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RESEARCH ARTICLE

Effects of Oral Administration of Essential Oil (Mix Oil®) on Growth Performance and Intestinal Morphometry of Japanese Quails (*Coturnix coturnix japonica)*

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ABSTRACT

The current study was performed to evaluate the potential effects of essential oil (MIX OIL®) on growth and histomorphometry of intestine in Japanese quail. The Mix Oil® is available as commercial product (Animal Wellness Product, Italy) which is a blend of different essential oils. This product contains eucalyptus oil, thyme oil, rosemary oil, savory oil, lemon oil, garlic oil, citric acid and oregano oil. A total of one hundred day-old quail chicks were purchased from a local hatchery. Birds were placed at temperature-controlled sheds at quail farm. The birds were separated randomly into four groups (A, B, C and D) containing 25 birds each. Birds were offered commercial basal corn-based diet and offered three different graded concentrations of Mix Oil®. Group-A was control while group B, C and D contain 0.33ml/2 liter of water 0.15%, 0.5ml/2 liter of water 0.25%, 1ml/2 liter of water 0.5%, respectively. On day 35, all birds were slaughtered and the samples of intestines were excised and prepared for histological studies. The significant differences (P<0.05) in body weight of birds, weight gain of quails and feed conversion ratio was recorded. Among the histomorphometric parameters of intestine, there was an increase observed in villus height and significant change in number of goblet cells among all treated groups. In conclusion, essential oil (MIX OIL®) was proved to be a potent growth promoter. The intestinal health was also improved by giving essential oil (MIX OIL®) that resulted in better growth in Japanese quails.

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INTRODUCTION

The use of essential oils is gaining much attention in modern livestock and poultry production systems because of having multidimensional benefits with the ultimate objective of maintaining overall health (Idris *et al*., 2017; Abbas *et al*., 2018; Khater *et al*., 2018; Ahmad *et al*., 2019; Fayaz *et al*., 2019).

In poultry industry, the essential oils usage is the center of attention particularly in case of morphology of intestinal tract and digestibility (Wenk, 2002).

The basic components of the essential oils are the volatile compounds with the complex and complicated mixtures obtained from plants (aromatic) as their secondary metabolite. Essential oils or volatile oils are aromatic oily liquids extracted by distillation from plant parts, such as flowers, buds, seeds, leaves (Adaszyńska *et al*., 2017) Most essential oils are derived from twenty to sixty compounds from a wide range of chemicals, primarily terpenes and their further derivatives. Mostly the essential oils antimicrobial activity is mainly by the interactions among these compounds and bioactivity of these oils is closely related with the main component (Valero and Salmeron, 2003). Along with their antimicrobial properties, they possess biological activities, for example as an antioxidant (Miura *et al*., 2002) and stimulatory effects on the digestive system of animals reported by Ramakrishna *et al*. (2003), to enhance the formation of digestive enzymes and better utility of products involved in digestion through improved functions of the liver (Hernandez *et al*., 2004).

Dietary interventions have markedly improved the morphology of the intestine in poultry. Good intestinal health performs key role in poultry to attain the maximum growth and feed efficiency (Montagne *et al.*, 2003). Intestinal epithelium is a natural filter against harmful bacteria and detrimental substances. The nutrients absorption has been improved by the alterations in intestinal morphology, such as increased villus height, decreased crypt depth, increased villus height to crypt depth ratio and eventually more surface area of small intestine offered for absorption (Awad *et al*., 2008). The alterations in gastrointestinal microflora also produce changes in the immune response of the birds (Choct, 2009). Antibiotic growth promoters when administered in the diet resulted in decreased thickness of the intestinal wall and increase in length of gastrointestinal tract (Dibner *et al*., 1996). Aromatic plants, their seeds and essential oils have been tested for their growth-promoting properties in broiler chickens (Alcicek *et al.*, 2003**)** as well as in layers (Bozkurt *et al*., 2012).

