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Research Article

Effects of Different Levels of Essential Oil Mixed (Peppermint-Thyme-Anise Oil) Supplementation in the Drinking Water on the Growth Performance, Carcass Traits and Histologic Structure of Terminal Ileum in Quails [1]

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Abstract

This study was conducted in order to define the effects of oregofarm (peppermint, thyme and anise oil) supplementation in the drinking water on the growth performance, carcass quality and histologic structure of terminal ileum in quails. A total of 348 Japanese quail chicks (*Coturnix coturnix japonica*) of both sexes were included in this study. They were divided into one control group and two experimental groups and each of them contained 116 Japanese quail chicks. Each group was further divided into four subgroups with 29 Japanese quail chicks. This study was finalized in six weeks. All groups were fed with basal diets and received fresh water during the experiment. The control group received non-supplemented water. The group 1 and 2 received 1.0 ml/5 L and 1.5 ml/5 L oil mixture, respectively. All experimental groups were fed with water and ad-libitum. As a result of the study, there were statistically significant differences between the feed consumption and efficiency ($P<0.001$) in the end of the three weeks. Similarly, there were also statistically significant differences between same parameters ($P<0.01$; 0.05) five weeks later. The body weights of quails were not significantly different from each other ($P>0.05$). At the end of the study, there were statistically differences in the warm and cold carcass parameters ($P<0.05$). Adding essential oil mixed were not affected on histological structure of terminal ileum ($P>0.05$). Conclusively, the supplementation of oregofarm (peppermint + thyme and anise oil) has no additional effect on quail performance.

Keywords: *Quail, Essential oil mixed, Performance, Carcass, Ileum*

İçme Suyuna Farklı Düzeylerde İlave Edilen Esansiyel Yağ Karışımının (Nane-Kekik-Anason Yağı) Bıldırcınlarda Büyüme Performansı, Karkas Parametreleri ve İleumun Histolojik Yapısı Üzerine Etkileri

Özet

Bu araştırma, bıldırcın içme suyuna oregofarm (nane-kekik-anason yağı) ilavesinin büyüme ve besi performansı, karkas özellikleri ve ileumun histolojik yapısı üzerine etkisini belirlemek amacıyla yapılmıştır. Araştırmada her iki cinsiyette toplam 348 adet Japon bıldırcın (*Coturnix coturnix japonica*) civcivi kullanılmıştır. Her grupta 116 civciv bulunan 1 kontrol 2 deneme grubu oluşturulmuştur. Gruplar kendi aralarında 4'erli alt gruba ayrılmıştır ve her alt grup 29 adet civcivden oluşturulmuştur. Deneme altı hafta sürdürülmüştür. Bütün deneme grupları temel rasyonla beslenmiştir ve deneme boyunca temiz içme suyu sağlanmıştır. Kontrol grubunun içme suyuna oregofarm ilavesi yapılmamıştır. 1. deneme grubu ve 2. deneme grubu içme suyuna sırasıyla 1.0 mg/5 L ve 1.5 mg/5 L. oregofarm ilavesi yapılmıştır. Tüm deneme gruplarına yem ve su ad-libitum olarak verilmiştir. Araştırma sonunda, yem tüketimi ve yemden yararlanma oranı bakımından 3. haftada istatistiksel açıdan önemli farklılıklar ($P<0.001$) gözlenmiş olup, benzer şekilde 5. haftada da aynı değerler bakımından farklılıklar tespit edilmiştir ($P<0.01$; 0.05). Denemede canlı ağırlıklar bakımından gruplar arasında istatistiksel olarak herhangi bir farklılık gözlenmemiştir ($P>0.05$). Denemenin sonunda gruplar arasında sıcak ve soğuk karkas parametreleri bakımından istatistiksel olarak farklılıklar bulunmuştur ($P<0.05$). Esansiyel yağ ilavesinin ileumun termal yapısı üzerine etkisi görülmemiştir ($P>0.05$). Sonuç olarak, bıldırcın içme suyuna oregofarm ilavesinin performansı artırıcı ilave bir etkisinin olmadığı tespit edilmiştir.

