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# Impacts of Essential Oils and Vitamin E Supplements on Gut Microbiota and Ileum Histomorphology of Broilers at Two Different Ages

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**Abstract** A study using one hundred and seventy-five, day-old male broiler chicks (Ross 308) –five chicks per experimental unit with seven replications – was conducted to evaluate the effects of various essential oils (grape seed, coriander, and laurel leaf oils) and vitamin E supplements as natural animal feed additives on the growth performance, gut microbiota, and ileum histomorphology of broiler chicks at different ages (the 21st and 42nd days). For this evaluation, experiments were made on five different dietary treatments, namely: 1) Control, 2) Grape seed Oil (200 mg/kg), 3) Laurel Leaf Oil (200 mg/kg), 4) Coriander Oil (200 mg/kg), and 5) Vitamin E (200 mg/kg). The performance results showed that there was no difference amongst the treatments at 21 days of age. However, the feed conversion ratio (FCR) improved only in the group fed with vitamin E at 42 days of age ($P<0.01$). Yeast, *Enterobacteriaceae*, lactic acid bacteria and coliform bacteria counts were determining in the ileum and cecum contents. In the experiment, the lowest ileum pH value at 42 days of age was observed in the animals fed by grape seed oil. Moreover, according to the microorganism counts in the ileum, the highest LAB count and the lowest *Enterobacteriaceae* and Coliform group bacteria counts were also observed in the same treatment group. Feeding with laurel leaf oil increased the villus height and the crypt depth but decreased the *laminamuscularis mucosae* at 21 days of age ($P<0.05$). However, this positive effect did not continue at 42 days of age. These results indicate that the essential oils may improve gut health; however, further research is deemed necessary to better understand their effects extensively.

**Keywords** Essential oils, vitamin E, gut microbiology, ileum, cecum

## 1. Introduction

The use of antibiotics as growth promoters in poultry diets in order to protect animal health and to increase the production efficiency started in the 1940s [1-2]. However, it was observed that they caused a stiff resistance in pathogenic bacteria and therefore taken out of hen diets on global scale, initiated by Swedish Authorities in 1985 [2-3]. The decision to eliminate antibiotics in poultry feeds by the European Union and their successive banned years are provided in Table 1 [2].

**Table 1:** Antibiotics as growth promoters in the European Union and their banned years [2]

| Active Component | Ban year |
|---|---|
| Avoparcin | 1996 |
| Spiramycin<br>Tylosin<br>Virginiamycin<br>Zinc Bacitracin | 1999 |
| Avilamycin<br>Flavomycin | 2006 |

Essential oils or plant extracts are reported to have antioxidant and antibacterial effects on broilers and they might be an alternative to the antibiotics since the use of latter have been banned in poultry feeds as growth promoters since early 2006 [4]. The alternative surveys focused on the prevention of the pathogenic bacteria propagation in the digestive tract and their replacement by the natural beneficial bacteria [3]. Researchers revealed that due to its benefiting from nutrients, the bowel microflora had crucial effects on the nutrition, health, and growth performance of the poultry and produce respective effects on the development of the digestive system and its interactions. It was reported that this interaction was very complicated and might lead to a dual, either positive or negative effect on animal health and growth depending on the composition and activity of the bowel microflora [3, 5-6]. Observations proved that the animals reared in a pathogen-free environment grew 15% faster than those reared by being exposed to bacteria and viruses under conventional conditions. In addition, the bowel microflora is critically thought to be a burden for feeding fast-growing broilers. The fact that the active microflora contents increase the animals' maintenance energy requirements and decrease their feed conversion ratios is shown as an evidence of this [3].

To sum up, the objective of this study was to examine the impacts of different essential oils (laurel leaf, coriander, and grape seed oils) and vitamin E-supplements on broiler diets with regards to their impacts on growth, feed conversion, gut microbiota, and ileum histomorphology.

## 2. Materials and Methods

### 2.1. Animals and Housing

Day-old male chicks (n = 175) of a commercial broiler strain, Ross 308 [7], were used to test the effects of essential oils (laurel leaf, coriander, and grape seed oils) and vitamin E on soybean- and maize-based basal diets. The chicks were weighed, sorted, and randomly placed in the broiler battery cages (100 × 60 cm). The broiler chicks were placed in three-tier broiler battery units with mesh floors. Five chicks were placed per cage. The cages were kept in controlled rooms considering the changing environmental conditions. Feed and water were provided *ad libitum*. The chicks were fed on experimental diets as starters for the first three weeks (Days 0-21) and as finishers for the next two weeks (Days 21-42). Lighting was provided for 23 h/day. The chicks were weighed individually on Days 1, 21, 35, and 42. The experiment was concluded on Day 42. The chicks were weighed and their feed consumption was calculated. The feed conversion ratio was measured and adjusted to the weight of the dead chicks. Body weight (BW) gain, feed intake, feed conversion ratio (FCR), lengths of intestinal parts, organ weights, ileum histomorphology, yeast, *Enterobacteriaceae*, lactic acid bacteria and coliform bacteria counts of the ileum and the cecum were verified at 21 and 42 days of age.

