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Effect of Dietary Synbiotic and Cinnamon Essential Oil on Growth Performance, Intestinal Morphology, Serum Indices and Intestinal Microbial Population in Japanese Quails

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Key words
Cinnamon oil, Japanese quail, Microbial population, Synbiotic, Serum indices

ABSTRACT

Antimicrobial resistance is an emerging problem. Alternatives to antibiotics such as synbiotics and essential oil are being used in poultry diets. The study was designed to investigate the effects of synbiotics and the essential oil of cinnamon (CEO) in feed supplementation at different levels on growth performance, gut histomorphometry, specific serum indices, and intestinal microbial populations in Japanese quails. A hundred Japanese day–old birds were randomly allocated into four treatments groups including the Control group (T1) with basal diet (BD), T2 (BD+CEO 200mg/kg of diet), T3 (BD+synbiotic 1g/kg of diet), and T4 (BD+ CEO 200mg/kg of diet + synbiotic 1g/kg of diet). The trials were conducted for 35 days with a basal diet and water ad libitum was provided with usual farming practices. After the completion of the trial (day 35), two birds per replicate (25 birds per group) were slaughtered for serum analysis. Improved growth performance and gut histomorphometry were observed in the supplemented groups. The triglyceride values were significantly ($P<0.05$) decreased in supplemented groups than the control group. The serum total cholesterol had also significantly ($P<0.05$) decreased in supplemented groups than the control group. The *E. coli* count was significantly ($P<0.05$) decreased in supplemented groups than the control group. However, the *Lactobacillus* count significantly ($P<0.05$) increased in supplemented groups than the control group and was maximum in group III. it is concluded that Synbiotic and CEO dietary inclusion improves the blood serum indices and intestinal beneficial bacteria and decreases pathogenic bacteria in Japanese quails.

INTRODUCTION

Pakistan's poultry industry is the most important agricultural sector, contributes 1.4 percent to the national GDP, provides employment to 1.5 million people, and also gives a low-cost source of protein in the form of a diet. Japanese quail *Coturnix coturnix japonica* farming is the smallest type of commercial poultry farming. Important genetic features like short generation intervals, low feed consumption, low rearing costs, high disease resistance, the requirement of little floor space, low investment, and sexual maturity at a young age are the motives to rear Japanese quail for commercial eggs and meat productions (Ojadapo and Amao, 2014; Manafi *et al*., 2016). High protein and contents of specialized mucus-secreting epithelial cells in the gastrointestinal tract of quail's digestive tract protect it from harmful microorganisms (Zaidi *et al*., 2017).

Antibiotic-resistant bacteria and the transmission of antibiotic deposits in meat and eggs, cause harmful effects on humans, which is a growing concern to restrict the use of antibiotic feed additives (Yang *et al*., 2019). Over several years, to maximize feed efficiency and lessen mortality, antibiotics as growth promoters (AGPs) have been used in the poultry industry (Afrin *et al*., 2021). Numerous products can be used as AGPs placement candidates, including synbiotics and plant extracts (essential oils). Supplementing poultry feeds with

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essential oils (EO) derived from aromatic plants could have been a promising and eco-friendly alternative to antibiotics. Cinnamon essential oils (CEOs) are utilized to make a variety of plant extracts that are used as feed additives in the poultry industry. The extract of oil contains nutrients such as carbohydrates, proteins, vitamins (A, K, C, B3), and minerals, as well as various bioactive molecules that have immunomodulatory, antioxidant, antiviral, antimicrobial, gastroprotective, and antibacterial activities (Saeed *et al.*, 2018). Cinnamaldehyde and eugenol, two of their components, have antibacterial properties against *Staphylococcus, Enterococcus faecalis, Salmonella* sp., *Staphylococcus aureus*, and *Escherichia coli*. The highest percentage of cinnamaldehyde has been observed in *Cinnamomum verum* (67.57 percent) Eugenol, 2'-hydroxy cinnamaldehyde, cinnamyl alcohol, and 2'-benzoloxycinnamaldehyde, cinnamyl acetate, and camphor are among the other constituents (Saeed *et al.*, 2018). Cinnamon includes antioxidant properties that may help to defend cell membranes from damage caused by free radicals (Saeed *et al.*, 2018). CEO has high antioxidant and hypocholesterolemia effects. In the gastrointestinal tract of poultry, plant extracts, and herbs inhibit the growth of numerous pathogenic bacteria while promoting the growth of beneficial bacteria (Abd El-Hack *et al.*, 2020).

