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Growth Performance, Carcass Characteristics and Intestinal Microflora of Broiler Chickens Fed Diets Containing *Carum copticum* Essential Oil

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Abstract

We evaluated the effects of dietary *Carum copticum* essential oil (CCEO) on growth performance, carcass characteristics and intestinal microflora of broiler chickens. A total of 240 Cobb broiler chickens were allocated to five dietary treatments, each with four replicates of 12 birds. Dietary treatments were prepared by formulating a corn-soybean meal-based diet free of antibiotics (Control) and supplementing the basal diet with three levels of CCEO at 150, 250, 350 mg/kg or antibiotic Virginiamycin at 200 mg/kg. Treatments were fed from 0 to 42 d of age. Body weight gain decreased linearly (P=0.035) with increasing CCEO while Virginiamycin increased body weight gain at 1 to 10 d compared to the control treatment ($P < 0.05$). Though feed consumption was not affected by CCEO, CCEO insignificantly improved feed conversion ratio (FCR) ($P > 0.05$). There were no differences in carcass characteristics among broiler chickens fed the control, CCEO and Virginiamycin diets ($P > 0.05$). Lactic acid bacteria in the cecum and ileum at 42 d of age were not influenced by the treatments ($P > 0.05$) but there was a linear increase of the log numbers of *E. coli* in the ileum (P=0.02) with increasing CCEO ($P < 0.05$). In conclusion, supplementing CCEO to diet at 150 mg/kg improve the growth performance, decrease undesirable intestinal bacteria in broiler chickens and is an adequate alternative to antibiotics.

Introduction

Several antibiotics as growth and health promoters (AGP) have been used in poultry feed for approximately 60 years in the world. However, the use of AGP has been restricted due to the development of bacterial resistance in humans, and the growing demand for antibiotic residue-free food products (Saleha *et al.*, 2009). Prohibition of using antibiotics has led to increasing attempts to find products that can be used in poultry diet as growth and health stimulators, without having adverse effects on consumers' health.

Recently, the use of essential oil has become popular due to its antimicrobial properties (Akin *et al.*, 2010). Herbal essential oils are compounds that originate from secondary metabolites in plants as products of water evaporation from the plant root, husk, flower, fruit or stem. Furthermore, essential oils are responsible for the formation of odor and color in plants (Lee *et al.*,

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2004; Zhang *et al*., 2005). The concentration of the biologically active components in essential oils are variable and dependent on the species, the part of the plant used, soil, environmental conditions, and time of harvest (Lee *et al*., 2004; Barreto *et al*., 2008). Aromatic herbs and essential oils derived from them have shown various biological functions such as antimicrobial, antiparasitic, antiviral and antioxidant properties in poultry. These compounds may affect the metabolism and digestibility of nutrients by stimulation of digestive enzymes and have beneficial effects on the gastrointestinal tract and microbial population. These compounds can also stimulate the immune system and endocrine glands (Jamroz *et al*., 2005; Jang *et al*., 2007).

Carum copticum or Ajwain belongs to the Apiaceous plants family and grows in the eastern parts of India, Iran, and Egypt. In Iran, the most important growth areas include Sistan-Baluchistan, Azerbaijan, Isfahan, Khuzestan, Fars, Kerman, and Khorasan (Zahin *et al*., 2010; Zareshenas *et al*., 2013). Its seeds are used extensively for medicinal purposes as a digestive stimulant and to treat liver disorders (Ishikawa *et al*., 2001). The plant's fruit (named Zenian in Persian) is small, oval, and brownish yellow in color, and has a penetrating scent like that of *Thymus vulgaris*. Medicinal properties of the plant include antioxidant, antispasmodic, antimicrobial and antifungal (Zareshenas, 2013). Khaje *et al*, (2004) reported the active ingredients of the plant include six major chemical compounds including 49% thymol, 30.8% γ-terpinene, 15.7% p-cymene, 2.1% b-pinene, 0.8% myrcene and 0.7% limonene. The antimicrobial effects of the essential oils against *Staphylococcus aureus* and *Bacillus subtilis* have been demonstrated (Kazemi Oskouee *et al*., 2011). Comparison of antimicrobial effects of aqueous and acetone extracts of Ajwain on 11 pathogenic bacterial species indicated that aqueous extract of Ajwain has greater antibacterial capacity in preventing the growth of *Enterococcus faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhimurium*, and *S. flexneri* (Kaur and Arora, 2009). Although this plant is indigenous to Iran and is an important component of traditional medicine, few studies have investigated its effects on broiler chickens. We aim to make a comparison between the effectiveness of CCEO and Virginiamycin (VIR) on the performance, carcass characteristics, and intestinal microbes of broiler chickens.

