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PAPER

Effects of oregano essential oil supplementation to diets of broiler chicks with delayed feeding after hatching. Morphological development of small intestine segments

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Abstract

The study aimed to investigate the effects of dietary supplementation of oregano essential oil (OEO) on the morphological development of small intestine of broilers with different feeding times (immediate, 24, 48 or 72 h post-hatching delayed feeding) from d 0 to 14. The diets were supplemented with: no, 250 or 500 mg/kg of the OEO (OEO250 and OEO500, respectively). Fasting for 72 h significantly increased the weight and length of small intestine segments of broilers on d 14. The OEO250 and OEO500 significantly increased the jejunum villus height of chickens fed immediately and the duodenum villus height of broilers fasted for 48 h. The duodenum villus surface area of chickens fasted for 48 h and the ileum villus surface area of broilers fasted for 24 h were significantly increased by the OEO250. The OEO500 significantly enhanced the duodenum villus surface area of broilers fasted for 24 h and their ileum villus surface area fasted for 48 h. The crypt depths of small intestine segments of broilers fasted for 72 h were significantly reduced by OEO250 and OEO500. In conclusion, the dose of phenolic compounds in OEO reaching the small intestine might be enough for protecting the intestinal epithelial cells from damages of toxins and for removing the negative effects of delayed feeding on the morphological development of all the small intestine segments of broiler chicks on d 14.

Introduction

In recent years, broiler chicks show a 40 to 45 times increase in body weight (BW) by 40 days of age or an even shorter period of time (Van Den Brand *et al.,* 2010). In order to realise this potential, they must be fed to meet their nutritional requirements and able to digest the feed and absorb its nutrients. These processes are directly correlated to the development of gastrointestinal tract, especially of the small intestine (Franco *et al.,* 2006). At hatching, the digestive system especially the small intestine of broiler chick is anatomically immature and its functional capacity is not fully developed. The small intestine undergoes morphological [villus height (VH) and area, surface area (VSA), crypt depth (CD), *etc*.] and physiological (pancreatic and digestive enzymes) developments including increased surface area of nutrient digestion and absorption during the post-hatch period (Yadav *et al.,* 2010). On the other hand, under practical conditions, a fasting period of 72 h after hatching during transportation to the broiler farm is generally common, due to variation in hatching time and logistics (Willemsen *et al.,* 2010). In conclusion, the morphological development of digestive system and small intestine is impaired when chicks have delayed access to diet after hatching (Sklan and Noy, 2000; Van Den Brand *et al.,* 2010).

Much research suggests that the early feeding of certain nutrients or feed additives has a great effect on the morphological development of the small intestine and growth performance (Kadam *et al.,* 2009; Yadav *et al.,* 2010). As a result, recent research has evaluated the use of feed supplements to decrease the negative effects of delayed feeding (Jamroz *et al.,* 2006; Peric *et al.,* 2010; Yadav *et al.,* 2010; Cheled-Shoval *et al.,* 2011). The limited use of antibiotic growth promoters has accelerated investigations on alternative feed additives for animal diets. Herbs, spices and their essential oils that have been used as phytogenic feed additives have gained attention in recent years (Steiner, 2009).

Oregano, a characteristic spice in Turkey obtained by drying leaves and flowers of *Origanum vulgare* and *onites* spp. grown in wild and cultivated. Oregano essential oil (OEO) extracted from oregano herb has two major phenols, *i.e.* carvacrol and thymol, comprising about 78 to 85% of essential oil (Roofchaee *et al.,* 2011). There are *in vivo* studies about the effects of essential oils or plant extracts on the morphological development of the small intestine in broilers (Jamroz *et al.,* 2006; Peric *et al.,* 2010; Yadav *et al.,* 2010; Amad *et al.,* 2013).

This study was planned to investigate whether the dietary supplementation of OEO removes the negative effects of delayed feeding on the morphological development of small intestine segments of broiler chicks on d 14.

