Effects of oregano or red pepper essential oil supplementation to diets for broiler chicks with delayed feeding after hatching. 1. Performance and microbial population

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Primary Audience: Nutritionists, Feed Additive Companies, Researchers, Veterinarians, Production Managers

SUMMARY

This study was conducted to investigate the effects of dietary supplementation of oregano or red pepper essential oil on the performance, digestive organs, serum biochemistry parameters, and microbial population of the small intestine of broilers with immediate, or 24- or 48-h posthatching delayed access to diet and water. The dietary treatments included (1) a non-supplemented corn-soybean meal diet (CONT), (2) CONT + 250 mg/kg of oregano essential oil (OO250), and (3) CONT + 250 mg/kg of red pepper essential oil (RPO250). Irrespective of dietary treatment, especially delayed access to diet and water for 48 h posthatch significantly decreased daily BW gain from 0 to 21 d and daily feed intake from 4 to 21 d and increased the relative weight of the yolk sac at 3 d of broilers. The relative weight of the liver or gizzard of chickens at 21 d was significantly decreased by delayed access to diet and water for 24 h post-hatch. The diet containing RPO250 significantly increased the relative weight of the pancreas at 21 d. Delayed access to diet and water for 24 or 48 h posthatch significantly reduced serum glucose levels at 21 d. The serum aspartate aminotransferase level in broilers given immediate access to feed and water was significantly decreased by the diet containing RPO250. Generally, the coliform bacteria and total yeast contents of the small intestine of chickens were significantly increased by extending the time to access to feed and water for broilers. Total aerobic bacteria contents of the small intestine of broilers with immediate, or 24- or 48-h posthatching delayed access to diet and water was significantly decreased by CONT, OO250, and RPO250 diets.

Key words: broiler, essential oil, microflora, oregano, performance, red pepper

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DESCRIPTION OF PROBLEM

In a commercial hatchery, a fasting period of 24 to 72 h after hatch to transportation to the broiler farm is generally common, due to variation hatching time and logistics [1]. This delay in the start of diet and water intake leads to dehydration and yolk depletion as birds are hauled

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and spend up to 72 h without access to feed or water [1, 2], which further increases the fasting period experienced by the chicks [3]. Any delay in diet and water intake can have detrimental effects on the performance of the chicks with respect to growth, immune system activation, organ development, digestive enzyme stimulation, and microbial population of the gastrointestinal system [1]. Newly hatched chicks become more susceptible to pathogens, increased weight loss, and the development of critical tissues is restricted with delayed access to diet and water [3]. At the time of hatch, the gastrointestinal tract of the chick is typically sterile. The establishment of the bacterial populations occurs posthatch; the number as well as diversity of these bacteria with feeding and age is changed and remained relatively stable thereafter [4]. The early development of the gastrointestinal tract and maintenance of a healthy gut microflora is essential to the future of well-being and growth performance of the chick during the rearing period.

In an attempt to reduce the negative effects of delayed feeding, recent researchers have evaluated the usage of feed supplements. The limited use of antibiotic growth promoters as the feed supplement has stimulated investigations on alternative feed additives in animal nutrition. Essential oils derived from herbs and spices have gained interest as alternative feed additives in recent years. The concept behind the early use of these supplements is to provide the early morphological and physiological development of the small intestine and activation of digestive enzymes of posthatched chicks. Two of these essential oils are oregano and red pepper essential oils [5–7].

Oregano, a characteristic spice of Turkey is obtained by drying leaves and flowers of *Origanum vulgare* and *onites* spp. growing in the wild and cultivating appears out of place. Oregano essential oil extracted from the oregano herb has 2 major phenols, which are carvacrol and thymol, constituting about 78 to 85% of the essential oil [7]. Thymol and carvacrol disrupt the membrane integrity of microbes, which affects the pH homeostasis and equilibrium of inorganic ions [8].

Red pepper was obtained locally from red pepper fruit. Red pepper essential oil extracted from *Capsicum annuum* L. has a series of compounds called capsaicinoids. Capsaicinoids possess antimicrobial activities against pathogenic bacteria, including *Escherichia coli*, *Clostridium perfringens*, and *Salmonella enteritidis* [9], and powerful antioxidant, digestion stimulant, antiinflammatory, and antidiarrheal activities promote energy consumption and suppress the accumulation of fats in the organism [10–12]. Scientific evidence exists that herbs and plant extracts stimulate the growth of beneficial bacteria and limit numerous pathogenic bacterial activities in the gut of poultry [13, 14].

The objective of this study was to investigate the effects of the supplementation of oregano or red pepper essential oil to the diet of broilers with immediate, or 24- or 48-h posthatching delayed access to diet on growth performance, growth of digestive organs, serum biochemistry profile, and microbial populations of the small intestine of broilers from hatching to 21 d of age.