Materials and Methods

Experimental design and husbandry

240 mixed-sex one-day old broiler chicks (Cobb 500) were obtained from a local commercial hatchery, weighed, and randomly assigned to five dietary treatments with four replicates, each with 12 birds. The experimental treatments were 1) basal diet, 2) basal diet supplemented with 200 mg/kg of virginiamycin (the dose suggested by the manufacturer), and 3, 4, and 5) basal diet supplemented with 150, 250, or 350 mg/kg of CCEO, respectively. Virginiamycin - a well-known antimicrobial growth promoter used in poultry – was used as a positive control.

Feed and water were provided *ad libitum*. Lighting schedule was 23L:1D. On arrival, the temperature was 32°C, and then gradually reduced by 3°C each week to reach constant at 22°C. The basal diet was formulated to meet or exceed Cobb 500 broiler nutrition specifications for nutrients (Table 1) and was prepared in mash form each week. The feed program consisted of 3 phases: 1-10 days of age, 11-22 days of age, and 23-42 days of age. Body weight and cumulative feed intake (FI) were measured and feed conversion ratio was calculated at the end of each stage of the experiment.

Preparations and analysis of chemical composition of essential oil

Air-dried powdered fruits of *Carum copticum* (100 g) were completely immersed in water and hydrodistilled in a full glass Clevenger-type apparatus. The extraction was carried out for 4 hrs and yielded oils that were light yellow in color. After the condensed material cooled, the water and essential oil were separated. The obtained essential oils were stored in dark glass bottles at −4°C until used for the experiment (Kazemi Oskouee *et al*., 2011). The essential oil was analyzed by GC/MS (Varian-2200, Australasia) column (VF-5MS, 30 mm × 0.32 mm fused silica capillary column, film thickness 0.25 µm) using a temperature program of 60-240ºC at a rate of 4ºC/min, an injector temperature of 220ºC, and with helium as the carrier gas. The constituents were identified by the comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by the comparison of retention indices with those of authentic compounds or with the literature (Oroojalian *et al*., 2010).

Table 1. Feed ingredients and composition of the basal diet

Ingredients (%)	Starter (0-10 d)	Grower (11-22 d)	Finisher (23-42 d)
Maize, yellow	55.45	60.47	63.88
Soybean meal	38.52	32.94	29.98
Soybean oil	2.16	2.82	2.84
Dicalcium phosphate	1.51	1.46	1.22
Calcium carbonate	1.05	1.00	0.95
Salt	0.45	0.44	0.42
Vitamin Premix[1]	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25
DL-Methionine	0.26	0.23	0.18
L-Lysine	0.10	0.14	0.03
Chemical composition (%)			
ME (Kcal/Kg)	2900	3000	3050
Crude protein	21	19	18
Calcium	0.86	0.81	0.73
Available phosphorus	0.43	0.41	0.36
Sodium	0.19	0.19	0.18
Lysine	1.26	1.15	1.00
Methionine	0.59	0.53	0.47
Methionine + Cystine	0.94	0.86	0.79
Threonine	0.82	0.75	0.70

[1]Contained per kilogram; Vitamin A: 5,500,000 IU; Vitamin D_3: 1,500,000 IU; Vitamin E: 15,000 mg; Vitamin K_3: 800 mg; Thiamine: 1000 mg; Riboflavin: 4000 mg; Niacin: 25,000 mg; Biotin: 30 mg; Folic acid: 500 mg; Pantothenic acid: 5000 mg; Pyridoxine: 1500 mg; Vitamin B_{12}: 15 mg.