Anahtar sözcükler: *Bıldırcın, Esansiyel yağ karışımı, Performans, Karkas, İleum*

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INTRODUCTION

For several years, the dietary use of antibiotics has been extensively used throughout the world as growth promoters in animal feeds, particularly in poultry products [1]. However, many countries have banned the use of antibiotics in animal feed as an additive since their use may lead to the antibiotic resistant bacteria which are harmful to humans [2-4]. Therefore, nutritionists and production managers have to find alternatives which have potential to alleviate the problems related to the withdrawal of antibiotics from diets and reduce enteric diseases in the poultry [5,6]. Various studies have currently focused on the research on natural feed additives in animal diets such as antioxidant [7,8], anticoccidial [9,10] or antimicrobial [11,12] plants. Recently, food additives with plant origin such as essential oils, have received considerable attention since substitutes for antibiotics are being used as a growth promoters [13]. Consequently, some plants have attracted increasing interest since they have an alternative feed additivies (antimicrobial, antiparaziter, anticancer, antioxidant, antistress and appetizing) and they can replace antibiotic growth promoters [14].

Peppermint is a member of the Labiatae family and it is possibly originated from Eastern Asia. It is used widely in herbal medicine and it is believed to be beneficial particularly for the immune system. The most abundant constituent of peppermint is menthol, which has antibacterial features [15]. Peppermint is also rich source of polyphenolic compounds and hence, it could possess strong antioxidant properties [16,17]. Anise *(Pimpinella anisum* L.) is an annual aromatic plant belonging to the Apiaceae family. It has been used over the years due to its antioxidant [18], antimicrobial [19], antibacterial [20] and antifungal [21] properties. *Thymus vulgaris* L. (thyme) is also an aromatic plant belonging to Lamiaccea family. It has been a subject of considerable interest as a medicinal and therapeutic agent worldwide [22]. The pharmacological effect of thyme is attributed to carvacrol and thymol which are main bioactive components of thyme [23]. Thymol and carvacrol have considerable antimicrobial and antifungal activity [24,25]. Furthermore, they have also been reported to have antioxidant properties as well as antibacterial activity against a wide range of bacteria [26,27].

In recent years, there have been numerous studies about essential oil supplementation of dietary on poultry [28-30]. But as we know, there have not been any study supplementation of essential oil mixed in drinking water on poultry performance. The aim of the present study is to evaluate the effects of oregofarm (peppermint, thyme and anise oils) supplementation in drinking water of quails on the growth performance and carcass quality.

MATERIAL and METHODS

This research was conducted after the approval of Kafkas University Animal Testing Local Ethics Council (Approval Number: KAÜ-HADYEK/2012-53).

Japanese quails *(Coturnix coturnix japonica)* bred in the test unit of Kafkas University Research and Application Center. A total of 348 Japanese quail chicks were used in this study. They were divided into one control group and two experimental groups each containing 116 Japanese quail chicks. Each group was divided into four subgroups and each subgroup contained 29 Japanese quail chicks. All groups were fed *ad-libitum* with a 23% crude protein and 3080 kcal/kg metabolize energy diet. The quails received fresh water during the experiment. The control group of quails received non-supplemented water. The group 1 and 2 received 1.0 and 1.5 ml/5 L oil mixture, respectively. The active components of mixture is presented in *Table 1* (Oregofarm, FARMAVET AS). It contains peppermint, thyme and anise. The composition of basal diet is presented in *Table 2*. The experimental period lasted in 42 days. The ratios were isocaloric and isonutrigenos. Diets were formulated to meet the NRC [31] nutrient requirements.

Table 1. *The active components of the essential oil mixture*
Tablo 1. *Esansiyel yağ karışımının aktif bileşenleri*

Components (mg/kg)	
Thymol (thyme oil)	2000
B-phellanderene (thymeoil)	1300
Limonene (thyme oil+pepermint oil)	3525
B-pinene (thyme oil+pepermit oil)	1977
Carvacrol (oreganum oil)	8910
Linalool (oreganum oil)	3645
Anethole (anise oil)	10712
Menthole (pepermint oil)	6375

Table 2. *Composition and calculated analysis of basal diet*
Tablo 2. *Temel rasyonun bileşimi ve hesaplanan analiz değerleri*

