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Original Article

Comparison the effects of probiotic and prebiotic as antibiotic alternatives on *Salmonella* colonization, performance, and egg quality in laying hens challenged with *Salmonella enterica* serotype Enteritidis

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Abstract

Background: Consumption of contaminated eggs with *Salmonella enterica* serotype Enteritidis (SE) cause gastroenteritis in human. **Aims:** The present study examined the effect of probiotic and prebiotic compared to antibiotic on the colonization of SE in the ceca, and the quantity and quality of produced eggs in the laying hens challenged with SE. **Methods:** One hundred Hy-Line W-36 laying hens with 44-week-olds were studied for 13 weeks in a randomized complete block design containing five treatments and four replicates with five birds in each replicate. Treatments included: negative control, positive control, and antibiotic: diets containing antibiotic (Oxytetracycline 0.15 g/kg diet), probiotic (Bactocell® 0.1 g/kg diet), and prebiotic (Diamond V Original XPC™ 1.25 g/kg diet). All experimental groups except negative control were challenged with 1 ml of suspension solution containing 1×10^7 CFU/ml SE by oral gavage at the start of the ninth week of the experiment. Laying performance traits and cecal bacterial population were measured at the end of each week. **Results:** Probiotic and prebiotic showed a greater effect in the reduction of yolk cholesterol and blood cholesterol level before and after challenge with SE, respectively ($P<0.05$). In pre-challenge period, treatments had no effect on the cecal bacterial population; but after the challenge, three dietary supplements decreased the colonization of SE in the ceca of laying hens, and prebiotic showed more preventive effect ($P<0.05$). **Conclusion:** The result of this study showed that the prebiotic can be effective in reducing and preventing SE colonization in laying hens and act as an alternative to antibiotics.

Key words: Antibiotic, Cecal bacterial population, Egg yolk cholesterol, Prebiotic, Probiotic

Introduction

Salmonella enterica serotype Enteritidis (SE) is a pathogen, which can infect humans and poultry causing fowl paratyphoid. SE colonizes in the intestinal tract of poultry, subsequently affects immune cells, especially macrophages, and encloses the pathogenic bacteria. This allows bacteria to penetrate into the macrophage's intracellular environment, survive and multiply. Infected macrophages migrate to internal organs such as the liver, heart, spleen, ovary, and oviduct (Gantois *et al*., 2009). The production of SE-contaminated eggs is a major source of human infections with *Salmonella*. Laying hens may be infected with SE but show no signs of clinical illness, and infected subclinical carriers may shed the bacteria within their eggs, and feces, contaminating the environment (Dhama *et al*., 2007). Consumption of contaminated eggs with SE in raw or undercooked form or food products containing contaminated eggs have negative impacts on human health causing salmonellosis (Staji *et al*., 2012).

Including antibiotics in diets and applying the principles of biosecurity are used to prevent intestinal infections such as SE infection in poultry flocks. Antibiotics are used in egg production for therapy, prophylactic, and to improve the performance of hens. The elimination of pathogens from the gastrointestinal tract, improvement in nutrient absorption, lower energy and protein expenditure, lower ammonia production, and a lower rate of food passage is the effects of the sub-therapeutic use of antibiotics in laying hen feed (Lander *et al*., 2012). Despite the beneficial effects of antibiotics, their residues in eggs can cause bacterial resistance, alteration of the normal intestinal microflora in consumers, and serious side effects on human health (Marshall and Levy, 2011).

The prohibition of antibiotic growth-promoters led to the development of alternatives to antibiotics such as organic acids, phytogenics, prebiotics, and probiotics (Bajagai *et al*., 2020). Prebiotics are a source of food for

gut-healthy bacteria that cause a reduction of intestinal pH, change the composition and activity of the gut microflora, and prevent colonization by enteric pathogens (Davani-Davari *et al*., 2019). Also, it can improve digestion and minerals absorption, stimulate specific mucosal immune functions, and have the potential to reduce serum cholesterol levels in laying hens and broilers (Jha *et al*., 2020). It has been reported that feeding laying hens with prebiotic supplemented diets increased the cecal populations of lactobacilli and bifidobacteria, and decreased the cecal *Escherichia coli* (Mookiah *et al*., 2014); and *Salmonella* population in the cecal and rectal intestinal contents (Ribeiro *et al*., 2007).

Probiotics are gram-positive, immobile, non-spore-forming anaerobes or facultative anaerobes bacteria such as *Lactobacillus* or *Bifidobacterium* that have been selected from members of the normal healthy intestinal microflora. New probiotic microbes from other species and genera can inhibit the growth of several species of harmful bacteria owing to producing an acidic environment through convert hexose sugars to lactic acid, (Makarova *et al*., 2006). Supplementation of probiotics promotes gut health and prevents pathogenic bacteria colonization in the gastrointestinal tract via mechanisms such as competitive exclusion, production of antimicrobial peptides (bacteriocins), stimulating mucus production of goblet cells and the immune system, reducing the production of the toxic metabolite (ammonia), and improving the intestinal mucosal barrier function (Amalaradjou and Bhunia, 2012). It has been reported that dietary prebiotic supplementation (ALPHAMUNE™) had an improvement effects on the histomorphological structure of the small intestine and lymphatic tissue of the cecal tonsil in broiler chickens (Majd *et al*., 2014).

Fermentation metabolites of Diamond V Original XPC™ (XPC) is a prebiotic-like compound and derived from yeast fermentation (post-fermentation growth medium residues, residual yeast cells, and yeast cell wall fragments). Mixed incubation of XPC with chicken ceca content *in vitro* anaerobic culture showed the presence of XPC resulted in a rapid reduction of *S*. Typhimurium population and an increase in short-chain fatty acid concentration (Rubinelli *et al*., 2016). Bactocell® is the trade name for a probiotic, based on viable cells of a strain of *Pediococcus acidilactici*. It has been reported that the addition of Bactocell® to the broiler diet decreased the total aerobic, coliforms, fecal coliforms and *E. coli* counts in both the small intestine and caecum; and numerical increase in Lactobacilli count (Youssef *et al*., 2017). The protective effect of probiotics and prebiotics against *Clostridium perfringens* (Ramlucken *et al*., 2020), aflatoxin (Asadi *et al*., 2018), and coccidiosis (Behnamifar *et al*., 2019) have been mentioned in previous studies.

