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Effect of Different Levels of Essential Oils of Wormwood (*Artemisia absinthium*) and Cumin (*Cuminum cyminum*) on Growth Performance Carcass Characteristics and Immune System in Broiler Chicks

Research Article

R. Habibi[1*], G. Jalilvand[1], S. Samadi[2] and A. Azizpour[3]

[1] Department of Animal Science, Faculty of Agriculture, University of Zabol, Zabol, Iran
[2] Department of Poultry Science, Faculty of Agriculture, Tabrbiat Modarres University, Tehran, Iran
[3] Department of Plant Science, Meshginshahr Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran

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*Correspondence E-mail: ramin.habibi@uoz.ac.ir

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ABSTRACT

This experiment had been done to evaluate the effects of different levels of essential oils (EOs) of wormwood (*Artemisia absinthium*) and cumin (*Cuminum cyminum*) on growth performance, carcass characteristics and immune system in broiler chicks. Three hundred ninety two Ross 308 male broiler chicks in a completely randomized design were divided into 7 experimental treatments. The experimental treatments were included a corn-soybean based diet as control and groups of containing 100, 200 and 300 ppm of wormwood and cumin EOs. Results showed that cumin and wormwood EOs supplementation to the diets had no significant effects on growth performance and carcass characteristics ($P>0.05$) of broiler chicks. EOs infusions significantly ($P<0.05$) increased the relative weight of immune organ such as bursa of fabricius, However there was not significant difference between treatments in the relative weight of spleen ($P>0.05$). Antibody titre against sheep red blood cell (SRBC), after 7 days injection, significantly increased by addition 200 ppm wormwood EOs and 200 and 300 ppm cumin EOs ($P<0.05$). In 14 days after SRBC injection, except treatment containing 100 ppm, all supplemented groups increased antibody titre compared to the control group ($P<0.05$). The results of this experiment suggest that inclusion wormwood and cumin EOs to the diets, tended to increase growth performance and improve immune system of broiler chickens.

KEY WORDS broiler, essential oil, immune system, performance.

INTRODUCTION

The use of antibiotics in food animals selects for bacteria resistant to antibiotics used in humans, and these might spread via the food to humans and cause human infection, hence the banning of antibiotic growth-promoters. The actual danger seems small and there might be disadvantages to human and to animal health. Although some antibiotics are used both in animals and humans, most of the resistance problem in humans has arisen from human use (Phillips *et al*. 2004), because of this medicinal plant extracts are becoming more popular. They act as antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal, anticoccidial and growth promoters. These plant extracts compete with the synthetic drugs. Majority of medicinal plants do not have the residual effects (Tipu *et al*. 2006). Use of various dietary herbs, plant extracts, especially essential oils (Eos), have been studied for their antimicrobial and growth promoter abilities (Cross *et al*. 2007; Ocak *et al*. 2008; Bampidis *et al*. 2005; Griggs and Jacob, 2005). EOs are volatile, aromatic compounds synthesised by plants for antimicrobial, antifungal, and antiviral purposes and to deter herbivorous insects and animals (Tiihonen *et al*. 2010). *Artemisia absinthium* is an aromatic plant of the

family *Asteraceae* that is known by the common name of wormwood and Cumin EOs yield is 1.3% (Rezaeinodehi and Khangholi, 2008) and also *Cuminum cyminum* (Cumin) of the *Umbelliferae* family is an important medical herb in Asia and has antioxidant, anticholestrol and antimicrobial properties (Aami-Azghadi *et al.* 2010). Platel (2000) reported that cumin enhanced bile activity and content and also Platel and Srinivasan (1996) showed that cumin increased small intestine digestive enzyme such as amylase, lipase, trypsine and chymotripsine in rats. Khaligh *et al.* (2011) indicated that using of 10 g/kg blend of alfalfa, senna, corn flower and absinthe in broiler diet improved antibody titer against Newcastle disease virus. Also, it was pointed out EO of cumin might be more useful than their synthetic counterparts, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), as additives for chicken patties to maintain oxidative / microbial stability and increase shelf life (Sarıçoban and Yilmaz, 2014). Nevertheless, information on the effect of wormwood and cumin EOs or its compounds on broiler performance and carcass characteristics in broiler chicken, is lacking. Therefore, objectives of the present study were to examine the performance and carcass characteristics of broiler chickens fed on a diet containing wormwood and cumin EOs.