Ingredients (%)		Values Analysed (%)	
Corn	50.62	Dry Matter	90.90
Soybean meal	42.16	Crude Protein	23.02
Oil	4.17	Crude Extract	6.56
Limestone	1.34	Crude Ash	3.37
Dicalcium phosphate	1.09	Crude Fiber	2.37
Salt	0.18	ME, kcal/kg**	3080.88
Vitamin-mineral prem.*	0.20	Ca	0.95
Sodium Bicarbonate	0.08	P	0.66
DL-methionine	0.14		
L-Lysine	0.02		
Total	100		

** Each 1 kg of Vit-min premix includes 20.000.000 IU Vit A, 3.000.000 IU Vit D_3, 25 g Vitamin E, 4 g Vitamin B_1, 8 g Vitamin B_2,2.5 g Vitamin B_6, 20 mg Vitamin B_{12}, 20 g Nicotinamid, 12 g Calcium-D-pantothenate, 200 g Choline Chloride, 50 g Mangane, 50 g Iron, 50 g Zinc, 10 g Copper, 0.8 g Iodine, 0.15 g Cobalt, 0.15 g Selenium; ** Calculated nutritional values*

Crude nutrients in feed and experimental ratio were analyzed using AOAC [32] method. The levels of metabolic energy were calculated by using the formula developed by TSE [33]. At beginning (0 day) and on the 7th 14th 21st, 28th, 35th and 42nd day of the study, the body weight and body weight gain of the quails were recorded. Meanwhile, the whole feed residues of the subgroups were weighed weekly in order to measure feed consumptions and feed conversion ratios.

Eleven male and eleven female birds from each subgroups, a total of 132 chicks were randomly selected and slaughtered in order to determine their carcass quality. Then, animals were gained again and warm carcass weight was recorded. Cold carcass weight was determined upon keeping the carcasses at +4ºC for 18 h. Cold carcass weight divided by the slaughter weight was calculated to determine cold carcass yield.

The ileums were taken from 6 animals of each group to examine histological. The tissues were fixed in alcohol formaldehyde then were passed series of solution which are used for routine histological process, and were embedded in paraffin. Sections taken from paraffin blocks were put on the slides and were stained with Hematoxylin Eosin (HE) and Periodic Acid Schiff (PAS) then slides were examined by light microscopy (Olympus BX-51) [34]. Length and width of villusses were measured by a micrometer and for this measurements were chosen 6 adjoining villusses at random per ileum. For villus length were taken into account between top point of ileal crypts and top of villus [35]. For width of villus was taken into account in the middle of the villus.

Statistical analyses were performed and the significance of the difference between the mean scores of the groups for body weight, body weight gain, feed consumption, feed conversion ratio, carcass weight and yield were determined by using the variance analysis method. Significant differences between the mean values of different treatments were determined by using the Duncan's multiple range tests. The statistical analysis was conducted by using the SPSS 16.0 (Inc. Chicago. IL. USA) program. For ileum samples One-Way ANOVA in PASW statistic 18 programe was used for statistical calculations.

RESULTS

The effects of drinking-water supplementation with peppermint+thyme and anise oils for 42 days on growth performance are shown in *Table 3*. The body weight of quail chicks was not significantly influenced by the essential oil supplementation (P>0.05). Body weight (BW), body weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) are shown *Table 4*. Feed consumption in control group which was not fed with essential oils was found to be higher compared to the feed consumption of experimental groups. Furthermore, the feed consumption was significantly affected by essential oils and it was found to be lower than the feed consumption of the control group (P<0.01; 0.001). In the fifth weeks, BWGs in group II were higher when compared to other experimental groups (P<0.01). When compared to other groups, feed conversion ratio was markedly low (P<0.05; 0.001) in the group II (1.5 ml/5 L oregofarm) during the 3rd and 5th weeks. The overall FCR, calculated for the 6 weeks, was significantly lower than the overall FCR of other experimental groups (P<0.05). As shown in *Table 5*, the drinking water supplementation with essential oils statistically affected the slaughter weight, warm and cold carcass (P<0.05).

It was observed that ileum tissues were histological similar in all groups *(Fig. 1, 2, 3)*. In *Table 6*, between groups, width and length of villusses were statistically determined to be similar in all groups (P>0.05).

