Original Article

Effect of Dietary Prebiotic Supplementation on the Immune Response and Intestinal Health Following Eimeria Infection of Broiler Chicken

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ABSTRACT

Background: Antibiotics are preferred drugs for controlling coccidiosis. However, prolonged use of ionophores will result in *Eimeria* resistance to these drugs.

Objectives: The present work was conducted to evaluate the possible substitution of prebiotic (nutri yeast [NY]) for antibiotics in mild-challenged broilers with *Eimeria*.

Methods: A total of 420 1-d-old male Ross 308 chicks were used in a completely randomized design with 7 treatments and 5 replicates. Experimental treatments included: 1) Negative control (NC) (without prebiotic and challenge); 2) Positive control (PC) (without prebiotic and challenged with sporulated oocysts of *Eimeria* [SO]); 3) 0.2% NY in starter, 0.1% in grower, 0.05 % in finisher, challenged with SO; 4) 0.2 % NY in starter, 0.1 % in grower, 0.05 % NY in finisher, without challenge; 5) 0.2% NY in the whole rearing period of chicks challenged with SO; 6) 0.2% NY in the whole rearing period of chicks without challenge; and 7) Salinomycin (0.05 % of diet). At 7 d of age, treatments 2, 3 and 5 were challenged with a 20-fold dose of *Eimeria* vaccine via oral gavage. Antibody levels against sheep red blood cell (SRBC) were measured at 31 and 41 days of age. On days 28 and 42, two birds per replicate were slaughtered to collect ileal digesta for microbial analysis. Samples were collected for blood metabolite parameters, carcass traits and visceral organ weight, intestinal morphology, and interleukin 6 (*IL-6*) gene expression on day 42.

Results: The results showed that NY supplementation increased the concentration of serum total protein (3.10 vs 2.57 g/dL) and decreased serum triglycerides (50.6 vs 57.3 mg/dL) and cholesterol (108.6 vs 133.9 mg/dL) levels compared to NC group (P<0.05). Serum antibody titers against Newcastle were higher in prebiotic treatments compared to control groups, and for the secondary immune response against SRBC broilers fed NY diet had the highest levels of total Ig (8.4 vs 6.4) and IgG (5.6 vs 4.2) compared to the control group (P<0.05). The inclusion of NY improved intestinal pH and the relative weights of immune organs, breast muscle, and small intestine compared to the NC treatment (P<0.05). Villus height (806.6

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vs 578.7 µm) and numbers of lactobacillus (8.77 vs 8.29 CFU/g) increased, while crypt depth (112.8 vs 144.9 µm) and numbers of coliforms (6.19 vs 6.61 CFU/g) decreased in broilers fed a diet containing NY compared to the NC group (P<0.05). Dietary supplementation of NY decreased *IL-6* gene expression in challenged and unchallenged birds compared to the control group (P<0.05).

Conclusion: The results of the current study confirmed our hypothesis that the use of prebiotic (NY) has protective activities against coccidiosis in broiler chicks.

Keywords: *Eimeria*, Immune response, Intestinal health, Prebiotic

Introduction

Coccidiosis is an intestinal disease caused by several distinct species of *Eimeria* parasites that damage the host's intestinal system. Parasitism of the intestinal tract is a major stress factor, and these parasitic infections can cause a wide range of harm to the infected host, resulting in poor nutrition absorption, reduced performance, diarrhea, abortion, and even death of severely infected animals (Alagbe et al., 2023). It has been estimated that coccidiosis causes an annual economic loss of 3 billion US dollars in the poultry industry (Teng et al., 2020).

Control of this disease is based essentially on chemoprevention using antibiotics or coccidiostats. Antibiotics are effective in increasing disease resistance in the modern poultry industry and eliminating antibiotics during the production cycle may cause negative effects on the conversion rate of diets (Salois et al., 2016). The broader use of these substances has contributed to the development of resistant bacteria, which can infuse into the soil, where they can survive and contaminate the environment (Mazhar et al., 2021). They are a potential risk to human health, so the European Union banned antibiotics as growth promoters in animal feed in 2006. Unfortunately, this ban led to many problems in production, such as increased feed conversion ratio and animal diseases (Leone & Ferrante, 2023). Thus, feed additives are antibiotic alternatives to control diseases and promote nutrient utilization (Barberis et al., 2015).

Several alternative strategies have proven their effectiveness in coccidiosis control with potential stimulatory effects on performance and immunity (Leone & Ferrante, 2023). They are mainly based on preserving the integrity of the intestinal barrier and stimulating the immune response (Kiarie et al., 2019). In recent years, prebiotics have been considered potential alternative antibiotics (Teng & Woo, 2018). In some studies, the inhibitory effects of prebiotics against *Eimeria* infection in poultry have been reported (Angwech et al., 2019; Elmusharaf et al., 2007). Yeast cultures as prebiotics were introduced into animal feed as an alternative approach to feed supplements after antibiotics were banned (Adhikari et al., 2018). Prebiotics are suggested to influence these aspects and comprehensively regulate the interaction between the host and the intestinal microbiota (Teng & Woo, 2018). Therefore, it was hypothesized that prebiotic supplementation would improve the immune system of broiler chickens challenged with *Eimeria* and modulate specific populations of bacteria in the gut. The prebiotic used in this study (nutri yeast [NY]) is a product of *Saccharomyces cerevisiae* yeast autolysis, which contains extract components and yeast cell wall (YCW). Its effective components include nucleotides, mannan oligosaccharides, beta-glucans, essential amino acids, and peptides.