Due to the beneficial effects of probiotics and prebiotics on intestinal health and immune function, the present study aimed to investigate the effects of these feed additives on quantitative and qualitative parameters of egg production and SE colonization. Therefore, two stages of the experiment were designed: under normal conditions and after the SE challenge. This made it possible to better understand the destructive effects of SE on laying hens and to better compare the prevention effects of prebiotic and probiotic with antibiotics on SE colonization.

Materials and Methods

Hens, husbandry, dietary treatments, and *Salmonella* challenge

A total of 150 laying hens (Hy-Line W-36) at 40 weeks old, and free of SE were housed in commercial battery cages, providing 645 cm^2 of floor space per hen. After four weeks of recording, the absence of *Salmonella* was confirmed using the method described by Gama *et al*. (2003), and 100 hens with similar body weight and laying rate (44 weeks old), in a randomized complete block design were divided into five treatment and four replication groups with five birds in each replication group for 13 weeks. Treatments included: negative control (NC), positive control (PC), antibiotic (ANT): 0.15 g oxyvet per kg of basal diet, probiotic (PRO): 0.1 g Bactocell® per kg of the basal diet (formulated with a specific live culture of lactic acid bacteria *Pediococcus acidilactici* MA185M; Lallemand Animal Nutrition SA, Blagnac, France), and prebiotic (PRE): 1.25 g Diamond V Original XPC™ per kg of basal diet [a common prebiotic-like compound, which includes post-fermentation growth medium residues, residual yeast cells, and yeast cell wall fragments (mannan-oligosaccharides and β-glucans); Cedar Rapids, IA, United States]. All three feed additives were added to the diet according to the manufacturer's recommendation. Mash feed was fed to meet the nutrient requirement of laying hens based on phase feeding (Table 1), light schedule (16L:8D, 30 lux), temperature (20-25°C), and environmental conditions in all experimental groups were applied according to the Hy-Line W-36 management guide (www.hyline.com).

The SE used in our study was obtained from the microbiology laboratory of the Department of Bacteriology, Faculty of Veterinary Medicine, University of Tehran (Tehran, Iran). The culture was prepared from an overnight culture previously transferred 3 times in Tetrathionate Broth Base with iodine-iodide solution (Merck, Darmstadt, Germany). Serially dilution in phosphate buffered saline (PBS) was used for preparing the challenge inoculum with a concentration of approximately 10^7 CFU/ml. The colony-forming units of the challenge inoculum were confirmed by plating on brilliant green agar with nalidixic acid (Dunkley *et al*., 2007). All experimental groups except negative control were challenged with 1 ml of suspension solution by oral gavage at the beginning of the ninth week of the experiment. Bacterial challenge concentration was determined based on our previous studies and Watarai (2005). Therefore, the experiment period divided into two stages: the pre-challenge stage for eight weeks, and

the post-challenge stage for five weeks.

Throughout the experiment period, all experimental groups had free access to water and feed. Birds were handled according to Hy-Line international welfare goals in the Poultry Research Center, Faculty of Agriculture, Tarbiat Modares University, Tehran, and good quality water and nutritionally balanced diets, comprehensive care, and handling procedures were prepared for them during the experiment period.

Table 1: Ingredients and composition of the basal diet of laying hens

Ingredients (%)	Weeks 40-48	Weeks 49-57
Corn	60.60	57.50
Soybean meal (44% protein)	21.50	22.50
Limestone	9.00	11.31
Corn gluten meal	2.75	2.00
Soybean oil	3.05	3.75
Di-calcium phosphate	1.60	1.45
Salt	0.25	0.25
$NaHCO_3$	0.15	0.15
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
DL-methionine	0.50	0.55
L-lysine	0.010	0.04
Contents by calculation		
ME (Kcal/kg)	2925	2875
Crude protein (%)	16.30	16.00
Calcium (%)	4.30	4.40
Sodium (%)	0.18	0.18
Available phosphorus (%)	0.47	0.44
Threonine (%)	0.63	0.61
Arginine (%)	0.89	0.85
Lysine (%)	0.84	0.81
Methionine + cysteine	0.78	0.75

[1, 2] Supplied the following per kg of diet: 9,000 IU of retinyl acetate, 2,000 IU of cholecalciferol, 12.5 IU of dl-α-tocopheryl acetate, 1.76 mg of menadione sodium bisulfite, 0.12 mg of biotin, 1.2 mg of thiamine, 3.2 mg of riboflavin, 6.4 mg of calcium d-pantothenate, 1.97 mg of pyridoxine, 28 mg of nicotinic acid, 0.01 mg of cyanocobalamine, 320 mg of choline chloride, 0.38 mg of folic acid, 60 mg of $MnSO_4.H_2O$, 80 mg of $FeSO_4.7H_2O$, 51.74 mg of ZnO, 8 mg of $CuSO_4.5H_2O$, 0.8 mg of iodized NaCl, and 0.2 mg of Na_2SeO_3

Performance and egg quality

During both experiment stages, egg production and egg weight were recorded daily, and feed intake was recorded at the end of each week by subtracting the feed residues weight from the amount of feed distribution. The percentage of egg production was calculated by dividing the number of eggs totalized by plot by the number of hens. Egg mass was calculated by multiplying the average egg weight by egg production percentage. Feed conversion ratio (FCR) was calculated by dividing the total feed consumed by the total egg mass.

Qualitative properties of eggs including shell thickness, shell strength, shell weight, Haugh unit, yolk weight, yolk color, and egg yolk cholesterol level were measured during the last week of each experimental stage. The egg yolks were weighed after separation from the albumen. Eggshells were weighed using the digital scale with ± 0.01 g error (Sartorius®, Germany) after being separated from the eggs, cleared, washed, and incubated for 24 h at room temperature. Shell thickness was gauged using a micrometer (Ultrasonic Thickness Gauge, Echometer 1062, ROBOTMATION Co., Ltd., Japan) at three points in the center of shells, and the average of the measured values was considered as the thicknesses of the shell. The shell strength was evaluated using the so-called Eggshell Force Gauge (Digital Egg Shell Force Gauge, model II, ROBOTMATION Co., Ltd., Japan). Haugh unit and yolk color were assessed using Egg Multi Tester (EM-5200, ROBOTMATION Co., Ltd., Japan) (Behnamifar *et al*., 2018).