DISCUSSION

According to our study, the oregofarm supplementation in the drinking water did not lead to statistically significant difference between experimental groups in terms of live body weights (P>0.05) *(Table 3)*. In terms of the results

Table 3. *Mean body weights of the groups (g)*
Tablo 3. *Gruplarda ortalama canlı ağırlıklar (g)*

Weeks	n	Control $X \pm S_x$	n	Group I $X \pm S_x$	n	Group II $X \pm S_x$	P
0	116	8.41±0.08	116	8.38±0.08	116	8.38±0.07	-
1	108	14.13±0.31	107	14.07±0.34	106	13.13±0.30	-
2	100	32.43±0.94	98	33.61±0.91	100	32.30±0.89	-
3	98	65.51±1.44	95	65.78±1.48	93	65.34±1.47	-
4	97	101.33±1.92	94	100.78±1.94	91	102.98±1.87	-
5	97	140.23±2.28	94	136.71±2.37	91	142.29±2.38	-
6	96	158.88±2.48	94	155.31±2.44	91	159.27±2.77	-

-: Differences among the groups were not statistically significant (P>0.05)

Table 4. *Mean feed consumption, weekly body weight gain and feed conversion rate* values of groups*
Tablo 4. *Gruplarda ortalama yem tüketimi, haftalık canlı ağırlık artışı ve yemden yararlanma oranı**

Weeks	Parameters	Control $X \pm S_x$	Group I $X \pm S_x$	Group II $X \pm S_x$	P
1	FC (g/chick) BWG (g) FCR	10.01±0.23 5.73±0.39 1.77±0.11	10.12±0.45 5.69±0.17 1.78±0.07	10.19±0.18 4.87±0.36 2.12±0.14	- - -
2	FC (g/chick) BWG (g) FCR	48.43±1.47 18.19±0.56 2.67±0.10	49.43±3.12 19.14±0.56 2.58±0.11	44.51±3.03 20.39±2.01 2.77±0.33	- - -
3	FC (g/chick) BWG (g) FCR	94.59±1.46^{a} 32.76±0.40 2.89±0.02^{a}	93.81±1.22^{a} 32.13±0.70 2.92±0.05^{a}	74.12±1.64^{b} 32.16±0.71 2.31±0.06^{b}	*** - ***
4	FC (g/chick) BWG (g) FCR	120.34±1.02 36.46±0.83 3.50±0.11	117.71±4.63 35.22±0.94 3.34±0.12	112.09±1.91 36.27±0.45 3.09±0.06	- - *
5	FC (g/chick) BWG (g) FCR	157.51±6.10^{a} 39.27±0.24^{ab} 4.01±0.14^{a}	141.99±1.50^{b} 35.98±0.80^{b} 3.95±0.08^{b}	144.17±2.32^{b} 40.46±1.89^{a} 3.58±0.12^{b}	** * *
6	FC (g/chick) BWG (g) FCR	141.16±1.01 18.72 ±1.42 7.80±0.66	139.72±4.94 18.35±1.24 7.72±0.57	137.91±1.81 20.77±4.30 7.32±1.10	- - -
1-6	FC (g/chick) BWG (g) FCR	574.04±8.15^{a} 150.44±3.02 3.82±0.09^{a}	552.77±12.12^{a} 146.94±1.67 3.76±0.05^{a}	522.97±3.25^{b} 155.34±6.89 3.38±0.13^{b}	** - *

a,b: *Mean values on the same row followed by different letters change significantly (P<0.05, 0.01, 0.001; respectively);* ***-:*** *Differences among the groups were not statistically significant (P>0.05);* ******* *(kg, feed consumption/kg, body weight gain);* ***FC:*** *Feed Consumption,* ***BWG:*** *Body Weight Gain,* ***FCR:*** *Feed Conversion Rate*

Table 5. *Mean slaughter weight , carcass weight and carcass yields of groups*
Tablo 5. *Grupların ortalama kesim ve karkas ağırlıkları ile karkas randımanları*