Very few studies are available on supplementation of poultry diet with essential oils and their capability to change intestinal morphology in terms of growth-promoting properties. The current study is intended to examine the essential oils (Mix Oil) effects supplemented through drinking water on growth performance including overall weight, feed consumption ratio, weight of the internal organs in relation to body weight, alterations in microarchitecture of intestine in Japanese quail (*Coturnix coturnix japonica*).

MATERIALS AND METHODS

Experimental design and diet plan: Present study involved one-hundred day-old quail chicks obtained from local hatchery and kept in experimental sheds at quail farm. These sheds were thoroughly cleaned with 10% Formalin and $KMnO_4$ before arrival of birds (Screenivasaiah, 2006). The birds were weighed and randomly divided into 4 equal groups A, B, C and D. Each group of 25 birds was kept in a separate shed.

Birds were fed with corn-based commercial basal diet. Birds were supplemented with three different levels (0.15%, 0.25% and 0.5% prepared in water) of essential oil (Mix Oil®) available as commercial product (Animal Wellness Product, Italy) administered through drinking water for five weeks. Group-A was control, Group-B was given basal diet + 0.15% Mix oil® (0.33 ml/2 liter of water), Group-C was given basal diet +0.25% Mix oil®. (0.5 ml/2 liter of water) and Group-D was given basal diet+ 0.5% Mix oil® (1 ml/2 liter of water).

Growth parameters and sampling: Body weights of the birds were recorded individually by weighing balance at the end of each week and feed intakes per pen were also calculated. Feed conversion ratio was calculated for each group. Mortality was also recorded during experiment. At 35 days of experiment, five Japanese quails were randomly slaughtered from each group and the segments of intestine were excised for histomorphometry.

Histomorphometry of intestine: The samples of intestine were fixed in 10% neutral buffered formalin solution (24 hours), paraffin-embedding was performed and sectioned at 5 µm. Slides were thoroughly studied under light microscope [LABOMED®, USA] attached with a computer. Histomorphometry of each sample was done by a commercial program (Prog Res® 2.1.1 Camera Control Software). Evaluation of the following parameters were performed: villus height, villus width, villus surface area, depth of villus, and mucosal layers of the intestine. The counting of differential goblet cells was also performed by staining with combination of Alcian blue-PAS. The blue-stained goblet cells were acidic, mucin (goblet cells) and mixed containing both neutral and acidic mucin showed purple stain (Bancroft *et al*., 2013).

Statistical analysis: The analysis of the experimental data was done by ANOVA technique using SPSS®. The difference in between the groups was evaluated by application of appropriate one-way ANOVA. The comparison of the means was performed by using post hoc test to identify the degree of significance at $P<0.05$.

RESULTS

Aromatic plants, their essential oils and seeds have been used earlier in research studies for their growth-promoting properties in poultry**.** They are also experimented for their role in digestion by investigating their effects on intestinal morphology (Alcicek *et al*., 2003).

Growth performance: The difference in body weight was recorded in all treated groups on weekly basis and found non-significant during the first week of experiment. In the second, third, fourth and fifth week of experiment, significant difference ($P<0.05$) was recorded (Table 1). The effect of essential oil (Mix oil) was recorded by calculating the weight gain per day in grams (g). The results of weight gain per day have been presented in Table 2. The difference in weight gain between groups supplemented with 0.15, 0.25, 0.5% and control group was significant ($P<0.05$) during the entire five weeks of experimental study (Table 2). The feed intake comparatively was in decreasing tendencies in control group and Group D in comparison to Group C and Group B (Table 3). The difference in feed conversion ratio FCR between groups was recorded non-significant in first week of experiment. In 2nd, 3rd, 4th and 5th week significant difference ($P<0.05$) in FCR was recorded among all experimental groups. The feed conversion ratio was comparatively in decreasing tendencies from control group to treated groups (Table 4). The decrease in feed conversion ratio indicates better feed conversion.

