



Effects of Essential Oils for Broiler Chicks with Delayed Feeding after Hatching 2. Morphological Development of Small Intestine

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ABSTRACT

The study investigated the effects of oregano- or red pepper-essential oil at delayed feeding (0, 24 and 48 h post-hatching) on performance and morphological development of small intestine segments. Female broilers were fed one of 3 rations including a control ration with no essential oil (CONT), the rations added with either oregano essential oil (OEO) or red pepper essential oil (RPEO) at 250 mg/kg to CONT. A total of 18 chicks from each treatment were used to measure the morphological parameters of the small intestine segments on the 14th day. Prolongation of accessing time to ration significantly decreased the body weights of broilers at 3rd, 7th and 14th d, feed intake (FI) from 4 to 7 d and improved feed conversion ratio (FCR) of broilers at the period of 4-7 d. Access to ration for 48 h post-hatching significantly decreased the body weight gains at the period of 4-7 and 7-14 d, FI from 7 to 14 d and improved FCR of broilers at the period of 7-14 d. OEO250 ration significantly increased villus height (VH) and villus surface area (VSA) of jejunum (J) and ileum (I) of broilers fed immediately and the IVH and IVSA of broiler accessed to ration for 48 h post-hatching. VH of duodenum (D), IVH and IVSA of broilers fed for 24 h post-hatching were significantly increased by RPEO250 ration. OEO250 and RPEO250 rations significantly reduced crypt depth (CD) of D and J of broiler accessed to ration for immediate and DCD of broiler fed for 24 h post-hatching. OEO250 and RPEO250 rations significantly increased IVH and IVSA and reduced DCD, JCD and ICD of broilers. In conclusion, OEO250 and RPEO250 rations affected positively VH and VSA of I and reduced CDs of small intestine segments of broilers accessed to ration at different times.

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Introduction

Broilers have been able to reach a market weight in a short time approximately 40 days of age due to genetic selection (Mahapatra et al., 2017). This situation is directly correlated to the development of especially the small intestine. Early nutrition leads to the morphological development of the small intestine and the increase in the growth performance of broilers. On the other hand, starvation of chicks until 72 h due to the variation of hatching time and logistics is impaired the morphological development of the small intestine (Prabakar et al., 2016). To alleviate the negative effects of delayed feeding, some researchers have investigated the effects of the early feeding of aromatic plants and their essential oils on the morphological development of the small intestine in recent times (Vidanarachchi et al., 2010; Al-Tememy et al., 2011; Sujatha et al., 2017). Oregano (OEO)- and red pepper- (RPEO) essential oils have antimicrobial activity due to their phenolic compounds (Zarringhalama et al., 2013; Teuchert, 2014).

The present study investigated the effects of the addition of OEO or RPEO to the ration of broiler chicks with immediate, 24- or 48-h post-hatching delayed feeding on the growth performance and morphological development of their small intestine segments from hatch to 14 d.

Materials and Methods

A total of 432 female broiler (Ross 308) chicks were obtained from a local hatchery. The broiler chicks were randomly allocated to nine groups of similar average weight each of which included three replicates of 16 chicks. The study was conducted in accordance with animal welfare at the Poultry Research Centre of Ankara University.

In the study being a randomized complete block design with a 3×3 factorial arrangement of treatments, chicks were accessed free to water and ration at three

different feeding times (0, 24 and 48 h post-hatching delayed feeding) and fed one of 3 different rations (23% crude protein, 3105 kcal ME/kg), which were corn-soybean meal based in mash form. The 3 experimental rations were a control ration (CONT) which contain no essential oil and the other rations added with either OEO250 or RPEO at a level of 250 mg/kg ration to the CONT ration. The rations were prepared as isocaloric and isonitrogenic. The ingredients and nutritional composition of the control ration are given in Table 1.

