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The Effects of *Enterococcus Faecium* and *Origanum Onites* Powder Supplementation to Diet on Growth, Gut Health, Intestinal Morphology, Meat Traits and Breast Meat MDA of Male Broiler Chicks*

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ABSTRACT

This study evaluated growth, gut health and meat traits effectiveness of *Enterococcus faecium* (EF) and *Origanum onites* powder (OOP) on male broilers. A total of 160 (ROSS 308) one day old male broiler chicks randomly distributed to four dietary treatments; basal diet (T_0), basal diet + 0.2% EF (T_1), basal diet + 0.5% OOP (T_2), basal diet + 1% OOP (T_3). BWG was higher in T_1 and T_2 groups than Control T_0 group (P<0.05). FI, FCR, mortality, internal organs, meat traits, breast meat MDA levels and LAB count did not change among the treatments. Villi length increased in T_1 and T_2 groups than control T_0 group (P<0.001) in jejunum and ileum. Crypt depth increased in T_2 and T_3 groups than T_0 and T_1 groups in jejunum and crypt depth increased T_2 group than compared to the other groups in ileum (P<0.001). Villi leng crypt depth ratio was found higher in T_1 group compared to the other groups in jejunum and ileum (P<0.001). *Enterococcus* sp. count increased in T_1 group and *E. coli* count decreased in T_2 group compared to other groups in cecum (P<0.05), Lactobacillus count was not differ among the groups (P>0,05). Gizzard weight was foun low in T_1 group than T_0 and T_2 groups (P<0.05). L* value of thigh meat increased in T_3 group than control T_0 group. The results of study showed that 0.5% OOP supplementation (T_2) improved the performance values like probiotic *Enterococcus faecium* supplementation (T_1) and have a potential as growth promoter in broilers. However, studies are needed to reveal the effects of OOP supplementation at different doses or under different stress factors.

*All animal care and experimental procedures were conducted following the guidelines of the Kırşehir Ahi Evran University Local Ethics Committee for Animal Experiments. 31.03.2021-68429034/06.

Enterococcus Faecium ve *Origanum Onites* Tozu İlavesinin Erkek Etlik Civcivlerde Büyüme, Bağırsak Sağlığı, Bağırsak Morfolojisi, Et Özellikleri ve Göğüs Eti MDA Düzeyleri Üzerine Etkileri

Araştırma Makalesi

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ÖZ

Bu çalışmada, erkek etlik piliçlerde *Enterococcus faecium* (EF) ve *Origanum onites* tozunun (OOP) büyüme, bağırsak sağlığı ve et özellikleri üzerine olan etkinlikleri değerlendirildi. Toplam 160 (ROSS 308) bir günlük erkek piliç civciv rastgele dört muamele uygulamasına dağıtıldı. Muameleler; bazal diyet (T_0), bazal diyet + %0,2 EF (T_1), bazal diyet + %0,5 OOP (T_2), bazal diyet + %1 OOP (T_3) olacak şekilde planlanmıştır. Canlı ağırlık artışı (CAA), T_1 ve T_2 gruplarında Kontrol (T_0) grubuna göre daha yüksek olarak saptanmıştır (P<0,05). Muameleler arasında yem tüketimi, yemden yararlanma oranı, ölüm oranı, iç organlar, et özellikleri, göğüs eti MDA seviyeleri ve LAB sayıları ise farklılık saptanmamıştır (P>0,05). Jejunum ve ileumda, Kontrol (T_0) grubuna göre T_1 ve T_2 gruplarında villi uzunluğu artmıştır (P<0.001). Kript derinliği jejunumda T_2 ve T_3 gruplarında T_0 ve T_1 gruplarına göre, ileumda ise T_2 grubunda diğer gruplara göre daha yüksek bulunmuştur (P<0,001).Villi uzunluğu/kript derinliği oranı jejunumda ve ileumda T_1 grubunda diğer gruplara göre daha yüksek bulunmuştur (P<0,001). Sekumda T_1 grubunda Enterokokkus sayısı artmış, T_2 grubunda *E. coli* sayısı azalmış (P<0,05), Laktobasilli sayıları bakımından ise bir farklılık oluşmamıştır (P>0,05). Taşlık ağırlığı T_1 grubunda, T_0 ve T_2 gruplarına göre daha düşük bulunmuştur (P<0,05). But etinin L* değeri, T_3 grubunda kontrol grubuna göre artmıştır. Çalışmanın sonuçları, %0,5 OOP takviyesinin (T_2) probiyotik (*Enterococcus faecium)* takviyesi (T_1) ile benzer şekilde performans değerlerini iyileştirdiğini ve piliçlerde büyümeyi destekleyici potansiyele sahip olduğunu göstermiştir. Ancak farklı dozlarda OOP ilavesinin veya farklı stres faktörleri altında etkilerinin ortaya konulmasına yönelik çalışmalara da ihtiyaç duyulmaktadır.