[2]Contained per kilogram; Cu: 12,000 mg; Fe: 35,000 mg; Zn: 25,000 mg; Co: 150 mg; I: 500 mg; Se: 120 mg; Mn: 38,000 mg.

Performance traits

Body weight gain, feed consumption, and feed conversion ratio were determined between each feed change periods (between days 1-10, 11–22 and 23–42 and also from days 1 to 42.

Carcass characteristics

On day 42, two birds per pen closest to the mean body weight of the pen were slaughtered and whole carcass, breast, thigh, abdominal fat pad (excluding the gizzard fat), gizzard, liver, heart and lymphoid organs (spleen and bursa of Fabricius) were excised and weighed individually. The carcass yields were calculated as a percentage of the pre-slaughter live body weights of broiler chickens. Weights of internal organs were expressed as a percentage of live body weight.

Microbial sampling and incubation

On day 42 of the experiment, two birds from each replicate were killed and the ileum (defined as the region between Meckel's diverticulum and the ileocecal junction) and cecum contents were collected. The contents of these intestinal segments were used for the microbial study. Cecum and ileum samples were homogenized in saline and series of decimal dilution (10^{-3} to 10^{-7}) were prepared. The total count of aerobic bacteria was determined on plate count agar (PCA) for 24 hrs at 37°C. *Escherichia coli* was quantified on the Eosin-methylene blue (EMB) agar. Plates were incubated for 24 hrs at 37°C. Colonies that had a green metallic surface color were considered to be *E. coli*. Lactic acid bacteria were counted on the De Man rogosa sharpe agar (MRS) medium. Plates were incubated in anaerobic conditions at 37°C for 48–72 hrs. Results were expressed as the log10 of colony forming units (CFU) per gram of ileal and cecal digesta.

Statistical analysis

A completely randomized design (CRD) was employed and data were analyzed using the General Linear Models (GLM) procedure of SAS (2001). Duncan's multiple range test were used to compare means. Statistical significance is based on $P < 0.05$. The linear and quadratic effects of dietary CCEO inclusion level were studied using polynomial contrasts.

Results and Discussion

Chemical composition of CCEO

15 compounds were identified in CCEO (Table 2), representing 99.8% of total oils. The main constituents were thymol (42%), γ -terpinene (30.2%), p-cymene (19%), and b-pinene (1.8%).

Khajeh *et al.* (2004) showed that there were six components including thymol (49.0%), γ-terpinene (30.8%), p-cymene (15.7%), b-pinene (2.1%), myrcene (0.8%), and limonene (0.7%). CCEO has previously been shown to have two chemotypes, thymol and carvacrol (Mohagheghzadeh *et al.*, 2007). Therefore, the *Carum copticum* oil used in the present study belonged to the thymol chemotype. These differences in chemical compositions of the oils could be attributed to the species, the part of the plant used, soil, environmental conditions, and time of harvest (Barreto *et al.*, 2008).

Table 2. Chemical composition of *Carum copticum* essential oil

No	Phytochemicals	Retention Index	%
1	α-Thujene	924	0.7
2	α-Pinene	932	0.3
3	Sabinene	969	0.7
4	β-pinene	974	1.8
5	Myrcene	988	1
6	δ-3-Carene	1008	0.1
7	α-Terpinene	1014	0.8
8	p-Cymene	1020	19
9	β-Phellandrene	1025	1
10	γ-terpinene	1045	30.2
11	Linalool	1095	0.1
12	Terpinene-4-ol	1174	0.2
13	trans-antol	1282	1.4
14	Thymol	1289	42
15	Carvacrol	1298	0.5
	Total identified		99.8

Growth performance

The effects of CCEO and VIR supplementation on body weight gain (BWG) of broiler chickens are shown in Table 3. In the initial period (1–10d), BWG decreased linearly (P=0.035) with CCEO supplementation concentrations while VIR yielded chickens with significantly higher BWG compared to chickens from the basal diet and 350 mg/kg CCEO treatments (P < 0.05). Moreover, during the same trial period (1 to 10 d of age), there was no significant difference between the 250 and 350 mg/kg CCEO with and control diet (P > 0.05). There were no differences in BWG from 11 to 22 d (grower) and 23 to 42 d (finisher) age between treatments (P > 0.05).

