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Addition of Capsicum Oleoresin, Carvacrol, Cinnamaldehyde and Their Mixtures to the Broiler Mixed Feed I. Effects On Growth Performance, Carcass Characteristics, Intestinal Microflora, Some Blood Parameters and IGF-1 Gene Expression Levels

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Abstract
The aim of this study was to evaluate the effects of dietary capsicum oleoresin (CAP, 150 mg/kg), carvacrol (CAR, 150 mg/kg), cinnamaldehyde (CIN, 150 mg/kg) and equal amount of mixtures (CAP+CAR+CIN, 50 mg/kg each, capsicum oleoresin + carvacrol + cinnamaldehyde) supplements on growth performance, carcass characteristics, intestinal microflora, some blood parameters and IGF1 gene expression levels in broiler chickens. In the experiment, four hundred Ross-308 day-old, both sexs broiler chicks were randomly distributed to five dietary treatments, each with five replicates. Among these five dietary treatment groups formed for the experiment, the control group was fed without feed additives (control), the second group with 150 mg capsicum oleoresin for each kg of feed, the third group with 150 mg carvacrol for each kg of feed, the fourth group with 150 mg cinnamaldehyde for each kg of feed, and the last group with 150 mg mixtures for each kg of feed. The experiment was maintained to six weeks. According to the research results, the effects of dietary treatments on the body weight and the body weight gain were found to be significant ($p<0.05$). Especially in the group fed on diet added CAP+CAR+CIN, the treatments had a significant effect on feed intake and feed conversion ratio as well as body weight and body weight gain when compared to the control group on a periodic basis ($p<0.05$). In addition, the effect of the treatments on livability is insignificant ($p>0.05$). Among carcass properties, only carcass yield and some organ-related properties were significantly affected from addition of capsicum oleoresin, carvacrol, cinnamaldehyde and their mixtures ($p<0.05$). Cinnamaldehyde and mixture groups caused to significantly ($p<0.05$) reduce AST levels, carvacrol, cinnamaldehyde and capsicum oleoresin groups led to decrease level of triglycerides, carvacrol and mixture groups importantly ($p<0.05$) affected LDL levels were detected. And also, especially in the mixture groups, the number of ileum *Lactobacillus* spp. was found to be significantly higher ($p<0.05$) than the other groups. The changes in dietary content were not accompanied by changes in IGF-1 gene expression levels ($p>0.05$). In conclusion, the supplementation carvacrol, cinnamaldehyde, capsicum oleoresin and especially their mixtures at the level of 150 mg/kg to broiler diets affected positively growth performance, slaughter characteristics, some examined intestinal microflora and blood parameters in broiler chickens.

Keywords: Broiler, capsicum oleoresin, carvacrol, cinnamaldehyde

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1. Introduction:

Food safety, food reliability and nutrition are leading subjects regarding all world countries recently (Dolekoglu Ozcicek, 2003). Nutrition is one and probably the only essential requirement for individuals in terms of physically, mentally and spiritually healthy and strong living, economic and social development, prosperity, happy, peacefully and safely continuing his existence (Yagmur and Gunes, 2010). It is predicted that for adequate and balanced nutrition, each adult should take 1 g protein each day per kg of body weight and 42-50 % of this amount should consist of animal proteins (Kaya, 1996). Another of the most important animal protein sources that should be consumed for physical and mental development and health and balanced nutrition is broiler. Broiler is an important and strategic nutritional source as it has low levels of cholesterol and fat, its digestibility is easy and it is a good protein source in terms of nutritional value thereof and also it is budget friendly compared to some other protein sources (Inci et al., 2014). On the other hand, however, in broiler farming, housing a great number of animals within the coop causes animals to become more vulnerable and unresisting and thus, causes them to be affected easily by immediate condition changes. Until last few years, antibiotics added to rations as development-enhancing feed additive played a remarkable role for the purpose of decreasing risk of animals' getting sick and enabling animals to reach a rapid living weight increase in a short time (Can and Celik, 2008). Nevertheless, as a result of long term use of antibiotics, it has been prohibited to use many antibiotics around the world, particularly European Union countries, as of 2006 because strains resistant to these antibiotics have evolved and risk of resistance formation has appeared (Simsek et al., 2005). Thus, researchers have started seeking a natural and safe development stimulating material as growth factor that may maintain these developments experienced during use of antibiotics in poultries and that may be alternative to overcome negativities that may occur in absence of antibiotics. (Alcicek, 2003; Ceylan et al., 2003). For this purpose, aromatic plants and essential oils derived from these plants and primary active ingredients thereof are used in pharmaceutical, cosmetics and feed fields due to many features such as antibacterial, antioxidant, antiviral, antifungal and digestive system-stimulating feature and its utilisation in animal feeding field is also gaining currency (Can and Celik, 2008). Research conducted by Al-Kassie (2009), investigated the effects of 100 and 200 ppm cinnamon and thyme oil addition to the mixed feed of broiler chicks (Arbor Acres) on body weight gain, feed intake, feed conversion rate and blood constituents, and thus revealed that the body weight gain and feed intake was higher; feed conversion rate was better and serum cholesterol levels were lower in 200 ppm group compared to the control group. Khattak et al. (2014), studied the effect of commercial essential oil mix (basil+caraway+laurel+lemon+oregano+ sage+tea and thyme) addition to mixed feed at different doses; i.e, 0-100-200-300-400-500 g/t, on performance, carcass weight, intestinal activity and blood biochemistry of broiler chicks (Ross, 308); and concluded that the body weight and carcass weight increased in accordance with the dosage, however no difference was observed among groups regarding blood biochemistry and intestinal activity. Sharifi et al. (2013), respectively added 15, 3, 2 and 2 g/kg of four different aromatic leaves in dried form (Cumin, peppermint, yarrow and poley-haired herba chamaedrys) to broiler chicks' (Arbor Acres) mixed feed, and investigated related effects on performance and blood constituents. 0.4 g/kg Flavomycin group was formed as the positive control. The experiment revealed that the body weight and triglyceride levels were lower in aromatic plant mixture group; that the total cholesterol levels increased in the group where cumin and peppermint were added; whereas it decreased in the group where yarrow and poley was added. It was stated that HDL and LDL values differed (increased or decreased) at different rates among different groups. It was observed that the *Bifidobacterium* and *Clostridium perfringens* (log 10/g) levels decreased in all groups except for the cumin group, whereas *Lactobacillus* spp. and *Coliform* levels remained the same. Research conducted regarding the subject suggests that the addition of various plant extracts enhanced the body weight gain Al-Mashhadani et al. 2011a, b); and the feed conversion rate (Jamroz et al. 2005), however had no effect on carcass characteristics (Şimşek et al. 2005; Kırkpınar et al. 2014); and on blood plasma lipids such as total cholesterol, trigylcerides and HDL cholesterol (Lee et al. 2004; Nafaji and Torki 2010); reduced the *Escherichia Coli* count in the intestines (Shanoon et al. 2012; Hashemipour et al. 2013) and on the other hand boosted the *Lactobacillus* spp. and *Bifidobacterium* counts in broiler chicks (Hashemipour et al., 2013). It should further be considered that the composition and quantity of essential oils obtained from different organs (root, stem, flower, etc.) and in different growth stages of plants growing under different climatic conditions might differ accordingly. This difference challenges the determination of effective dose of the plant itself or the essential oils to be used directly as feed additives. Hence, usage of primary active ingredients isolated from volatile essential oils is favored as feed additives (Kahraman, 2015). Consequently, following constituents were used for the purpose of this research: carvacrol and cinnamaldehyde for their appetite enhancing characteristics, along with their antimicrobial and antifungal

properties (Saad et al., 2013; Kahraman, 2015); capsicum oleoresin for its endocrine and immune system stimulating (Jamroz et al., 2005; Catala-Gregori et al., 2008) and antioxidizing (Viktorija et al., 2014) properties; and lastly the combination group for the assumption of synergetic effects of various combinations of the above mentioned active ingredients (Nevvcomb, 1999). This study aims to investigate and determine the effects of the addition of synthesized, commercially available (and intended antibiotic alternatives) 150 mg/kg carvacrol, cinnamaldehyde, naturally obtained capsicum oleoresin, and the mixtures containing equal amounts of them (50 mg/kg capsicum oleoresin + 50 mg/kg carvacrol + 50 mg/kg cinnamaldehyde) to the mixed feeds of broiler chickens on performance characteristics (body weight, body weight gain, feed intake and feed conversion ratio), livability, carcass characteristics (carcass yield, breast ratio, leg ratio and abdominal fat ratio), organ weights, intestinal microflora, some blood parameters and IGF1 gene expression.