MATERIALS AND METHODS

Chicks and Housing

A total of 432 female Ross 308 broiler chicks (Ross Breeders Anadolu, Elmadağ/Ankara, Turkey) were obtained from a local hatchery, where time of hatch was defined as time of clearing the shell. Then, the chicks were wing banded, weighed, and randomly assigned to 9 groups of similar mean weight, each of which included 16 chicks for each of the 3 replicates. The chicks were kept in wire cages [(105 × 70 cm) and 5 cm of feeder space per bird] equipped with nipple drinkers under standard environmental conditions throughout the experiment. A continuous lighting program was provided during the experiment. Relative humidity throughout the experiment was 60 to 70%. The temperature was set at 28.6 to 30.5°C on the first day, 27.6 to 29.5°C on d 3, 26.6 to 28.5°C on d 6, 25.6 to 27.5°C on d 9, 23.8 to 25.0°C on d 12, 22.5 to 24°C on d 15, 21.5 to 23.0°C on d 18, and 20.5 to 22.0°C on d 21, respectively. The experimental producers used in this trial were approved by the University of Ankara Institutional Animal Care and Use Committee (Ankara, Turkey) and were in compliance with recommended guidelines.

Dietary Treatments

In a 3 × 3 factorial arrangement, broiler chicks were given access to water and diet at

3 different feeding times (immediate, or 24- or 48-h posthatching delayed feeding) and fed 1 of 3 different diets, which contained corn-soybean meal based in mash form. Prior to experimental diet formulation, feed ingredients were analyzed for their DM, CP, EE, CF, starch, and total sugar content according to the methods of AOAC International [15]. The ME of feed ingredients was calculated based on analyzed values of feedstuffs [16]. All values were expressed on a DM basis.

During the rearing period that lasted 21 d, the experimental diets and drinking water were supplied ad libitum. The ingredients and nutritional composition of the commercial basal diet are given in Table 1. The diets were formulated to meet or exceed minimum NRC [17] standards for all ingredients. The 3 experimental diets were as follows: diet 1 (control diet, **CONT**) was a commercial diet that contained no essential oil, diet 2 (**OO250**) supplemented CONT with oregano essential oil at 250 mg/kg, and diet 3 (**RPO250**) supplemented CONT with red pepper essential oil at 250 mg/kg.

The red pepper (*Capsicum annuum* L.) was harvested in August 2009 at the Department of Sericulture, University of Gaziosmanpasa (Tokat, Turkey). The red pepper was washed and dried in a drying oven at 50°C for 6 to 8 h. Then, it was cut into pieces of 4.0 ± 0.2 mm in thickness. The dried material was ground to a fine powder, passed through a 60-mesh sieve and kept in an airtight container at 4°C until further use. The essential oil was distilled from the ground samples using a Clevenger distillation apparatus [18] in accordance with the US Pharmacopeia and the National Formulary (1995), following US Pharmacopoeia methods. First, 100 g of ground sample was submitted to water distillation for 2 h using the Clevenger apparatus to obtain essential oil.

The oregano essential oil was provided by the Altes Agricultural Products Ltd. Company (Antalya, Turkey). Oregano essential oil also obtained by steam distillation using the Clevenger distillation apparatus and derived from *Origanum onites* spp. growing wild in Turkey was used in the study.

The 2 most-active compounds of the essential oils were determined by a gas chromatography-mass spectrometry (HP 6890GC/5973 MSD [19]) system. The essential oil obtained was diluted with *n*-hexane (1:100) and injected into the gas chromatography-mass spectrometry system [injection temperature: 250°C; injection split: 1/100; column: DB-17 30 m, 0.25 μm, 0.32 mm [20]; oven program: initial temperature, 70°C, rate 8°C/min, final temperature: 200°C; injection vol.: 1 μL].

The carvacrol and thymol contents, which are the most active compounds of oregano es-

Table 1. The ingredients and chemical composition of the basal diet

Item	Amount, g/kg
Ingredient	
Maize	549.05
Soybean meal (46% CP)	360.87
Fish meal (65% CP)	15.00
Sunflower oil	37.08
Limestone	11.41
Dicalcium phosphate	14.89
Salt	3.50
Vitamin premix[1]	2.50
Trace mineral premix[2]	1.00
DL-Methionine	3.20
L-Lysine-HCL	1.50
Total	1,000.00
Analyzed chemical composition	
DM, %	88.64
CP, % of DM	22.94
CF, % of DM	3.10
Crude ash, % of DM	5.92
Crude fat, % of DM	6.11
Calcium, % of DM	0.98
Total phosphorus, %	0.73
Calculated composition	
ME, kcal/kg	3,105
CP, %	23.01
Calcium, %	1.01
Available phosphorus, %	0.45
Lysine, %	1.35
Tryptophan, %	0.25
Arginine, %	1.49
Methionine, %	0.62
Methionine + cystine, %	0.99
Threonine, %	0.86