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Introduction

Origanum onites is a medicinal aromatic plant in the genus of Origanum, which grows up in Turkiye, Greece and Sicily (Wikipedia, 2023). Plant height is 40-45 cm; leaves contain 1.88-3.06 essential oils; carvacrol is the main component of essential oils, leaves contain varying amounts of cineol, borneol, linalool and γ- terpinene; they have antioxidant (Özkan and Erdoğan, 2011; Yılmaz et al., 2019) and antimicrobial activities (Kacar et al., 2006; Sarac and Ugur, 2008). Despite these benefits, the number of studies using Origanum onites (OO) essential oils as a phytobiotic in poultry is finite. In these studies, Sarıca et al., (2014) showed that 500 mg/kg dietary OO essential oil prevented a performance decrease in 14 day old broilers fasting for 72 hours. Also Corduk et al., (2013) reported that 250 mg/kg OO essential oil supplementation prevented a performance decrease in 21 day old broilers fasting for 48 hours. Sarıca et al., (2018), reported that 250 mg/kg OO essential oil supplementation

increased villi length, jejunal and ileal crypt depth in 14 day old broiler chicks fasting for 48 hours. There are also studies reporting that 500mg/kg (Basmacıoğlu et al. 2010), and 300 mg/kg (Sarica et al. 2009; Kırkpınar et al. 2011) dietary OO essential oil supplementation did not affect broiler performance parameters. In previous studies, only OO essential oil was used as a phytobiotic in poultry, and there is no study in which the effects of Origanum onites powder (OOP) on performance, gut morphology and meat characteristics of broilers were determined. Moreover, besides determining the effect on performance increase, no study was found in which the possible effect was compared with proven commercial probiotics. Probiotics have proven in previous scientific studies that they can be an alternative to antibiotics in poultry. One of these probiotics is *Enterococcus faecium* (EF). It was shown that the dietary supplementation of EF to broiler diets improves performance (Samli et al. 2007; Castañeda et al. 2020; Bassiony et al. 2021; Simonová et al. 2022). Therefore this study aimed to evaluate the impacts of dietary 0.2% EF and 0.5-1% OOP on growth, internal organ development, intestinal histology, cecum microbiota, meat traits and breast meat MDA of male broilers for 21 days.

Material and Methods

Ethical approval

All animal care and experimental procedures were conducted following the guidelines of the Kırşehir Ahi Evran University Local Ethics Committee for Animal Experiments. 31.03.2021-68429034/06.

Animal, Diet and Management

In the study, one day old 160 healthy ROSS 308 male broiler chicks purchased from a commercial hatchery (Ross Breeders Anadolu, Ankara, Türkiye) with standardized live weights were placed in 4 flour chick rearing cages in an experimental room. The study distributed broiler chicks to treatments with 4 replicates (10 chicks). Treatment groups were T_0) as control, T_1) basal diet + 0.2% EF as positive control, T_2) basal diet + 0.5% OOP, T_3) basal diet + 1% OOP. Cages (100cmx50cmx40cm) have wire floors, feeders and nipple drinkers. Feed and water were offered *ad-libitum* and a 23-hour lighting program was applied throughout the experiment. Room temperature set up 32 ^{0}C for 1 week and 2 ^{0}C reducing every week. The experiment was continued for 21 days. No vaccination was applied to the birds. During the study, broiler starter diets with 22.39% CP and 3080 kcal/kg ME were purchased from a commercial feed company in Kayseri, Türkiye (Table 1).

