Research Article

Anise (*Pimpinella anisum*) Enhances the Growth Performance, Immunity and Antioxidant Activities in Broilers

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ABSTRACT

The beneficial effects of anise in broiler ration were evaluated through investigation of growth performance, immunostimulant and antioxidant status by determination of the serum immunoglobulins (IgA, IgM and IgG), interferon gamma (INF-γ) and interleukins (IL-2 and IL-10). Moreover, malondialdehyde (MDA) and glutathione reduced (GSH) levels in the breast and thigh muscles were determined. There are numerous patents suggesting the use of anise as feed additive and remedy supplement. To achieve this objective, 180 of one-day old Cobb chicks were allocated into four equal groups as control group that supplemented with the basal diet, anise I that fed on basal diet containing (0.25 g anise seed powder/kg diet), anise II that fed on basal diet containing (0.50 g anise seed powder/kg diet) and anise III that fed on basal diet containing (0.75 g anise seed powder /kg diet). The GC-MS analysis of methanolic extract of anise evidenced the presence of anethole (34.55 %) as a major active ingredient of antioxidant potential. Anise significantly increased the immunoglobulins, INF-γ, IL-2, IL-10 and muscle GSH levels. Whereas, muscle MDA levels were significantly decrease. From the obtained results we can stated that anise I promotes enhancement in broiler's performance, feed conversion ratio and protein efficiency in relation to the other anise treated and control groups with enhancement of immunity and antioxidant activities.

Keywords: Anise, Performance, Immunity, Antioxidant status.

INTRODUCTION

Plant active principles are important task for developing therapeutic agents[1]. Herbal products greatly safe in compare to the synthetics[2]. Herbal components are extracted from different parts like leaves, bark, roots, flowers and seeds[3]. Herbal essential oil is an alternative to synthetic feed additives. This practice is of important values in broiler chickens[4] and laying hens[5] that assist in enhancement of the beneficial gastrointestinal tract flora[6]. Besides their antimicrobial properties[7], they also exhibit antioxidant[8-10], antifungal[11] and digestion-stimulating activities[12,13]. Patently, anise oil was also been used in veterinary drug formulae[14]. Anise (*Pimpinella anisum*), also called aniseed, belongs to the *Umbelliferae* family of 30-50 cm in length and grows in the West Asia, Middle East, Mexico, Egypt, and Spain[15]. Anise is a herb have numerous traditional uses and pharmacological potentials[16]. Anise has been used as antiparasitic[17], antitussive [18] antibacterial[19], and antifungal effect[20]. Anethole the major active ingredient of anise was purified from the aqueous phase[21]. *Trans*-anethole of aniseed constitutes about 1.5–6.0 % of a volatile oil[22]. *cis*-anethole, estragole, *p*-anisaldehyde, anisketone, linalool and β-farnesene are also active constituents of anise[23].

Therefore, the effects of anise on broiler growth performance, immune response and antioxidant status were investigated through determination of the serum immunoglobulins (IgA, IgM and IgG), interferon gamma (INF-γ), interleukin-2 (IL-2) and interleukin 10 (IL-10). The antioxidant potential of anise also was monitored by determination of the levels of malondialdehyde (MDA) and glutathione reduced (GSH) in thigh and breast muscles.

MATERIALS AND METHODS

Birds, accommodation and management

One hundred and eighty of one-day old Cobb broiler chicks were randomly allocated into four equal groups (each one allocated into three replicates each replicates contains 15 birds). The housing of chicks was done in a clean well-ventilated room. The room temperature was adjusted according to age by electric heaters. Furthermore, the birds were vaccinated by Hitchner IB (7th day), Gumbro (14th day) and Gumbro and Clone (21st day) through eye drop.

Diet and experimental design

The chicks were fed on the two phases feeding program (Starter and Grower) from 1st to 21st days on starter and from 22nd to 35th days on grower diets[24]. The control diet composition was represented in Table 1 and analyzed according to AOAC[25]. In addition, Lysine and Methionine were calculated according to Kamel[26].

Anise seeds were washed, grinded and refined. The ground powder was mixed with the ration by the

concentration of 0.5% in anise I, 1% in anise II and 1.5% in anise III groups, while control one was fed on basal diet that with water were accessed *ad libitum*.