### 2.2. Diets

The experiments were conducted on five dietary treatments with seven replications, namely 1) Control, 2) Grape seed Oil (200 mg/kg), 3) Laurel Leaf Oil (200 mg/kg), 4) Coriander Oil (200 mg/kg) and 5) Vitamin E (200 mg/kg). Both standard (NRC, 1994) starter (22% crude protein; 3,050 Kcal/kg of ME) and grower (20% crude protein; 3,200 Kcal/kg of ME) broiler diets based on maize and soybean meals were supplemented with the essential oils and vitamin E separately. The list of experimental diets is provided in Table 2. The aromatic oils used in the experiments were included in the dietary oil, thereby obtaining a homogeneous mixture. On further stages of the experiment, it was added to the feed mix with the help of a mixer.

### 2.3. Organ Weights and Carcass Yield Percentages

At 21 and 42 days of age, the chicks were weighed, and their feed consumption was calculated. A chick was selected randomly from each cage. For each treatment, seven separately weighed chicks were taken and killed by cervical dislocation. The digestive tract and organs were weighed after removing the content and their ratios to 100 g of live weight were measured. Furthermore, the digestive tract lengths were recorded.

### 2.4. Intestinal Microbiology

The abdominal cavity was opened after cervical dislocation and all digestive contents of the ileum and the cecum were immediately collected under aseptic conditions into sterile glass bags and put on ice until they were transported to the laboratory for the assessment of microbial populations. VRBD (MERCK, 1.10275) agar was used for *Enterobacteriaceae*, MRS agar (MERCK, 1.10660) for lactic acid bacteria (LAB) and malt extract agar (MERCK, 1.05398) for yeast as the incubation media. LAB and yeast counts of the ileum or cecum contents were obtained at 30 °C following the three-day incubation period [8]. Coliform was grown on VRB agar (MERCK, 1.01406) aerobically at 37 °C for 24-48 hours [9]. The bacterial colonies were counted and based on per gram of original ileal and cecal contents. The average number of live bacteria was calculated. All quantitative data were converted into logarithmic colony forming units (cfu/g).

**Table 2:** Chemical composition of the basal diet (as fed)

| Ingredients | Starter | Finisher |
|---|---|---|
| Maize | 57.25 | 52.50 |
| Soybean meal (48%) | 28.24 | - |
| Soybean meal (44%) | - | 30.11 |
| Full fat soybean | 8.00 | 8.00 |
| Soya oil | 1.65 | 5.67 |
| Dicalcium phosphate | 2.01 | 1.68 |
| Limestone | 1.22 | 1.15 |
| L-lysine HCl | 0.64 | - |
| DL-methionine | 0.29 | 0.20 |
| Salt | 0.35 | 0.34 |
| Premix[a] | 0.35 | 0.35 |
| Calculated Nutrient Content[b] | | |
| ME (Kcal/g) | 3.05 | 3.20 |
| Crude protein, % | 22.00 | 20.0 |
| Crude fiber, % | 2.80 | 3.79 |
| Ether extract, % | 5.55 | 9.34 |
| Ca, % | 1.00 | 0.90 |
| $P_{av}$, % | 0.50 | 0.45 |
| Na, % | 0.16 | 0.16 |
| Lysin, % | 1.65 | 1.21 |
| Methionine, % | 0.61 | 0.53 |
| Met+Cys, % | 0.97 | 0.88 |
| Tryptophan, % | 0.29 | 0.26 |

[a]Provided the following per kg of diet; vitamin A 15,000 IU, vitamin $D_3$ 3,000 IU, vitamin E 100 mg, vitamin $K_3$ 5 mg, vitamin $B_1$ 3 mg, vitamin $B_2$ 6 mg, vitamin $B_6$ 6 mg, vitamin $B_{12}$ 0.02 mg, niacin 50 mg, folic acid 1.5 mg, calcium D-Pantothenate 15 mg, biotin 0.15 mg; Fe 60 mg, Cu 5 mg, Mn 80 mg, Co 0.2 mg, Zn 60 mg, I 1 mg, Se 0.15 mg.

[b]Based on NRC (1994) values for feed ingredients

### 2.5. Histomorphological Measurements

At the midst and end of the study, the ileal segments of seven randomly selected chicks (per treatment) (4 cm each, from Meckel's diverticulum to the distal end) were removed, rinsed with the formalin solution, cut into pieces and placed into 10% neutral buffered formalin until further processing. For each sampling, the tissues were cut into 0.5cm sections. The tissue sections were processed, embedded in paraffin, cut (5 µm) by a microtome and floated onto slides. After staining with hematoxylin and eosin, three cross-sections from each ileal sample were prepared using routine paraffin embedding procedures [10]. The slides were examined on an Olympus CX31 microscope (Olympus Corporation, Tokyo, Japan) fitted with a digital video camera. Three intact and well-oriented crypt-villus units were selected for each intestinal cross section (21 measurements for each treatment). Afterwards, crypt depth, thickness of the *Lamina muscularis mucosae*, villus height and villus width were measured using an image processing and analysis system (Motic Images Plus 2.0).