Intestinal histomorphometry: The effect of essential oil supplementation on different segments of small intestine duodenum, jejunum and ileum was recorded in terms of alteration in morphometry of these parts of small intestine such as measurement of villus height, villus width, villus surface area, villus height to crypt depth, crypt depth ratio, thickness of muscularis mucosa and thickness of tunica muscularis.

Duodenum: The experimental study revealed that there was significant increase in villus height,villus height/crypt depth ratio and tunica muscularis thickness ($P<0.05$) while the difference in other morphological parameters width of villus, crypt depth and thickness of muscularis mucosae was found non-significant (Table 5a, Fig. 1).

Table 1: Effects of essential oil (MIX OIL®) on body weight (g per bird) of Japanese quail (*Coturnix japonica*)

Weeks	Group A Control	Group B (0.15%)	Group C (0.25%)	Group D (0.5 %)	*p-value*
1st week	29.3±2.2^{aE}	31.1±2.0^{aE}	29.9±3.1^{aE}	31.5±3.2^{aE}	0.710
2nd week	51.8±1.0^{bD}	58.8±1.3^{abD}	57.8±4.2^{abD}	59.1±3.1^{aD}	0.035
3rd week	87.8±2.3^{bC}	96.0±2.3^{aC}	94.7±3.1^{abC}	103.1±2.9^{aC}	0.001
4th week	127.8±2.8^{cB}	139.6±2.9^{bB}	143±3.9^{bB}	156±2.1^{aB}	0.000
5th week	167.1±2.6^{cA}	185.1±1.3^{bA}	194.1±3.1^{aA}	211.1±3.9^{aA}	0.000

Different small alphabetic superscript within each row and different capital alphabetic superscript within each column showing the significant difference P<0.05 while similar superscript showing the non-significant difference. Values are Mean±SEM.

Table 2: Effects of essential oil (MIX OIL®) on weight gain (g per day) in Japanese quails (*Coturnix japonica*)

Weeks	Group A Control	Group B (0.15%)	Group C (0.25%)	Group D (0.5 %)	*p-value*
1st week	2.88±0.12^{bB}	3.23±0.1^{aD}	2.98±0.05^{abE}	3.21±0.15^{aE}	0.011
2nd week	3.21±0.2^{bB}	3.96±0.14^{aC}	3.98±0.21^{aD}	3.94±0.11^{aD}	0.001
3rd week	5.1±0.5^{bA}	5.32±0.2^{bB}	5.27±0.22^{bC}	6.28±0.19^{aC}	0.006
4th week	5.7±0.51^{cA}	6.21±0.21^{bcB}	6.9±0.15^{abB}	7.64±0.1^{aB}	0.000
5th week	5.61±0.1^{dA}	6.5±0.14^{cA}	7.28±0.1^{bA}	7.91±0.15^{Aa}	0.000
P-value	0.000	0.000	0.000	0.000	-

Different small alphabetic superscripts within each row and different capital alphabets within each column showing the significant difference P<0.05 while similar superscript showing the non-significant difference. Values are Mean±SEM.

Table 3: Effects of essential oil (MIX OIL®) on feed intake (g per day) in Japanese quails (*Coturnix japonica*)

Weeks	Group A Control	Group B (0.15%)	Group C (0.25%)	Group D (0.5 %)	*p-value*
1st week	8.0±0.1^{bcE}	8.5±0.16^{aE}	8.3±0.2^{abE}	7.9±0.12^{cE}	0.004
2nd week	11.9±0.11^{aD}	11.5±0.21^{abD}	10.9±0.2^{cD}	11.1±0.1^{bcD}	0.000
3rd week	17.6±0.2^{aC}	16.9±0.1^{bC}	18.1±0.3^{aC}	16.7±0.1^{bC}	0.000
4th week	22.5±0.11^{abB}	21.9±0.12^{bB}	23.1±0.21^{aB}	22.9±0.4^{aB}	0.001
5th week	23.1±0.1^{bA}	24.1±0.3^{abA}	24.5±0.5^{aA}	23.8±0.5^{abA}	0.013
P-value	0.000	0.000	0.000	0.000	-

Different small alphabetic superscript within each row and different capital alphabets within each column showing the significant difference P<0.05 while similar superscript showing the non-significant difference. Values are Mean±SEM.

