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Effects of Adding Ethanol Extract of Propolis and Cumin Essential Oil to Diet on the Performance, Blood Parameters, Immune Response and Carcass Traits of Broiler Chicks

Research Article

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ABSTRACT

This study was conducted to determine the effects of adding ethanol extract of propolis (EEP) and cumin essential oil (CEO) to diets of broiler chicks on the performance, blood parameters, immune response and carcass traits. A total of 240 day-old Ross-308 broiler chicks were randomly allocated to four treatments with six replicates and 10 birds in each replicate in a completely randomized design. Each of four iso-caloric and iso-nitrogenous corn-soybean-based experimental diets including control, diet with 0.2 g/kg EEP, diet with 0.8 g/kg CEO and diet with 0.2 EEP g/kg as well as 0.8 g/kg CEO were offered to the birds during a 6-week trial period. Body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were determined throughout starter and grower periods (1-21 and 22-42 days of age, respectively). Plasma levels of albumin, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triacylglyceride (TG), uric acid, glucose and leukocyte profile were determined on day 42 of age. At the end of the experimental period, carcass traits were also determined. Diet inclusion of EEP and / or CEO significantly improved BW, BWG and FCR. The birds fed diet containing EEP at 0.2 g/kg had significantly lower plasma concentration of glucose compared to the control group. The use of EEP or CEO significantly decreased plasma concentration of cholesterol compared to the control group. The use of EEP or CEO also increased plasma concentration of albumin compared to the control group. Diet inclusion of EEP and/or CEO did not significantly influence the antibody titters against Newcastles vaccine at 31 and 41 days of age, H/L ratio and also carcass characteristics. Results of this study showed that EEP and CEO can be used in diets of broiler chickens at 0.2 g/kg and 0.8 g/kg, respectively with positive effects on performance and blood parameters.

KEY WORDS blood parameters, cumin essential oil, immune response, performance, propolis.

INTRODUCTION

Since usage of most growth promoter antibiotics as feed additives has been banned by the European Union due to cross-resistance against pathogens and residues in tissues, scientists have searched for alternatives to antibiotics. In this view, aromatic plants and essential oils extracted from these plants are becoming more important due to their antimicrobial effects and the stimulating effect on animal digestive system (Ciftci *et al.* 2005). Many plants contain extensive variety of phytochemical compounds with antimicrobial activity (Cowan, 1999). *Cuminum cyminum* (Cumin), an annual plant of the Umbelliferae family, is an important medical herb with antioxidant, anticholesterol and antimicrobial effects (Sagdic *et al.* 2002). According to Shetty *et al.* (1994), fungi and yeast were more sensitive to

CEO when compared to bacteria. Cumin enhanced the activity and excretion content of bile acids (Platel, 2000; Ayasan, 2011) and also increased pancreas enzymes such as amylase, trypsin, chymotrypsin and lipase in rats (Platel and Srinivasan, 2000). Other researchers have also reported an increase in BW and FCR, with a decreaseing in hematological values hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) when using 2% of cumin in broiler diets (Ibrahim *et al.* 2007). In another experiment, Sema *et al.* (2007) indicated the potential role of plant volatile oils for the control of some diseases caused by *E. coli* infections.

Propolis (bee glue) is an adhesive, dark yellow to brown colored balsam which smells like resin. It is collected from the buds, leaves and similar parts of trees and other plants like pine, oak, eucalyptus, poplar, chestnut, and so on by bees and mixed with their wax (Seven *et al.* 2010). More than 200 constituents have been identified in different propolis samples (Orsolić *et al.* 2004). The composition of raw propolis is generally composed of 50% resin and vegetal balsam, 30% beeswax, 10% essential and aromatic oils and 5% other organic substances (Kumova *et al.* 2002). Based on the literature, propolis increased FI, BW, flavonoid content, taste improvement, antioxidant and antimicrobial properties (Shalmany and Shivazad, 2006). Antioxidative, cytostatic, anti-mutagenic and immunomodulatory properties of propolis are based on its rich flavonoid, phenolic acid and terpenoid contents (Wang *et al.* 2004). In addition, based on the study in which the effect of some bee products on immune response of chicken infected with virulent Newcastle disease virus (NDV) evaluated, the mortality rate was reduced in groups infected with virulent NDV and subsequently treated either with propolis or honey when compared with the infected control group (Hegazi *et al.* 1995). Undoubtedly, plant extracts and propolis, which are considered as alternatives to antibiotics, have a wide range of potential uses. Therefore, determining the effects of these products on human and animal health are of significant importance at present due to the increasing practice of organic agriculture and increasing importance attached to safe nutrient production. So the purpose of this study was to evaluate the effect of dietary CEO and EEP on the performance, blood parameters, immune response and carcass traits of broiler chicks to find out any probable interaction (especially synergic effect) between these two feed additives.