### 2.6. Statistical analyses

The collected data were recorded on a weekly basis and statistical one-way analysis of variance (ANOVA) was conducted using a statistical software program [11]. Differences between the means were separated by Duncan's multiple range test.

## 3. Results and Discussion

### 3.1. Performance

The average live weight of the chicks on the first-day was 45.6 g. No statistical difference was observed in the performance results at 21 days of age (P>0.05). However, the lowest feed conversion ratio numbers (FCR) (1.358) and the highest live weight gain figures (LWG) (744.5g) were observed in the groups fed by vitamin E supplements (Table 3). Furthermore, this effect on the FCR value continued at 42 days of age and the difference was found statistically significant (P<0.01).

**Table 3:** Performance results at 21 and 42 days of age

| Treatments | 21 days of age | | | 42 days of age | | |
|---|---|---|---|---|---|---|
| | FI (g) | WG (g) | FCR (g/g) | FI (g) | WG (g) | FCR (g/g) |
| Control | 1011.2 | 696.9 | 1.451 | 4194.3 | 2534.1 | 1.655 **a** |
| Grape seed oil | 1032.4 | 724.1 | 1.426 | 4187.3 | 2551.6 | 1.641 **a** |
| Laurel leaf oil | 994.6 | 708.0 | 1.405 | 4188.3 | 2528.9 | 1.656 **a** |
| Coriander oil | 1031.1 | 742.3 | 1.389 | 4288.8 | 2617.6 | 1.639 **a** |
| Vitamin E | 1011.0 | 744.5 | 1.358 | 4125.5 | 2571.3 | 1.604 **b** |
| SEM* | 11.453 | 13.086 | 0.019 | 35.743 | 22.569 | 0.006 |
| P levels | 0.853 | 0.739 | 0.592 | 0.732 | 0.754 | 0.004 |

Values with different letters (a, b) in the same column differ significantly (P<0.01; n=7).

*SEM: Standard error of means; FI: Feed intake; WG: Live weight gain; FCR: Feed conversion ratio.

Unlike the results of the study, Botsoglou et al. [12] observed that two different levels of marjoram oil (50 and 100 mg/kg of feed) and alpha-tocopherol acetate (200 mg/kg of feed) had no effect on performance. In another study by them, Botsoglou et al. [13] the female broiler diet supplements with α-tocopherol acetate (200 mg/kg) and a commercial essential oil mix (0.5 g/kg and 1.0 g/kg) showed no difference in the performance parameters. Lee et al. [14] searched the effects of *thymol* (100 ppm), *cinnamaldehyde* (100 ppm) and a commercial aromatic oil mixture containing *thymol* on the performance of female broilers and claimed that there was no difference. Jang et al. [15] and Tekeli [16] reported that the diet supplements with antibiotics and different levels of aromatic oils did not have any significant effect on the performance of animals.

### 3.2. Internal Organs' Measurement Results

#### *3.2.1. Internal Organ Measurement Results at 21 days of age*

In Table 4, the digestive tract lengths of the animals killed at 21 days of age are provided with their ratios to 100 g of live weights of the animals and no statistical difference was observed among the treatments (P>0.05). Nevertheless, the numerically highest ileum length and the lowest duodenum (3.050 cm), jejunum (7.910 cm) and cecum lengths (1.835 cm) were observed in the group which consumed grape seed oil. On the other hand, the lowest ileum length was detected in those having consumed vitamin E.

**Table 4:** Digestive tract lengths on the 21st day (cm/ 100 g of LW)

| Treatments | Duodenum, cm | Jejunum, cm | Ileum, cm | Cecum, cm |
|---|---|---|---|---|
| Control | 3.293 | 8.144 | 6.063 | 1.989 |
| Grape seed oil | 3.050 | 7.910 | 6.127 | 1.835 |
| Laurel leaf oil | 3.250 | 8.000 | 6.007 | 2.030 |
| Coriander oil | 3.426 | 8.644 | 5.890 | 1.944 |
| Vitamin E | 3.341 | 7.956 | 5.690 | 2.063 |
| SEM* | 0.074 | 0.140 | 0.135 | 0.049 |
| P levels | 0.572 | 0.479 | 0.876 | 0.618 |

The difference among the treatments is insignificant (P>0.05; n=7).

*SEM: Standard error of means.

Whilst no statistical difference was observed in the digestive tract weights of the animals killed at 21 days of age (P>0.05), numerically parallel results to their respective lengths were found. The highest duodenum and jejunum weights (1.44 and 2.46 g, respectively) were recorded in the groups consuming coriander oil, whereas the lowest duodenum weight and the highest ileum weight were found in those consuming grape seed oil (Table 5).

**Table 5:** Digestive tract weights on the 21st day (g/ 100 g of LW)

| Treatments | Duodenum, g | Jejunum, g | Ileum, g | Cecum, g |
|---|---|---|---|---|
| Control | 1.31 | 2.19 | 1.80 | 1.39 |
| Grape seed oil | 1.22 | 2.30 | 1.99 | 1.19 |
| Laurel leaf oil | 1.35 | 2.31 | 1.98 | 1.39 |
| Coriander oil | 1.44 | 2.46 | 1.82 | 1.14 |
| Vitamin E | 1.37 | 2.22 | 1.87 | 1.28 |
| SEM* | 0.032 | 0.042 | 0.065 | 0.088 |
| P levels | 0.322 | 0.305 | 0.856 | 0.857 |

The difference among the treatments is insignificant (P>0.05; n=7).
*SEM: Standard error of means.