[1]Vitamin premix provided the following (per kilogram of diet): *trans*-retinol, 3,600 μg; cholecalciferol, 15.0 μg; α-tocopherol acetate, 50 mg; vitamin K_3, 5 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; niacin, 25 mg; Ca-D-pantothenate, 12 mg; folic acid, 1 mg; D-biotin, 0.05 mg; apocarotenoic acid ester, 2.5 mg; and choline chloride, 400 mg.

[2]Trace mineral premix provided the following (per kilogram of diet): Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.20 mg; I, 1 mg; and Se, 0.15 mg.

sential oil, were determined to be 84.02 and 1.78%, respectively. The quercetin and luteolin contents, which are the most active compounds of red pepper essential oil, were determined to be 20.65 and 8.80%, respectively.

Oregano or red pepper essential oil was added to an amount of sunflower oil and homogenized by mixer and then the mixture was pulverized with the maize. Maize with essential oil was added to the premixture. Finally, the premixture was added to the main mixture. Diets were prepared weekly and stored in airtight containers.

Measurements

Body weights of broilers in each experimental treatment were measured on d 0, 3, and 21. Chicks were weighed to ± 0.001 g at hatch and ± 1 g thereafter. During the period of 4 and 21 d, feed intake was recorded to the nearest gram and FCR was calculated as daily feed intake (**DFI**; g) per daily BW gain (**DBWG**; g). Mortality was recorded daily.

On d 3 and 21, 3 female chicks whose BW were similar to the group average were selected from each experimental group and slaughtered by severing the jugular vein to determine the weight of the yolk sac and the digestive organs. The digestive organs (liver, gall bladder, pancreas, proventriculus, gizzard, and small intestine) and yolk sac were dissected and weighed to the nearest 0.001 g. Organ weights were presented as relative to BW (g/100 g of BW).

To prevent coagulation, blood samples were collected in test tubes without anticoagulant and centrifuged at 1,800 × *g* for 15 min. After centrifugation, serum was collected and stored at −20°C for further serum biochemical profile analysis. Serum biochemistry parameters (total protein, glucose, cholesterol, triglyceride, aspartate aminotransferase, alanine aminotransferase, uric acid, and creatinine) were measured spectrophotometrically using commercial kits (audit autoanalyzer test kits [21]) as described by the manufacturers.

On d 21, 9 chickens from each treatment were slaughtered and the small intestine (from the distal end of the duodenum to the ileocecal junction) was removed from each bird and put on ice until it was transported to the laboratory to determine the number of coliform bacteria, total aerobic bacteria, and total yeast. The small intestine was rapidly opened longitudinally, the mucosal surface and digesta were scraped with a sterile surgical knife, and then samples of the small intestine were transferred under aseptic conditions into sterile tubes. One gram of intestinal content was diluted 1:9 (wt/vol) with physiological salt water ($\log_{10}$). Samples were serially diluted from 10^{-1} to 10^{-6} to determine total yeast concentration in the intestine. Using these samples, the total yeast was enumerated on Sabouraud dextrose agar after incubation at 25°C for 48 h. Samples were serially diluted from 10^{-1} to 10^{-9} to determine the total aerobic bacteria concentration in the intestine and enumerated on nutrient agar after incubation at 37°C for 48 h. Samples for *E. coli* were diluted serially from 10^{-1} to 10^{-3} to determine *E. coli* concentration in the small intestine and enumerated on IMViC (indole, methyl red, Voges-Proskauer, and citrate) agar after incubation at 37°C for 48 h [22, 23].

Statistical Analysis

A GLM using the SPSS (17.0) statistic package [24] was applied to data with a model including essential oil and access time to diet and water and interaction between essential oil and access time to diet and water. Significant differences between treatment means were separated using Duncan's multiple range test [25]. Results were presented at least squares means and standard error of means. All statements of significance were based on $P \leq 0.05$.