Table 1 The ingredients and chemical composition of the control ration as fed basis

| Ingredients | Amount, g/kg |
|--|--------------|
| 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* | 2.50 |
| Trace mineral premix** | 1.00 |
| DL-Methionine | 3.20 |
| L-Lysine HCL | 1.50 |
| Total | 1000.00 |
| Analysed chemical composition, % of DM | |
| Dry matter | 88.64 |
| Crude protein | 22.94 |
| Crude fiber | 3.10 |
| Crude ash | 5.92 |
| Crude fat | 6.11 |
| Calcium | 0.98 |
| Total phosphorus | 0.73 |
| Calculated chemical composition, % of DM | |
| Metabolizable energy, kcal/kg | 3105 |
| Crude protein | 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 |

*Vitamin premix provided (per kg of ration): 3600 µg of *trans*-retinol, 15.0 µg of cholecalciferol, 50 mg of α -tocopherol acetate, 5 mg of vitamin K₃, 3 mg of vitamin B₁, 6 mg of vitamin B₂, 5 mg of vitamin B₆, 0.03 mg of vitamin B₁₂, 25 mg of niacin, 12 mg of Ca-D-pantothenate, 1 mg of folic acid, 0.05 mg of D-biotin, 2.5 mg of apo-carotenoic acid ester, 400 mg of choline chloride.

**Trace mineral premix provided (per kg of ration): 80 mg of Mn, 60 mg of Fe, 60 mg of Zn, 5 mg of Cu, 0.20 mg of Co, 1 mg of I, 0.15 mg of Se.

Before experimental diet formulation, nutrient values (dry matter, crude protein, crude fat, starch and total sugar) of feed ingredients were analyzed according to the methods of AOAC (2007). The rations were formulated to meet minimum requirements (NRC 1994) standards for all ingredients. The essential oils of the red pepper (*Capsicum annuum* L.) (RPEO) and the oregano (*Origanum onites* spp) (OEO) were obtained from the samples by steam-distillation using Clevenger distillation

apparatus according to U.S. Pharmacopoeia methods (1995). The major two phenolics (carvacrol and thymol) of these essential oils were determined by GC/MS (Shimadzu, QP2010-Ultra Model).

The carvacrol and thymol content of OEO were 84.02 and 1.78%, respectively. The quercetin and luteolin content of RPEO also were 20.65 and 8.80%, respectively. OEO or RPEO were added to an amount of sunflower oil and then the mixture was added to corn.

The broiler chicks were kept in wire cages with nipple drinkers under standard environmental conditions with the controlled temperature and humidity throughout the experiment. The lighting regime was 23 hours light and 1-hour darkness in the experiment. Ambient temperature was gradually decreased from 32°C on d 0 to 25°C on d 14.

A total of 18 female broiler chicks from each treatment (six chicks from each replicate) that were nearest to the average weight were selected and slaughtered by severing the jugular vein and measured the morphological parameters of the small intestine segments (duodenum, jejunum and ileum) on the 14th day.

To analyse the morphological structure of the small intestine, the intestinal content was firstly removed. Then, the samples were flushed with 0.9% (wt/vol) NaCl and handled according to Uni et al. (2003). Histological examinations were carried out using a computer-assisted image analysis according to the method of Uni et al. (1998). Villus height (VH) 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 (VSA) was calculated from the villus height and width at half height.

Statistical Analysis

Linear Model using the SPSS (17.0)[®] statistic package (2007) was applied to data with a model including OEO250 or RPEO250 and accessing time to ration and interaction between essential oils and accessing time to ration. Significant differences between treatment means were separated using Duncan's multiple range test (1955). All statements of significance were based on P<0.05.