When the digestive organ weights were examined, the proventriculus and liver weights were found to be the highest in the groups fed with laurel leaf oil supplements as compared with the other groups and the difference was found statistically significant (P<0.05). The lowest value among the liver weights in the treatments was seen in the animals in the control group (P<0.05). Additionally, the numerically highest gizzard and heart weights were also observed in the groups consuming laurel leaf oil (Table 6). The stay of the nutrient content in the proventriculus for a longer time might be the reason of the proventriculus' heaviness.

**Table 6:** Digestive organ weights on the 21st day (g/ 100 g of LW)

| Treatments | Gizzard | Proventriculus | Abd. Fat | Heart | Liver | Pancreas |
|---|---|---|---|---|---|---|
| Control | 2.46 | 0.56 **b** | 0.88 | 0.61 | 2.56 **b** | 0.35 |
| Grapeseed oil | 2.32 | 0.56 **b** | 0.82 | 0.64 | 2.70 **ab** | 0.37 |
| Laurel leaf oil | 2.73 | 0.74 **a** | 0.85 | 0.66 | 2.99 **a** | 0.37 |
| Coriander oil | 2.39 | 0.54 **b** | 0.77 | 0.61 | 2.80 **ab** | 0.36 |
| Vitamin E | 2.51 | 0.61 **ab** | 0.93 | 0.60 | 2.74 **ab** | 0.36 |
| SEM* | 0.072 | 0.028 | 0.034 | 0.011 | 0.057 | 0.012 |
| P levels | 0.412 | 0.138 | 0.709 | 0.502 | 0.252 | 0.985 |

a-b: The difference among the averages with different letters in the same column is significant (P<0.05; n=7).
*SEM: Standard error of means.

In their study, Lee et al. [14] fed female broilers with *thymol* (100 ppm), *cinnamaldehyde* (100 ppm) supplements and a commercial aromatic oil mixture (containing *thymol*). As a result of this, the liver weight (g/100 g of live weight) averages at 21 days of age were found to be the highest in the groups supplemented with *thymol*, whereas this effect was not observed at 40 days of age. This result resembles the outcomes of the study.

### *3.2.2. Internal Organ Measurement Results at 42 days of age*

The numerical results observed at 21 days of age in the groups supplemented with grape seed oil remained unchanged at 42 days of age and the statistically lowest duodenum and cecum lengths were detected (P<0.05; Table 7). One of the reasons why the lengths were proportionally shorter might be the stay of the digestive contents in these sections for a shorter period, i.e. as a result of the faster passage of the digestive contents through these sections. The highest duodenum length (1.235 cm) was observed in the groups consuming laurel leaf oil, while the highest cecum length (0.857 cm) was recorded in those consuming coriander oil (P<0.05). The statistical difference in jejunum and ileum lengths between the treatments (P>0.05) was found insignificant.

**Table 7:** Digestive tract lengths on the 42nd day (cm/ 100 g of LW)

| Treatments | Duodenum, cm | Jejunum, cm | Ileum, cm | Cecum, cm |
|---|---|---|---|---|
| Control | 1.116 **ab** | 2.964 | 2.140 | 0.791 **ab** |
| Grapeseed oil | 1.059 **b** | 3.101 | 2.191 | 0.741 **b** |
| Laurel leaf oil | 1.235 **a** | 3.120 | 2.265 | 0.835 **ab** |
| Coriander oil | 1.186 **ab** | 3.023 | 2.111 | 0.857 **a** |
| Vitamin E | 1.136 **ab** | 3.193 | 2.117 | 0.793 **ab** |
| SEM* | 0.024 | 0.058 | 0.050 | 0.015 |
| P levels | 0.184 | 0.798 | 0.886 | 0.146 |

a-b: The difference among the averages with different letters in the same column is significant (P<0.05; n=7).
*SEM: Standard error of means.

The digestive tract weights of the animals killed at 42 days of age are shown in Table 8. The difference in ileum weights was found statistically significant (P<0.05). The highest weight group (1.16 g) was recorded to be the animals consuming coriander oil, whereas the lowest value (0.88 g) was detected in animals consuming vitamin E.

**Table 8:** Digestive tract weights on the 42nd day (g/ 100 g of LW)

| Treatments | Duodenum, g | Jejunum, g | Ileum, g | Cecum, g |
|---|---|---|---|---|
| Control | 0.53 | 1.04 | 0.97 **ab** | 0.67 |
| Grapeseed oil | 0.48 | 0.93 | 0.93 **ab** | 0.55 |
| Laurel leaf oil | 0.48 | 1.02 | 0.95 **ab** | 0.65 |
| Coriander oil | 0.53 | 0.96 | 1.16 **a** | 0.70 |
| Vitamin E | 0.53 | 0.94 | 0.88 **b** | 0.58 |
| SEM* | 0.012 | 0.020 | 0.036 | 0.031 |
| P levels | 0.415 | 0.433 | 0.122 | 0.557 |

a-b: The difference among the averages with different letters in the same column is significant (P<0.05; n=7).
*SEM: Standard error of means.

