC and H/L ratio in broiler .

Our results on the WBCs level cleared that, there was a significant variation ($P < 0.05$) among different treatment groups where the high level of WBCs observed in group 2 that treated with standard diet with 2g anise powder /Kg diet as its level reached to 22.21 X 10^3 /mm^3 dl followed by group 4 that treated with standard diet with 1 g anise powder and 0.5 ml anise oil as its level reached to 22.21 X 10^3 /mm^3 dl. While, the lower WBCs level observed in control group that reached to 20.58 X 10^3 /mm^3 dl and in group 3 that treated with standard diet with 1 ml anise oil/Kg diet as its level reached to 21.70 X 10^3 /mm^3 dl.

These findings corroborated those noted that a further potential effect of anise on the physiological condition and immunity of chicken is the high quantity of WBCs. Because of the activation of macrophages and leucocytes, the immunized broilers displayed WBCs that can fend off bacterial infection.(23)

Our results on the H/L level cleared that, there was a no significant differences ($P > 0.05$) among different treatment groups in its level of H/L where the high level of H/L observed in control group as its was 0.33 followed by the group 2 that treated with with standard diet with 2g anise powder /Kg diet as its level reached to 0.32 followed by group 4 that treated with standard diet with 1 g anise powder and 0.5 ml anise oil as its level reached to 0.32 and the lower H/L level observed in the group 3 that treated with standard diet with 1 ml anise oil/Kg diet as its level reached to 0.30.

These variations between the groups could be the result of an active element, such as the digestive stimulant of the anise (11). Additionally, anise the essential oils have been shown to have antioxidant, antifungal, anticoccidial, and antibacterial properties (24).

C- Effect of using anise seed and its oils on ND and IBD antibody titers of broiler .

The data obtained in Table 4 revealed that the ND and IBD antibody titers of broilers were significantly different among different treatment groups ($P < 0.05$).

The results cleared that, the higher ND titer observed in group 4 that treated with standard diet with 1 g anise powder and 0.5 ml anise oil as its level reached to 6869, followed by the ND titer in group 3 that treated with standard diet with 1 ml anise oil/Kg diet as its level reached to 6703 while, the lower ND titer observed in control group as its level reached to 2971 and in group 2 that treated with treated with standard diet with 2g anise powder /Kg diet as its level reached to 6643.

Also, the results cleared that, the IBD higher titer level observed in group 4 that treated with standard diet with 1 g anise powder and 0.5 ml anise oil as its level reached to 7004, followed by the IBD titer in group 3 that treated with standard diet with 1 ml anise oil/Kg diet as its level reached to 6716 while, the lower IBD titer observed in control group as its level reached to 5838 and in group 2 that treated with treated

with standard diet with 2g anise powder /Kg diet as its level reached to 7004.

This is explained by the presence of phenols and essential oils in anise, which enhanced mucin secretion in the intestinal epithelial layer and local intestinal immunity, resulting in the epithelium cells' high functioning in the absorption of nutrients that have been digested (16).The diversity of advantageous microorganisms in the broiler gut increased as a result of the inclusion of herbal medications and their extracts in feed (25), consequently enhancing intestinal immunity and digestive ability. Additionally, broilers treated with anise showed better (viscerasomatic index, VSI) biometric indicators.

The VSI refers to the lengthened period of fish intestines compared to the fork period, which is related to anise's helpful role in enhancing the fitness of the digestive system. It is therefore possible to suggest that nutritional anise is beneficial for improving intestinal fitness and digestive potential, which lead to increased feed utilization and performance.

These outcomes are similar , to outcomes of (26, 110 , 27) whose mentioned that the suggest antibody titer in opposition to ND and IBD confirmed A appreciably extended withinside the anise seeds remedy and stepped forward immune device of the broiler chickens and (28) whose stated that the aniseed addition to basal weight loss plan on the price of 0.five g/kg and 1 g/kg of feed had best immunomodulatory interest both for humoral and mobile immune response , Anise's favorable outcomes on blood's mobile traits is probably because of upgrades in organic and metabolic processes, in addition to Anise seeds have a high-quality impact on nutrient digestibility, improving protein, cellulose, and fats digestion, enhancing ileal digestibility of vitamins, growing pancreatic lipase and amylase sports, having antioxidant interest, stopping fatty acid oxidation, and growing nutrient usage (10).

The characteristics of the anise seeds in this test can also having triggered the immune system of the broilers. Better feed utilization inside the fish carcass might be compared to the number of vitamins accumulated (29). The results showed a substantial increase in the crude protein content of the European sea bass carcass treated with nutritional anise. The findings take into account earlier study that found higher levels of protein inside the carcasses of birds treated with anise (9).