Table 1. Ingredients and compositions of starter diet (g/100g).

Ingredients	%
Corn	44.00
Soy bean meal (44%)	41.15
Meat and bone meal (35%)	4.00
Soy oil	6.50
DCP	2.50
L-lysine HCl	0.70
DL-methionine	0.35
Salt	0.30
Vitamin premix*	0.25
Mineral premix#	0.25
Composition	
ME [kcal/kg]	3080
Crude protein	22.39
Crude fibre	2.80
Ether extract	8.50
Ca	1.60
Available P	3.80

* Vitamin A, 12.000 IU; Vitamin D_3, 2.400 IU; Vitamin E, 4 mg; Vitamin B_1, 30 mg; Vitamin K_3, 7 mg; Vitamin B_6, 3 mg; Vitamin B_2, 15 µg; Niacin, 5 mg; Vitamin B_{12}, 25 mg; Fe, 1 mg; Pantotenic acid, 80 mg; Folic acid,10 mg; Biotin, 125000 mg; Cu, 45 mg; Colin, 5 mg; Mn, 60 mg; Se, 150 µg.80 mg; Zn, 60 mg.

Live weight, feed intake and mortality were recorded weekly. On the 21st day of the study, 2 chicks were slaughtered from each replicate close to the group average weight and their internal organ weights were determined. On the day of slaughter, the cecum contents of slaughtered animals from each replicate were taken and *Enterococcus* spp., LAB, *E. coli* were detected same day in these samples. The lightness (L*), redness (a*), and yellowness (b*) of breast and thigh meat were measured with a Minolta Chrometer CR410. pH values of breast and thigh meat were measured by pH meter Testo 205 at slaughtered birds. Breast meat samples collected from each slaughtered birds for MDA analyses in a refrigator for 3 days.

This study used the probiotic *Enterococcus faecium* NCIMB 10415 (DSM Nutritional Products Ltd., Birsfelden, Switzerland CYLACTIN® LBC ME10). *Origanum onites* were provided by a local herbalist in Kırşehir Province. For the trial, *Origanum onites* were ground at a thickness of 0.1 mm in a mill.

Determination of TBARS Value in Breast Meat of Broiler Chicks

The lipid oxidation level in the samples was determined using the 2-thiobarbituric acid method. A sample of 10 grams was taken from the samples, 50 ml of distilled water at 50^0C was added and homogenized for 2 minutes in ultraturrax (IKA-T18). The mixture was taken into the tubes of the distillation machine, 47.5 ml of more pure water was added, and 2.5 ml

of HCL (4 N) solution was added and paraffin was placed to prevent foaming, and boiling stones to facilitate boiling were placed in the distillation device. The distillation apparatus was adjusted for low steam power, and distillation was continued until 50 ml of distillate was collected. 5 ml of the total distillate was taken into flasks, and 5 ml of TBA reagent was added. Blanks were prepared by mixing 5 ml of distilled water and 5 ml of TBA reagent. The samples, which were thoroughly mixed by vortexing, were kept in a boiling water bath for 35 minutes, after being removed from the water bath, they were cooled in water for 10 minutes and readings were made against the blind at a wavelength of 538 nm in the spectrophotometer (Shimadzu UVmini-1240). The obtained absorbance value was multiplied by 7.8 and the result was found to be mg malondialdehyde (MDA) per kg of sample (Tarladgis et al. 1960).

Determination of Duodenum, Jejunum, and Ileum Histology

On the 21st day of the study, duodenum, jejunum and ileum samples taken from slaughtered animals in each treatment group were placed in 10% formaldehyde. In the histology analysis of the study, paraffin blocks were prepared and the samples were cut with a thickness of 5 microns and the tissues were adhered to the slide. The tissues on the slide were freed from paraffin by passing them through xylene, and then they were passed through alcohol and xylene was removed from the tissues. The cleaned tissue samples were stained with hematoxylin and eosin dye and photographed with a digital camera microscope equipped with an AxioCam ERc 5s camera (ZEISS Primo Star, Germany) for proper imaging. The photographs obtained for each treatment group and each sample were measured with the ZEN 2012 SP2 image processing and analysis program.

