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Evaluation of the Effects of Different Natural Dietary Feed Additives on Performance and Intestinal Histomorphology in Quails

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ABSTRACT: The aim of the present study was to investigate the influence of a commercial probiotic and a commercial essential oil blend and their mixture, as a natural feed additive, on growth parameters live weight (LW), live weight gain (LWG), feed intake (FI), feed conversion ratio (FCR), carcass yield (CY) and small intestinal histomorphology of quails. A total of 200 1-day-old Japanese (*Coturnix coturnix Japonica*) quails, including both males and females, were divided into four groups containing 50 quails and treated as follows: (1) a control treatment without medication (2) 18 g ton^{-1} probiotic; (3) 300 g ton^{-1} essential oil blend and (4) 18 g ton^{-1} probiotic plus 300 g ton^{-1} essential oil blend. Each experimental group consisted of 5 replicated groups. The results of the research show that the additives added to quail diets do not have a significant effect on the performance parameters of LW, LWG, FI and FCR ($P > 0.05$). However, significant differences were found in the CY, which is one of the performance parameters ($P < 0.05$). A probiotic addition of 18 g ton^{-1} (treatment 1) to the quail diet resulted in a significant increase in carcass yield ($P < 0.05$). Moreover, the addition of natural feed additives such as probiotics, essential oil blends and mixture to quail diets caused significant ($P < 0.001$) increases in villus heights and crypt depth in the duodenum and ileum segments. In addition, significant enlargements were found in the villus surface area in the experimental groups compared to the control group ($P < 0.05$). In conclusion, in this study, it was determined that the addition of feed additives, which are developed as an alternative to antibiotics to the quail diet did not have a negative effect on performance and caused significant differences in the effective parameters in the evaluation of intestinal health.

Keywords: crypt depth; essential oil; gut health; Japanese quail, performance; probiotic

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INTRODUCTION

Intestinal health is an important concept for maintaining efficient and effective gastrointestinal (GIT) physiology (Choct, 2009; Salois et al., 2016). The integrity of the gastrointestinal tract (GIT) and the gut microbial community play vital roles in the absorption of nutrients, the development of immunity, and disease resistance. In addition, in the Gastrointestinal system (GIT), the development of the microbiota community in favor of beneficial microorganisms and the preservation of cellular structure integrity are very important for animal health and then performance (Shang et al ., 2018). In the small intestine is the site of absorption of nutrients, so maintaining gut health is very important for animal performance (Pan and Yu, 2014). The small intestine epithelium is a very important structure that secretes hormones and acts as a physiological barrier against pathogens by producing glycoprotein and defensin, containing nutrient carriers and digestive enzymes (Peterson and Artis, 2014). The folds formed by the inward folding of the small intestinal epithelium form the intestinal crypts, while the outward folds that are directly exposed to the lumen environment form the intestinal villi (Crosnier et al., 2006). The density and size of villi of small intestine are directly related with absorption capacity of the poultry. Villi height is the best measurement to show changes on surface area of absorptive cells (Silva et al., 2007). Performance parameters are the most widely used way of evaluating nutritional studies. However, today it is known that many additives can affect the development of intestinal villi and animal performance is evaluated by examining the histological structure of the gastrointestinal system.

Natural feed additives with plant origins are generally believed to be safer, healthier and less hazardous. Special herbs have been used as food for medicinal purposes for centuries and some of them have played a significant role in maintaining human health and improving the quality of human life for thousands of years (Kassie, 2008). Many herbs and plant extracts have both antimicrobial and antioxidant activities that make them useful as natural feed additives for animals (Yesilbag et al., 2013; Yesilbag et al., 2014; Cengiz et al., 2015; Lee et al., 2015; Tufarelli et al., 2021). Moreover, herbs contain active substances that can improve digestion and metabolism. They also stimulate antibacterial and immunostimulant activities in animals (Giannenas et al., 2003; 2005). The use of probiotics stimulates the growth of beneficial microorganisms rather than the growth of potentially pathogenic bacteria (Galdeano et al.,2019). Many studies have examined the effects of probiotics added to the poultry diet on the presence of intestinal microorganism and performance (Souza et al., 2010; Dominguez et al., 2014; Ramos et al., 2014; Patel et al., 2015). However, the effects of probiotics on intestinal histological structure have been less studied.

MATERIAL AND METHODS

The trial was conducted in the Uludag University Animal Health and Production, Research and Application Centre Poultry Research Unit. The animal protocol was approved by the Animal Ethics Committee of Bursa Uludağ University Animal Experiments Local Ethics Committee (2020-02/05).

Animals, Managements and Experimental Design

A total of 200 1-day-old (8.87±0.08) Japanese (*Coturnix coturnix japonica*) quails, including both males and females, were obtained from Uludag University Animal Health and Production, Research and Application Centre quail breeding unit. The quails were randomly allocated to one control and three treatment groups containing 50 quails. Each group was randomly divided into five subgroups, comprised of 10 quails each and quails were tried to be distributed equally to each study group. Newly hatched chicks in all of the groups were reared under the same growing conditions, in brooding cages (colony type) in an open-sided house with mechanical ventilation. Gender determination was made in the third week of the trial. The quails were transferred randomly at the fourth week of age from the growing cages to laying cages (100 cm wide, 45 cm deep, 21 cm high in front, and 17 cm high in the rear, 112.5 cm^2 per quail) and housed there until the end of the study. All of the chicks were brooded and reared at 28 °C for the 1st wk, 27 °C for the 2nd wk, 24 °C for the 3rd wk, and 18-21 °C from the 28th day until the quails reached 42 days of age. The lighting schedule was 23 hr light with 1 hr dark period during the first 3 days, after then birds were subjected to 16 hr light with 8 hr dark until the end of the experiment.