Results and Discussion

Growth Performance

Body weights of broilers at 3rd, 7th and 14th days were not affected by RTs (Table 2). On the other hand, prolongation of accessing time to ration from immediate to 24 and 48 h post-hatching significantly decreased the body weights of broilers at 3rd, 7th and 14th days (P<0.01). Body weight gains of broilers during the period of 4-7 and 7-14 days were not significantly influenced by RTs. Access to ration for 48 h post-hatching significantly decreased the body weight gains at the period of 4-7 and 7-14 days compared to immediate and 24 h post-hatching (P<0.01). These results showed that broiler chickens could not compensate for the retardation of their body weight gain during the period of 4-7 and 7-14 d when chickens accessed to ration for 48 h post-hatch. Results of this study related to body weight gain are in agreement with the findings of Bigot et al. (2003) who reported that feed deprivation for 2 d post hatching of broilers was not sufficient to compensate for the retardation of body

weight gain and reduced. Moreover, Barreto et al. (2008) showed that there was not any significant difference between the control ration and ration supplemented with red pepper extract at 200 ppm in terms of the body weight gain of broilers throughout 21 d. Prolongation of accessing time from immediate to 24 and 48 h post-hatching to ration of broilers significantly reduced feed intake and improved feed conversion ratio (FCR) of broilers at the period of 4-7 d (P<0.01). Moreover, delaying feeding for 48 h post-hatching significantly decreased feed intake and improved FCR of broilers at the

period of 7-14 d compared to accessing time to ration for immediate and 24 h post-hatching (P<0.01). This result is not in agreement with the finding of Al-Harhi (2002) indicated that the dietary supplementation of 0.2% red pepper extract resulted in the best growth and FCR compared to the control group. RTs did not significantly affect the growth performance through all experimental period (Table 2). As a result, it can be said that their any significant effect will not show when essential oils were supplemented to ration at insufficient rates (Lee et al., 2003; Cross et al., 2007).

Table 2 Effects of treatments on the performance parameters of broiler chicks (n=3)

| RT | AT | Body weights, g/day | | | | Body weight gains, g/day | | Feed intake, g/day | | Feed conversion ratio, g/g | |
|---------|-----------|---------------------|---------------------|---------------------|--------------------|--------------------------|--------------------|--------------------|--------------------|----------------------------|-------|
| | | Initial | 3. | 7. | 14. | 4-7 | 7-14 | 4-7 | 7-14 | 4-7 | 7-14 |
| CONT | Immediate | 50.00 | 83.95 | 174.40 | 430.70 | 22.62 | 36.62 | 28.85 | 49.43 | 1.276 | 1.350 |
| | 24 h | 48.60 | 73.34 | 159.00 | 397.90 | 21.41 | 34.13 | 26.41 | 46.67 | 1.234 | 1.368 |
| | 48 h | 49.50 | 61.10 | 133.50 | 368.90 | 18.10 | 33.64 | 21.29 | 42.79 | 1.176 | 1.272 |
| OEO250 | Immediate | 50.00 | 79.24 | 159.60 | 385.60 | 20.09 | 32.28 | 26.51 | 43.26 | 1.320 | 1.340 |
| | 24 h | 48.90 | 72.86 | 158.00 | 395.90 | 21.29 | 33.98 | 25.28 | 46.02 | 1.188 | 1.354 |
| | 48 h | 49.25 | 61.29 | 137.10 | 367.10 | 18.95 | 32.85 | 21.91 | 43.19 | 1.156 | 1.315 |
| RPEO250 | Immediate | 50.00 | 83.38 | 173.70 | 410.30 | 22.59 | 33.79 | 28.73 | 47.56 | 1.272 | 1.408 |
| | 24 h | 49.00 | 71.64 | 156.10 | 396.90 | 21.12 | 34.39 | 24.36 | 47.59 | 1.153 | 1.384 |
| | 48 h | 49.40 | 61.52 | 138.90 | 363.90 | 19.34 | 32.15 | 22.39 | 43.70 | 1.158 | 1.359 |
| SEM | | 1.67 | 3.81 | 9.59 | 0.65 | 1.06 | 0.90 | 1.65 | 0.023 | 0.018 | |
| RT | CONT | 72.79 | 155.60 | 399.20 | 20.71 | 34.79 | 25.52 | 46.30 | 1.232 | 1.331 | |
| | OEO250 | 71.13 | 151.60 | 382.80 | 20.11 | 33.04 | 24.57 | 44.16 | 1.222 | 1.337 | |
| | RPEO250 | 72.18 | 156.20 | 390.40 | 21.02 | 33.45 | 25.16 | 46.28 | 1.197 | 1.384 | |
| AT | Immediate | 82.19 ^a | 169.20 ^a | 408.90 ^a | 21.76 ^a | 34.23 ^a | 28.03 ^a | 46.75 ^a | 1.288 ^a | 1.366 ^a | |
| | 24 h | 72.61 ^b | 157.70 ^b | 396.90 ^b | 21.27 ^a | 34.17 ^a | 25.35 ^b | 46.76 ^a | 1.192 ^b | 1.369 ^a | |
| | 48 h | 61.30 ^c | 136.50 ^c | 366.60 ^c | 18.80 ^b | 32.88 ^b | 21.86 ^c | 43.23 ^b | 1.163 ^c | 1.315 ^b | |
| SEM | | 0.241 | 0.96 | 2.20 | 5.54 | 0.38 | 0.61 | 0.52 | 0.95 | 0.019 | 0.011 |
| P value | | | | | | | | | | | |
| RT | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| AT | | ** | ** | ** | ** | ** | ** | * | ** | ** | ** |
| RT × AT | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