Administration of 150 mg/kg of CCEO significantly improved overall body weight gain by 2.28 and 2.6%, when compared to control and 350 mg/kg of essential oil, respectively, and were similar to antibiotic treatment at 1 to 42 d (P < 0.05). Throughout the period of study (1-42d), supplementing broiler chickens with VIR had a negligible effect on growth performance compared to the control diet (P > 0.05). Our findings suggest that CCEO may reverse effects and decrease growth performance at high levels (350 mg/kg0. Therefore, CCEO supplementation above 250 mg/kg is not recommended in broiler diet.

Table 3. Effect of dietary CCEO and VIR on body weight gain (g) in broiler chickens

Treatment	1-10 d	11-22 d	23-42 d	1-42 d
Control	212.48^{b}	498.96	1597.92	2276.75^{b}
VIR[1]	233.50^{a}	497.91	1606.25	2304.88^{ab}
CCEO[2] (150 mg/kg)	234.33^{a}	481.24	1642.71	2328.84^{a}
CCEO (250 mg/kg)	226.20^{ab}	497.91	1594.79	2288.21^{ab}
CCEO (350 mg/kg)	212.04^{b}	488.54	1597.92	2268.42^{b}
SEM[3]	9.35	13.11	32.40	25.04
P-value				
Anova	0.03	0.83	0.98	0.032
Linear	0.035	0.63	0.71	0.05
Quadratic	0.064	0.68	0.68	0.43

[1]Virginiamycin; [2]*Carum copticum* Essential Oil; [3]Standard Error of Means.

Means within a column having different superscripts are significantly different (P < *0.05*).

The addition of VIR to the broiler chicken diets only had significant effects on weight gain in the first phase of rearing. Antibiotics eliminate harmful bacterial populations and their growth-reducing metabolites, thereby reducing competition over access to food among bacteria living in the host's gastrointestinal tract (Esteve-Garcia *et al*., 1997). Moreover, by reducing the thickness of intestinal mucosal wall (Miles *et al*., 2006) and increasing the ratio of intestinal villus length to the crypt depth, these compounds increase digestion and absorption of nutrients in the intestine, and thereby increase body weight and improve performance of poultry (Mountzouris *et al*., 2011). In congruence with this study, Yakhkeshi *et al*. (2011) demonstrate that body weight gain was significantly higher in diets containing antibiotics compared to control treatment. Mills *et al*., (2006) proposed that the reaction of broiler chickens to antibiotics is affected by different factors such as age, genetics, and environmental factors. For example, since younger animals are more sensitive to antibiotics and in better hygienic conditions during rearing, growth-stimulating effects of antibiotics may not be obvious.

In the present study, broilers given diets supplemented with 150 mg/kg CCEO significantly increased body weight gain compared to the control treatment during the initial and the whole trial period. Mathlouthi *et al*. (2012) investigated how weight gain of Arbor Acres broiler chicks was influenced by the antibiotic Avilamycin (44 mg), rosemary essential oil (100 mg), oregano essential oil (100 mg), and a combination of rosemary and oregano essential oils (50 mg each), and 1000 mg of commercial products containing essential oils. Chicks were fed essential oils and antibiotics in the first period had significantly higher weight gain compared to the control group. In contrast, Jang *et al*., (2007) reported that addition of 25 and 50 mg commercial compound containing herbal essential oils to broiler diets did not have a significant effect on body weight gain, feed intake, and feed conversion ratio. Herbal essential oils improve digestion and absorption capacity of the gastrointestinal tract by increasing production and secretion of enzymes such as trypsin, lipase, and intestinal amylase. They can also increase the size of intestinal microvilli and decrease crypt depth, leading to accelerated growth in animals (Jamroz *et al*., 2005; Windisch *et al*., 2008).