Synbiotics are a combination of probiotics and prebiotics that improve intestinal microbial balance and increase performance in the GI tract of the host. Probiotics are single or mixed cultures of live bacteria that balance the microbiome and leave no residues in food obtained from animals or birds, thus they do not promote antimicrobial resistance (AMR) (Mehrabi *et al.*, 2012; Mehdipour and Afsharmanesh, 2018). Probiotics are mainly composed of beneficial bacteria, hence *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are the most common probiotics. These probiotic microorganisms prevent the production of harmful pathogens in the intestine by lowering the load of *E. coli*, enteric bacterial streptococci and staphylococci infections from spreading (Ahiwe *et al.*, 2021). In Japanese quail, the effects of probiotics on enteric histology and intestinal microbial population were limited, along with small intestine pH measurements (Afrin *et al.*, 2021). The purpose of this study was to determine the effect of synbiotics and CEO individually, and in combination growth performance, jejunum, and ileum histomorphometry, ileocecal microbial population, and serum indices in Japanese quails.

MATERIALS AND METHODS

Birds and experimental design

A total of 100 days old unsexed Japanese quails were reared at Avian Research and Training Centre, Lahore for 35 days. These birds were randomly divided into 04 equal groups, with five replicates in each group. Group I was the control group fed with basal diet (no additives in feed), Group II was supplemented with CEO 200mg/kg of diet, Group III was supplemented with basal diet and synbiotic 1g/kg of diet Group IV was supplemented with CEO at 200mg/kg of diet + synbiotic 1g/kg of diet (Mehdipour and Afsharmanesh, 2018). The treatments were given from day 1 to the 35 days of the experiment. On the 35th day of the trial, two birds selected per replicate, slaughtered and their blood samples were collected for serum biochemical analysis.

Growth performance

The birds were weighed weekly with the help of a weighing balance. The body weight gain (g) was determined on a weekly basis.

Body weight gain (g) = final body weight (g) – initial body weight (g)

Feed conversion ratio (FCR) was calculated on a weekly basis:

Feed conversion ratio = feed intake (g) / body weight gain (g)

Intestinal histomorphometry

A one-centimeter segment was incised from the mid of the jejunum and ileum of each bird and preserved in 10% formalin solution before paraffin embedding. Hematoxylin and Eosin (H and E) stains were used for slide preparation by the method adopted by (Bancroft *et al.*, 2013). The slides were analyzed at 10× under a microscope (Olympus, NV, Aartselaar, Belgium) with a digital imaging system and a commercial morphometry program Pixel Pro for jejunum and ileum villus height, the width of villus, crypt depth, villus height to crypt depth ratio and depth of lamina propria as described by (Ashraf *et al.*, 2017).

Biochemical parameters

Total cholesterol (TC) and triglyceride of serum were estimated by colorimetric test (Ornatska *et al.*, 2011).

Enumeration of intestinal bacteria

For the counting of the ileocecal microbial population, a 1gm sample was collected immediately after slaughtering two birds from each replicate then prepared serial dilutions. After serial dilution 0.1ml per dilution were cultured on standard plate count agar for total bacterial count (TBC), Mac Conkey agar for *E. coli* and De Man, Rogosa and Sharpe (MRS) for *Lactobacillus* bacterial colony counts after anaerobic incubation at 38°C for 48 h (Sugiharto *et al.*, 2018; Afrin *et al.*, 2021). The colony-forming unit can be calculated by using the formula:

Colony forming unit (cfu) = number of colonies x dilution factor/volume of sample spread on the plate.

Statistical analysis

Normally distributed data were analyzed by using SPSS Inc. Version.20 through one-way analysis of variance (ANOVA). The normality of data was checked by the Kolmogorov Smirnov test. The difference between the groups was tested by Tukey's test. The results were statistically significant (P<0.05).

RESULTS

Growth performance

The effects of supplementation of CEO, synbiotic and their combination on growth parameters are shown in Table I. The body weight gain was significantly (P< 0.05) improved in all supplemented groups than the control group. The highest value of body weight gain was observed in Group II (CEO group). The feed intake was significantly (P<0.05) improved in all supplemented groups than the control group. The highest value of feed intake was observed in Group IV. FCR was improved significantly (P<0.05) in Group IV (combination group) than other three groups.