Diets and Experimental Treatments

A basal diet was formulated and considered as control according to recommendation of NRC (1994). The composition of the diets and the content of nutrients are shown in Table 1. Group feeding was used in all treatment groups. Three experiment diets were formulated by supplemented the basal control diet with probiotic (CYLACTIN® LBC ME20 plus; DSM) (18 g ton^{-1}), essential oil blend (CRINA® Poultry plus; DSM) (300 g ton^{-1}) and mixture of probiotic and essential oil blend, respectively. The level of natural feed additives were based on the manufacturer recommendations. Cylactin LBC ME20 plus (DSM Nutritional Products Ltd., Basel, Switzerland) is a commercial micro-encaptulated microbial feed additive (probiotic) for the stabilization of the intestinal microbiota. The probiotic contains *Enterococcus faecium* in the amount of 2.0×10^{10} colony forming units (CFU) per gram. Crina poultry (DSM Nutritional Products Ltd., Basel, Switzerland) is a commercial essential oil. According to the information obtained from the manufacturer, the EO components used in this study mainly include thymol, eugenol, piperine and benzoic acid. It is a carefully balanced combination of essential oil compounds of high purity with a content of 872 g/kg in their natural/ nature-identical form on a carrier. The diet was fed to the quails in the form of mash and water *ad libitum* throughout the entire experimental period (42 d).

The nutritional composition of the diets was determined according to the AOAC (2000). Metabolisable energy (ME) levels of diets were estimated using the equation of Carpenter and Clegg (Leeson and Summers 2001): ME, kcal/kg = 53+ 38 [(CP, %) + (2.25 x ether extract, %) + (1.1 x starch, %) + (sugar, %)].

Performance Parameters

The quails were weighed individually at the beginning of the experimental period, after which the animals were weighed weekly to calculate live weight gains (LWG). Mortality was recorded when it occurred. Feed intake was recorded weekly and expressed as g per quail per week. The feed conversion ratio (FCR) was calculated as kg feed per kg body weight gain. Feed was weighed weekly to evaluate intake and FCR per cage. At the end of the experimental period, the sex ratio was established in each group and 20 male quails of each group (4 male quails from each replicate) were randomly selected and weighed to determine the carcass yield (CY). The quails were slaughtered by severing the jugular vein. The inert organs, heads and feet were removed after the carcasses were passed through a poultry defeathering machine. The chilled carcass weights were determined after allocation 18 hours at 4^{o}C, and then the CY was calculated.

Intestine Histomorphology

At the end of the feeding trial (d 42), 4 quails per pen (20 quails/group/male female mixed) were slaughtered and collect duodenum and, ileum for a histological examination. Intestinal segment samples (approximately 2 cm in length) were excised and flushed with 0.9% saline to remove the contents. Gut segments were fixed in 10% neutral-buffered formalin for histology. The tissues were dehydrated with increasing alcohol concentrations, cleared in xylene and embedded in paraffin. Five µm thick sections were cut from paraffin blocks, mounted on slides, and dried overnight. After dewaxing and rehydration, sections were used for Crossman's trichrome staining to determine morphological changes in tissue samples by light microscopy (Nikon 80i microscope) according to Culling et al. (1985). The morphometric indices evaluated were villus height from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, and the villus height to crypt depth ratio (Zhang et al., 2009). Villus absorptive surface area was calculated using the formula: Villus absorptive surface area = 2 π x (average villus width/2) x villus height.

Statistical Analysis

Statistical analysis was performed using the SPSS (1997) software package for Windows (SPSS Inc., Chicago, IL, USA). One way ANOVA was used to evaluate the effects of natural feed additives on performance. For intestine Duncan's test was used as a post-hoc test. The effects of plant volatile oil combinations on the sex ratio in quails were evaluated by the chi-square (0.117) test and the level of significance used in all of the tests was $p< 0.05$ (Dawson and Trapp, 2001). A Kruskal-Wallis test was used for the comparison of numerical variables among the groups for histological examination.

RESULTS

The ingredient and chemical composition of the diets is presented in Table 1. The mortality rate and the respective numbers of males and females did not significantly ($P > 0.05$) differ between the experimental groups (Table 3). Although there was no difference in the mortality rate between the groups, animal deaths were observed in the control group. The effects of using probiotic and essential oil blend separately and together on LW, LWG, feed intake, FCR and carcass characteristics are shown in Table 3. In the study, the addition of probiotic and essential oil blend to quail diet did not cause any difference in LW, LWG, FI and FCR parameters. In contrast, CY obtained at the end of the study was statistically significant between the control and experimental groups ($P < 0.001$). The highest carcass yield ($P < 0.001$) and carcass weight ($P > 0.05$) were measured in the experimental group animals with probiotics.

The effects of dietary treatment on the duodenum and ileum villus heights, crypt depths, villus surface areas and villus heights to crypt depth in the study are shown in Table 4. In addition, duodenum and ileum villus heights (Figure 1, Figure 3) and crypt depths (Figure 2, Figure 4) of the control and experimental groups, respectively, were examined histomorphologically and presented as figures. These parameters, which are an indicator of intestinal health, differed significantly ($P < 0.001$) between the groups. In the study, duodenal the villi heights and crypt depths were measured in the highest experimental groups compared to the control group. The highest villus height and crypt depths were determined in the experimental group materials in which probiotic and essential oil blend were used together. The largest villus surface area in the duodenum was detected in the experimental group animals with probiotics. Measurements in the parameters we specified showed the same results in the ileum section.