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Materials and methods

A total of 720 male broiler chicks (Ross-308) were obtained from the hatchery where time of hatch was defined as time of clearing the shell. Then, the chicks were wing-banded, weighed and randomly assigned to twelve groups of similar mean weight each of which included three replicates of 20 chicks. During the rearing period, the experimental diets and drinking water were supplied *ad libitum*. The chicks were kept in wire cages (105x70 cm) equipped with nipple drinkers under standard environmental conditions throughout the experiment. A 23 L 1 D lighting programme was provided during the experiment. Temperature and humidity (55%) in the room were controlled throughout the experiment. Ambient temperature was gradually decreased from 32°C on d 0 to 25°C on d 14.

In a 4×3 factorial arrangement, broiler chicks were accessed to water and diet at four different feeding times (immediate, 24, 48 or 72 h post-hatching delayed feeding) and fed one of 3 different diets, which were corn-soybean meal based in mash form. The 3 experimental diets were as follows: diet 1 (CONT), a basal diet which contained no OEO; diet 2

(OEO250), supplemented with OEO at a level of 250 mg/kg; and diet 3 (OEO500), supplemented with OEO at a level of 500 mg/kg. Prior to experimental diet formulation, feed ingredients were analysed for their contents of dry matter, crude protein, crude fat, starch and total sugar according to the methods of the Association of Analytical Chemists (AOAC, 2007). Metabolisable energy (ME) of feed ingredients was calculated based on analysed values of feedstuffs (WPSA, 1989). All values were expressed on a dry matter basis. The basal diet based on corn-soybean meal contained 230.0 g/kg crude protein and 12.99 MJ/kg ME. The ingredients and nutritional composition of the basal diet are given in Table 1. The diets were formulated to meet or exceed minimum National Research Council (1994) standards for all ingredients.

Oregano essential oil was provided by the Altes Agricultural Products Ltd. Company (Antalya, Turkey). Oregano essential oil also obtained by steam-distillation using Clevenger distillation apparatus and derived *Origanum onites* spp. growing wild in Turkey was used in the study. The composition of the OEO was determined by gas chromatography-mass spectrometry (GC/MS) (HP 6890GC/5973MSD) system. The essential oil obtained was diluted with n-hexane (1/100) and injected into a GC/MS (HP 6890GC/5973MSD) system (injection temperature: 250°C; injection split: 1/100; column: DB-17 30 m, 0.25 m, 0.32 mm; oven programme: initial temperature, 70°C, rate 8°C/min, final temperature: 200°C, injection vol. 1 L; Agilent Technologies, Santa Clara, CA, USA). The carvacrol and thymol contents, which are the most active compounds of OEO, were determined as 84.02 and 1.78%, respectively. Oregano essential oil was supplemented to an amount of sunflower oil and homogenised by mixer and then the mixture was blended with the corn. Corn with essential oil was added to pre-mixture. Finally, the pre-mixture was supplemented to the main mixture, prepared weekly and stored in airtight containers.

Body weights of broilers in each experimental treatment were measured on d 0, 3, 4 and 14. Chicks were weighed to ±0.001 g at hatch and ±1 g thereafter.

Male broiler chicks fed as described above were killed by severing the jugular vein and measured the relative weights on d 3, 4 and 14 and the morphological development of small intestine segments (9 chicks per treatments for each period) on d 14 of ages. Segments were removed from the duodenum, jejunum and ileum as follows: i) intestine from the gizzard to pancreatic and bile ducts was referred to as the duodenum; ii) midway was between the point of entry of the bile ducts and Meckel's diverticulum (jejunum); iii) 10 cm proximal to the ileo-caecal junction (ileum). The weights of small intestine segments were weighed to the nearest 0.001 g and values with the help of electronic weighing balance, while their lengths were measured with non-stretchable thread and scale. The relative weights and lengths of the small intestine segments are presented as g/100 g BW and cm/100 cm small intestine length, respectively. Then, the samples were handled as described by Uni *et al.*

Table 1. Ingredients and chemical composition of the control diet (as fed basis).