RESULTS AND DISCUSSION

Performance Parameters

The average initial BW of chicks at hatching day was not significantly different among experimental treatments (shown in Table 2). The effects of dietary treatments (**DT**) and access time (**AT**) to feed and water on the DBWG of broilers at the period of 0 and 21 d are given in Table 2. The DT did not significantly affect the DBWG of chickens during the period between 0 and 21 d. As indicated in Table 2, the DBWG at 0 and 21 d and DFI from 4 to 21 d of chickens were significantly decreased when broilers were

Table 2. The effects of oregano or red pepper essential oil supplementation and delayed access to diet and water on performance parameters of broilers[1]

Item[2]	AT	IBW, g	DBWG, g	DFI, g	FCR, g:g
DT					
CONT	Immediate	50.00	37.17	59.54	1.437
	24 h posthatch	48.60	36.82	57.14	1.375
	48 h posthatch	49.50	32.84	51.42	1.365
OO250	Immediate	50.00	34.85	54.63	1.408
	24 h posthatch	48.90	35.21	55.25	1.390
	48 h posthatch	49.25	32.98	51.87	1.373
RPO250	Immediate	50.00	35.64	57.69	1.454
	24 h posthatch	49.00	35.37	56.84	1.418
	48 h posthatch	49.40	32.34	52.04	1.405
SEM		0.241	0.958	1.887	0.048
DT					
CONT			35.61	56.03	1.392
OO250			34.35	53.92	1.390
RPO250			34.35	55.53	1.426
AT					
Immediate			35.89[a]	57.28[a]	1.433
24 h posthatch			35.80[a]	56.41[a]	1.395
48 h posthatch			32.72[b]	51.78[b]	1.381
SEM			0.550	1.094	0.028
P-value					
DT			NS	NS	NS
AT			**	**	NS
DT × AT			NS	NS	NS

[a,b]Values within a column not sharing a common superscript differ significantly ($P \leq 0.01$).

[1]AT = access time; IBW = initial BW; DBWG = daily BW gain; DFI = daily feed intake.

[2]DT = dietary treatment; CONT = control diet (contained no essential oil); OO250 = oregano essential oil (250 mg/kg); RPO250 = red pepper essential oil (250 mg/kg).

**$P \leq 0.01$.

given access to feed and water at 48 h posthatch. Our results related to DBWG concur with the findings of Bigot et al. [26], who reported that feed deprivation for 2 d posthatching of broilers reduced their BW gain (**BWG**). Based on these results, broiler chickens could not compensate for the retardation of DBWG during the period of 0 to 21 d when chickens accessed to diet and water for 48 h posthatch. Alternatively, our finding related to feed intake is not in agreement with the results of Pinchasov and Noy [27]. They reported that posthatch birds deprived of food and water for 24 h had a significant decrease in feed intake. As shown in Table 2, both DT and AT to feed and water did not influence FCR of broilers from 4 to 21 d.

Supplementation of oregano essential oil to broiler diets [28–30] at a level of 50 and 100, 150 and 300 mg/kg, or 1,000 mg/kg, respectively, had no beneficial effect on growth performance. Likewise, Barreto et al. [31] reported that there was no significant effect of dietary supplementation with red pepper extract at 200 ppm on the BWG, feed intake, and FCR of broilers throughout 21 d. However, Roofchaee et al. [32] reported that inclusion of 600 and 1,200 mg/kg of oregano essential oil to the broiler diet significantly improved the FCR compared with the control diet. In addition, Al-Kassie et al. [33] reported that the supplementation of a mixture of black pepper and hot red pepper at the level of 0.25% significantly improved the BWG gain, the feed intake, and the FCR of broilers.

The lack of effect of the oregano essential oil may relate to the composition of the basal diet or environmental conditions, or both [34]. Likewise, it is known that well-nourished, healthy chicks do not respond to oregano essential oil, as they are housed under clean and disinfected conditions. However, according to the findings of some authors [13, 31], dietary supplementation of essential oils or their active compounds

in combination obtained from different herbs may give better results in poultry [32, 33].

Weight of the Yolk Sac and Digestive Organs

The effects of DT and AT to feed and water on the relative weight of the yolk sac at 3, 7, or 14 d posthatch are summarized in Table 3. The relative weight of the yolk sac of chicks at 7 or 14 d was not significantly influenced by the experimental treatments. However, the relative weight of yolk sac of chicks at 3 d was significantly increased by AT to feed and water for the 48-h posthatch treatment. The impaired development of the gastrointestinal tract, and the lower metabolism and overall growth may have reduced the energy needs of broiler chicks when the AT to feed and water was extended. As a result of this, yolk sac utilization was lower than or equal to that in chicks with immediate access to the diet [35]. In addition, Noy and Sklan [36] concluded that the effect of the feed intake on yolk utilization may be attributed to enhanced transport of the yolk to the gastrointestinal tract due to increased intestinal motility and activity after feed and water are ingested.

The effects of the experimental treatments on the relative weights of digestive organs of broiler chicks at 3 and 21 d are shown in Table 4. The relative weight of the gizzard of chicks at 3 d was significantly decreased by immediate access to feed and water. The relative weight of the gizzard of broilers at 21 d was significantly decreased by providing access to feed and water at 24 h posthatch compared with immediate access.