Table 4: Effects of essential oil (MIX OIL®) on feed conversion ratio (FCR) in Japanese quail (*Coturnix japonica*)

Weeks	Group A Control	Group B (0.15%)	Group C (0.25%)	Group D (0.5 %)	*p-value*
1st week	2.7±0.21^{aD}	2.63±0.15^{aC}	2.7±0.11^{aB}	2.46±0.12^{aD}	0.253
2nd week	3.7±0.11^{aBC}	2.90±0.2^{bBC}	2.73±0.13^{bB}	2.81±0.10^{bBC}	0.000
3rd week	3.45±0.2^{aC}	3.17±0.1^{aB}	3.43±0.15^{aA}	2.65±0.10^{bC}	0.000
4th week	3.94±0.1^{aAB}	3.52±0.2^{bA}	3.34±0.12^{bcA}	2.99±0.12^{cAB}	0.000
5th week	4.11±0.2^{aA}	3.69±0.1^{bA}	3.36±0.1^{cA}	3.01±0.05^{dA}	0.000
P-value	0.000	0.000	0.000	0.000	-

Different alphabetic small superscript within each row and different capital alphabets within each column showing the significant difference P<0.05 while similar superscript showing the non-significant difference. Values are Mean±SEM.

Jejunum: The experiment results obtained revealed significant increase in villus height and thickness of tunica muscularis (P<0.05) while the difference in other morphological parameters villus width, crypt depth, villus surface area and muscularis mucosae thickness was found non-significant (P>0.05) in jejunum. (Table 5b, Fig. 1).

Ileum: The experiment results revealed that there was significant (P>0.05) increase in villus height, villus width, muscularis mucosae thickness and thickness of tunica muscularis (P<0.05) while the changes in other morphological parameters crypt depth, villus surface area and villus height/crypt depth ratio was found non-significant (P>0.05) in ileum. (Table 5c, Fig. 1).

Histochemistry of goblet cells: The total number of goblet cells, acidic and mixed goblet cells were counted per villus in all three segments (duodenum, jejunum and ileum) of small intestine in four experimental groups. The acidic goblet cells stained blue whereas mixed goblet cells stained purple. The acidic, mixed and total goblet cells counted in all treatment groups presented in Table 6 and Fig. 1b. There was significant change in goblet cell numbers among all treatment group in comparison to control group.

DISCUSSION

Antibiotics are now replacing from other natural and safe products to increase the performance of birds in poultry industry. By considering the versatile actions of essential oils, they can be used as growth promoter. In present experimental study, body weight and weight gain was significantly affected by the essential oil (Mix Oil®) supplementation. The body weight and weight gain was significantly increased in group D supplemented with 0.5% concentration of essential oil in comparison to control and group B supplemented with 0.15% concentration of essential oil. The FCR was also improved in essential oil supplemented groups predominantly in group D in comparison to other groups. These results are in accordance with the Alcicek *et al*. (2003) who performed an experimental stud by giving six different essential oils mixture (oregano oil, sage leaf oil, myrtle leaf oil, fennel seed oil and citrus peel oil) and reported increased body weight and weight gain in broiler. This positive effect on weight gain as well as growth of Japanese quails is probably due to the presence of plant extracts in essential oils with their growth-promoting properties such as their antimicrobial activity (Sies *et al*., 1979; Ouwehand *et al*., 2010) antioxidant activity (Hashemipour *et al*., 2013), immune-stimulating (Awaad *et al*., 2010; Gopi *et al*., 2014) and anti-inflammatory effects (Krishan and Narang, 2014) of essential oil. The improved FCR and weight gain achieved with supplementation of essential oil could be attributed because of the beneficial effects on nutrient digestibility and digestion stimulating effects of essential oil as described by Langhout (2000).

