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Effectiveness of Phytogenic Feed Additive as Alternative to Bacitracin Methylene Disalicylate on Hematological Parameters, Intestinal Histomorphology and Microbial Population and Production Performance of Japanese Quails

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ABSTRACT: This study was conducted to evaluate the effects of phytogenic additive and antibiotic growth promoter in laying Japanese quails. One hundred and sixty five quails were divided into three groups of 5 replicates and 11 quails (8 females and 3 males) in each replicate. Treatment 1 was fed control diet, treatment 2 was fed control diet supplemented with 0.05% bacitracin methylene disalicylate as antibiotic growth promoter and treatment 3 was fed control diet supplemented with 0.1% phytogenic feed additive (PFA) for two periods of 3 weeks each from 37 to 42 weeks of age. Results showed that egg production, eggshell strength, eggshell weight, villus height and villus height to crypt depth ratio were significantly ($p\leq0.05$) increased and feed consumption, feed conversion ratio, albumen, Haugh unit, cholesterol, low-density lipoprotein, alanine transaminase, gamma glutamyltransferase, alkaline phosphatase, high-density lipoprotein, triglyceride, number of goblet cell, crypt depth and intestinal bacterial population of Coliforms, Salmonella and *E. coli* were significantly ($p\leq0.05$) decreased in PFA fed group. It is concluded that addition of PFA containing phytomolecules and organic acids as main ingredients could significantly improve the production parameters and the general health of laying quails as an alternative to antibiotic growth promoters. (**Key Words:** Feed Antibiotic, Blood Constituents, Performance, Phytogenic Feed Additive, Japanese Quails)

INTRODUCTION

Due to the growing demand for the use of herbal ingredients in human daily food, a tendency to minimize the chemical feed additives in poultry diets is among the interests of the producers. Considering the ban of feed antibiotics in many countries including Iran, their removal from the diet may negatively affect profitability of the animals (Manafi, 2015).

The beneficial effects of different bioactive compounds are found to enhance poultry productivity. The term ‘phytogenic compound’ refers to the plant parts (*e.g.* garlic, oregano, thyme, rosemary, coriander, and cinnamon) as well as to their respective extracts in the form of essential oils. Many beneficial properties of phytogenic compounds originate from their bioactive molecules (*e.g.* carvacrol, thymol, cineole, linalool, anethole, allicin, capsaicin, allylisothiocynate, and piperine) (Grashorn, 2010). A large number of *in vitro* and *in vivo* studies have established a wide range of phytobiotic activities in poultry nutrition like stimulation of feed consumption (FC), antimicrobial, coccidiostatic, anthelmintic, immunestimulating (Manafi, 2015) antibacterial and antioxidant functions (Windisch et al., 2008), enhancement of digestive enzyme activities (Jamroz et al., 2006), positive effects on performance and feed conversion ratio (FCR), quality and carcass meat safety and lowering the levels of cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Stanaćev et al., 2011) in birds.

Recently, rearing of quail has gained importance in the poultry industry. The consumption of quail eggs and meat has significantly increased and there is scope for increasing

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healthy quail products in the market. Quail meat has extremely low skin fat and low cholesterol value. It is rich in micronutrients and a wide range of vitamins including the B complex, E and K and folate. The effects of individual essential oils have been studied earlier in quails; however, to the author's knowledge, there have been limited studies investigating the effects of bioactive components combination in laying quails. Therefore, this study was planned and performed to investigate the effects of phytomolecules bioactive compounds (allicin, peppermint, thymol, and carvacrol) and organic acids (propionic acid and fumaric acid) in comparison with antibiotic growth promoter in production performance, serum biochemistry, illeal bacterial status, immune response and intestinal morphology of Japanese laying quails.

