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Effect of pennyroyal, savory and thyme essential oils on Japanese quail physiology

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Abstract

An experiment was conducted to show the effects of different levels of pennyroyal, thyme and savory essential oils dietary supplementation on performance, organs weight, intestinal morphology, and serum lipids in quails. A total of 550 day-old Japanese quail chicks were allocated into 11 dietary treatments of 5 replications (10 birds in each cage) under a randomized experimental design. The treatments were a basal diet (Control), or 3 levels (200, 300, and 400 ppm) of thyme essential oil (TO), or 3 levels (200, 300, and 400 ppm) of savory essential oil (SO), or 3 levels (200, 300, and 400 ppm) of pennyroyal essential oil (PO) added separately to the basal diet, or also the basal diet supplemented with 100 ppm of flavophospholipol. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured weekly and calculated totally for 35 d. The organs weight and morphology of intestine parts of one bird from each cage were measured on 35 d. At the same day, blood samples were collected and cholesterol and triglyceride values were separately determined for male and female quails. Although the addition of different levels of essential oils in quail's diet did not affect BWG, a significant decline in FI was observed in the group

supplemented with 400 ppm of TO. At the same time, FCR significantly improved ($P < 0.05$) in the same group as well as in the group supplemented with the antibiotic. However, the organs weight was not significantly affected by the experimental treatments. Nevertheless, villi height of duodenum, jejunum and ileum was significantly increased and crypt depth was significantly decreased in the quails fed diets supplemented with different levels of TO and SO. The values of serum triglycerides decreased in both sexes in the groups that received diets supplemented with different levels of essential oils whereas the values of cholesterol decreased only in males of the essential oils supplemented groups. From the present observations it can be concluded that thyme and savory essential oils can improve FCR by decreasing FI through boosting the absorption of nutrients in intestine. These plant essential oils can replace antibiotic growth parameters without having any adverse effect on quail's health with thyme essential oil exerting the most effective activity.

Keywords: Agriculture, Veterinary medicine, Food science, Zoology, Plant biology, Physiology

1. Introduction

With the explosive growth of the human population in the last century, the nutritional needs are continuously increased. The supply of protein products has a special significance among food sources. Poultry meat is a very important source of protein; therefore, in many countries increased capital has been invested in the poultry industry and especially in quails. The quail is the smallest bird of the pheasant family. The amount of protein in the meat is 5–10 percent more than other birds and ruminants (Genchev et al., 2008). Quail meat is also a good source of niacin, phosphorus, copper, thiamine, riboflavin, vitamin B6, zinc, iron, and selenium (Hamm and Ang, 1982; Santhi and Kalaikannan, 2017).

Nowadays, human diet is related with several diseases and as a result, people's desire to use natural products increased. Therefore, researchers are seeking alternatives to replace synthetic sources used in poultry farming, such as growth promoting antibiotics that are used for increasing the efficiency of poultry production and the improvement of meat quality and health status. One of the proposed ingredients for the replacement of these substances is the use of essential oils. The main effects of essential oils and their modes of action in poultry production have already been reviewed by Brenes and Roura (2010). They proposed four mechanisms that describe the action of essential oils, i.e., sensorial, metabolic, antioxidant and antimicrobial. In addition, it was noted that the type and the applied level of the essential oil have the major influence on the performance and nutrient availability in the birds (Chowdhury et al., 2018). For example, supplementation with 0.3 g of cinnamon oil

per kg of diet could improve growth performance and nutrient utilization in broiler chickens while 0.6 g/kg of clove oil and 0.4 g/kg ajwain oil in broiler diets did not show any effect (Chowdhury et al., 2018).

Although a lot of studies exist describing the effects of essential oils on poultry, scarce data exist on the effects of Lamiaceae family essential oils on quail production. Therefore, the present study is designed to investigate and compare the effects of thyme, savory and pennyroyal essential oils in quail nutrition by emphasizing on performance, relative weight of organs, intestinal morphology and the value of triglyceride and cholesterol contents at grower and developer phases.