MATERIALS AND METHODS

Care of laboratory animals

All experimental protocols adhered to the guidelines of, and were approved by, the Animal Ethics Committee of Razi University (Kermanshah, Iran) and were in accordance with the guidelines on animal welfare.

Propolis origins

Hand-collected propolis samples were kept dried in a dark place until processing. Propolis samples were extracted for two weeks with 100 ml of 96% ethanol, at room temperature to obtain the extract (Krell, 1996). After two weeks, the solution was filtered; the liquid portion was stored in a dark green or dark brown bottle in a cool, dry and dark place. The ethanolic extract solution was filtered through a whatman 1 filter paper and restored to the original volume with 96% ethanol.

Experiment design and treatments

A total number of 240 day-old commercial broiler chicks (Ross-308) were individually weighed and randomly allocated to the experimental dietary treatments (average initial body weights= 44.5±3 g). The experiment was carried out with four treatments and six replicates of 10 birds per each. The chicken received the corn-soybean meal basal diet with different concentrations of EEP at 0 or 0.2 g/kg and CEO at 0 or 0.8 g/kg or combinations of them, throughout the starter (1-21 d of age) and grower (22-42 d) periods. Doses of feed additives were determined based on previous studies (Shalmany and Shivazad, 2006; Aami-Azghadi *et al.* 2010). The corn-soybean meal diets were formulated to meet or exceed requirements (NRC, 1994). The composition of the basal diet is presented in Table 1. Feed and water was available *ad libitum*. The BW and FI in each group of birds were determined four h after feed removal and FCR was calculated throughout all stages of experimental period. Daily probable mortalities were recorded and used to correct performance criteria.

Blood biochemicals

On day 42 of age, six chicks from each treatment (n=24) were selected at random to collect blood samples via the wing vein, then plasma was separated by centrifugation for 15 min at 3000 rpm and stored at -20 ˚C until assayed for albumin, cholesterol, HDL, LDL, TG, uric acid, and glucose by enzymatic colorimetric methods using a commercial kit supplied by Pars Azmoon® Company, Karaj, Iran (Mohammadi and Ansari-Pirsaraei, 2014).

White blood cell profile

On day 42, blood samples were collected via wing vein in tubes with heparin. Blood samples were taken from one randomly selected bird from each replicate (n=24). Briefly, two drops of blood were placed on a slide, spin was prepared and stained with May-Grunwald-Giemsa stain.

All slides were coded and white blood cells were counted. Relative percentage of the various groups of white blood cells as well as the ratio of heterophiles to lymphocytes per each blood sample was calculated.

Table 1 Ingredients and chemical composition (g/kg) of starter and grower diets

Ingredients	Starter (0-21 days)[1]	Grower (22-42 days)
Corn	619.5	677.4
Soybean meal (44% crude protein)	341.6	283.6
Vegetable oil	0.4	4.7
Limestone	12.5	13.3
Di-calcium phosphate	13.9	10.3
Iodine common salt	4	2.9
Sand	0.8	0.8
Vitamin permix[2]	2.5	2.5
Mineral permix[3]	2.5	2.5
Lysin-HCL	1	1.4
DL-methionine	1.3	0.6
Calculated composition (%)		
Metabolizable energy (kcal/kg)	2850	2950
Crude protein	20.49	18.44
Ether extract	2.664	3.22
Crude cellulose	3.754	3.48
Calcium	0.891	0.83
Available phosphorus	0.401	0.32
Lysine	1.15	1.04
Methionine	0.445	0.35