Table 1. Ingredients (%) and chemical composition of the basal diet

Ingredients, g/kg	Basal diet
Corn, grain	433.0
Soybean meal, CP 44%	360.0
Wheat	80.0
Corn gluten, CP 43%	60.0
Vegetable oil	30.0
$CaCO_3$	16.0
Dicalcium phosphate, DCP	6.20
Salt	3.00
L Lysine	3.50
DL Methionine	4.00
L Threonine	0.80
Vitamin mineral premix*	3.50
Analyzed Values, g/kg	
Metabolisable energy **, MJ/kg	12.56
Crude protein	243.6
Crude fiber	38.7
Ether extract	51.4
Ash	72.4
Dry matter	882.3

* Supplied the following per kilogram of diet: 3.000.000 IU vitamin A, 1.200.000 IU vitamin D_3, 0,36 g vitamin E, 1 mg vitamin K, 3 mg vitamin B1, 4 mg vitamin B2, 3 mg vitamin B6, 0.003 mg vitamin B12, 10 mg pantethonic acid, 20 mg niacin, 40 mg folic acid, 1 g choline, 0.3 mg biotin, 6 mg Cu, 300 mg I, 100 mg Fe, 0.2 mg Se, 60 mg Mn, 50 mg Zn

** Metabolisable energy content of diets was estimated using the equation of Carpenter and Clegg (Leeson and Summers, 2001)

Table 2. Study design for control and treatment groups of the feed additives

Feed Additives	**Control**	**Treatment 1**	**Treatment 2**	**Treatment 3**
CYCLACTIN LBC ME 20 Plus (probiotic) †	-	18 g ton^{-1}	-	18 g ton^{-1}
CRINA Poultry Plus (essential oil blend) ††	-	-	300 g ton^{-1}	300 g ton^{-1}

†: CYLACTIN® LBC ME 20 Plus is the best protected micro-encapsulated form, Enterococcus faecium NCIMB 10415 Min: $2x10^{10}$ cfu per gram

††: CRINA® Poultry Plus is the additive comprising a specific blend of essential oil components including thymol, eugenol and piperine. (benzoic acid 80%+ essential oil blend 10%).

Table 3. Live weight (LW), live weight gain (LWG), feed intake (FI), feed conversion ratio (FCR) and carcass characteristic of quails fed diets containing probiotic, essential oil blend and a mixture of both

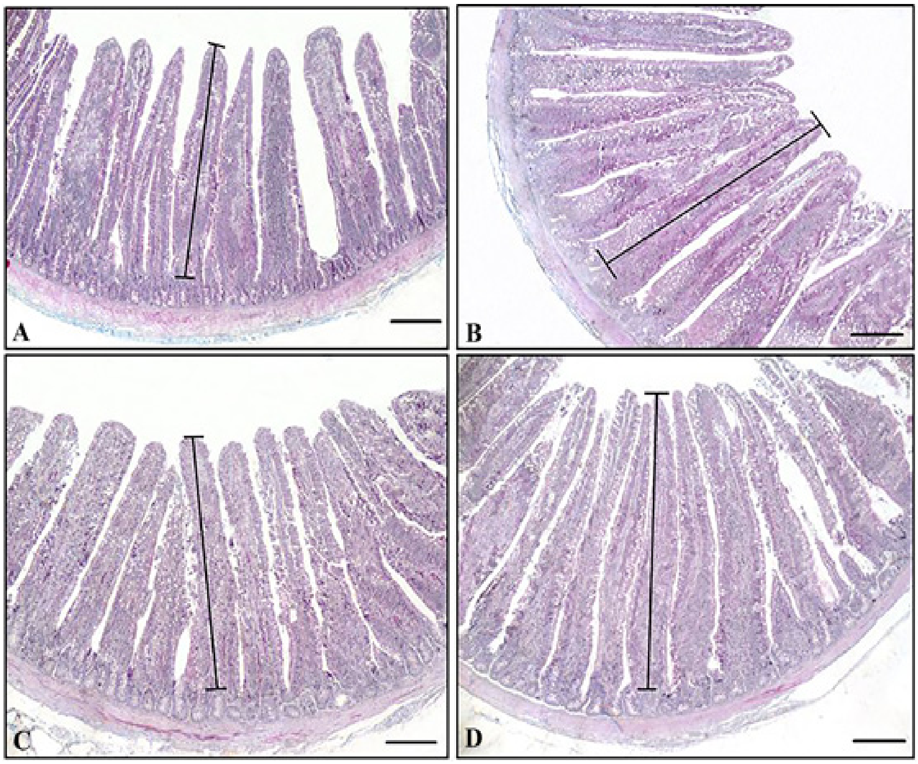
	Control	Treatment 1	Treatment 2	Treatment 3	P- values
Mortality (%)	4.00	0	0	0	0.261
Number of death	2	0	0	0	
Sex ratio (M/F)	29/19	31/19	30/20	30/20	1.00
LW (g)(n=50)					
1 d	8.84 ± 0.16	8.84 ± 0.16	9.12 ± 0.14	8.68 ± 0.15	0.264
21 d	99.34 ± 1.80	98.68 ± 2.15	99.22 ± 1.77	98.36 ± 1.61	0.979
42 d	191.00 ± 3.81	194.54 ± 3.59	200.75 ± 4.03	192.74 ± 3.27	0.267
LWG (g)(n= 5)					
1 to 21 d	90.51 ± 2.59	90.12 ± 4.13	90.26 ± 2.45	89.86 ± 1.60	0.999
22 to 42 d	91.25 ± 3.41	96.76 ± 2.77	101.52 ± 4.41	94.37 ± 2.17	0.235
1 to 42 d	181.76 ± 5.05	186.86 ± 3.09	191.78 ± 2.60	184.24 ± 4.24	0.332
FI (g) (n=5)					
1 to 21 d	287.82 ± 9.75	340.54 ± 11.74	333.84 ± 24.66	353.24 ± 17.56	0.070
22 to 42 d	500.16 ± 26.56	497.68 ± 13.87	482.80 ± 18.65	493.76 ± 15.75	0.923
1 to 42 d	787.98 ± 29.43	838.22 ± 9.76	816.64 ± 16.07	847.00 ± 20.92	0.214
FCR (g/g) (n=5)					
1 to 21 d	3.19 ± 0.18	3.82 ± 0.28	3.72 ± 0.34	3.94 ± 0.26	0.271
22 to 42 d	5.49 ± 0.29	5.17 ± 0.26	4.81 ± 0.35	5.23 ± 0.14	0.390
1 to 42 d	4.34 ± 0.17	4.48 ± 0.071	4.26 ± 0.11	4.60 ± 0.17	0.349
Carcass characteristic (n=20)					
Body weight (g)	193.84 ± 6.28	194.84 ± 5.45	203.46 ± 8.78	193.81 ± 5.49	0.687
Carcass weight (g)	136.79 ± 3.22	142.43 ± 3.57	138.67 ± 4.69	136.34 ± 3.46	0.658
CY (%)	71.00 ± 0.98^{b}	73.26 ± 0.58^{a}	68.77 ± 0.92^{b}	70.48 ± 0.42^{b}	0.001