2. Materials & methods

2.1. Essential oils

Hydro-distillated thyme (*Thymus vulgaris*) essential oil, savory (*Satureja hortensis*) essential oil, and pennyroyal (*Mentha pulegium*) essential oil were purchased from Barij essence Kashan company in Iran, analyzed by GC/MS technique for determining their components [29 compounds were found in thyme oil (main compounds: thymol, 35.40%, durenol, 31.09%, and p-cymene, 6.70%)], 19 compounds were found in savory oil (main compounds: thymol, 33.06%, 4,4'-diapophytoene, 20.72%, and carvacrol 11.45%), and 29 compounds were found in pennyroyal oil (main compounds: pulegone, 45.67%, 3,3'-dimenthol, 18.85%, and (E)-p-mentha-2-en-1-ol, 12.15%] and kept in dark glassy bottles in 4 °C temperature until their use.

2.2. Pens preparation, trial treatments and performance data collection

The experimental protocols were approved by the Research Ethics Committee of the Shahid Bahonar University of Kerman, Iran. The present study was carried out in standard quail breeding halls in Yazd agricultural and natural resources research center (Yazd, Iran). For the breeding period, the two halls (A and B) were washed and disinfected. A total of 550 day-old Japanese quail chicks (mixed sex by the ratio of 3 female: 2 male) were allocated to 11 dietary treatments of 5 replications (10 birds in each cage with the size of 40 × 90 × 25 cm) under a randomized experimental design. The treatments were a basal diet (Control), or 3 levels (200, 300, and 400 ppm) of thyme essential oil (T200, T300, T400), or 3 levels (200, 300, and 400 ppm) of savory essential oil (S200, S300, S400), or 3 levels (200, 300, and 400 ppm) of pennyroyal essential oil (P200, P300, P400) added separately to the basal diet, or also the basal diet supplemented with 100 ppm of flavophospholipol as a growth promoter antibiotic. Experimental diets were formulated using nutritional requirements of quails proposed by Brazilian tables for poultry and swine during growing and developing phases (Rostagno et al., 2011) (Table 1). The essential

Table 1. Feed ingredients and nutrient composition of basal diets during different production phases.

Ingredient (%)	Grower and developer phases
Corn, yellow	55.33
Soybean meal	39.20
Soybean oil	1.91
Limestone	1.21
Dicalcium phosphate	1.33
Common salt	0.40
Vitamin premix ¹	0.25
Mineral premix ²	0.25
DL-Methionine	0.07
Nutrient composition	
ME _n (kcal/kg)	2900
Crude protein (%)	22.00
Calcium (%)	0.90
Non-phytate P (%)	0.37
Na (%)	0.17
Methionine (%)	0.42
Lysine (%)	1.19
Threonine (%)	0.79
Tryptophan (%)	0.21
Arginine (%)	1.19

¹ Vitamin premix provided the followings per kilogram of diet: vitamin A, 12000 IU; cholecalciferol, 5000 IU; vitamin E, 45 IU; vitamin K₃, 2.4 mg; thiamine, 2.6 mg; riboflavin, 6.6 mg; pantothenic acid, 25 mg; niacin, 55 mg; choline chloride, 500 mg; biotin, 0.1 mg; folic acid, 1.5 mg; pyridoxine 5.5 mg; vitamin B₁₂, 0.015 mg; BHT, 1 mg.

² Mineral premix provide the followings per kilogram of diet: iron, 50 mg; zinc, 85 mg; manganese, 90 mg; iodine, 1 mg; copper, 10 mg; selenium, 0.25 mg.

oils were first mixed with the required oil of the diets and then added to the basal diet (mash diet). In the first 15 d, chicks were kept in the hall A at a temperature of 36 °C and the cages were equipped with 40 watts lamps, baby drinkers and chicken feeding trays. After 15 d, chicks were removed to the hall B equipped with automatic linear feeders and nipple drinkers at 32 °C temperature. The hall temperature was dropped by 2° weekly and maintained at 24 °C in the last weekend. Chicks were maintained on a 24 h lighting regimen and had free access to water and to the experimental diets throughout the trial. Birds and the feed remnants per cage were weighed weekly and BWG, FI and FCR were calculated at the end of the trial (35 d). Mortality was daily recorded to adjust performance data.