[1] The levels of 0 (control); 0.2 g/kg (EEP); 0.8 g/kg () and 200 (EEP) + 800 (CEO).
[2] Vitamin premix per kg of premix: vitamin A: 7.2 g; vitamin D: 7 g; vitamin E: 14.4 g; vitamin K_3: 1.6 g; vitamin B_1: 0.72 g; vitamin B_2: 3.3 g; Ca-D-pantotenat: 12 g; Niacin: 12.6 mg; vitamin B_6: 6.2 mg; B_{12}: 0.6 g; Biotin: 0.2 g and Choline chloride: 440 mg.
[3] Mineral premix per kg: Mn: 64 g; Zn: 44 g; Fe: 100 g; Cu: 16 g; I: 0.64 g; Co: 0.2 g and Selenyum: 8 g.
EEP: ethanol extract of propolis and CEO: cumin essential oil.

Antibody response to Newcastle disease virus (NDV)

To evaluate the humoral immune response of the broiler chicks, all the birds were vaccinated by 0.1 mL Newcastle vaccine at days 16 and 31.

On days 31 and 41, one chick from each replicate was randomly selected (n=24) and blood samples were collected via brachial vein. The separated sera by centrifugation (3000 rpm, 15 minutes) were used for antibody titration against Newcastle vaccine by standard HI procedure (Allan *et al.* 1978).

Carcass characteristics

At the end of the experimental period (day 42), six randomly selected birds from each treatment (n=24) were weighed and slaughtered to determine carcass, breast, legs, liver, heart, abdominal fat pad, gall bladder, gizzard, bursa of fabricius, spleen and pancreas weights, and the length of duodenum, jejunum, ileum; besides, their relative percentages of live body weight were calculated.

Statistical analysis

Data were analyzed by the General Linear Model (GLM) procedure of SAS (2002). Significant differences among means were found, and differentiated using Duncan's multiple range test. Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Performance parameter

The effects of adding CEO and EEP to diet on the performance parameters of broiler chicks are shown in Table 2. In this study, significant effects of EEP and CEO on BW, BWG and FCR throughout experimental period were detected ($P<0.05$).

There was no significant effect of dietary treatment on FI ($P>0.05$), which is agreement with previous report (Mansoori *et al.* 2006). Eyng *et al.* (2014) reported negative effects of EEP supplementation on body weight gain (BWG) and FI in 7-day old broilers (Eyng *et al.* 2014).

Considering the studies about the use of propolis in poultry diets, dietary supplementation by propolis had no effect on BWG (Biavatti *et al.* 2003; Ziaran *et al.* 2005). Contrary to these studies, there are some reports indicating the positive effects of plant extracts and/or propolis on BWG (Shalmany and Shivazad, 2006; Aami-Azghadi *et al.* 2010; Alcicek *et al.* 2004; Eclache and Besson 2004; Roodsari *et al.* 2004).

The findings of our study show that these two additives (CEO and/or EEP) can significantly improve BWG compared to the control group ($P<0.05$), at least in part due to the formation of a more stable intestinal flora (Tekeli, 2007).

In accordance with our results, addition of black cumin seed to broiler chick diet, significantly increased BW on days 31 and 41 of age (Khalaji *et al.* 2011). Based on other studies, cumin seed may increase bile acids and bile salts synthesis and secretion (Platel and Srinivasan, 2000). In addition, the use of cumin seed provided the higher concentration and secretion of digestive enzymes in pancreas and small intestine (Platel and Srinivasan, 2000), simultaneously; transit time of feed in gastrointestinal tract was reduced (Platel and Srinivasan, 2001). Besides, 21-day-old broiler chickens receiving 0.5% ethanolic extract of propolis, showed a higher sucrase activity in the jejunum, whereas pancreatic enzymes activity was not influenced (Amaral Duarte *et al.* 2011). These factors could improve nutrient digestibility and performance (Platel and Srinivasan, 2004), which may be the reasons for improved performance in the current study. In the present study, FCR was significantly improved by adding CEO and / or EEP to diets ($P<0.05$).

