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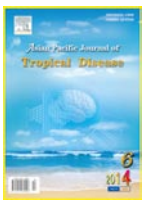

Document heading doi:10.1016/S2222-1808(14)60604-6

Anise seed (*Pimpinella anisum* L.) as an alternative to antibiotic growth promoters on performance, carcass traits and immune responses in broiler chicks

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PEER REVIEW

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Comments

This is a good study in which the authors evaluated the anise seed levels to antibiotic growth promoter and immune response in poultry nutrition. The experiment is designed well. The results of this study are useful, and the results can be applied in all breeding birds.
Details on Page 450

ABSTRACT

Objective: To evaluate the effect of inclusion of three levels of anise seed (*Pimpinella anisum* L.) as an antibiotic growth promoter substitute on growth performance, carcass traits, and immune responses in broiler chickens.

Methods: Two hundred and forty, 1-day-old, hatched Ross broilers received a maize-soybean meal basal diet and were allocated randomly in the following five experimental treatments for 6 weeks: basal diet-no additives, basal diet containing 1 g anise/kg diet, basal diet containing 5 g anise/kg diet, basal diet containing 10 g anise/kg diet and basal diet containing flavophospholipol at 4.5 mg/kg diet. At Day 42, two birds per replicate were slaughtered for determination of carcass and organ weights. At Day 28, serum antibody titers against avian influenza virus were measured by the hemagglutination inhibition test.

Results: Bodyweight of broilers fed basal diet was higher at 42 d of age than other groups but it was not statistically significant ($P>0.05$). Broilers receiving basal diet had higher feed intake compared to broilers receiving difference levels of anise seed ($P<0.05$). The most efficient feed conversion throughout the study was observed in chicks fed diets supplemented with 1 g anise/kg ($P<0.05$). Most of the carcass characteristics of broilers slaughtered at Day 42 were not influenced by treatments but carcass yield significantly increased ($P<0.05$) in broilers supplemented with 10 g anise/kg compared to antibiotic group. Antibody titer against avian influenza virus increased in the group treated with 10 g anise/kg diet compared with other groups ($P<0.05$).

Conclusions: The results suggested that dietary inclusion of 10 g anise/kg can be applied as alternatives to in-feed antibiotics for broiler diets.

KEYWORDS

Anise seed, Carcass traits, Immune responses, Performance, Broilers

1. Introduction

Antibiotic growth promoters have been successfully used at subtherapeutic doses in poultry production to promote growth and protect health of the birds[1,2]. However, in 1969 the Swann Committee recommended that use of antibiotics as a supplement in animal diet should be restricted to those with little or no application as therapeutic agents for humans and animals, which would not impair the efficacy of therapeutic antibiotics through the development of resistant strains of organisms. Antimicrobials like avoparcin, ardacin, zinc bacitracin, virginiamycin, tylosin, spiramycin, carbadox and olaquindox were withdrawn within the period 1997-1999. Four others (monensin sodium, salinomycin sodium, avilamycin and flavophospholipol) were still permitted for use as growth promoters in animal feed in the

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Foundation Project: This study was supported by Islamic Azad University, Khorasgan Branch, Iran (Grant No. 3256).

Article history:
Received 30 Apr 2014
Received in revised form 6 May, 2nd revised form 18 May, 3rd revised form 31 May 2014
Accepted 17 Jun 2014
Available online 8 Jul 2014

European Union. Since January 2006, they have been banned as well. Thus, probiotics and prebiotics, herbs, spices and various plant extracts have received increased attention as possible antibiotic growth promoter substitutions[3].

Aromatic plants and herbal products have been used worldwide as natural additives for medicinal purposes because they have been accepted by consumers as natural additives[4-6]. Various biological activities, such as antioxidative[7,8], anticoccidial[8,9], immunostimulator[10] or antimicrobial[11] properties have been identified in these plants.