Table 5 (a): Effect of essential oil (MIX OIL®) on the duodenum of Japanese quail (*Coturnix japonica*)

Duodenum	Group A Control	Group B 0.15% E.O	Group C 0.25% E.O	Group D 0.5% E.O	p-value
VH (μm)	478.48±31.42^{c}	602.86±59.92^{b}	716.10±32.36^{ab}	775.81±36.58^{a}	0.000
VW (μm)	94.27±14.29	85.14±6.49	85.55±8.29	86.96±6.48	0.888
CD (μm)	185.37±16.711	217.51±51.90	183.31±21.013	153.13±19.28	0.566
MMT(μm)	14.69±0.87	11.64±1.14	12.99±1.22	15.05±1.24	0.137
VSA (μm^2)	13956±2879	15663±2061	18653±1828	20604±1510	0.137
MET (μm^2)	30.74±1.36^{b}	38.12±3.63^{ab}	37.85±1.76^{ab}	45.22±4.08^{a}	0.014
VH:CD	2.97±0.42^{b}	3.67±0.54^{b}	4.23±0.42^{ab}	5.41±0.62^{a}	0.012

$^{a-c}$Within the same row, means having different superscripts present significantly different values (p<0.05). Values are Mean±SEM. VH: Villus height, VW: Villus width, CD: Crypt depth, MMT: Muscularis mucosa thickness, VSA: Villus surface area, MET: Muscularis externa thickness, VH:CD: Villus height/crypt depth ratio.

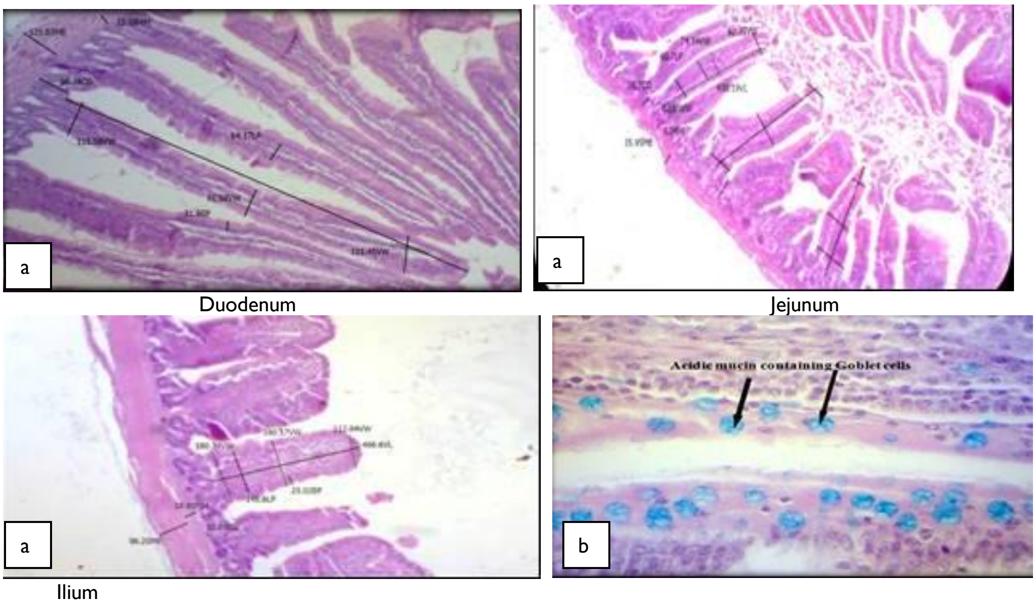

Fig. 1 (a): Photo-micrographs of different segments of small intestine of Japenese quails (*Coturnix japonica)* (H&E; 100X*)*.Ileum showing mucosal layers for measuring villi height, width and crypt depth in micrometer (H&E; 100X*)*. VH: Villus height, VW: Villus width, CD: Crypt depth, MMT: Muscularis mucosa thickness, VSA: Villus surface area, MET: Muscularis externa thickness, VH:CD: Villus height/crypt depth ratio. **(b):** Morphometery of intestine showing different type of goblet cells (PAS stain) at 400 X.