Table 2 Effects of dietary supplemental ethanol extract of propolis and cumin essential oil on body weight, body weight gain, feed consumption and feed conversion ratio on broiler performance for 6 weeks

Treatment groups		Body weight (g/bird)		Body weight gain (g/bird/day)			Feed consumption (g/bird/day)			Feed conversion ratio (g feed:g gain)		
CEO (g/kg)	EEP (g/kg)	21	42	0-21	22-42	0-42	0-21	22-42	0-42	0-21	22-42	0-42
0	0	540.67^{b}	1856.25^{c}	23.63^{b}	62.65^{c}	43.14^{c}	47.39	134.55	90.97	2.01^{a}	2.15^{a}	2.10^{a}
0.8	0	620.33^{a}	1984.67^{b}	27.43^{a}	64.97^{bc}	46. 2^{b}	48.06	133.19	90.63	1.75^{b}	2.05^{b}	1.96^{b}
0	0.2	624.75^{a}	2098.17^{a}	27.65^{a}	70.16^{a}	48.90^{a}	47.60	134.36	90.98	1.72^{b}	1.91^{c}	1.86^{c}
0.8	0.2	602.00^{a}	2012.83^{b}	26.55^{a}	67.18^{b}	46.86^{b}	47.53	135.18	91.35	1.79^{b}	2.01^{b}	1.95^{b}
SEM		8.78	20.83	0.419	0.697	0.496	0.185	0.485	0.237	0.029	0.022	0.021
P-values		0.0002	0.0001	0.0002	0.005	0.0001	0.34	0.28	0.48	0.0004	0.0024	< 0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
CEO: cumin essential oil and EEP: ethanol extract of propolis.

There are other reports showing that the dietary supplementation by plant extract and propolis did not have significant effect on FCR (Ziaran *et al.* 2005; Demir *et al.* 2003); however, in line with our results, Roodsari *et al.* (2004) reported that FCR of broiler was improved along with increased levels of propolis; besides, it has been demonstrated that supplementing rations with 200 and 250 ppm alcohol extract of propolis (Shalmany and Shivazad, 2006) and also 1% black cumin seeds (Khalaji *et al.* 2011), resulted in improved FCR in broilers.

It has also been shown that EEP may improve microflora within the gut of broiler chicks under heat stress condition (Abdel-Mohsein *et al.* 2014); in addition, significant improvements in villus height in jejunum, crypt depth in duodenum, jejunum and ileum, the villus height/crypt depth ratio in jejunum and number of goblet cells in both duodenum and ileum in broiler chicks received ethanolic extract of propolis, have been shown. These positive effects may result in more efficient digestion (Khaleghi Miran *et al.* 2013a).

Blood biochemicals

The effects of adding CEO and EEP to diet on blood biochemicals of broilers at day 42 are shown in Table 3. There was no significant effect of EEP and/or CEO on blood concentrations of HDL, LDL, TG and uric acid (P>0.05). EEP and / or CEO adding significantly influenced glucose, cholesterol and albumin levels (P<0.05). The highest plasma cholesterol content (133.26 mg/dL) was recorded in the control group and the lowest plasma cholesterol content was determined in the CEO (113.01 mg/dL) and EEP (113.35 mg/dL) groups. Cumin seed inhibits hepatic 3-hydroxy-3-ethylglutaryl CoA (HMG-CoA) reductase activity (Crowell, 1999), which is a key regulator enzyme in cholesterol synthesis. In line with our results, TG level was not influenced but cholesterol was significantly suppressed in broiler chickens receiving 300 ppm EEP; however, the same report showed a significant decrease and increase in LDL and HDL figures, respectively (Khaleghi Miran *et al.* 2013b).

In other studies, in line with our results, it has been reported that plant extract and propolis lead to a decrease in plasma levels of cholesterol and glucose (Lee *et al.* 2003; Fuliang *et al.* 2005). The highest and the lowest plasma glucose concentrations were identified in the control (236.86) and EEP (207.22) groups, respectively. It may be assumed that the significant decrease in plasma glucose level in the EEP group, possibly is a result of insulin release. It has been reported that essential oils are the secondary metabolites of the plant, enhancing release of insulin or insulin-like substances (Greathead, 2003). It is also reported that increasing doses of EEP (0 to 5000 ppm), decreased duodenal and increased jejunal sucrase activity in broilers at 7 and 21 days of age (Eyng *et al.* 2014). Insulin stimulates glucose transport into liver cells and leads to a decrease in blood level of glucose. Subsequently, glucose entering liver cells is firstly converted to pyruvate and then to acetyl-CoA, and utilized as substrate in the synthesis of fatty acids.