Ingredients, g/kg	
Corn	510.00
Soybean meal (46% CP)	285.00
Fullfat soybean	140.00
Sunflower oil	24.90
Limestone	10.50
Dicalcium phosphate	20.50
Salt	3.50
Vitamin premix°	2.50
Trace mineral premix#	1.00
DL-methionine	2.10
Total	1000.00
Calculated chemical composition	
Metabolisable energy, MJ/kg	12.99
Crude protein, %	23.00
Calcium, %	1.01
Phosphorus (available), %	0.45
Methionine, %	0.62
Met+Cys, %	0.94
Lysine, %	1.29
Analysed chemical composition, %	
Dry matter	89.82
Crude protein	23.01
Crude fat	6.98
Crude fibre	3.96
Crude ash	1.96

CP, crude protein; Met, methionine; Cys, cysteine. °Vitamin premix provided per kg of diet: trans-retinol, 3600 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; apo-carotenoic acid ester, 2.5 mg; choline chloride, 400 mg. #Trace mineral premix provided the following (per kg diet): Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.20 mg; I, 1 mg; Se, 0.15 mg.

Table 2. Effects of experimental treatments on the body weights (g) of broiler chicks on d initial, 3, 4 and 14.

DT	AT	Days			
		Initial	3	4	14
CONT	0 h	37.6	65.66	72.47	252.27
	24 h	37.2	57.71	64.94	227.44
	48 h	37.0	45.24	52.76	217.30
	72 h	37.5	30.09	42.63	213.26
OEO250	0 h	37.3	64.07	72.84	231.36
	24 h	37.3	56.76	66.03	244.24
	48 h	37.1	44.08	52.10	233.36
	72 h	37.7	29.72	39.50	180.58
OEO500	0 h	37.1	63.13	69.95	230.00
	24 h	37.0	56.04	63.85	212.12
	48 h	37.2	42.04	50.84	209.24
	72 h	37.6	30.15	42.27	206.05
DT	CONT		49.68	58.20	227.57
	OO250		48.66	57.62	222.39
	OO500		47.84	56.73	214.35
AT	0 h		64.29^{a}	71.75^{a}	237.88^{a}
	24 h		56.84^{b}	64.94^{b}	227.93^{a}
	48 h		43.79^{c}	51.90^{c}	219.97^{ab}
	72 h		29.99^{d}	41.47^{d}	199.96^{b}
Pooled SEM		0.097	2.231	2.045	4.315
P					
DT			ns	ns	ns
AT			**	**	**
DTxAT			ns	ns	ns

DT, dietary treatments; AT, accessing time to diet and water; CONT, not containing oregano essential oil; OEO250, oregano essential oil, 250 mg/kg; OEO500, oregano essential oil, 500 mg/kg; ns, not significant. $^{a-d}$Values in the same column not sharing a common superscript differ significantly (**P≤0.01).

(2003) to the morphological analysis of small intestine segments. The samples were gently flushed with 0.9% (wt/vol) NaCl to remove the intestinal contents and were fixed in fresh 4% (vol/vol) buffered formaldehyde. Samples were dehydrated, cleared and embedded in paraffin. Serial 5 m sections were cut at and placed on glass slides. For all assays, sections were deparaffinised in xylene and rehydrated in a graded alcohol series. Sections were examined by light microscopy and the height and width of the villus were measured using a computer assisted image analysis described by Uni *et al.* (1998). Villus height was measured from the tip of villus to the crypt-villus junction, whereas villus width (VW) was defined as the distance from the outside epithelial edge along a line passing through the vertical midpoint of the villus. Villus surface area was calculated from the villus height and width at half height.

Statistical analysis

Linear model using the SPSS (17.0)® statistic package (SPSS, 2007) was applied to data with a model including OEO and accessing time to diet and water (AT) and interaction between essential oil and AT. Significant differences between treatment means were separated using Duncan's multiple range test (Duncan, 1955). All statements of significance were based on P≤0.05.

Results and discussion

Body weights of broiler chicks

Body weights of broilers on d initial, 3, 4 and 14 are given in Table 2. As shown in Table 2, the BWs of broiler chicks on d 3 and 4 were significantly decreased by extending of access time to diet and water from immediately to 72 h post-hatching (P≤0.01). On the other hand, the BWs of broiler chicks on d 14 were significantly reduced by delayed feeding for especially 72 h post-hatching compared to those of chicks fed immediately and after 24 h post-hatching (P≤0.01).

