



THE DIETARY IMPACT OF CLOVE AND CINNAMON POWDERS AND OIL SUPPLEMENTATIONS ON THE PERFORMANCE, ILEUM MORPHOLOGY, AND INTESTINE BACTERIAL POPULATION OF QUAILS

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Abstract

The present study was aimed at assessing the impacts of dietary cinnamon or clove powders and oils on productive performance, morphology, and intestine bacterial population of quails. For this purpose, 420 quail chicks aged one day were randomly assigned into 7 treatments. Each treatment consisted of 15 females and 5 males and included 3 replicates. The chicks were reared for a period of 12 weeks. According to the results, compared to the powder treatments, the oil treatments led to significantly higher improvement in the quails' body weight, sexual maturity age, egg production (H.D%), feed intake, feed conversion ratio (FCR) (g feed / g egg), FCR (g feed /egg) and egg weight (g), bacterial population of *Lactobacillus*, and ileum morphology (villus, crypt depth, goblet cell numbers) of small intestine. Moreover, supplementations of cinnamon and clove oils resulted in a decrease in *E. coli*, enterococcus, and fungi. Cinnamon and clove essential oils were better than their powders in terms of improving the studied characteristics of the quails.

Key words : Clove, Cinnamon, Reproductive performance, Ileum morphology, Bacterial counts.

Introduction

As enhancer of poultry performance, feed supplementations are initially utilized to improve hatchability, fertility, and performance of egg production and raise use of the limited feed allowance, and since there has been a decrease in the consumers' acceptance of antibiotic feed supplementation and an increase in multiple resistance of bacteria (Schwarz *et al.*, 2001; Bach Knudsen, 2001), there has been a gradual drop in utilization of antibiotics as feed supplementations, and they were banned in the European Union since 2006 (Plail, 2006).

Over the past few years, there has been an increasing interest in studying different aspects of replacing antibiotics with new primitive additives in animal diets. Such studies have led to utilization of products in animal nutrition, such products contain essential oils that are obtained from some special herbs and spices and are used as additives to promote growth and performance of animals (Brenes

and Roura, 2010).

Research has demonstrated that different compounds that are derived from plants have different antimicrobial activities that are synthesized by the plants' secondary metabolism and produced by the interactions between animals, microbes, and plants (Reichling, 2010).

One of the herbs that is widely used in food industry is cinnamon (*Cinnamomum zylanicum*) which has special aroma. Moreover, cinnamon has been reported to have hypo-cholesterolamic, antioxidant, antiulcer, and anticandidal activities and strong antibacterial properties (Lin *et al.*, 2003). Another herb that is used both in industry and as a spice is clove. This herb is usually used in Indian cuisine to add a special taste to foods. It has also been reported that clove has inhibitory impacts on a large number of food-borne pathogens (Sunil, 2006). The inhibitory impacts of clove on microbes are attributed the fact that this herb contains essential oils which in turn are composed of components such as eugenol (Asha *et*

al., 2014). Nowadays, utilizing these herbs is being paid much attention especially in the industries of quails (Mustafa *et al.*, 2017) and laying hens (Çabuk *et al.*, 2006). Herbal essential oils are also used to help beneficial microbes colonize inside the gastrointestinal tract (Jang *et al.*, 2007). Such oils are also antifungal (Shin and Lim, 2004), stimulate digestion, develop intestine morphology, and promote enzymatic activities (Jamroz *et al.*, 2005; Mustafa *et al.*, 2017).

The present study was carried out in order to examine the benefits of using essential oils derived from clove and cinnamon spices in the diet of quails on improving their ileum morphology, bacterial population, and egg production during hot months.