Contrary to the findings of this study, Demir *et al.* (2003) and Lee *et al.* (2003) reported that plant extracts used as growth promoters had no effect on plasma lipid and glucose concentrations. In contrary to the present finding, Galal *et al.* (2008) reported that supplemental propolis in quail rations did not have significant effect on albumin. Total protein levels in the serum has been proved to be a sensitive biochemical indicators in broilers (Kubena *et al.* 1990), besides, total serum protein has been reported as an indicator of the retained protein in the animal body (Esonu *et al.* 2001). The positive effects of CEO and EEP on albumin level in the present study may be attributed to the synergic effect between CEO and EEP.

White blood cell profile and antibody response to Newcastle disease virus (NDV)

The effects of adding CEO and EEP to diet on H/L ratio at day 42 of age and relative antibody responses to NDV of the broilers at days 31 and 41 of age are shown in Table 4. Adding CEO and / or EEP to diet had no significant effect on H/L ratio of the broiler chicks (P>0.05).

Table 3 Effects of dietary supplemental ethanol extract of propolis and cumin essential oil on blood biochemicals of broiler chicks at d 42 of age

Treatment groups		Glucose	Cholesterol	Albumin	HDL	LDL	Triglycerides	Uric acid
CEO (g/kg)	EEP (g/kg)	(mg/dL)	(mg/dL)	(mg/dL)	(m mol/L)	(m mol/L)	(mg/dL)	(mg/dL)
0	0	236.86^{a}	133.26^{a}	1.212^{b}	5.318	0.232	52.64	4.205
0.8	0	219.06^{ab}	113.01^{b}	1.643^{a}	5.498	0.087	53.89	3.462
0	0.2	207.22^{b}	113.35^{b}	1.604^{a}	5.035	0.212	53.89	3.683
0.8	0.2	224.25^{ab}	120.36^{ab}	1.307^{ab}	5.405	0.205	46.92	3.27
SEM		3.92	3.23	0.069	0.168	0.029	4.69	0.281
P-values		0.021	0.031	0.008	0.791	0.231	0.685	0.779

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
CEO: cumin essential oil; EEP: ethanol extract of propolis; HDL: high density lipoprotein and LDL: low density lipoprotein.

It is reported that addition of 1% black cumin seeds significantly decreased monocytes percentage but increased red blood cells, hematocrit, and hemoglobin (Khalaji *et al.* 2011). Since in circulating blood, 80 to 90% of the lymphocytes are T lymphocytes, the increased lymphocyte percentage might be partly due to an increase in T lymphocyte number.

Table 4 Effects of dietary supplemental of propolis ethanol extracts and cumin essential oil on antibody response to NDV (Anti-NDV) titers (log_2) and heterophile / lymphocyte ratio (H/L ratio) of broiler chicks

Treatment groups		Anti-NDV	Anti-NDV	H/L
CEO (g/kg)	EEP (g/kg)	(d 31)	(d 41)	
0	0	4.33	4.33	0.416
0.8	0	4.67	5.67	0.351
0	0.2	5.00	6.00	0.276
0.8	0.2	5.83	7.00	0.224
SEM		0.175	0.23	0.0235
P-values		0.385	0.488	0.865

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
CEO: cumin essential oil and EEP: ethanol extract of propolis.

Secretion of interleukin-2 and -4 (IL-2 and -4) from T lymphocytes and diffusion to B lymphocyte is necessary for antibody production. These cytokines cause B lymphocytes to convert into plasma cells which can synthesize antibody (Jain, 1993). The immunostimulant effect of cinnamic acid on IL-2 and -4 production and lymphocyte proliferation has been described (Park *et al.* 2004). Thus, in our study a possible explanation for this immunostimulant activity could be attributed to the presence of the cinnamic acid or to a synergic effect between the components. In birds, the heterophils are phagocytic cells whose main function is protection against invading microorganisms, whereas primary functions of lymphocytes are cell-mediated and humoral immunity. Since heterophil and lymphocytes numbers increase and decrease respectively in stress condition, the H/L value is used as an index of response to a stressor (Gross and Siegel, 1983; Gross and Siegel, 1985).

