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EFFECT OF COLD-PRESS CARROT SEED OIL ON THE PERFORMANCE, CARCASS CHARACTERISTICS, AND SHELF LIFE OF BROILER CHICKENS

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ABSTRACT

Present study aims to assess the effects of cold-press carrot seed oil (CSO) on performance, gut microflora, some serum biochemical parameters, meat color and meat shelf life of broiler chickens by experimenting with young broiler chicks. Accordingly, hundred fifty day-old Ross PM 308 broiler chicks were randomly given three different dietary treatments supplemented with 0 (control), 100mL/kg or 200 mL/kg (CSO). Five replicates of 10 birds each. It was observed that the supplementation of CSO led to an increase in weight gain, hot carcass and carcass yield on day 42. Addition of CSO supplement in the basal diet had no effect on serumbiochemical parameters. The number of lactic acid bacteria was higher in the group of chicks fed on the diet supplemented with 200 ml/kg CSO than in the other groups. Compared to the control group, dietary CSO supplement reduced the MDA values in leg muscles. L^* (lightness) and b^* (yellowness) values of the breast muscles increased in the chicks fed with 200 ml/kg CSO. Dietary 100 ml/kg CSO supplementation decreased a^* (redness) values of the breast and leg muscles.In conclusion, supplementation of CSO had resulted in positive changes on weight gain,carcas yield, lactic acid bacteria count and breast tissue shelf life. As a result, this study suggests that cold-press carrot seed oil can be used as a dietary source of natural antioxidant for broilers.

Key words: carrot seed oil, broiler, gut microflora, malondialdehyde, meat lightness.

INTRODUCTION

Since the use of antibiotics was prohibited in order to enhance the quality as well as to increase the yield of the products, researchers have started to focus on phytogenics as non-antibiotic growth promoters. There are many studies investigating the use of essential oils as growth promoters. Accordingly,aromatic plants can increase feed intake, feed conversion ratio, weight gain and can improve the oxidative stability of tissues (Erhan *et al*., 2012; Ürüşan and Bölükbaşı, 2017).

Alçiçek *et al*., (2003, 2004) recorded that the supplementation of the mixture of herbs (thyme, bay, sage, myrtle leaf, fennel and citrus essential oil) to the broiler´s diets, significantly improved live weight, feed efficiency and carcass characteristics of the broilers.

Bozkurt *et al*., (2007) found that citrus sinensis peel mixtures of organic acid and essential oils (thyme, bay leaf, fennel, sage, myrtle and orange peel oils) increased live body weight and feed conversion ratio in broilers

Carrot (Daucus carota) is a commonly consumed vegetable species belonging to the family Apiacea, which grows in temperate regions of Europe, Asia and Africa (Hammam, 2014). Carrot contains a lot of active ingredients such as steroids, tannins, flavonoids, and carotene (Mahran *et al*., 1991; Jasicka-Misiak *et al*., 2005; Vasudevan *et al*., 2006). It has been reported that carrot seeds are rich in antioxidants (Yu *et al*., 2005) and contain a lot of active ingredients such as camphene, b-pinene, a-pinene, myrcene, sabinene, y-terpinene, limonene, b-bisabolene, geranyl acetate, and carotol.

Health benefits and reliability of cold press carrot seed oil as a plant based supplement have been reported by Yu *et al*. (2005) because it is not exposed to a chemical process and heat treatment. In addition, it was also stated that the cold-press carrot seed oil is a natural antioxidant which protects important protein molecules from radical-mediated damage (Yu *et al*., 2005). Another study by Moghadam *et al.* (2012) has revealed that carrot seed extract acts as an antioxidant on liver tissue of rats.

Color is an important quality criteri on that affects consumer satishfaction in food products. Products that differ in colour from the original product are often rejected by consumers.This has been a problem for the poultry industry for many years (Qiao *et al*., 2001). For this reason, it has been suggested to use lightness (L^*) values as a marker of meat quality in industrial poultry (Owens *et al*., 2000; Woelfel *et al*., 2002).