No statistical difference was observed among the digestive organ weights of the animals at the end of the performance period (at 42 days of age) (P>0.05; Table 9). This result shows parallelismto Lee et al [14].

**Table 9:** Digestive organ weights on the 42nd day (g/ 100 g of LW)

| Treatments | C/LW | Gizzard | Proventriculus | Abd. Fat | Heart | Liver | Pancreas |
|---|---|---|---|---|---|---|---|
| Control | 0.73 | 1.23 | 0.33 | 1.72 | 0.44 | 1.49 | 0.18 |
| Grape seed oil | 0.74 | 1.19 | 0.31 | 1.54 | 0.43 | 1.47 | 0.16 |
| Laurel leaf oil | 0.73 | 1.26 | 0.28 | 1.49 | 0.43 | 1.54 | 0.17 |
| Coriander oil | 0.73 | 1.06 | 0.35 | 1.58 | 0.43 | 1.50 | 0.16 |
| Vitamin E | 0.74 | 1.18 | 0.31 | 1.48 | 0.46 | 1.60 | 0.16 |
| SEM* | 0.002 | 0.036 | 0.011 | 0.061 | 0.010 | 0.031 | 0.007 |
| P levels | 0.235 | 0.507 | 0.359 | 0.757 | 0.783 | 0.646 | 0.936 |

The difference among the treatments is insignificant (P>0.05; n=7).
*SEM: Standard error of means; C/LW: Carcass/Live weight.

When hatching is concerned, the digestive system organs increase at a faster pace than the other organs, tissues and body weight. The increase observed in growth is at the maximum level at 4-6 days of age and continues with a decrease till the 10th day. An increase downturn is observed with age in the proportional size of the digestive system [17]. In this study it was also noted thatthe ratios of the organ weights at 21 days of age to 100 g of live weight were higher than those at 42 days of age. Only the abdominal fat ratio increased proportionally with the ages of the animals.
In the study conducted by Jang et al. [15] the liver, pancreas and small intestine weights were not affected by the antibiotics and different levels of essential oil supplements, which shows similarity to the results of the study.

### 3.3. Ileum and Cecum pH and Microbiological Culture Results

#### *3.3.1. Ileum and cecum pH results (at 21 and 42 days of age)*

The ileum and cecum pH values of the animals measured at 21 and42 days of age are demonstrated in Table 10 (P>0.05). When the ileum and cecum pH values at 42 days of age were examined, the difference in ileum pH was found statistically significant (P<0.05; Table 10). The lowest pH in the ileum (5.02) was observed in the animals consuming grape seed oil. While no statistical difference was seen in cecum pH, the numerically highest pH was seen in the groups consuming grape seed oil.

**Table 10:** Ileum and cecum pH results at 21 and 42 days of age

| | 21 days of age | | 42 days of age | |
|---|---|---|---|---|
| **Treatments** | Ileum | Cecum | Ileum | Cecum |
| Control | 6.56 | 5.82 | 6.22 **ab** | 6.07 |
| Grapeseed oil | 7.03 | 6.26 | 5.02 **b** | 6.48 |
| Laurel leaf oil | 6.25 | 6.63 | 6.65 **a** | 6.43 |
| Coriander oil | 6.49 | 7.02 | 6.89 **a** | 6.20 |
| Vitamin E | 6.74 | 6.05 | 7.19 **a** | 6.29 |
| SEM* | 0.139 | 0.200 | 0.204 | 0.081 |
| P levels | 0.578 | 0.387 | 0.153 | 0.622 |

Values with different letters (a, b) in the same column differ significantly (P<0.05; n=2).
*SEM: Standard error of means.

#### *3.3.2. Microorganism counts in the ileum and the cecum at 21 days of age*

The variation in the ileum microorganism counts of the animals at 21 days of age is provided in Table 11. The highest number of colonies in the *Enterobacteriaceae* and coliform groups were observed in the groups consuming grape seed oil. However, the lactic acid bacteria (LAB) and yeast counts were found to be the lowest in the above mentioned. These differences were found statistically significant (P<0.05).

**Table 11:** Microorganism counts in the ileum on the 21st day (log10 cfu/g)

| Treatments | Enterobacteria | LAB | Yeast | Coliform |
|---|---|---|---|---|
| Control | 5.38 **b** | 5.63 **ab** | 6.10 **a** | 5.87 **b** |
| Grapeseed oil | 6.58 **a** | 4.73 **b** | 4.99 **b** | 6.59 **a** |
| Laurel leaf oil | 5.61 **a** | 6.12 **a** | 6.29 **a** | 6.08 **ab** |
| Coriander oil | 5.98 **a** | 5.70 **a** | 6.06 **a** | 5.68 **b** |
| Vitamin E | 6.15 **a** | 5.82 **a** | 6.02 **a** | 6.16 **ab** |
| SEM* | 0.156 | 0.104 | 0.085 | 0.101 |
| P levels | 0.222 | 0.089 | <0.001 | 0.109 |

Values with different letters (a, b) in the same column differ significantly (P<0.05; n=7).
*SEM: Standard error of means.