Interleukin (IL)-6 is a multifunctional cytokine that plays a vital role in many acute-phase reactions, autoimmune diseases, and hematopoietic mechanisms, particularly inflammatory bowel disease in broilers (Yu et al., 2019). Swaggerty et al. (2015) reported that selection for the pro-inflammatory mediators, including *IL-6*, produces chickens more resistant to *Eimeria*. This study aimed to investigate the effects of prebiotic NY supplementation on the intestinal morphology, gut microbiome, immunological response, blood parameters, carcass characteristics and *IL-6* gene expression following *Eimeria* infection of broiler chickens.

Materials and Methods

Birds, diets and management

A total of 420 1-d-old male Ross 308 broiler chicks, with an average body weight of 46.8±0.8 g, were used in this study. The chicks were distributed into 35 homogenous groups of floor pens (1×1 m) according to their initial weight. They were allocated to a completely randomized design experiment with 7 treatments and 5 pen replicates

(12 birds per pen). All chicks were vaccinated based on a routine program. Diets were formulated according to the Ross 308 nutrition specification booklet (2019). Feed and freshwater were offered ad libitum throughout the experiment. Table 1 describes the diet ingredients and nutrient contents of the basal diets. Experimental treatments included: 1) Negative control (NC; unchallenged), 2) Positive control (PC); challenged with sporulated oocysts of *Eimeria* (SO), 3) Basal diet+0.2 % NY (Persian Kimiazyme Co., Tehran, Iran) in starter, 0.1 % NY in grower and 0.05% NY in finisher challenged with SO (NYC0.2S%0.1%G0.05%F), 4) Basal diet+0.2% NY in starter, 0.1% NY in grower and 0.05% NY in finisher without challenge (NY0.2S%0.1%G0.05%F), 5) Basal diet+0.2% NY in the whole breeding period with SO(NY0.2%WPC), 6) Basal diet+0.2% NY in the whole breeding period without challenge (NY0.2%WP) and 7) Basal diet+salinomycin (0.05 % of diet). All the dietary

Table 1. Composition and calculated nutrient composition of the basal diets

Items		Starter (d 0–10)	Grower (d 11–24)	Finisher (d 25–42)
Ingredients (%)	Corn	58.32	59.75	63
	Soybean meal (CP: 44%)	31.93	33.84	29
	Corn gluten meal	4	0	0
	Soybean oil	0.6	1.94	3.1
	Dicalcium phosphate	1.92	1.55	1.41
	Calcium carbonate	1.13	1.03	0.95
	Sodium bicarbonate	0.15	0.15	0.15
	Common salt	0.23	0.24	0.24
	Mineral and vitamin premix*	0.5	0.5	0.5
	DL-Methionine	0.26	0.27	0.27
	L-Lysine	0.4	0.22	0.22
	L-Threonine	0.16	0.1	0.06
	Choline	0.05	0.05	0.05
	Filler and prebiotic	0.4	0.41	1.05
	Total	100	100	100
Nutrient composition	Metabolizable energy (kcal/kg)	2900	2950	3050
	Crude protein (%)	22.24	20.64	18.85
	Lysine (%)	1.37	1.24	1.12
	Methionine+cysteine (%)	1.04	0.95	0.88
	Threonine (%)	0.95	0.85	0.74
	Calcium (%)	0.94	0.83	0.76
	Available phosphorus (%)	0.48	0.41	0.38
	Sodium (%)	0.16	0.16	0.16

*Supplied per kg diet: Vitamin A: 11000 U; vitamin D3: 5000 U; vitamin E: 36.75 U; vitamin K3: 3.4 mg; vitamin B1: 1.98 mg; vitamin B2: 5.25 mg; pantothenic acid: 10.5 mg; niacin: 31.5 mg; vitamin B6: 2.87 mg; folic acid: 1.2 mg; vitamin B12: 0.024 mg; biotin: 0.105 mg; choline: 800 mg; manganese: 120 mg; zinc: 100 mg; iron: 50 mg; copper: 12 mg; I: 1.3 mg; selenium: 0.3 mg; antioxidant: 100 mg.

treatments were fed continuously for 42 days from 1 day old. At 7 days of age, treatments 2, 3 and 5 were challenged with 20-fold doses of the EIMERIAVAX 4m (Bioproperties Pty Ltd Co., Ringwood, Australian) via oral gavage with 0.5 mL. This vaccine contains viable oocysts of *Eimeria acervulina*, *Eimeria maxima*, *Eimeria necatrix*, and *Eimeria tenella* suspended in phosphate-buffered saline (PBS). According to the product catalog of the manufacturer's company, each dose comprises a minimum of *E. acervulina* 50 oocysts, *E. maxima* 100 oocysts, *E. necatrix* 100 oocysts and *E. tenella* 150 oocysts, with a minimum predicted titer of 1.6×10^4 oocysts per mL at the end of the shelf-life.

Collection of samples

At 31 and 41 days of age, two birds per pen were selected for blood collection. At 28 and 42 days of age, two birds were slaughtered humanely by knife at the agricultural experiment station of Guilan University to collect ileal digesta for microbial analysis.