To our knowledge, there is no study that has investigated the effects of cold-press carrot seed oil supplement on broiler chickens. Therefore, we have examined carrot seed oil in order to assess its effects on performance, intestinal microflora, some serum biochemical parameters, meat color and meat shelf life of broilers.

MATERIALS AND METHODS

Experimental Design: The experiments were performed with 150 day-old (75 male and 75 female) Ross 308 broiler chicks. The broilers were divided into 3 groups, each gropu has 50 chicks with 5 subgroups. Carrot seed oil, which comprises 45% carotol and 6% daucene, was purchased from a commercial company. The control group was fed with basal feed, and the experiment groups were fed with rations which includes 100 and 200 ml/ kg carrot seed oil per day for 42 days. The chickens were fed *ad libitum* with feed and water. The research protocol was approved and regulated according to the Animal Ethics Committee Guidelines of Atatürk University (No: 2010/41). Feed intake and body weight were recorded by weighing the chicks. FCR was calculated with the obtained data.

At the final stage of the experiment, blood samples (3 male, 3 female) collected from the chickens were centrifuged at 4000 g for 10 minutes at 20 °C. Then, the serum samples had been kept at -20 °C until the analysis has been made. Biochemicial parameters were determined in serum by commercial kits (Roche). From every treatment group, six chickens (3 male and 3 female) were randomly selected and slaughtered. The carcasses were plucked and the heads, necks, shanks as well as the feet were removed; the liver and heart were removed from the viscera. Then, the carcass yield was calculated.

The leg and breast muscles were used to determine the meat color and the pH of the muscles. All samples were stored at 4 °C until the further analysis. Colour properties of the tissues (L *, a * and b*) were measured using a colorimeter (Minolta Chroma Meter Measuring Head CR-200, Minolta, Osaka, Japan). After the slaughtering at 24 hours the pH values of the muscles were measured using direct probe of pH meter (SCHOTT L 6880, Lab Star pH).

The intestinal contents were removed and 2 g contents from jejunum to the sterile plastic tubes. Microbial population were counted on the same day. Lactic acid bacteria was counted by using the method reported by Baumgart *et al*. (1993) and *E.coli* was counted by using the method reported by Halkman *et al*. (1994).

Data Analyses: A variance analysis was applied to the data using SPSS (1999) statistical package programme for Windows version 10.0. Mean values that significantly differ were separated by Duncan's multiple comparison test at $\alpha = 0.01$ and 0.05 levels, respectively.

Table 1. The constituents of the experimental diet (g/kg).

Item	Starter diet (1-21 d)	Finisher diet (22-42 d)
Corn	562	556
Soybean Meal	189	120
Full-Fat Soybean	160.00	229.35
Animal by-products	69.00	69.00
Soybean Oil	4.00	12.00
Salt and Soda	3.30	4.05
Lysine	3.00	2.10
Methionine	2.00	1.30
Limestone	2.00	2.20
Vitamin Mixture[1]	2.00	2.00
Mineral Mixture[2]	1.50	1.50
DCP (dicalcium phosphate)	2.20	0.50
Composition (%)		
Crude Protein (analysed)	22.70	20.99
Crude Fat (analysed)	8.13	10.15
Crude Fibre(analysed)	3.96	4.21
Crude Ash (analysed)	5.24	5.29
Dry Matter (analysed)	88.94	90.08
ME (kcal/kg)	3040	3240
Ca	1.05	0.99
P	0.56	0.53
Methionine	1.22	0.94
Lysine	1.50	1.25

[1:] Provided per kilogram: 12 000 IU Vitamin A., 3 500 IU Vitamin D3, 100 g Vitamin E., 3 mg Vitamin K3, 2.5 mg. Vitamin B1, 6 mg Vitamin B2, 25 mg Niacin, 12 mg Ca-D-Pantothenate, 4 mg Vitamin B6., 0.015 mg Vitamin B12., 1.5 mg Folic Acid,150mg D-Biotin., 100 mg Vitamin C., 450 mg Colin chloride.

[2:] Provided per kilogram: 100 mg Mangan., 25 mg Iron., 65 mg Zink., 15 mg Copper., 0.25 mg Cobalt., 1 mg Iodine., 0.2 mg Selenium.