MATERIALS AND METHODS

EOs isolation and analyses

The EOs were extracted using a Clevenger-type apparatus. Samples of 50 g of dried wormwood leaves and cumin seeds herbs after distillation (for 1.5 h) were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4 ˚C. EOs was incorporated in diets by dissolving it in the dietary oil. Gas chromatography/mass spectrometry analysis (GC/MS) of the EOs was performed using apparatus of Gas chromatography–mass spectrometry, 7890A Network GC system/5975C InertXL, mass selective detector, Agilent Technologies Company, Sanata Clara, California, USA. The capillary column was a CP-Sil 8 CB LB with a 30 m long fused silica, an interior diameter of 0.32mm and a film thickness of 0.25 µm. For GC/MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium at a flow rate of 1.2 mL/min was used as carrier gas (Adams, 2007).

Chickens, diets and management

A total of 392 d-old Ross-308 male broilers were randomly allocated to 7 experimental treatments. Each treatment consisted of 4 replicates with 14 birds each. A pen was considered as a replicate experimental unit. Corn-soybean meal basal diet was formulated to meet the nutrient levels suggested by NRC (1994).

The experimental treatments were: a corn-soybean based diet as control and groups that received the basal diet supplemented with 100, 200 and 300 ppm of wormwood or cumin Eos. The ingredients and the nutrient composition of the basal diets are presented in Table 1.

Table 1 Ingredients and the nutrient composition of the basal diets

Ingredients (g/kg)	1-21 d	21-42 d
Corn grain	636.2	695.2
Soy meal (44%)	297.9	242.8
Fish meal	31.7	30
Soy oil	0.5	4.7
Calcium bicarbonate	11.2	11.8
Dicalcium phosphate	10.9	6.3
Salt	3.6	2.7
Vitamin permix[1]	2.5	2.5
Mineral permix[2]	2.5	2.5
DL-methionine	1.5	0.4
L-lysine HCL	1.5	1.1
Analysis results (g/kg)		
Energy (kcal/kg)	2900	3000
Crude protein	208.1	187.5
Calcium	9.4	8.4
Available phosphor	4.2	3.3
Methionine	5.2	3.9
Methionine + cystein	8.3	6.7
Lysine	12.9	11.1
Sodium	1.7	1.4

[1] Vitamin premix provides per kg diet: vitamin A: 3600000 IU; vitamin D_3: 800000 IU; vitamin E: 7200 IU; vitamin k_3: 800 IU; vitamin B_6: 2640 mg; Pantothenic acid: 4000 mg; Nicotinic acid: 12000 mg; Niacin: 1200 mg; Folic acid: 6 mg; Biotin: 720 mg; Choline chloride: 100000 mg and Antioxidant: 40000 mg.
[2] Mineral premix provides per kg diet: Manganese: 40000 mg; Iron: 20000 mg; Copper: 4000 mg, Iodine: 400 mg; Selenium: 80 mg; Zinc: 33880 mg and Choline chloride: 100000 mg.

Standard management practice (lighting, ventilation and spaces) of commercial broiler production was applied. During the feeding period of 42 d, feed and water were provided *ad libitum*. The condition and standards of care used in this research were in accordance with the guide for the care and use of laboratory animals (Clark, 1996).

Data collection

Broilers performance was assessed by measuring feed intake (FI) and average body weight (ABW) and then average daily gain (ADG) and feed conversion ratio (FCR) were calculated. Body weight and FI per pen were recorded at 21 and 42 d of age. At 42 d of age, two birds per pen, were randomly selected and killed for dissection purposes. Organs (spleen, gizzard, bursa, heart, liver, thigh and breast) were removed and weighed from each bird. Weight of the organs were expressed as a percentage of BW. The sheep red blood cell (SRBC) test was performed to quantify the specific antibody titre. To do SRBC test, PBS solution was completely mixed with 10cc of SRBC and 0.5 cc of the obtained solution was injected into the breast muscle of 4 birds per treatment in 21 and 28 days of age.