Table 5 (b): Effects of essential oil (MIX OIL®) on the jejunum of Japanese quail (*Coturnix japonica*)

Jejunum	Group A Control	Group B 0.15% E.O	Group C 0.25% E.O	Group D 0.5% E.O	p-value
VH (μm)	394.85±44.79	411.56±50.15	414.52±41.46	536.93±22.77	0.067
VW (μm)	92.61±8.23	93.61±5.60	97.16±9.28	74.77±3.96	0.786
CD (μm)	103.01±18.67	135.92±18.85	116.10±20.23	149.71±33.83	0.523
MMT (μm)	18.62±1.15	16.28±0.93	18.53±1.11	17.83±2.01	0.342
VSA (μm^2)	11660±3345	12450±1455	13471±1813	13551±1143	0.908
MET (μm^2)	44.68±4.75[ab]	34.95±2.30[b]	38.51±3.04[ab]	48.03±2.64[a]	0.035
VH:CD	4.14±0.67	3.35±0.62	3.88±0.51	4.50±0.72	0.626

[a-c]Within the same row, means having different superscripts present significantly different values (P<0.05). Values are Mean±SEM. VH: Villus height, VW: Villus width, CD: Crypt depth, MMT: Muscularis mucosa thickness, VSA: Villus surface area, MET: Muscularis externa thickness, VH:CD: Villus height/crypt depth ratio.

Table 5 (c): Effect of essential oil (MIX OIL®) on the morphometry of ileum of Japanese quail (*Coturnix japonica)*

Ilium	Group A Control	Group B 0.15% E.O	Group C 0.25% E.O	Group D 0.5% E.O	p-value
VH (μm)	265.14±43.63[bc]	225.61±13.69[c]	341.35±28.24[ab]	408.32±48.53[a]	0.0041
VW (μm)	115.71±10.59[a]	89.72±5.12[b]	92.96±8.38[b]	76.17±4.31[b]	0.0021
CD (μm)	167.85±14.747	90.08±14.079	174.94±22.47	156.50±47.30	0.125
MMT(μm)	15.13±0.99[a]	22.15±3.06[b]	18.65±1.16[ab]	22.86±2.39[a]	0.046
VSA (μm^2)	97153±22062	58348±36218	92523±10361	893345±11139	0.186
MET (μm^2)	36.34±3.39[b]	41.83±4.87[b]	52.78±4.18[a]	37.00±2.33[b]	0.011
VH:CD	2.72±0.36	4.27±1.42	3.26±0.31	4.05±0.81	0.104

[a-c]Within the same row, means having different superscripts present significantly different values (P<0.05).Values are Mean±SEM. VH: Villus height, VW: Villus width, CD: Crypt depth, MMT: Muscularis mucosa thickness, VSA: Villus surface area, MET: Muscularis externa thickness, VH:CD: Villus height/crypt depth ratio.

Table 6: Histochemistry of Goblet cell in various parts of small intestine of Japanese quail (*Coturnix japonica*).