Two birds per pen (at 42 days of age) were randomly selected to assess intestinal morphology. Their ileum was collected in cryotubes, immediately stored in liquid nitrogen, and then transferred to a freezer for storage at -70 ˚C

Humoral immune response

Chicks were immunized by intramuscular injection of 0.1 mL of 25% sheep red blood cell (SRBC) in PBS on days 20 and 34 to assess the systemic antibody response. Blood samples were collected from two birds of each replicate via the wing vein on days 31 and 41 of age. After segregating serum by centrifugation at 3000×g for 15 minutes, sera were decanted and frozen (-80 ˚C) until serological examination. Antibody titers for SRBC were determined by micro hemagglutination. Samples were incubated at 56 ˚C for 30 min to inactivate the complement. The titers of IgG were determined by incubating the serum with an equal volume (50 µL) of 1.4%, 2-mercaptoethanol (2-ME; Sigma, St, Louis, MO, USA) in PBS at 37 ˚C for 30 min before hemagglutination test. The 2-mercaptoethanol-sensitive antibody titers (IgM) were determined by subtracting the 2-ME-resistant antibody titer (total Ig minus IgG titers). The antibody titers were expressed as $\log_2$ of the highest dilution of serum that agglutinated an equal volume of 0.5% red blood cells. Newcastle disease vaccine was administered in drinking water at 8 (V4 strain), 16 and 24 days (LaSota strain) of age for all groups. On day 41, two chickens from each pen were randomly selected and blood samples were collected into 5-mL vacuum tubes. The sera were stored at -20 ˚C until analysis. Antibody response was measured by the hemagglutination inhibition (HI) technique, according to Hassanpour et al. (2013). Briefly, 25 µL of serum-containing antibody was serially diluted into a 96-well plate with PBS (pH 7.4, 4 ˚C). The same volume of Newcastle disease virus (NDV) antigen (4 HA unit) was added to react and bind with the antibody for the HI test.

The addition of 2% red blood cell solution in each well should show the ability of NDV left to agglutinate with red blood cells. If enough antibodies were bound to the virus during incubation, hemagglutination would be completely inhibited. The titers were expressed as $\log_2$ of the reciprocal of the highest serum dilution, showing HI (Salehimanesh et al., 2016).

Intestinal morphology

Two birds per pen were selected to obtain small intestine tissue to measure villus height and crypt depth. Fragments of approximately 5 cm in length were obtained from the ileum between the Meckel diverticulum and the anterior portion of the ileocecal junction. The excised fragments were immersed in a phosphate-buffered formalin solution. Two portions per sample were cut perpendicular to the longitudinal axis of the intestine and embedded in paraffin wax. Transverse sections were cut (3~5 µm). The morphometric study captured images using a light microscope and a system that analyzes computerized images (Bio-Rad Microscience, UK). Villus height and crypt depth (µm) were measured using an image-analysis system under a light microscope according to the Eftekhari et al. (2015) method. The villus height: crypt depth ratio was then calculated.

Bacterial enumeration of the ileal digesta

On days 28 and 42, two birds per pen were randomly selected, weighed, and slaughtered. Samples of the proximal ileum contents were collected and stored in 15 mL tubes and kept under 4 ˚C for 24 h until analysis. One gram of sample was used and submitted to serial 10-fold dilutions with saline solution (0.85%). After preparing different dilutions, each sample was inoculated on MRS agar and MacConkey agar medium at 37 ˚C for 24 or 48 h. *Lactobacillus* and *coliform* colonies were counted after finishing the incubation period. The concentration of microflora was finally expressed as log10 colony-forming units per gram of intestinal content (Wu et al., 2019).

Gastrointestinal tract pH measurement

At 28 and 42 days of age, the crop, ileum, and cecal pH were measured using a digital pH meter (HI99161, Hanna, Villafranca Padovana, Italy) after mixing 1 g of digesta of each gastrointestinal tract segment with 3 mL of distilled water according to the method of Moss et al. (2018). Each sample was measured 3 times.

Serum biochemistry

At 42 days of age, 4 mL of blood was collected from the wing veins of 10 birds in each treatment to measure blood metabolite parameters. The collected blood samples were centrifuged for 10 min at 3000 rpm, and the serum was separated and then stored at -20 ˚C until assayed to measure serum biochemical analysis. Serum levels of triglyceride, cholesterol, albumin, total protein (TP), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were measured by spectrophotometer using commercial test kits (Pars Azmoon kit, Pars Azmoon Inc., Tehran, Iran) according to the manufacturer's protocols.

Internal organs and small intestine

At 42 days of age, 10 chickens from each treatment (2 chickens from each replicate) were taken out randomly from each pen and were killed humanely by a knife to study carcass characteristics and organ weight of the broilers. Then, their organs were harvested. Hot carcass, heart, gizzard, liver, breast and thigh muscles, abdominal fat, pancreas, ceca, spleen, bursa of Fabricius, thymus, duodenum, jejunum, and ileum were weighed. The length of the small intestine was also measured. The ileum was defined as the region from the Meckel diverticulum to 40 mm proximal to the ileocecal junction. The jejunum was defined as the portion of the intestine extending from the bile duct entrance to the Meckel diverticulum.

Gene expression

In our study, quantitative real-time PCR (qRT-PCR) was applied to detect the relative mRNA expression levels of the pro-inflammatory cytokine *IL-6* in the ileum tissue of birds. The differences in relative expression levels of this gene were compared between the infection and control groups, and correlations of the relative expression levels were analyzed.

RNA isolation and quality assessment

According to the manufacturer's instructions, total RNA from chicken tissues was isolated using the column RNA isolation kit (Dena Zist, Iran). A NanoPhotometer spectrophotometer (NanoDrop 2000, USA) was used to assess the total RNA's optical density value (A260/A280). RNA degradation was monitored with 1% agarose gel. Qualified RNA samples were diluted to 100 ng/mL and stored at -70 ˚C.