^{a,b,c} Value within a column not sharing a common superscript differ significantly (*P<0.05; ** P<0.01); Different letter superscripts indicate means that were significantly different (P<0.05), RT: ration treatments; AT: accessing time to ration; CONT: no contained essential oil; OEO250: oregano essential oil, 250 mg/kg; RPEO250: red pepper essential oil, 250 mg/kg; NS: Not Significant; SEM: Standard error of the means

Morphological Development of Small Intestine Segments

The effects of RTs and accessing time ATs to ration on the morphological development of duodenum, jejunum and ileum of small intestine in broilers on the 14th day were given in Table 3.

RTs significantly affected the villus height (DVH) (P<0.01) and the villus surface area (DVSA) (P<0.001) of duodenum. The CONT ration significantly increased DVH and DVSA compared with the OEO250 and RPEO250 rations. Moreover, access immediately to ration also enhanced DVH and DVSA of broilers (P<0.001).

There is an interaction between RTs and ATs to ration in terms of the DVH, DVSA and DCD of broilers on the 14th day (Table 3). The DVH and DVSA of the chickens fed the CONT and OEO250 rations were increased (P<0.001) by feeding immediately. On the other hand, the DVH and DVSA of broilers fed the RPEO250 ration were enhanced by 24 h post-hatching (P<0.001).

As investigated the interaction between ATs to ration and RTs, the DVH and DVSA of broilers accessed to ration immediately were increased (P<0.001) by feeding

the CONT ration. Result of this study did not similar with the findings of Akbarian et al. (2013) and Teuchert (2014) who found no significant differences between the control ration and ration supplemented with the oregano extract on villus height of duodenum of broilers. The DVH of broiler fasted for 24 h post-hatching was increased by the RPEO250 ration (P<0.001). The DVSA of broilers fasted for 24 or 48 h post-hatching, respectively, was increased (P<0.001) by the CONT and, CONT and OEO250 rations, respectively.

The crypt depth (CD) of duodenum was significantly decreased by OEO250 and RPEO250 rations (P<0.001). DCD of broilers was significantly increased by prolongation of accessing time to ration (P<0.001).

There is a significant interaction between RTs and ATs in terms of DCD of chickens (P<0.001). The DCD of broilers fed CONT ration was significantly reduced by access to ration for immediate and 48 h post-hatching (P < 0.001). On the other hand, DCDs of broilers fed OEO250 and RPEO250 rations were significantly decreased by feeding for immediate and 24 h post-hatching (P<0.001).