2. Materials and Methods

Chicks, Experimental Design and Diets: In the study, 400 daily mixed gender broiler chicks (Ross-308) were used as animal material. Chicks were purchased from a commercial hatchery (EGETAV). Experiment was conducted in a total of 5 groups, 1 control and 4 treatment groups. Each group was again divided into 5 groups, each containing 16 chicks. Crumble feed material used in the study was obtained from a commercial feed company. Rations used in the study were prepared in accordance with nutrient requirements stated in NRC (1994). During study, chicks were given broiler chick feed from day 1 to day 21 and broiler grower feed from day 22 to slaughter day (day 42). Compositions and nutrient contents of mixed feeds used in the study were given in *Table 1*. Ration of control group was prepared not to contain any additives. First treatment group ration was prepared by daily 150 mg/kg capsicum oleoresin addition to control group ration, second treatment group ration was prepared to contain daily 150 mg/kg carvacrol, third treatment group ration was prepared by daily 150 mg/kg cinnamaldehyde addition and fourth treatment group ration was fed with crumble feeds containing 150 mg mixture (50 mg each, capsicum oleoresin + carvacrol + cinnamaldehyde). To provide feed homogeneous mixture of carvacrol, cinnamaldehyde and capsicum oleoresin, firstly premixture with zeolite (g/kg) was prepared and then this mixture was added to main mixture (to crumble feed). In the study, synthetically obtained and commercially sold 99 % carvacrol, 98 % cinnamaldehyde, 99 % capsicum oleoresin and mixture thereof were used. Carvacrol, cinnamaldehyde and capsicum oleoresin level added to mixed feeds were determined in parallel with levels stated in studies (Eldeeb et al., 2006; Lee et al., 2003a; 2003b) pertaining to various effective materials and essential oils in poultries.

Chicks Housing and Management: Group feeding was applied for animals in each compartment (1.2 m x 1.1= 1.32 m^2), feed and water were presented *ad libitum*. Wood flour was used as base in the experiment and the study was carried out in environmentally controlled closed experiment coops in Facilities of Animal Science Department, Faculty of Agricultural, Ege University. In the first week of experimental chick feeder and waterer were used and then suspended bucket type round feeder and automatic suspended waterers were used. During the course of experiment 23-hour-light and 1-hour dark plan (day light and ampoules) was applied. It was noted to keep ambient temperature in chick level at 34 °C during the first 5 days and this temperature was dropped to until minimum 23 °C gradually on other days of the study. During the trial animals were monitored daily and death/deaths were recorded. Experiment was maintained for 6 weeks.

Feed material Analaysis: The amount of nutrients in the broiler feed (apart from cellulose) used in the study was determined according to Weende analysis method (Naumann and Bassler, 1993), whereas raw cellulose was determined according to Lepper method (Bulgurlu and Ergul, 1978). Additionally, metabolical energy content was calculated using the regression equation recommended by TSI (Turkish Standards Institute) standard No. 9610 (TSI, 1994).

Performance: The animals have been weighted individually at the same day and same hour once every week in the days of 7., 14., 21., 28., 35. and 42 of trial and beginning of the experiment with an objective to determine the characteristics of the performance and consumption of feed of each sub group have been recorded. The feed conversion ratio has been calculated by dividing feed intake values determined between 0-7, 7-14, 14-21, 21-28, 28-35 and 35-42 days of each sub group by the body weights gained in these terms. Necessary adjustments have been made in calculations as per the compartment occupancy of deceased animals and the feed amount consumed by such.

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Carcass Characteristics: Fifty animal in total, 5 females and 5 males from each group, each 1 male and 1 female has been selected randomly from the repetition in the 42th day of the experiment have been slaughtered by shedding blood and plucking and their heads and foots are separated. After that, their internal organs and abdominal fat have been removed and hot carcass has been weighted and leg (from Art. coxea) and breast (Art. sternocostalisten) meat have been separated from the carcass. Carcass parts have been weighted without skin to specify the carcass features on the date of slaughter and the results have been proportioned to the body weight (%) and it's been given by proportional values. The carcass yield has been calculated by proportioning the hot carcass weight to the body weight.

Table 1. **Ingredients and chemical composition of experimental diets (as-fed basis)**

Feed stuff, %	Broiler chick (0-21. Days)	Broiler (22-42. Days)
Corn	46.96	48.91
Soybean meal	20.88	12.37
Wheat	0.00	5
Full-fat soybean	14.57	15
Corn bran	4.5	3
Sunflower seed meal 34 % CP	4	4.5
DDGS	2.5	3
Poultry meal	2.29	4
Marble powder	1.12	0.93
DCP 18 %	1.08	0.71
Lysine sulfate 70 %	0.51	0.46
Commerical fat	0.5	1.15
Commerical methionine	0.32	0.23
[1]Vitamin premix-001	0.2	0.00
[2]Vitamin premix-002	0.00	0.2
Salt	0.19	0.16
Sodium sulfate	0.12	0.10
[3]Mineral	0.10	0.1
L-threonine	0.06	0.04
Liquid choline 75 %	0.06	0.06
Coccidiostat	0.05	0.05
Calculated values, g/kg		
Metabolizable energy, kcal/kg	3025	3150
Lysine	14.57	12.72
Methionine + cystine	10.97	9.99
Avaliable P	5.00	4.5
Composition (analysed), g/kg		
Dry matter	880.29	880.85
Crude protein	237.65	217.67
Ether extract	56.96	66.06
Crude ash	59.73	52.69
Crude fiber	37.06	36.70
Calcium	10.50	9.00
Total phosphorus	6.40	5.69
Starch	344.30	376.12
Sugar	41.24	35.65

[1]Vitamin premix-001 per kg diet: 11000 IU Vitamin A; 5000 IU Vitamin $D_{3;}$ 0.069 mg 25-OH-$D_{3;}$ 150 mg Vitamin E; 3 mg Vitamin $K_{3;}$ 3 mg Vitamin $B_{1;}$ 8 mg Vitamin $B_{2;}$ 4 mg Vitamin $B_{6;}$ 0.02 mg Vitamin $B_{12;}$ 60 mg Niacin; 15 mg D-Pantothenic; 2 mg Folic acid; 0.2 mg Biotin; 100 mg Vitamin C; 400 mg choline,
[1]Vitamin premix-002 per kg diet: There are 10000 IU Vitamin A; 5000 IU Vitamin $D_{3;}$ 0.069 mg 25-OH-$D_{3;}$ 50 mg Vitamin E; 3 mg Vitamin $K_{3;}$ 3 mg Vitamin $B_{1;}$ 8 mg Vitamin $B_{2;}$ 4 mg Vitamin $B_{6;}$ 0.02 mg Vitamin $B_{12;}$ 60 mg Niacin; 12 mg D-Pantothenic; 2 mg Folic acid; 0.2 mg Biotin; 100 mg Vitamin C; 400 mg choline,
[3]Mineral per kg diet: 150 g Mn, 120 g Fe, 150 g Zn, 14 g Cu, 0,4 g Co, 3 g Se.