2.3. Blood sampling and organs weighing

At final day of trial (35 d), feed was removed 3 h before slaughtering. One bird per cage nearest to the average weight of each replicate was selected to evaluate organs weight and also two birds (male and female) were separately selected to evaluate serum lipids. Each bird was exsanguinated by cutting the jugular vein and 5 mL of blood samples were collected by small plastic tubes. Viscera were removed immediately, thereafter the weights of liver, heart, tights, lean, breast, and gastrointestinal tract were measured by a sensitive digital scale. Carcass yield and relative weights of organs were calculated as a percentage of live body weight. After transferring blood samples to the lab, they were centrifuged at 3000 rpm for 10 min (HETTICH Rota-fix32A, Germany) and serum samples were stored at -20°C until further analysis. Serum triglyceride and cholesterol concentrations were measured using a biochemical analyzer (Autolab, PM 4000, Auto analyzer, Medical System, Rome, Italy) according to recommended procedures of producing company.

2.4. Tissue sampling

During visceral separation, 1-cm segments of the duodenum (proximal intestine), jejunum (before the Meckel's diverticulum) and ileum (distal intestine) were excised, washed in physiological saline solution, and fixed in 10% buffered formalin. The tissue samples were later embedded in paraffin, and a $2\ \mu\text{m}$ section of each sample was placed on a glass slide and stained with hematoxylin and eosin according to the procedure described by [Baurhoo et al. \(2007\)](#).

2.5. Statistical analysis

All data were subjected to one-way analysis of variance using ANOVA procedures of SAS statistical software ([SAS, 1999](#)). The following model was used for the analysis of all traits. $Y_{ij} = \mu + A_j + e_{i(j)}$, where Y_{ij} is the observed value for a particular variable, μ is the overall mean, A_j is the effect of A treatment level j, and $e_{i(j)}$ is the random error associated with the ij^{th} recording. The treatment means were compared by using Duncan's multiple range tests at the $P < 0.05$ significant level.

3. Results & discussion

3.1. Performance traits (BWG, FI, FCR)

The performance data of quails fed with different levels of thyme, savory and pennyroyal essential oils is illustrated in [Table 2](#). Birds that were fed with the diets supplemented with 400 ppm of TO and antibiotic had numerically higher values for BWG than the control and the other treatments, but differences were not significant ($P > 0.05$). FI was significantly decreased in T400 group ($P < 0.05$). These results

Table 2. Effect of different levels of pennyroyal, savory, thyme essential oils and antibiotic on performance of quail chicks during 0–35 d of rearing.

Treatments*	FI (g/d per bird)	BWG (g/d per bird)	FCR (g feed/g gain)
Control	18.40 ^b	6.29	2.92 ^{ab}
Antibiotic	18.32 ^{bc}	6.45	2.84 ^c
P200	18.40 ^b	6.20	2.96 ^{ab}
P300	18.41 ^b	6.23	2.95 ^{ab}
P400	18.41 ^b	6.21	2.96 ^{ab}
S200	18.49 ^{ab}	6.33	2.92 ^{ab}
S300	18.37 ^{bc}	6.19	2.97 ^{ab}
S400	18.33 ^{bc}	6.22	2.94 ^{ab}
T200	18.61 ^a	6.14	3.03 ^a
T300	18.47 ^{ab}	6.20	2.98 ^{ab}
T400	18.24 ^c	6.39	2.82 ^c
P-Value	0.01	0.38	0.05
SEM	0.06	0.09	0.04

^{abc} Different superscripts indicate significant differences ($p < 0.05$) within the same column.

* Antibiotic is 100 ppm Flavophospholipol. P200, P300, P400 indicate the levels of 200, 300 and 400 ppm of *Mentha pulegium* essential oil. S200, S300, S400 indicate the levels of 200, 300 and 400 ppm of *Satureja hortensis* essential oil. T200, T300, T400 indicate the levels of 200, 300 and 400 ppm of *Thymus vulgaris* essential oil.