The relative weight of the liver of chicks at 21 d was significantly increased by providing access to feed and water immediately or 48 h posthatch compared with 24 h posthatch. This finding does not concur with that reported by Corless and Sell [37], who reported that a delayed feeding of 54 h posthatch had no adverse effect on the liver relative weight of poults.

Table 3. The effects of oregano or red pepper essential oil supplementation and delayed access to diet and water on the relative weight of yolk sac of broilers

Item[1]	AT	3 d	7 d	14 d
DT				
CONT	Immediate	0.84	0.06	0.000
	24 h posthatch	0.69	0.01	0.637
	48 h posthatch	1.64	0.02	0.017
OO250	Immediate	1.06	0.07	0.013
	24 h posthatch	0.86	0.39	0.017
	48 h posthatch	2.15	0.08	0.103
RPO250	Immediate	0.50	0.07	0.010
	24 h posthatch	1.35	0.03	0.013
	48 h posthatch	0.88	0.02	0.000
SEM		0.308	0.134	0.200
DT				
CONT		1.06	0.03	0.218
OO250		1.36	0.18	0.044
RPO250		0.91	0.04	0.008
AT				
Immediate		0.80[b]	0.07	0.008
24 h posthatch		0.97[b]	0.14	0.222
48 h posthatch		1.56[a]	0.04	0.040
SEM		0.178	0.077	0.116
P-value				
DT		NS	NS	NS
AT		*	NS	NS
DT × AT		NS	NS	NS

[a,b]Values within a column not sharing a common superscript differ significantly ($P \leq 0.05$).

[1]DT = dietary treatment; CONT = control diet (contained no essential oil); OO250 = oregano essential oil (250 mg/kg); RPO250 = red pepper essential oil (250 mg/kg); AT = access time.

*$P \leq 0.05$.

Lee et al. [38] also reported that dietary thymol supplementation significantly increased the relative weight of the liver in broilers for 21 d. The RPO250 significantly increased the relative weight of pancreas at 21 d compared with CONT. As shown in Table 4, there is a significant interaction between DT and AT to feed and water in terms of the relative weight of the proventriculus of broilers 21 d. The relative weight of the proventriculus of broilers fed OO250 and RPO250 was significantly decreased by delaying access to diet and water for 24 h posthatch. The OO250 or RPO250 supplementation to the diet might have shortened the staying time of the feed in the proventriculus due to the increased digestibility of the nutrients in the broiler proventriculus. The relative weight of the proventriculus of broilers given immediate access to feed and water was significantly increased by OO250. The relative weight of the proventriculus of broilers given access to feed and water at 48 h posthatch was significantly decreased by RPO250 compared with OO250.

In general, the dietary supplementation of oregano or red pepper essential oil had no any effects the relative weights of the liver, gall bladder, proventriculus, or small intestine. These results are similar to those reported by Al-Harthi [39, 40].

Serum Biochemistry Parameters

The effects of DT and AT to feed and water on the serum biochemistry parameters of broilers at 21 d are shown in Table 5. As shown in Table 5, experimental treatments did not significantly affect the serum total protein, cholesterol, triglyceride, alanine aminotransferase enzyme, uric acid, and creatinine levels, except glucose and aspartate aminotransferase enzyme (**AST**) levels. These findings concur with the results of Al-Harthi [41] who reported that a mixture of cardamom, cumin, and red and black pepper at 2 or 4 g/kg did not have any significant effects on plasma total protein, total cholesterol, and alanine aminotransferase enzyme levels. These findings are in agreement with the results of Lee et al. [42], who reported that the dietary carvacrol supplementation at the 200 ppm level did not affect plasma cholesterol in female broiler chickens. Likewise, Lee et al. [43] reported that dietary supplementation of thymol, cinnamaldehyde and a commercial preparation of essential oil components did not change plasma lipid (total cholesterol and triglyceride) concentrations of female broiler chickens at 21 d. Likewise, Al-Kassie et al. [33] pointed out that the supplementation of a mixture of black pepper and hot red pepper at the level of 0.25% did not significantly influence the serum cholesterol level of broilers. However, Case et al. [44] reported that the feeding of thymol at a dietary concentration of 150 ppm to Leghorn chickens for 21 d decreased serum cholesterol concentration by 9%. In addition, Srinivasan and Satyanarayana [45] reported that capsaicin, the active component of red pepper, fed to female rats lowered serum triglyceride levels.

Delayed access to diet and water for 24 or 48 h posthatch significantly reduced serum glucose levels at 21 d compared with immediate access. Newly hatched chicks have limited resources of the liver and muscle glycogen and consume these resources as soon as possible. On the other hand, chicks subjected to a delay in access to carbohydrates during the first 2 to 3 d after hatching lose BW. The BW losses reduce the energy requirement for the living value of chickens. As a result, first the liver and muscle glycogen resources and then the blood glucose level are decreased [46].