Cecum Microbiology

One g of cecal samples in 9 ml in peptone water was mixed with a vortex to obtain a homogeneous distribution. Serial dilutions (1/10) were prepared at specific ratios from the stock materials. MRS agar was used for lactic acid bacteria (MERCK, 1.10660) at 37^0C degrees following 3 days in an incubator and BEA (Bile Aesculin Azide Agar) Agar (Merck 100072 was used for *Enterococcus spp*. at 37^0C degrees following 3 days in an incubator. EMB (Eosin Methylene Blue) Agar (Merck 101347) was used for *E. coli* at 37^0C for 2 days in an incubator. The microbial colonies were counted and expressed as $\log_{10}$ colony forming units (CFU) per gram of caecal content.

Statistical Analysis

The analysis of the data obtained from the study was carried out with one-way analysis of variance (ANOVA). The differences between the means were subjected to the Duncan multiple comparison test and the results were recorded. Statistical analyses in the study were made using SPSS 15.0 (SPSS Inc., Chicago, IL, USA) for Windows evaluation version statistical package program.

Results

Growth Performance

In this study, dietary 0.2% EF (T_1) and 0.5% OOP (T_2) supplementation increased live weight gain according to the control (T_0) group (P<0.05). Feed consumption, feed conversion ratio (FCR) and mortality were not differed between the groups. Dietary supplementation of *Enterococcus faecium* and *Origanum onites* to male broiler chicks resulted in a numerical decrease or improvement in FCR compared to control group (Table 2).

Table 2. Differentiation of growth, feed intake, feed conversion ratio (FCR) with the supplementation of *Enterococcus faecium* and *Origanum onites* powder to 21 day old male broiler diets.

	Treatments					
Parameters	T_0	T_1	T_2	T_3	SEM	P value
LWG g/bird	615.76[b]	660.66[a]	650.09[a]	641.06[ab]	6.01	0.03
Feed intake g/bird	923.20	926.13	935.10	912.85	7.44	0.81
FCR	1.50	1.40	1.44	1.43	0.016	0.19

[a-b]. Superscripts represent the degree of significance of values between inline means for each variable (P<0.05). LWG= Live weight gain SEM= Standard error of means; T_0= Control basal diet; T_1= 0.2% EF; T_2=0.5% OOP; T_3= 1% OOP.

Gut Histology

The effects of dietary EF (T_1) and OOP (T_2, T_3) on intestinal histology were shown in Table 3. Dietary supplementation of OOP and EF to broiler diets had no effect on duodenum morphological parameters (P>0.05).

In jejunum, the villi length was higher in T_1 and T_2 groups than control (T_0) group (P<0.001). Especially, T_1 group's villi length was higher than all groups. Dietary supplementation of 0.5% OOP (T_2) and 1% OOP (T_3) increased the crypt depth than those of control (T_0) and EF (T_1) group. The villi length/crypt depth ratio of the EF (T_1) group was higher than all groups.

In ileum, the villi lengths were higher in T_1 and T_2 groups than control (T_0) and T_3 groups. Crypt depth was the highest in the T_2 group compared to the other treatments. The

villi length/crypt depth ratio was higher in the T_1 and T_3 groups compared to control group, and the highest level was found in T_1 group (P<0.001).

Table 3. Differentiation of intestinal morphology with the supplementation of *Enterococcus faecium* and *Origanum onites* powder to 21 day old male broiler diets.

Groups	T_0	T_1	T_2	T_3	SEM	P value
			Duodenum			
Villi length (µ)	1095.35	1118.15	1117.55	1118.54	17.69	0.960
Crypt depth (µ)	64.50	66.56	67.77	68.38	1.32	0.747
VL/CD	16.98	16.80	16.49	16.36	0.37	0.900
			Jejunum			
Villi length (µ)	1175.52^{b}	1526.18^{c}	1292.62^{a}	1205.38^{ab}	24.05	0.001
Crypt depth (µ)	78.71^{b}	75.29^{b}	91.05^{a}	90.22^{a}	2.36	0.001
VL/CD	14.93^{b}	20.27^{b}	14.13^{b}	13.36^{b}	0.41	0.001
			Ileum			
Villi length (µ)	546.16^{c}	700.13^{b}	745.75^{a}	579.03^{c}	11.28	0.001
Crypt depth (µ)	66.22^{b}	60.40^{b}	77.99^{a}	59.48^{b}	1.72	0.001
VL/CD	8.25^{c}	11.59^{a}	9.56^{bc}	9.73^{b}	0.29	0.001