Anise (*Pimpinella anisum* L.), a member of the Apiaceae family, is an annual aromatic plant, native to Iran, India, Turkey and many other warm region in the world. Anise seed possesses eugenol *trans*-anethole, methylchavicol, anisaldehyde, estragole, coumarins, scopoletin, umbelliferone, estrols, terpene hydrocarbons, polyenes, and polyacetylenes[12]. Most of the plant parts such as fruits, seeds, and essential oil contain compounds with proven antiparasitic and digestion stimulating[13], antifungal[14] and antipyretic[15], antioxidant[12], antimicrobial[16], anthelmintic[17] and hypocholesterolemic[18] properties.

Furthermore, Ciftci *et al.* observed the beneficial influence of anise oil on body weight gain and feed conversion ratio (FCR) in broiler chickens[19]. In another study, the incorporation of anise seeds in the feed of laying Japanese quail modified egg yolk color had a cholesterol lowering effect in blood serum, without having any adverse effect on performance and the other egg quality characteristics[20]. Despite these findings, there has been a dearth of information on the possible immunopotentiating effects of anise seed powder on broiler chickens. The present study was designed to compare the efficacy of three inclusion levels of anise seed as an antibiotic growth promoter on growth performance, carcass characteristics, and immune responses in broiler chickens when used as supplements in the diet.

2. Materials and methods

2.1. Animals and dietary treatments

Two hundred and forty, one-day-old broiler chickens of mixed sex (Ross-308) were weighed and randomly allocated to each of the 5 treatment groups, each with 4 replicate of 12 chicks. A commercial antibiotic growth promoter was supplemented to basal diet with no additives. The dietary treatments were basal diet (control), control+4.5 mg flavophospholipol/kg, and control+1, 5 or 10 g anise seed powder/kg diet.

Table 1 lists the basal diet formulated to meet or exceed the nutrient requirements of broilers[21]. The birds were fed a starter diet from Day 0 to Day 14, a grower diet from Day 15 to Day 28, and finisher diet from Day 29 to Day 42. All the dietary treatments were added to the basal diets at the expense of corn. Chicks were raised on floor pens (120 cm×120 cm×80 cm) for 6 weeks and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness. The ambient temperature in experimental house was maintained at 32 °C during the first week and gradually decreased by 3 °C in the second and third weeks, and finally fixed at 22 °C thereafter.

Table 1

The ingredient and calculated composition of basal starter, grower, and finisher diets.

Item		Starter	Grower	Finisher
Ingredient (g/kg)	Corn	537.3	533.0	561.5
	Soybean meal	400	396	370
	Soybean oil	20	35	35
	Dicalcium phosphate	19.3	17.1	15.6
	$CaCO_3$	10.5	8.7	8.5
	NaCl	3.5	3.0	3.0
	Trace mineral premix[1]	2.5	2.5	2.5
	Vitamin premix[2]	2.5	2.5	2.5
	DL-methionine	3.1	2.0	1.4
	L-lysine	1.3	-	-
Calculated composition	Metabolizable energy (kcal/kg)	2870	2980	3000
	Crude protein (g/kg)	221	220	210
	Calcium (g/kg)	8.6	7.5	7.0
	Available phosphorus (g/kg)	4.9	4.4	4.1
	Methionine+cysteine (g/kg)	10.1	8.9	8.0
	Lysine (g/kg)	13.3	11.9	11.3
	Threonine (g/kg)	8.3	8.3	6.3
	Tryptophan (g/kg)	3.2	3.2	3.0

[1]Provided the following per kg of diet: Mg: 56 mg; Fe: 20 mg; Cu: 10 mg; Zn: 50 mg; Co: 125 mg; I: 0.8 mg.

[2]Provided the following per kg of diet: vitamin A: 10000 IU; vitamin D3: 2000 IU; vitamin E: 5 IU; vitamin K: 2 mg; riboflavin: 4.2 mg; vitamin B12: 0.01 mg; pantothenic acid: 5 mg; nicotinic acid: 20 mg; folic acid: 0.5 mg; choline: 3 mg.