Histomorphometry parameters

Jejunum

The effect of supplementation of CEO, synbiotic and their combination on villus height, villus width, crypt depth, VH: CD and lamina properia thickness of jejunum are shown in the Table I. Villus height, villus width and crypt depth of jejunum were significant among different treatment groups (P<0.05) (Fig. 1). The villus height of jejunum was improved significantly (P<0.05) in treated groups than control group. Villus height of jejunum was highest in synbiotic supplemented group than other groups. Villus width of jejunum had increased significantly (P<0.05) in supplemented groups than control group. Villus width was highest in Group IV (combination group). Crypt depth was improved slightly significant (P<0.043) in supplemented groups than control group. Crypt depth was increased in Group III than other groups. VH: CD results were non-significant (P<0.801). The thickness of lamina properia was non significan (P<0.609).

Table I. Effect of supplementations of cinnamon essential oil (CEO) and synbiotic on growth performances, histo-morphometry of jejunum, ileum and TG and TC of quail birds (Mean ± SD).

Parameter	Control (Goup I)	Treatments			P value
		CEO (Goup II)	Synbiotic (Goup III)	CEO + Synbiotic (Goup IV)	
Growth performances					
Weight gain (g)	41.35±0.62	42.55±1.48	41.88±2.49	41.39±1.03	0.005
Feed intake (g)	90.81±12.77	99.93±4.37	93.94±13.10	100.89±10.66	0.001
FCR (g)	90.81±1.77	99.93±2.37	93.94±0.10	100.89±4.80	0.002
Jejunum histo-morphometry					
VH* (µm)	539.20±28	555.60±32.	616.40±13	610.00±51	0.005
Avrg.VW** (µm)	51.87±12	68.10±12	81.43±12	86.10±12	0.002
CD*** (µm)	95.32±5	103.28±4.	113.96±10	107.96±14	0.043
VH:CD (µm)	5.66±0.38	5.38±0.33	5.45±0.57	5.75±1	0.801
Lamina properia thickness (µm)	27.44±4	29.62±8	27.30±5	31.96±5	0.609
Ileum histo-morphometry					
VH (µm)	247.20±35.79	464.80±8.67	304.80±34.80	445.40±60.83	0.000
Avrg.VW (µm)	85.17±14.36	72.10±7.77	92.76±11.16	67.19±9.72	0.008
CD (µm)	74.60±5.71	107.14±6.09	116.74±14.66	91.67±18.27	0.000
VH:CD (µm)	3.33±0.55	4.34±0.22	2.624±0.24	5.12±1.69	0.003
Lamina properia thickness (µm)	23.30±.00	27.30±5.47	25.30±4.47	27.30±5.47	0.455
TG	133.90±22.99	84.60±16.52	86.50±10.25	70.80±6.97	0.000
TC	160.50±11.40	128.60±22.81	141.00±20.42	151.50±7.67	0.001

VH, villus height; VW, villus width; CD, crypt depth, TG, triglyceriod; TC, total cholesterol.

Ileum

The effect of supplementation of CEO, synbiotic and their combination on the villus height, villus width, crypt depth, VH: CD and lamina properia thickness is also shown in Table I. The villus height was improved significantly ($P<0.05$) in supplemented groups than control group (Fig. 1). The villus height was highest in group II (CEO group). The villus width was improved significantly in supplemented groups. The villus width was highest in Group III (synbiotic group) than other groups. The crypt depth significantly ($P<0.05$) increased in supplemented groups. Crypt depth was highest in Group III (synbiotic group). VH: CD improved significantly ($P<0.05$). VH: CD was highest in Group IV. The thickness of lamina properia was non significantly ($P<0.455$).

Total cholesterol and triglycerides

The effects of supplementation of CEO, synbiotic and their combination on TC and triglycerides (TG) of serum sample of japanese quail are shown in Table I. The results indicated that there was a significant ($P<0.05$) difference in serum TC and TG. The TG values decreased significantly ($P<0.05$) in supplemented groups than control group. The lowest value of TG were found in group IV. The serum TC had also significantly ($P<0.05$) decreased in supplemented groups than control group. The lowest value was in the Group II.

Bacterial count enumeration

The effect of supplementation of CEO, synbiotic and their combination on total bacterial count (TBC), *E. coli* and lactic acid bacteria (LAB) in the sample of ileum and cecum of Japanese quail are shown in Table II. The results were significantly different among different groups. The TBC had decreased significantly than control and lowest value in Group II of ileum. The *E. coli* count decreased significantly ($P<0.05$) in supplemented groups than control Group. *E. coli* count had lowest values in Group II in ileum. LAB also significantly ($P<0.05$) increased in supplemented groups than control group. The count was maximum in Group III of ileum.