Primer design

Based on the published chicken *IL-6* gene sequences in GenBank (2025), qRT-PCR primer for the target gene was designed using the Primer software, version 5 (Premier Biosoft, Palo Alto, CA). The gene expression level of *IL-6* was analyzed using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as an endogenous housekeeping control. All primers were synthesized by Sinaclon Biotech Co. (Tehran, Iran), and the primer information is presented in Table 2.

cDNA synthesis

The extracted total RNA was reverse transcribed with Sina Green HS-qPCR Master Mix, 2x (Sinaclon, Tehran Bio Inc, Iran) according to the manufacturer's instructions. Reverse transcription was carried out in a final volume of 20 µL assembled on ice, containing RNA template, 1 µL Oligo(dT)18, 1 µL dNTP mix and diethylpyrocarbonate (DEPC)-treated water. The mixture was incubated at 70 ˚C for 2 min, then 4 µL 5X buffer M-MuLV and 1.2 µL Reverse Transcriptase (RT) enzyme mix added to 20 µL. The reaction conditions were 50 °C for 60 min and 70 ˚C for 15 s. The samples were stored at -20 ˚C.

Quantitative real-time PCR

Fluorescence quantitative analysis was performed using the Sina Green HS-qPCRMix kit (Sinaclon, Tehran Bio Inc, Iran) with a total volume of 12.5 µL, which contained 6.25 µL of Sina Green HS-qPCRMix (Sinaclon, Iran), 4.65 nuclease-free water, 0.3 µL each of upstream and downstream primer and 1 µL of cDNA template. Also, qRT-PCR was carried out as follows: Preliminary denaturation at 95 ˚C for 15 min, followed by 40 cycles of denaturation for 20 s at 95 ˚C, annealing for 30 s at 56 ˚C and extension for 30 s at 72 ˚C. Data at multiple points were collected for dissolution curve analysis. Each sample was analyzed in triplicate.

Statistical analyses

All data were analyzed as a completely randomized design with 7 treatments and 5 replications using SAS's general linear model procedure (SAS Institute, 2009). The statistical model for data analysis was as Equation 1:

1. $Y_{ij}=\mu+T_i+e_{ij}$

Table 2. qRT-PCR primers used in this study

Gene Amplified	Primer Sequence (5'-3')	Amplification Size (bp)	GenBank Accession No.
IL-6	F: CTTCGACGAGGAGAAATGCC R: TGACTTCAGATTGGCGAGGA	229	NM_204628.2
GAPDH	F: GGAGTCCACTGGTGTCTTCA R: GACCCTCCACAATGCCAAAG	233	NM_204305.2

Where Y_{ij} is the trait of interest for chicken, μ is the overall mean, T_i denotes the treatment effect and e_{ij} is the residual error. The data's normal distribution of residuals and variance homogeneity were tested using the univariate procedure and Levene's test, respectively. Differences among means were considered statistically significant at $P<0.05$. Significant differences between means were separated by the Tukey test. We used the $2^{-\Delta\Delta Ct}$ method (Yu et al., 2019) to analyze the qRT-PCR results.

Results

Immune response

Data for the immune response against SRBC and NDV are shown in Table 3. Regarding the primary immune response against SRBC, total Ig was lowest in birds fed the PC diet (4.2) and there was a significant difference between challenged birds fed the NY0.2%WPC diet and the PC group ($P<0.05$). The IgM titer was lower in the PC group (2.4) than in the other groups, but there was no significant difference between unchallenged birds and the NC group. Also, no significant difference existed between the challenged birds and the PC group. No significant effects were observed on IgG, but the IgG was highest in broilers fed the NY0.2%WP diet and lowest in birds fed the PC diet. For the secondary immune response, broilers fed the PC diet had the lowest total Ig, IgM and IgG titers, whereas broilers fed the NY0.2%WP diet had the highest total Ig and IgM levels. There was no significant difference in antibody titers against SRBC between birds fed with salinomycin and birds fed with NY diets (unchallenged and challenged). For the primary response against NDV at 31 days of age, birds that received a salinomycin-supplemented diet showed the highest NDV antibody titers. Still, no significant difference existed between birds fed with salinomycin and birds fed with NY diet. NDV antibody titers for the secondary response were lowest in birds fed the PC diet. Chicks fed with NY0.2%S0.1%G0.05%F diet had the highest NDV antibody titers.

Table 3. Effect of dietary treatments on primary (day 31) and secondary (day 41) antibody response against SRBC and NDV

Items	31 Day				41 Day			
	Total Ig	IgM	IgG	NDV	Total Ig	IgM	IgG	NDV
NC[1]	5.2^{ab}	3.4^{ab}	1.8	3.4^{bc}	6.4^{b}	2.2	4.2^{b}	5.8^{ab}
PC[2]	4.2^{b}	2.4^{b}	1.6	2.8^{c}	6^{b}	2	4^{b}	4^{b}
NYC0.2%S0.1%G0.05%F[3]	5.2^{ab}	3.2^{ab}	2	3. 6^{abc}	7.4^{ab}	2.4	5^{ab}	5.6^{ab}
NY0.2%S0.1%G0.05%F[4]	6.2^{a}	3.8^{a}	2.4	4.4^{ab}	8.2^{a}	2.4	5.8^{a}	6.8^{a}
NY0.2%WPC[5]	5.8^{a}	3.4^{ab}	2.4	3.8^{abc}	7.2^{ab}	2.4	4.8^{ab}	5. 8^{ab}
NY0.2%WP[6]	6.4^{a}	3.8^{a}	2.6	4. 6^{ab}	8.4^{a}	2.8	5.6^{a}	6.6^{a}
Salinomycin[7]	6.2^{a}	4. 8^{a}	2. 2	4.8^{a}	8.2^{a}	2.4	5.8^{a}	6.2^{a}
SEM8 (n=10)	0.159	0.131	0.109	0.155	0.193	0.092	0.15	0.202
P	0.0001	0.013	0.147	0.0007	0.0002	0.0454	0.0001	0.0013

[1]Negative control: Unchallenged; [2]PC: Challenged with SO; [3]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher challenged with SO; [4]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher without challenge; [5]0.2% NY in the whole breeding period challenged with SO; [6]0.2% NY in the whole breeding period without Eimeria challenge; [7]Salinomycin (0.05% of diet); [8]Standard error of the mean.

