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Citrus Peel Oils Supplementation in Broiler Diet: Effects on Performance, Jejunum Microflora and Jejunum Morphology

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Broiler, citrus peel oil, microflora, performance, villus.

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ABSTRACT

This study was carried out to determine citrus peel oils (orange, lemon, bergamot) as food supplementation in different levels on performance, microflora and morphology in broilers. The trial consisted of 1-day-old male and female Ross 308 chicks (250 male 250 female). The study consisted of 10 groups in total and each group was replicated five times. Experimental diets were prepared by adding orange, lemon and bergamot peel oil levels (1,2 and 3 mL/kg) to basal diet. It was observed that the supplementation of citrus peel oils to the diet improved significantly the feed conversion rate. Especially the lowest feed intake and the best feed conversion ratio were received from broilers which were fed 3 mL/kg of orange peel oil. The study shows that the count of *Escherichia coli* (*E.coli*) and lactic acid bacteria in the jejunum decreased significantly with the supplementation of bergamot peel oil to broilers diets. Citrus peel oil, in contrast to bergamot peel oil, significantly increased lactic acid bacteria count. The highest lactic acid bacteria count was determined in birds fed 3 mL/kg with orange peel oil. In the result of this study, both the length and density of villi and the density of blood and lymphatic capillary in the jejunum increased significantly by dietary 3 mL/kg orange peel oil supplementation. Finally, our results indicate that, especially 3 mL/kg orange peel oil has positive effects on performance, jejunum microflora, and jejunum morphology. It could be advised to supply broilers feed with 3 mL/kg orange peel oil as feed additives.

INTRODUCTION

In the present decade, antibiotics as an alternative in feed, has been researched by many scientists. It is known that the essential oils extracted from spices and herbs can be used as replacement for antibiotic feed additives. It is reported that herbal extracts or active substances may increase feed intake and may improve secretion of endogenous digestive enzymes (Craig, 1999; Jamroz *et al*., 2003). Besides that, it has been reported that essential oils have antibacterial effects in chickens (Cowan, 1999; Faleiro *et al*., 2003; Bölükbaşi *et al*., 2008, 2009; Giannenas *et al*., 2014; Skoufos *et al*., 2016).

Orange (*Citrus sinensis*), lemon (*Citrus lemon*) and bergamot (*Citrus bergamia*) aromatic herbs, are members of the Rutaceae family. By using a cold-pressing or steam distillation method, the essential oil is extracted from the peel of the fruit. Citrus essential oils contain high amounts of limonene and linalool. It is reported that the chemical composition of these oils was strongly influenced by harvesting time (Kırbaşlar *et al*., 2001; Zantar *et al*., 2015).

Some researchers 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 (Alçiçek *et al.,* 2003,2004).

It is reported 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 (Bozkurt*et al*., 2007).

It was determined that feed conversion rate significantly improved when laying hens were supplemented 1.5 mL/kg bergamot oil (citrus bergamia) in their feed (Bölükbaşı *et al*., 2010).

In some studies, different essential oils such as thyme, sage, rosemary and nigella sativa were seen to be more active against strains of *E. coli* in layers (Bölükbaşı *et al*., 2007, 2008, 2009) and broilers (Erhan *et al*., 2012). Jamroz *et al*. (2003) found that plant extracts decreased the number of *E. coli* and *Clostridium perfringens* in total in the intestines of broiler chickens.

Pourhossain *et al*. (2012) reported that dried citrus sinensis peel increased lactic acid bacteria count in ileum of broilers at 14 d.

Antioxidative and antimicrobial features of plant oils have been well documented by many researchers. However, a few studies have been conducted on supplementing diets with plant extracts to investigate the action mode of active plant oils, morphologically and histologically in animals (Hassan *et al*., 2011; Murakami *et al*., 2014). Therefore, the present study was carried out to determine the effects of citrus peel oils (bergamot, orange, lemon) in different levels on performance, jejunum morphology and intestinal microflora in broilers.

MATERIAL AND METHODS

Materials*:* Ross 308 broiler chickens were used in the study which were 1-day-old and 500 (250 male 250 female) in total. The chicks were randomly assigned to 10 treatment groups; five replicates with ten chicks in every cage. Ten treatments consisted of a control (basal diet), 1 mL/kg orange peel oil, 2 mL/kg orange peel oil, 3 mL/kg orange peel oil, 1 mL/kg lemon peel oil, 2 mL/kg lemon peel oil, 3 mL/kg lemon peel oil, 1 mL/kg bergamot peel oil, 2 mL/kg bergamot peel oil, 3 mL/kg bergamot peel oil (citrus oils were purchased from a commercial company, in Turkey).