^{a,b,c} Mean values with different superscripts in the same row differ significantly ($p< 0.05$) M: Male F: Female LW: Live weight LWG: Live weight gain FI: Feed intake
FCR: Feed conversion rate CY: Carcass Yield Mortality and M/F: Groups were tested by homogeneity test (chi-square)
Treatment1: probiotic Treatment2: essential oil blend Treatment3: probiotic+essential oil blend

Table 4. Histomorphological characteristics of dudenum and ileum of quails fed diets containing probiotics, essential oils, and a mixture of both

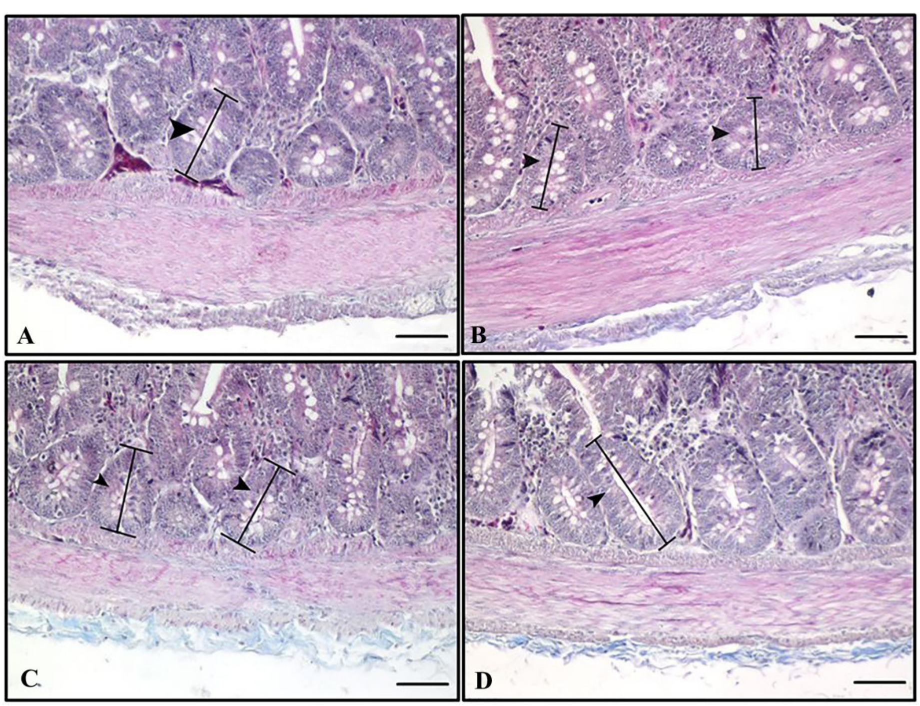
Parameters	Control	Treatment 1	Treatment 2	Treatment 3	P- values
DUODENUM (n=20)					
Villus height, µm	824.75 ± 18.66^{a}	992.00 ± 32.55^{c}	886.80 ± 13.52^{b}	1137.95 ± 15.52^{d}	0.001
Crypt depth, µm	364.25 ± 15.49^{a}	445.71 ± 24.99^{bc}	438.20 ± 14.89^{b}	475.95 ± 22.12^{bcd}	0.001
Villus surface area, mm^2	0.47 ± 0.04^{a}	0.83 ± 0.04^{c}	0.64 ± 0.037^{b}	0.78 ± 0.04^{c}	0.05
Villus height to crypt depth	2.34 ± 0.11^{b}	2.35 ± 0.14^{ab}	2.06 ± 0.06^{a}	2.49 ± 0.12^{bc}	0.05
ILEUM (n=20)					
Villus height, µm	890.00 ± 19.96^{b}	955.65 ± 13.42^{c}	829.05 ± 17.75^{a}	1021.55 ± 12.21^{d}	0.001
Crypt depth, µm	238.40 ± 15.30^{a}	266.75 ± 7.71^{b}	307.65 ± 12.29^{c}	314.70 ± 13.89^{cd}	0.001
Villus surface area, mm^2	0.82 ± 0.004^{a}	0.95 ± 0.05^{ab}	0.95 ± 0.06^{ab}	1.00 ± 0.04^{b}	0.05
Villus height to crypt depth	3.95 ± 0.22^{c}	3.63 ± 0.10^{bc}	2.76 ± 0.10^{a}	3.36 ± 0.15^{b}	0.05

^{a,b,c,d} Mean values with different superscripts in the same row differ significantly ($p< 0.05$)
Treatment1: probiotic Treatment2: essential oil blend Treatment3: probiotic+essential oil blend