Materials and Methods

Experimental plan

To carry out the present study, 420 quails aged one day were randomly assigned into 7 treatments. Each treatment consisted of 3 replicates which included 15 females and 5 males each. The chicks were reared for 17 weeks. The quails were obtained from Animal Resources Department, the College of Agriculture, University of Salahaddin - Erbil/Iraq. They were reared in particularly-designed cages with dimensions of 65 cm long, 60 cm wide, and 50 cm high. During that period, the temperature of the room was controlled and kept as 35-39°C in the afternoon and 28-30°C at night and in the morning. The control quails (T_0) were provided with standard diet, standard diet + 2 g clove powder/ kg diet was given to the treatment 1 (T_1), standard diet + 1 ml clove oil / kg diet to treatment 2 (T_2), standard diet + 2 g cinnamon powder to treatment 3 (T_3), standard diet + 1 ml cinnamon oil / kg diet to treatment 4 (T_4), standard diet + 1 g clove powder + 1 g cinnamon powder/ kg diet to treatment 5 (T_5), and standard diet + 0.5 ml clove oil + 0.5 ml cinnamon oil/ kg diet to treatment 6 (T_6). The laying quails aged 6-17 weeks were fed a diet that included corn 48.43%, soybean meal 27.6%, wheat 15%, sunflower oil 1.0%, limestone 6.0%, di-calcium phosphate (DCP) 1.15%, vitamin premix 0.10%, mineral premix 0.10%, methionine 0.17%, lysine 0.45%, and the chemical calculated of the diet (per kg) included metabolized energy 2800 (kcal), crude protein 18.52%, crude fiber 4.3%, methionine 0.45%, lysine 1.02%, Ca 2.61%, available P 0.354%, and Na 0.176%.

Nutrient analysis of clove buds and cinnamon bark

Laboratory nutrient analysis of the 100 gram powder of clove buds used in the present study indicated that it contained 308 kcal energy, carbohydrate 44.01%, water

9.53%, protein 6.28%, total fat (lipid) 6.93%, total fiber 33.25%, cholesterol 0.0%, calcium 0.748 g, phosphor 0.107 g, and total carotenes 451 µg. And the cinnamon bark powder that was used included 256 kcal energy, carbohydrate 39.51%, water 9.97%, protein 7.43%, total fat (lipid) 5.07%, total fiber 38.02%, cholesterol 0.0%, calcium 0.983 g, phosphor 0.071 g, and total carotenes 483 µg.

Chemical analysis of Fatty acids and volatile oils in clove and cinnamon oils

The two samples of clove and cinnamon oils for were analyzed in laboratory by Gas Chromatography. The results of the analysis revealed that the important fatty acids in clove bud oil were SF 6.67%, MUFA 2.38%, PUFA 11.90%, Omega-3 259 mg, and Omega-6 148 mg, and the cinnamon bark oil contained SF 0.27%, MUFA 0.14%, PUFA 1.55%, Omega-3 1.3 mg, and Omega-6 3.17 mg. Moreover, the clove bud oil included some volatile oils such as eugenol 80.71%, eugenyl acetate 13.60%, and b-caryophyllene 4.45% and the cinnamon bark oil contained E-cinnamaldehyde 78.10%, copaene 8.12%, and eugenol 7.26%.

Production performance

At the end of the study, the egg weight, feed conversion ratio (FCR), feed intake, egg production (H.D%), sexual maturity age (SMA), and the body weight of the females were calculated.

Ileum microbial determination

The ileum contents were cautiously kept in sterile petri dishes at -20°C until they were analyzed in the laboratory. For this purpose, from each homogenized sample, 1 gram of was obtained and transferred into 10 ml sterile saline solution for dilution. Afterwards, each sample was spread on selective agar plates as follows. For *lactobacillus* bacteria, MRS agar medium was used, and for *E. Coli* and *Enterococcus*, Nutrient and McConkey agars were utilized (Harrigan and McCance, 1976). Also, fungi were cultured in Sabouraud Dextrose Agar which was incubated at 37°C for 24 hours, and the colonies were determined.

Morphology of small intestine

At the end of the study, cervical dislocation was used to kill the birds. Afterwards, the abdominal cavity was cleaved, and the small intestines were weighed. In order to measure pH, the contents of the duodenums were collected in plastic bottles. Samples of intestinal duodenum were dehydrated, cleared, and embedded in paraffin. Serial sections were cut at 5 µm and placed on glass slides. For all assays, sections were deparaffinized in

xylylene and rehydrated in a graded alcohol series. Sections were examined by light microscopy (Uni *et al.*, 1998), and a special digital camera was used to determine the

number of goblet cells, villus height, crypt depth, and villus-to-crypt (V/C) ratio.

Table 1: The influence of using clove or cinnamon powders and their oil supplementations as antistressor in the diets of quails on their performances and characteristics.