Orange peel oil contains: 97.46% limonene, 1.98% beta-myrecene, 0.22% linalool. Bergamot peel oil contains: 35.66% Dl-limonene, 20.28% linalool, 20.89% linalyl acetate, 2.13% beta-terpinly acetate, 14.96% alpha terpinenly acetate. Lemon peel oil contains: 1.92% beta-myrecene, 95.28% limonene, 1.32% E-citral, 0.94% Z-citral). In Table 2, ingredients and chemical compositions of the basal diet are shown. Feed and water were given ad libitum for 42 days.

Experimental procedures: Body weights of the chickens were recorded on days 1 and 42, and feed intake was measured over this period on day 42. The research protocol was approved and conducted in accordance with the Animal Ethics Committee Guidelines of Atatürk University (protocol number: 2009/55).

Five birds selected randomly from each treatment were slaughtered (neck cutting). The carcasses were plucked and the heads, necks, shanks as well as feet were removed. The liver and heart were dissected from the viscera. The carcass yield was calculated.

The carcasses of the birds were opened, and 2 g of intestinal contents from jejunum were transferred to sterile plastic bags. All samples were processed within the same day for enumeration of microbial populations. The number of lactic acid bacteria and *E. coli* were determined by using the methods reported by Merck (1998) and Baumgart *et al.* (1986), respectively.

Dissecated jejunum was sent to the laboratory for histopathologic analyses by putting into the tubes with 10% formalin. Jejunum tissues were rapidly kept in 10% buffered formalin for 24–48 hours for histopathological examination. After fixation, each liver-tissue sample was then routinely processed and embedded in paraffin. After embedding, 5- µm sections were taken from the tissue´s blocks and then stained with hematoxylin-eosin (H&E). These sections were stained with H&E and all jejunums were examined by light microscopy for histological evaluation of the following parameters in Table 1(Pellegrino *et al.,* 1979).

To analyse the data, one-way analysis of variance (ANOVA) was applied by using the statistical package SPSS for Windows (SPSS, 1999).

Table 1 – Histopathological scoring system for tunica mucosa.

	Density Score (DS)		Lenght and Density Score (LDS)
Blood Capillaries and Lymphatic Capillaries	3: Very Dense	Villus	3: Very Long and Dense
	2: Dense		2: Long and Dense
	1: Rare		1: Short and Rare

Table 2 – Composition of experimental diets (g/kg).

Ingredient (g/kg)	0-14 d	15-21d	22-35 d	36-42 d
Corn	575.50	575.50	600.02	635.02
Soybean meal (CP 48%)	175.50	110.00	67.18	28.72
Full Fat Soy (CP 38%)	160.00	219.50	240.00	240.00
Chicken Meal (CP 60%)	35.00	35.00	35.00	35.00
Meat Bone Meal (CP 35%)	34.00	34.00	34.00	34.00
Vegetable Oils	4.00	10.00	13.63	17.18
Salt	1.80	1.70	2.00	2.00
Lysine	3.00	2.23	1.99	1.80
Methionine	2.00	1.37	1.22	1.02
Limestone	2.00	2.70	-	-
Vitamin premix[1]	2.00	2.00	2.00	2.00
Mineral premix[2]	1.50	1.50	1.50	1.50
Sodium Bicarbonate	1.50	1.50	1.46	1.76
Dicalcium Phosphate	2.20	2.50	-	-
Total	1000	1000	1000	1000
Calculated composition (%)				
Crude protein (%)	23.00	21.80	20.05	19.40
Crude oil (%)	8.03	9.79	10.50	11.00
Crude fiber(%)	4.22	4.41	4.43	4.45
Crude ash(%)	5.59	5.25	5.13	4.98
ME(kcal/kg)	3040	3200	3180	3241
Ca(%)	1.05	0.99	0.98	0.95
P(%)	0.56	0.53	0.53	0.51
Methionine	1.22	0.98	0.90	1.00
Lysine	1.50	1.30	1.20	1.10
Analyzed composition (%)				
Crude protein (%)	22.70	21.10	20.89	19.11
Crude oil(%)	8.13	10.10	10.20	11.91
Crude fiber(%)	3.96	4.20	4.22	4.23
Ash(%)	5.24	5.00	5.57	4.61
Dry matter(%)	88.94	90.31	89.84	89.82