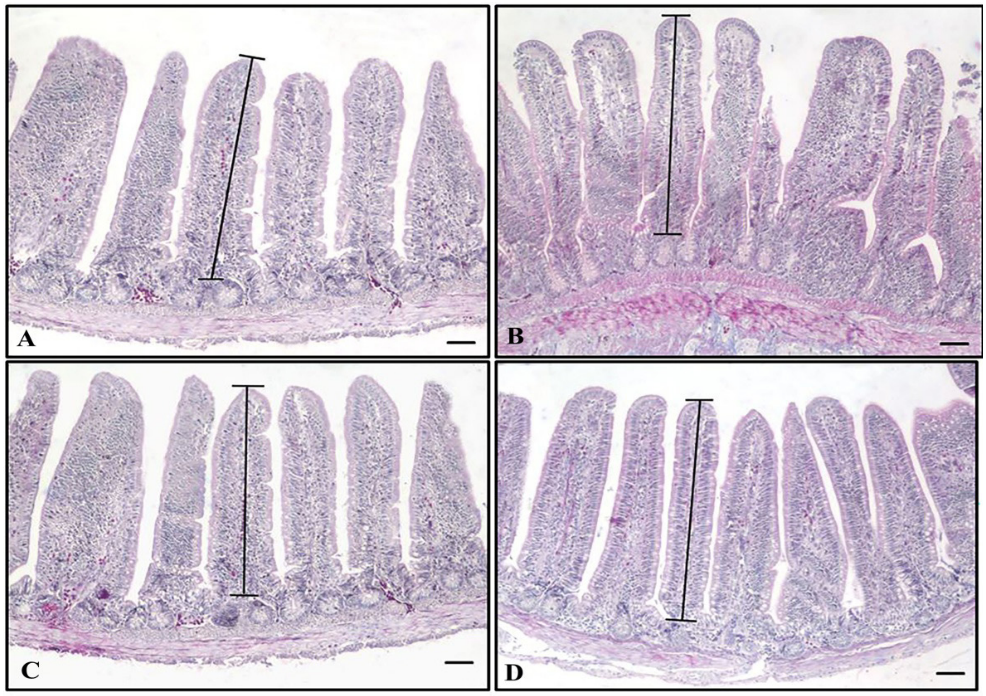
Control (A), Probiotic (B), Essential oil blend (C) and Probiotic + Essential oil blend (D) groups.
Crossman's triple stained. Bar: 25 µm.

Figure 1. Morphological evaluation of mean villi height for duodenal cross sections for control and experimental groups



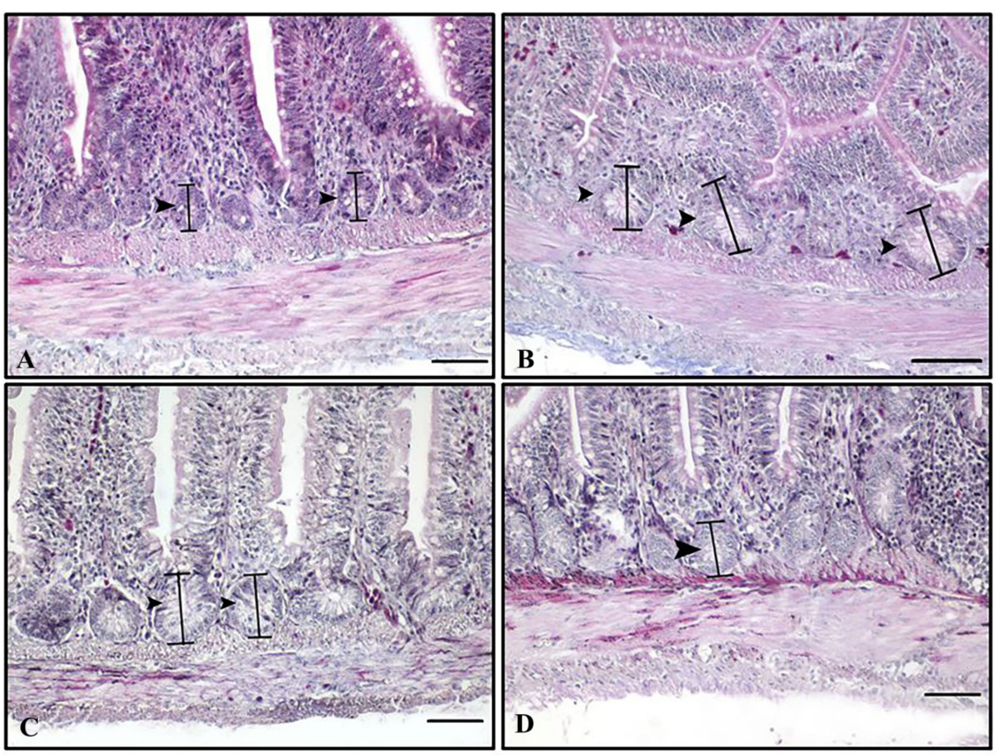
Control (A), Probiotic (B), Essential oil blend (C) and Probiotic +Essential oil blend (D) groups.
Crossman's triple stained. Arrow head (crypt depth measurement) Bar: 50 µm.

Figure 2. Morphological evaluation of mean crypt depths for duodenal cross sections for control and experimental groups.



Control (A), Probiotic (B), Essential oil blend (C) and Probiotic + Essential oil blend (D) groups.
Crossman's triple stained. Bar: 25 µm.

Figure 3. Morphological evaluation of mean villi height for ileum cross sections for control and experimental groups



Control (A), Probiotic (B), Essential oil blend (C) and Probiotic + Essential oil blend (D) groups.
Crossman's triple stained. Arrow head (crypt depth measurement) Bar: 50 µm.

Figure 4. Morphological evaluation of mean crypt depths for ileum cross sections for control and experimental groups

DISCUSSION

After most anticoccidial and performance enhancing antibiotics were banned, the poultry industry faced problems such as growth performance and disease control. It should not be forgotten that disease control can only be achieved with a healthy digestive tract. If the digestive tract is healthy, an improvement in performance can be observed by obtaining maximum benefit from nutrients. Therefore, new period research has focused on feed additives that are natural, have no residue and are effective in intestinal health. Probiotic and essential oils which are among these additives, form the basis of this research. For this purpose, it was aimed to investigate the use of probiotics, essential oil blend and their combined use, primarily performance data, digestive tract histomorphological structure and digestion-absorption areas.