Organ Weights: On the 42th day of the experiment, the animals that are selected with an objective to specify the carcass features are slaughtered and liver, heart, spleen, pancreas, bursa fabricius, grandual stomach, gizzard, small intestine, cecum and large intestine have been taken our carefully and weighted at the 0.01 g sensitive balance. The data has been proportionated to body weight (%) and they are calculated in proportional values and the results have been given in such way.

Intestinal Microflora and Some Blood Analaysis: At the end of the study (day 42), 4-5 cc of blood was taken to heparin-containing tubes from brachial veins of 50 animals (2 animal/repetition, 10 animal/treatment group). Blood samples were transferred to respective laboratories by separating serums by 20 minute centrifugation in 4000 rpm in a ventilated centrifuge (+ 4°C). Biochemical parameters of Alanine Transaminase (ALT), Aspartate Transaminase (AST), albumin, total protein, glucose, triglyceride, total cholesterol, High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) levels were spectrophotometrically detected using related commercials kits (Roche cobas 501). For microbiological analyses, intestines of animals were quickly removed and the ileum part of the small intestines was separated. The ileum samples taken for microbiological analyses were transferred to the Directorate of Bornova Veterinary Control Institute Poultry Disease Diagnostic Laboratory in an icebox preserving the +4 ºC temperature condition and a *Salmonella* spp. positive/negative test was performed on the ileum samples (TSI, 2005a). The samples were shortly delivered to the Ege University Microbiological Analysis Laboratory (EGEMIKAL) for *Clostridium perfringens*, *Staphylococcus aureus* and *Lactobacillius* spp. counts in the ileum content. The *Lactobacillius* spp. count was performed by ISO 15214, the *Staphylococcus aureus* detection was performed by ISO 6888-1 (ISO, 1998a; 1998b), the *Salmonella* spp. positive/negative test was performed by ISO 6579 and the *Clostridium perfringens* detection was performed by the ISO 7937 method (TSI, 2005a; 2005b).

Sample Collection, Total RNA and cDNA Isolation: At the end of the study (42 days), to specify the expression level of the IGF 1 gene, 4-5 cc blood have been taken from the brachial vein of the 50 animals in total (2 animals/repeated, 10 animals/treatment group) to the EDTA tubes. The samples have been brought to the lab within an ice bag lymphocyte separation has been conducted for RNA isolation from the whole blood. Total RNA was isolated from each blood sample using the RNeasy Mini Kit (QIAGEN) according to the manufecturer's protocal. Total RNA was quantified spectrophotometrically at 260/280 nm and stored at -80 °C. The reverse transcription (RT) was carried out following the RT First Strand Kit (QIAGEN) protocol (QIAGEN, 2013).

Primer Design, Sample Loading and Quantification of Gene Expression by Real Time PCR: To design the primers, related studies have rewiewed and showed that all consequences were compared to NCBI data center (Lu et al., 2009; MacKinnon et al., 2009). The primer and genbank accession numbers of the target genes are included in *Table 2*. Expression of Insulin Like Growth Factor-1 (IGF-1) and a housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), were determined in blood by quantitative RT-PCR. For each sample, reaction mixture for both IGF1 gene and also GADPH gene that we used as a reference housekeeping gene were prepared by mixing 12.5 µl 2x RT^2 SYBR Green Mastermix, 1 µl RT^2 qPCR Primer Assay (10 µM stock solution) and 10.5 µl RNase-free water in a microcentrifuge tube of 1.5 ml. In each well, 24 µl of reaction mixture and 1 µl of DNA sample was mixed to achieve a total volume of 25 µl. Samples prepared in this way were placed in Rotorgene Corbett Research device. Replication curves were simultaneously followed from the device monitor. Data analysis was performed using the software in analysis subsection of the online website: http://www.sabiosciences.com. In addition to graphical expression of the results, evaluation was made with comparative threshold (C_T) method. In this method, initially the C_T values of samples from both treatment and control groups were calculated for each of the GADPH and IGF1. Then, GAPDH C_T value

was subtracted from the IGF1 C_T value to calculate the ΔC_T (Delta C_T) value in each of the treatment and control groups. Then, mean ΔC_T value of the control group was subtracted from the mean ΔC_T value of the treatment group in order to determine the $\Delta\Delta C_T$ value. This value was applied to the formula $2^{-\Delta\Delta Ct}$ in order to determine the relative folds of increase or decrease of IGF1 gene expression in the treatment group in comparison to the control group, while taking GADPH gene expression as the internal control (ABI PRISM 7700, 1997). ΔC_T ve $\Delta\Delta C_T$ values were calculated as follows:

$$\Delta C_{T,Control} = (Control_{IGF1} - Control_{GAPDH})$$

$$\Delta C_{T,Treatment} = (Treatment_{IGF1} - Treatment_{GAPDH})$$

$$\Delta\Delta C_{T,Treatment} = \text{with respect to control} = (\Delta C_{T,Treatment} - \Delta C_{T,Control})$$

$$\text{Fold expression with respect to control} = 2^{-\Delta\Delta C_T}$$

Table 2. Real-time RT-PCR primer and Genbank accession numbers of the target genes.

Target Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Genbank Accession
GAPDH	GGGCACGCCATCACTATCTTC	ACCTGCATCTGCCCATTTGAT	NM_204305
IGF-I	GTATGTGGAGACAGAGGCTTC	TTTGGCATATCAGTGTGGCGC	NM_001004384

Statistical analyses: Statistical analyses of data obtained was performed in SPSS 21,0 (Inc., Chicago, II, USA) program. One-way variance analysis (ANOVA) was applied for difference between statistical calculations of groups and mean values of groups and Duncan test for significance of difference among groups and significance levels ($p<0.05$) were determined (SPSS, 2012). Findings, means of groups and standard deviation of differences among groups and significance level (p) obtained at the end of the study are presented in tables.

3. Results and Discussion

3.1. Growth Performance

Considering *Table 3*, it may be seen that there was no significant difference among the experiment groups in terms of body weight (BW) in the first week and the 14th day of the trial ($p>0.05$); and there was a significant difference in the 7th, 21st, 28th, 35th, and 42nd days ($P<0.05$). In the 7th day, the highest mean body weight of the chicks was reached both in the CAP+CAR+CIN group and the CAP group, while only the CAP+CAR+CIN group reached it in the 21st, 28th, 35th, and 42nd days. At the end of the trial, it was seen that the CAP+CAR+CIN experiment group had a higher mean body weight than the control group and the other experiment groups. In the 42nd day, the experiment group fed with a 150 mg/kg daily addition of CAP+CAR+CIN to the feed had gained 4.6% more body weight than the control group. Considering previous studies on the effects of essential oils and main components on body weight, there were conflicts. Accordingly, it was stated by Fotea et al. (2010) that addition of 1% carvacrol in the 0-42nd days, by Al Mashhadani et al. (2011a) that addition of 200 mg/kg thyme + 200 mg/kg anise in the 28-38th days and by Awaad et al. (2014) that addition of 100 g/ton CAP+CAR+CIN (+111 g) improved live weight significantly. According to Kucukyılmaz et al. (2012), addition of a commercial mixture containing thyme by 48 mg/kg in the 0-42nd days, and according to Celikbilek et al. (2014), addition of a commercial oil mixture by 2 g/kg, did not create an effect on body weight. Nafaji and Torki (2010) found that addition of cinnamon oil to the ration did not change live weight in the 0-21st days and the 43-49th days, but reduced it in the 23-42nd days.