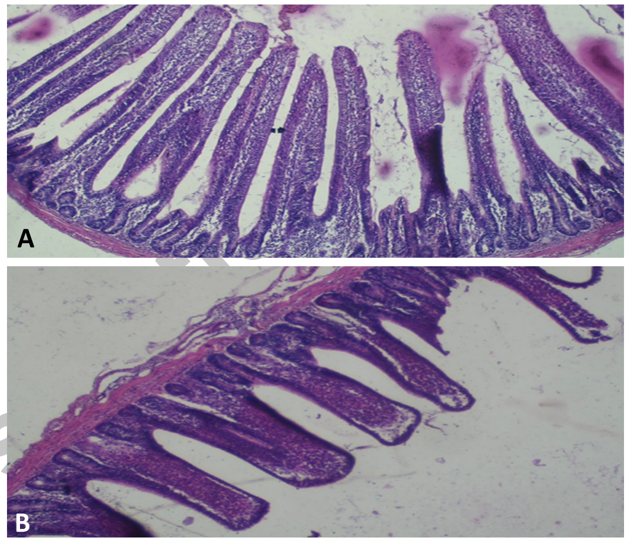

Fig. 1. Effect of synbiotic and CEO on histological structure of jejunum (A) and ileum (B) of Japanese quails. Magnification: 10x; Stain: H & E.

Table II. Effect of supplementations of CEO and SYN on total bacterial count (TBC), *E. coli* and lactic acid bacteria (LAB) in ileum and caecam of quail birds (Mean ± SD).

Parameters	Control (Goup I)	Treatments			P value
		CEO (Goup II)	Synbiotic (Goup III)	CEO + Synbiotic (Goup IV)	
Bacteria in ileum					
TBC	9.55±0.04	9.22±0.10	9.30±0.12	9.32±0.14	0.002
E. coli	7.10±0.26	4.52±0.19	5.38±0.23	6.08±0.47	0.000
LAB	7.77±0.38	7.93±0.18	8.51±0.17	7.33±0.53	0.001
Bacteria in caecam					
TBC	8.99±1.01	9.15±0.56	7.94±0.29	7.61±0.25	0.002
E. coli	6.55±0.14	4.10±0.56	5.19±0.58	4.75±0.99	0.000
LAB	7.83±0.49	7.79±0.63	7.81±0.45	7.36±0.36	0.414

DISCUSSION

The quail's digestive tract contains high protein content, and specialized epithelial cells which produce mucus that protects it from harmful microorganisms (Zaidi *et al*., 2017). In the present study, the effect of CEO, synbiotic (biomin) and their combination were investigated to observe its rule on the growth performance, intestinal histomorphometry, ileocecal microbial population, and serum TC and TG in Japanese quail. This research is an attempt to introduce alternatives to AGPs. Due to potential public health issues, antibiotic-free meat and egg production are demanded worldwide by different poultry stakeholders (Cervantes, 2015). To our knowledge, dietary synbiotic and CEO alternative to antibiotics is used scarily in quails, and interestingly, improved growth performance and gut health was observed in the present study.

A positive and improved effect on growth performance and FCR were noticed by the supplementation of CEO and their combination in the feed of quail birds. Similarly Manafi *et al*. (2016) and Tufan and Bolacali (2017) reported improved growth performance in broiler. Hamasalim (2016) reported that the addition of synbiotics to feed increase digestion and absorption of protein, which may have positive effects on growth performance in broiler. Mehdipour and Afsharmanesh (2018) reported active ingredients in cinnamon, which increases body weight gain and feed conversion.

Increased villus height provides increased surface area for nutrient absorption (Bogucka *et al*., 2019). In current study, villus height of jejunum and ileum and crypt depth showed significant increase in the supplemented groups. Bogucka *et al*. (2019) cited decrease in crypt depth is due to lesser repair ability of the intestinal cells. In present investigations, increase in the crypt depth was observed, so it can be inferred that supplementation of CEO, synbiotic and their combination improve intestinal cells repair. Supplementation of synbiotic improves the gut health by decreasing the colonization of harmful bacteria and beneficial bacteria in the gut increased the acidity in the gut environment. Bifidobacteria and lactobacilli were nonpathogenic bacteria that digest prebiotics, decreasing intestinal pH, raising osmotic potential, and promoting proton exchange. Butyrate was one of the most important fatty acids produced because it regulates epithelial cell formation and promotes differentiation and death. These properties help the intestine to absorb nutrients while also protecting and improving its microstructure. When villi height and the villi height/crypt depth ratio rise, the region of nutrient absorption is known to increase (Awad *et al*., 2008). Probiotic such as *Lactobacillus* and *Bifidobacterium*, as well as *Enterococcus faecium*, increased villus height and decreased villus crypt depth in the jejunal villus. Shorter villi and deeper crypts can lead to lower nutrient absorption, increased gastrointestinal secretion, and performance. If there was less repairing of intestinal epithelial cell then because of less mitotic activity in the crypt depth decrease in crypt depth can be expected (Awad *et al*., 2009).