Table 3 The effects of different reach times to the rations containing different essential oil on the development of small intestine morphology of broilers on the 14th day

| RT | AT | Duodenum | | | Jejunum | | | Ileum | | |
|------------|-----------|--------------------------------|-------------------------------------|-------------------------------|--------------------------------|-------------------------------------|-------------------------------|-------------------------------|-------------------------------------|-------------------------|
| | | VH (μm) | VSA ($10^{-4}, \mu\text{m}^2$) | CD (μm) | VH (μm) | VSA ($10^{-4}, \mu\text{m}^2$) | CD (μm) | VH (μm) | VSA ($10^{-4}, \mu\text{m}^2$) | CD (μm) |
| CONT | Immediate | ^a 1494 ^A | ^a 84.1 ^A | ^a 158 ^B | ^b 910 ^C | ^b 42.1 ^C | ^a 146 ^B | ^b 700 ^B | ^c 28.1 ^C | 150 |
| | 24 h | ^b 1276 ^B | ^a 70.2 ^B | ^a 164 ^A | ^a 1236 ^A | ^a 57.5 ^A | ^b 146 ^B | ^b 728 ^A | ^b 33.0 ^A | 152 |
| | 48 h | ^a 1242 ^B | ^a 65.0 ^C | ^b 154 ^B | ^a 1076 ^B | ^a 51.7 ^B | ^b 152 ^A | ^b 704 ^B | ^b 31.1 ^B | 148 |
| OEO250 | Immediate | ^b 1320 ^A | ^b 73.7 ^A | ^b 146 ^B | ^a 1054 ^B | ^a 50.6 ^B | ^b 132 ^B | ^a 732 ^B | ^a 31.7 ^B | 146 |
| | 24 h | ^c 1212 ^B | ^b 64.0 ^B | ^c 148 ^B | ^b 1130 ^A | ^b 53.4 ^A | ^a 158 ^A | ^b 730 ^B | ^c 31.7 ^B | 145 |
| | 48 h | ^a 1244 ^B | ^a 62.7 ^B | ^a 166 ^A | ^b 1108 ^B | ^b 48.0 ^C | ^a 162 ^A | ^a 794 ^A | ^a 36.3 ^A | 144 |
| RPEO250 | Immediate | ^c 1212 ^B | ^c 57.1 ^B | ^b 146 ^B | ^b 894 ^B | ^c 40.2 ^B | ^b 136 ^B | ^b 694 ^B | ^b 30.6 ^B | 144 |
| | 24 h | ^a 1306 ^A | ^b 62.5 ^A | ^b 150 ^B | ^c 910 ^B | ^c 40.3 ^B | ^c 132 ^B | ^a 802 ^A | ^a 36.2 ^A | 148 |
| | 48 h | ^a 1248 ^B | ^b 58.1 ^B | ^a 166 ^A | ^a 1052 ^A | ^b 47.6 ^A | ^c 142 ^A | ^b 712 ^B | ^c 30.3 ^B | 146 |
| RT | CONT | 1337 ^a | 73.1 ^a | 159 ^a | 1074 ^a | 50.5 ^a | 151 ^a | 711 ^c | 30.7 ^c | 150 ^a |
| | OEO250 | 1259 ^b | 66.7 ^b | 153 ^b | 1097 ^a | 50.7 ^a | 148 ^b | 752 ^a | 33.2 ^a | 145 ^b |
| | RPEO250 | 1255 ^b | 59.2 ^c | 154 ^b | 952 ^b | 42.7 ^b | 137 ^c | 736 ^b | 32.4 ^b | 146 ^b |
| | Immediate | 1342 ^a | 71.6 ^a | 150 ^c | 953 ^b | 44.3 ^b | 138 ^c | 709 ^b | 30.1 ^b | 147 |
| AT | 24 h | 1265 ^b | 65.5 ^b | 154 ^b | 1092 ^a | 50.4 ^a | 145 ^b | 753 ^a | 33.6 ^a | 148 |
| | 48 h | 1245 ^b | 61.9 ^b | 162 ^a | 1079 ^a | 49.1 ^a | 152 ^a | 737 ^a | 32.6 ^a | 146 |
| Pooled SEM | | 20.460 | 8.434 | 2.567 | 12.478 | 3.265 | 2.896 | 8.075 | 2.896 | 1.985 |
| P value | | | | | | | | | | |
| RT | | ** | *** | *** | *** | *** | *** | *** | * | ** |
| AT | | *** | *** | *** | *** | *** | *** | *** | *** | NS |
| RT × AT | | *** | *** | *** | *** | *** | ** | ** | *** | NS |