Relative weight and length of small intestine segments

The effects of dietary treatments (DTs) and AT on the duodenum relative weight (DRW) and length (DRL) of broiler chicks on d 3, 4 and 14 are summarised in Table 3.

There is not any significant effect of DT and the interaction between DT and AT on the DRW and DRL on d 3. Access to diet and water of broiler chicks for immediate and 24 h post-hatching significantly increased the DRW on d 3 (P≤0.01). In addition, the DRL of broiler chicks on d 3 was significantly increased by feeding for immediate and 72 h post-hatching (P≤0.05).

There is also a significant interaction between experimental treatments in terms of the DRW of broiler chicks on d 4 (P≤0.05). The DRW of broiler chicks fed CONT and OEO250 was significantly the highest when they were fasted for 24 h post-hatching. On the other hand, OEO500 significantly increased the DRW of broiler chicks fed immediately and fasted for 24 and 48 h post-hatching on d 4. The DRW

Table 3. The effects of dietary oregano essential oil supplementation to diets and delayed access to diet and water on relative weight (g/100 g) and relative length (cm/100 cm) of small intestine segments in broiler chicks on d 3, 4 and 14.

DT	AT	DRW			JRW			IRW			DRL			JRL			IRL		
		3	4	14	3	4	14	3	4	14	3	4	14	3	4	14	3	4	14
CONT	0 h	2.40	2.07 ^{Aab}	1.72	2.95	2.89	1.95	2.27	2.47	1.50	21.8	20.1	9.02	46.6	44.2^{Ba}	17.4	46.9	37.5	18.6
	24 h	2.14	2.40 ^{ABa}	1.60	3.13	3.05	2.18	2.42	2.72	1.75	23.9	23.6	9.26	48.5	43.9^{Aa}	19.0	47.0	46.3	19.1
	48 h	1.50	2.03^{Aab}	1.60	2.45	3.57	2.30	2.18	2.68	1.56	27.4	28.3	9.06	44.4	47.3^{Aa}	20.4	47.3	58.9	19.7
	72 h	1.22	1.51^{Bb}	1.80	2.28	2.69	2.21	2.04	2.55	1.79	26.7	27.2	8.96	59.2	53.4^{Aa}	18.8	46.5	54.7	19.5
OEO250	0 h	1.87	1.94^{Ab}	1.48	2.68	2.86	2.17	1.81	2.27	1.41	20.6	21.4	9.16	53.1	46.5^{ABa}	20.8	37.5	43.9	20.1
	24 h	2.06	2.55^{Aa}	1.25	2.85	2.93	1.86	2.23	3.38	1.47	24.4	22.6	7.54	48.9	46.7^{Aa}	16.7	42.6	41.1	18.0
	48 h	1.40	1.91^{Ab}	1.52	1.92	3.26	2.06	1.70	3.24	1.47	23.9	23.2	8.92	47.9	46.1^{Aa}	19.9	45.0	47.7	21.3
	72 h	1.05	1.98^{Ab}	1.79	1.66	3.19	2.29	1.58	3.29	1.91	23.6	30.1	10.7	61.2	54.1^{Aa}	24.2	51.7	53.6	24.7
OEO500	0 h	2.33	2.07^{Aa}	1.42	2.99	3.12	2.10	2.55	2.39	1.32	21.1	23.3	8.86	53.3	53.0^{Aa}	20.5	47.9	45.2	21.3
	24 h	2.05	1.92^{Ba}	1.42	2.94	2.63	2.02	2.26	2.27	1.47	22.6	22.3	9.27	55.9	48.1^{Aab}	20.8	47.9	48.7	21.4
	48 h	1.40	2.26^{Aa}	1.49	2.93	3.31	1.87	2.14	2.52	1.46	22.0	25.3	9.30	49.7	50.8^{Aa}	20.9	45.0	46.4	21.6
	72 h	1.81	1.31^{Bb}	1.94	2.23	2.35	2.29	2.27	2.86	1.87	30.7	24.8	13.0	54.1	36.8^{Ab}	26.3	56.7	56.1	26.8
DT	CONT	1.60	2.00	1.68	2.70	3.05	2.16	2.23	2.60	1.65	24.9	24.8	9.07	49.7	47.2	18.9	46.9	48.1	19.2^{b}
	OO250	1.81	2.09	1.51	2.28	3.06	2.10	1.83	3.05	1.57	23.1	24.3	9.08	52.8	48.4	20.4	44.2	46.6	21.0^{ab}
	OO500	1.90	1.89	1.57	2.77	2.86	2.07	2.30	2.51	1.53	24.1	23.9	10.1	53.3	47.2	22.1	49.3	49.1	22.8^{a}
AT	0 h	2.20^{a}	2.03^{a}	1.54^{b}	2.88	2.96	2.08	2.21	2.38	1.41^{b}	27.2^{a}	21.6^{c}	9.01^{b}	51.0	47.9	19.6^{b}	44.1	42.2^{c}	20.0^{b}
	24 h	2.08^{a}	2.29^{a}	1.42^{b}	2.98	2.87	2.02	2.30	2.79	1.56^{b}	23.6^{b}	22.9^{bc}	8.69^{b}	51.1	46.2	18.8^{b}	45.8	45.4^{bc}	19.5^{b}
	48 h	1.43^{b}	2.07^{a}	1.54^{b}	2.43	3.38	2.08	2.01	2.81	1.50^{b}	24.4^{b}	25.6^{ab}	9.09^{b}	47.3	48.1	20.4^{ab}	45.7	49.3^{ab}	20.9^{ab}
	72 h	1.36^{b}	1.60^{b}	1.84^{a}	2.06	2.74	2.27	1.96	2.90	1.86^{a}	27.0^{a}	27.4^{a}	10.9^{a}	58.1	48.1	23.1^{a}	51.6	54.8^{a}	23.6^{a}
Pooled SEM		0.57	0.397	0.264	0.781	0.520	0.323	0.632	0.596	0.288	4.298	3.726	1.725	9.262	7.152	3.694	6.564	8.032	3.600
P																			
DT		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
AT		**	**	**	ns	ns	ns	ns	ns	**	*	**	*	ns	ns	*	ns	**	*
DTxAT		ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns

DT, dietary treatments; AT, accessing time to diet and water; DRW, duodenum relative weight; JRW, jejunum relative weight; IRW, ileum relative weight; DRL, duodenum relative length; JRL, jejunum relative length; IRL, ileum relative lenght; CONT, not containing oregano essential oil; OEO250, oregano essential oil, 250 mg/kg; OEO500, oregano essential oil, 500 mg/kg; ns, not significant. $^{a-c,A-B}$Values in the same column not sharing a common superscript differ significantly (*P≤0.05 and **P≤0.01), with lowercase letters showing the interaction between dietary treatments and accessing time to diet and water, while uppercase letters showing the interaction between accessing time to diet and water and dietary treatments.

of broiler chicks fasted for 24 h post-hatching was significantly higher by feeding OEO250 than that of broiler chicks fed OEO500 on d 4. The DRW of chicks fasted for 72 h post-hatch on d 4 was significantly increased by feeding OEO250 compared to OEO500. Fasting of chicks for 72 h post-hatching significantly increased the DRL on d 4 compared to those of broiler chicks accessed to diet and water for immediate and 24 h post-hatching (P≤0.01).

Especially fasting of chicks for 72 h post-hatching significantly increased their DRW (P≤0.01) and DRL (P≤0.05) on d 14.

The experimental treatments did not significantly influence the jejunum relative weight (JRW) on d 3, 4 and 14, and jejunum relative length (JRL) on d 3 (Table 3). On the other hand, there is a significant interaction between DTs and AT in terms of the JRL on d 4 (P≤0.05). Feeding OEO500 significantly reduced the JRL on d 4 by fasting of chicks for 72 h post-hatching compared to those of broiler chicks accessed to diet and water for immediate and 48 h post-hatching (P≤0.05).