Table 3. The effect of the capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture added to the compound feed of broiler on body weights, g (x±SEM).

a,b,c: The differences between means in the same row with different letters are important, p<0.05

	Treatment Groups					
Day	Control	CAP	CAR	CIN	CAP+CAR+CIN	P
0	33.83±0.31	33.97±0.30	34.13±0.32	33.99±0.36	33.68±0.23	0.0871
7	139.00±1.69^{c}	154.47±1.65^{a}	142.53±1.63^{c}	145.16±1.96^{b}	158.27±1.42^{a}	0.000
14	439.49±4.26	442.36±5.38	443.11±4.81	436.73±5.15	445.10±4.25	0.760
21	927.75±9.05^{ab}	924.11±10.26^{ab}	918.30±9.79^{b}	903.077±10.07^{b}	949.14±9.92^{a}	0.024
28	1578.58±16.72^{ab}	1547.20±18.43^{b}	1523.38±18.80^{b}	1558.75±20.83^{ab}	1606.04±18.18^{a}	0.025
35	2323.83±30.05^{ab}	2303.47±31.56^{ab}	2232.67±31.43^{b}	2287.48±33.44^{ab}	2365.00±28.56^{a}	0.046
42	2992.18±40.83^{b}	2988.73±41.42^{b}	2910.42±43.00^{b}	2967.80±45.51^{b}	3130.78±39.61^{a}	0.005

Considering *Table 4*, the differences among the groups in terms of the mean body weight gains (BWG) were not found statistically significant in the 7-14th and the 28-35th days (P>0.05), while they were significant in the 14-21st, the 21-28th, and the 35-42nd days (P<0.05). According to the obtained findings, the highest mean BWG was found in the CAP+CAR+CIN and CAP groups between the 1st and the 7th days, and in the CAP+CAR+CIN, control and CIN groups in the 21-28th days. The highest BWG was seen only in the CAP+CAR+CIN in the 14-21st and the 35-42nd days. According to the findings regarding the BWG means computed for periods, the differences among the experiment groups in the 0-21st, 22-42nd and 0-42nd days were significant (p<0.05), while the highest BWG was seen in the CAP+CAR+CIN group. It was found that addition of 150 mg/kg CAP+CAR+CIN into the feed in the 0-42nd days increased the mean BWG by 4.3% in comparison to the control group. Accordingly, the results obtained from the study agreed with those of other studies. Awaad et al. (2014) reported that a 100 g/ton addition of CAP (2.18%) + CAR (5.04%) + CIN (2.90%) reduced the BWG in comparison to the control in a 1-week period, did not change it in a period of 1-3 weeks, and increased it in a period of 3-5 weeks. Bravo et al. (2011) reported that, the CAR (5%) + CIN (3%) + CAP (2%) mixture was not effective by 100 mg/kg combination in 1-22nd days on BWG, while Karadas et al. (2014) found that addition of the same mixture and dosage to the feed increased BWG by 86 g in comparison to the control in the period of 0-21st days. According to Babaoglan and Kutlu (2008), addition of natural or synthetic thymol:carvacrol in the ratio of 1:1, and according to Alcicek et al. (2004), addition of a commercial essential oil mixture containing thyme by 48 mg/kg improved BWG in comparison to control; while Lee et al. (2004) reported that addition of 200 ppm CAR into the feed decreased it by 4.6%.

Table 4. The effect of the capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture added to the compound feed of broiler on body weight gain, g (x±SEM).

Day	Treatment Groups					
	Control	CAP	CAR	CIN	CAP + CAR + CIN	P
0-7	105.24±2.74^{b}	119.06±3.01^{a}	109.11±1.81^{b}	105.00±3.69^{b}	124.71±1.57^{a}	0.000
7-14	300.47±3.51	289.34±3.61	300.58±5.26	292.92±4.94	286.83±4.14	0.118
14-21	488.26±3.85^{b}	481.63±5.11^{b}	475.19±5.30^{b}	477.22±7.04^{b}	506.17±4.26^{a}	0.003
21-28	650.82±7.73^{a}	622.98±10.88^{ab}	605.49±13.30^{b}	644.99±10.64^{a}	654.89±8.51^{a}	0.014
28-35	745.25±29.95	756.12±17.29	709.15±21.91	730.12±24.86	759.21±10.03	0.488
35-42	685.58±34.09^{b}	697.57±30.00^{ab}	672.60±18.88^{b}	657.51±34.69^{b}	772.10±8.04^{a}	0.000
0-21	879.47±3.18^{b}	890.02±5.58^{b}	884.17±8.73^{b}	891.13±10.75^{b}	917.61±8.05^{a}	0.030
22-42	2081.65±45.92^{b}	2076.68±36.36^{b}	1987.23±24.87^{b}	2050.62±14.28^{b}	2186.21±17.73^{a}	0.003
0-42	2975.63±48.23^{b}	2966.70 ±38.17^{bc}	2871.41±27.96^{c}	2930.40±12.79^{bc}	3103.82±21.56^{a}	0.001

a,b,c: The differences between means in the same row with different letters are important, $p<0.05$

Considering *Table 5*, while there was no statistically significant difference among group in terms of feed intake in the 7-14th, 21-28th and 35-42nd days ($p>0.05$); the difference was significant in the 0-7th, 14-21st and 28-35th days ($p<0.05$). The highest feed intake value in the 0-7th days was found in the control and CAP group, while it was found in the CAP+CAR+CIN group in the 14-21st and 28-35th days. Considering periods of the 0-21st, 22-42nd and 0-42nd days, the trial had significant effects on feed intake ($p<0.05$), while the highest consumption was found to be in the CAP+CAR+CIN group. At the end of the trial (42nd day), while the CAP+CAR+CIN experiment group had a mean per-animal feed intake that is 53.28 higher than that of the control group, the difference was not significant ($p>0.05$). Finding the feed intake rates among groups significantly difficult was explained by, essential oils reducing pathogenic bacteria population and improving intestinal microflora (Alcicek et al., 2004), stimulating effects on the digestive system (Mukhtar, 2011) and appetizing effects (Babaoglan, 2008). It was reported by Fotea et al. (2010) that 1% carvacrol addition and by Al-Mashdani et al. (2011b) that 1% coriander oil increased feed intake; it was reported by Babaoglan (2008) that thymol/carvacrol addition and by Mukhtar (2011) that 400 mg/kg clove oil addition did not affect feed intake rates; while Akyurek and Yel (2011) stated that addition of 250 mg/kg thymol + 250 mg/kg carvacrol reduced the rate of feed intake.

Table 5. The effect of the capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture added to the compound feed of broiler on feed intake, g ((animal/day), x±SEM).