MATERIALS AND METHODS

Experimental birds and diets

One hundred and sixty five layer Japanese quails (*Coturnixcoturnix japonica*) at 37 weeks of age with average initial body weight of 224±5 g were divided into 3 treatment groups, with 5 replicates and 11 quails (8 females and 3 males) in each replicate. Data were recorded from the quail when they were aged between 37 to 39 and 40 to 42 weeks, according to Malayer University approved animal care rules and protocols. Quails were housed in thermostatically controlled batteries (152.4×45.6×26.7 cm) with raised wire floors in an environmentally controlled building having forced air ventilation for 6 weeks duration. Treatment groups were fed a corn-soybean meal basal diet (control); control plus 0.05% bacitracin methylene disalicylate (BMD) and control plus 0.1% phytogenic feed additive (PFA). The PFA used in this study was Natusol, a combination of phytomolecules bioactive compounds (allicin, peppermint, thymol, and carvacrol) and organic acids (propionic acid and fumaric acid) from natural sources, provided by a commercial company (Zeus Biotech Limited, Mysore, India). The basal commercial quail breeder diet was fed as mash and prepared with the same batch of ingredients. Diet was formulated isonitrogenic and isoenergic. The ingredients and chemical composition of the coccidiostat-free basal diets are shown in Table 1. All quails were fed *ad libitum* feed and water throughout the study period and exposed to 17 h light. The PFA and BMD were procured from local market in powder form and added on top to the basal diet and were included into the basal diet according to experimental treatments. The feeding and collection protocols used in the present experiment were approved by the bioethical committee of Malayer University (protocol No. 84/5-1-246) under the guidelines of animal protection used for experimental and other scientific purposes. The quails were also raised and cared based on the recommendation of Iranian Council on Animal Care.

Table 1. Ingredients and chemical composition of the basal diet of layer Japanese quail (37 to 42 weeks of age)

Item	%
Maize	38.00
Soybean meal (44% CP)	25.00
Sunflower meal (34.7% CP)	10.00
Meat-bone meal (58.5% CP)	2.00
Barley	12.70
Vegetable oil	4.00
Dicalcium phosphate	0.4
Limestone	7.300
Sodium chloride	0.30
Vitamin premix[1]	0.15
Mineral premix[2]	0.15
Calculated composition	
ME (kcal/kg)	2819
CP (%)	20.5
Dig Lys (%)	1.08
Dig Met (%)	0.35
Dig Met+Cys (%)	0.75
Dig Thr (%)	0.79
Ca (%)	3
Available P (%)	0.40

CP, crude protein; ME, metabolizable energy.

[1] Each kg of vitamin premix contains 6,000,000 IU vit A; 600,000 IU vit D_3; 20,000 IU vit E; 2 g vit K; 1.2 g vit B_1; 2.4 g vit B_2; 2 g vit B_6; 12 mg vit B_{12}; 10 g niasin; 300 mg folic acid; 4 g calcium panthotenate; 50 mg D-Biotin.

[2] Each g of mineral premix contains 80 g Mn; 30 g Fe; 60 g Zn; 5 g Cu; 0.5 g Co; 2 g I; 235.68 g Ca.

Production parameters

Prior to start of the experiment, two weeks pre-experimental feed was given to quails for adaptation. The production parameters measured were hen day egg production (EP, %), egg weight (EW, g), FC (g/week) and FCR. FC was calculated as the average of each replicate and the FCR was expressed as grams of feed consumed per gram of egg produced (g feed/g egg). Daily EP and EW (g) were recorded during 6 weeks. Treatment means for these traits were not different at the start of experiment ($p>0.05$). Mass EP was calculated (EW×EP), accordingly.

Egg characteristics

At the end of the experiment (week 42), ten eggs per treatment (two eggs per replicate) were randomly chosen, examined and measured for eggshell thickness (mm), eggshell strength (kgf/cm^2), albumen (mm), Haugh unit scores and eggshell weight (g). Eggshell thickness (without inner and outer shell membranes) was measured at 3 different points (top, middle, and bottom) using an ultrasonic micrometer (Sanovo Technology A/S, Odense NV, Denmark) without cracking the eggshell. Likewise, eggshell thickness was calculated from the middle of the egg using a micrometer instrument (Norouzi et al., 2013). Albumen (height) was measured using standard micrometer (CE 300). The Haugh unit score was calculated using Roush's formula (Roush, 1981). Eggshell weight was measured after depleting internal contents and placing them at room temperature for 48 h by digital pan balance with 0.001 g accuracy.