The ileum relative weight (IRW) on d 3 and 4 and the ileum relative length (IRL) on d 3 were not significantly influenced by the experimental treatments (Table 3). Fasting of broiler chicks for 72 h post-hatching significantly increased the IRW on d 14 (P≤0.01). The IRL of broiler chicks on d 4 (P≤0.01) and d 14 (P≤0.05) was significantly increased by fasting of chicks for 72 h post-hatching compared to those of broiler chicks accessed immediately or 24 h post-hatching. In addition, feeding OEO500 significantly enhanced the IRL of chickens on d 14 compared to the CONT (P≤0.05).

The results related to BWs and relative weights and lengths of the small intestine segments of broiler chicks showed that extending of accessing time to the diet and water from 0 to 72 h significantly reduced relative weights of small intestine segments, and simultaneously increased their relative lengths on d 3 and 4. Fasting for especially 72 h of broiler chicks significantly increased the relative weights and lengths of small intestine segments on d 14 although their decreasing BWs on d 14. Although these increases in the relative weights and lengths of all small intestine segments on especially d 14 are suggestive of a compensatory response to nutrient intake by delayed feeding, they did not alleviate the retardant effects of delayed access to diet and water on the dietary nutrients' absorption and utilisation, resulting in the reduced BW of broiler chicks on d 14 (Corless and Sell, 1999). On the other hand, the dietary OEO supplementation did not significantly affect the relative weights and lengths of small intestine segments.

Morphological development of small intestine segments

The effects of DT and AT on the morphological development of the small intestine segments in broiler chicks on d 14 are given in Table 4, still, they could not be discussed in the manuscript because the interaction between DT and AT was significant.

There is a significant interaction between

Table 4. The effects of dietary oregano essential oil supplementation to diets and delayed access to diet and water on the morphological development of small intestine segments in broiler chicks on d 14.

DT	AT	Duodenum			Jejunum			Ileum		
		VH (μm)	VS (μm^2) x10^4	CD (μm)	VH (μm)	VS (μm^2) x10^4	CD (μm)	VH (μm)	VS (μm^2) x10^4	CD (μm)
CONT	0 h	1045^{Aa}	37.5^{Aab}	110^{Bc}	704^{Ba}	20.8^{ABb}	88.0^{Bb}	638^{Ab}	19.4^{Ab}	03^{Ba}
	24 h	932^{ABb}	28.5^{Bb}	145^{Aa}	810^{Ab}	21.7^{Ab}	87.0^{Ab}	564^{Ac}	15.6^{Bc}	90^{Ab}
	48 h	860^{Bb}	28.6^{Bb}	97.0^{Cd}	842^{Ab}	22.3^{Ab}	98.0^{ABa}	647^{Ab}	15.9^{Cc}	103^{Aa}
	72 h	1106^{Aa}	43.6^{Aa}	125^{Ab}	820^{Ab}	26.8^{Aa}	105^{Aa}	698^{Aa}	21.5^{Aa}	112^{Aa}
OEO250	0 h	1057^{Aa}	35.5^{Ab}	128^{Aa}	790^{Aa}	22.3^{Aa}	98.0^{Aab}	592^{Ba}	17.2^{Bb}	111^{Aa}
	24 h	995^{Aa}	31.5^{Bc}	118^{Bb}	712^{Bb}	22.6^{Aa}	88.0^{Ac}	591^{Aa}	18.7^{Aa}	92^{Ac}
	48 h	1032^{Aa}	41.8^{Aa}	131^{Aa}	717^{Bb}	21.2^{Aa}	102^{Aa}	604^{ABa}	18.2^{Bab}	100^{Ab}
	72 h	1034^{ABa}	36.3^{ABb}	117^{Ab}	697^{Bb}	20.8^{Ba}	93.0^{Bab}	512^{Bb}	14.6^{Bc}	87^{B}
OEO500	0 h	961B^{a}	35.0^{Ab}	119^{ABa}	750^{ABa}	19.3^{Bb}	99.0^{Aa}	609^{ABa}	17.2^{Bb}	96^{Ca}
	24 h	865^{Bb}	43.4^{Aa}	99.0^{Cc}	789^{Aa}	23.3^{Aa}	88.0^{Ab}	395^{Bc}	9.58^{Cd}	79^{Bc}
	48 h	1038^{Aa}	36.2^{Ab}	114^{Bab}	773^{Ba}	20.8^{Ab}	95.0^{Ba}	588^{Ba}	20.1^{Aa}	89^{Bb}
	72 h	965^{Ba}	33.6^{Bb}	105^{Bbc}	773^{Aa}	20.2^{Bb}	97.0^{ABa}	525^{Ab}	15.0^{Bc}	86Bb
DT	CONT	986^{ab}	34.5	119^{a}	794^{a}	22.9	94.5	637^{a}	18.1^{a}	102^{a}
	OO250	1030^{a}	36.3	124^{a}	729^{b}	21.7	95.3	575^{b}	17.2^{b}	97.5^{b}
	OO500	957^{b}	37.1	109^{b}	771^{a}	20.9	94.8	529^{c}	15.5^{c}	87.5^{c}
SEM		16.850	9.900	1.581	10.384	3.965	1.287	7.068	2.337	1.186
AT	0 h	1021^{ab}	36.0	119	748	20.8	95.0^{a}	613^{a}	18.0^{a}	103^{a}
	24 h	930^{c}	34.5	121	770	22.5	87.7^{b}	517^{c}	14.6^{c}	87^{c}
	48 h	977^{bc}	35.5	114	777	21.4	98.3^{a}	613^{a}	18.1^{a}	97.3^{b}
	72 h	1035^{a}	37.8	116	763	22.6	98.3^{a}	578^{b}	17.0^{b}	95^{b}
SEM		19.457	11.431	1.826	11.991	4.578	1.486	8.162	2.699	1.369
P										
DT		*	ns	***	***	**	ns	***	***	***
AT		**	ns	ns	ns	*	***	***	***	***
DTxAT		***	***	***	***	***	***	***	***	***