For the determination of egg yolk cholesterol concentration, 1 g of pooled yolks of each replication was added to 9 ml of 2% NaCl solution and was shaken for 2 h. Then, 1 ml of the diluted yolk was re-diluted 10 times, and 10 µL of it was mixed with 100 µL of salt solution and 1 ml of the enzymatic reagent. The same procedure was also implemented for the standard of cholesterol. As the blank sample, 10 µL of deionized water was used instead of the sample or standard of cholesterol. The samples were incubated in a water bath at 37°C for 15 min and then the light absorbance was read at the wavelength of 500 nm (Pasin *et al*., 1998).

Egg yolk MDA as an indicator of lipid oxidation was determined by thiobarbituric acid reactive substances. For this purpose, the yolk sample was vortexed with trichloroacetic acid and 2.5 ml of butylene hydroxytoluene. After centrifugation, the hexane layer on the surface of the solution was discarded and the aqueous phase in the tubes was filtered with a Whatman paper No. 1. After volumizing with trichloroacetic acid, the tubes were put in a Ben Murray with 70°C for 30 min. After cooling, the amount of light absorption was measured by a spectrophotometer at 521 nm (Botsoglou *et al*., 1994).

Blood parameters

In the last week of the experiment, 1 ml blood was taken from 10 hens in each experimental unit through a wing vein to determine blood parameters. In the lab, blood samples were centrifuged at 4000 g (revolutions per min) for 5 min. Total protein, uric acid, glucose, cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels in blood serum samples were measured using commercial diagnostic kits (Zistshimi Co.) and a spectrophotometer (Jenway Genova MK3, UK) (Mohamadzade *et al*., 2020).

Bacterial population and SE colonization

To assess the microbial population, one bird of each replicate was killed at 7 days after challenge (Adhikari *et al*., 2017). The contents of ceca were collected in sterile dishes and were immediately transferred to the lab in an ice pack and prepared for microbial culture. One g of cecal contents was serially diluted and 10 µL of each dilution was spot on each plate containing plate count agar, MRS agar, and MacConkey agar media to count total aerobes, facultative anaerobic microbes (*Lactobacillus*), and *E. coli*, respectively. After

incubation for 24 h at 37°C, the bacteria were counted and the number of bacteria in the initial volume was determined in the colony-forming unit (CFU)/g (Mahmoud *et al*., 2014; Behnamifar *et al*., 2020).

Also in the post-challenge stage, cecal contents from one bird of each replicate were collected, pooled, weighed, enriched in Tetrathionate Broth Base with iodine-iodide solution (Merck, Darmstadt, Germany) and serially diluted (1:10, 100, 1000) with PBS. Twenty µL of each dilution were cultured in brilliant green agar with nalidixic acid (100 µg/ml) and incubated at 37°C for 24 h. Finally, the numbers of *Salmonella* colony-forming units were expressed as $\log_{10}$ *Salmonella* per gram of cecal content (de Barros Moreira Filho *et al*., 2015).

Statistical analysis

The data obtained through the experiment was analyzed using the general linear models (GLM) procedure in SAS 9.1 software and the significant difference in treatments was determined using Duncan's multiple range test (P=0.05).

Results

The feed additives had no effect on the quantitative performance of laying hens pre- and post-challenge, including egg production, egg weight, egg mass, feed intake, and FCR. Also, the challenge with SE in the PC group showed no significant effect on measured quantitative indices (Table 2).

The effects of dietary supplements on egg quality indicators before and after the challenge with SE are presented in Table 3. All egg quality indicators included shell thickness, shell strength, shell weight, Haugh unit, yolk color, relative yolk weight, yolk cholesterol level, and egg yolk MDA concentration were not affected by challenge with SE. Yolk cholesterol was the only qualitative indicator that was affected by the applied treatments (P<0.05). Probiotic in the pre-challenge stage, and prebiotic in the post-challenge stage showed a greater effect in the reduction of yolk cholesterol (P<0.05).

The effect of treatment on blood parameters after challenge with SE is presented in Table 4. All treatments showed no overall effect on total protein, uric acid, glucose, and HDL in hens' serum. Antibiotic, probiotic, and prebiotic decreased the LDL, triglyceride, and cholesterol levels of serum (P<0.05), but the effect of prebiotic was more than the others. Also, the challenge with SE had no effect on blood parameters (P>0.05).

All treatments showed no overall effect on the cecal bacterial population before the challenge with SE (Table 5). After the challenge, the PC group showed greater colonization of SE in the ceca (P<0.05). While the NC group was free of any *Salmonella* infection, applied treatments decreased the colonization of SE in the ceca of laying hens, and the prebiotic showed a more prominent effect (P<0.05). Also, bacteria, facultative anaerobic microbes (*Lactobacillus*), and *E. coli* population were not affected by all three feed additives after the challenge (P>0.05, Table 5).

Table 2: Effects of antibiotic, probiotic, and prebiotic on the performance of laying hens before/after challenge with *Salmonella enterica* serotype Enteritidis (SE)

Treatments	SE challenge									
	Egg production (%)		Egg weight (g)		Egg mass (g/hen/day)		Feed intake (g/hen/day)		FCR	
	before/after		before/after		before/after		before/after		before/after	
NC	91.97	88.92	62.39	64.04	57.55	54.82	94.05	87.99	1.60	1.58
PC	92.27	89.82	61.49	63.69	56.88	55.59	96.69	89.44	1.62	1.56
ANT	92.05	88.75	60.45	65.09	55.61	53.42	92.34	81.93	1.59	1.60
PRO	91.92	89.11	59.45	66.24	54.65	55.20	93.26	85.86	1.61	1.57
PRE	91.16	88.57	62.67	64.72	57.21	53.26	94.17	84.58	1.63	1.59
SEM	1.44	2.08	0.48	0.71	0.25	0.15	2.05	2.41	0.03	0.04
P-value	1.08	1.68	0.12	0.14	0.21	0.25	0.32	0.31	0.18	0.22