[1] Per kg diet was supplemented with: 12 000 IU vitamin A, 3500 IU vitamin D3, 100 IU vitamin E, 3 mg vitamin K3, 2.5 mg vitamin B1, 6 mg vitamin B2, 25 mg niacin, 10 mg calcium D-pantothenate, 4 mg vitamin B6, 0.015 mg vitamin B12, 1.5 mg folic acid, 0.150 mg D-biotin, 100 mg vitamin C,0.450 mg choline chloride.

[2]Per kg diet was supplemented with: 100 mg Manganese, 25 mg Iron, 65 mg Zinc, 15 mg Copper., 0.25 mg Cobalt., 1 mgg Iodine, 0.2 mg Selenium.

RESULTS AND DISCUSSION

The growth performance, total feed intake, carcass yield, weight of liver and hearth of the broilers are shown in Table 3. The supplementation of citrus peel oil (bergamot, lemon, orange) in the diet had no effects on weight gain, carcass yield or weight of liver and hearth. But, total feed intake of the chicks ($p<0.05$) reduced significantly with bergamot, lemon and orange peel oil. Dietary citrus peel oil suplementation significantly ($p<0.01$) improved feed conversion rate. The best feed conversion rate was obtained in the group fed the diet containing 3 mL/kg orange peel oil.

The results of this study showed that supplementing the diet with citrus peel oil had no effect on gaining weight. In contrast to the present findings, Alçiçek *et al*. (2004) reported that adding a mixture of herbal essential oils to the diet, increased body weight gain of the broilers. Dietary supplementation of citrus peel oil significantly improved feed conversion rate and decreased feed intake. Similarly, Çabuk *et al*. (2006) reported that the feed conversion rate improved and feed intake was decreased by the mixture of herbal essential oil supplements in breeder flocks. Erhan *et al*. (2012) found that the supplementation of pennyroyal to broiler diet decreased feed intake and improved feed conversion ratio. They also say that pennyroyal antimicrobial substances may inhibit intestinal pathogenic organisms and improve digestion and absorption, thus, acting as a growth promoter. The improvement in feed conversion rate in this study was in accordance with results which were reported by Alçiçek *et al.* (2003). However, in contrary to these findings, Botsoglou *et al*. (2004), Demir *et al.* (2003), Eclache & Besson (2004) and Hernández *et al.* (2004) found that the addition of an essential oil isolated from oregano to

Table 3 – Effects of dietary supplementation of citrus peel oils on performance and some carcass characteristics of the broilers.

	Total feed intake (g)	Body weight gain (g)	Feed conversion rate (g:g)	Hot carcass yield (%)	Liver Weight (g)	Heart weight (g)
Control	3714.22^{a}	2067.94	1.80^{a}	77.20	57.2	11.6
1 mL/kg Bergamot	3553.41^{ab}	2205.40	1.61^{bc}	76.11	48.0	14.8
2 mL/kg Bergamot	3538.81^{b}	2148.99	1.65^{bc}	78.31	56.4	14.4
3 mL/kg Bergamot	3509.52^{b}	2081.63	1.69^{ab}	76.52	42.0	12.0
1 mL/kg Lemon	3560.70^{ab}	2144.87	1.66^{bc}	78.93	41.6	14.8
2 mL/kg Lemon	3528.71^{b}	2150.94	1.64^{bc}	74.66	53.2	16.8
3 mL/kg Lemon	3461.52^{b}	2092.22	1.66^{bc}	74.62	50.4	14.4
1 mL/kg Orange	3480.43^{b}	2146.26	1.62^{bc}	75.54	54.4	14.0
2 mL/kg Orange	3577.47^{b}	2256.29	1.59^{bc}	77.98	50.0	15.2
3 mL/kg Orange	3410.76^{b}	2249.95	1.52^{c}	77.45	46.0	14.4
SEM	18.54	16.45	0.02	0.42	1.44	0.50
P	0.03	0.83	0.01	0.83	0.126	0.55

$^{a, b, c}$: Column means with no common superscript differ significantly

SEM: standarderrorofmeans.

broiler diet, had no beneficial effect on feed conversion ratio. The reason of this can be the fact that the level of active ingredients of the citrus peel oil was different and it was added to the feed in different levels, and also the fact that different races were used.