Serum biochemical parameters

At the end of the experiment (week 42), ten quails per treatment (two quails per pen) were selected, and individual blood samples were collected separately in non-heparinized tubes. The serum was separated, stored at –20°C for the further use and later was analyzed for glucose, cholesterol, LDL, HDL, alanine transaminase (ALT), gamma glutamyltransferase (GGT), alkaline phosphatase (ALP), and triglyceride by enzyme-linked immunosorbent assay technique using commercial kits (Boehringer Mannheim Hitachi 704 automatic analyzer, Tokyo, Japan). The methodology and the set of reagents for each parameter were recommended by the manufacturer of the analyzer system. Treatment-wise means of values were computed and presented.

Intestinal morphology parameters

At the end of the trial, upon obtaining the permission of Ethical Committee of the University, ten birds from each treatment (two quails per replicate) were randomly selected, stunned and killed by cutting the jugular vein. The abdominal cavity was then opened and the digestive tract with contents was removed aseptically and each carcass was subjected to detailed postmortem examination. Ileum tissue samples were separated from the Meckel's diverticulum up to 1 cm proximal to the ileocecal junction and then dried with desiccant paper. A 2-cm section of ileum was taken from the middle of the ileum and gently flushed with phosphate-buffered saline (pH 7.2). Tissue sections were immediately fixed in 10% neutral buffered formalin and changed 3 times to complete the fixation process. A single 0.5 cm sample was cut from each ileal section, dehydrated with increasing concentrations (70%, 80%, 95%, and 100%) of ethanol, cleared with xylene and placed into polyfin embedding wax. Tissue sections (2 μm) were cut by microtome (Leitz-1512 Microtome, Leitz, Wetzlar, Germany), floated onto slides and stained with hematoxylin (Gill no. 2, Sigma, St. Louis, MO, USA) and eosin (Sigma). To measure villus height and crypt depth, images from samples were taken using a digital camera with light microscopy. Twelve images from 4 tissue sections of each ileal section were taken and 24 villus heights and crypt depths were measured by imaging software. Measurements for each villus length were taken from the tip of the villus to the valley and measurements for crypt depth were taken from the valley to the basolateral membrane (Xu et al., 2003). For determining the number of goblet cells in 1mm of villus length, all samples were dehydrated in ethanol and embedded in paraffin wax. Sections were stained with Alcian blue and periodic acid-Schiff to visualize goblet cells (Rohana and Thomas, 2009).

Selected intestinal bacterial population

To determine the changes in some selected microbial populations in the gastrointestinal tract of quails at week 42, cecal contents immediately after killing the quails were gently collected in sterile sampling tube and transferred on ice to the laboratory for microbial study. The cecal contents of each bird were pooled for serial dilution. Microbial populations were determined by serial dilution (10^{-4} to 10^{-6}) of cecal samples in anaerobic diluents before inoculation onto Petri dishes of sterile agar as described by Bryant and Burkey (1953). *E. coli* was grown on eosin methylene blue agar, Salmonella in *Salmonella Shigella* (Merck, Darmstadt, Germany) and Coliforms on McConkey agar (Darmstadt, Germany). *E. coli* was incubated aerobically at 37°C. Plates were counted between 24 and 48 h after inoculation. Colony forming units were defined as distinct colonies measuring at least 1mm in diameter. Then, 9 sterile test tubes with lids containing 9 mL of phosphate buffer solution as diluent were prepared. Approximately 1 g of the cecal contents taken by sterile swab and homogenized for 3 min before transferring to microbiology lab in cold condition and mixed (Bryant and Burkey, 1953). Then 1ml out of 10 mL of buffer plus cecal sample in the test tube was removed by 1,000 μL sampler and was transferred to the tubes and was mixed thoroughly. Similarly, it was transferred to other new tubes and this procedure was repeated until a dilution of 9 was completed. Later, 1ml of contents of each test tube was transferred to one of three selective media agar in petri plates, respectively, and each petri was plate incubated in 37°C for 24 h. Finally, the intestinal bacterial colony populations formed in each plate was counted and adjusted to $\times 10^9$ manually and then was reported.