NC: Negative control, PC: Positive control, ANT: Antibiotic (0.15 g per kg of basal diet), PRO: Probiotic (0.1 g per kg of basal diet), and PRE: Prebiotic (1.25 g per kg of basal diet). FCR: Feed conversion ratio. SEM: Standard error of the mean

Table 3: Effect of antibiotic, probiotic, and prebiotic on egg quality of laying hens before/after challenge with *Salmonella enterica* serotype Enteritidis (SE)

Treatments	SE Challenge															
	Shell thickness (mm)		Shell strength (kg/cm^2)		Shell weight (g)		Haugh unit		Yolk color (DSM)		Relative yolk weight (%)		Yolk cholesterol (mg/g)		Egg yolk MDA (µg/g)	
	before/after		before/after		before/after		before/after		before/after		before/after		before/after		before/after	
NC	0.33	0.30	1.61	1.60	9.52	9.53	81.54	80.87	4.75	4.91	27.52	30.13	11.04^a	12.04^a	0.090	0.098
PC	0.32	0.30	1.58	1.32	9.42	923	80.87	82.24	4.58	4.89	28.13	28.16	10.88^a	11.99^a	0.092	0.092
ANT	0.31	0.32	1.63	1.33	9.39	9.33	82.24	81.93	4.82	4.85	27.60	28.74	10.01^{ab}	11.47^{ab}	0.089	0.088
PRO	0.32	0.29	1.32	1.72	9.46	9.30	79.35	83.57	4.74	4.92	25.59	29.85	9.11^b	11.22^{ab}	0.090	0.091
PRE	0.31	0.31	1.33	1.06	9.31	9.74	83.96	80.93	4.98	4.92	26.66	27.85	9.44^{ab}	10.98^b	0.093	0.087
SEM	0.01	0.006	0.21	0.19	0.22	0.13	2.40	2.31	0.14	0.15	0.46	0.51	0.19	0.24	0.003	0.002
P-value	0.17	0.15	0.99	0.18	0.65	0.20	0.09	0.88	0.73	0.11	0.08	0.09	0.02	0.03	0.110	0.091

NC: Negative control, PC: Positive control, ANT: Antibiotic (0.15 g per kg of basal diet), PRO: Probiotic (0.1 g per kg of basal diet), and PRE: Prebiotic (1.25 g per kg of basal diet). SEM: Standard error of the mean. Different superscript (^{a}, ^{b}) in column show significant difference P<0.05

Table 4: Effects of the antibiotic, probiotic, and prebiotic on the blood parameters of laying hens after challenge with *Salmonella enterica* serotype Enteritidis

Treatments	Total protein (g/dl)	Uric acid (mg/dl)	Glucose (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Cholesterol (mg/dl)
NC	5.62	6.01	164.02	1191.62^{a}	151.42^{a}	56.85	191.47^{a}
PC	5.60	6.22	166.93	1181.09^{a}	147.21^{a}	59.68	185.39^{a}
ANT	5.49	5.99	162.66	1135.07^{ab}	125.97^{ab}	54.90	173.61^{ab}
PRO	5.84	6.41	165.99	1142.63^{ab}	94.56^{b}	61.10	162.91^{b}
PRE	5.77	5.95	163.99	1069^{b}	86.79^{b}	63.84	145.41^{c}
SEM	0.05	0.13	14.33	148.26	52.9	6.23	84.18
P-value	0.51	0.07	0.81	0.02	0.01	0.07	0.02

NC: Negative control, PC: Positive control, ANT: Antibiotic (0.15 g per kg of basal diet), PRO: Probiotic (0.1 g per kg of basal diet), and PRE: Prebiotic (1.25 g per kg of basal diet). SEM: Standard error of the mean. Different superscript (^{a}, ^{b}, ^{c}) in column show significant difference P<0.05

Table 5: Effects of the antibiotic, probiotic, and prebiotic on cecal bacterial community in laying hens before/after challenge with *Salmonella enterica* serotype Enteritidis

Treatments	Total count of aerobic bacteria (log CFU/g)		Lactic acid bacteria (log CFU/g)		Coliforms (log CFU/g)		*Salmonella* spp. (log CFU/g)
	before/after		before/after		before/after		after
NC	7.65	7.20	7.48	7.57	6.18	6.60	0.00^{d}
PC	7.74	8.02	7.40	7.64	6.23	6.53	6.65^{a}
ANT	8.01	7.39	8.08	7.90	6.66	6.66	6.25^{b}
PRO	7.35	7.10	7.42	8.03	6.24	6.61	6.20^{b}
PRE	7.34	7.38	7.61	7.12	6.02	6.58	5.65^{c}
SEM	0.24	0.34	0.55	0.29	0.52	0.03	0.01
P-value	0.44	0.81	0.82	0.95	0.93	0.61	0.04

NC: Negative control, PC: Positive control, ANT: Antibiotic (0.15 g per kg of basal diet), PRO: Probiotic (0.1 g per kg of basal diet), and PRE: Prebiotic (1.25 g per kg of basal diet). SEM: Standard error of the mean. Different superscript (^{a}, ^{b}, ^{c}, ^{d}) in column show significant difference P<0.05

Discussion

As shown in Table 2, the production parameters were not affected with all three feed additives and challenge with SE. These results are coordinate with Ramasamy *et al*. (2009), Shang *et al*. (2010), and Mahfuz *et al*. (2018) which have reported that the inclusion of probiotic, prebiotic, and antibiotic had no significant effect on egg production parameters, respectively. Also, it has been reported that hen-day egg production and egg mass were not affected by the inclusion of prebiotics in the laying hens' diet (Martinez *et al*., 2018). It has been mentioned that a diet supplemented with probiotics did not affect feed intake and egg production of laying hens (Mikulski *et al*., 2012). Contrary to the results of the present study, a reduction in feed intake with a probiotic (*Saccharomyces cerevisiae*) was reported in broiler chicken due to an improvement in indigestibility of energy and amino acid (Kaushal *et al*., 2019).