$^{a-b}$. Superscripts represent the degree of significance of values between inline means for each variable (P<0.05). SEM= Standard error of means; T_0= Control basal diet; T_1= 0.2% EF; T_2=0.5% OOP; T_3= 1% OOP; µ= Micron

Caecal Microbiota

Differentiation of caecal microbiota with the supplementation of EF and OOP to broiler diets was shown in Table 4.

Table 4. Differentiation of caecal microbiota with the supplementation of *Enterococcus faecium* and *Origanum onites* powder to 21 day old male broiler diets (log 10/CFU).

Groups	T_0	T_1	T_2	T_3	SEM	P value
Enterococcus spp.	6.96^{b}	7.40^{a}	6.80^{b}	7.28^{ab}	0.10	0.037
E. coli	7.00^{a}	6.99^{a}	6.30^{b}	7.21^{a}	0.08	0.004
Lactobacillus spp.	7.49	7.59	7.93	8.08	0.11	0.189

$^{a-b}$. Superscripts represent the degree of significance of values between inline means for each variable (P<0.05). SEM= Standard error of means; T_0= Control basal diet; T_1= 0.2% EF; T_2=0.5% OOP; T_3= 1% OOP; CFU= Colony forming units.

Dietary supplementation of EF (T_1) to broiler diets increased *Enterococcus* spp. and 0.5% OOP (T_2) supplementation decreased *E. coli* count in cecum. Dietary supplementation of EF and OOP had no effect on the number of *Lactobacillus* spp.

Internal Organs

Dietary supplementation of OOP and EF at different to broiler diets (Table 5) had no effect on internal organ weights. It was determined that the addition of EF (T_1) decreased the weight of gizzard compared to the control (T_0) and 0.5% OOP (T_2) supplemented groups.

Table 5. Differentiation of internal organs with the supplementation of *Enterococcus faecium* and *Origanum onites* powder to 21 day old male broiler diets (g-cm/100gr LW)

Groups	T_0	T_1	T_2	T_3	SEM	P value
Gut length	21.74	21.47	21.62	21.36	0.26	0.968
Gizzard	3.35[a]	2.88[b]	3.23[a]	3.16[ab]	0.06	0.044
Heart	0.72	0.74	0.70	0.73	0.01	0.567
Liver	2.45	2.65	2.61	2.58	0.04	0.216
Proventriculus	0.64	0.61	0.64	0.64	0.01	0.649
Bursa of Fabricius	0.35	0.32	0.38	0.37	0.01	0.328
EIO	6.51	6.26	6.54	6.47	0.07	0.544

[a-b]. Superscripts represent the degree of significance of values between inline means for each variable (P<0.05). SEM: Standard error of means. T_0= Control basal diet; T_1= 0.2% EF; T_2=0.5% OOP; T_3= 1% OOP; EIO= edible internal organs.

Meat Traits

The effects of dietary EF and OO on thigh and breast meats' L* (lightness), a* (redness), b* (yellowness) and pH levels are given in Table 6. In the study the L* value of the T_3 group's was higher than the control group. It was determined that dietary EF and OOP had no effect on other parameters (P>0.05). In the study, it was determined that the supplementation of OOP did not have negative effects on the color values of thigh and breast meat. Breast meat was not affected by dietary treatments. However, dietary supplementation of 0.5%OOP (T_2) and 1%OOP (T_3) numerically decreased 3-day refrigerator stored breast meat MDA value (P>0.05).

Table 6. Differentiantion of L*, a*, b*, MDA and pH meats with the supplementation of *Enterococcus faecium* and *Origanum onites* powder to 21 day old male broiler diets.