RESULTS

The results of the performance values are indicated in Table 2. According to the results, the prescribed diet did not affect the consumption of total feed and feed conversion ratio, however, there was a significant increase ($P < 0.05$) in the body weight and weight gain after the supplementation of carrot seed oil (CSO) at the end of the experiment.

The effects of different dietary treatments on some carcass characteristics of the broilers are presented in Table 3. Carrot seed oil supplement led to a remarkable increase in hot carcass, hot carcass yield of the broilers. No effect of CSO has been observed on the ratio of heart and liver of broiler chicks.

The differences in *E.coli* bacteria *count* between the groups were not significant. However, the number of lactic acid bacteria was significantly (P<0.05) affected by the supplementation of carrot seed oil. Accordingly, as can be seen in Table 4, the highest lactic acid bacteria count was observed in the group that received 200 ml/kg carrot seed oil per day.

The effects of prescribed diets on some of the blood metabolites of broilers are illustrated in the table below (Table 5). As can be seen in the table, there is no effect of the dietary factors on serum ALT, AST, glucose, TG, total cholesterol, LDL and HDL concentration.

The results of different dietary treatments in terms of TBARS values of leg and breast tissues are shown in Table 6. Supplementation of dietary carrot seed oil did not change TBARS values of breast tissues on the 1st, 3rd and 5th days. None of the dietary consitutents changed the TBARS values of leg tissue on the 1st and 3rd days. However, MDA values of leg tissue of the group fed with 200 ml/kg carrot seed oil per day were significantly (P < 0.01) lower than the other groups on the 5th day. The correlation between Group x Days and TBARS values of leg and breast tissues was not significant (P > 0.05).

When we compare the properties of breast and leg meat, significant differences were found for all parameters excluding leg L^* and b^* values (Table 7). The L^* and b^* values of breast tissue were higher in the group fed on 200 ml/kg carrot seed oil per day than the other groups. The breast and leg tissue of birds fed 100 ml/kg carrot seed oil had significantly lower a^* value than the other groups. The difference in the L^* and b^* values in the leg tissues between the groups were not significant.

Table 2.The effect of adding carrot seed oil on performance values in broilers.

Groups	Body weight 1 d (g/bird)	Body weight 42 d (g/bird)	Weight gain (g/bird)	Total feed consumption (g)	Feed conversion ratio (g:g)
Control	36.25	2120.55[b]	2084.30[b]	3516.94	1.69
CSO 100 mL/kg	35.83	2448.33[a]	2412.50[a]	3858.88	1.60
CSO 200 mL/kg	36.02	2461.25[a]	2425.25[a]	3745.67	1.55
SE	1.24	62.50	58.17	68.09	0.025
P	NS	*	*	NS	NS

[a, b]: The column average is significantly different
SE: standard error *: p<0.05 NS: Not significant

Table 3. **Effects of dietary carrot seed oil on some carcass characteristics of broilers.**

Groups	Hot carcass (g)	Hot carcass yield (g)	Heart ratio (%)	Liver ratio (%)
Control	1541.25[b]	72.68[b]	0.62	2.32
CSO 100 mL/kg	1873.33[a]	76.51[a]	0.58	2.88
CSO 200 mL/kg	1822.50[a]	77.92[a]	0.62	2.54
SE	64.49	0.69	0.025	0.13
P	*	*	NS	NS

[a, b]: The column average is significantly different
SE: standard error, **: p<0.01, *:p<0.05, NS: Not significant

Table 4.The effect of carrot seed oil on jejunum microflora in broilers.

Groups	*E. coli* (MPN/g)	Lactic acid bacteria (cfu/g)
Control	1100	$55.00x10^2$b
CSO 100 mL/kg	1100	$51.34x10^2$b
CSO 200 mL/kg	780	$121.55x10^2$a
SE	91.42	11.17
P	NS	*

[a, b]: The column average is significantly different
SE: standard error, *: p<0.05 NS: Not significant

Table 5. Effects of dietary supplementation of carrot seed oil on blood serum biochemical parameters of the broilers.