Although a decrease in egg production and egg quality is expected during SE infection, there was no effect on the quantity and quality of eggs produced in the positive control group in the present study. Also, no mortality has been observed in laying hens after the challenge with SE. There are usually no clinical signs in birds infected with SE to inform farmers that eggs are contaminated (Sutherlin and Swerdlow, 1997). In general, SE is not very pathogenic in chickens and it is a silent infection in the bird. Also, infection of hens by SE has been reported to increase daily egg production in some experiments (Guard-Petter, 2001). Before and after the challenge with SE, egg quality indicator except for yolk cholesterol was not affected by the applied treatments which are in consent with the study done by Shalaei *et al*. (2014) who reported eggshell quality was not significantly affected by antibiotic, organic acid, probiotic, and prebiotic supplementation. In contrast to our results, Martinez *et al*. (2018) reported feeding dietary a prebiotic in laying hens increased yolk weight, percentage of yolk yield, and percentage of yolk solids. Also, it has been reported that egg weight, eggshell thickness, eggshell relative weight, and egg specific gravity were increased by the supplementation of a probiotic (Mikulski *et al*., 2012). The beneficial effect of probiotic and prebiotic feeding on egg eggshell quality probably related to a favorable environment in the intestinal tract that helps to assimilate more nutrients such as the increase in calcium retention (Panda *et al*., 2008). An induced acidic environment by lactic acid bacteria in the digestive tract improves the ionization of minerals and their absorption (Haddadin *et al*., 1996), which was not observed in the present study.

According to Table 5, cecal counts of *Lactobacillus* spp. were not affected by the applied treatments before and after the challenge with SE. In agreement with our results, Pineda-Quiroga *et al*. (2017) reported that probiotics did not change the cecal microbial population and *Lactobacillus* spp. cecal counts in laying hens. As

well as, it has been reported antibiotics (neomycin and oxytetracycline) and prebiotics (inulin) supplements had no effect on cecum *Lactobacilli*, *Enterococcus*, and *Salmonella* in broiler chicken (Kareem *et al*., 2017).

After the challenge, a reduction of cecal SE prevalence by applied treatment was a significant issue in the present study. Based on Table 5, feeding the dietary prebiotic has shown the best effect in this regard. In agreement with our results, Roto *et al*. (2017) reported that broilers receiving prebiotic exhibited a lower *Salmonella* prevalence in comparison with the control group. Also, a reduction in *Salmonella* fecal shedding of the broilers (Ohimain and Ofongo, 2012; Feye *et al*., 2016; Kimminau *et al*., 2021), and in the lung and air sac *Mycoplasma gallisepticum* lesion scores of the layer pullets (Elliott *et al*., 2020) has been reported by feeding prebiotic-supplement diets. Anaerobic incubation of the cecal contents of broiler chickens with a prebiotic reduces *Campylobacter* survival (Feye *et al*., 2020) and *S.* Typhimurium level (Rubinelli *et al*., 2016) in comparison to the control culture. Inhibitory activities of fructooligosaccharide on *S.* Enteritidis before or together with cecal microbiota inoculation have been reported in previous studies (Donalson *et al*., 2007; Rubinelli *et al*., 2016). Most of the bacteria in the cecum include species belonging to groups such as *Lactobacilli*, *Bifidobacterium*, *Propionibacterium*, and *Methanogens* that are capable of growing anaerobically and fermenting fructooligosaccharide. Enteric pathogens cannot use fructooligosaccharide as carbon source and it is nondigestible for them (Salanitro *et al*., 1974; Oyarzabal and Conner, 1995; Lu and Walker, 2001; Ricke *et al*., 2004; Donalson *et al*., 2007; Saengkerdsub *et al*., 2007). Therefore, prebiotics promote the coexistence and stability of the cecal microbial ecosystem and develop natural resistance to infections produced by intestinal pathogens. Al-Zenki *et al*. (2009) reported that probiotics reduced *Salmonella* contamination on the exterior body and in the ceca of broiler chickens at different ages. Probiotic is a feed additive based on live lactic acid bacteria (*Pediococcus acidilactici*). Bacterial interactions (competitive exclusion) and stimulation of a host's innate immune response are two mechanisms for the reduction of *Salmonella* by probiotics (Higgins *et al*., 2007). Also, competition for receptor sites, production of volatile fatty acids that are inhibitory of certain enteric pathogens, competition with pathogens and native flora for limiting nutrients, and production of bacteriocins are proposed methods for probiotics that are able to exclude enteric pathogens (Mead, 2000). Lactic acid bacteria produce soluble antimicrobial peptides (bacteriocins) that inhibit the growth of several pathogenic organisms from genera including *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Listeria*, *Clostridium*, and *Bacillus*; and non-pathogenic bacterium such as *Lactococcus* and *Pediococcus in vitro* condition (Bogovič-Matijašić *et al*., 1998; Ocaña *et al*., 1999).

Blood lipid levels and egg yolk cholesterol were affected by applied treatments. These findings were in disagreement with the results of Mohebbifar *et al*. (2013) who observed using probiotics and prebiotics had no significant effects on the blood parameters and egg cholesterol. Similarly, as in this experiment, Mikulski *et al*. (2012) and Tang *et al*. (2017) reported that dietary probiotic and prebiotic (isomaltooligosaccharide, IMO) supplementation decreased egg yolk cholesterol concentrations by more than 10% compared with the control group; and the serum total cholesterol and serum LDL cholesterol, respectively. The differences between results can be attributed to the strain of bacteria, concentration and the form of bacteria used (viability, dryness, or their products) and differences in the ages of hens (Mohebbifar *et al*., 2013). Laying hens eliminate remarkable amounts of cholesterol in the egg and egg cholesterol originates from serum cholesterol (Andrews Jr *et al*., 1968). Fermentation of prebiotics by gut microbiota produces short-chain fatty acids, and prebiotics can be a good substrate for the growth of probiotic microbes (Rahminiwati *et al*., 2014). Short-chain fatty acids are able to suppress hepatic cholesterol synthesis and stimulate bile acid synthesis. Also, some of the microorganisms present in the probiotic preparation could precipitate the cholesterol with deconjugated bile salts and assimilate the cholesterol present in the gastrointestinal tract for their own cellular metabolism (Kurtoglu *et al*., 2004; Tang *et al*., 2015).