In the study, the addition of probiotic, essential oil blend and combination use of 1-day-old quails to the diet for 6 weeks did not affect mortality, LW, LWG, FI, FCR and carcass weight. Although the mortality and number of death did not make a significant difference between the control and experimental groups, the highest number of deaths was determined in the control group. Awad et al. (2009) found in their study that the probiotic addition of lactobacillus to broiler diets caused a decrease in mortality compared to the control group. Through competitive exclusion, probiotics could potentially reduce mortality with a negative effect on enteric pathogens, but in trials with broilers given Lactobacillus preparations, the effects on mortality were inconsistent (Jin et al., 1998, 2000; Zulkifli et al., 2000). The addition of essential oils to diets has a positive effect on the mortality rate in animals due to their antimicrobial effects (Zhai et al., 2018; Sevim et al., 2021). However, many studies have been conducted with essential oils, and studies have found that their effectiveness on performance is variable. The main reasons for this variability are related to essential oils, animals, diets and the environment.

In this study, the addition of probiotics into quail's feed had no effect on the performance, contrary to previous studies that have showed improvements. Ramos et al. (2014) reported increased FI and WG with the probiotics utilization (*Lactobacillus acidophilus, Bifidobacterium bifidum and Streptococcus faecium*). Cengiz et al. (2015) reported that dietary probiotic increased the FI and WG in broilers as compared to those fed un-supplemented diets. On the other hand, other researchers have not verified the effect of probiotic supplementation on performance (Shargh et al., 2012; Nosrati et al., 2017). Among the performance parameters in the study, only the carcass yield was found to be significantly higher in the experimental group animals that were supplemented with probiotics. Jin et al. (1998) considered that the variation in the efficiency of probiotics might occur due to different bacterial species, strains, or method of preparation of the probiotic supplement. Also, it can be explained in terms of variation in product stability, viability, type, and dose. Including this study, probiotics belonging to Enterococcus species have a distinct advantage over performance. These are highly competitive mainly due to their resistance to a wide range of pH and temperature. Their competitiveness also owes their ability to produce bacteriocins, known for their wide-ranging action on pathogenic bacteria (Hanchi et al., 2018).

In the current digesstudy, the effects of two different natural feed additives presented as an alternative to antibiotics on intestinal morphology were also examined. Among these natural additives, probiotics, essential oil mixes and their combined use caused significant differences on the small intestine morphology. The small intestine is an important organ responsible for the digestion and absorption of nutrients from the diet. The duodenum and ileum, where nutrients are absorbed extensively from the small intestine, were examined in the study. As a result, the use of nutrients in the feed structure is closely related to the intestinal structure and villus health. In the study, villus heights in both small intestine segments were found to be higher in the experimental groups compared to the control group. Among the experimental groups, the highest small intestine villi heights were found in the animals in which feed additives were used together. In the study, the surface area of the samples with high villi height was determined to be wide. It is clear that longer villi lead to increased villus surface area in the small intestine. This is in agreement with the findings of Pelicano et al. (2005) who observed a greater crypt depth in the intestinal mucosa of broilers fed probiotics based on Bacillus spp. In another study, supplementations of probiotic, prebiotic and synbiotic groups increased the length and surface area of villi in the ileum and jejunum, compared with the positive control and antibiotic groups (Al-Baadani et al., 2016). These findings are in line with the results of Samanya and Yamauchi (2002), who reported that *B. subtilis* led to an increase in villus height in the small intestine. Abdel-Raheem et al. (2012) and Sen et al. (2012) reported that dietary supplementations of *B. subtilis* increased villus length in comparison with the dietary control. Unlike these studies Chichlowski et al. (2007) demonstrated that the villus width was not affected by probiotic and antibiotic flavomycin compared to the basal diet of broilers.

Essential oils significantly improved gut morphology (Du et al., 2016). The height of intestinal villi is connected with the capacity of the bird to absorb nutrients from feed. Longer villi are typically associated with excellent gut health and high absorptive efficiency. Studies show that organic acids such as benzoic acid (Giannenas et al., 2014; Tzora et al., 2017; Gong et al.,2021) and essential oils obtained from aromatic herbs (Mohammadi et al.,2014; Zeng et al., 2015; Zhang et al., 2021) are widely used as effective additives to improve gut health and thus performance in monogastric animals. Giannenas et al. (2014) determined in their studies that the addition of a mixture of essential oil and benzoic acid to the diet caused significant increases in the height of the jejunal and ileal villi. Studies have shown that an increase in villus length is typically excellent gut health and performance (Giannenas et al., 2014; Tzora et al., 2017; Gong et al.,2021), whereas short villi and deep crypts may lead to poor nutrient absorption, increased toxin secretion in the gastrointestinal tract and worse performance (Miles et al., 2006). Yang et al. (2018) determined that the addition of thymol mixture with fumaric and sorbic acid to the diet during broiler growth period caused a significant increase in villi height. In this study, a significant increase was noted in the duodenal and ileal villus heights of the experimental groups. The lowest villi height was measured in the samples of the control group. However, the difference in villus height did not cause a significant effect on performance parameters. Histological parameter values were found to be higher especially in the experimental groups in which the probiotic and essential oil mixture was used together.

CONCLUSIONS

As a result, the combination of probiotic and essential oil, which are feed additives effective on intestinal health by affecting the intestinal histomorphology, strengthened the effect of each other and led to more significant results. In short, it was concluded that the use of feed additives separately and together for this study is effective in maintaining the important intestinal histomorphological structure in the intestinal balance

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest

REFERENCES

Abdel-Raheem SM, Abd-Allah SM, Hassanein KM (2012) The Effects of Prebiotic, Probiotic and Synbiotic Supplementation on Intestinal Microbial Ecology and Histomorphology of Broiler Chickens. Int J Agro Vet Med Sci 6(????): 277-289.

Al-Baadani HH, Abudabos AM, Al-Mufarrej SI, Alzawgari M (2016) Effects of Dietary Inclusion of Probiotics, Prebiotics and Synbiotics on Intestinal Histological Changes in Challenged Broiler Chickens. South African J Anim Sci 46 (2): 157-165.

AOAC (2000) Official Methods for Analysis. 17th ed. Maryland USA: Association of Official Analytical Chemists.