2.2. Performance and carcass components

Body weights of broilers were determined at 1, 14, 28, and 42 d of age. Feed intake and weight gain were recorded in different periods and FCR was calculated. Mortality was recorded as it occurred and was used to adjust the total number of birds to determine the total feed intake per bird and FCR. At 42 d of age, two birds per replicate were randomly chosen, based on the average weight of the group and slaughtered through cutting carotid arteries and partial slicing of the neck by a manual neck cutter. Carcass yield was calculated by dividing eviscerated weight by live weight. Abdominal fat, liver, gizzard, heart, proventriculus, small intestine, cecum, were collected, weighed and calculated as a percentage of live body weight and also carefully examined to detect any pathological lesion or damages. Small intestine length and cecum length was measured.

2.3. Immune responses

The commercially available oil-adjuvant injectable emulsion against newcastle disease viruse (NDV) and avian influenza viruse (AIV) (H9N2 subtype) were used for vaccinating broiler chicks, and they were injected subcutaneously with 0.2 mL per chick at 9 d of age. Antibody titers against AIV, and relative weight of lymphoid organs were measured as immune responses. At 28 d of age, two male broilers from each pen were randomly selected, and blood samples were taken by puncture of the brachial vein for analysis of antibody titers against AIV. Serum antibody titers against AIV were measured by the hemagglutination inhibition test, and hemagglutination inhibition antibodies were then converted to $\log_2$.

At 42 d of age, three birds per replicate were randomly chosen, based on the average weight of the group and slaughtered through cutting carotid arteries and partial slicing of the neck by a manual neck cutter; bursa and spleen were collected, weighed and calculated as a percentage of live bodyweight.

2.4. Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the general linear model procedures of SAS Institute (2009). Means were compared using Tukey test. Statements of statistical significance are based on $P<0.05$.

3. Results

3.1. Performance and carcass traits

As Table 2 indicates bodyweight of broilers fed basal diet was higher than other groups at 42 d of age but it was not statistically significant ($P>0.05$). Broilers receiving basal diet had higher feed intake compared to broilers receiving different levels of anise seed ($P<0.05$). Feed intake of broilers in other periods was not markedly affected, but considering the entire experimental period (0-42 d) it tended to be lower in broilers receiving different levels of anise seed. Broilers receiving 1 g anise/kg had lower FCR compared to broilers receiving basal diet or basal diet supplemented with 5 g anise/kg ($P<0.05$). The most efficient feed conversion throughout the study was observed in chicks fed diets supplemented with 1 g anise/kg ($P<0.05$). No difference was observed because treatment effects were observed on mortality.

Table 3 shows carcass, abdominal fat, and relative organ weights as a percentage of live weight at slaughter, and absolute small intestine and cecum lengths. Most of the carcass characteristics of broilers slaughtered at Day 42 were not influenced by treatments but carcass yield significantly increased ($P<0.05$) in broilers supplemented with 10 g anise/kg compared to antibiotic group.

Table 2

Effect of experimental diets on performance indices of broilers at different ages.

Dietary treatments	Body weight (g)			Daily feed intake (g/d)				Feed: gain (g: g)			
	14 d	28 d	42 d	0-14 d	14-28 d	28-42 d	0-42 d	0-14 d	14-28 d	28-42 d	0-42 d
Control	261.50	1030.30	2210.60	25.92	95.15^{a}	160.75	91.75	1.41	1.80^{a}	2.00	1.80^{ab}
Flavophospholipol	274.50	1062.00	2090.50	26.17	94.27^{ab}	159.72	91.12	1.39	1.75^{ab}	2.10	1.90^{a}
1 g anise seed/kg	275.20	1053.70	2116.50	25.75	89.62^{bc}	154.57	88.87	1.40	1.70^{b}	2.00	1.70^{b}
5 g anise seed/kg	274.00	1041.50	2125.70	25.00	88.45^{c}	158.12	88.65	1.40	1.80^{a}	2.10	1.80^{ab}
10 g anise seed/kg	273.20	1035.00	2129.70	25.50	89.91^{bc}	159.00	88.27	1.41	1.75^{ab}	2.10	1.80^{ab}
SEM[1]	5.37	25.94	54.41	0.53	1.58	3.08	1.40	0.03	0.04	0.05	0.05

Values in the same column not sharing a common superscript differ significantly ($P<0.05$). [1]: Standard error of mean.