Traits	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	MSE
BW g	200.3 ^b	212.6 ^b	237.0 ^a	221.3 ^{ab}	245.2 ^a	214.8 ^b	240.3 ^a	19.33
SMA (d)	36 ^a	35 ^{ab}	34 ^b	35 ^b	33 ^b	35 ^{ab}	34 ^b	1.50
EP- (H.D %)	71.16 ^c	76.33 ^b	78.11 ^{ab}	78.48 ^{ab}	81.41 ^a	77.90 ^b	80.23 ^{ab}	7.13
FI (g/day/bird)	26.49 ^b	26.07 ^b	27.20 ^{ab}	27.06 ^{ab}	28.12 ^a	26.86 ^{ab}	27.40 ^{ab}	2.19
FCR (g feed / g egg)	3.40 ^a	3.03 ^{ab}	2.90 ^b	2.97 ^b	2.72 ^c	2.97 ^b	2.77 ^{bc}	0.326
FCR (g feed /egg)	26.52 ^a	26.07 ^a	27.20 ^a	26.92 ^a	28.12 ^a	26.86 ^a	27.33 ^a	2.38
Egg Weight (g)	10.95 ^c	11.27 ^{bc}	12.01 ^{ab}	11.61 ^b	12.70 ^a	11.61 ^b	12.33 ^{ab}	1.92

T₀=control (SD; standard diet), T₁= SD+2 g clove powder/kg diet, T₂= SD+1 ml clove oil/kg diet, T₃=SD+2 g cinnamon powder, T₄= SD+1 ml cinnamon oil/kg diet, T₅= SD+1 g clove powder+1 g cinnamon powder/kg diet, T₆=SD+0.5 ml clove oil+0.5 ml cinnamon oil/kg diet, SE= mean of standard error, IBW= Initial body weight, BW= Body weight, FI= Feed Intake, FCR= Feed conversion ratio, SMA= Sexual maturity age. statements with the same letters mean non-significant. ^{a-c} Means within columns with different superscripts differ significantly at (P≤0.05).

Table 2: The effect of extracts and antibiotics on some bacterial count (CFU×10⁵/ g) numbers in ileum contents of the broilers.

Traits	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	MSE
Lactobacillus	6.09 ^c	10.52 ^{ab}	11.15 ^a	9.44 ^b	9.69 ^b	10.13 ^{ab}	11.00 ^a	0.45
<i>E. coli</i>	7.15 ^a	3.62 ^c	3.33 ^d	5.03 ^b	4.16 ^{bc}	3.82 ^c	3.25 ^d	0.31
Enterococcus	6.93 ^a	3.28 ^{bc}	3.07 ^{bc}	3.96 ^b	3.45 ^a	3.08 ^{bc}	2.88 ^c	0.29
Fungi	6.77 ^a	3.48 ^{bc}	2.65 ^c	4.13 ^b	3.36 ^{bc}	3.62 ^{bc}	2.75 ^c	0.37

T₀=control (SD; standard diet), T₁= SD+2 g clove powder/kg diet, T₂= SD+1 ml clove oil/kg diet, T₃=SD+2 g cinnamon powder, T₄= SD+1 ml cinnamon oil/kg diet, T₅= SD+1 g clove powder+1 g cinnamon powder/kg diet, T₆=SD+0.5 ml clove oil+0.5 ml cinnamon oil/kg diet. MSE= mean of standard error. ^{a-d} Means within columns with different superscripts differ significantly at (P≤0.05).

Table 3: The influence of using clove and cinnamon powders and oil supplementations as anti-stressors in the diets of quails on their small intestine profile and ileum morphology.

Treatments	Small intestine length (cm)/body weight (g)	Relative weight of small intestine (g)	Ileum morphology			
			No. Goblet cells ¹	Villus height (mm)	Crypt depth (mm)	V/C ratio
T ₀	12.85 ^c	0.231 ^b	7.89 ^c	517 ^c	79.16 ^c	6.531 ^c
T ₁	13.48 ^b	0.250 ^b	10.05 ^b	683 ^b	84.65 ^b	8.06 ^b
T ₂	13.83 ^b	0.289 ^{ab}	11.68 ^{ab}	725 ^b	88.91 ^{ab}	8.15 ^b
T ₃	13.73 ^b	0.304 ^a	11.90 ^{ab}	718 ^b	85.07 ^b	8.44 ^{ab}
T ₄	14.13 ^{ab}	0.338 ^a	12.48 ^a	813 ^{ab}	91.17 ^{ab}	8.92 ^a
T ₅	13.98 ^{ab}	0.332 ^a	10.75 ^{ab}	796 ^{ab}	86.39 ^b	9.21 ^a
T ₆	15.02 ^a	0.347 ^a	12.33 ^a	845 ^a	93.18 ^a	9.07 ^a
MSE	1.67	0.029	0.843	86.9	9.73	0.692

T₀=control (SD; standard diet), T₁= SD+2 g clove powder/kg diet, T₂= SD+1 ml clove oil/kg diet, T₃=SD+2 g cinnamon powder, T₄= SD+1 ml cinnamon oil/kg diet, T₅= SD+1 g clove powder+1 g cinnamon powder/kg diet, T₆=SD+0.5 ml clove oil+0.5 ml cinnamon oil/kg diet. MSE= mean of standard error. ^{a-d} Means within columns with different superscripts differ significantly at (P≤0.05).