DT, dietary treatments; AT, accessing time to diet and water; VH, villus height; VS, villus surface; CD, crypt depth; CONT, not containing oregano essential oil; OEO250, oregano essential oil, 250 mg/kg; OEO500, oregano essential oil, 500 mg/kg; ns, not significant. $^{a-d,A-C}$Values in the same column not sharing a common superscript differ significantly (*P≤0.05; **P≤0.01; ***P≤0.001), with lowercase letters showing the interaction between dietary treatments and accessing time to diet and water, while uppercase letters showing the interaction between accessing time to diet and water and dietary treatments.

DT and AT to diet and water in terms of the duodenum villus height (DVH), villus surface area (DVSA) and crypt depth (DCD) of broiler chicks on d 14 (P≤0.001) (Table 4). The DVH and DVSA of the broiler chicks fed CONT were the highest by fasting for 72 h post-hatching (P≤0.001). The DVSA of the broiler chicks fed OEO250 was significantly increased by fasting for 48 h post-hatching without affecting their DVH (P≤0.001). The DVH of broiler chicks fed OEO500 was the highest by accessing immediately and fasting for 48 and 72 h post-hatching, on the other hand, their highest DVSA was recorded by fasting for 24 h post-hatching (P≤0.001). By investigating these data in terms of the interaction between AT and DT, the DVH of the broiler chicks accessed immediately and fasted for 24 h post-hatching to diet and water turned out to be significantly increased by their feeding CONT and OEO250, on the other hand, feeding OEO250 and OEO500 resulted in the significant high DVH for broiler chicks fasting for 48 h. In contrast, the DVH of the chicks fasted for 72 h was significantly the highest by feeding CONT. It was found that the DVSA of chicks fasted for 24, 48 and 72 h, respectively, was significantly increased by feeding OEO500, OEO250 or OEO500, and CONT, respectively. The DCD of the chicks accessed to diet and water for immediate and 48 h post-hatching was significantly decreased by CONT. On the other hand, OEO500 significantly reduced the DCD of the chicks fasted for 24 and 72 h post-hatching (P≤0.001).