Day	Treatment Groups					
	Control	CAP	CAR	CIN	CAP + CAR + CIN	P
0-7	173.81±2.53^{a}	170.57±3.30^{a}	147.32±2.33^{c}	156.28±6.27^{bc}	165.35±6.63^{b}	0.004
7-14	319.84±5.62	325.40±4.45	327.34±6.21	322.43±6.20	324.16±4.10	0.886
14-21	609.44±6.56^{b}	616.92±5.75^{ab}	601.91±8.75^{b}	605.56±5.48^{b}	632.81±12.80^{a}	0.026
21-28	965.35±8.89	953.78±8.80	978.33±6.86	954.44±4.97	979.29±7.05	0.120
28-35	1222.11±19.33^{b}	1218.00±13.55^{b}	1249.04±16.06^{ab}	1197.24±23.07^{b}	1280.09±14.23^{a}	0.031
35-42	1396.78±19.91	1403.38±36.57	1384.48±25.29	1390.27±11.96	1450.06±8.25	0.295
0-21	1103.09±10.64^{ab}	1112.89±10.72^{ab}	1062.58±10.18^{c}	1084.25±13.20^{bc}	1122.33±46^{a}	0.018
22-42	3601.84±49.53^{ab}	3520.69±22.06^{b}	3548.92±61.48^{b}	3541.95±37.06^{b}	3714.10±21.15^{a}	0.024
0-42	4704.93±54.52^{bc}	4659.29±20.55^{b}	4722.14±83.83^{bc}	4626.20±30.08^{b}	4835.13±26.97^{a}	0.044

a,b,c: The differences between means in the same row with different letters are important, P<0.05

Table 6. The effect of the capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture added to the compound feed of broiler on feed conversion ratio, ((g feed/ g BWG), x±SEM).

Day	Treatment Groups					
	Control	CAP	CAR	CIN	CAP + CAR + CIN	P
0-7	1.66±0.06^{b}	1.43±0.02^{a}	1.36±0.02^{a}	1.43±0.06^{a}	1.33±0.06^{a}	0.001
7-14	1.07±0.01^{a}	1.15±0.01^{b}	1.09±0.01^{a}	1.13±0.02^{b}	1.13±0.00^{b}	0.000
14-21	1.24±0.00^{a}	1.29±0.01^{c}	1.28±0.01^{bc}	1.27±0.01^{abc}	1.25±0.01^{ab}	0.019
21-28	1.48±0.01^{a}	1.53±0.02^{b}	1.54±0.01^{b}	1.57±0.02^{b}	1.50±0.02^{ab}	0.044
28-35	1.72±0.06^{b}	1.61±0.04^{a}	1.74±0.03^{b}	1.64±0.02^{ab}	1.68±0.01^{ab}	0.002
35-42	2.05±0.09	2.12±0.17	2.15±0.11	2.11±0.13	1.93±0.04	0.659
0-21	1.32±0.02^{b}	1.28±0.01^{ab}	1.24±0.01^{a}	1.27±0.03^{ab}	1.24±0.02^{a}	0.018
22-42	1.74±0.02^{ab}	1.70±0.03^{a}	1.78±0.03^{b}	1.70±0.01^{a}	1.69±0.01^{a}	0.040
0-42	1.53±0.02^{b}	1.49±0.01^{ab}	1.51±0.02^{b}	1.50±0.01^{ab}	1.46±0.01^{a}	0.029

a,b,c: The differences between means in the same row with different letters are important, p<0.05

Considering *Table 6*, there was no significant difference among the groups in terms of feed conversion in the 35-42nd days (p>0.05), while the difference was found significant in the 0-7th, 7-14th, 14-21st, 21-28th and 28-35th days (p<0.05). Additionally, in the 0-7th and 7-14th days, feed conversion values for some experiment groups were found similar to that in the control group. The best feed conversion rate was found in the control group in both the 14-21st and 21-28th days, and in the CAP group in the 28-35th days. According to the findings on feed conversion ratios based on periods, the values calculated for the periods of 0-21st, 22-42nd and 0-42nd day were significantly different among each other (p<0.05). The best feed conversion ratio was found in the CAP+CAR+CIN and CAR groups in the 0-21st days, and in the CIN, CAP and CAP+CAR+CIN groups in the 22-42nd days. Additionally, the best feed conversion

ratio in the 0-42nd days was found in the CAP+CAR+CIN group. Results of relevant studies showed that addition of essential oils or bioactive substances to broiler chicken feed mixtures has varying effects. It was stated by Babaoglan (2008) that addition of 300 ppm thymol/carvacrol in the 0-3rd weeks, by Al-Mashdani et al. (2011b) that addition of 0.5% and 1% coriander oil, by Fotea et al. (2010) that addition of 1% carvacrol, and by Kucukyilmaz (2012) that addition of 48 mg/kg essential oil mixture increased the ratio of feed conversion; while it was reported by Shanoon et al. (2012) that addition of 10, 20 or 40 mg/kg/day clove oil and by Akyurek and Yel (2011) that addition of 250 mg/kg thymol + 250 mg/kg carvacrol did not have an effect on feed conversion ratio. Alçiçek et al. (2004) reported that addition of essential oil mixture to the feed did not change feed conversion in a period of 0-21 days, but increased it in a period of 0-42 days. Based on the results obtained in the study, it is possible to say that addition of CAP, CAR, CIN or CAP+CAR+CIN increased the ratio of feed conversion in broiler chickens.

The effects of the trial on livability are given in *Table 7*. Along the trial (0-42), a total of 9 animals died including 1 in the control group, 1 in the CAP group, 2 in the CAR group, 4 in the CIN group, and 1 in the CAP+CAR+CIN group. Accordingly, it may be argued that the addition of 150 mg/kg capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture (50 mg each) into the feed did not have negative effect on livability. It was seen that the results obtained from the study were in agreement with those of previous studies (Alcicek et al., 2004; Shanoon et al., 2012; Kucukyilmaz et al., 2012; Celikbilek et al., 2014).

Table 7. The effect of the capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture added to the compound feed of broiler on livability, % (x±SEM).

Treatment Groups	Livability**, %****
Control	98.75
CAP	98.75
CAR	97.50
CIN	95
CAP+CAR+CIN	98.75
*P	0.265

*p<0.05
** Chi-square analysis was carried out,

3.2. Carcass Characteristics

Considering *Table 8*, the differences among the groups were statistically insignificant in terms of carcass characteristics (P>0.05) except carcass yield. The highest carcass yield was found in the CAP group, while the lowest was found in the CAR and control groups (p<0.05). The findings of the study were found to agree with those of Alcicek et al. (2004), Simsek et al. (2005), Shanoon et al. (2012), Duarte et al. (2013), Khattak et al. (2014) and Kirkpinar et al. (2014). Additionally Babaoglan and Kutlu (2008) reported that addition of thymol:carvacrol (1:1) did not affect the carcass yield, but reduced the abdominal fat ratio. According to Al-Kassie (2009), addition of 200 ppm carvacrol reduced the abdominal fat ratio, while addition of 200 ppm cinnamon oil increased it. Awaad et al. (2014) reported that the addition of CAP+CAR+CIN to the feed reduced the abdominal fat by 0.27% in comparison to the control group.

Table 8. The effect of the capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture added to the compound feed of broiler on carcass characteristics, (x±SEM).

Characteristic	Treatment Groups					
	Control	CAP	CAR	CIN	CAP + CAR + CIN	*P
Slaughter weight (g)	2916.00±101.8	2948.00±104.98	2892.40±106.7	2928.60±100.97	3049.90±116.24	0.857
Carcass weight (g)	2167.50±83.34	2228.10±79.78	2121.00±78.78	2182.50±75.66	2271.30±88.15	0.733
Carcass yield (%)	73.26±0.46[b]	74.95±0.41[a]	73.33±0.43[b]	74.54±0.46[ab]	74.46±0.36[ab]	0.027
Breast, (g)	789.40±40.06	789.70±32.42	748.50±21.63	805.20±27.77	813.80±30.93	0.631
Breast, (%)**	26.98±0.62	26.88±0.77	25.96±0.39	27.54±0.52	26.71±0.37	0.340
Leg, (g)	546.10±28.06	544.20±30.81	540.20±27.93	548.30±23.93	577.00±30.46	0.895
Leg, (%)**	18.67±0.47	18.37±0.54	18.63±0.51	18.69±0.30	18.86±0.44	0.964
Abdominal fat, (g)	22.00±2.51	22.38±2.69	15.10±2.88	19.00±2.53	20.30±3.30	0.377
Abdominal fat, (%)**	0.79±0.11	0.63±0.14	0.54±0.11	0.67±0.10	0.70±0.13	0.676

a,b: The differences between means in the same row with different letters are important, *p<0.05
** The body weights have been used for calculation of the proportional weights.