Groups	AST(U/L)	ALT(U/L)	TG(mg/dl)	Cholesterol(mg/dl)	HDL(mg/dl)	LDL(mg/dl)	Glucose(mg/dl)
Control	258.40	7.40	30.20	120.60	101.40	26.60	204.00
CSO 100 mL/kg	276.00	8.00	35.60	129.80	102.40	32.00	209.40
CSO 200 mL/kg	318.80	8.20	36.20	121.60	90.40	34.40	201.80
SE	15.49	0.27	1.86	5.20	3.72	3.42	5.49
P	NS	NS	NS	NS	NS	NS	NS

SE: standard error, NS: Not significant

Table 6. The effects of dietary carrot seed oil on the TBARS values (mg MDA/kg tissue) in legs and breast of the broilers.

Groups	TBARS					
	Leg			Breast		
	1st Day	3rd Day	5th Day	1st Day	3rd Day	5th Day
Control	0.009	0.026	0.031[b]	0.008	0.023	0.030
CSO 100 mL/kg	0.015	0.027	0.035[a]	0.016	0.024	0.026
CSO 200 mL/kg	0.011	0.025	0.027[c]	0.006	0.023	0.025
SE	0.0018	0.0010	0.0011	0.002	0.002	0.002
P value	NS	NS	**	NS	NS	NS
Days		NS			NS	
Group x Days		NS			NS	

a, b: The column average is significantly different
SE: standard error, **: p<0.01 NS: Not significant

Table 7.The effect of dietary carrot seed oil on the color properties (L*, a* and b*) of legs and breast of the broilers.

Groups	Breast			Leg		
	Lightness (L*)	Redness (a*)	Yellowness (b*)	Lightness (L*)	Redness (a*)	Yellowness (b*)
Control	49.19[b]	4.93[a]	4.32[a]	52.01	6.89[a]	6.75
CSO 100 mL/kg	50.47[b]	3.39[b]	2.44[b]	55.47	3.50[b]	5.64
CSO 200 mL/kg	54.85[a]	5.10[a]	5.86[a]	48.44	6.33[a]	5.38
SE	0.95	0.34	0.57	1.67	0.53	0.348
P	**	*	*	NS	**	NS

a, b: The column average is significantly different
SE: standard error, *: p<0.05 **:p<0.01 NS: Not significant

DISCUSSION

Carrot seed oil supplementation did not change the feed intake and feed conversion ratio but significantly increased the body weight and body weight gain. To the best of our knowledge, there is no research conducted on the effects of carrot seed extract as dietary intake on the broilers, thus, the findings of this study may be the first in this field.

Erhan *et al*. (2012) found out that the dietary supplementation of pennyroyal reduced the FI and improved the FCR in broilers. In this study, dietary carrot seed oil (CSO) increased the BWG. Similarly, some other studies on broiler chickens (Al-Mashhadani, 2015; Hussein, 2013; Naderi *et al*., 2014) and Japanese quail (Çiftçi *et al*., 2016) discovered that the dietary supplementation of essential oils increases the weight gain. On the contrary, Erhan and Bölükbaşı (2017) found out that supporting the diet with citrus peel oil did not change the weight of the broilers. According to Babic *et al*., (1994); Yu *et al*., (2005), the obtained results in this study may be attributed to antioxidant and antimicrobial properties of CSO.

We may claim that the improvements in the BWG, which were observed in many studies, occurred because of the appetizer properties of plant extracts by increasing the gastric digestion liquor (William and Rosa, 2001) and forming more balanced intestine flora with their antimicrobial effects (Bölükbaşı and Erhan, 2007; Erhan *et al*., 2012).

CSO increased the weight of hot carcass and hot carcass yield, but did not affect the ratio of heart and liver of the chickens in this study.

Yarru *et al*., (2009) reported that thymol and turmeric powder did not affect the liver weight. However, Bölükbaşı *et al*., (2006) claimed that supplementation of thymol reduced the liver weight of broilers.