Similar to other herbal remedies, anise feeding can speed up the metabolism of proteins, causing an abnormal buildup of proteins inside the fish's body (34). The crude protein level, however, points to smaller values than the initial level following the study. The increased fish sizes at the conclusion of the study may have contributed to the reduced protein level by causing high moisture levels and coffee protein content. The conclusions take into account various studies that demonstrated a notable antibacterial effect of anise against harmful microorganisms. Additionally, the majority of therapeutic plants have the ability to fight against dangerous microorganisms with their antibacterial properties (9, 10 and 29).

The decreased Vibrio spp. and faecal coliform levels inside the intestines, which are an immediate result of active intestinal digesting

ability, offer a justification for the increased feed use. The body's immune system and local intestinal immunity were also activated as a result of the decreased number of dangerous microorganisms in the stomach (16).

These results imply that broilers fed nutritional anise are able to fend off the anticipated diseases, leading to low death rates. The findings support earlier studies that demonstrated strong and regular blood values in anise-treated birds (10). No prior studies on fish examined how anise affected blood characteristics, but the addition of medicinal flora resulted in elevated hemoglobin, hematocrit, RBCs, and WBCs (30).

There is a considerable risk of invasion during the growing season because multiple environmental stressors endanger their immunity (31). Fish without the problem and with less capacity for dangerous microbes thanks to phagocytic and lysozyme activities (32).

Additionally, studies have demonstrated that the antibacterial characteristics of medicinal herbs' natural extracts can benefit fish by lessening the effects of pathogenic germs (33). According to the findings, anise-fed broilers displayed active lysozyme and phagocytic responses. The results support numerous research that demonstrate the superior effect of medicinal flora in enhancing lysozyme and phagocytic activities (34). Anise also contains essential oils that stimulate leucocyte and macrophage interest, resulting in an increase in WBCs and efficient lysozyme and phagocytic activities (6).

The typical outcomes of the prevailing have a look at open the door for in addition research at the effect of anise at the productives of broiler species and different animal. This has to don't forget making use of anise primarily based totally on species-precise way the use of superior analytical strategies to make clear the viable mode of action. Also, the antibacterial potential of anise needs to be evaluated via way of means of detecting resistance in opposition to pathogenic contamination. In this way, anise may be proposed to update antibiotics in in hen and animal production.

Table 3. Effect of using anise seed and its oils on RBC, PCV and Hb in broiler (mean±SE)

Treatment	RBC (*10^6/mm^3)	PCV (%)	Hb (mg/dl)
T1	2.10 ± 0.09b	31.33 ± 0.94b	10.44 ± 0.31b
T2	2.15 ± 0.05b	32.09 ± 2.36b	10.69 ± 0.78b

T3	2.09 ± 0.01b	32.00 ± 1.01b	10.66 ± 0.31b
T4	2.11 ± 0.02b	31.83 ± 2.21b	10.61 ± 0.73b
P-value	NS	NS	NS

- T1: control, T2 standard diet with 2 g anise powder/ kg diet, T3 standard diet with 1 ml anise oil / kg diet and T4 standard diet with 1 g anise powder and 0.5 ml anise oil.
- Means within the same column of the different superscripts are significantly differ at ($P < 0.05$)
- NS no significant effect ($P \geq 0.05$)

Table 4. Effect of using anise seed and its oils on WBC and H/L ratio in broiler (mean±SE)

Treatment	**WBC (*10^3/mm^3)**	**H/L**
T1	20.58 ± 0.14 b	0.33 ± 0.03
T2	22.21 ± 0.38 a	0.30 ± 0.01
T3	21.70 ± 0.35 ab	0.29 ± 0.02
T4	22.17 ± 0.23 a	0.30 ± 0.01
P-value	*	NS

T1 : control , T2 standard diet with 2 g anise powder/ kg diet , T3 standard diet with 1 ml anise oil / kg diet and T4 standard diet with 1 g anise powder and 0.5 ml anise oil .

NS no significant effect ($P \geq 0.05$).

Means within the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 5. Effect of using anise seed and its oils on ND and IBD antibody titers of broiler (mean±SE)

Treatment	ND Titer	IBD Titer
T1	2971 ± 734.39 b	5838 ± 720 b
T2	6643 ± 44.67 a	6529 ± 353 ab
T3	6703 ± 146.65 a	6716 ± 156 a
T4	6869 ± 402.96 a	7004 ± 184 a
P-value	*	*

- T1 : control , T2 standard diet with 2 g anise powder/ kg diet , T3 standard diet with 1 ml anise oil / kg diet and T4 standard diet with 1 g anise powder and 0.5 ml anise oil .
- NS no significant effect ($P \geq 0.05$).
- Means in the same row with various superscripts differ considerably ($P \leq 0.05$).

Conclusion

Generally, the addition of Anise seeds powder and oil as herbal additive to ration will increase immunity and WBC in broiler. Further research on the use of anise and other medicinal plants, synthetic antioxidants, and immunostimulants in hen farms is concurrently advised by us.

Conflict of interest

The authors declare no conflict of interest.

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