were reflected in positive effects on FCR so that the best FCR was also observed in T400 group, similar to that of the antibiotic supplemented group ($P < 0.05$). The effects of thyme and savory in different shapes (powder, extracts, and essential oils) on poultry performance have been widely reported in previous studies whereas the information about the effects of their main ingredient, i.e., thymol on poultry is scarce. [Abdel-Wareth et al. \(2012\)](#) observed a quadratic effect of thyme powder on BWG of broilers and the highest values were observed at levels of 15 and 20 g/kg diet. Also, in another experiment the BWG of quails was significantly increased when they were fed with diets supplemented with 1 g/kg of TO ([Khaksar et al., 2012](#)). However, other authors did not observe neither positive nor negative effect of these essential oils on poultry performance ([Hoffman-Pennesi and Wu, 2010](#); [Mehdipour et al., 2014](#); [Montazeri et al., 2014](#)). Similar to our findings, [Hashemipour et al. \(2013\)](#) examined the effects of a diet containing 60, 100 and 200 mg/kg of thymol and carvacrol combination on broiler chicks. They found that this combination significantly reduced feed intake linearly, although the highest weight gain and feed efficiency were observed in chicks fed a diet supplemented with 200 mg/kg thymol and carvacrol combination. However, the carvacrol content of our thyme and savory essential oils was low compared to thymol.

According to [Brenes and Roura \(2010\)](#), essential oils stimulate oronasal sensing and digestive secretions such as saliva, intestinal mucosa, bile acids, and pancreatic

enzymes. Birds have lower taste bud numbers than mammals which has been assumed as a proof of lower taste acuity (Roura et al., 2013). Therefore, the observed reduction in feed intake in 400 ppm TO cannot be fully related to the taste of the essential oil. It seems that the positive effects of thyme on performance may be, in part, attributed to the content of thymol and its effects on improvement of feed efficiency. Some authors observed evidence of antioxidant and antimicrobial activity (Botsoglou et al., 2002; Jang et al., 2007), improvement in digestibility (Hernandez et al., 2004) and stimulation of digestive and pancreatic enzymes (Lee et al., 2003; Jang et al., 2007) in response to oral intake of feed additives containing thymol and carvacrol. Therefore, in our experiment addition of 400 ppm TO may improve birds' performance by reducing feed intake as a result of an improved absorption of nutrients in intestine. Few studies have been done on the effects of PO and its main ingredient (i.e. pulegone) on poultry production. In this study, PO did not improve performance in quails which may be due to PO effective dose (our used doses may be little) and the less influence of pulegone than thymol. However, a previous report indicated that supplementation with 0.5% of pennyroyal powder in broiler diets resulted in improvement of FCR by reducing feed intake (Erhan et al., 2012).

3.2. Organs weight

In this study, different levels of essential oils of pennyroyal, savory, thyme and even antibiotic had no marked effect on the relative weight of the liver, heart, carcass (skinless), breast, thighs, and gastrointestinal tract (Table 3). Only some of the treatments influenced the organs weights slightly. In agreement with our findings, some authors investigated the effects of pennyroyal, savory and thyme powders, extracts or essential oils on poultry weight of organs and reported no significant effects (Ghalamkari et al., 2012; Nobakht et al., 2011; Hernandez et al., 2004). On the other hand, Khaksar et al. (2012) revealed higher carcass and breast percentages in Japanese quail fed diets supplemented with thyme essential oil.

3.3. Serum lipids

Changes in the pattern of blood components in male and female quails at 35 d are shown in Table 4. The present findings indicated that the cholesterol and triglyceride levels at 35 d of age in male and female quails were affected by the treatments. The level of triglyceride in male birds tended to decrease in treatments containing different levels of all essential oils compared to control and antibiotic supplemented group ($p = 0.087$). In females, the amount of triglyceride in treatments containing 300 and 400 ppm SO and 400 ppm TO decreased significantly compared to control, antibiotic, P300, and T300 treatments ($P < 0.05$). Also, blood cholesterol levels were affected by treatments in both sexes. In males, the highest level of blood cholesterol was related to 300 ppm PO and the lowest blood cholesterol was related to 200

Table 3. Effect of different levels of pennyroyal, savory, thyme essential oils and antibiotic on weight of organs in 35- d aged quail chicks (expressed as % of live body weight).