Diet fatty acid profile provided to birds influence the blood and tissues fatty acid profile which disturbs the metabolism and consequently accumulation of consumed lipids (Crespo and Esteve-Garcia, 2001; Jankowski *et al*., 2012). More dietary fat consumption causes metabolic disturbances, glucose intolerance dyslipidemia and hepatic steatosis. Dietary fat feeding in mammal's outcome in metabolic ailments and adjustments in glucose tolerance (Honors *et al*., 2010; Fraulob *et al*., 2010), dyslipidaemia (Khan and Mahboob, 2014), and hepatic steatosis (Fraulob *et al*., 2010; Gauthier *et al*., 2006). Balanced feed should be designed to decrease blood fatty acid profile. The serum biochemistry of CEO, synbiotic and combination treated groups in current investigations showed a significant decrease in TC levels and TG during serum analysis. Similar results are reported in broilers (Tufan and Bolacali, 2017) that the addition of synbiotic to the diet caused TC levels to decrease. The mechanism behind is, by lowering its absorption in the gastrointestinal tract and cholesterol synthesis in the liver. It can be concluded that the TC are reduced in Japanese quails by the mechanism cited in broilers (Tufan and Bolacali, 2017). Other studies in broilers reported analogous to our findings that showed cinnamon affects the biochemical profile of the blood, including antioxidant activity on immunity. Rashid *et al*. (2020) conducted an experiment in poultry birds and reported that the cinnamon-based PFAs group showed significant variations in TC and TG concentrations.

The normal microbial population in the intestine of a poultry bird is important source of healthy diet and also plays significant roles in the physiology and health of the host (Pan and Yu, 2014; Hooper *et al*., 2012). The lactobacilli are bacteria normally present in fruits, milk, and soil. These bacteria are identified to retain the natural equilibrium of the microflora in the chicken intestine (Chen *et al*., 2005, 2017). Different studies have established that the fruitful effects of lactobacilli species for the poultry birds, like these bacteria lower fat concentrations in the serum and body of poultry, thereby increases proficiency of poultry, gut epithelial cells have high affinity for lactobacilli binding, therefore act as a competitive exclusive bacterium, boost the immune system and are healthy symbionts (Heravi, 2011). Different species of *Lactobacillus* in different counts are reported to be a healthy alternative for antibiotic as a growth promoter (Chichlowski, 2007; Zakeri and Kashefi, 2011). Our finding showed significant (P<0.05) reduction in *E. coli* and increased *Lactobacillus* count in ileum and cecal region of intestine in the supplemented groups as compared to control group. *E. coli* was ruled out to be a high cause of first week mortality in baby chicks (Nolan *et al*., 2020). Similarly, Chao *et al*. (2000) reported that in the ileum of birds, dietary cinnamon enhanced Lactobacillus spp. growth while suppressing *Campylobacter* spp. and *E. coli* growth. It was stated by Chao *et al*. (2000) that the *Lactobacillus* spp. fermentation produces SCFAs that's responsible for the intestinal environment's stability and the protection of pH sensitive harmful bacteria. Cinnamon's bioactive components had antimicrobial properties against *E. coli* Gram negative bacteria. Hence, it is concluded that the dietary cinnamon supplementation increases the microbial counts of *Lactobacillus* species.

CONCLUSION

It can be concluded that the supplementation of synbiotic, CEO and their combination decreases the serum TC and triglyceride and ileaocecal microbial population in Japanese quail, and increased the beneficial bacterial counts.

DECLARATIONS

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Ethical approval

Ethical approval for the conduction of this experiment was acquired from the Avian Research and Training Centre (ARTC), University of Veterinary and Animal Sciences, (UVAS), Lahore, and the Directorate of Advanced Studies, UVAS.

Statement of conflict of interest

The authors have declared no conflict of interest.

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