Table 9. The effect of the capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture added to the compound feed of broiler on some circulation and secretory organ weights, (x±SEM).

Characteristic	Treatment Groups					
	Control	CAP	CAR	CIN	CAP + CAR + CIN	P
Liver, (g)	49.80±2.73	48.90±2.28	50.00±3.38	52.40±2.65	47.40±2.74	0.785
Liver, (%)**	1.70±0.05	1.67±0.07	1.73±0.09	1.79±0.08	1.55±0.06	0.181
Heart, (g)	13.40±1.11	13.10±1.04	13.50±0.79	12.90±1.20	13.10±1.00	0.994
Heart, (%)**	0.46±0.03	0.44±0.02	0.47±0.02	0.44±0.03	0.43±0.02	0.821
Spleen, (g)	3.35±0.35	3.14±0.37	2.84±0.28	2.73±0.21	2.33±0.15	0.114
Spleen, (%)**	0.11±0.01	0.11±0.01	0.10±0.01	0.10±0.01	0.08±0.01	0.024
Pancreas, (g)	6.11±0.39	5.48±0.27	5.07±0.41	5.39±0.36	5.83±0.41	0.343
Pancreas, (%)**	0.21±0.02	0.19±0.01	0.18±0.01	0.18±0.01	0.19±0.01	0.361
Bursa Fabricius, (g)	4.82±0.39	4.49±0.39	3.86±0.35	4.86±0.50	5.38±0.61	0.236
Bursa Fabricius, (%)**	0.16±0.01	0.15±0.01	0.14±0.01	0.17±0.02	0.18±0.02	0.448

*P<0.05,
**The body weights have been used for calculation of the proportional weights.

Considering *Table 9*, the difference among the groups in terms of the circulation and secretion organs obtained from the slaughtered animals and the ratios of these in total live weight was not significant (p>0.05). Langhout (2000) stated that essential oils stimulate digestive system, increase pancreatic enzymes and improve liver functions in poultry. Yildiz (2007) found that 1–2 kg/ton carvacrol + thymol + rosmarinic acid combination did not have effects on the weights of bursa, liver and pancreas, and the spleen weight decreased by the increased dosage. Al-Kassie (2009) reported that an addition of 100–200 ppm thyme or cinnamon extract increased liver weight in line with dosage, heart weight decreased by 100 ppm addition of both extracts, while it increased by 200 ppm addition. Shanoon et al. (2012) stated that addition of 20–40 mg/kg ginger oil did not have an effect on heart and pancreas

weights, but liver weight increased in line with the dosage. According to the results of this study, it was observed that the addition of CAP, CAR, CIN or CAP+CAR+CIN had varying effects on the organs of broiler chickens.

Considering *Table 10*, the difference among the groups in terms of glandular stomach and proportional glandular stomach weights, gizzard and proportional gizzard weights, total intestine and proportional intestine weights was statistically significant ($p<0.05$). While the lowest glandular stomach and proportional glandular stomach weights were found in the CIN group, there was no statistically significant difference among other experiment groups. The highest gizzard weight was in CIN and CAP+CAR+CIN, the highest proportional gizzard weight was in CIN, and the highest total intestine and proportional total intestine was found in the control group. When the groups were analyzed in terms of weights regarding the sections of the intestines (duodenum, jejunum and ileum) their proportions, the difference among the means was found statistically significant ($p<0.05$). The highest duodenum weight and proportion, jejunum proportion and ileum weight were observed in the control group ($p<0.05$). Additionally, the highest jejunum weigh was found in control and CAR, and the highest ileum proportion was found in control and CAP ($p<0.05$). When the groups' large intestine and proportional large intestine weights were analyzed, it was seen that the differences for both parameters were statistically significant ($p<0.05$). Both large intestine weight and large intestine proportion were found the highest in the control group. Accordingly, it may be stated that addition of CAP, CAR, CIN and CAP+CAR+CIN into the feed mixture had a reducing effect on the weights of digestion organs ($p<0.05$). Nafaji and Torki (2010) found that addition of 200 mg/kg thyme, cinnamon and clove oil did not have an effect on the weights of duodenum, jejunum and ileum, while according to Kucukyilmaz (2012), a commercial mixture of essential oils did not have an effect on small and large intestines. Thus, it was seen that the results obtained in this study were different to those in previous studies.

Table 10. The effect of the capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture added to the compound feed of broiler on digestive organ weights, (x±SEM).

Characteristic	**Treatment Groups**					
	Control	CAP	CAR	CIN	CAP + CAR + CIN	*P
Grandular stomach, (g)	9.50 ± 1.46^{a}	8.40 ± 0.48^{a}	9.30 ± 1.04^{a}	5.39 ± 0.36^{b}	9.00 ± 0.63^{a}	0.013
Grandular stomach, (%)**	0.32 ± 0.04^{a}	0.29 ± 0.01^{a}	0.32 ± 0.03^{a}	0.18 ± 0.01^{b}	0.30 ± 0.02^{a}	0.003
Gizzard, (g)	29.40 ± 2.27^{ab}	26.70 ± 2.03^{ab}	23.60 ± 1.35^{b}	31.80 ± 2.02^{a}	31.20 ± 2.16^{a}	0.032
Gizzard, (%)**	1.36 ± 0.10^{ab}	1.21 ± 0.10^{b}	1.11 ± 0.05^{b}	1.52 ± 0.09^{a}	1.38 ± 0.10^{ab}	0.030
Total intestine, (g)	76.50 ± 3.16^{a}	66.20 ± 2.32^{b}	70.70 ± 3.82^{ab}	68.22 ± 3.90^{ab}	61.30 ± 2.70^{b}	0.021
Total intestine, (%)**	2.65 ± 0.13^{a}	2.25 ± 0.03^{bc}	2.44 ± 0.08^{ab}	2.49 ± 0.18^{ab}	2.01 ± 0.06^{c}	0.003
Duodenum, (g)	12.50 ± 0.70^{a}	9.60 ± 0.83^{b}	9.30 ± 0.87^{b}	9.00 ± 5.00^{b}	9.70 ± 0.68^{b}	0.010
Duodenum, (%)**	0.43 ± 0.03^{a}	0.32 ± 0.02^{b}	0.32 ± 0.03^{b}	0.36 ± 0.03^{ab}	0.32 ± 0.02^{b}	0.013
Jejenum, (g)	38.20 ± 1.62^{a}	33.40 ± 1.54^{ab}	38.25 ± 1.68^{a}	35.44 ± 2.31^{ab}	30.38 ± 1.93^{b}	0.021
Jejenum, (%)**	1.32 ± 0.07^{a}	1.15 ± 0.07^{ab}	1.30 ± 0.06^{a}	1.18 ± 0.08^{ab}	1.00 ± 0.05^{b}	0.023
İleum, (g)	8.92 ± 0.57^{a}	7.00 ± 0.62^{b}	6.40 ± 0.65^{b}	8.00 ± 0.50^{ab}	7.30 ± 0.54^{ab}	0.038
İleum, (%)**	0.41 ± 0.02^{a}	0.42 ± 0.04^{a}	0.30 ± 0.03^{b}	0.37 ± 0.03^{ab}	0.33 ± 0.03^{ab}	0.043
Large intestine, (g)	18.29 ± 0.97^{a}	14.80 ± 0.77^{bc}	17.12 ± 0.91^{ab}	16.57 ± 0.92^{abc}	14.00 ± 0.86^{c}	0.009
Large intestine, (%)**	0.91 ± 0.08^{a}	0.67 ± 0.04^{b}	0.75 ± 0.03^{b}	0.71 ± 0.06^{b}	0.62 ± 0.04^{b}	0.003

a,b,c: The differences between means in the same row with different letters are important, *$p<0.05$