Treatments*	Liver	Heart	Carcass (skinless)	Breast	Tights	Gastrointestinal tract**
Control	2.25	0.80	59.39	29.97	20.75	8.28
Antibiotic	2.00	0.85	61.44	29.71	20.89	8.73
P200	2.59	0.85	62.86	30.28	22.55	7.65
P300	2.49	0.95	59.74	29.66	20.90	7.78
P400	2.80	0.88	60.68	29.52	21.12	8.95
S200	2.55	0.88	63.11	30.49	20.87	7.76
S300	2.56	0.75	58.99	29.12	20.88	8.43
S400	2.19	0.87	60.59	29.65	20.89	8.30
T200	2.07	0.84	62.02	30.74	21.10	7.39
T300	2.41	0.84	59.03	29.24	20.75	8.88
T400	2.56	0.82	58.66	27.81	20.30	8.00
P-Value	0.72	0.13	0.60	0.54	0.70	0.78
SEM	0.29	0.03	1.74	0.83	0.65	0.66

* Antibiotic is 100 ppm Flavophospholipol. P200, P300, P400 indicate the levels of 200, 300 and 400 ppm of *Mentha pulegium* essential oil. S200, S300, S400 indicate the levels of 200, 300 and 400 ppm of *Satureja hortensis* essential oil. T200, T300, T400 indicate the levels of 200, 300 and 400 ppm of *Thymus vulgaris* essential oil.

** Gastrointestinal tract includes the total weight of the crop, gizzard and intestines.

ppm SO ($P < 0.05$). Anyway, the cholesterol level in S200 was not significantly different with T200, T300, T400, S300, S400, P200 and antibiotic. The cholesterol level in the females decreased significantly in P300 compared to S300, S400, T200, T300, and T400 treatments ($P < 0.05$). However, there was no difference in the triglyceride and cholesterol between the antibiotic and essential oils groups. Hypolipidemic effects of some medical plants, their essential oils or their main components have been reported in several studies (Khaksar et al., 2012; El-Ghousein and Al-Beitawi, 2009; Ali et al., 2007; Akbari and Toriki, 2014; Ghazaghi et al., 2014; Mehri et al., 2015). However, few studies have been performed to identify the potential effects of essential oils on lipid metabolism in chickens. In this case, a study on a mentha plant (peppermint) revealed that the concentrations of triglyceride and total cholesterol linearly decreased in quails received graded levels of dietary peppermint (Mehri et al., 2015).

It has been stated that the essential oils and some of their individual constituents can reduce levels of plasma cholesterol and triglyceride (Edris, 2007). This fact may be a result of the inhibition of enzymes involved in synthesis of these lipids by monoterpenes (Goldstein and Brown, 1990; Elson, 1995). However, Lee et al. (2003) concluded that carvacrol and thymol dietary supplementation did not have any

Table 4. Effect of different levels of pennyroyal, savory, thyme essential oils and antibiotic on triglyceride and cholesterol (mg/dl) based on the sex of quail chicks in 35 d.

Treatments*	Triglyceride		Cholesterol	
	Male	Female	Male	Female
Control	255.70	1564.00 ^a	215.93 ^{ab}	268.07 ^{ab}
Antibiotic	243.33	1161.10 ^{abcde}	188.60 ^{bc}	257.73 ^{ab}
P200	248.33	1456.90 ^{ab}	185.27 ^{bc}	271.87 ^{ab}
P300	233.53	1110.00 ^{bcde}	243.87 ^a	207.57 ^b
P400	166.43	1224.00 ^{abcde}	209.83 ^{ab}	256.67 ^{ab}
S200	211.97	1346.50 ^{abc}	157.97 ^c	253.80 ^{ab}
S300	221.17	829.20 ^e	176.77 ^{bc}	310.67 ^a
S400	184.27	893.30 ^{de}	193.40 ^{bc}	299.67 ^a
T200	170.07	1245.70 ^{abcd}	189.10 ^{bc}	316.50 ^a
T300	156.27	1091.70 ^{bcde}	179.33 ^{bc}	298.67 ^a
T400	143.93	999.00 ^{ede}	185.90 ^{bc}	309.00 ^a
P-Value	0.08	0.008	0.02	0.01
SEM	28.60	123.50	13.64	18.40

^{abcde} Different superscripts indicate significant differences ($p < 0.05$) within the same column.

* Antibiotic is 100 ppm Flavophospholipol. P200, P300, P400 indicate the levels of 200, 300 and 400 ppm of *Mentha pulegium* essential oil. S200, S300, S400 indicate the levels of 200, 300 and 400 ppm of *Satureja hortensis* essential oil. T200, T300, T400 indicate the levels of 200, 300 and 400 ppm of *Thymus vulgaris* essential oil.

hypocholesterolemic activity in female broiler chickens, but dietary carvacrol lowered plasma triglycerides alone.