Parameters	Control $X \pm S_x$	Group I $X \pm S_x$	Group II $X \pm S_x$	P
Slaughter weight (g)	153.89±3.68^{a}	141.62±3.51^{b}	143.75±3.90^{ab}	*
Warm carcass (g)	114.04±2.45^{a}	105.26±2.59^{b}	106.40±2.52^{b}	*
Cold carcass (g)	111.80±2.43^{a}	102.87±2.54^{b}	103.74±2.47^{b}	*
Warm carcass percentage (%)	74.32±0.01	74.39±0.01	74.40±0.01	-
Cold carcass percentage (%)	72.84±0.01	72.67±0.01	72.53±0.01	-

a,b: *Mean values on the same row followed by different letters change significantly (P<0.05);* ***-:*** *Differences among the groups were not statistically significant (P>0.05)*

Table 6. *The effect of different levels essential oil mixed on histologic structure of terminal ileum in Japonese quails*
Tablo 6. *Japon bıldırcınlarında farklı düzeylerde essensiyel yağ karışımı ilavesinin ileumun termal yapısı üzerine etkisi*

Treatment	n	Villus Length (µm)
Control ($X \pm S_x$)	36	315.72±61.50
Group I ($X \pm S_x$)	36	298.00±43.49
Group II ($X \pm S_x$)	36	311.27±56.54
SEM		0.359
Treatment	n	Villus Width (µm)
Control ($X \pm S_x$)	36	84.47±15.40
Group I ($X \pm S_x$)	36	83.41±14.64
Group II ($X \pm S_x$)	36	79.77±13.69
SEM		0.362

Differences among the groups were not statistically significant (P>0.05)

of the live body weight gain, there was a statistically significant difference between the groups in the 5th week (P<0.05). These findings are different from the results of the study performed by Biricik et al.[13] in which they examined the effects of essential oil supplementation in the quail diets on the live body weight. However, live body weight gain results were similar to each other. In the study conducted by Parlat et al.[36] in which they searched the effects of the supplementation of virginiamycine and thyme essential oils in the diet. According to their results, the best results were obtained from quail groups which were fed with thyme essential oils. Denli et al.[37] performed a study with Japan quails and it has been shown that the thyme essential oil supplementation enhanced live body weight as well as live body weight gain according to the control group. The supplementation of the thyme + anise and rosemary extracts was shown by Tucker et al.[38]