Awad WA, Ghareeb K, Abdel-Raheem S, Bohm J (2009) Effects of Dietary Inclusion of Probiotic and Synbiotic on Growth Performance, Organ Weights, and Intestinal Histomorphology of Broiler Chickens. Poult Sci 88(??): 49-56.

Cengiz SS, Yesilbag D, Meral Y, Cetin I, Biricik H (2015) Juniper Oil Improves Oxidative Stability and Eggshell and Albumin Quality of Quail Eggs. Br Poult Sci 56(1): 58-65.

Chichlowski M, Croom J, Mcbride B, Daniel L, Davis G, Koci M (2007) Direct-Fed Microbial PrimaLac and Salinomycin Modulate Whole-Body and Intestinal Oxygen Consumption and Intestinal Mucosal Cytokine Production in the Broiler Chick. Poult Sci 86(6): 1100-1106.

Choct M. (2009) Managing Gut Health through Nutrition. Br Poult Sci 50(1): 9-15.

Crosnier C, Stamataki D, Lewis J (2006) Organizing Cell Renewal in the Intestine: Stem Cells, Signals and Combinatorial Control. Nat Rev Genet 7: 349-359.

Culling CFA, Allinston RT, Barr WT (1985) Cellular Pathology Technique: 642-647 ed. Butterworths, London, UK: Connective Tissue.

Dawson B, Trapp RG (2001) Basic and Clinical Biostatistics. 3rd ed. New York, USA: Lange Medical Books.

Domingues CH, Santos ET, Castiblanco DC, De Quadros TCO, Petrolli TG (2014) Avaliação do Desempenho e Rendimento de Carcaça de Frangos de Corte Alimentados com Dietas Contendo Probiótico nas Diferentes Fases de Criação. Rev Agrocientífica 1(1): 7-16.

Du E, Wang W, Gan L, Li Z, Guo S, Guo Y (2016) Effects of Thymol and Carvacrol Supplementation on Intestinal Integrity and Immune Responses of Broiler Chickens Challenged with Clostridium Perfringens. J Animal Sci Biotechnol 7: 19.

Galdeanoa CM, Cazorlaa SI, Dumit JML, Vélez E, Perdigóna G (2019) Beneficial Effects of Probiotic Consumption on the Immune System. Ann Nutr Metab 74:115-124.

Garcia V, Catala-Gregori P, Hernandez F, Megias MD, Madrid J (2007) Effect of Formic Acid and Plant Extracts on Growth, Nutrient Digestibility, Intestine Mucosa Morphology and Meat Yield of Broilers. J Appl Poult Res 16: 555-562.

Giannenas I, Florou-Paneri P, Botsoglou NA, Christaki E, Spais AB (2005) Effect of Supplementing Feed with Oregano and/or a-Tocopheryl Acetate on Growth of Broiler Chickens and Oxidative Stability