^{a,b,c}, A, B,C Value within a column not sharing a common superscript differ significantly (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Different letter superscripts indicate means that were significantly different ($P < 0.05$)

^{A, B, C} Value within a right column with different capital letters show difference between ration treatments and accessing time to ration

^{a, b, c} Value within a left column with different small letters show difference between accessing time to ration and ration treatments

SEM: Standard error of means; NS: Not Significant; RT: ration treatments; AT: accessing time to ration; CONT: no contained essential oil; OEO250: oregano essential oil, 250 mg/kg; RPEO250: red pepper essential oil, 250 mg/kg; VH: Villus Height; VSA: Villus Surface Area; CD: Crypt Depth

There is also a significant interaction between ATs and RTs in terms of DCD of broiler ($P < 0.001$). DCDs of chickens fed immediate or 24 h post-hatching were significantly decreased by OEO250 and RPEO250 rations ($P < 0.001$). On the other hand, DCD of broilers accessed to ration for 48 h post-hatching was significantly reduced by CONT ration ($P < 0.001$).

This finding did not in agreement with the results of Akbarian et al. (2013) and Teuchert (2014) who reported that the plant extracts did not affect any significant effect on the crypt depth of duodenum in broilers.

The essential oils and their phenolic compounds have an antibacterial activity due to combating pathogenic bacteria in the small intestine of poultry (Ayman et al. 2016). As a result of this, the total pathogenic bacteria in the intestinal wall, the production of toxic compounds and the damage to intestinal epithelial cells like deeper crypts were decreased by the essential oils (Ghazanfari et al., 2014).

The CONT and OEO250 rations significantly enhanced the villus height (JVH) and villus surface area (JVSA) of jejunum of broilers compared to the RPEO250 ration ($P < 0.001$). In addition, access to ration for 24 and 48 h post-hatch significantly increased JVH and JVSA of broilers compared with immediately access ($P < 0.001$).

There is a significant interaction ($P < 0.001$) between RTs and ATs to ration in terms of the villus height (JVH), villus surface area (JVSA) and crypt depth (JCD) of jejunum of broilers on the 14th day (Table 3). The JVH and JVSA of broilers fed the CONT and OEO250 rations were increased by feeding after 24 h post-hatching

($P < 0.001$). Moreover, the JVH and JVSA of chickens fed with the RPEO250 ration were enhanced by feeding after 48 h post-hatching ($P < 0.001$).

The interaction between ATs to ration and RTs, the JVH and JVSA of broilers immediately accessed to ration were the highest ($P < 0.001$) when broilers were fed the OEO250 ration.

This finding agrees with the results of Jamroz et al. (2006) who reported that the dietary supplementation of a plant extract blend caused to major increase in JVH of chickens. Sujatha et al. (2017) pointed out villus height of jejunum of chicks fed with neem supplementation was significantly increased compared to that of control chicks.

On the contrary, the results related to the JVH did not in agreement with the findings of Teuchert (2014) who found no significant differences between the control ration and ration supplemented with an oregano extract on villus height of jejunum in broilers.

On the other hand, the JVH and JVSA of broilers fasted for 24 h post-hatching were increased by the CONT ration ($P < 0.001$). There are no differences among the rations in terms of JVH of chickens accessed to ration for 48 h post-hatching. JVSA of chickens accessed to ration for 48 h post-hatching was significantly increased by the CONT ration ($P < 0.001$).