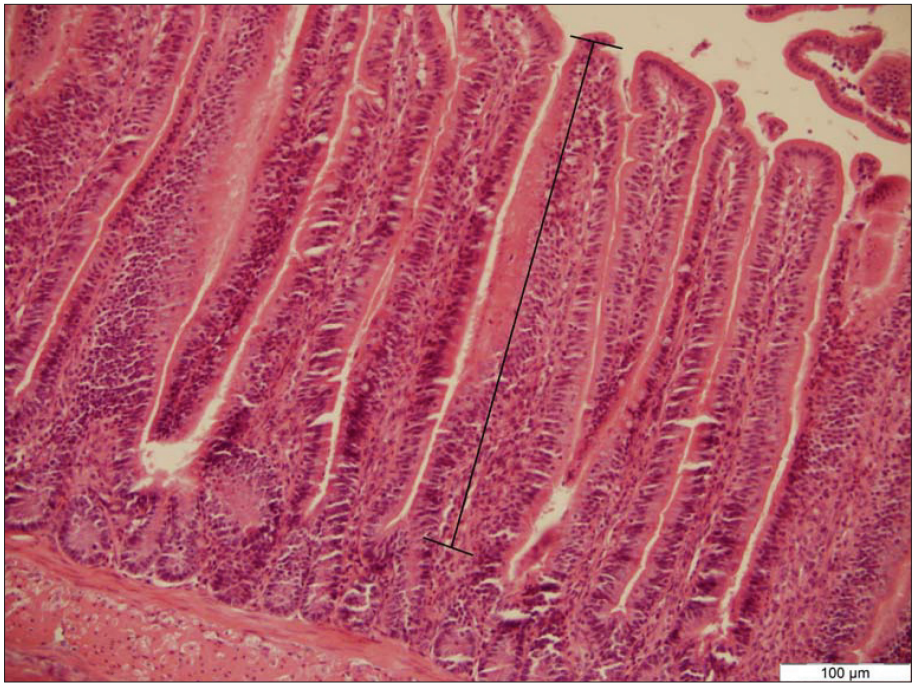
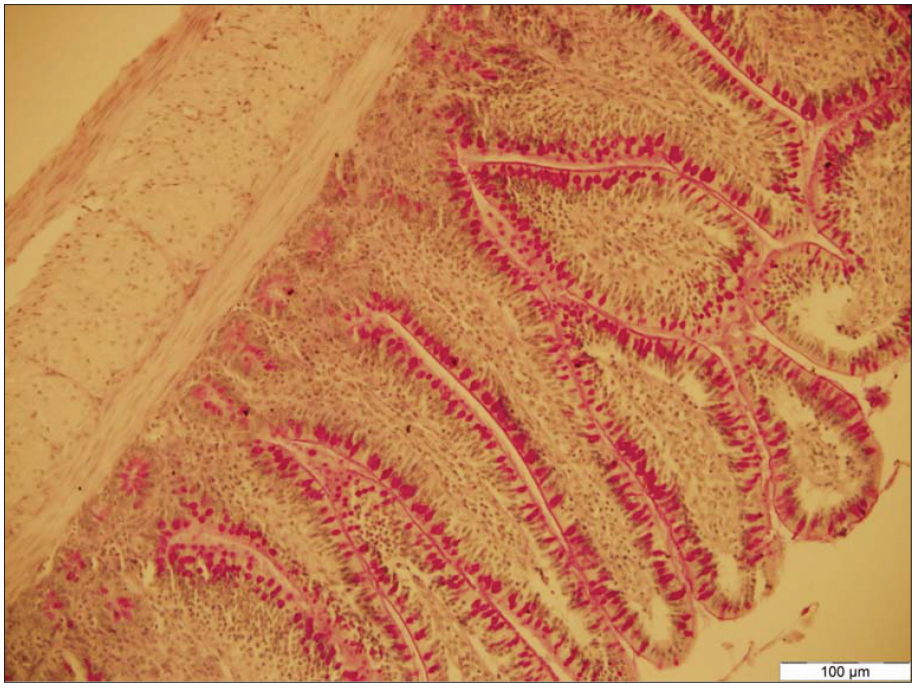


Fig 1. Villusses could be observed in group 1. HE, X20. Bar 100 µm

Şekil 1. Grup 1'de villusların görünümü. HE, X20. Bar 100 µm

Fig 2. Vilusses and goblet cells could be observed in group 2. PAS, X20. Bar 100 µm

Şekil 2. Grup 2'de villusların ve kadeh hücrelerinin görünümü. PAS, X20. Bar 100 µm



that they increased the live body weight of broilers and decrease the death rate. Celik [39] have indicated that the different amounts of essential oil mixtures (mint + juniper + rosemary + thyme) supplemented in the diet of broilers did not affect the live body weight whereas enhanced the death rate. These results are similar to the results of our study.

In a study, different doses of anise essential oil as an alternative to antibiotics were supplemented in the diet of broilers and it has been shown that the maximum live body weight was reached when the maximum dose of anise oil was supplemented. Therefore, they stated that anise oil can be used as a growth factor [40]. When the thyme oil was added to the diet as an alternative to antibiotics, the maximum live body weight was obtained in a group in which the thyme oil and antibiotics were supplemented together [41]. The differences between the studies can be due to the different species of animals, different usage of the extracts as well as the diets.

When the feed consumption and the rate of the feed of groups were assessed, it has been seen that there was a significant differences between groups starting from the 3rd