Egg-based products are the main source for the spread of foodborne outbreaks of *Salmonella* infection. Since infection of laying hens with SE is not usually associated with clinical symptoms, according to the results of the present study, it seems that probiotics and especially prebiotics can be effective in reducing colonization of SE in the ceca of laying hens. It is necessary to mention that host-related factors, such as age, production system, breed, sex, feed, and rearing conditions have important effects on the development and composition of gut microbiota (Khan *et al*., 2020), and the efficiency of feed additives can be affected by these factors.

It appears that inflamed epithelial cells have adhesion receptor sites that are exploited only by pathogenic bacteria (Khan *et al*., 2020). Since the gut microbiota constitutes after the chicken's hatch, so it seems that an earlier introduction to non-pathogenic microorganisms can enhance the digestive tract (Jha *et al*., 2020). Using probiotics and prebiotics in newly hatched chicks/pullets may have better effects on improving performance, egg quality, and colonization resistance against intestinal pathogens especially SE, which is suggested in future studies. Also, it is possible that a combination of probiotic and prebiotic was the more effective treatment in decreasing the SE colonization in the cecum and improving production parameters in infected herds. Therefore, the combined use of these feed additives for the control of SE is recommended in future studies.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Adhikari, P; Cosby, DE; Cox, NA and Kim, WK (2017). Effect of dietary supplementation of nitrocompounds on *Salmonella* colonization and ileal immune gene expression in laying hens challenged with *Salmonella* Enteritidis. Poult. Sci., 96: 4280-4286.

Al-Zenki, SF; Al-Nasser, AY; Al-Saffar, AE; Abdullah, FK; Al-Bahouh, ME; Al-Haddad, AS; Alomirah, H and Mashaly, M (2009). Effects of using a chicken-origin competitive exclusion culture and probiotic cultures on reducing *Salmonella* in broilers. J. Appl. Poult. Res., 18: 23-29.

Amalaradjou, MAR and Bhunia, AK (2012). Modern approaches in probiotics research to control foodborne pathogens. Adv. Food Nutr. Res., 67: 185-239.

Andrews Jr, JW; Wagstaff, RK and Edwards Jr, HM (1968). Cholesterol metabolism in the laying fowl. Am. J. Physiol., 214: 1078-1083.

Asadi, B; Fakhraei, J; Hosseini, A; Yarahamdi, HM and Aghashahi, A (2018). Dietary inclusion of commercial toxin binders and probiotics alleviate adverse effects of aflatoxin on growth performance, immune responses, and blood biochemical parameters of broiler chicks. Eur. Poult. Sci., 82: 1-13.

Bajagai, YS; Alsemgeest, J; Moore, RJ; Van, TT and Stanley, D (2020). Phytogenic products, used as alternatives to antibiotic growth promoters, modify the intestinal microbiota derived from a range of production systems: an *in vitro* model. Appl. Microbiol. Biotechnol., 104: 10631-10640.

Behnamifar, A; Rahimi, S; Akhavizadegan, MA; Karimi Torshizi, MA and Maleki, A (2020). Isolation and Identification of Microorganisms in Eggs of a Commercial Ostrich Breeder Farm. J. Anim. Sci. Res., 4: 16-22.

Behnamifar, A; Rahimi, S; Karimi Torshizi, MA and Mohammad Zade, Z (2018). Effect of chamomile, wild mint and oregano herbal extracts on quality and quantity of eggs, hatchability, and some other parameters in laying Japanese quails. JMPB., 7: 173-180.

Behnamifar, AR; Rahimi, S; Kiaei, MM and Fayazi, H (2019). Comparison of the effect of probiotic, prebiotic, salinomycin and vaccine in control of coccidiosis in broiler chickens. Iran. J. Vet. Res., 20: 51-54.

Bogovič-Matijašić, B; Rogelj, I; Nes, IF and Holo, H (1998). Isolation and characterization of two bacteriocins of *Lactobacillus acidophilus* LF221. Appl. Microbiol. Biotechnol., 49: 606-612.

Botsoglou, NA; Fletouris, DJ; Papageorgiou, GE; Vassilopoulos, VN; Mantis, AJ and Trakatellis, AG (1994). Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. J. Agric. Food Chem., 42: 1931-1937.

Davani-Davari, D; Negahdaripour, M; Karimzadeh, I; Seifan, M; Mohkam, M; Masoumi, SJ; Berenjian, A and Ghasemi, Y (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods. 8: 92-119.

de Barros Moreira Filho, AL; de Oliveira, CJB; de Oliveira, HB; Campos, DB; Guerra, RR; Costa, FGP and Givisiez, PEN (2015). High incubation temperature and threonine dietary level improve ileum response against post-hatch *Salmonella* Enteritidis inoculation in broiler chicks. PLoS One. 10: 0131474-0131187.

Dhama, K; Mahendran, M and Tomar, S (2007). Probiotics and prebiotics: A safer way towards improving health and productivity in poultry. Poult. World. 2: 28-32.

Donalson, LM; Kim, WK; Chalova, VI; Herrera, P; Woodward, CL; McReynolds, JL; Kubena, LF; Nisbet, DJ and Ricke, SC (2007). *In vitro* anaerobic incubation of *Salmonella enterica* serotype Typhimurium and laying hen cecal bacteria in poultry feed substrates and a fructooligosaccharide prebiotic. Anaerobe. 13: 208-214.

Dunkley, KD; McReynolds, JL; Hume, ME; Dunkley, CS; Callaway, TR; Kubena, LF; Nisbet, DG and Ricke, SC (2007). Molting in *Salmonella* Enteritidis challenged laying hens fed alfalfa crumbles. I. *Salmonella* Enteritidis colonization and virulence gene hilA response. Poult. Sci., 86: 1633-1639.

Elliott, KEC; Branton, SL; Evans, JD; Leigh, SA; Kim, EJ; Olanrewaju, HA; Pharr, GT; Pavlidis, HO; Gerard, PD and Peebles, ED (2020). Growth and humoral immune effects of dietary Original XPC in layer pullets challenged with *Mycoplasma gallisepticum*. Poult. Sci., 99: 3030-3037.