3.4. Morphology of different parts of intestine tissue

Table 5 presented the effects of different levels of essential oils and antibiotic on the morphology of duodenum, jejunum and ileum cells. The results indicate that the villi height, villi width, and the crypt depth of the intestine cells were influenced by the experimental treatments. In comparison to control, the villi height and width were increased in the essential oils and antibiotic treatments. The data showed a significant increase in duodenal villi height ($P < 0.0001$) in treatments containing different levels of PO and 400 ppm TO compared to control, antibiotics and other essential oil levels. In jejunum, villi height increased significantly in treatments containing different levels of PO and TO than control and antibiotics ($P < 0.0001$). However, villi height in ileum showed a greater fluctuation among treatments so that it was significantly increased in essential oil supplemented groups apart from T400 group ($P < 0.001$). The duodenum, jejunum and ileum villi width were also affected by experimental treatments so that in duodenum a significant decrease in villi width

Table 5. Effect of different levels of pennyroyal, savory, thyme essential oils and antibiotic on intestine morphology of quail chicks in 35 d.

Treatments*	Villi height (μm)			Villi width (μm)			Crypt dept (μm)		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
Control	993.3 ^g	806.0 ^d	750.0 ^d	93.3 ^{ab}	86.6 ^d	85.3 ^{bc}	84.0 ^{bc}	74.6	68.6
Antibiotic	1007.3 ^{fg}	864.0 ^c	808.6 ^{cd}	94.6 ^{ab}	89.3 ^{bcd}	89.3 ^{abc}	84.0 ^{bc}	77.3	68.0
P200	1084.0 ^a	911.3 ^{ab}	855.3 ^{abc}	96.0 ^a	90.6 ^{abcd}	85.3 ^{bc}	89.3 ^a	77.3	69.3
P300	1084.0 ^a	933.3 ^a	892.6 ^a	94.6 ^{ab}	88.6 ^{bcd}	92.0 ^a	88.0 ^{ab}	77.3	64.0
P400	1087.3 ^a	936.0 ^a	888.0 ^{ab}	95.3 ^{ab}	92.0 ^{ab}	88.0 ^{abc}	84.0 ^{bc}	78.0	70.6
S200	1009.3 ^{fg}	870.0 ^c	822.0 ^c	92.6 ^{abc}	87.3 ^{cd}	90.0 ^{ab}	82.0 ^{cd}	74.6	70.6
S300	1022.6 ^{ef}	872.0 ^c	825.3 ^{bc}	94.0 ^{ab}	92.0 ^{ab}	88.6 ^{abc}	79.3 ^{cd}	76.6	66.0
S400	1033.3 ^{de}	883.3 ^{bc}	846.0 ^{abc}	90.6 ^{bcd}	94.6 ^a	89.3 ^{abc}	78.0 ^d	78.6	64.0
T200	1051.3 ^{cd}	904.6 ^{ab}	848.6 ^{abc}	84.0 ^e	87.3 ^{cd}	84.6 ^c	56.0 ^f	72.6	62.6
T300	1059.3 ^{bc}	918.0 ^a	870.6 ^{abc}	86.0 ^{de}	90.6 ^{abcd}	90.6 ^a	62.0 ^e	78.0	68.0
T400	1074.6 ^{ab}	924.6 ^a	758.0 ^d	88.0 ^{cde}	91.3 ^{abc}	88.0 ^{abc}	60.0 ^{ef}	74.6	66.0
P-Value	<.0001	<.0001	0.0003	<.0001	0.0043	0.021	<.0001	0.185	0.376
SEM	6.89	10.50	19.50	1.55	1.25	1.40	1.61	1.51	2.57

^{abcdefg} Different superscripts indicate significant differences ($p < 0.01$) within the same column.