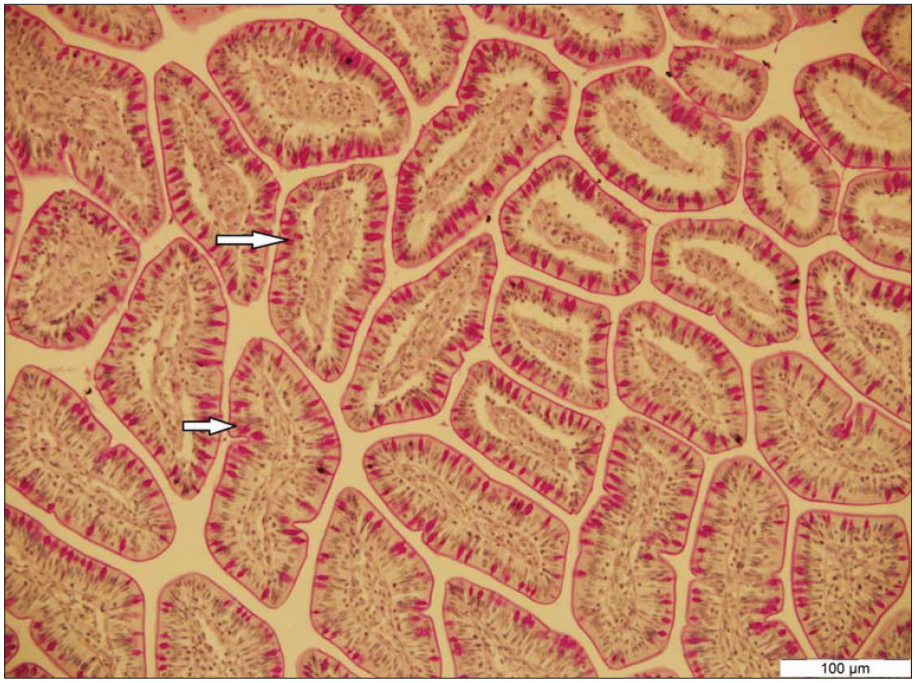


Fig 3. Cross-section of villusses, and goblet cells *(arrows)* could be observed in control group. PAS, X20. Bar 100 µm

Şekil 3. Kontrol grubu villusların enine kesiti ve kadeh hücrelerinin *(oklar)* görünümü. PAS, X20. Bar 100 µm

week (P<0.001; 0.01) *(Table 4)*. It has been detected that the feed consumption decreased with the supplementation of the mint, thyme and anise essential oil mixture in the drinking water. Harnandez et al.[42] have reported that the different two plant extract mixtures did not have a prominent effect on the feed consumption and the rate of feed. Similarly, Tucker et al.[38] have specified that the thyme and mint oil mixtures did not have an impact on the rate of feed of the broilers. Biricik et al.[13] performed a study in which they have indicated that essential oil supplementation in quail diet did not influence statistically the feed consumption. Our results are not consistent with these findings. According to the study performed by Halle et al.[43] thyme and thyme essential oil supplementation in the diets of broilers decreased the feed consumption and the essential oil prominently enhanced the rate of feed. Our study is in agreement with the study obtained by Ghazaghi et al.[44] in which they worked with quails. Similarly, Sengün et al.[45] added thyme extract in the diet of quails and they observed the improvement in the rate of feed.

When the quails were evaluated about their carcass characteristics, there was a statistically significant difference between experimental and control groups in terms of slaughter, hot and cold carcass weights (P<0.05) *(Table 5)*. According to Ghazaghi et al.[44] there was no difference between the quail groups in terms of their slaughter weights. There was no significant difference between groups related to their hot and cold carcass yields (P>0.05). Likewise, Biricik et al.[13] have reported that the essential oil supplementation in the diet of quails did not influence the hot and cold carcass yields. Because of these differences, due to adding different essential oil composition and added shape.

The length and the width of villus and villus goblet cell number are shown figures 1 to 3. It has been observed that there was no statistically significant difference between the experimental and the control groups related to the length and the width of the villus when the essential oil mixed are added to the drinking water of quails (P>0.05) *(Table 6)*. Our results are similar to the results of Cabuk et al.[46]. In contrast to the present result, Denli et al.[37] reported that including thyme and black seed essential oil in the diet increases intestinal weight and intestinal length in quails. These differences, due to adding different essential oil composition and added shape.

Conclusively, essential oils or their mixtures have been supplemented in the diet in studies have been performed till now. In these studies, it has been aimed to examine their effects by adding them in the drinking water of animals. It has been shown that the addition of the essential oil mixtures in the drinking water of the quails decreases the feed consumption, enhances the rate of feed, and decreases the death rate. The supplementation do not have so much effect on live body weight and carcass yield whereas it has positive and prominent effects on the digestive system under the poor environmental conditions and malnutrition. Thus, further studies should be performed with different doses and under different animal husbandry systems.

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