Note: Within a column with different superscripts are significantly different ($P<0.05$).

Table 4. Effect of NY on the morphology of the ileum sample in broilers on day 42

Items	Villus Height (µm)	Crypt Depth (µm)	Villus Height: Crypt Depth
NC[1]	578.74^{c}	144.97^{ab}	4.05^{c}
PC[2]	235.44^{d}	163.52^{a}	1.46^{d}
NYC0.2%S0.1%G0.05%F[3]	260.76^{d}	151.12^{ab}	1.76^{d}
NY0.2%S0.1%G0.05%F[4]	772.43a^{b}	111.46^{c}	7.05^{a}
NY0.2%WPC[5]	670.28^{bc}	122.35^{bc}	5.55^{b}
NY0.2%WP[6]	806.63^{a}	112.89^{c}	7.19^{a}
Salinomycin[7]	591.12^{c}	134.81^{abc}	4.63^{bc}
SEM8 (n=10)	26.31	3.21	0.27
P	0.0001	0.0001	0.0144

[1]Negative control: Unchallenged; [2]PC: Challenged with SO; [3]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher challenged with SO; [4]0.2% NY in the starter, 0.1% NY in the grower and 0.05% NY in the finisher without challenge; [5]0.2% NY in the whole breeding period challenged with SO; [6]0.2% NY in the whole breeding period without Eimeria challenge; [7]Salinomycin (0.05% of diet); [8]Standard error of the mean.

Note: Within a column with different superscripts are significantly different (P<0.05).

Intestinal morphology

The results of the intestinal morphology of broiler chickens at 42 days of age are presented in Table 4. The results showed that dietary supplementation of NY in unchallenged birds significantly affected the ileum's villus height, crypt depth, and height: Crypt depth ratio. They were higher than those in the NC group. Villus height and height: Crypt depth ratio was significantly higher, and crypt depth was significantly lower for the birds fed with NY0.2%WPC diet than the PC group. There was no significant difference between birds fed with salinomycin and birds fed with NY in crypt depth. In contrast, the villus height and the ratios of the villus height to crypt depth significantly differed between unchallenged birds fed with NY and birds receiving a salinomycin-supplemented diet (P<0.05). NY supplementation improved the villus height, crypt depth, and villus height: Crypt depth in the ileum (P<0.05).

Intestinal microflora

Regarding the bacteria enumeration analysis (Table 5), unchallenged birds had lower concentrations of *coliforms* and higher *lactobacillus* counts in the ileum than the other groups. At 28 and 42 days of age, the birds fed with the NY0.2%WPC diet had similar concentrations of *lactobacillus* when compared to birds fed with the NY0.2%S0.1%G0.05%F diet, and differences between the PC group and birds fed with NYC0.2%S0.1%G0.05%F diet was not significant. At 28 days of age, the supplemented group had the same concentration of *lactobacillus* as the unchallenged group (NY0.2%S0.1%G0.05%F) and the challenged group (NY0.2%WPC). The concentration of *coliforms* in the PC group was the highest and significantly different from the challenged group fed with NY0.2%WPC. At 42 days of age, there was a significant difference between unchallenged birds fed with NY and NC groups. Birds fed with NY0.2%WPC had a significantly higher *lactobacillus* concentration and lower *coliforms* than the PC group. NY supplementation increased the number of *Lactobacillus* and decreased coliform numbers in the ileum.

Digestive tract pH

The effect of dietary NY supplementation on the pH of the digestive tract is shown in Table 6. The results showed no significant difference in the pH of the crop among the different treatments (P<0.05). Dietary supplementation of NY significantly affected the ileum and cecum pH in challenged and unchallenged birds compared to the NC group (P<0.05). However, no significant difference existed between birds fed with salinomycin and birds fed with NY in the ileum and cecum pH. There was no significant difference between challenged birds fed with NY and PC group for the ileum and cecum pH.

Table 5. Effect of NY on the intestinal microflora of broilers (log10 CFU/g)

Treatments	28 Days		42 Days	
	Lactobacillus	Total *Coliforms*	*Lactobacillus*	Total *Coliforms*
NC[1]	8.61[b]	6.70[b]	8.29[c]	6.61[c]
PC[2]	7.79[c]	7.10[a]	7.66[d]	7.16[a]
NYC0.2%S0.1%G0.05%F[3]	7.90[c]	6.96[a]	7.78[d]	6.91[b]
NY0.2%S0.1%G0.05%F[4]	8.79[b]	6.40[cd]	8.73[a]	6.22[c]
NY0.2%WPC[5]	8.60[b]	6.58[bc]	8.54[ab]	6.58[c]
NY0.2%WP[6]	8.87[a]	6.23[d]	8.77[a]	6.19[d]
Salinomycin[7]	8.62[b]	6.68[b]	8.36[bc]	6.57[c]
SEM8 (n=10)	0.051	0.037	0.052	0.042
P	0.0001	0.0001	0.0001	0.0001

[1]Negative control: Unchallenged; [2]PC: Challenged with SO; [3]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher challenged with SO; [4]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher without challenge; [5]0.2% NY in the whole breeding period challenged with SO; [6]0.2% NY in the whole breeding period without Eimeria challenge; [7]Salinomycin (0.05% of diet); [8]Standard error of the mean.