The JCD of broilers was significantly decreased by OEO250 and RPEO250 rations ($P < 0.001$). Prolongation of accessing time to ration significantly increased JCD of broilers at 14 days ($P < 0.001$).

There is a significant interaction between RTs and ATs in terms of JCD of broilers ($P < 0.01$). JCDs of

broilers fed CONT or RPEO250 rations were significantly reduced by access to ration for immediate or 24 h post-hatching ($P<0.01$). On the other hand, JCD of broilers fed OEO250 ration was significantly reduced by feeding immediately ($P<0.01$).

There is also a significant interaction between ATs and RTs in terms of JCD of broilers ($P<0.01$). JCD of broilers accessed to ration immediately was significantly decreased by OEO250 and RPEO250 rations ($P<0.01$). On the other hand, JCD of chickens fed after 24 or 48 h post-hatching was significantly reduced by RPEO250 ration ($P<0.01$).

This result did not in agreement with the findings of Garcia et al. (2007), Akbarian et al. (2013) and Teuchert (2014) who pointed out supplementation of a dietary plant extract blend or oregano extract to ration did not influence the JCD of chickens.

Feeding OEO250 and RPEO250 rations significantly increased IVH and IVSA of broilers compared to those of broilers fed CONT ration. Moreover, prolongation of accessing time to ration from immediate to 24 and 48 h post-hatching significantly increased IVH and IVSA of broilers ($P<0.001$).

This finding is in agreement with the results of Skoufos et al. (2016) who reported that the supplementation of oregano essential to ration significantly increased IVH compared to the CONT group. On the contrary, Teuchert (2014) found that oregano extract supplementation to ration did not influence IVH of broilers compared to the control ration.

There is a significant interaction between RTs and ATs in terms of IVH ($P<0.01$) and IVSA ($P<0.001$) of broilers. IVHs of broilers fed CONT and RPEO250 rations were significantly increased by feeding after 24 h post-hatching ($P<0.01$). On the other hand, IVH ($P<0.01$) and IVSA ($P<0.001$) of broilers fed OEO250 ration was significantly enhanced by access to ration for 48 h post-hatching.

There is also a significant interaction between ATs and RTs in terms of IVH ($P<0.01$) and IVSA ($P<0.001$) of broilers. IVH of broilers fed immediately or 48 h post-hatching was significantly increased by OEO250 ration ($P<0.01$). On the other hand, IVH ($P<0.01$) and IVSA ($P<0.001$) of broilers accessed to ration for 24 h post-hatching was significantly enhanced by RPEO250 ration. The results in this study indicate that feeding essential oils enriched in phenolic compounds due to their high antimicrobial activities increased villus height that is associated with enhanced digestive and absorptive surface area and nutrient absorption (Ghazanfari et al., 2015).

OEO250 and RPEO250 rations significantly decreased ICD of broilers compared to that of broilers fed CONT ration ($P<0.01$). On the other hand, ATs did not influence ICD of broilers.

Oregano- and red pepper- essential oils and their phenolic compounds reduced the microbial load of pathogenic bacteria and their toxins on the small intestine. As a result of this, the crypt depths of small intestine segments were decreased, which prevented the degeneration of absorptive epithelial cells (Al-Mashhadani et al., 2013).

Conclusion

RTs did not significantly affect the growth performance through all experimental period. Prolongation of accessing time to ration from immediate to 24 and 48 h post-hatching significantly decreased the body weights of broilers at 3rd, 7th and 14th days, feed intake from 4 to 7 d and improved feed conversion ratio (FCR) of broilers at the period of 4-7 d. Access to ration for 48 h post-hatching significantly decreased the body weight gains at the period of 4-7 and 7-14 days, feed intake from 7 to 14 d and improved FCR of broilers at the period of 7-14 d compared to accessing time to ration for immediate and 24 h post-hatching. The supplementation of oregano- and red pepper-essential oils to ration at 250 mg/kg level affected positively the villus height and villus surface area of ileum and reduced crypt depths of small intestine segments of broiler accessed to ration at different times.

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