Table 1: The starter and grower diet's ingredients percentage and calculated composition (as fed basis)

Ingredients	Starter diet	Grower diet
Corn	52.55	60.27
SBM (CP 44%)	34.26	29.31
Corn gluten (CP 60%)	5.5	3.0
Corn oil	3.3	3.26
Limestone	1.35	1.53
Dicalcium phosphate	1.74	1.47
L-Lysine	0.11	0.13
Dl-methionine	0.39	0.23
Vitamins and minerals premix	0.3	0.3
NaCl	0.5	0.5
Total	100	100
Composition		
ME (Kcal/Kg diet)	3061.2	3119.35
CP %	23.0	20.0
Calorie/protein ratio	133.1	155.97
Lysine %	1.3	1.16
Methionine %	0.8	0.58
Calcium %	1.0	0.9
Av. (P) %	0.45	0.40
NaCl	0.15	0.15

SBM= Soybean meal, ME = Metabolizable Energy, CP = crude protein, Av. (P) = Available phosphorous

*L-lysine 99% feed grade

**Dl-methionine 99% feed grade China

***Vitamin and mineral premix (Hero mix) produced by Heropharm and composed (per 3 kg) of vitamin A 12000000 IU, vitamin D3 2500000 IU, vitamin E 10000 mg, vitamin K3 2000 mg, vitamin B1 1000 mg, vitamin B2 5000 mg, vitamin B6 1500 mg, vitamin B12 10 mg, niacin 30000 mg, biotin 50 mg, folic acid 1000 mg, pantothenic acid 10000 mg, manganese 60000 mg, zinc 50000 mg, iron 30000 mg, copper 4000 mg, iodine 300 mg, selenium 100 mg and cobalt 100 mg.

Anise extract preparation

The fine powder of anise seeds were extracted by methanol according to the method of Shyamala[27] with some modifications.

Briefly, 15 g of dried anise were mixed with 100 mL of methanol and kept for 24 h with occasional shaking. The extract was filtered and evaporated to dryness in vacuum.

Gas chromatography–mass spectrometry (GC-MS) analysis

Trace GC Ultra-ISQ mass spectrometer with a direct capillary column TG–5MS (30 m×0.25 mm×0.25 µm) was injected by 10 µl of anise methanolic extract. The column oven temperature was started 60 °C and then increased by 5 °C /minute till reach 280 °C. The injector and detector (MS transfer line) temperatures were kept at 250 °C. Helium flow rate of 1 ml/minute was used as carrier gas for 37.83 minutes. The solvent delay was 2 minute and diluted samples of 1 µl were injected automatically using auto-sampler AS3000 coupled with GC in the splitless mode. The ion source and quadrupole temperatures were set at 200 and 150°C, respectively. The mass spectra of the identified components were determined by comparison to NIST 11 mass spectral database.

Performance parameters

The basal diets of both starter and grower phases were formulated according to the recommendation of National Research Council Nutrient Requirements for Broilers[28]. Performance parameters include the final body weight, feed intake, feed conversion ratio (FCR)[29] and protein efficiency ratio[30] those were determined weekly throughout the experimental period.

Biochemical analysis

The blood samples at 1st, 2nd, 3rd, 4th and 5th weeks were collected from wing vein by the sterile sharp needle with wide pore. Each blood sample was left to coagulate at room temperature and centrifuged at 3000 RPM for 5 minutes. The collected sera were subjected to determination of IgA, IgM, IgG, INF-γ, IL-2 and IL-10 by ELISA kits manufactured by Elabscience Co.

At 5th week five birds of each replicate are sacrificed and samples of 10 g each were taken from breast and thigh muscles. Muscle samples were homogenized and centrifuged at 3000 RPM for 15 minutes. The clear supernatants were subjected to determination of MDA[31] and GSH[32].

Statistical Analysis

By SPSS software package v.20, the obtained data were analyzed by one way analysis of variance (ANOVA), with Duncan's multiple range tests for significant between means.

RESULTS

The data presented in Figure 1 and illustrated in Table 2 revealed the chemical composition of one sample was carried out using the GC–MS analysis led to the identification of eleven different components; anethole (34.55 %), varidiflorene (7.36 %), eicosane (5.14 %), docosane (4.92 %), nonadecane (9.29 %), pentadecane (6.41 %), butanoic acid (5.25 %), heneicosane (10.95 %), octacosane (7.21 %), Hexadecane (5.14 %) and cyclohexane (3.78 %), respectively.

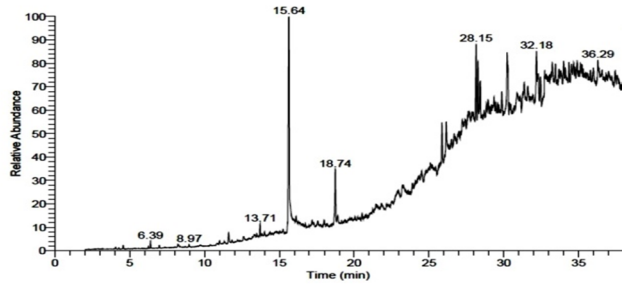

Figure 1: GC-MS chromatogram of anise methanolic extract

The data obtained in Table 3 revealed that the average body weight of broilers were significantly increased (P<0.05) in the anise I group at 5th week in relation to control and other anise treated groups.