Note: Within a column with different superscripts are significantly different (P<0.05).

Table 6. Effect of NY on the digestive tract pH of broilers

Treatments	pH at 28 Days			pH at 42 Days		
	Crop	Ileum	Cecum	Crop	Ileum	Cecum
NC[1]	5.96	6.75[a]	6.86[a]	6.02	6.82[a]	6.88[a]
PC[2]	5.53	6.15[b]	6.32[b]	5.56	6.22[b]	6.33[b]
NYC0.2%S0.1%G0.05%F[3]	5.64	6.37[b]	6.42[b]	5.68	6.41[b]	6.43[b]
NY0.2%S0.1%G0.05%F[4]	5.41	6.22[b]	6.33[b]	5.44	6.28[b]	6.34[b]
NY0.2%WPC[5]	5.60	6.35[b]	6.43[b]	5.62	6.42[b]	6.44[b]
NY0.2%WP[6]	5.46	6.29[b]	6.31[b]	5.48	6.33[b]	6.32[b]
Salinomycin[7]	5.34	6.33[b]	6.37[b]	5.36	6.35[b]	6.38[b]
SEM8 (n=10)	0.057	0.032	0.030	0.056	0.031	0.030
P	0.0635	0.0001	0.0001	0.0371	0.0001	0.0001

[1]Negative control: Unchallenged; [2]PC: Challenged with SO; [3]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher challenged with SO; [4]0.2% NY in the starter,0.1% NY in the grower, and 0.05% NY in the finisher without challenge; [5]0.2% NY in the whole breeding period challenged with SO; [6]0.2% NY in the whole breeding period without Eimeria challenge; [7]Salinomycin (0.05% of diet); [8]Standard error of the mean.

Note: Within a column with different superscripts are significantly different (P<0.05).

Blood metabolites

The effect of dietary treatments on blood metabolites is presented in Table 7. The results showed that the concentration of serum TP and albumin increased (P<0.05), and cholesterol and triglycerides concentration decreased (P<0.05) in unchallenged birds fed with NY compared to the control group. Moreover, NY-supplemented in unchallenged birds (NY0.2%WP) had lower cholesterol and higher TP concentrations (P<0.05) compared with those of the control and antibiotic-supplemented groups. No significant differences were observed in triglycerides, cholesterol, HDL, LDL and VLDL levels between unchallenged birds fed with NY (NY0.2%S0.1%G0.05%F) and salinomycin treatments. There is a decrease in serum VLDL in birds fed with NY compared to the control. There was no significant difference in serum total cholesterol, triglycerides, TP, albumin, HDL, LDL, and VLDL between unchallenged birds fed with NY (NY0.2%S0.1%G0.05%F) and challenged birds fed with NY (NY0.2%WPC). This finding indicates that the NY can improve blood metabolite parameters even in the condition of *Eimeria* challenge.

Internal organs

The results for the relative weights of internal organs are shown in Table 8. The dietary treatments did not affect the relative weights of the carcass, gizzard, liver, heart, pancreas, ceca, spleen, tight and abdominal fat. However, there is an increase in tight weight and a decrease in abdominal fat in birds fed with NY compared to the control. There was a significant difference in breast weight among treatment groups (P<0.05). There is also a significant increase in the relative weight of the immune organs (bursa of Fabricius and thymus) and an increase in the spleen in NY-supplemented broilers.

Different sections of the small intestine

The results for the small intestine weight and length are shown in Table 9. There was no significant difference between treatments for duodenum, jejunum, and ileum length. The weight of the duodenum and ileum were significantly higher in the prebiotic-supplemented group compared with the control (P<0.05). No significant differences were observed in duodenum and ileum weights between unchallenged birds fed with NY and salinomycin diet (P>0.05). There was no significant difference between treatment groups fed with NY and salinomycin diet for the jejunum weight (P>0.05).

Table 7. Effect of NY on blood parameters of broilers

Treatments	Blood Parameters						
	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Total Protein (g/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Albumin (g/dL)
NC[1]	57.32^{b}	133.9^{b}	2.578^{cd}	46.72^{a}	71.24^{bc}	11.48^{ab}	1.274^{bc}
PC[2]	63.12^{a}	139.4^{a}	2.246^{d}	41.12^{b}	78.98^{a}	12.64^{a}	1.164^{d}
NYC0.2%S0.1%G0.05%F[3]	59.70^{ab}	138.5^{a}	2.440^{cd}	41.68^{b}	74.68^{b}	11.68^{ab}	1.194^{cd}
NY0.2%S0.1%G0.05%F[4]	51.46^{c}	124.5^{c}	3.108^{a}	46.98^{a}	65.18^{d}	10.92^{bc}	1.502a
NY0.2%WPC[5]	54.52^{bc}	128.8^{bc}	2.888^{ab}	45.66^{ab}	67.72^{cd}	11.94^{ab}	1.432^{ab}
NY0.2%WP[6]	50.68^{c}	108.6^{d}	2.946^{ab}	47.14^{a}	53.96^{e}	10.16^{c}	1.466^{a}
Salinomycin[7]	54.52^{bc}	130.8^{bc}	2.474^{cd}	45.50^{ab}	68.46^{cd}	10.30^{c}	1.220^{cd}
SEM8 (n=10)	0.84	1.72	0.048	0.53	1.302	0.17	0.023
P	0.0001	0.0001	0.0001	0.0003	0.0001	0.0001	0.0001

[1]Negative control: Unchallenged; [2]PC: Challenged with SO; [3]0.2%NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher challenged with SO; [4]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher without challenge; [5]0.2% NY in the whole breeding period challenged with SO; [6]0.2% NY in the whole breeding period without Eimeria challenge; [7]Salinomycin (0.05% of diet); [8]Standard error of the mean.