There is no beneficial effect of the citrus peel oils in the liver and hearth weight of the broiler chicks in this study. Likewise, Lee *et al.* (2003) reported that thymol did not influence liver weight. This, however, contradicts the findings of Bölükbaşı *et al.* (2006) who reported that thymol resulted in reduced liver weight.

Broilers fed the diet with citrus peel oil had significantly ($p<0.05$) decreased *E. coli* in the jejunum (Table 4). 3 mL/kgbergamot and 1mL/kg lemon group had the lowest average number of *E. coli* in the jejunum.

Table 4 – Effects of dietary supplementation of citrus peel oils on microflora in jejunum of the broilers.

Group	*Escherichia coli* (MPN/g)	Lactobacillus bacteria (CFU/g)
Control	1100^{a}	57.5× 10^{2d}
1 mL/kgBergamot	673^{ab}	39.9× 10^{2e}
2 mL/kgBergamot	673^{ab}	12.4× 10^{2f}
3 mL/kgBergamot	460^{b}	6.9× 10^{2f}
1 mL/kgLemon	460^{b}	84.2× 10^{2c}
2 mL/kgLemon	1100^{a}	134.9× 10^{2b}
3 mL/kgLemon	886^{ab}	116.8× 10^{2b}
1 mL/kgOrange	886^{ab}	41.9× 10^{2e}
2 mL/kgOrange	1100^{a}	90.6× 10^{2c}
3 mL/kgOrange	886^{ab}	191.6× 10^{2a}
SEM	58.89	110.9
P	0.031	0.00

$^{a, b, c,d,e,f}$: Column means with no common superscript differ significantly

SEM: standarderrorofmeans.

The addition of orange and lemon peel oils to the broiler's diet led to a significant ($p<0.01$) increase in the lactic acid bacteria count. Supplementation of bergamot peel oil resulted in a lower lactic acid bacteria, 2 and 3 mL/kg bergamot group had the lowest average count of lactic acid bacteria in the jejunum, compared to other groups. The highest lactic acid bacteria count was determined in bird feed 3 mL/kg orange peel oil. Besides, feed conversion rate was the best in this group. Therefore, we can say that citrus peel oil improved feed conversion rate by increasing the number of lactic acid bacteria.

There are many studies that show that essential oils might inhibit pathogenic bacteria in the small intestine. It is reported that herbal extract has antimicrobial effect againts *E. coli* in poultry and pigs (Bölükbaşı *et al*., 2007, 2009; Bruggeman *et al*., 2002; Kamel 2001; Mitsch *et al.,* 2004). The results of trials, which those researchers conducted, fit the findings which we carried out. Erhan *et al.* (2012) found that pennyroyal supplementation in the diet of broilers, increased the lactic acid count in the jejunum, improved the microflora balance, and decreased the *E. coli* count. Therefore, in this study, increased Lactobacilli counts could be due to antibacterial effect of citrus peel oils. Similar to the results of our study, Tucker (2002) reported that blends of essential oils increased the number of lactic acid in broilers. But, some researchers reported that herbal dietary extracts (Demir *et al*., 2003) and essential oils (Cross *et al*., 2007; Kırkpınar *et al*., 2011) have no effect on the population of microorganisms of broilers. The reason of this, is that essential oil was added to the feed in different levels.

The density of blood and lymphatic capillaries; and the length and density of villus of jejunum are shown in Table 5. It is observed that the density score of blood

Table 5 – Effects of dietary supplementation of citrus peel oils on in jejunum morphology of the broilers.