The efficiency of nutrient consumption in the first period of a broiler's life is low due to an underdeveloped gastrointestinal tract and low secretion of digestive enzymes such as lipase, amylase, and trypsin (Sklan *et al*., 2000). As the chicks grow and their gastrointestinal tract becomes larger, the secretion of these digestive enzymes also increases. Therefore, the addition of essential oils to chicks' diet in the early stage of their lives would increase enzyme secretion increase weight gain. In addition, the effective herbal compounds increase the length of intestinal microvilli, thereby increasing absorption of nutrients *via* the intestine (Zhang *et al*., 2005). Ali *et al*. (2003) and Rama Krishna *et al*. (2003) found that the use of *Carum copticum* in the diet of rats stimulates the secretion of protease, amylase and lipase enzymes from the pancreas. Therefore, based on the findings of this study, weight gain of broilers in the initial stage of their life and in the entire rearing period with 150 mg/kg of CCEO, is likely attributed to the secretion of digestive enzymes. However, Botsoglou *et al*. (2002) found no change in body weight gain and feed conversion ratio of broiler chickens fed 50 and 100 mg/kg of oregano essential oil for a period of 38 days. Effect of essential oils on growth performance is very diverse. This may be due to differences in the herbal additives, concentrations, and biological activities. Moreover, the reaction of chickens to a herbal additive may be influenced by type of diet, age, health, and environmental factors (Amad *et al*., 2011). It is reported that in critical situations and adverse dietary conditions for chickens, essential oils can show growth-stimulating effects (Zhang *et al*., 2005).

Interestingly, 350 mg/kg of CCEO negatively affected weight gain of broilers. Traesel *et al*. (2011) investigated the effects of essential oils in broiler diets on performance and secretion of digestive enzymes and showed that addition of 100 mg/kg mixture of oregano, sage, rosemary and pepper sage essential oils/increased body weight in all rearing periods. They suggested that higher levels of essential oils may cause renal nephritis and decrease liver function, leading to adverse performance in the broilers. Plantamed (2009) reported sage and rosemary at high doses can cause renal failure, and prolonged use can cause nephritis. It would appear that a ketone known as *thujone* in sage oil is responsible for kidney and liver impairment. Analysis of compounds in CCEO in this experiment indicated the presence of *thujone*. Therefore, high levels of CCEO in broiler diets can negatively affect performance by kidney and liver impairment. Based on the findings of Lee *et al*. (2004), adding high levels of essential oil to the broiler's diet can have adverse effects on their performance. Thus, poultry performance is affected by the dosage of essential oil in the diet.

There were no significant differences in feed intake between the treatment groups ($P > 0.05$; Table 4). Botsoglou *et al*. (2002), Hernandez *et al*. (2004) and Lee *et al*. (2004) similarly reported that addition of plant extracts or essential oils to broiler diets had no effect on feed intake. In contrast, Valiollahi *et al*., (2014) showed that feed intake increased significantly in 0.02% *Carum copticum* powder in comparison to control treatment.

Table 4. Effect of dietary CCEO and VIR on feed intake (g) in broiler chickens

Treatment	1-10 d	11-22 d	23-42 d	1-42 d
Control	250.43	768.81	3297.19	4316.57
VIR[1]	261.66	760.31	3220.94	4242.92
CCEO[2] (150 mg/kg)	250.66	745.72	3174.06	4170.11
CCEO (250 mg/kg)	259.58	746.04	3306.04	4311.67
CCEO (350 mg/kg)	254.68	757.29	3311.46	4323.44
SEM[3]	5.48	14.73	122.75	130.08
P-value				
Anova	0.49	0.63	0.91	0.92
Linear	0.81	0.80	0.52	0.58
Quadratic	0.93	0.10	0.49	0.52

[1]Virginiamycin; [2]*Carum copticum* essential oil; [3]Standard Error of Means.

There were no differences in FCR among experimental treatments ($P > 0.05$; Table 5). Oregano and rosemary addition (5 g/kg of diet) to diet also did not have an effect on FCR in laying hens (Botsoglou *et al*., 2005). In contrast, Hernandez *et al*. (2004) reported positive effects of essential oil mixtures on nutrient digestibility and feed efficiency. Cross *et al*. (2007) reported that gut passage rate, movement of the gallbladder, and peristaltic contraction of gastrointestinal tract increased in the presence of herbal essential oils, resulting in rapid digestion and metabolic activity. *Carum copticum* can increase the amount of gastric acid and bile secreted in rats (Platel and Srinivasan, 2001) and can reduce the feed transit time through the digestive tract, decreasing the intestinal contractions (Hejazian *et al*., 2007). As a result, feed remains in contact with the enzymes for longer periods, which leads to improved feed efficiency and weight gain.