Note: Within a column with different superscripts are significantly different (P<0.05).

Table 8. Effect of NY on organ weights of broilers (g)

Organ	Dietary Treatment							SEM[8] (n=10)	P
	NC[1]	PC[2]	NYC0.2%S 0.1%G0.05%F[3]	NY0.2%S 0.1%G0.05%F[4]	NY0.2%WPC[5]	NY0.2%WP[6]	Salinomycin[7]		
Carcass[9]	63.15	62.40	63.23	63.44	63.64	64.38	63.62	0.209	0.344
Abdominal fat[9]	1.532	1.638	1.440	1.332	1.380	1.228	1.370	0.0581	0.610
Liver[9]	2.57	2.53	2.58	2.63	2.67	2.96	2.72	0.044	0.206
Gizzard[9]	1.376	1.338	1.338	1.380	1.390	1.428	1.442	0.0200	0.895
Heart[9]	0.392	0.41	0.41	0.414	0.426	0.432	0.460	0.0069	0.332
Pancreas[9]	0.199	0.214	0.203	0.205	0.210	0.193	0.197	0.0034	0.720
Spleen[9]	0.120	0.110	0.122	0.140	0.124	0.142	0.126	0.0044	0.823
Bursa[9]	0.108^{cd}	0.080^{d}	0.118^{bc}	0.21^{a}	0.108^{cd}	0.190^{a}	0.152^{b}	0.008	0.0001
Thymus[9]	0.308^{b}	0.182^{c}	0.250^{bc}	0.478^{a}	0.280^{bc}	0.418^{a}	0.214^{bc}	0.018	0.0001
Breast[10]	42.772^{b}	36.722^{b}	39.416^{b}	50.860^{a}	38.928^{b}	51.038^{a}	41.782^{b}	1.029	0.0001
Thigh[10]	28.666	28.540	29.300	30.956	29.652	30.780	30.462	0.264	0. 37
Cecum[9]	8.97	8.66	8.29	10.17	9.86	9.67	9.38	0.243	0.366

[1]Negative control: Unchallenged; [2]PC: Challenged with SO; [3]0.2% NY in the starter, 0.1%NY in the grower, and 0.05% NY in the finisher challenged with SO; [4]0.2% NY in the starter, 0.1%NY in the grower, and 0.05% NY in the finisher without challenge; [5]0.2% NY in the whole breeding period challenged with SO; [6]0.2% NY in the whole breeding period without Eimeria challenge; [7]Salinomycin (0.05% of diet); [8]Standard error of the mean; [9]% of live weight; [10]% of carcass weight.

Note: Within a column with different superscripts are significantly different (P<0.05).

Table 9. Effect of NY on weight and length of the small intestine of broilers

Organ	Dietary Treatment							SEM[8] (n=10)	P
	NC[1]	PC[2]	NYC0.2%S 0.1%G0.05%F[3]	NY0.2%S 0.1%G0.05%F[4]	NY0.2%WPC[5]	NY0.2%WP[6]	Salinomycin[7]		
Duodenum weight*	12.74d[e]	11.712^{e}	13.66^{cd}	17.10^{a}	14.766^{bc}	16.97^{a}	15.76^{ab}	0.351	0.0001
Jejunum weight*	29.090^{ab}	27.796^{b}	29.208^{ab}	31.680^{ab}	30.618^{ab}	34.728^{a}	30.418^{ab}	0.596	0.0397
Ileum weight*	20.690^{bc}	17.95^{c}	21.816^{b}	27.058^{a}	21.144^{bc}	27.044^{a}	25.104^{a}	0.605	0.0001
Duodenum length**	33.124	32.422	33.300	39.626	33.326	34.036	37	0.941	0.363
Jejunum length**	85.686	83	85.864	87.266	89.438	92.498	92.600	1.004	0.0641
Ileum length**	86	82.806	86.304	86.538	88.950	91.176	91.474	1.056	0.288

[1]Negative control: Unchallenged; [2]PC: Challenged with SO; [3]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher challenged with SO; [4]0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher without challenge; [5]0.2% NY in the whole breeding period challenged with SO; [6]0.2% NY in the whole breeding period without Eimeria challenge; [7]Salinomycin (0.05% of diet); [8]Standard error of the mean; *g; **cm.

Note: Within a column with different superscripts are significantly different (P<0.05).