Statistical analysis

Data were analyzed as a completely randomized design manner using the general linear model procedure of SAS (SAS Institute, 2007) for those traits which were measured only once during the experimental period. MIXED procedure of SAS (SAS Institute, 2007) was used for analysis of traits that were measured repeatedly throughout the experimental period with age considered as a new main factor and initial values considered as a covariate effect in the model. Differences between treatment means were tested using Duncan's multiple comparison tests for main effects and Tukey's test for interactions. Statistical significance was declared at p≤0.05.

RESULTS AND DISCUSSION

Production parameters

Mean EP, EW, FC, and FCR in control, BMD and PFA groups are shown in Table 2. In period I (weeks 37 to 39), EP and FCR values showed a significant (p≤0.05) improvement by addition of PFA, whereas, BMD group showed no significant changes compared with control. However, FCR was significantly similar in all studied treatment groups. In period II (weeks 40 to 42), the values for EP and FCR were significantly (p≤0.05) higher in BMD and PFA fed quails when compared with control group. In both periods, EW and FC values remained statistically non-significant (p≤0.05).

In current experiment, the PFA treated group consumed a similar amount of feed while producing more eggs due to utilization of feed more efficiently than control. In many previous studies—in agreement to our findings—addition of phytogenic/herbal compounds showed performance and FCR improvements (Sahin et al., 2010) and a better economic efficiency in Japanese quails (Christaki et al., 2011), commercial broilers (Tiihonen et al., 2010) and laying hens and broiler breeders (Bozkurt et al., 2012). However, these findings contradict some other reports (Bölükbaşi et al., 2008). In current experiment, EW did not vary among different treatments. These findings are in accordance with those of Bozkurt et al. (2012). In contrast, increased EW of layer hens by addition of essential oils has been reported by Bölükbaşi et al. (2008).

While exploring the current experiment data, it is worth mentioning that in period II, the EP drastically decreased compared with period I. This is also mainly due to the aging of the quails, though many factors like stock density, nutritional manipulation and environmental conditions can be the cause for these changes. González (1995) reported that the quail EP was significantly lowered by advancing quail age.

The PFA product used in this study contains phytomolecules (allicin, carvacrol, paprika, cinnamaldehyde, peppermint, and thymol) and organic acids (propionic acid and fumaric acid). The possible mechanism of action of herbal components might be due to the increased digestibility and improved utilization of feed thanks to their antioxidant properties and phenolic compounds. The intestinal availability of essential nutrients may result in better absorption through stimulating the secretion of pancreatic and endogenous digestive enzymes (Lovkova et al., 2001). In addition, the active substances of essential oils present in PFA had a diversifying power to synergistically improve nutrient utilization. As a result, energy and protein digestibilities are improved leading to optimized performance of laying quails (Sultan et al., 2015). Some other possible ways include increasing feed palatability due to their aromatic characteristics which promote FC, regulating of the gut microbial flora through changing the gastrointestinal bacterial load and modifying mucin biosynthesis (Windisch et al., 2008). On the other hand, organic acids present in PFA may favorably affect the host by lowering pH through inhibiting the pathogenic intestinal bacteria and decreasing the level of their toxic products. Modulation of the gut microbiota has a critical role in maintaining host health (Tollba et al., 2012). Organic acid in combination with essential oil showed beneficial impacts on digestive enzyme activities of both pancreas and intestinal mucosa, leading to increased growth performance of broilers (Jang et al., 2007). In turkeys, a mixture of essential oils with a blend of organic acids increased body weight and decreased FCR (Mikulski et al., 2008). The boosting effect of BMD could be attributed to the active ingredients of bacitracin which can inhibit the synthesis of bacterial cell membrane of gram positive bacteria. BMD can also enhance the antibacterial activity and increase the

Table 2. Effect of dietary feed additives on performance parameters of layer Japanese quail (37 to 42 weeks of age)