** The body weights have been used for calculation of the proportional weights.

3.3. Intestinal Microflora

On the 42nd day of the trial, *Lactobacillus* spp., *Staphylococcus aureus, Clostridium perfringens* counts and *Salmonella* spp. (positive/negative) test were performed on the ileum part of the intestines of the animals taken for each group and the results are given in *Table 11*. When *Table 11* is examined, *Salmonella* spp. was isolated in 5 out of 5 samples taken for the control group and only 1 out of each group in the samples taken for the treatments groups. It was determined that the addition of capsicum oleoresin, carvacrol, cinnamaldehyde and their combinations to the mixed feeds did not affect the number of *Staphylococcus aureus, Clostridium perfringens* ($p>0.05$), while it affected the *Lactobacillus* spp. count in a significant level ($p<0.05$). When the groups were evaluated in terms of *Staphylococcus aureus, Clostridium perfringens* numbers, the both microorganisms were found to be at uncountably low levels in all groups. When the average values for the ileum *Lactobacillus* spp. counts were evaluated, it was detected that the ileums of the treatment group animals fed with CAP+CAR+CIN added feeds contained a significantly higher amount of *Lactobacillus* spp. content ($P<0.05$) with an average value of 4.48 ($\log_{10}$) CFU/g compared to the ileums of the control, CIN, CAP and CAR group animals with average values of 3.74, 3.56, 3.25 and 3.24 ($\log_{10}$) CFU/g respectively. The control, CIN, CAP and CAR groups on the other hand were similar in nature and there was no statistically significant difference between them ($p>0.05$). At the end of the trial, the intestines of the animals were removed, microbiological analyzes were conducted in the ileum part and while there was a significant difference in *Lactobacillus* spp. counts between the groups $p<0.05$), no significant difference was observed in *Staphylococcus aureus, Clostridium perfringens* numbers ($p>0.05$) (see *Table 11*). When the results obtained in this study are compared to the results of the studies where other herbal extracts and bioactive components that affect intestinal microflora of broilers were used; while they show similarity with the results indicating that the thymol:carvacrol (1:1) mix added to broiler feeds by 200 mg/kg increased *Lactobacillus* spp. and *Bifidobacterium* numbers (Hashemipour et al., 2013), they differ from the results of another study conducted by Sharifi et al. (2013) where it was shown that the addition of 4 different aromatic plants in dried form (cumin, mint, yarrow and poley-haired herba chamaedrys) to the mix broiler (Arbor Acres) feeds by 15, 3, 2 and 2 g/kg respectively significantly reduced the *Clostridium* (log 10/g) numbers in the groups except for the cumin group, and did not change *Lactobacillus* numbers in all the treatments groups. Shanoon et al. (2012) reported that the addition of ginger oil to the mix feed of broilers (Ross 308) consisting of male and female groups with different doses (0, 10, 20, 40 mg/kg/day) significantly reduced *Escherichia coli, Enterobacteria, Staphylococci, Salmonella* and *Shigella* spp. numbers; Zhou et al. (2007) reported that the combination of cinnamladehyde (100 mg/l) + carvacrol (200 mg/l) significantly reduced decreased the development of *Salmonella typhimurium* in bacteria culture (Mueller Hinton); Xu et al. (2008) reported that depending on the increasing doses (0, 100, 200, 400, 800 mg/l), the addition of carvacrol:thymol (1-1) combinations to *Escherichia coli* bacteria culture (Mueller Hinton broth, 1×107 CFU/ml) significantly reduced the development of *E.coli*; Yossa et al. (2012) treated *E.coli* (0157:H7) and *Salmonella* spp. bacteria cultures (Luria-Bertani broth, 1×107 CFU/ml) with cinnamaldehyde in two different doses (800 ve 1000 ppm) and at the end of 1 hour incubation they reported that bacteria populations were decreased to an unidentifiably low degree in both doses.

Table 11. Effects of capsicum oleoresin, carvacrol, cinnemaldehyde and mixtures added to broiler mixed feeds on intestinal microflora ($\log_{10}$) CFU/g, (x±SEM).

	***Treatment groups**					
Microorganism	**Control**	**CAP**	**CAR**	**CIN**	**CAP+CAR+ CIN**	**P**
Lactobacillus spp.	3.74 ± 0.38^{b}	3.25 ± 0.18^{b}	3.24 ± 0.07^{b}	3.56 ± 0.20^{b}	4.48 ± 0.18^{a}	0.021
S. aureus	<10	<10	<10	<10	<10	-
C. perfringens	<10	<10	<10	<10	<10	-
Salmonella spp.	Positive	Negative	Negative	Negative	Negative	-

a,b,c: Means within rows with different superscript differ at $p<0,05$, *Control: no feed additive, CAP: 150 mg/kg capsicum oleoresin, CAR: 150 mg/kg carvacrol, CIN: 150 mg/kg cinnamaldehyde and CAP+CAR+CIN: 150 mg/kg mixture (50 mg each, capsicum oleoresin + carvacrol + cinnamaldehyde).

3.4. Some Blood Parameters

Findings of some biochemical parameters detected in blood samples taken from brachial veins of animals before slaughter to be laid open for carcass features on day 42 of trial are given in *Table 12*. When *Table 12* was examined, it was determined that bioactive secondary plant metabolite addition insignificantly ($p>0.05$) affected glucose, ALT, total cholesterol and HDL values, cinnamaldehyde and mixture group decreased AST values, carvacrol cinnamaldehyde and capsicum oleoresin group decreased triglyceride value, carvacrol and mixture group decreased LDL values and cinnamaldehyde group increased albumin and total protein levels significantly ($p<0.05$). Among examined biochemical parameters, AST 337-500 U/L, ALT 5.33-6.89 U/L, glucose 232.22-241.33 mg/dl, triglyceride 31.11-60.44 mg/dl, total cholesterol 106.70-118.33 mg/dl, HDL 94.80-99.22 mg/dl, LDL 3.76-7.85 mg/kg, total protein 3.01-3.52 mg/dl and albumin 1.52-1.65 mg/dl has exhibited change from mean values. When biochemical analysis result belonging to blood parameters such as glucose, ALT, total cholesterol and HDL, it was determined that differences among treatment groups were not statistically significant $p>0.05$). When treatment groups were compared to control group, it is seen that cinnamaldehyde and mixture group has decreased AST values while carvacrol group has increased it, carvacrol, cinnamaldehyde and capsicum oleoresin group has decreased triglyceride value, carvacrol and mixture group has decreased LDL values and cinnamaldehyde group has increased albumin and total protein levels significantly ($p<0.05$) (see *Table 12*). Calislar et al. (2009) revealed that addition of oregano essential oil (81.89 % carvacrol, 5.1% x-terpinene, 3.76 % cymen, 2.42 % thymol) at levels of 300, 500 and 700 ppm to broiler mixed feeds did not affect total cholesterol, HDL and triglyceride levels. Similarly, in a study made in 2004, Lee et al. added carvacrol and cinnamaldehyde and mixtures thereof (carvacrol and cinnamaldehyde of 100 ppm each) in 200 ppm levels to the mixed feeds of female broiler feeding on feeds containing carboxymethyl cellulose, and as a result they stated that treatment group as compared to control group did not affect plasma lipid levels (total cholesterol, HDL, triglyceride and phospholipid). Najafi and Torki (2010) revealed that addition of thyme, cinnamon and clove essential oil in 200 ppm levels to broiler mixed feeds did not affect total cholesterol, triglyceride and HDL values, difference between the highest and the lowest hematocrit (HCT) and red blood cell (RBC) values were seen in groups fed with cinnamon and clove essential oils, however they revealed that difference relating to this values in groups fed with thyme essential oil and control group were not present.