* Antibiotic is 100 ppm Flavophospholipol. P200, P300, P400 indicate the levels of 200, 300 and 400 ppm of *Mentha pulegium* essential oil. S200, S300, S400 indicate the levels of 200, 300 and 400 ppm of *Satureja hortensis* essential oil. T200, T300, T400 indicate the levels of 200, 300 and 400 ppm of *Thymus vulgaris* essential oil.

were observed in the treatments containing different levels of TO, and the maximum width of the villi related to the treatment containing 200 ppm PO, though, it was not significantly different compared to the controls. The highest villi width in jejunum related to 400 ppm SO, which was similar to S300, T300, T400, P200 and P400 treatments statistically. The highest villi width in ileum was observed in 300 ppm PO which differed significantly from control, T200 and P200 groups ($P < 0.05$). The crypt dept showed only significant differences in the duodenum ($P < 0.0001$). The lowest crypt dept was related to the treatment contained 200 ppm TO which statistically was similar to the levels of 300 and 400 TO. On the other hand, the highest crypt depth in the duodenum was related to 200 ppm PO which showed a significant difference compared to the control, antibiotics and other treatments ($P < 0.0001$). The crypt dept in jejunum and ileum did not show any significant difference.

Different parts of small intestine are the areas for absorption of various nutrients and the structure of them is related to their function. So, changes in feed and feed additives may affect the morphology of intestine different parts. In this case, [Mohiti-Asli and Ghanaatparast-Rashti \(2018\)](#) compared the effects of a combined phytogetic feed additive (oregano, anise and citrus peel) with oregano essential oil on intestinal morphology in broilers. They reported that broilers fed 300 ppm individual oregano essential oil in their diet had larger villi height, villi surface area, villi height to crypt dept ratio and lower crypt dept in jejunum than those fed either control diet or combined phytogetic feed additive. Also, similar to our findings, [Hashemipour et al. \(2013\)](#) reported that the inclusion of 100 and 200 ppm thymol and carvacrol increased villi height, surface area and villi height to crypt dept ratio of jejunum and ileum in broilers. Villi development provided greater absorption surface for availability of nutrients ([Awad et al., 2008](#)). It is well known that improvement of nutrient absorption occurs by increasing the villi height and decreasing crypt depth. In addition, increased intestinal villi height and height to crypt depth ratio and decreased crypt depth by essential oils may be attributed to the decreased renewal rate of intestinal cells as a result of antioxidant properties and antimicrobial actions of essential oils ([Patra et al., 2018](#)). Therefore, thyme, savory and pennyroyal essential oils may increase the absorption of nutrients by affecting the height, width and crypt dept of the intestine villi. In the current study, 200, 300, and 400 ppm TO reduced villi width in duodenum of quails. In contrast, the results of a study on rats showed that the villi width significantly increased in duodenum and jejunum of the rats fed thyme volatile oil ([Sepehri Moghadam et al., 2014](#)). Moreover, crypt dept was not significantly influence by the treatments in jejunum and ileum but only in duodenum. Crypt dept in duodenum was decreased significantly in different levels of TO and SO treatments while 200 and 300 ppm PO increased crypt depth compared to control and antibiotic treatments. Based on these results, it seems that supplementation of different levels of TO and SO in quails feed due to their

thymol and carvacrol content improved intestinal morphology whereas the effects of PO were limited. Accordingly, Du et al. (2016) reported that in the pathogen challenged birds, thymol and carvacrol increased significantly the villi height to crypt depth ratio compared to the basal diet.

4. Conclusions

This study draws attention to compare three essential oils from Lamiaceae family as feed additives in quail's nutrition with the intention to improve performance, organs weight, serum lipids and intestinal morphology. Based on the results obtained in the present study, it can be concluded that the selected plant essential oils may have the potential to improve quails performance by increasing nutrient absorption in intestine and lowering feed intake. Among these essential oils, thyme and savory were more effective due to their greater level of thymol compared to pennyroyal. In addition, thyme essential oil could compete with antibiotic growth parameter in all aspects. Moreover, the decrease of the level of serum lipids by essential oils may lead to a reduction in the accumulation of abdominal fat which can improve the quality of the meat. As the result, these plant essential oils can replace antibiotic growth promoters without having any adverse effect on quails' health and among them thyme essential oil is more effective.

Declarations

Author contribution statement

M. Afsharmanesh, M. Salarmoini: Conceived and designed the experiments.

N. Dehghani: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

H. Ebrahimnejad, A. Bitaraf: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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