¹number of goblet cells per mm of villus length.

Statistical analysis

The collected data were analyzed through Complete Randomize Design (CRD) and Statistical Analysis System (2005). Moreover, Duncan's multiple range test was used at a significant level of 0.05 to determine significant differences among treatment means.

Results

The effects of using clove and cinnamon powders and oil supplementations as anti-stressors in the diets of quails on their performances and characteristics are presented in (Table 1). As seen, using clove and cinnamon powders and oils as dietary supplementations led to significantly higher BW (g), SMA (d), EP- (H. D%), FI (g/day/bird), FCR (g feed / g egg), FCR (g feed /egg) and egg weight (g) (Pd≤0.05) (See Table 1).

As presented in table 2, the ileum count of *Lactobacillus* was significantly higher in all

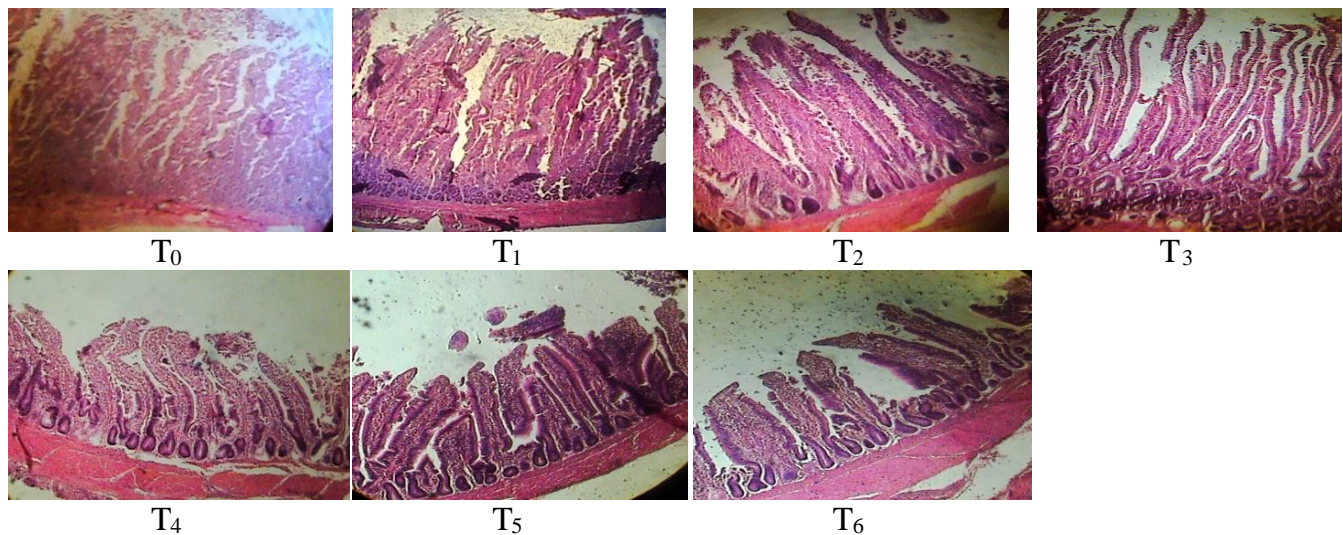


Fig. 1: The influence of using clove and cinnamon powders and oil supplementations as anti-stressors in the diets of quails on their small intestine and ileum morphology.

treatments of clove and cinnamon powders and oils ($p \leq 0.05$). Moreover, adding the same spices and their oils led to a significant decrease in the harmful bacteria (*E. Coli* and *Enterococcus*) and fungi in ileum compared to the control quails. It is obvious that the clove oil and the combination of clove and cinnamon oils led to a greater effect on ileum bacterial population (See Table 2).