There is a significant interaction between DT and AT to feed and water in terms of serum AST level of broilers at 21 d. Serum AST level of broilers fed CONT was significantly reduced by delaying access to diet and water for 24 h posthatch, whereas delaying access to diet and water for 24 h posthatch significantly increased the serum AST level in broilers fed RPO250. Serum AST level of broilers with immediate access to feed and water was significantly decreased by feeding RPO250 compared with that of broilers fed CONT. In general, the serum AST level of broilers with access to feed and water at 24 h posthatch was significantly increased by OO250 and RPO250 compared with CONT.

This finding does not agree with the results of Traesel et al. [12], who reported that the serum AST levels in the group supplemented with essential oils from oregano, sage, rosemary, and pepper crude extract at 150 mg/kg were significantly higher than in the control group. In ad-

Table 4. The effects of oregano or red pepper essential oil supplementation and delayed access to diet and water on carcass parameters

		Liver		Gall bladder		Pancreas		Proventriculus		Gizzard		Small intestine	
Item[1]	AT	3 d	21 d	3 d	21 d	3 d	21 d	3 d	21 d	3 d	21 d	3 d	21 d
DT													
CONT	Immediate	3.70	2.94	0.42	0.07	0.46	0.35	1.47	$0.60^{A,z}$	6.93	3.39	7.16	5.04
	24 h posthatch	3.55	2.41	0.26	0.09	0.49	0.33	1.39	$0.65^{A,y}$	8.00	2.59	7.86	4.91
	48 h posthatch	4.16	2.97	0.28	0.11	0.40	0.33	1.39	$0.68^{A,yz}$	7.73	3.08	6.32	5.21
OO250	Immediate	3.70	2.71	0.36	0.05	0.47	0.39	1.49	$0.77^{A,y}$	6.71	3.36	5.71	5.05
	24 h posthatch	3.90	2.51	0.27	0.07	0.50	0.35	1.48	$0.62^{B,y}$	7.27	2.62	7.30	5.21
	48 h posthatch	3.91	2.74	0.25	0.11	0.45	0.33	1.29	$0.73^{A,y}$	8.04	2.88	7.64	5.06
RPO250	Immediate	3.65	2.75	0.38	0.10	0.48	0.43	1.34	$0.66^{A,z}$	6.91	3.40	7.47	5.43
	24 h posthatch	3.67	2.55	0.41	0.14	0.49	0.42	1.41	$0.58^{B,y}$	8.06	2.78	7.61	4.31
	48 h posthatch	4.54	2.63	0.23	0.09	0.42	0.37	1.25	$0.63^{A,z}$	7.76	3.18	7.68	5.05
SEM		0.279	0.124	0.062	0.017	0.046	0.030	0.092	0.029	0.326	0.278	0.554	0.361
DT													
CONT		3.80	2.77	0.32	0.09	0.45	0.34^{b}	1.42	0.64	7.56	3.02	7.11	5.05
OO250		3.84	2.65	0.29	0.08	0.47	0.36^{ab}	1.42	0.71	7.34	2.95	6.88	5.11
RPO250		3.96	2.64	0.34	0.11	0.46	0.41^{a}	1.33	0.62	7.57	3.12	7.59	4.93
AT													
Immediate		3.68	2.80^{a}	0.39	0.07	0.47	0.39	1.44	0.68	6.85^{a}	3.38^{a}	6.78	5.17
24 h posthatch		3.71	2.49^{b}	0.31	0.10	0.49	0.37	1.43	0.61	7.78^{b}	2.66^{b}	7.59	4.81
48 h posthatch		4.20	2.78^{a}	0.25	0.10	0.42	0.35	1.31	0.68	7.84^{b}	3.04^{ab}	7.21	5.11
SEM		0.162	0.072	0.040	0.009	0.027	0.018	0.053	0.017	0.188	0.160	0.319	0.208
P-value													
DT		NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
AT		NS	*	NS	NS	NS	NS	NS	NS	**	*	NS	NS
DT × AT		NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS

[a,b]Values within a column not sharing a common superscript differ significantly ($P \leq 0.01$).

[A,B]Values within a column with different capital letters show differences between essential oil sources ($P \leq 0.05$).

[y,z]Values within a column with different letters show differences between access times to feed and water ($P \leq 0.05$).

[1]DT = dietary treatment; CONT = control diet (contained no essential oil); OO250 = oregano essential oil (250 mg/kg); RPO250 = red pepper essential oil (250 mg/kg); AT = access time.