Treatments		Egg production* (%)		Egg weight (g)		Feed consumption (g/week)		Feed conversion ratio (g feed/g egg)	
BMD	PFA	37-39 week	40-42 week	37-39 week	40-42 week	37-39 week	40-42 week	37-39 week	40-42 week
0	0	72.73[bc]	66.14[c]	11.54	11.74	322.30	323.50	2.46[a]	2.72[a]
0.05%	0	77.20[a]	73.76[a]	11.93	11.83	321.70	320.33	2.22[b]	2.30[b]
0	0.1%	74.47[b]	71.92[b]	11.85	11.82	320.25	331.28	2.31[ab]	2.44[b]
ANOVA									
SEM		0.525	0.810	0.181	0.136	13.992	17.986	0.037	0.058
p value		≤0.0001	≤0.0001	0.6800	0.9654	0.0721	0.0625	0.0195	0.0040

BMD, bacitracin methylene disalicylate (antibiotic growth promoter); PFA, phytogenic feed additive; ANOVA, analysis of variance; SEM, pooled standard error of column wise means comparison.

* Hen day mass egg production (%).

Feed conversion ratio was calculated as kilograms of feed consumed per kilogram of egg produced.

Means with different letters within the same column are significantly different (p≤0.05).

product stability after combination with bacitracin (Attia et al., 2003).

Egg characteristics

The effect of BMD and PFA supplementation on the egg quality parameters of quails at 42 weeks of age is given in Table 3. Notably, eggshell thickness was not influenced by dietary treatments; whereas, eggshell strength and eggshell weight were increased and albumen and Haugh unit scores were reduced significantly ($p\leq0.05$) by addition of PFA into the basal diet. Incorporation of BMD also revealed almost similar results with PFA, when compared with control group. PFA was found to be more effective than BMD at the end of trial (week 42).

Previous literature reveals contradictory results about the effects of herbal feed additives/phytogenic components on egg quality characteristics of laying birds. It was reported that Haugh unit scores, shell thickness, eggshell weight, yolk color, yolk weight and albumen and yolk indices were not altered by supplementing essential oils into the daily diet of laying quails (Bozkurt et al., 2012). The achievement on eggshell strength in current study is supported by the findings of Kaya et al. (2013), who reported that plant extract of *Origanum vulgare*, *Thymus vulgaris*, thyme oil, origanum oil, garlic oil, anise oil and fennel oil enhanced eggshell strength and eggshell thickness. However, Bozkurt et al. (2012) reported that essential oils supplementation had no effect on eggshell strength and eggshell thickness of laying hens.

There are several factors influencing the quality characteristics of quail eggs. The improvement of the eggshell strength found in current trial by consumption of PFA in quails could be attributed to the stimulatory role of the essential oils on enzymes secretion and amino acids production, which are required for the formation of eggshell (Nazligul et al., 2001). Digestibility of most amino acids in ileum may increase by addition of essential oils into the diet (Maenner et al., 2011). The other possible reason is the influence of essential oils on the metabolic activity of beneficial bacteria, within the intestine, which positively influences mineral absorption rate, especially those of Ca^{2+} and Mg^{2+} (Roberfroid, 2000).

On the other hand, organic acids may affect the integrity of microbial cell membranes and interfere with nutrient transport and energy metabolism (Hedayati et al., 2014). It is also believed that lactic acid may decrease pathogenic microorganisms in the crop leading to less competition for nutrients between quails and bacteria. In addition, there is a report stating the lactic acid is palatable for birds which increases FC leading to enhance egg quality characteristics (Laury et al., 2009). More so, it is presumed that the organic acid mixture contributes to the production of longer and wider quail eggs with thicker shells, larger surface areas and increased breaking strength in quails.

Serum biochemical parameters

The effect of BMD and PFA feed supplements on the blood biochemical parameters of laying quails are presented in Table 4. It was found that PFA addition significantly ($p\leq0.05$) decreased cholesterol, LDL, HDL, ALP, and triglyceride. By contrast, increased GGT levels were observed by inclusion of PFA with no impact on ALT levels, compared with control group. Likewise, the BMD also showed less effectiveness than PFA on all above-mentioned parameters. The glucose content was significantly ($p\leq0.05$) decreased in BMD group and remained unchanged among PFA and control treatments, but it was significantly higher in PFA than control and BMD fed groups.