Table 3

Effect of experimental diets on carcass yield and internal relative organ weight of broilers on Day 42.

Dietary treatments	Relative organ weight									
	Carcass (%)	Abdominal fat (%)	Liver (%)	Gizzard (%)	Heart (%)	Proventriculus (%)	Small intestine (%)	Small intestine (cm)	Cecum (%)	Cecum (cm)
Control	70.60^{ab}	1.58	2.440	2.61	0.500	0.51	4.56	1950	0.63	37.00
Flavophospholipol	70.50^{b}	1.64	2.570	2.76	0.550	0.49	4.60	195.6	0.64	40.00
1 g anise seed/kg	70.70^{ab}	1.58	2.480	2.46	0.550	0.56	4.95	204.0	0.79	41.00
5 g anise seed/kg	72.00^{ab}	1.66	2.410	2.49	0.530	0.52	4.54	198.0	0.71	40.00
10 g anise seed/kg	72.40^{a}	1.67	2.500	2.49	0.500	0.47	4.43	198.1	0.64	39.00
SEM[1]	0.25	0.04	0.013	0.05	0.007	0.01	0.30	4.8	0.02	0.45

Values in the same column not sharing a common superscript differ significantly ($P<0.05$). [1]: Standard error of mean.

3.2. Immune responses

The effects of treatments on immune related parameters are shown in Table 4. According to Table 4 spleen's weight was not affected by dietary treatments although bursa weights were signed ($P<0.05$) higher for birds fed diets supplemented with 1 g anise/kg. Antibody titer against AIV increased in the group treated with 10 g anise/kg diet compared with other groups ($P<0.05$) (Table 5). Antibiotic had not any significant effect on antibody titers against AIV but it tended to be higher.

Table 4

Effect of experimental diets on lymphoid organs on Day 42.

Dietary treatments	Bursa*	Spleen*
Control	0.180^{b}	0.090
Flavophospholipol	0.210^{ab}	0.090
1 g anise seed/kg	0.250^{a}	0.100
5 g anise seed/kg	0.220^{ab}	0.090
10 g anise seed/kg	0.220^{ab}	0.080
SEM[1]	0.006	0.002

Values in the same column not sharing a common superscript differ significantly ($P<0.05$). *: Percentage of live body weight. [1]: Standard error of mean.

Table 5
Effect of experimental diets on antibody titers against AIV on Day 28.

Dietary treatments	Influenza (log_2)
Control	3.37^{d}
Flavophospholipol	4.25^{cd}
1 g anise seed/kg	5.12^{bc}
5 g anise seed/kg	4.12^{cd}
10 g anise seed/kg	7.00^{a}
SEM[1]	0.36

Values in the same column not sharing a common superscript differ significantly ($P<0.05$). [1]: Standard error of mean.