According to the results presented in table 3, there were significant differences between the control quails and those that received cinnamon and clove powders and oils as supplementation in terms of percentages of small intestine length (cm)/ body weight (g) ($P \leq 0.05$). The highest value was observed in T_6 , and no significant difference was observed among T_1 , T_2 , T_3 , T_4 , and T_5 ; however T_0 and T_6 were significantly different. This results are also shown in Fig. 1.

Discussion

Quail farming is a widely practiced activity in the poultry sector. Compared to other types of farming, it is a quite simple activity which can be started with a limited initial fund. Recently, there has been an increase in the consumers' demand for quail eggs and meat, which has encouraged farmers to launch the activity or raise their productions. There also been an increase in the consumers' concern about the quality of quail products. In order to meet these requirements of the market, researchers have studied different food supplementations that can improve the quality of final products without having any negative impact of the quails feed efficiency (Nunes *et al.*, 2010). In line with this spectrum of research and in order to add to the body of the related knowledge, the present study focused on examining the

effect of clove and cinnamon powders and oils on the performance, ileum morphology, and intestine bacterial population of quails.

The results of the present study indicated that adding clove and cinnamon powders and oils as supplements to the quails' diets led to a significant increase in BW (g), SMA (d), EP- (H. D%), FI (g/day/bird), FCR (g feed / g egg), FCR (g feed /egg) and egg weight (g). This finding is in line with relevant research that has demonstrated that additives such as essential oils, herbal extracts, symbiotics, prebiotics, probiotics, organic acids, and antioxidants have various characteristics that help maintain the final product's quality (Biondo *et al.*, 2017). Also, in line with the results of the present study, Tung *et al.*, (2008) reported that cinnamon is a significant phytochemical additive in diets for farm animals that can enhance the quality of the final product and some characteristics of the animals because this herb has antioxidant, antimicrobial, and antiseptic properties (Tung *et al.*, 2008).

Similar to the findings of the present study, research on cinnamon has shown that it has a positive impact on broilers and laying hens due to its antimicrobial and antifungal properties (Oliveira *et al.*, 2010; Sang *et al.*, 2013). Moreover, Oliveira *et al.*, (2010) have shown that there are high levels of carotenoids in cinnamon; therefore, compared to diets containing only corn, diets with cinnamon additives have higher levels of pigments, resulting in the significant increase in the yolk color.

The observed improvements caused by cinnamon and clove powders and oils can be attributed to the interaction many factors including the valuable nutrients contained in cinnamon and clove powders such as carbohydrate,

protein, Ca, P, and carotenes. Moreover, cinnamon and clove oils contain some volatile oils, and saturated and unsaturated fatty acids which have a positive impact on body performance, egg production, and FCR. The combination of all these components leads to a decrease in mortality of the birds. In addition, clove essential oil can effectively eliminate parasites from the digestive system. It has been reported that a 0.05% eugenol solution is sufficient to kill bacillus tuberculosis (Milind and Deepa, 2011). Similar to our results are the findings of Park (2008) and Gbenga *et al.*, (2009) who reported that the broilers that were given diets containing cinnamon extract had significantly higher average daily gain and lower feed-to-gain ratio over a period of 6 weeks compared to the control birds.

According to the results of the present study, there was a significant increase in the ileum count of *Lactobacillus* in all treatments of clove and cinnamon powders and oils. Moreover, there was a significant decrease in the harmful bacteria (*E. Coli* and *Enterococcus*) and fungi in ileum compared to the control quails. Therefore, clove oil and the combination of clove and cinnamon oils resulted in a greater effect on ileum bacterial population. Similar to these results, it has been reported that gut microflora significantly affects host nutrition and health and growth performance by interacting with utilization and development of the gut system of the host which is dependent on the activity and composition of the gut microflora of birds (Glannenasa *et al.*, 2012). Moreover, as concluded by Dalkiliç *et al.*, (2009), a decrease in total *coliform* microorganism counts of broiler small intestine at the ages of 21 and 42 days was obtained by utilizing antibiotic supplementation and clove extract. It has also been revealed that the total bacterial and Gram negative bacterial counts can significantly decrease in the broiler chickens as a result of using organic acid mixture decreases (Gunal *et al.*, 2006). Moreover, it has been shown that the butyric acid leads to a decrease in the virulent gene expression and invasiveness in *Salmonella* Enteritidis, which reduces its colonization in the caeca of broiler chicken (Van Immerseel *et al.*, 2004).