$^{**}P \leq 0.01$; $^{*}P \leq 0.05$.

Table 5. The effects of oregano or red pepper essential oil supplementation and delayed access to diet and water on serum chemistry parameters of broilers[1]

Item[2]	AT	Total protein, mg/dL	Glucose, mg/dL	Cholesterol, mg/dL	Triglyceride, mg/dL	AST, U/L	ALT, U/L	Uric acid, mg/dL	Creatinine, mg/dL
DT									
CONT	Immediate	4.20	272.67	92.33	66.00	$21.00^{A,y}$	8.67	4.73	1.90
	24 h posthatch	4.63	257.00	95.00	68.67	$12.00^{B,z}$	12.67	5.47	1.43
	48 h posthatch	4.37	246.00	90.00	73.67	$15.33^{AB,y}$	11.00	5.03	0.73
OO250	Immediate	3.93	275.33	102.33	71.67	$15.00^{A,yz}$	10.00	5.33	1.70
	24 h posthatch	4.23	261.33	96.00	66.00	$18.33^{A,y}$	13.00	3.93	1.63
	48 h posthatch	4.43	266.00	105.33	70.67	$21.00^{A,y}$	9.67	5.50	1.40
RPO250	Immediate	4.43	281.00	108.33	65.67	$13.00^{B,z}$	12.67	4.33	1.57
	24 h posthatch	4.60	268.33	88.00	68.67	$20.33^{A,y}$	14.33	4.77	1.83
	48 h posthatch	4.03	258.00	98.33	74.00	$18.67^{AB,y}$	11.33	4.93	1.63
SEM		0.234	7.222	8.962	5.249	2.040	1.689	0.745	0.279
DT									
CONT		4.40	258.56	92.44	69.44	16.11	10.78	5.08	1.36
OO250		4.20	267.56	101.22	69.44	18.11	10.89	4.92	1.58
RPO250		4.36	269.11	98.22	69.44	17.33	12.78	4.68	1.68
AT									
Immediate		4.19	276.33^{a}	101.00	67.78	16.33	10.44	4.80	1.72
24 h posthatch		4.49	262.22^{b}	93.00	67.78	16.89	13.33	4.72	1.63
48 h posthatch		4.28	256.67^{b}	97.89	72.78	18.33	10.67	5.16	1.26
SEM		0.135	4.170	5.174	3.031	1.178	0.975	0.430	0.161
P-value									
DT		NS	NS	NS	NS	NS	NS	NS	NS
AT		NS	*	NS	NS	NS	NS	NS	NS
DT × AT		NS	NS	NS	NS	**	NS	NS	NS

[a,b]Values within a same column not sharing a common superscript differ significantly ($P \leq 0.05$).

[A,B]Values within a column with different capital letters show differences between essential oil sources ($P \leq 0.01$).

[y,z]Values within a column with different letters show differences between access times to feed and water ($P \leq 0.01$).

[1]AST = aspartate aminotransferase enzyme; ALT: alanine aminotransferase enzyme.

[2]DT = dietary treatment; CONT = control diet (contained no essential oil); OO250 = oregano essential oil (250 mg/kg); RPO250 = red pepper essential oil (250 mg/kg); AT = access time.

*$P \leq 0.05$; **$P \leq 0.01$.

dition, essential oils are quickly metabolized in the liver, the main detoxifying organ, and this can overload the liver, causing damage, suggesting that the increasing serum AST was due to an initial hepatic injury. Based on our results related to the serum AST level, dietary supplemented level and prolonged use of essential oils was safe in terms liver and renal function. Based on reports, essential oils can produce toxic effects in chickens when administered in high doses; therefore, more studies are needed to define safety levels. In our study, OO250 and RPO250 did not significantly influence the serum uric acid and creatinine levels associated with kidney disease of chickens. Therefore, the supplemented level and the prolonged use of essential oils could not cause renal failure and nephritis [12].

Microbial Population of the Small Intestine

Effects of experimental treatments on microbial populations of the small intestine of chickens at 21 d are given in Table 6. There was a significant interaction between DT and AT to feed and water in view of total aerobic bacteria and coliform contents of the small intestine of broilers at 21 d. Delaying access to diet and water for 48 h posthatch significantly increased the coliform content of small intestine of broilers at 21 d by all dietary treatments. Treatments OO250 and RPO250 significantly reduced the coliform content of the small intestine when broilers had access to diet and water delayed for 24 h posthatch compared with CONT. However, the coliform contents of the small intestine of broilers with immediate access, or 24- and 48-h post-

Table 6. The effects of oregano or red pepper essential oil supplementation and delayed access to diet and water on the microbial population of the small intestine