In the experiment, supplementation of CSO increased the number of lactic acid bacteria, however, did not change the number of E. coli bacteria. A series of studies were carried out in order to determine the effect of essential oils on gut microflora in small intestine and significant improvements on gut microflora have been observed in some of the studies (Namagirilakshmi, 2005; Bölükbaşı and Erhan, 2007; Bölükbaşı *et al*., 2009), while the improvements observed in the other studies were not significant (Demir *et al*., 2003; Cross *et al*., 2007; Kırkpınar *et al*., 2011). It was reported that supplementation of turmeric powder (Ürüşan and Bölükbaşı, 2017), pennyroyal (Erhan *et al*., 2012) and citrus peel oil (Erhan and Bölükbaşı, 2017) in the diet of broilers increased the ratios of lactic acid bacteria and decreased the ratio of E.coli in jejunum. Steenfeldt *et al*., (2007) reported that coliform and lactic acid bacteria count of small intestines were not influenced with the incorporation of carrot into diet of laying hens.

CSO supplement did not change the levels o f serum ALT, AST, glucose, TG, total cholesterol, LDL and HDL concentration. The activity of AST and ALT enzymes is an important indicator for liver injury. Moghadam *et al*., (2012) found out that 400ml/kg carrot seed extract significantly decreased aspartete aminotransferase (AST) in serum of rats. Moreover, they reported that daily treatment of carrot seed extract help remarkably regulate the biochemical status of rats.

Hamman (2014) found that ALT and AST were significantly increased by CCI_4, but 5ml/kg carrot juice + 1.25ml/kg CCl4 significantly decreased AST and ALT in serum of rabbits, which was attributed to the hepatoprotective activity of the carrot juice.

Supplementation of 200 mL/kg carrot seed oil per day significantly reduced the MDA value of leg tissue compared to the other groups on 5th day. It has been suggested that some essential oils may play a role as a dietary antioxidant in decreasing the lipid peroxidation in blood and tissues (Lado *et al*., 2004; Bölükbaşı *et al*., 2006; Chikhi *et al*., 2012). Many researchers have examined the antioxidant properties of carrot (Fuhrman *et al*., 2000; Yu *et al*., 2005; Sesso, 2006). In this study, it was determined that incorporating the CSO in the ration of the broiler chicks decreased the amount of the oxidation in legs on the 5th day. It is estimated that this decrease in the lipid oxidation rate is caused by carotenoid, which is the main component of CSO. It was reported that carrot seeds contain carotenoids which are high in natural antioxidants and pigments and carry lipophilic properties (Yu *et al*., 2005). Furthermore, Yu *et al*. (2005) stated that oxygen radical absorbance capacity of cold-press carrot seed oil was 160 µmol TE/g and it had 60 % DPPH -scavenging capacity.

The other important component of carrot seed oil is the antioxidant lemonene (Roberto *et al*., 2009). Çiftçi *et al*., (2016) observed that adding orange peel extract, which contains limonene, to the Japanese quail diet reduced the MDA levels in liver and heart tissues under cold-stressed conditions. Moghadam *et al*., (2012) found out that the addition of 200 and 400 mL/kg carrot seed extract in the diet significantly decreased the MDA level in liver tissue of rats.

Addition of 100 mL / kg of carrot seed oil decreased the a^* value in the breast and leg tissues, while 200 mL / kg of the carrot seed oil increased the L^* and b^* values of the breast tissue. It was reported that adding oregano oil to the broiler diet had no effect on breast meat (Lopez-Bote *et al.,*1998). Pirmohamammadi *et al*., (2016) found out that mentha and thyme powder did not change the redness and yellowness of broiler meat under heat stress.

In conclusion, addition of CSO to the basal diet increased the weight gain, hot carcass weight and carcass yield. An increase in the number of lactic acid bacteria in jejunum, L^* (lightness) and b^* (yellowness) values of the breast muscles have been observed in the group of chicks fed on 200 ml/kg CSO per day when compared to the other groups, and MDA values of the leg muscles decreased in chicks fed on 200 mL/kg CSO on the 5th day. As a result, carrot seed oil can be added in the diet of broilers as a beneficial dietary supplement which contains natural antioxidants.

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