The result of this study showed that antibody titers against Newcastle's vaccine in days 31 and 41 were not significantly influenced by dietary CEO and EEP (P>0.05). Khalaji *et al.* (2011) has demonstrated that addition of 1% black cumin seeds to broiler chick diet significantly decreased antibody response against SRBC. Flavonoids (one of the functional ingredient of propolis) stimulate the production of antibodies in a yet poorly known fashion (Goodwin and Webb, 1980). Other researchers have observed that administration of propolis (extracts or ingredients) in mice cause an augmentation of IL-1, -2 and -4 (Park *et al.* 2004) that can, in turn, increase antibody production.

It has been shown that propolis polysaccharide, as a Chinese herbal ingredient, positively stimulates chicken immune system; besides, specific dose of propolis polysaccharide was found to be more potent than astragalus polysaccharide and isatis root polysaccharide, in promoting chicken humoral immune response (Kong *et al.* 2006); besides, antiviral (Vynograd *et al.* 2000) and anti-inflammatory effects (Borrelli *et al.* 2002) of propolis can influence immune response. The relation between cellular and humoral immunity should be considered because lymphocytes increase in viral infections before the antibody production (Jain, 1993). For instance, Merz *et al.* (1981) reported that after vaccination of chickens with NDV, cell-mediated immunity comes first; then humoral immunity follows.

Carcass characteristics

The effects of dietary supplemental CEO and EEP on carcass characteristics of broilers at day 42 are shown in Tables 5. Dietary supplemental CEO and EEP did not have any significant effect on carcass characteristics (P>0.05), which is in line with previous report (Eyng *et al.* 2014).

It is reported that the relative weight of small intestine, liver and pancreas of broiler chickens were not significantly affected by different levels of EEP; in the same report, crypt depth in duodenum and jejunum were found significantly shorter in the birds receiving 0.29 and 0.32% of EEP. 0.30% EEP had also significant positive effect on villus/crypt ratio in duodenum (Eyng *et al.* 2011). Broilers with 1% black cumin seed in their feed had significantly more gizzard relative weight (Khalaji *et al.* 2011).

Table 5 Effects of dietary supplemental ethanol extract propolis ethanol extracts and cumin essential oil on carcass characteristics (%, relative weight to body weight) of broiler chicks at d 42

Treatment groups		% Carcass characteristics (relative weight to body weight)						
CEO (g/kg)	CEO (g/kg)	Carcass weight	Breast	Legs	Liver	Heart	Abdominal fat pad	Gall bladder
0	0	96.29	19.69	17.66	2.543	0.616	1.206	0.056
0.8	0.8	96.04	21.45	17.52	2.598	0.578	1.103	0.062
0	0	96.65	20.52	17.92	2.812	0.618	0.812	0.060
0.8	0.8	96.44	20.63	18.30	2.488	0.554	0.973	0.067
SEM		0.235	0.558	0.245	0.081	0.014	0.081	0.004
P-values		0.976	0.524	0.636	0.295	0.644	0.428	0.984

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
CEO: cumin essential oil and EEP: ethanol extract of propolis.

Aami-Azghadi *et al.* (2010) reported that dietary supplemental CEO and prebiotic fermacto did not affect the organ weights of broiler chicks. It was proved by Golian *et al.* (2010) that cumin seed and cumin seed meal additive decreased abdominal fat pad in broilers compared to control and prebiotic fermacto groups. In a study by Tekeli *et al.* (2011), *Z. officinale* when combined with propolis led to a significant decrease in liver weight; but abdominal fat weight significantly increased in propolis and *Z. officinale* groups. Tekeli *et al.* (2008); Tekeli *et al.* (2011) also reported dietary antibiotic, *Z. officinale* and propolis extract effect on the weights and/or lengths of broiler chick digestive system. Cabuk *et al.* (2006), stated that essential oil mixtures did not have any effect on small intestine length in broiler. All in all, the above mentioned literature has proved not significant effects of EEP and cumin seed on carcass characteristics, which are in agreement with our results.

CONCLUSION

In conclusion, dietary EEP and CEO supplementation caused an increase in BW, BWG, FCR, serum albumin and decrease in cholesterol and glucose. In addition, dietary supplemental EEP significantly decreased H/L ratio (P>0.05) and increased antibody titers against Newcastle's vaccine on days 31 and 41 of age, but antibody titers against Newcastle's vaccine on day 41 of age was increased by dietary CEO supplementation.

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