Groups	T_0	T_1	T_2	T_3	SEM	P value
Breast meat						
L*	50.92	50.22	49.80	51.18	0.30	0.356
a*	14.35	13.86	14.42	13.60	0.19	0.349
b*	14.01	13.71	13.02	13.07	0.15	0.259
pH	5.86	5.94	5.91	5.94	0.03	0.824
MDA	0.19	0.18	0.17	0.15	0.01	0.746
Thigh meat						
L*	55.89[b]	56.99[ab]	57.18[ab]	58.68[a]	0.32	0.020
a*	14.95	14.76	14.97	14.20	0.15	0.269
b*	15.07	14.76	15.59	15.55	0.16	0.212
pH	6.20	6.11	6.13	6.19	0.023	0.305

[a-b]. Superscripts represent the degree of significance of values between inline means for each variable (P<0.05). SEM= Standard error of means. T_0= Control basal diet; T_1= 0.2% EF; T_2=0.5% OOP; T_3= 1% OOP; MDA= Malondialdehyde.

Discussion

The results of this study revealed that dietary *Origanum onites* powder and *Enterococcus faecium* affected similarly the growth of broilers. The results of this study resemble to the results of studies reported that adding *Origanum vulgare* leaf powder or essential oil to the diet has positive effects on growth (Silva Vázquez et al. 2018; Giannenas et al. 2016; Ariza Nieto et al. 2018; Méndez Zamora et al. 2017; Abdel Wareth et al. 2022;

Amer et al. 2021; Ampode and Mendoza, 2022). However, there are studies reported that they have no positive effects on performance values (Basmacıoğlu et al. 2010; Fonseca-García et al. 2017; Vlaicu et al. 2018; Hernández Coronado et al. 2019; Parvizi et al. 2020; Özel et al. 2022). *Enterococcus faecium* was chosen as a positive control group in this study. In earlier studies, it was demonstrated that *Enterococcus faecium* had been shown to increase the growth of broilers (Samli et al. 2007; Castañeda et al. 2020) and rabbits (Simonová et al. 2022).

Histological results showed that dietary supplementation of OOP and EF significantly increased the villi length in the intestines of broilers and provided better digestion, and especially supplementation of probiotic EF increased the villi length, jejunal and ileal villi/crypt ratio. The results of this study resemble the results of previous studies. Amer et al., (2021) showed that dietary oregano essential oil supplementation of (OEO) increased the villi lengths in all intestine parts statistically at all levels than the control group, and increased the crypt depths in the duodenum and jejunum. Also, Tzora et al., (2016) reported that dietary supplementation with OEO increased jejunal villi length. Fonseca-García et al., (2017) reported that dietary supplementation with OEO did not affect on 42-day performance values, but increased villi sizes in intestine parts (duodenum, jejunum and ileum). Zhang et al., (2021), reported that 200 mg/kg natural OEO to broiler diets increased villi length, villi/crypt ratio and decreased crypt depth in duodenum, jejunum and ileum. Ding et al., (2020) reported that dietary supplementation of OEO increased the villi length numerically, decreased the crypt depth, and increased the villi-crypt ratio statistically in the jejunum in Peking ducks. Also, Özel et al., (2022) reported that 50 mg/kg OEO supplementation increased trout villi length. Similarly, Krauze et al., (2020) and Samli et al., (2007) reported that dietary *Enteroccocus durans* and *faecium* respectively increased ileal villi length of broilers. It was reported that increased villi length enhances digestive surface area capable of more absorption of digested nutrients (Awad et al. 2011; Krauze et al. 2020). Yason et al., (1987) reported that crypts are villi producing factories and deeper crypts are responsible for renewal of decreased villi in case of inflammations, pathogens, toxins and some stress factors. The result of this study showed that OOP and EF supplementations increased villi length, villi/crypt ratio, digestion and gut health of broiler chicks and thus had a positive effect on performance.