Feye, KM; Anderson, KL; Scott, MF; McIntyre, DR and Carlson, SA (2016). Inhibition of the virulence, antibiotic resistance, and fecal shedding of multiple antibiotic-resistant *Salmonella* Typhimurium in broilers fed Original XPC™. Poult. Sci., 95: 2902-2910.

Feye, KM; Rubinelli, PM; Chaney, WE; Pavlidis, HO; Kogut, MH and Ricke, SC (2020). The preliminary development of an *in vitro* poultry cecal culture model to evaluate the effects of Original XPCTM for the reduction of *Campylobacter jejuni* and its potential effects on the microbiota. Front. Microbiol., 10: 3062-3076.

Gama, NMSQ; Berchieri Jr, A and Fernandes, SA (2003). Occurrence of *Salmonella* sp in laying hens. Braz. J. Poult. Sci., 5: 15-21.

Gantois, I; Ducatelle, R; Pasmans, F; Haesebrouck, F; Gast, R; Humphrey, TJ and Van Immerseel, F (2009). Mechanisms of egg contamination by *Salmonella* Enteritidis. FEMS Microbiol. Rev., 33: 718-738.

Guard-Petter, J (2001). The chicken, the egg and *Salmonella* Enteritidis. Environ. Microbiol., 3: 421-430.

Haddadin, MSY; Abdulrahim, SM; Hashlamoun, EAR and Robinson, RK (1996). The effect of *Lactobacillus acidophilus* on the production and chemical composition of hen's eggs. Poult. Sci., 75: 491-494.

Higgins, JP; Higgins, SE; Vicente, JL; Wolfenden, AD; Tellez, G and Hargis, B (2007). Temporal effects of lactic acid bacteria probiotic culture on *Salmonella* in neonatal broilers. Poult. Sci., 86: 1662-1666.

Jha, R; Das, R; Oak, S and Mishra, P (2020). Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. Animals. 10: 1863-1381.

Kareem, KY; Loh, TC; Foo, HL; Asmara, SA and Akit, H (2017). Influence of postbiotic RG14 and inulin combination on cecal microbiota, organic acid concentration, and cytokine expression in broiler chickens. Poult. Sci., 96: 966-975.

Kaushal, S; Sharma, RK; Singh, DV; Shukla, SK; Kumar, S; Palod, J and Singh, MK (2019). Performance, carcass characteristics and economics of broiler chickens fed dietary enzymes and probiotic. Iran. J. Vet. Res., 20: 293-298.

Khan, S; Moore, RJ; Stanley, D and Chousalkar, KK (2020). The gut microbiota of laying hens and its manipulation with prebiotics and probiotics to enhance gut health and food safety. Appl. Environ. Microbiol., 86: 1-18.

Kimminau, EA; Karnezos, TP; Berghaus, RD; Jones, MK; Baxter, JA and Hofacre, CL (2021). Combination of probiotic and prebiotic impacts *Salmonella* Enteritidis infection in layer hens. J. Appl. Poult. Res., 30: 100200-100208.

Kurtoglu, V; Kurtoglu, F; Seker, E; Coskun, B; Balevi, T and Polat, ES (2004). Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. Food Addit. Contam., 21: 817-823.

Landers, TF; Cohen, B; Wittum, TE and Larson, EL (2012). A review of antibiotic use in food animals: perspective, policy, and potential. Pub. Health Rep., 127: 4-22.

Lu, L and Walker, WA (2001). Pathologic and physiologic interactions of bacteria with the gastrointestinal epithelium. Am. J. Clin. Nutr., 73: 1124S-1130S.

Mahfuz, S; Song, H; Wei, J; Chen, M; Zhen, D; Nahar, J and Liu, Z (2018). Organic egg production, egg quality, calcium utilization, and digestibility in laying hens fed with mushroom (*Flammulina velutipes*) stem waste. Braz. J. Poult. Sci., 20: 717-724.

Mahmoud, MA; Abdel-Mohsein, HS and Mahmoud, UT (2014). Effect of Chinese propolis supplementation on Ross broiler chicks: microbial population in fecal matter and litter. J. Adv. Vet. Res., 4: 77-84.

Majd, NE; Mayahi, M and Moghadam, AS (2014). The effect of alphamune and biomin on histomorphological structure of small intestine and caecal tonsil lymphoid tissue in broiler chicken. Iran. J. Vet. Res., 15: 30-35.

Makarova, K; Slesarev, A; Wolf, Y; Sorokin, A; Mirkin, B; Koonin, E; Pavlov, E; Pavlova, N; Karamychev, V; Polouchine, N and Mills, D (2006). Comparative genomics of the lactic acid bacteria. Proc. Natl. Acad. Sci., 103: 15611-15616.

Marshall, BM and Levy, SB (2011). Food animals and antimicrobials: impacts on human health. Clin. Microbiol. Rev., 24: 718-733.

Martinez, JS; Blount, RL; Park, J; McIntyre, DR; Pavlidis, HO and Carey, JB (2018). Effects of feeding original XPCTM to laying hens on egg production, component yield and composition. J. Appl. Poult. Res., 27: 603-608.

Mead, GC (2000). Prospects for 'competitive exclusion' treatment to control *Salmonellas* and other foodborne pathogens in poultry. Vet. J., 159: 111-123.

Mikulski, D; Jankowski, J; Naczmanski, J; Mikulska, M and Demey, V (2012). Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poult. Sci., 91: 2691-2700.

Mohamadzade, Z; Rahimi, S; Karimi Torshizi, MA and Behnamifar, A (2020). Effect of Prebiotic and Chamomile, Oregano, and Marjoram Extracts on Laying Performance, Blood Parameters, and Intestinal Histomorphology in Laying Hens in the Late Phase of Production. Anim. Prod., 22: 583-593.

Mohebbifar, A; Kashani, S; Afsari, M and Torki, M (2013). Effects of commercial prebiotic and probiotics of diet on performance of laying hens, egg traits and some blood parameters. Annu. Res. Rev. Biol., 921-934.

Mookiah, S; Sieo, CC; Ramasamy, K; Abdullah, N and Ho, YW (2014). Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J. Sci. Food Agric., 94: 341-348.

Ocaña, VS; de Ruiz Holgado, AAP and Nader-Macías, ME (1999). Characterization of a bacteriocin-like substance produced by a vaginal *Lactobacillus salivarius* strain. Appl. Environ. Microbiol., 65: 5631-5635.