Groups	Density score of blood capillaries*	Density score of lymphatic capillaries*	Density and length score of villus**	Total score
Control	2	2	2	6
1 mL/kg Bergamot	1	1	1	3
2 mL/kg Bergamot	2	2	3	7
3 mL/kg Bergamot	2	2	3	7
1 mL/kg Lemon	1	1	1	3
2 mL/kg Lemon	2	2	2	6
3 mL/kg Lemon	2	2	3	7
1 mL/kg Orange	1	1	1	3
2 mL/kg Orange	2	2	2	6
3 mL/kg Orange	3	3	3	9

*:1: rare 2: dense 3: very dense. **: 1;short and rare, 2; long and dense, 3; very long and very dense.

and lymphatic capillaries of the birds fed with the diet containing 3 mL/kg orange peel oil, was very dense. The density score of blood capillaries and lymphatic capillaries of the groups consuming 1mL/kg citrus peel oil (bergamot, lemon and orange) were determined as rare. The increase of nutrient absorbtion surface and the amount of nutrient absorbtion is related to the increase of the density of blood capillaries. However,

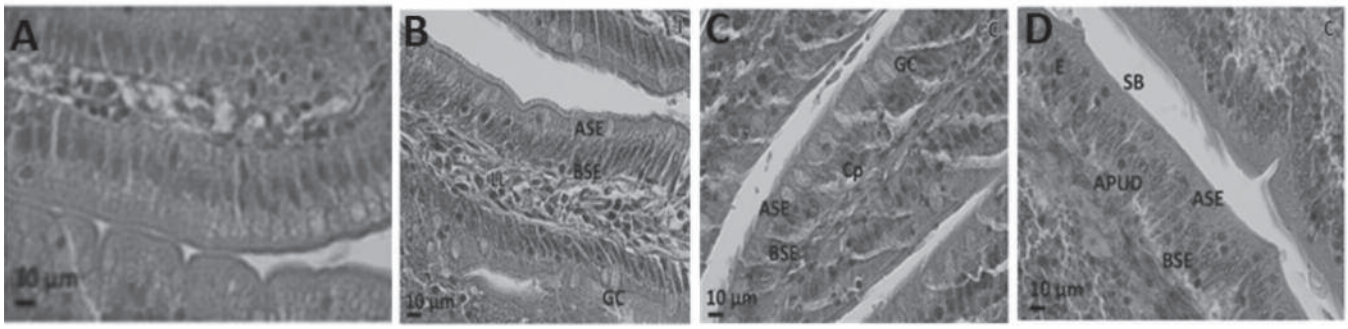

Figure 1 – Histopathological micrographs of control (A),1 mL/kg Bergamot (B),2 mL/kg Bergamot (C),3 mL/kg Bergamot (D) groups. ASE: Apical Side of Enterocytes, BSE: Basal Side of Enterocytes, GC: Goblet Cell, Cp: Capillary, SB: Striated Border, APUD Cell: Amine Precursor Uptake and Decarboxylation Cell.

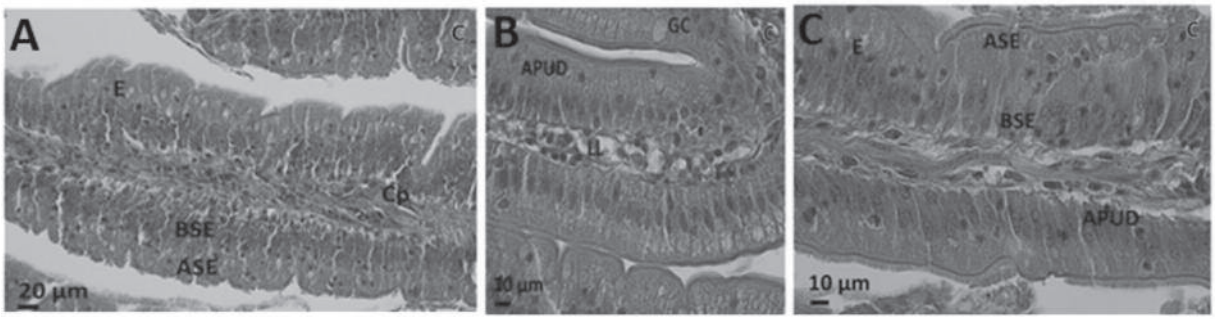

Figure 2 – Histopathological micrographs of 1 mL/kg Lemon (A), 2 mL/kg Lemon (B), 3 mL/kg Lemon (C) groups. ASE: Apical Side of Enterocytes, BSE: Basal Side of Enterocytes, GC: Goblet Cell, Cp: Capillary, SB: Striated Border, APUD Cell: Amine Precursor Uptake and Decarboxylation Cell.ASE: Apical Side of Enterocytes, BSE: Basal Side of Enterocytes, Cp: Capillary, APUD Cell: Amine Precursor Uptake and Decarboxylation Cell, GC: Goblet Cell.