Table 5. Effect of dietary CCEO and VIR on feed conversion ratio in broiler chickens

Treatment	1-10 d	11-22 d	23-42 d	1-42 d
control	1.18	1.54	2.08	1.89
VIR[1]	1.12	1.53	2.01	1.84
CCEO[2] (150 mg/kg)	1.08	1.55	1.93	1.79
CCEO (250 mg/kg)	1.15	1.50	2.08	1.88
CCEO (350 mg/kg)	1.20	1.55	2.07	1.90
SEM[3]	0.05	0.03	0.09	0.06
P-value				
Anova	0.56	0.31	0.31	0.76
Linear	0.16	0.46	0.41	0.35
Quadratic	0.14	0.65	0.39	0.32

[1]Virginiamycin; [2]*Carum copticum* essential oil; [3]Standard Error of Means.

Carcass characteristics

Relative weights of the carcass, breast, thigh, abdominal fat, gizzard, liver, lymphoid organs, and heart at 42 days were not affected by dietary treatments ($P > 0.05$). These findings are in accordance with the findings of Hernandez *et al*. (2004) who reported that the weights of gizzard, liver, and pancreas were not influenced by essential oils (two mixtures of oregano, cinnamon, and pepper essential oils, and another mixture of sage, thyme, and rosemary essential oil extracts) in broiler chickens. In contrast, Mahmoodi Bardzardi *et al*. (2014) reported that relative carcass weight increased when diet was supplemented with antibiotic and essential oil from *Myrtus communis*, especially at 300 mg/kg. Windisch *et al*. (2008) attributed the variation to differences in animal physiology, rearing environments, illness, diet compounds, and the ingredients of the essential oils.

Table 6. Effect of dietary CCEO and VIR on carcass composition in broiler chickens (% of live body weight)

Treatment	Carcass	Breast	Thigh	AF[3]	Liver	Heart	Gizzard	Spleen	BF[4]
Control	63.78	19.85	17.39	1.78	2.61	0.65	1.95	0.11	0.21
VIR[1]	65.34	21.83	18.10	1.68	2.57	0.59	1.86	0.13	0.24
CCEO[2] (150 mg/kg)	66.02	21.18	18.24	1.61	2.61	0.58	1.86	0.15	0.20
CCEO (250 mg/kg)	64.62	21.42	17.60	1.45	2.47	0.60	1.88	0.11	0.18
CCEO (350 mg/kg)	64.02	20.03	17.76	1.52	2.44	0.69	1.89	0.10	0.22
SEM[5]	1.28	1.08	0.70	0.19	0.17	0.04	0.17	0.04	0.05
P-value									
Anova	0.62	0.63	0.45	0.60	0.94	0.35	0.96	0.21	0.84
Linear	0.63	0.92	0.87	0.65	0.92	0.78	0.86	0.15	0.96
Quadratic	0.51	0.91	0.98	0.86	0.81	0.61	0.95	0.17	0.84

[1]Virginiamycin; [2]*Carum copticum* essential oil; [3]Abdominal fat; [4]Bursa of Fabricius; [5]Standard Error of Means.

Ileum and cecum microflora count

There were no significant differences in counts of both total aerobic bacteria and lactic acid bacteria in the ileum and cecum ($P > 0.05$; Table 7). Nonetheless, at the age of 42 d, CCEO concentrations increased linearly with the ileum concentration of *E. coli* (P=0.02), and the count of *E. coli* in the ileum was significantly lower in birds receiving VIR ($P < 0.05$).