Table 12 shows the variation in the cecum microorganism counts of the animals at 21 days of age. The lowest number of colonies in the coliform group was observed in the groups consuming coriander oil, whereas the yeast count was found to be the lowest in the groups consuming grape seed oil, as in the ileum microbiota (P<0.05). No difference in *Enterobacteriaceae* and LAB counts was observed in between the treatments (P>0.05).

**Table 12:** Microorganism counts in the cecum on the 21st day (log10 cfu/g)

| Treatments | Enterobacteria | LAB | Yeast | Coliform |
|---|---|---|---|---|
| Control | 6.22 | 6.38 | 5.87 **ab** | 6.37 **a** |
| Grapeseed oil | 6.43 | 6.26 | 5.49 **b** | 6.59 **a** |
| Laurel leaf oil | 6.30 | 6.10 | 6.12 **a** | 6.54 **a** |
| Coriander oil | 6.30 | 5.98 | 5.81 **ab** | 5.90 **b** |
| Vitamin E | 6.08 | 6.27 | 6.14 **a** | 6.36 **a** |
| SEM* | 0.092 | 0.047 | 0.064 | 0.073 |
| P levels | 0.810 | 0.326 | 0.095 | 0.035 |

a-b: The difference among the averages with different letters in the same column is significant (P<0.05; n=4).
*SEM: Standard error of means.

### 3.3.3. Microorganism counts in the ileum and the cecum at 42 days of age

The ileum microorganism counts in the animals at 42 days of age are shown in Table 13 and as seen proves no statistically significant differences (P<0.01). The highest yeast count was observed in the control group (6.31 cfu/g) and amongst those consuming the feed with grape seed oil supplements (6.29 cfu/g) whereas the highest *Enterobacteriaceae* count was recorded in the control group (6.78 cfu/g). However, all treatments were significantly different in statistics amongst the LAB and coliform groups (P<0.001).

**Table 13:** Microorganism counts in the ileum on the 42nd day (log10 cfu/g)

| Treatments | Enterobacteria | LAB | Yeast | Coliform |
|---|---|---|---|---|
| Control | 6.78 **a** | 5.75 **c** | 6.31 **a** | 6.71 **b** |
| Grape seed oil | 6.11 **c** | 6.15 **a** | 6.29 **a** | 6.04 **e** |
| Laurel leaf oil | 6.51 **ab** | 5.88 **b** | 6.14 **b** | 6.86 **a** |
| Coriander oil | 6.37 **bc** | 5.49 **d** | 6.09 **b** | 6.18 **d** |
| Vitamin E | 6.44 **b** | 5.92 **b** | 5.65 **c** | 6.34 **c** |
| SEM* | 0.058 | 0.048 | 0.052 | 0.064 |
| P levels | 0.003 | <0.001 | <0.001 | <0.001 |

a-e: The difference among the averages with different letters in the same column is significant (P<0.01; n=7).
*SEM: Standard error of means.

Some researchers [4, 18] reported that the ileum and cecum pH values in poultry were around 6.3 and 5.7, respectively. In this study, the lowest ileum pH value (5.02) was seen in the animals fed with grape seed oil supplements (Table 10). Nevertheless, the highest LAB count and the lowest *Enterobacteriaceae* and Coliform group bacteria counts were also observed in the same treatment group (Table 13). Lactic acid bacteria are thought to have an antagonistic effect on other microorganisms due to their production of organic acids such as lactic and acetic acids; $H_2O_2$, bacteriocin or bacteriocin-like metabolites; and such metabolites as diacetyl, alcohol, and $CO_2$. While the LAB indicates an inhibitory effect against most bacteria at a low pH, they remain ineffective against yeast and molds [19]. This result coincides with the results.

Unlike the results in the study, in a study conducted by Jang et al. (2007) it was stated that the LAB count in the digestive contents collected from the ileocecum had not been affected by supplementation of antibiotics or different levels of essential oils (25 or 50 mg/kg of feed).

As a result of the microbiological analysis of the ceca of the animals killed at the end of the performance period, the *Enterobacteriaceae* and LAB counts were found to be the lowest in the groups supplemented with grape seed oil and laurel leaf oil. On the other hand, the highest LAB and coliform counts were observed in the control group. Moreover, the yeast count was found to be the highest in the group consuming laurel leaf oil, but at its lowest in the control group and in the groups supplemented with grape seed oil (P<0.001; Table 14).

**Table 14:** Microorganism counts in the cecum on the 42nd day (log10 cfu/g)

| Treatments | Enterobacteria | LAB | Yeast | Coliform |
|---|---|---|---|---|
| Control | 6.88 **a** | 6.45 **a** | 5.88 **c** | 6.93 **a** |
| Grapeseed oil | 6.39 **b** | 6.13 **c** | 5.80 **c** | 6.44 **c** |
| Laurel leaf oil | 6.43 **b** | 6.10 **c** | 6.21 **a** | 6.67 **b** |
| Coriander oil | 6.84 **a** | 6.25 **b** | 6.17 **ab** | 6.29 **d** |
| Vitamin E | 6.72 **a** | 6.30 **b** | 6.10 **b** | 6.27 **d** |
| SEM* | 0.052 | 0.029 | 0.035 | 0.052 |
| P levels | <0.001 | <0.001 | <0.001 | <0.001 |

a-d: The difference among the averages with different letters in the same column is significant (P<0.001; n=4).
*SEM: Standard error of means.