4. Discussion

Anise seed and antibiotic had not any positive effect on body weight, but the most efficient feed conversion throughout the study was observed in chicks fed diets supplemented with 1 g anise/kg. Soltan *et al.* reported that, anise supplementation at 0.5 and 0.75 g/kg of diet improved body weight gain, performance index and relative growth rate of broiler chickens, while had no significant effect on feed intake and FCR when compared with the control[22]. The favorable effects of adding anise seed to the basal diet on FCR may be due to active ingredient such as anothole, eugenol, methylchavicol, anisaldehyde and estragole in anise. Cabuk *et al.* reported that crushed anise seed stimulate animal digestive system, particularly protein, fats and cellulose digestion[13]. Also it seems that the positive effect of anise seeds on FCR may be due to the improvement of apparent whole tract and ileal digestibility of the nutrients and increasing the effect of pancreatic lipase and amylase[23,24]. In this experiment, broilers receiving 1 g anise/kg had lower FCR compared to broilers received higher dosage of anise seed. In agreement with our results, Ertas *et al.* reported that addition of 200 mg/L essential oil mix derived from oregano, clove and anise improved body weight, while addition of 400 mg/L reduced live body weight when compared with the negative control of broiler chickens[25]. The reason of reducing FCR with the higher level of anise seed supplementation may be affected negatively digestive system. Landy *et al.* reported that high dosage of medicinal plants in the diet may have an adverse effect on some beneficial microbial population, such as *Lactobacillus*, preventing the herb from exhibiting its positive influence on digestive system[26]. In the present trial most of the carcass characteristics of broilers were not influenced by treatments but carcass yield increased in broilers supplemented with 10 g anise/kg compared to other groups. Adjustment with our results, Hamodi and Al-khalani reported a significant increase in relative weight of breast and thigh of broilers when broilers were supplemented with anise seed[27].

Use of antibiotic, flavophospholipol, failed to have any effect on antibody titer against AIV, and relative weight of lymphoid organs in comparison with control groups. Dafwang *et al.* reported a slight response of broiler antibodies against SRBC to diets supplemented with oxytetracycline, lincomycin, penicillin, bambermycins or tylan[28]. Also in our another trial, humoral immune responses of broilers fed diet supplemented with flavophospholipol were not affected by dietary treatments[29]. In the present study, antibody titer against AIV increased in the groups treated with 1 or 10 g anise/kg diet. Also, bursa weights were signed ($P<0.05$) higher for birds fed diets supplemented with 1 g anise/kg. As anise has been reported to have antimicrobial[16], antifungal[14] and antioxidant activities[12], an increase in immune responses of chicks was anticipated. Jaffe found that birds with larger bursa of fabricius have more resistance against diseases[30]. Yamamoto and Glick reported that the synthesis of immunoglobulins was higher in chicken with larger bursa of fabricius[31]. Our data are in agreement with Al-Beitawi, who stated that addition of mixture derived from thyme, black seed and anise improved antibody titers against infectious bronchitis, NDV and infectious bursal diseases[32]. Bayram *et al.* showed that anise seed potentiate the humoral immune response to NDV[33]. Unfortunately, no other reports are available on the effects of anise seed on bird immunity.

In conclusion, the results suggested that dietary inclusion of 10 g anise/kg can be applied as alternatives to in-feed antibiotics for broiler diets.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This study was supported by Islamic Azad University, Khorasgan Branch, Iran, and resulted from M.Sc thesis of Faramarz Fekri Yazdi (Grant No. 3256).

Comments

Background

Antibiotic growth promoters have been successfully used at subtherapeutic doses in poultry production to promote growth and protect health of the birds. However, there is the fear that the continuous subtherapeutic use of in-feed antibiotics could lead to the development of antibiotic resistant bacteria, which can be harmful to humans.

Research frontiers

The study was designed to compare the efficacy of three inclusion levels of anise seed as an antibiotic growth promoter on growth performance, carcass characteristics, and immune responses in broiler chickens when used as supplements in the diet.

Related reports

A lot of researches regarding anise seed as an antibiotic growth promoters have been reported. The beneficial influence of anise oil on body weight gain and FCR in broiler chickens is reported as well.

Innovations & breakthroughs

The study makes an attempt to investigate the inclusion of three levels of anise seed as an antibiotic growth promoter substitute on growth performance, carcass traits, and immune responses in broiler chickens.

Applications

The results of the present study can be applied in poultry types and remove antibiotics for production of healthy meat.

Peer review

This is a good study in which the authors evaluated the anise seed levels to antibiotic growth promoter and immune response in poultry nutrition. The experiment is designed well. The results of this study are useful, and the results can be applied in all breeding birds.

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