7 days after each sensitization (on days 28 and 35), the injected birds were bled for assessing haemagglutination (HA) titre against SRBC by using freshly prepared one percent SRBC. Whole sheep blood collected in solution (Dextrose 5.125 g, sodium citrate 2 g, sodium chloride 1.05 g in 250 mL distilled water) was washed three times in phosphate buffered saline (PBS) with pH= 7.4 and diluted in PBS to 25%.

The test serum (25 µL) was serially diluted 1:2 with PBS in microtiter plates. After dilution, 25 µL of 1% SRBC was added to each and was mixed thoroughly. The plate was incubated at 37 ˚C for 1 hour and haemagglutination titre was expressed as the log2 of the reciprocal of the highest dilution showing 100 percent agglutination (Yamuna and Thangavel, 2011).

Statistical analysis

All data were analyzed according to the general linear model (GLM) procedure of SAS (2004) as a completely randomized design experiment. When significant differences were found, means were separated using Tukey's range test.

All statements of significance are based on a probability of less than 0.05. Three linear contrasts between experimental treatments were used to test differences between groups of treatments.

RESULTS AND DISCUSSION

The chemical composition of the wormwood and cumin EOs is shown in Table 2. The major components of the wormwood and cumin EOs were found to be β-pinene and γ-terpinene, their contents being 24.2 and 26.24%, respectively.

Growth performance

The effects of wormwood and cumin EOs on performance of broiler chicks presented in Tables 3 and 4. There was no significant differences between different dietary treatments with regard to ABW, ADG, FI or FCR (P>0.05). However, there was a tendency for broilers consuming an essential oil-supplemented diet to grow faster compared to those fed the control group. This results was in agreement with Hernandez *et al*. (2004) who observed an improvement in body weight gain of chicks fed diets supplemented with medical plant extracts and / or EOs. Lee *et al*. (2003) investigated the effect of thymol and carvacrol on female broiler chick performance. The dietary carvacrol reduced BWG, whereas feed: gain ratio improved as compared to control and / or thymol fed birds. Cross *et al*. (2007) reported that thyme oil and yarrow herb has positively influenced growth performance of broilers as it occurred with our birds fed cumin essential oil diets, positive performance results by dietary essential oil supplementation were also demonstrated by Alcicek *et al*. (2003) who showed that essential oil combination from wild herbs increased the BW and improved FCR of broilers.

Table 2 Chemical composition of wormwood and cumin essential oils

Constituents	Percent in oil	
	Cumin	Wormwood
Linalool	0.12	3.9
α-thujone	0.4	1.1
β-thujone	-	19.4
Iso-3-thujanol	-	1.3
Trans pinocarveol	-	0.8
Terpinen-4-ol	0.43	1.4
α-pinene	0.85	3.6
Sabinene	0.5	9.2
β-pinene	11.8	24.2
Myrcene	0.6	4.2
α-phellandrene	-	3
α-terpinene	-	0.3
ρ-cymene	21.5	2.1
β-phellandrene	-	0.9
1,8-cineole	0.3	0.5
β-ocimene	-	0.6
ɣ-terpinene	26.24	0.9
Myrtenal	-	0.4
Germacrene	-	3.3
β-salinene	-	0.6
α-dehydro-ar-himachalene	-	4.1
ɣ-dehydro-ar-himachalene	-	0.8
Neryl isovalerate	-	0.5
Geranyl isovalerate	-	2.3
Cubenol	-	4.6
α-cadinol	-	1.9
Charnazulene	-	1.2
Limonene	0.7	-
Terpinolene	0.1	-
α-terpineol	0.45	0.4
Cuminal	19.8	-
2-caren-10-al	7.8	-
Carbicol	4.5	-

That suggested that dietary EOs enhance production of digestive secretions, stimulate blood circulation, exert antioxidant properties, reduce levels of pathogenic bacteria and may enhance immune status (Brenes and Roura, 2010). However, the performance effects are likely to be dependent on the quality and quantity of EOs used in each study.

Carcass characteristics

The effect of cumin and wormwood EOs on relative organ weights of broiler chicks at d 42 are shown in Table 5. The different levels of cumin and wormwood EOs in diets did not have an effect on carcass yield and relative weights of liver, intestine, heart, gizzard, thigh, breast and abdominal fat in broiler chicks at d 42 (P>0.05). In orthogonal comparison, any comparison was not significant (P>0.05).