In many studies, it is reported that essential oils from different plant origins have increased serum triglyceride, total cholesterol and glucose of Japanese quail (Soltan et al., 2008; Khaksar et al. 2012). In contrary, there is evidence of reduced serum cholesterol, plasma triglyceride and phospholipids in birds with the incorporation of plant derivatives into the diets (Babazadeh et al., 2011).

Previous reports have demonstrated the antioxidant and antimicrobial activity roles of PFA. Furthermore, other effects such as anti-inflammatory, anti-fungal, anti-infectious and anti-toxigenic have been reported in many studies (Swiatkiewicz and Arczewska-Wlosek, 2012). Blood metabolites indices of liver, renal functions and hematological contents may provide good instances of

Table 3. Effect of dietary feed additives on egg characteristics of layer Japanese quail at 42 weeks of age

Treatments		Eggshell thickness (mm)	Eggshell strength (kgf/cm²)	Albumen height (mm)	Haugh unit	Eggshell weight (g)
BMD	PFA					
0	0	0.20	2.10[b]	4.91[a]	91.33[a]	1.08[b]
0.05%	0	0.20	2.26[a]	4.24[c]	86.10[c]	1.14[a]
0	0.1%	0.22	2.28[a]	4.46[b]	89.33[b]	1.13[a]
ANOVA						
SEM		0.010	0.26	0.79	0.552	0.10
p value		0.4413	0.0132	≤0.0001	≤0.0001	0.0067

BMD, bacitracin methylene disalicylate (antibiotic growth promoter); PFA, phytogenic feed additive; ANOVA, analysis of variance; SEM, pooled standard error of column wise means comparison.

Means with different letters within the same column are significantly different ($p\leq0.05$).

Table 4. Effect of dietary feed additives on serum biochemical of layer Japanese quail at 42 weeks of age

Treatments		Glucose	Cholesterol	LDL	HDL	ALT	GGT	ALP	Triglyceride
BMD	PFA	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)	(Iu/L)	(Iu/L)	(U/L)	(mg/dL)
0	0	304.29[a]	356.04[a]	115.45[a]	99.33[a]	2.01[a]	264.13[b]	3,660.67[a]	382.32[a]
0.05%	0	293.50[b]	224.52[b]	98.20[b]	96.78[b]	2.01[a]	300.33[a]	2,997.04[b]	293.11[b]
0	0.1%	304.65[a]	218.24[c]	84.29[c]	90.81[c]	1.95[b]	253.66[c]	2,486.83[c]	281.67[c]
ANOVA									
SEM		1.26	6.25	3.09	0.97	0.01	3.20	116.81	3.42
p value		≤0.0001	≤0.0001	≤0.0001	≤0.0001	0.0032	≤0.0001	≤0.0001	≤0.0001

LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; BMD, bacitracin methylene disalicylate (antibiotic growth promoter); PFA, phytogenic feed additive; ANOVA, analysis of variance; SEM, pooled standard error of column wise means comparison.
Means with different letters within the same column are significantly different (p≤0.05).

animal health status and physiological condition (Toghyani et al., 2010). The lipophilic property and chemical structure of phenolic compounds present in PFA could play a role in manipulation of the enzyme activities inside the body of broilers (Attia et al., 2003). The lower serum cholesterol content may be the result of an increased lipid digestibility due to a higher secretion of bile and digestive enzymes indicating enhanced nutrient supply and transport (Manafi, 2015). The increased nutrient supply for growth is reflected in enhanced nutrient transport in the blood. Moreover, the PFA could cause a circulatory enzyme elevation. Generally GGT and ALT are considered as liver enzymes which are increased at the time of liver damage (heptato-cellular degeneration), so the decrease in these enzymes may provide proof for the occurrence of hepato-protective effect of essential oils present in PFA. Brenes and Roura (2010) have reported that serum total protein, albumin concentrations and AST activities significantly enhanced in laying hens receiving organic acids but lipid metabolism markers (cholesterol, HDL, LDL, triglyceride, and total lipid concentrations) remained unchanged. The glucose level in blood samples is a biochemical indicator of stress that decreases in quails fed PFA compared with BMD in current study and could be justified by the stress-lowering effect of this feed additive (Yesilbag and Coplan, 2006).