Table 12. Effects of capsicum oleoresin, carvacrol, cinnemaldehyde and mixtures supplementation to broiler mixed feeds on some blood parameters (x±SEM).

BLOOD PARA-METERS	***Treatment groups**					
	Control	**CAP**	**CAR**	**CIN**	**CAP+CAR+CIN**	**P**
Glucose (mg/dl)	232.22±4.26	235.78±5.56	237.00±3.77	241.33±6.13	239.56±4	0.716
AST (U/L)	$428.60±6.35^{b}$	$500.00±33.1^{c}$	$444.50±8.2^{bc}$	$367.29±16.2^{a}$	$337.00±41^{a}$	0.000
ALT (U/L)	5.33±0.44	6.56±0.87	6.60±0.65	6.33±0.62	6.89±1.06	0.655
Triglyceride (mg/dl)	$35.67±5.6^{a}$	$40.00±4.77^{ab}$	$42.20±7.65^{ab}$	$60.44±9.49^{b}$	$31.11±5.3^{a}$	0.009
Total Cholesterol (mg/dl)	108.89±3.47	115.44±6.35	106.70±2.57	118.33±8.56	109.89±3.2	0.494
HDL (mg/dl)	97.11±2.43	98.33±5.16	94.80±2.69	98.67±3.13	99.22±2.66	0.884
LDL (mg/dl)	$7.85±1.35^{b}$	$5.4±0.31^{ab}$	$3.76±0.43^{b}$	$5.48±0.69^{ab}$	$4.96±0.75^{a}$	0.000
Total Protein (mg/dl)	$3.17±0.08^{b}$	$3.20±0.05^{b}$	$3.16±0.10^{b}$	$3.52±0.18^{a}$	$3.01±0.05^{b}$	0.021
Albumin (mg/dl)	$1.61±0.03^{abc}$	$1.63±0.03^{ab}$	$1.52±0.03^{c}$	$1.65±0.05^{a}$	$1.53±0.03^{bc}$	0.025

a,b,c: Means within rows with different superscript differ at p<0,05, *Control: no feed additive, CAP: 150 mg/kg capsicum oleoresin, CAR: 150 mg/kg carvacrol, CIN: 150 mg/kg cinnamaldehyde and CAP+CAR+CIN: 150 mg/kg mixture (50 mg each, capsicum oleoresin + carvacrol + cinnamaldehyde).

3.5. IGF1 Gene Expression Levels

Considering the *Table 13*, in animals fed with feeds containing 150 mg/kg capsicum oleoresin, carvacrol, cinnamaldehyde or the mixture of these in equal proportions, the differences among groups in terms of IGF1 expression were insignificant (p>0.05). C_T, ΔC_T, $\Delta\Delta C_T$ and fold-change values of GADPH and IGF1 genes are presented in *Table 13*. Comparison of control, CAP, CAR, CIN and CAP+CAR+CIN groups in terms of mean C_T and mean ΔC_T values of GAPDH and IGF1 genes did not show any statistically significant difference between the groups (p>0.05). Whether treatment caused any increase or decrease in IGF1 expression was determined by calculation of its fold-change. Accordingly, in comparison to the control group, IGF1 gene expression was increased by 18 folds in CAP group, 8.88 folds in CAR group, 38.05 folds in CAP+CAR+CIN group, where as it was unchanged in the CIN group. There are no studies in the literature similar to these findings, while Kita et al. (1996) reported that the broilers fed with low-protein rations did not show different IGF1 gene expression in liver tissue in comparison to the control group. Kim et al. (2010), as a result of their study investigating the expression levels of genes on the intestinal tissue with addition of CAR (5 mg/kg), CIN (3 mg/kg) and CAP (2 mg/kg) into feed mixtures of broiler chickens, observed that 26 genes increased and 48 genes decreased among 72 in the group with CAR addition, 31 increased and 31 decreased among 62 in the group with CIN addition; while in the group with the highest amount of changes, the group with CAP addition, 156 genes increased and 98 genes decreased. As there are no studies in the literature regarding the effects of CAP, CAR, CIN addition on IGF1 gene expression, this study discussed other studies about IGF1 gene expression. While Heck et al. (2003) found that the IGF1 expression in the ovaries of broiler breeding chicken fed as ad libitum and with restriction did not change with age, Guernec et al. (2004) observed that the IGF1 mRNA levels in the *Pectoralis major* (PM) and *Sartorius* (SART) muscles of broilers that were left hungry between 0 and 2 days (48 h hungry, 48 h free) at 4 weeks of age for 16, 24 and 48 hours and fed freely for 16, 24 and 48 hours, increased in comparison to the group left hungry in the free-feeding group.

Table 13. C_T, ΔC_T, $\Delta\Delta C_T$ and fold-change values of GADPH and IGF1 genes in control and treatment Groups.

Genes	**Treatment Groups**					
	Control	CAP	CAR	CIN	CAP + CAR + CIN	*P
GADPH Average C_T	14.91±0.58	14.99±0.37	14.69±0.42	14.41±0.32	14.91±0.16	0.832
IGF1 Average C_T	34.20±2.22	30.43±0.27	30.83±1.06	33.62±1.79	29.49±0.64	0.194
ΔC_T IGF1-GAPDH	19.49±2.10	14.58±0.27	16.79±1.59	19.25±2.07	14.62±0.45	0.189
**$\Delta\Delta C_T$ ΔC_T - ΔC_T,Control	0.00	-4.71	-3.15	-0.07	-5.25	-
IGF1 Fold Expression	1.00	18.00	8.88	1.05	38.05	-

*p<0.05,** Relative quantification of $\Delta\Delta C_T$ and IGF1 were determined by calculation, and their mean values are presented.

4. CONCLUSION

In conclusion, based on the findings of the present study, individual positive and/or negative effects of capsicum oleoresin, carvacrol, cinnamaldehyde and their mixture addition to the mixed feeds of broiler chickens on the investigated characteristics and criteria have been observed, and it was thought that these negative effects might be caused by the high level of bioactive substance doses used. However, it was detected that the addition of mixture (50 mg/kg capsicum oleoresin+50 mg/kg carvacrol+50 mg/kg cinnamaldehyde) to the mixed feeds of broiler chickens improved the performance, carcass yield, some

organ weights, intestinal microflora and some blood parameters in broiler chickens. And it can be concluded that these bioactive secondary plant metabolites can be used in feeding of broilers as natural feed additive. Especially as this mixture created positive effects on broiler performance, it can be recommended for use in practice. Moreover, determination of levels of use of combination of this bioactive materials with different doses, research of effects thereof on amount and quality of animal products, improvement of practical application and repetition with mixed feed having different structure in feeding applications.

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Availability of data and materials

Authors approved the data and materials availability.

Ethics approval

All experimental procedurs involving animals were conducted in accordance with the Animal Experimental Guidelines provided by the Ege University Ethical Committee (No: 2014-013).

Conflict of interest statement

Authors declare no conflict of interest.

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