Segments	Group A Control	Group B 0.15% E.O	Group C 0.25% E.O	Group D 0.5% E.O	p-value
Duodenum					
Acidic	54.66±2.40[b]	64.11±2.76[ab]	69.61±5.16[a]	69.46±4.70[a]	0.032
Mixed	57.69±1.85[a]	44.84±3.09[b]	44.69±3.37[b]	54.59±4.68[ab]	0.021
Total	112.35±4.25[c]	108.95±5.75[ab]	114.30±8.53[a]	124.05±9.38[b]	0.000
Jejunum					
Acidic	52.57±2.15[c]	56.11±2.56[bc]	65.41±4.71[ab]	74.46±3.60[a]	0.000
Mixed	51.79±4.72[ab]	33.09±1.99[c]	40.19±5.15[bc]	53.19±3.77[a]	0.0031
Total	104.36±6.87[b]	89.20±4.55[b]	105.50±9.86[b]	127.65±7.37[a]	0.0042
Ileum					
Acidic	42.36±11.17[c]	52.06±5.60[b]	52.26±11.45[b]	69.67±3.71[a]	0.000
Mixed	46.58±3.69[a]	33.14±1.75[b]	36.26±3.40[b]	48.01±3.46a	0.000
Total	88.94±14.86[b]	85.20±7.35[b]	84.52±14.85[b]	117.68±7.17[a]	0.001

Within the same row, means having different superscripts present significantly different values (P<0.05). Values are Mean±SEM.

Hernandez *et al*. (2004) also concluded similar positive effect of essential oil on weight gain and performed an experimental study by adding the plant extracts mixed in feed and produced higher body weight in broiler chicken. Furthermore, several other group of researchers have reported the same growth-promoting action of essential oils in quails reported by Denli *et al*. (2004) and broilers (Jamroz *et al*., 2003; Ciftci *et al*., 2005). In contrast to present study, another group of scientists concluded from their studies performed on broiler chicken that the essential oils or mixture of different essential oils does not always show improvement in production performance and body weight gain or even make it worse (Papageorgiou *et al*., 2003; Zhang *et al*., 2005). These differences in results may be due to involvement of weak birds, influence of environmental factors such as bedding, presence of rodents, equipments, light factors and violation of biosafety rules.

Essential oils have a positive effect on the avian digestive system, since they help to restore the microbiota balance and increase nutrient absorption (Adaszyńska *et al.*, 2007). Mucosa of intestine is mainly responsible for the digestion of food and absorption of digestive nutrients which further regulate the growth in animals (Cheng *et al*. 2015). Shorter villi and deeper crypts may be a reason of absorption of poor nutrient, higher toxins secretion in the intestinal tract, and poor performance as reported by Xu *et al*. (2003).

In duodenum, the present experimental study revealed that villus height, villus height /crypt depth ratio and thickness of tunica muscularis increased significantly ($P<0.05$) while in jejunum height of villus and thickness of tunica muscularis significantly different from control group. In ileum, villus height, width of villus, muscularis mucosae thickness and thickness of tunica muscularis ($P<0.05$) found significantly increased in essential oil supplemented group. The results of experiment are in line with Denli *et al*. (2004) who observed that supplementation of thyme and black seed essential oil increase villi height of small intestine. These results are also in accordance with Hernandez *et al*. (2004) who investigated that the essential oils supplementation improved nutrient digestibility of ileum and increased secretion of digestive enzymes and improved feed utility due to increased liver function of broilers.

The present results are in accordance with Cabuk *et al*. (2014) who concluded from their experiment that adding up essential oil to the diet as a supplement increased goblet cell size and its number. Same type of results was reported by Reisinger *et al*. (2011) who uses blend of essential oil as supplementation resulted in increases the goblet cell number. Goblet cells are liable for the formation of mucin secreted from intestine which is the main constituent of the mucous membrane that form a coating in the chicken intestine. This layer is responsible for the protection of the host against microflora of lumen, cause prevention of gastrointestinal pathogenesis and takes part in nutrient absorption and digestion (Garcia *et al*., 2007).

It is conceivable from the present data that essential oil (MIX OIL®) has served as a potent growth promoter. Concurrently, the intestinal health has also improved by using essential oil (MIX OIL®) as feed additive in Japanese quails.

Authors contribution: ASQ conceived and designed the project and finalized the manuscript, AM conducted the experiment, applied the statistics, interpreted the results and prepared the manuscript. RS and HJ revised and approved the final manuscript.

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