Table 3 Effect of dietary treatments on ABW and ADG in broilers

Treatments	ABW (g)		ADG (g/d)		
	21 d	42 d	1-21 d	21-42 d	1-42 d
C	749.88	2303.94	33.73	75.25	53.87
W100	758.09	2344.37	34.12	75.53	54.83
W200	794.56	2376.59	35.85	75.33	55.95
W300	768.15	2326.89	34.61	74.22	54.42
C100	759.61	2348.45	34.18	75.66	54.92
C200	757.79	2343.40	34.10	75.29	54.80
C300	778.99	2360.20	35.11	75.29	55.20
SEM	4.68	15.96	0.22	0.78	0.38
P-value	NS	NS	NS	NS	NS
Contrasts	P-value				
Wormwood EO *vs*. control	NS	NS	NS	NS	NS
Cumin EO *vs*. control	NS	NS	NS	NS	NS
Wormwood EO *vs*. Cumin EO	NS	NS	NS	NS	NS

C: control group; W100, W200 and W300: containing 100, 200 and 300 ppm wormwood essential oil and C100, C200 and C300: containing 100, 200 and 300 ppm of cumin essential oil, respectively.
EO: essential oil; ABW: average body weight and ADG: average daily gain.
SEM: standard error of the means.
NS: non significant.

Table 4 Effect of dietary treatments on feed intake (FI) and feed conversion ratio (FCR) in broilers

Treatments	FI (g/d)			FCR (g/g)		
	1-21 d	21-42 d	1-42 d	1-21 d	21-42 d	1-42 d
C	53.78	157.79	105.79	1.59	2.05	1.91
W100	53.88	156.05	104.96	1.58	2.07	1.92
W200	55.51	160.46	107.99	1.54	2.13	1.94
W300	54.43	154.23	104.34	1.57	2.07	1.92
C100	52.92	155.60	104.27	1.55	2.06	1.91
C200	52.10	160.53	106.32	1.52	2.12	1.94
C300	54.87	152.93	103.90	1.56	2.03	1.88
SEM	0.36	1.77	0.89	0.011	0.014	0.012
P-value	NS	NS	NS	NS	NS	NS
Contrasts	P-value					
Wormwood EO *vs*. control	NS	NS	NS	NS	NS	NS
Cumin EO *vs*. control	NS	NS	NS	NS	NS	NS
Wormwood EO *vs*. Cumin EO	NS	NS	NS	NS	NS	NS

C: control group; W100, W200 and W300: containing 100, 200 and 300 ppm wormwood essential oil and C100, C200 and C300: containing 100, 200 and 300 ppm of cumin essential oil, respectively.
EO: essential oil.
SEM: standard error of the means.
NS: non significant.

Table 5 Effect of dietary treatments on carcass characteristics (% of live weight) in broiler chicks

Treatment	Carcass	Liver	Intestine	Heart	Gizzard	Thigh	Breast	Abdominal fat
C	61.17	2.35	2.34	0.56	1.89	18.49	27.14	1.51
W100	62.01	2.31	2.25	0.58	1.98	18.35	27.56	1.10
W200	62.69	2.38	2.39	0.46	1.69	18.22	27.91	1.08
W300	62.80	2.32	2.63	0.45	1.97	19.27	27.82	0.91
C100	60.61	2.42	2.63	0.54	1.91	18.16	27.11	1.06
C200	61.92	2.39	2.72	0.54	2.09	18.67	26.75	1.15
C300	62.27	2.41	2.44	0.65	1.72	18.13	27.74	1.01
SEM	0.33	0.04	0.05	0.02	0.05	0.19	0.23	0.04
P-value	NS	NS	NS	NS	NS	NS	NS	NS
Contrast	P-value							
Wormwood EO *vs*. control	NS	NS	NS	NS	NS	NS	NS	NS
Cumin EO *vs*. control	NS	NS	NS	NS	NS	NS	NS	NS
Wormwood EO *vs*. Cumin EO	NS	NS	NS	NS	NS	NS	NS	NS

C: control group; W100, W200 and W300: containing 100, 200 and 300 ppm wormwood essential oil and C100, C200 and C300: containing 100, 200 and 300 ppm of cumin essential oil, respectively.
EO: essential oil.
SEM: standard error of the means.
NS: non significant.