The effect of the clove and cinnamon oils on the performance, ileum morphology, and intestine bacterial population of quails can be justified by considering the properties of the organic acids that they contain. In this regard, it has been stated that due to time of dissociation, organic acids have a superior bactericidal capacity than HCL which is a strong acid and its dissociation happens more quickly than weak organic acids. Also, the antimicrobial impact of an acid is dependent on its pH or dissociation constant (pKa), at which half of the total

acid is undissociated. The molecule undissociated part is related to the antimicrobial impact (Davidson and Taylor, 2007) because the molecules that are undissociated penetrate into the cells. Furthermore, the chain length leads to an increase in the activity, which indicates that the organic compound itself has a direct action. However, compared to strong acids, weak acids possess higher undissociated portions, and they can efficiently penetrate through the microorganism's cytoplasmic membrane (lipid bilayer) (Davidson and Taylor, 2007). The results of the present study can be more justified by the reports of Iji *et al.*, (2001) who concluded that adding viscous products to the diet results in deeper crypts in broilers at the age of 14 days because the viscous properties of grain can directly influence the morphology of the animals, result in improvement in their digestive system along with the changes that occur in the ileum wall (Mathlouthi *et al.*, 2002). Inclusion of multi-enzymes in the diet might prevent the growth of ileum morphometry. According to relevant research, supplementation with enzymes can result in a potential drop in the rate of cell proliferation in the crypt depth (Silva and Smithard, 2002). As reported by Sarica *et al.*, (2005), a decrease in bacterial populations with whole wheat diets could be responsible for lower crypt depths. Moreover, decrease in crypt depth can be attributed to a lower activity of secretion such as production of mucus, and the fact that goblet cells are specifically placed in the crypt depth (Langhout *et al.*, 1990).

The results of the current study also showed a significant difference between the quails that were given cinnamon and clove powders and oils and those with the standard diet in terms of percentages of small intestine length (cm)/ body weight (g). Among the experimental quails those in treatment 6 (T_6) which received a combination of clove and cinnamon oils as their supplementation had the highest percentages of small intestine length (cm)/ body weight (g). This significant difference is related to utilizing the combination of clove and cinnamon oils, which allowed for the stretch of the small intestine, which led to larger surface area for absorption and digestion of the nutrients entering the digestive system of birds and also to the significant difference between the control and the birds receiving oil treatments in terms of their liver relative weight (Botsoglou *et al.*, 2004). Similar results have been reported by Rajput *et al.*, (2013) who reported that supplementing broiler diets with curcumin led to an improvement in the utilization of apparent metabolizable energy.

Furthermore, supplementing the diets of broiler

chickens with essential oils can enhance activities of trypsin and amylase in pancreas and small intestine. T6 which was supplementation with both types of essential oils was the best treatment for the proprieties of ileum morphology. As reported by Reisinger *et al.*, (2011) and Budd *et al.*, (2017), adding essential oils to the diet leads to a rise in the number of goblet cells which are responsible for producing intestinal mucins which are the major components of the mucus layer that coats the chicken intestine. However, the most appropriate level for the inclusion of clove powder in poultry diets has been proposed to be 1% (10 g/kg of diet), which is not different from the histological changes and growth rate of the intestines in broiler chickens (Al-Mufarrej *et al.*, 2019). This layer plays a role in digestion and absorption of the nutrients, prevents gastrointestinal pathology, and protects the host against luminal microflora. Agostini *et al.*, (2012) found that *Lactobacilli* counts increased for broiler chickens that were fed with supplementation of clove, this change in the ratio might be attributed to a positive selection of eugenol towards lactic acid bacteria. Similarly, Wu *et al.*, (2004) reported that given their organic fatty acids some, spices and their oil supplementations diets affect ileum morphometry positively. Moreover, it has been stated that adding clove or cinnamon oil or powders in non-starch polysaccharides (NSP) can lead to changes in gut physiology status and bacterial profiles, a decrease in apparent nutrient digestibility, and a rise in the viscosity of the feed to be digested (Buraczewska *et al.*, 2007).

Conclusion

According to the results of the present study, dietary supplementation with 2 g/kg of clove or cinnamon powders and 1 ml/kg clove and cinnamon oils can lead to improvement in broiler performance, without causing any evident adverse effects. Moreover, there was improvement in small intestine - ileum *Lactobacillus* count, goblet cells numbers, villus height, and crypt depth. The proposed supplementation also led to a decrease in the harmful bacteria (*Enterococcus*, *E. Coli*) and fungi. Finally, the studied characteristics of the birds were improved with cinnamon and clove essential oils better than their powders.

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