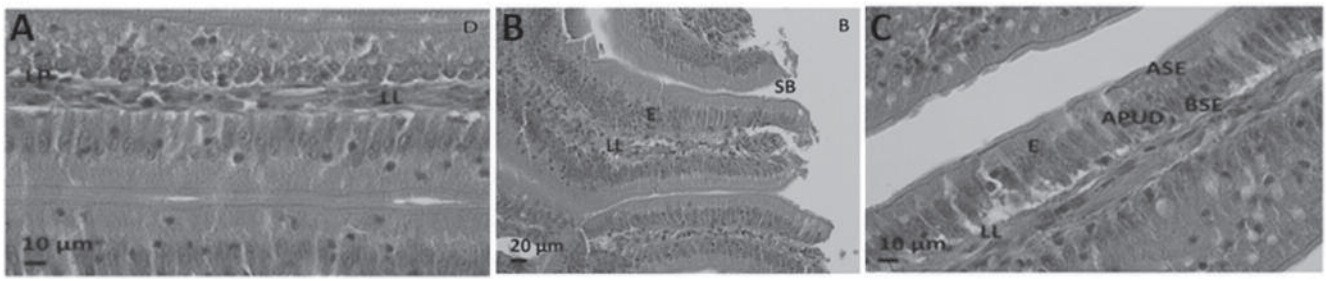

Figure 3 – Histopathological micrographs of 1 mL/kg Orange (A), 2 mL/kgOrange (B), 3 mL/kgOrange (C) groups. LP: Lamina Propria, LL: Lumen of Lacteal, E: Enterocyte, SB: Striated Border, ASE: Apical Side of Enterocytes, BSE: Basal Side of Enterocytes,APUD Cell: Amine Precursor Uptake and Decarboxylation Cell.

increasing the density of lymphatic capillaries causes the increase, both in the absortion surface and the amount of oil. The lowest density and length of villus was determined in birds fed with 1mL/kg of bergamot, lemon and orange peel oils. Density and length of villus of the control groups added 2mL/kg lemon and 2 mL/kg orange were found long and dense. Supplementation of broilers diets with a relatively high concentration of bergamot, lemon and orange peel oil (3mL/kg) increased the density and the length of the villus in the jejunum. The villi were found to be very long and dense in these groups. The increase of villi length and density is an indicator of increased intestinal absorbtion surface. Similar to our study, Hassan *et al*. (2011) found that the addition of 0.5 and 1% of essential oils to broilers diets, increased villus height significantly. Also, Murakami *et al.* (2014) reported that adding essential oil to the broiler´s ration increased villus height in the jejunum on day 14. In contrast, it was reported that supplementing essential oils to the broiler´s diets had no effect on villus height in the jejunum statistically, but it increased villus height numerically (Jamroz *et al.,* 2006). In this study, the performance values such as feed consumption and feed conversion ratio were determined to be best in the 3 mL/kg of orange peel oil, depending on the increase, both in the length and density of villi and the density of blood and lymphatic capillary. Some researchers reported that the morphological feature of villius was corresponded to the increase of feed intake and rapid growth rate of the broilers, showing a possible relationship between intestinal function and intestinal villus histological alterations (Yamauchi & Isshiki, 1991). It was reported that the microvilli might be protected by some components of spice plants. Intrinsic antioxidative activity, at both cell and tissue levels, have had effects on these functions of the intestinal mucosa layer (Rhodes 1996; Best 2000; Tschirch 2000). It was reported that reducing intestinal microbial load, by antimicrobial agents, caused decrease of toxins associated with negative changes in the intestinal morphology (Xu *et al*., 2003).

The highest total score was obtained from the group fed with the diet containing 3mL/kg orange peel oil. The supplementation 1 mL/kg level of bergamot, lemon and orange peel oil resulted in a lowest total score compared to the other groups.

Consequently, citrus peel oils had positive effects on the performance, intestinal micro flora and jejunum morphological properties. Supplementation of 3 mL/kg orange peel oil improved feed conversion rate and increased amount of lactic acid bacteria and villus dense in jejunum compared to other groups. Based on the results of this study, it can be recommended to supply broiler´s feed with 3 mL/kg orange peel oil.

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