As in our results, Lee *et al*. (2003) found that broiler chickens given dietary carvacrol and thymol at 200 mg/kg diet showed no differences in the relative weight of the liver compared with the control birds. Also Khaligh *et al.* (2011) reported that supplementation of broiler diets with medicinal plant blends did not altered liver and gizzard weight as compared to control birds. Habibi *et al*. (2014) reported that broiler carcass relative weight was not affected by the inclusion of 0.75 and 1.5% ginger root powder in the diet that consistent with present results.

Immune response

Results of effects of experimental groups on relative weights of lymphoid organs and antibody response values against SRBC are shown in Table 6. Addition of cumin and wormwood EOs to diet not affected the relative weight of the spleen (P>0.05).

However thyme-treated broilers showed the highest bursa of fabricius relative weights relative to the control broilers (P<0.05), except treatments containing of 100 mg/kg cumin and wormwood EOs.

Table 6 Effect of dietary treatments on immune parameters in broiler chicks

Treatment	Lymphoid organs (% of live weight)		Sheep red blood cell (SRBC)	
	Spleen	Bursa of fabricius	Day 7 after injection (28 d of age)	Day 14 after injection (35 d of age)
C	0.12	0.04^{c}	3.54^{b}	3.85^{b}
W100	0.14	0.05^{abc}	3.61^{b}	4.20^{b}
W200	0.15	0.06^{ab}	5.18^{a}	5.93^{a}
W300	0.13	0.06^{ab}	4.86^{ab}	5.45^{a}
C100	0.14	0.05^{bc}	4.81^{ab}	5.50^{a}
C200	0.14	0.07^{a}	5.29^{a}	5.66^{a}
C300	0.12	0.07^{a}	5.23^{a}	5.82^{a}
SEM	0.004	0.003	0.197	0.203
P-value	NS	0.006	0.028	0.009
Contrast				
Wormwood EO *vs*. control	NS	NS	NS	NS
Cumin EO *vs*. control	NS	NS	NS	NS
Wormwood EO *vs*. Cumin EO	NS	NS	NS	NS

C: control group; W100, W200 and W300: containing 100, 200 and 300 ppm wormwood essential oil and C100, C200 and C300: containing 100, 200 and 300 ppm of cumin essential oil, respectively.
The means within the same column with at least one common letter, do not have significant difference (P>0.01).
EO: essential oil.
SEM: standard error of the means.
NS: non significant.

There was a significant difference (P<0.05) in the HA titre against SRBC among treatment groups after day 7 and 14 (P<0.05) after SRBC injection. Treatments of 200 mg/kg wormwood essential oil and 200 and 300 mg/kg cumin essential oil significantly increased HA titre against SRBC in after day 7 (P<0.05). In day 14 after SRBC injection, All treatments (except 100 mg/kg wormwood essential oil) significantly increased HA titre compared with control broilers (P<0.05). These results were in consistent to report by Kong *et al.* (2006) who indicated that 4 Chinese herbal ingredients may be applied as immune stimulators for an active vaccine in chickens at both *in vitro* and *in vivo* assays. However Sadeghi *et al*. (2012) reported that infusions (5 grams per liter) of cinnamon, thyme and turmeric to diet had no effects on antibody titers to Newcastle disease virus vaccine. Also Aami-Azghadi *et al.* (2010) indicated addition of 0.2, 0.4 and 0.8 g/kg cumin essential oil to broiler diet did not affected total anti SRBC, IgG and IgM titres. The study of the immune system showed that wormwood and cumin EOs was most effective in immune system improvement.

Increasing response to SRBC in EOs groups was expected, because this herb increased stimulation of nonspecific immune system. Herbs that are rich in such flavonoids as cumin and wormwood extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function (Manach *et al*. 1996). On the other hand results of the GC/MS analysis of wormwood and cumin EOs in the present study show that compounds such as β-pinene, β-thujone, ρ-cymene and ɣ-terpinene, thus it is thought that immune enhancement of used EOs is provided by like components.

CONCLUSION

Although growth performance of broiler chicks tended to increase by the wormwood and cumin EOs supplementation but the effects were not statistically significant. EOs supplementation significantly improved the immune response of birds to the SRBC injection. Further research is required to fully understand the effect of wormwood and cumin EOs on immune responses of broiler chicks.

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