Item[1]	AT	Coliform, log ×10^5 cfu	Total aerobic bacteria, log ×10^6 cfu	Total yeast, log ×10^4 cfu
DT				
CONT	Immediate	0.90^{C,y}	6.48^{A,z}	0.69
	24 h posthatch	1.10^{B,x}	6.50^{A,y}	0.81
	48 h posthatch	2.12^{A,y}	6.28^{A,x}	1.10
OO250	Immediate	1.10^{B,x}	7.50^{A,x}	0.70
	24 h posthatch	0.90^{C,y}	5.50^{C,z}	0.70
	48 h posthatch	2.30^{A,x}	6.28^{B,x}	1.21
RPO250	Immediate	1.20^{B,x}	6.78^{B,y}	0.70
	24 h posthatch	0.88^{C,z}	7.60^{A,x}	0.69
	48 h posthatch	2.10^{A,y}	5.13^{C,y}	1.10
SEM		0.056	0.089	0.037
DT				
CONT		1.37	6.42	0.87
OO250		1.43	6.42	0.87
RPO250		1.39	6.50	0.83
AT				
Immediate		1.07	6.92	0.69^{b}
24 h posthatch		0.96	6.53	0.74^{b}
48 h posthatch		2.17	5.90	1.14^{a}
SEM		0.033	0.051	0.021
P-value				
DT		NS	NS	NS
AT		NS	NS	***
DT × AT		***	***	NS

^{a,b}Values within a column not sharing a common superscript differ significantly ($P \leq 0.001$).

$^{A–C}$Values within a column with different capital letters show differences between essential oil sources ($P \leq 0.001$).

$^{x–z}$Values within a column with different letters show differences between access times to feed and water ($P \leq 0.001$).

[1]DT = dietary treatment; CONT = control diet (contained no essential oil); OO250 = oregano essential oil (250 mg/kg); RPO250 = red pepper essential oil (250 mg/kg); AT = access time.

***$P \leq 0.001$.

hatching delayed access to diet and water were significantly decreased by CONT and RPO250, respectively. Treatment OO250 significantly increased the coliform content of the small intestine when chickens had delayed access to diet and water for 48 h posthatch compared with CONT and RPO250.

As presented in Table 6, there was a significant interaction of DT and AT to feed and water in terms of total aerobic bacteria content of the small intestine of chickens at 21 d. The total aerobic bacteria content of the small intestine of broilers with delayed access to diet and water for 24 h posthatch was significantly reduced by OO250, whereas the total aerobic bacteria content of the small intestine of chickens with delayed access to diet and water for 48 h posthatch was significantly decreased when broilers were fed RPO250. In addition, total aerobic bacteria content of the small intestine of broilers with immediate access, or 24- and 48-h posthatching delayed access to diet and water was significantly decreased by CONT, OO250, and RPO250, respectively.

As presented in Table 6, delayed AT to the diet and water significantly affected the total yeast content of the small intestine. Delaying access to diet and water for 48 h posthatch significantly increased the total yeast content of the small intestine of broilers. The acidity in the small intestine of poultry changes to a pH of between 3 and 5 due to the increasing digestive enzyme activities in the small intestine with feed intake, and the main bacterial species are *Lactobacillus*, *Bacteroides*, and *Fusobacterium*. On the other hand, the starvation of poultry in terms of feed and water prevented secretion of the digestive enzymes and increased the pH of

the small intestine. The increasing pH might have been increased the total yeast number and decreased the *Lactobacillus* number in the small intestine of poultry [47]. No mortality was recorded throughout the experiment.

CONCLUSIONS AND APPLICATIONS

1. Without regard to dietary treatments, delayed access to diet and water for 48 h posthatch significantly decreased DBWG from 0 to 21 d and DFI from 4 to 21 d and increased the relative weight of the yolk sac at 3 d of broilers.
2. The relative weight of the liver or gizzard of chickens at 21 d was significantly decreased by delaying access to diet and water for 24 h posthatch. The diet containing RPO250 significantly increased the relative weight of the pancreas at 21 d.
3. Delaying access to diet and water for 24 or 48 h posthatch significantly reduced serum glucose levels at 21 d. The serum AST level of broilers with immediate access to feed and water was significantly decreased by the diet containing RPO250.
4. Generally, the coliform bacteria and total yeast contents of the small intestine of chickens was significantly increased by delayed access to diet and water. Total aerobic bacteria contents of the small intestine of broilers with immediate access, or 24- and 48-h posthatching delayed access to diet and water was significantly decreased by CONT, OO250, and RPO250 diets, respectively.
5. Further studies are needed to investigate the effects of the supplementation of the essential oils or their active compounds in combination to diets of broiler chicks delayed access to diet and water on the growth performance and microbial population content of the poultry gastrointestinal system.

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