Morphology of intestine

This study revealed a significant (p≤0.05) increase in illeal villus height and villus height to crypt depth ratio (VH/CD), decrease in crypt depth and number of goblet cell in 1 mm of villus height in quails fed both PFA and BMD, compared to respected control groups (Table 5). The variation between the data of different treatments and studied parameters are more evident in PFA fed group.

The findings of present experiment in intestinal morphology parameters are in consistence with reports of Adibmoradi et al. (2006), who stated that jejunal villus height was increased and crypt depth was decreased leading to increased villus height to crypt depth ratio in birds fed graded levels of garlic in the diet. In contrast, some reports stating that the addition of essential oils did not affect villus height (Jamroz et al., 2006; Reisinger et al., 2011). Moreover, Garcia et al. (2007) reported an increase in crypt depth when essential oil was supplemented into the broiler diet. In contrast to our results, inclusion of essential oils in the diet of broilers increased goblet cell numbers reported by Garcia et al. (2007), Reisinger et al. (2011).

Villus height and crypt depth are considered as indicators of a well-functioning intestine. Increased villus height provides a bigger surface area for nutrients absorption and therefore performance enhancement (Awad et al., 2008). On the other hand, a drop in villus height can

Table 5. Effect of dietary feed additives on morphology of intestine of layer Japanese quail at 42 weeks of age

Treatments		Villus height	Number of goblet cells[1]	Crypt depth	Villus height to crypt
BMD	PFA	(µm)		(µm)	depth ratio
0	0	5.14[b]	10.19[a]	0.96[a]	5.34[c]
0.05%	0	6.48[a]	9.29[b]	0.76[b]	8.51[a]
0	0.1%	6.16[a]	9.38[b]	0.79[b]	8.81[b]
ANOVA					
SEM		0.155	0.104	0.024	0.034
p value		≤0.0001	≤0.0001	≤0.0001	≤0.0001

BMD, bacitracin methylene disalicylate (antibiotic growth promoter); PFA, phytogenic feed additive; ANOVA, analysis of variance; SEM, pooled standard error of column wise means comparison.
[1] Number of goblet cells in each 1 mm of villus length.
Means with different letters within the same column are significantly different (p≤0.05).

reduce nutrient absorption by decreasing the intestinal surface area for absorption. Hence, reduction in nutrient absorption will lead to decreased resistance to diseases and diminished performance. Escalation in secretion of digestive tract is the negative consequence of deeper crypt and shorter villi (Xu et al., 2003). Goblet cells are liable for the production of intestinal mucins that are the major component of the mucus layer coats of the broilers intestine. Many scientists believe that essential oils have the ability to reduce the growth of *E. coli* and *C. perfringens* and increase the numbers of *Lactobacillus* spp. when fed to birds (Brenes and Roura, 2010).

The PFA products stimulate gut hormones exerting diverse actions in the gastro intestinal tract leading to intestinal growth, enhancing mucosal blood flow and most importantly nutrient assimilation and thereby changing FC pattern by providing a feedback signal to the brain (Guban et al., 2006). On the subject of the intestinal morphology, normally, heavier broilers are linked with longer villi, greater villus width and higher villus surface area (Adibmoradi et al., 2006). It has been proposed that longer villi would result in an increased surface area and higher absorption of nutrients (Incharoen et al., 2010). This higher absorptive ability of the intestine in PFA fed birds is supported by higher blood nutrient concentrations. According to Yanishlieva et al. (1999), essential oils seem to have positive effects on blocking the radical chain process by interaction with peroxide radicals. Organic acids have direct effects on the poultry performance. In study of Antongiovanni et al. (2007) inclusion of organic acids in broiler diets increased the weight and length of small intestine. On the contrary, Vieira et al. (2008) reported that inclusion of some organic acids did not significantly influence the villus height at any age of broiler's life. Sultan et al. (2015) also demonstrated that organic acids improve the protein digestibility by decreasing endogenous nitrogen loss and by producing ammonia which ultimately lead to better intestinal morphological properties of birds.