It has been determined that EF and OOP have an effect by improving intestinal health by increasing the useful bacteria and decreasing pathogenic bacteria respectively. Turcu et al., (2018), reported that dietary supplementation of OEO decreased the numbers of *Enterobactericaea, E. coli* and *Staphylococcus* in broilers. Kırkpınar et al., (2011) reported

that 150 mg/kg OEO supplementation to broiler diets increased *Lactobacillus s*pp. and suppressed coliforms colonization numerically. Contrary to these results, Criste et al., (2017) reported that dietary supplementation of OEO did not affect the *E. coli* and *Enterobacteriaceae* populations. Xu et al., (2003) reported that pathogenic bacteria suppress digestive enzyme secretion by damaging villi and microvilli of gut mucosa and useful bacteria as competitive exclusion inhibit the proliferation of pathogens to increase digestive enzyme secretion in guts. In our study, the LAB count did not change, but numerically increased in the 0.5% OOP (T_2) and 1% OO (T_3) groups. Dietary supplementation of 0.5% OOP (T_2) decreased *E. coli* and dietary supplementation of EF increased *Enterococcus* spp. It can be said that 0.5-1% OOP supplementations increased gut health like commercial probiotic EF.

Dietary supplementation of OOP and EF to broiler diets had no detrimental effect on internal organ weights. They were normal size and weight. It was determined that the addition of EF decreased the weight of the gizzard compared to the control (T_0) and (T_2) groups. The result of this study was in agreement with the result of Samli et al., (2010). They reported that dietary supplementation of EF decreased gizzard weight. Fonseca et al., (2010) found that dietary probiotic supplementation decreases crop pH and this contributes to the decrease of pathogenic bacteria and digestion in intestine. Decreasing pH in crop may have increased digestion by breaking down the cellulose units of the diet. The increase in diet digestion should also have led to less muscle formation by providing more digestible feed entry to the gizzard. Similarly, Willis and Reid, (2008) and Abdel-Hafeez et al., (2017) found that the weight of the gizzard increased with the increase of indigestible content in the gizzard.

The effects of dietary supplementation of OOP and EF in broiler chicks on thigh and breast meat L* (brightness), a* (red), b* (green) and pH are given in Table 6. At the end of the study, the L* value of the T_3 group was higher than the control (T_0) group. It was determined that the addition of EF and OOP had no effect on other parameters. Dietary supplementation of OOP did not have negative effects on the color values of thigh and breast meat. Ri et al., (2017), reported that the dietary supplemented with oregano 150 mg/kg (*Origanum vulgare*) powder did not affect to L, a*, b*, pH and cooking loss in meats at 21 and 42 days. Our results were similar to the results of Ri et al., (2017).

Dietary supplementation with OOP numerically decreased breast meat MDA levels (Table 6). The results of this study are similar to the results of previous studies. Diler et al., (2017) reported that the addition of *Origanum vulgare* essential to trout feeds increased antioxidant enzyme activity in meat. Tzora et al., (2016) found that oregano essential oil supplementation increases breast and tight meat total phenol content. Ri et al., (2017) reported

that the addition of 150 mg/kg of oregano (*Origanum vulgare*) powder to broiler diets statistically reduced MDA formation in broiler meat compared to control. Although Ognik et al., (2017) reported that dietary EF supplementation decreased MDA formation, MDA of breast meat was not different. Dietary supplementation of OOP and EF did not affect L*, a*, b* and pH of breast and thigh meat of broilers. 1% OOP (T_3) supplementation increased L* value than in the control groups.

Conclusion

This study showed that the dietary supplementation of 0.2% EF and 0.5% OOP in broiler chicks improved daily weight gain compared to the control group, increasingincreased the digestive surface area by increasing the villi length in the jejunum and ileum. It has been shown to positively affecton gut health, primarily by increasing the villi/crypt ratio in the jejunum and ileum. EF supplementation increased *Enterococcus* spp. in cecum. Enterococcus species are lactic acid bacteria and used as probiotics. It has been shown that 0.5% OO supplementation positively affectsperformance by reducing the number of *E.coli*. Dietary supplementation of EF and OOP did not hurtinternal organ developmentor thigh and breast meat properties. The results of this study show that 0.5% supplementation of OOP to broiler diets can be used safely like commercial probiotics for performance improvement. However, tryingdifferent doses of *Origanum onites* powder or under different stress factors.

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Authors’ contributions

Planning, design of the experiments, methodology and statistical analysis, manuscript writing, reviewing and gut histology: Isa Coskun; Experiments and feeding studies and meat properties: Husamettin Celik and Huseyin Cayan; Microbiota detection: Huseyin Cayan.

Disclosure statement

No conflicts of interest was reported by authors.

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