of Meat. J Anim Feed Sci 14(3):521-535.
Giannenas I, Florou-Paneri P, Papazahariadou M, Botsoglou NA, Christaki E, Spais AB (2003) Effect of Dietary Supplementation with Oregano Essential Oil on Performance of Broilers After Experimental Infection with Eimeria Tenella. Arch Anim Nutr 57(2): 99-106.
Giannenas I, Papaneophytou C, Tsalie E, Mavridis S, Triantafillou E, Kontopidis G, Tontis D (2014) Effect of Benzoic Acid and Essential Oil Compounds on Performance of Turkeys, Intestinal Microbiota, Intestinal Morphology and Antioxidant Status. Asian-Australas J Anim Sci 27(2): 225-236.
Gong H, Yang Z, Celi P, Yan L, Ding X, Bai S, Zeng Q, Xu S, Su Z, Zhuo Y, Zhang K, Wang J (2021) Effect of benzoic acid on production performance, egg quality,intestinal morphology, and cecal microbial community of laying hens. Poult Sci 100(1): 196-205.
Hanchi H, Mottowea W, Sebei K, Hammami R (2018) The Genus Enterococcus: Between Probiotic Potential and Safety Concerns—An Update. Front Microbiol 9: 1971. Issue not found
Jin LZ, Ho YW, Abdullah N, Jalaludin S (1998) Growth Performance, Intestinal Microbial Populations and Serum Cholesterol of Broilers Fed Diets Containing Lactobacillus Cultures. Poult Sci 77: 1259-1265.
Kassie GAM (2008) The Effect of Anise and Rosemary on Broiler Performance. Int J Poult Sci 7:243-245.
Lee KW, Kim J, Oh S, Kang CB (2015) Effects of Dietary Sanguinarine on Growth Performance, Relative Organ Weight, Cecal Microflora, Serum Cholesterol Level and Meat Quality in Broiler Chickens. J Poult Sci 52(1): 15-22.
Leeson S, Summers JD (2001) Nutrition of the Chicken. 4th ed. Guelph, ON: University Books.
Miles RD, Butcher GB, Henry PR, Littell RC (2006) Effect of Antibiotic Growth Promoters on Broiler Performance, Intestinal Growth Parameters, and Quantitative Morphology. Poult Sci 85: 476-485.
Mohammadi Z, Ghazanfari S,. Moradi MA (2014) Effect of supplementing clove essential oil to the diet on microflora population, intestinal morphology, blood parameters and performance of broilers Eur Poult Sci 78: 1-11.
Nosrati M, Javandel F, Camacho LM, Khusro A, Cipriano M, Seidavi A (2017) The Effects of Antibiotic, Probiotic, Organic Acid, Vitamin C, and Echinacea Purpurea Extract on Performance, Carcass Characteristics, Blood Chemistry, Microbiota, and Immunity of Broiler Chickens. J Appl Poultry Res 26 (2): 295-306.
NRC (1994) Nutrient Requirements of Poultry. 9th ed. Washington, DC: National Academy Press.
Pan D, Yu Z (2014) Intestinal Microbiome of Poultry and its Interaction with Host and Diet. Gut Microbes 5: 1088-19.
Patel SG, Raval AP, Bhagwat SR, Sadrasaniya DA, Patel AP, Joshi SS (2015) Effects of Probiotics Supplementation on Growth Performance, Feed Conversion Ratio and Economics of Broilers. J Anim Res 5(1): 155-160.
Pelicano ERL, Souza PA, Souza HBA, Figueuredo DFII, Boiago MMI, Carvalho SRI, Bordon VFI (2005) Intestinal Mucosa Development in Broiler Chickens Fed Natural Growth Promoters. Br J Poult Sci 7(4):221-229.
Peterson LW, Artis D (2014) Intestinal Epithelial Cells: Regulators of Barrier Function and Immune Homeostasis. Nat Rev Immunol 14(3): 141-153.
Ramos LSN, Lobes JB, Ribeiro MN, Silva FES, Merval RR, Albuquerque DMN (2014) Aditivos alternativos a antibióticos para frangos de corte no período de 22 a 42 dias de idade. Rev Bras Saúde Prod Anim 15(4): 897-906.
Salois MJ, Cady RA, Heskett EA (2016) The Environmental and Economic Impact of Withdrawing Antibiotics from US Broiler Production. J Food Distrib Res 47(1): 1-2.
Samanya M, Yamuachi K (2002) Histological Alterations of Intestinal Villi in Chickens Fed Dried Bacillus Subtilis var. Natto. Comp Biochem Physiol A Mol Integr Physiol 133(1): 95-104.
Sen S, Ingale SL, Kim YW, Kim JS, Kim KH, Lohakare JD, Kim EK, Kim HS, Ryu MH, Kwon IK, Chae BJ (2012) Effect of Supplementation of Bacillus Subtilis LS 1-2 to Broiler Diets on Growth Performance, Nutrient Retention, Caecal Microbiology and Small Intestinal Morphology. Res Vet Sci 93(1): 264-268.
Sevim B, Gümüş E, Harman H, Ayaşan T, Başer E, Altay Y, Akbulut K (2020) Effects of Dietary Rosemary Essential Oil on Growth Performance, Carcass Traits and Some Hematological Values of Chukar Partridge. Turkish JAF Sci Tech 8(2):430-435
Shargh MS, Dastar B, Zerehdaran S, Khomeiri M, Moradi A (2012) Effects of Using Plant Extracts and a Probiotic on Performance, Intestinal Morphology, and Microflora Population in Broilers. J Appl Poult Res 21(2): 201-208.
Shang Y, Kumar S, Oakley B, Kim WK (2018) Chicken Gut Microbiota: Importance and Detection Technology. Front Vet Sci 5:254.
Silva MAN, Barbosa Filho JAD, Silva CJM, Rosario MF, Silva IJO, Coelho AAD, Savino VJM (2007) Avaliação do estresse térmico em condição simulada de transporte de frangos de corte. R Bras Zootec 36 (4):1126-1130.
SPSS (1997) Statistical Software for Windows Version 7.5 Microsoft Windows Chicago, IL.
Souza LFAD, Araujo DN, Astoplhi JLDL, Ambiel AC, Santos LS, Carmo AJ, Silva PCG (2010) Probiótico e antibiótico como promotores de crescimento para frangos de corte. Colloq Agr 5(1) 33-39.
Tufarelli V, Baghban-Kanani P, Azimi-Youvalari S, Hosseintabar-Ghasemabad B, Slozhenkina M, Gorlov I, Seidavi A, Ayaşan T, Laudadio V (2021). Effects of Horsetail (Equisetum arvense) and Spirulina (Spirulina platensis) Dietary Supplementation on Laying Hens Productivity and Oxidative Status. Animals 11: 335.
Tzora A, Giannenas I, Karamoutsios A, Papaioannou N, Papanastasiou D, Bonos E, Skoufos S, Bartzanas T, Skoufos I (2017) Effects of Oregano, Attapulgite, Benzoic Acid and their Blend on Chicken Performance, Intestinal Microbiology and Intestinal Morphology. J Poult Sci 54(3): 218-227.
Yang X, Xin H, Yang C, Yang X (2018) Impact of Essential Oils and Organic Acids on the Growth Performance, Digestive Functions and Immunity of Broiler Chickens. Anim Nutr 4(4): 388-393.
Yesilbag D, Biricik H, Gezen SS, Meral Y (2013) Effect of a Rosemary and Oregano Volatile Oil Mixture on Performance, Egg Traits and Oxidative Stability of Eggs in Laying Quails. Br Poult Sci 54(2): 231-237.
Yesilbag D, Cengiz SS, Cetin I, Meral Y, Biricik H (2014) Influence of Juniper (Juniperus Communis) Oil on Growth Performance and Meat Quality as a Natural Antioxidant in Quail Diets. Br Poult Sci 55(4): 495-500.
Zeng Z, Zhang S, Wang H, Piao X (2015) Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. J Anim Sci Biotechno (2015) 6(7): 2-10.
Zhai H, LIU H, Wang S, Wu J, Kluenter, AM (2018) Potential of Essential Oils for Poultry and Pigs. Anim Nutr 4(2): 179-186.
Zhang JJ, Piao XS, Huang DS, Wang JJ, Ma X, Ma YX (2009) Effects of Feed Particle Size and Feed Form on Growth Performance, Nutrient Metabolizability, and Intestinal Morphology in Broiler Chickens. Asian- Aust J Anim Sci 22(1):107-112.
Zhang LY, Peng QY, Liu YR, Ma QG, Zhang JY, Guo YP, Xue Z, Zha LH (2021) Effects of Oregano Essential Oil as an Antibiotic Growth Promoter Alternative on Growth Performance, Antioxidant Status, and Intestinal Health of Broilers. Poult Sci 100 (7):101-163.
Zulkifli I, Abdullah N, Azrin NM, HO YW (2000) Growth Performance and Immune Response of Two Commercial Broiler Strains Fed Diets Containing Lactobacillus Cultures and Oxytetracycline Under Heat Stress Conditions. Br Poult Sci 41(5):593-597.