Selected intestinal bacterial population

The bacterial population of intestine of quails fed BMD and PFA is shown in Table 6. Results showed that addition of BMD and PFA significantly ($p\leq0.05$) lowered the cecal number of coliforms, *Salmonella*, and *E. coli*, with more reduction emphasis in PFA fed group. Addition of PFA decreased the population of cecal bacterial count of coliforms, *Salmonella*, and *E. coli* significantly when compared with control. At present, there are prominent studies indicating that essential oils could control the common intestinal pathogen growth of poultry (Dorman and Deans, 2000). Addition of thyme essential oil in Japanese quail diet significantly ($p\leq0.05$) increased the number of Lactobacillus, decreased *E. coli* population in the ileum and inhibited the growth of *Salmonella typhimurium* due to its antimicrobial properties (Dorman and Deans, 2000). Siragusa et al. (2008) reported that addition of Humuluslupulus decreased *C. perfringens* population in the broiler intestine. A blend of essential oils containing carvacrol and thymol is reported to have the ability to decrease *E. coli* and *C. perfringens* in broilers (Jang et al., 2007). In study of Jamroz et al. (2006), a significant reduction in *E. coli* numbers was reported following addition of natural plant extract in broiler diet. However, there are a few studies conversely reporting that dietary essential oils had no effect on the intestinal microflora populations (Hermans et al., 2011). Organic acids including short chain fatty acids (SCFAs) such as acetate, propionate and butyrate have been found to control *Salmonella enteritidis*, showing growth promoting impact on the beneficial intestinal microflora (Hansen et al., 1997). Positive beneficial effects of organic acids on production performance and carcass traits of broilers were reported by Leeson et al. (2005) and Antongiovanni et al. (2007).

Table 6. Effect of dietary feed additives on intestinal bacterial count ($\times10^9$) of layer Japanese quail at 42 weeks of age

Treatments		Coliforms	Salmonella	*E. coli*
BMD	PFA			
0	0	3.55^a	7.15^a	3.25^a
0.05%	0	2.31^b	4.25^c	2.12^b
0	0.1%	2.43^b	5.42^b	2.22^b
ANOVA				
SEM		0.155	0.292	0.126
p value		≤0.0001	≤0.0001	≤0.0001

BMD, bacitracin methylene disalicylate (antibiotic growth promoter); PFA, phytogenic feed additive; ANOVA, analysis of variance; SEM, pooled standard error of column wise means comparison.
Means with different letters within the same column are significantly different ($p\leq0.05$).

Poultry caeca have a range of microorganisms with strong impacts on the performance and the health of broilers. Therefore, the aim of PFA addition into the diet is to create a favorable gut microflora by decreasing pathogenic bacteria (Apajalahti et al., 2001). It is assumed that gut microflora reduces nutrients availability to host animal by enforcing the intestinal cell turnover and thereby increasing the intestinal requirement for nutrients. Moreover, intestinal microflora and epithelial cells have to compete for nutrients (Dibner and Richards, 2005). A higher absorptive capacity in the intestine of PFA fed animals is also supported by higher blood nutrient concentrations of those quails, as observed in the present study. However, many factors influence the effectiveness of dietary supplementation of phyto-additives such as dose of extracts in the mucus layer in the intestine (Hermans et al., 2011). More so, fatty acids cause changes in intracellular pH of intestine. Butyric acid is considered the prime enterocytes energy source necessary for the correct development of the gut associated lymphoid tissue (Antongiovanni et al., 2007). In conclusion, the outcomes of current study revealed that this novel eubiotic feed additive having phytomolecules and organic acids as main ingredients, at an inclusion level of 0.1%, did improve the performance, most of studied egg characteristics, serum biochemical parameters, immunity, morphology of intestine and reduced population of harmful bacteria in quails. It can be concluded that addition of combined bioactive components of herbal plants along with organic acid as PFA at the dosage of 0.1% can be used as an alternative to antibiotic growth promoters in laying Japanese quails.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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