The data illustrated in Table 4 showed that the average body weight gain of broilers were increased significantly (P<0.05) in the anise I at 4th- 5th week and 1st-5th weeks in relation to control and other anise treated groups.

The data illustrated in Table 5 revealed that the average feed intake of broilers were significantly elevated (P<0.05) in the control group at 2nd - 5th week in relation to other anise treated groups. Moreover, anise II is the lowest feed intake (2970.67 g).

The average feed conversion ratio of broilers were increased significantly (P<0.05) in the anise I at 2nd - 5th week followed by anise II and anise III in comparison to control group (Table 6).

The average protein efficiency ratio of broilers were statistically increased (P<0.05) at 2nd - 5th week in the anise I in relation to control group while the average protein efficiency ratio of broilers were slightly increased in the anise I at 2nd - 5th week in relation to other anise treated groups (Table 7).

The data obtained in Table 8 revealed that the serum IgA, IgG, IL-2 and IL-10 of broilers were significantly increased (P<0.05) in the anise I, anise II and anise III at 3rd and 5th week whereas, the serum IgM and INF-γ were significantly increased (P<0.05) in the anise I at 3rd week in relation to control.

The data obtained in Table 8 revealed that the serum IgM, IgG, IL-2 and INF-γ of broilers were increased statistically (P<0.05) in the anise I, anise II and anise III and the most high results in anise I while at 5th week, the serum IgA and IL-10 were significantly increased (P<0.05) in the anise III in relation to control.

MDA levels were significantly decreased (P<0.05) in anise I; anise II and anise III in breast and thigh muscles in relation to control group of broilers, the data showed also GSH were significantly increase (P<0.05) in anise I, anise II and anise III in breast and thigh muscles in relation to control group (Table 9).

Table 2: GC-MS analysis of anise methanolic extract

	Compound Name	RT (Minutes)	Area %	Molecular Formula
1	Anethole	15.63	34.55	$C_{10}H_{12}O$
2	Varidiflorene	18.73	7.36	$C_{15}H_{24}$
3	Eicosane	25.87	5.14	$C_{20}H_{42}$
4	Docosane	4.92	4.92	$C_{22}H_{46}$
5	Nonadecane	26.16	9.29	$C_{19}H_{40}$
6	Pentadecane	28.28	6.41	$C_{15}H_{32}$
7	Butanoic acid	28.43	5.25	$C_{15}H_{20}O_3$
8	Heneicosane	30.23	10.95	$C_{21}H_{44}$
9	Octacosane	32.18	7.21	$C_{28}H_{58}$
10	Hexadecane	32.27	5.14	$C_{20}H_{42}$
11	Cyclohexane	32.45	3.78	$C_{26}H_{50}$

Table 3: Effect of anise dietary supplementation on average body weight (g) in broilers

	Control	Anise I	Anise II	Anise III
1st week	156.61±3.33^{a}	162.24±2.62^{a}	158.67±2.60^{a}	160.34±2.23^{a}
2nd week	462.14±11.09^{a}	452.41±6.72^{a}	440±7.08^{ab}	420.34±6.48^{b}
3rd week	793.75±18.20^{ab}	820.52±10.03^{a}	810.33±13.35^{ab}	772.59±13.49^{b}
4th week	1114.46±22.65^{a}	1171.72±12.41^{a}	1172±21.42^{a}	1159.48±21.73^{a}
5th week	1536.43±31.41^{b}	1651.21±19.96^{a}	1562.83±27.21^{b}	1554.14±34.38^{b}

Means carrying different superscripts within the same row are significantly different (P<0.05)

Table 4: Effect of anise dietary supplementation on average body weight gain (g) in broilers

	Control	Anise I	Anise II	Anise III
1st-2nd week	305.54±7.84^{a}	290.17±4.18^{b}	281.33±4.64^{b}	260±4.37^{c}
2nd-3rd week	331.61±7.80^{b}	368.1±3.78^{a}	370.33±6.58^{a}	352.24±7.36^{a}
3rd-4th week	320.71±6.10^{c}	351.21±3.89^{b}	361.67±8.37^{b}	386.9±9.26^{a}
4th-5th week	421.96±10.19^{b}	479.48±8.27^{a}	390.83±6.61^{c}	394.66±14.44^{bc}
1st-5th week	1379.82±28.45^{b}	1488.97±17.44^{a}	1404.17±24.72^{b}	1393.79±32.20^{b}