In the study conducted by Cross et al. [20] they demonstrated that the diet supplements of floor-reared animals with various plants (10 g/kg) and their essential oils (1 g/kg) had no effect on the coliform, lactic acid bacteria, total anaerobes, and *C. Perfringens* counts in the fecal and cecal contents at 28 days of age.

It was observed that a colonized stable microorganism population in poultry might reduce the effects of the pathogens that entered the body at advancing ages. Nevertheless, the presence of an unstable microflora or subclinical enteritis can damage the ecosystem by causing an excessive increase in *Clostridium* by increasing pH and the ratio of mucins produced by goblet cells [21]. In addition, such bacterial diseases as dysbacteriosis, non-specific enteritis and wet litter problem might also be observed in the event of propagation of pathogenic microorganisms in the population [22].

### 3.4. Results about Ileum Histomorphology

The changes which are likely to occur in the microflora of the digestive system and which would prevent the adherence of pathogens might lead to a profound effect on the structure of the bowel wall. For instance, with the studies performed, it was revealed that changes in the bowel morphology such as shorter villi and thicker crypts could be observed due to the presence of stress factors like bacterial toxins. Therefore, it is thought that the composition of the bowel microflora has a significant beneficial, harmful or neutral effect on the digestive system [23-24].

**Table 15:** Ileum histomorphology at 21 days of age

| **Treatments** | **Villus height, μm** | **Villus width, μm** | **Crypt depth, μm** | **VH/CD[1]** | ***Lamina muscularis mucosae*, μm** |
|---|---|---|---|---|---|
| Control | 986.07 **ab** | 148.30 **b** | 95.95 **b** | 10.27 **a** | 186.24 **b** |
| Grape seed oil | 1061.78 **ab** | 153.68 **ab** | 110.80 **ab** | 9.70 **ab** | 206.89 **ab** |
| Laurel leaf oil | 1110.70 **a** | 152.61 **ab** | 122.83 **a** | 9.09 **ab** | 182.24 **b** |
| Coriander oil | 1022.51 **ab** | 163.53 **ab** | 96.73 **b** | 10.77 **a** | 201.33 **ab** |
| Vitamin E | 897.90 **b** | 171.80 **a** | 112.84 **ab** | 8.02 **b** | 219.34 **a** |
| SEM* | 31.295 | 3.198 | 2.947 | 0.323 | 4.496 |
| P levels | 0.263 | 0.124 | 0.009 | 0.055 | 0.046 |

a-b: The difference among the averages with different letters in the same column is significant (P<0.05; n=7).
*SEM: Standard error of means; 1VH/CD: Villus height to crypt depth ratio.

The data about the ileum histomorphology of the animals in the growth period (21 days of age) are shown in Table 15 (P<0.05). The highest values in crypt depth and villus height were seen in the animals supplemented with laurel leaf oil. Furthermore, when the ratio of villus height to crypt depth was calculated, the smallest value was observed in those consuming vitamin E, whereas the highest values were seen in the control group and amongst those consuming coriander oil (P<0.05).

**Table 16:** Ileum histomorphology at 42 days of age

| **Treatments** | **Villus height, μm** | **Villus width, μm** | **Crypt depth, μm** | **VH/CD[1]** | ***Lamina muscularis mucosae*, μm** |
|---|---|---|---|---|---|
| Control | 1166.78 | 149.28 | 91.42 **b** | 12.86 | 268.36 **ab** |
| Grapeseed oil | 1007.15 | 173.38 | 94.95 **b** | 10.57 | 282.95 **a** |
| Laurel leaf oil | 1060.95 | 152.57 | 100.63 **ab** | 10.90 | 230.25 **ab** |
| Coriander oil | 1201.33 | 158.88 | 116.07 **a** | 10.80 | 240.33 **ab** |
| Vitamin E | 1082.01 | 176.83 | 96.31 **b** | 11.19 | 216.24 **b** |
| SEM* | 37.805 | 5.371 | 4.484 | 0.415 | 9.528 |
| P levels | 0.413 | 0.509 | 0.074 | 0.546 | 0.181 |

a-b: The difference among the averages with different letters in the same column is significant (P<0.05; n=7).
*SEM: Standard error of means; 1VH/CD: Villus height to crypt depth ratio.