Ohimain, EI and Ofongo, RT (2012). The effect of probiotic and prebiotic feed supplementation on chicken health and gut microflora. J. Anim. Vet. Adv., 4: 135-143.

Oyarzabal, OA and Conner, DE (1995). *In vitro* fructooligosaccharide utilization and inhibition of *Salmonella* spp. by selected bacteria. Poult. Sci., 74: 1418-1425.

Panda, AK; Rama Rao, SS; Raju, MV and Sharma, SS (2008). Effect of probiotic (*Lactobacillus sporogenes*) feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders. J. Sci. Food Agric., 88: 43-47.

Pasin, G; Smith, GM and O'mahony, M (1998). Rapid determination of total cholesterol in egg yolk using commercial diagnostic cholesterol reagent. Food Chem., 61: 255-259.

Pineda-Quiroga, C; Atxaerandio, R; Zubiria, I; Gonzalez-Pozuelo, I; Hurtado, A; Ruiz, R and Garcia-Rodriguez, A (2017). Productive performance and cecal microbial counts of floor housed laying hens supplemented with dry whey powder alone or combined with *Pediococcus acidilactici* in the late phase of production. Livest. Sci., 195: 9-12.

Rahminiwati, M; Rahmatullah, S; Batubara, I and Achmadi, SS (2014). Potency of turmeric rhizome extract as prebiotic agent for *Lactobacillus plantarum* growth promoter *in vitro*. JIFI., 12: 37-42.

Ramasamy, K; Abdullah, N; Jalaludin, S; Wong, M and Ho, YW (2009). Effects of *Lactobacillus* cultures on performance of laying hens, and total cholesterol, lipid and fatty acid composition of egg yolk. J. Sci. Food Agric., 89: 482-486.

Ramlucken, U; Ramchuran, SO; Moonsamy, G; Lalloo, R; Thantsha, MS and van Rensburg, CJ (2020). A novel *Bacillus* based multi-strain probiotic improves growth performance and intestinal properties of *Clostridium perfringens* challenged broilers. Poult. Sci., 99: 331-341.

Ribeiro, AML; Vogt, LK; Canal, CW; Cardoso, MRDI; Labres, RV; Streck, AF and Bessa, MC (2007). Effects of prebiotics and probiotics on the colonization and immune response of broiler chickens challenged with *Salmonella* Enteritidis. Braz. J. Poult. Sci., 9: 193-200.

Ricke, SC; Woodward, CL; Kwon, YM; Kubena, LF and Nisbet, DJ (2004). Limiting avian gastrointestinal tract *Salmonella* colonization by cecal anaerobic bacteria, and a potential role for methanogens. In: Beier, RC; Pillai, SD and Phillips, TD (Eds.), *Preharvest and postharvest food safety: contemporary issues and future directions*. (1st Edn.), Ames, IA: Blackwell Publishing Professional. PP: 141-150.

Roto, SM; Park, SH; Lee, SI; Kaldhone, P; Pavlidis, HO; Frankenbach, SB; McIntyre, DR; Striplin, K; Brammer, L and Ricke, SC (2017). Effects of feeding Original XPC™ to broilers with a live coccidiosis-vaccine under industry conditions: Part 1. Growth performance and *Salmonella* inhibition. Poult. Sci., 96: 1831-1837.

Rubinelli, P; Roto, S; Kim, S; Park, SH; Pavlidis, HO; McIntyre, D and Ricke, SC (2016). Reduction of *Salmonella* Typhimurium by fermentation metabolites of Diamond V Original XPC in an *in vitro* anaerobic mixed chicken cecal culture. Front. Vet. Sci., 3: 83-90.

Saengkerdsub, S; Anderson, RC; Wilkinson, HH; Kim, WK; Nisbet, DJ and Ricke, SC (2007). Identification and quantification of methanogenic archaea in adult chicken ceca. Appl. Environ. Microbiol., 73: 353-356.

Salanitro, JP; Blake, IG and Muirhead, PA (1974). Studies on the cecal microflora of commercial broiler chickens. Appl. Microbiol., 28: 439-447.

Shalaei, M; Hosseini, SM and Zergani, E (2014). Effect of different supplements on eggshell quality, some characteristics of gastrointestinal tract and performance of laying hens. Vet. Res. Forum., 5: 277-286.

Shang, HM; Hu, TM; Lu, YJ and Wu, HX (2010). Effects of inulin on performance, egg quality, gut microflora and serum and yolk cholesterol in laying hens. Br. Poult. Sci., 51: 791-796.

Staji, H; Ghazvinian, K; Javaheri Vayeghan, A; Salimi, MR and Mahdavi, A (2012). Prevalence of *Salmonella* spp. in the quail egg interior contents: A provincial study. Iran. J. Vet. Med., 6: 191-196.

Sutherlin, W and Swerdlow, D (1997). Epidemiology and control of egg-associated *Salmonella* Enteritidis in the United States of America. Rev. Sci. Tech. Off. Int. Epiz., 16: 542-553.

Tang, SGH; Sieo, CC; Kalavathy, R; Saad, WZ; Yong, ST; Wong, HK and Ho, YW (2015). Chemical compositions of egg yolks and egg quality of laying hens fed prebiotic, probiotic, and synbiotic diets. J. Food Sci., 80: 1686-1695.

Tang, SGH; Sieo, CC; Ramasamy, K; Saad, WZ; Wong, HK and Ho, YW (2017). Performance, biochemical and haematological responses, and relative organ weights of laying hens fed diets supplemented with prebiotic, probiotic and synbiotic. BMC Vet. Res., 13: 1-12.

Watarai, S (2005). Eliminating the carriage of *Salmonella enterica* serovar Enteritidis in domestic fowls by feeding activated charcoal from bark containing wood vinegar liquid (Nekka-Rich). Poult. Sci., 84: 515-521.

Youssef, IM; Mostafa, AS and Abdel-Wahab, MA (2017). Effects of dietary inclusion of probiotics and organic acids on performance, intestinal microbiology, serum biochemistry and carcass traits of broiler chickens. J. World Poult. Res., 7: 57-71.