Means carrying different superscripts within the same row are significantly different (P<0.05)

Table 5: Effect of anise dietary supplementation on average feed intake (g/bird/week) in broilers

	Control	Anise I	Anise II	Anise III
2nd week	412^{a}	393^{c}	410.67^{b}	373.33^{d}
3rd week	436.21^{d}	457^{b}	456.33^{c}	460^{a}
4th week	897.24^{b}	902.67^{a}	855.33^{d}	857.33^{c}
5th week	1375.86^{a}	1244^{d}	1248.33^{c}	1263^{b}
2nd-5th week	3121.31^{a}	2996.67^{b}	2970.67^{c}	2953.67^{d}

Means carrying different superscripts within the same row are significantly different (P<0.05)

Table 6: Effect of anise dietary supplementation on average feed conversion ratio (FCR) in broilers

	Control	Anise I	Anise II	Anise III
2nd week	1.38±0.05^{ab}	1.36±0.02^{b}	1.47±0.02^{a}	1.45±0.03^{ab}
3rd week	1.34±0.03^{a}	1.25±0.01^{b}	1.24±0.02^{b}	1.32±0.03^{a}
4th week	2.82±0.05^{a}	2.58±0.03^{b}	2.40±0.05^{c}	2.26±0.06^{d}
5th week	3.31±0.08^{a}	2.62±0.04^{b}	3.22±0.05^{a}	3.33±0.13^{a}
2nd-5th week	2.29±0.05^{a}	2.02±0.02^{c}	2.13±0.04^{bc}	2.15±0.05^{b}

Means carrying different superscripts within the same row are significantly different (P<0.05)

Table 7: Effect of anise dietary supplementation on average protein efficiency ratio (PER) in broilers

	Control	Anise I	Anise II	Anise III
2nd week	3.22±0.08^{a}	3.21±0.05^{a}	2.98±0.05^{b}	3.03±0.05^{b}
3rd week	3.31±0.08^{c}	3.50±0.04^{ab}	3.53±0.06^{a}	3.33±0.07^{bc}
4th week	1.70±0.03^{d}	1.85±0.02^{c}	2.01±0.05^{b}	2.15±0.05^{a}
5th week	1.46±0.04^{b}	1.84±0.03^{a}	1.49±0.03^{b}	1.49±0.05^{b}
2nd-5th week	2.42±0.05^{b}	2.60±0.03^{a}	2.50±0.04^{ab}	2.50±0.05^{ab}

Means carrying different superscripts within the same row are significantly different (P<0.05)

Table 8: Effect of anise dietary supplementation on serum IgA (ng/mL), IgM (ng/mL), IgG (µg/mL), INF-γ (pg/ml), IL-2 (pg/mL) and IL-10 (pg/dl) in broilers

		Control	Anise I	Anise II	Anise III
3rd week	IgA	87.09±6.51^{b}	106.82±5.44^{a}	113.75±1.63^{a}	113.13±1.17^{a}
	IgM	50.69±1.75^{b}	57.36±2.54^{a}	53.76±1.89^{ab}	53.08±0.98^{ab}
	IgG	6.72±0.33^{a}	7.32±0.75^{a}	7.58±0.21^{a}	7.63±0.36^{a}
	INF-γ	79.61±2.48^{b}	84.72±0.55^{a}	81.42±0.94^{ab}	81.50±0.74^{ab}
	IL-2	125.11±0.89^{b}	138.47±3.27^{a}	133.19±0.51^{a}	135.84±1.90^{a}
	IL-10	38.17±4.12^{b}	51.31±3.60^{a}	51.56±1.20^{a}	51.01±1.95^{a}
5th week	IgA	94.31±2.01^{b}	113.92±1.53^{a}	115.54±1.36^{a}	117.72±1.17^{a}
	IgM	55.18±1.92^{b}	78.31±4.78^{a}	77.31±1.17^{a}	75.41±1.01^{a}
	IgG	6.45±0.19^{b}	8.45±0.25^{a}	8.42±0.12^{a}	8.42±0.03^{a}
	INF-γ	83.96±1.57^{b}	95.67±0.40^{a}	94.09±1.23^{a}	95.03±1.22^{a}
	IL-2	131.14±2.99^{b}	147.57±1.10^{a}	145.50±1.42^{a}	145.44±1.04^{a}
	IL-10	43.38±0.80^{b}	53.71±1.79^{a}	55.16±1.87^{a}	56.02±1.40^{a}

Means carrying different superscripts within the same row are significantly different (P<0.05)