The results concerning the ileum histomorphology of the animals used in the experiment at 42 days of age are shown in Table 16. The crypt depth in the growth period (21 days of age) was observed to be the lowest in the control group and in the groups supplemented with coriander oil, while the highest crypt depth at the end of the performance period (42 days of age) was seen in the animals consuming a feed supplemented with coriander oil (P<0.05). The numerically highest villus height was observed in the same treatment (P>0.05). On the other hand, *Lamina muscularis mucosae* thickness was found to be the highest in those having consumed grape seed oil (282.95 μm) but the lowest in the group supplemented with vitamin E (216.24 μm) (P<0.05). Vis à vis the calculation of the ratio of villus height to crypt depth, no differences were observed in the values obtained from

the treatments (P>0.05). However, when compared with the case at 21 days of age, the VH/CD values were also found to be higher as the villus heights of the animals were higher at 42 days of age.
While no difference in ileum length was seen among the treatments on the 42nd day, those with feeds supplemented with coriander oil were found to be the highest in terms of weight, but those supplemented with vitamin E were found to be the lowest in terms of weight. These data coincide with the results about theileum histomorphology on the 42nd day.
In the studies performed [23, 25-26], it was observed that while the bowel wall thickness and the bowel weight decreased in the animals fed on antibiotic-containing feeds, the length increased. On the other hand, in their study, Miles et al. [6] argued that the bowel wall thickness, weight and length decreased and claimed that the effects were far more obvious in the ileum than in the duodenum. Some of the underlying reasons might be searched in the decrease of the chronically low level of infection with the competing endogenous microflora for nutrients. Decreases in the villus height and the crypt depth were observed in the animals grown in a hygienic and microorganism-free environment for seven days, which reveals the fact that the bowel microflora is also effective on the normal development of the digestive tract [5,21].
The changes seen in the morphology of the digestive tract such as thicker crypts and shorter villi were found relevant to the stress factors and toxins caused by nutrition [21, 27]. There occurs a decrease in the absorption surface of nutrients as the villi shorten. However, crypts may be treated as villus factories and a wide crypt may be an indication of a high demand for rapid tissue production and new tissues. The histological examination shows that crypts are the renewal regions of the epithelium. Some researchers found a close correlation between the rate of increase in epithelial cells and the crypt depth [28]. Narrow and long villi might indicate that the propagation in a crypt is faster as a result of the shorter life cycles of the epithelial cells due to faster progress of cells from the crypt to the villi. A higher villus height was reported to be a sign of the fact that the functions of bowel villi were active [29].

## 4. Conclusions

With the ban on the use of antibiotics as growth promoters in animals, researchers concentrated on studies using alternative natural feed additives like aromatic oils. Despite numerous studies conducted on use of aromatic oils, the results vary significantly, and they may have many aspects which have not yet been investigated. These differences can also be a result of the fact that the active substance contents of each aromatic oil are affected by production region, year, season, mode of harvesting and the production and storage conditions at the establishments and laboratories where they are obtained.
When the performance results obtained in the research were examined, no difference was observed at 21 days of age, whereas the lowest FCR value at 42 days of age was seen in the groups consuming vitamin E (P<0.01). However, no difference was recorded for the other treatments.
Amongst the ileum samples collected from the animals at the end of the performance period, the lowest pH was observed in those consuming grape seed oil and the difference was found statistically significant (P<0.05). The highest value of the LAB count was seen in those consuming grape seed oil. Nevertheless, the lowest *Enterobacteriaceae* and coliform group counts were also detected in those consuming grape seed oil. As a result of this study, the number of beneficial microorganisms in those consuming grape seed oil was found high whereas the number of possible pathogenic microorganisms was found low. This might be a result of the increased lactic acid bacteria count even though the pH was found lower than those of the other groups. It was reported that lactic acid bacteria could show an inhibitory effect against most bacteria by producing metabolites with an antagonistic effect at a low ph. Stillit is believed to be ineffective against yeast and molds.
When the ileum histomorphology of the animals in the growth period (21 days of age) was examined, the villus height and the crypt depth were found to be the highest but *lamina muscularismucasae* the lowest in those consuming laurel leaf oil (P<0.05). Nevertheless, the crypt depth was found to be the highest in the animals supplemented with coriander oil at 42 days of age; however, no statistical difference in the measurement values of the animals supplemented with laurel leaf oil was reported (P>0.05). The presence of nutrient rich contents and the great depth of the crypts– also called villus factories – in the ileum might be interpreted as an indication of a high demand for rapid tissue production and new tissues.

To conclude, vitamin E supplementation positively affected the feed conversion ratio out of the performance parameters, whereas grape seed oil affected the microorganism contents of the ileum in favor of beneficial microorganisms depending on the decrease in pH at the end of the performance period. In the same period, lower LAB, *Enterobacteriaceae* and coliform counts were detected in the cecum in all treatments as compared to the control group. This might be considered a positive effect when it is thought that microorganisms are in competition with the host for nutrients. In addition to these results, the positive effects of aromatic oils and vitamin E might have been found less obvious than those of the control group due to the hygienic and optimum cage conditions under which the animals led their lives. The potential results of the same experiment conducted amongst the broilers reared under farm conditions or on the litter are reserved as the topic of a separate future research study.

In some studies, however, there are reports that the diet consumed by animals and the contained raw materials cover the difference. In the study conducted by Lee et al. [14] they observed that aromatic oils had no effect on performance and thus it should not be disregarded that when the animals were fed a less hygienic and less digestible diet there might be positive effects. The diet we used in the experiment was a soybean- and maize-based mix and the potential effects of a diet prepared with raw materials such as wheat and barley may be addressed as a separate research subject.

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