There is also a significant interaction between DT and AT in terms of the jejunum villus height (JVG), villus surface area (JVSA) and crypt depth (JCD) of broiler chicks on d 14 (P≤0.001) (Table 4). Feeding CONT significantly resulted the highest JVH on d 14 for the chicks fasted for 24, 48 and 72 h post-hatching. The JVH of the chicks fed OEO250 on d 14 was significantly increased by immediately access to diet and water. The JVSA of the chicks fed CONT was significantly enhanced by fasting for 72 h post-hatching while the chicks fed OEO500 had significantly the highest JVSA by accessing to diet and water for 24 h post-hatching. As investigated these data in terms of the interaction between AT and DTs, the JVH and JVSA of chicks immediately accessed to diet and water were significantly the highest by feeding OEO250. On the other hand, feeding with CONT was enough in terms of the JVH and JVSA of the chicks fasted for 24, 48 and 72 h post-hatching. Feeding CONT, OEO500 and OEO250, respectively, significantly decreased JCD of the chicks accessed immediately, 48 h and 72 h post-hatching, respectively, to diet and water (P≤0.001).

A significant interaction (P≤0.001) between DT and AT in terms of the ileum villus height (IVH), villus surface area (IVSA) and crypt depth (ICD) of broiler chicks on d 14 is summarised in Table 4.

The IVH and IVSA of the broiler chicks fed CONT on d 14 were significantly the highest by fasting for 72 h post-hatching. Feeding with OEO250 siginificantly enhanced the IVH and IVSA of the chicks fasted for 24 h post-hatching. The chicks fed OEO500 had the significant highest IVH and IVSA when they were accessed to diet and water for 48 h post-hatching. The ICD of the chicks accessed to diet and water for immediate, 24 and 48 h post-hatching was significantly decreased by feeding especially OEO500. On the other hand, feeding OEO250 significantly reduced the ICD of the chicks fasted for 72 h post-hatching (P≤0.001).

As summarised the results related to the morphological development of small intestine segments, feeding OEO250 and OEO500 significantly increased the JVH of broiler chicks fed immediately and the DVH of broiler chicks fasted for 48 h post-hatching on d 14. The result related to the JVH did not concur with the findings of Yakhkeshi *et al.* (2011). reported that no significant differences were observed between the control diet and diet supplemented with herbal extracts in terms of the villus height of jejunum throughout the experiment. The DVSA of broiler chicks fasted for 48 h post-hatching and the IVSA of broiler chicks fasted for 24 h post-hatching were significantly increased by the OEO250. Feeding OEO500 significantly enhanced the DVSA of broiler chicks fasted for 24 h post-hatching and their IVSA fasted for 48 h post-hatching.

The ICDs of fed immediately and fasted for 24 h and 48 h post-hatching and the DCD of fasted for 24 h post-hatching on d 14 were significantly decreased by feeding OEO500. On the other hand, feeding OEO250 and OEO500 also significantly enhanced the JCD of the chicks fed immediately compared to CONT on d 14. The findings are not in agreement with the results of Jamroz *et al.* (2006) who pointed out that the dietary plant extract supplementation at the level of 100 mg/kg decreased the JCD compared to the control diet. In addition, the CD of small intestine segments of broiler chicks fasted for 72 h post-hatching on d 14 were significantly reduced by the OEO250 and OEO500.

It is reported that changes in the intestinal morphology such as shorter villi and deeper crpyts have been associated with the presence of toxins (Samadian *et al.,* 2013). It can be said that the dietary OEO supplementation at the increasing doses might have reduced the adhesion to epithelium and number of the total harmful bacteria in the intestinal wall, thus reducing production of toxic compounds and damage to intestinal epithelial cells of broiler chicks (Garcia *et al.,* 2007; Yakhkeshi *et al.,* 2011; Samadian *et al.,* 2013).

Conclusions

The dietary supplementation of OEO at the levels of 250 and/or 500 mg/kg improved the VH and VSA of small intestine segments of broiler chicks delayed access to diet and water for immediate, 24 and 48 h.

In addition, the CD of small intestine all segments of broiler chicks fasted for 72 h post-hatching on d 14 was significantly reduced by the OEO250 and OEO500.

As a result, the dose of the phenolic compounds in OEO in the small intestine might be enough for protecting the intestinal epithelial cells from damages of toxins and for removing the negative effects of delayed feeding on the morphological development of all the small intestine segments of broiler chicks on d 14.

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