Table 7. Effect of dietary CCEO and VIR on ileum and cecum microflora count (CFU/G) in broiler chickens at d 42

Treatment	cecum			ileum		
	Total aerobic bacteria	Lactic acid bacteria	*E.coli*	Total aerobic bacteria	Lactic acid bacteria	*E.coli*
Control	8.16	7.76	7.76	7.76	7.76	6.66[a]
VIR[1]	7.96	8.26	7.36	7.80	7.96	5.71[b]
CCEO[2] (150 mg/kg)	7.96	8.26	7.46	7.96	8.04	5.84[b]
CCEO (250 mg/kg)	8.36	8.16	7.45	7.86	8.06	6.41[ab]
CCEO (350 mg/kg)	8.06	7.76	7.56	8.36	7.16	6.71[a]
SEM[3]	0.19	0.23	0.28	0.29	0.36	0.36
P-value						
Anova	0.10	0.16	0.42	0.27	0.73	0.01
Linear	0.16	0.42	0.07	0.73	0.95	0.02
Quadratic	0.81	0.52	0.39	0.12	0.09	0.54

[1]Virginiamycin; [2]*Carum copticum* Essential Oil; [3]Standard Error of Means.
Means within a column having different superscripts are significantly different ($P < 0.05$).

Performance improved in chickens fed 150 mg/kg of CCEO probably due to the reduction of harmful *E. coli* bacteria in the ileum. The antimicrobial effects of CCEO have been proved *in vitro*. Effects of CCEO against *Staphylococcus aureus* and *Bacillus subtilis* have been demonstrated by Kazemi Oskouee *et al*. (2011). Kırkpınar *et al*. (2011) investigated the effect of 300 mg/kg thyme, 300 mg/kg garlic, and 150 mg/kg of garlic and thyme combinations in diets of Hubbard broilers and found no significant effect on *coliform* and *Lactobacilli* in the intestine. However, the *Clostridium* population in the intestines of the broilers fed thyme, garlic, and their combination significantly decreased. Jamroz *et al*., (2005) reported that adding commercial mixtures of essential oils to corn and soybean-based diets reduces the population of *E. coli* and increases the number of *lactobacilli* in the small intestine. However, with reduction of *Clostridium perfringens* and *E. coli* at the age of 14 days, the number of *Lactobacilli* decreased as well. The population of bacteria in the digestive tract plays an important role in nutrient digestion, stimulation of the immune system and maintenance of the intestine's health. Essential oils contribute to the stability of the digestive tract's bacterial ecosystem by reducing populations of pathogenic microorganisms in the digestive tract and consequently, decrease the production of unwanted microbial metabolites such as biogenic amines and urea (Windisch *et al*., 2008).

The antimicrobial properties of essential oils are largely attributed to phenolic compounds which have a phenyl ring with a hydroxyl group. In addition to the transition of ions through the plasma membrane (Ultee *et al*., 2002), the hydroxyl group is also effective in deactivation of bacterial enzymes (Burt, 2004). Therefore, these compounds may have antimicrobial activity against pathogens in the digestive tract without having negative effects on beneficial bacteria such as *bifidobacteria* and *lactobacillus* (Si *et al*., 2006). Essential oils are hydrophobic compounds with low molecular weight, and can penetrate the lipid membranes of bacteria and change the structure of the membrane or protein functions (Burt, 2004). In addition, these compounds can disrupt the vital activities of the bacterial cells, thereby disrupting the ion concentration gradient of H^+ and K^+. This process leads to leakage of the cell contents, ultimately resulting in cell death (Si *et al*., 2006). The antimicrobial properties of CCEO are attributed to its two main ingredients – thymol and carvacrol. γ-terpinene and p-cymene monoterpenes in CCEO have limited antimicrobial activity compared to phenol monoterpenes (Dorman and Deanis, 2000). Ultee (2002) proposed that antimicrobial activity of p-cymene is realized through its accumulation in the plasma membrane, which leads to ion leakage through the membrane.

Conclusion

The results of this study showed that the addition of 150 mg/kg of CCEO in broiler's diets reduced *E. coli* populations in the ileum which were associated with improved performance of broiler chickens. This concentration of the essential oil proved to have similar effects as the VIR antibiotic treatment on weight gain and bacterial population in the ileum. Therefore, CCEO can be used as an alternative to antibiotics. However, CCEO levels higher than 250 mg/kg is not recommended due to the negative effects on growth performance of broiler chickens.

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