Table 9: Effect of anise dietary supplementation on MDA (nmol/g tissue) and GSH (mmol/g tissue) levels in broiler's breast and thigh muscles

		Control	Anise I	Anise II	Anise III
Breast	MDA	9.29±0.18^{a}	7.68±0.10^{b}	7.43±0.34^{b}	7.70±0.62^{b}
	GSH	0.90±0.07^{b}	1.88±0.04^{a}	1.86±0.01^{a}	1.85±0.003^{a}
Thigh	MDA	8.69±0.58^{a}	6.67±0.15^{b}	7.13±0.34^{b}	6.92±0.09^{b}
	GSH	0.77±0.05^{b}	1.16±0.08^{a}	1.14±0.01^{a}	1.15±0.02^{a}

Means carrying different superscripts within the same row are significantly different (P<0.05)

DISCUSSION

Continuous use of antibiotics in poultry diets has evoked numerous problems such as cross-resistance and environmental pollution. So that the search for alternative growth promoting substances to replace classical antibiotics in poultry diets has to be continued[33]. Therefore, vegetables, herbs, spices and edible plants were suggested as non-traditional feed additives in animal nutrition[34].

The GC-MS analysis of anise cleared the anethole (4-methoxy propen-1-yl benzene) content of 34.55 % area in chromatogram. Anethole is the major active ingredient of anise was analyzed in different percentage according to the region of cultivation[35,36]. Moreover, anethole was purified from aqueous emulsion and crystallized in a patent new process. The process provides a purified anethole having improved odor and taste[37]. It significantly increased blood glutathione of male albino rats received 300 mg of anise oil/kg orally[38].

The average body weight, average body weight gain, feed conversion and average protein efficiency were statistically increased in the anise I group in relation to other treated anise and control groups. While the average feed intake increased in the control group in relation to anise treated group. These results are in agreement with that obtained by Soltan[39]. Moreover, Mahmood[40] stated that the birds showed good performance when aniseed was fed at lower doses however, the birds showed poor performance at higher levels of aniseed. Further, the broiler chicks those received aniseed infusion at the rate of 40 ml/L of water shown better growth performance, immunity and gross return[41]. Daily live weight gain was improved by approximately 15 % in a group supplemented by 400 mg anise oil/kg diet in relation to control group. This effect may be regard to the anethole present in anise oil that has digestive stimulating effects and antimicrobial activity against pathogens that affect the gastrointestinal tract[42].

The obtained data showed the immunostimulant effect of anise through a significant increase in the levels of IgA, IgG, IL-2 and IL-10 in anise I, anise II and anise III, while the serum IgM and INF-γ levels were significantly increased in the anise I at 3rd week in relation to control. In addition to, the serum IgM, IgG, IL-2 and INF-γ levels were significantly increased in the anise I, anise II and anise III with the highest results in anise I. The serum IgA and IL-10 were significantly increased in the anise III at 5th week in comparison to control one. This results come in accordance with that obtained in the study of

Mahmood[40] whose stated that the aniseed addition to basal diet at the rate of 0.5 g/kg and 1 g/kg of feed had best immunomodulatory activity both for humoral and cellular immune response.

The balance between reactive oxygen species (ROS) and the intrinsic antioxidant defenses decides the cellular antioxidant activity[43]. Lipid peroxidation leads to the formation of various products as the MDA. Therefore, blood MDA level is often determined in some studies as an indicator of lipid peroxidation in the body[44]. Our results showed that the supplementation of aniseed to the diet reduced blood MDA concentration, which indicates a decreased lipid peroxidation. In contrast, GSH levels in them were significantly increased in comparison to control one[45]. The antioxidant potential of aniseeds ethanolic extract exhibited scavenging activity against nitric oxide and superoxide radicals and reducing power in a concentration-dependent manner[46,47]. Furthermore, HepG2 cells and rat's liver those treated by n-hexane extract of anise seed showed higher levels of GSH and lower levels of MDA[48].

As the oxidative stress markers were ameliorated by anise seeds that may regard to its antioxidant constituents[48]. Anise seed possess a potent antioxidant activity that may be attributed to many polyphenol compounds have been detected in extracts from different anise species and anethole[49,50].

CONCLUSION

From the obtained results we can conclude that anise I (0.5 g of anise/ Kg diet) gives the best performance, feed conversion ratio and protein efficiency in relation to the other anise treated and control groups.

Generally, the addition of anise to ration increases immunity and increases antioxidant activity in broiler.

Concomitantly, we recommend further studies on the combinations of anise and other medicinal plants and synthetic antioxidants and immunostimulants in poultry farms.

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