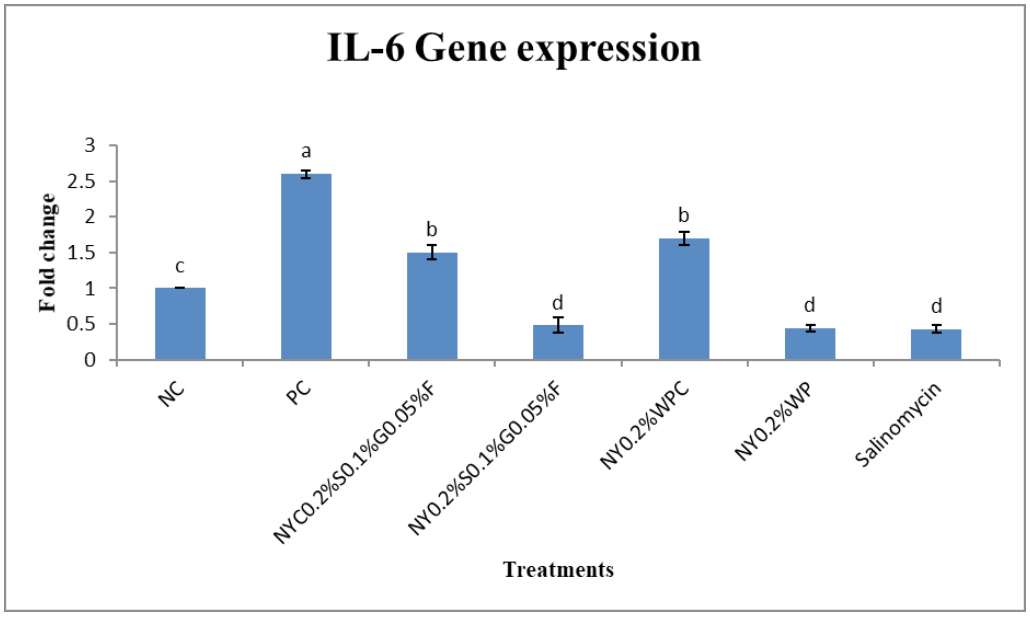

Figure 1. Relative interleukin-6 expression in the ileum on day 42

NC: Negative control: Unchallenged; PC, PC: Challenged with SO; NYC0.2%S0.1%G0.05%F, 0.2% NY in the starter, 0.1% NY in the grower and 0.05% NY in the finisher challenged with SO; NY0.2%S0.1%G0.05%F, 0.2% NY in the starter, 0.1% NY in the grower, and 0.05% NY in the finisher without challenge; NY0.2%WPC, 0.2% NY in the whole breeding period challenged with SO; NY0.2%WP, 0.2% NY in the whole breeding period without Eimeria challenge; Salinomycin, Salinomycin (0.05% of diet).

Note: Data are presented as Means±SE, n=10.

IL-6 gene expression

The results of *IL-6* gene expression in the ileum of broiler chickens at 42 days of age are presented in Figure 1. The results showed that *IL-6* gene expression was significantly lower in broilers fed with NY0.2%WP and salinomycin diets. No significant difference existed between birds fed with salinomycin and unchallenged birds fed with NY. Broilers fed with the PC diet showed the highest *IL-6* gene expression. The results showed a significant difference between challenged birds receiving NY and PC diets. Similarly, there was a significant difference between unchallenged birds that received NY and NC group diets.

Discussion

Developing new strategies for controlling coccidiosis is essential for the poultry industry. Due to environmental conditions during production, chickens are highly at risk for coccidial infections. High animal densities (>25000 chickens per building) on floor pens and warm surroundings favor a high transmission, replication, and accumulation of *Eimeria* spp. Moreover, the current practices for animal production create intense selective pressure on coccidia parasites to develop anticoccidial drug resistance. Prebiotics were supplemented in a poultry diet to prevent diseases (Elgeddawy et al., 2020). The hypothesis that prebiotic supplementation can enhance the immune response is based on the premise that prebiotics alter the gastrointestinal tract microflora by creating favorable conditions for beneficial bacteria to flourish while discouraging the proliferation of pathogenic bacteria. It has been reported that supplementing broiler diets with prebiotics (mannan oligosaccharide) reduced coccidiosis lesions caused by *Eimeria* species due to improving immune function (Elmusharaf et al., 2007).

YCW as a prebiotic has the potential of dietary supplementation to enhance immune responses and to protect the birds against coccidial infections (Alagbe et al., 2023), which is in line with the results of the current study where dietary supplementation of NY increased immunoglobulins concentrations against SRBC and antibody production against NDV.

Intestinal morphology, including villi height (VH), crypt depth (CD), and the VH/CD ratio, is an essential indicator of intestinal health, recovery, and functionality in broiler chickens. It plays a significant role in nutrient digestion and absorption (Celi et al., 2017). Prebiotics and YCW supplementation can improve broilers' intestinal mucosal development (Ricke, 2018; Micciche et al., 2018; Kim et al., 2019). The use of yeast derivatives

stimulates the length of intestinal villi (in the jejunum, duodenum, and ileum), which results in an enlargement of the absorption surface (Al-Mansour et al., 2011). Lepine and de Vos (2018) demonstrated that the responses of prebiotics were not limited to the effects on gut microbiota but could also occur directly via stimulating intestinal epithelial cells and immune cells. Intestinal cell proliferation, increased villi height, the villi: Crypt ratio, and the intestinal epithelial barrier are all promoted by strengthening tight junctions by prebiotic fermentation into short-chain fatty acids (SCFA), especially butyric acid (Swaggerty et al., 2019).

Investigations have shown that broiler chicks' lactic acid and other SCFAs created by the commensal bacteria prevent the growth of *Salmonella typhimurium*, *Clostridium perfringens*, and *Escherichia coli* through decreased pH (Bodie et al., 2019; Kumar et al., 2019). Resident bacteria boost mucosal defense mechanisms, inducing mucus production and the number of goblet cells. Enhancements in the morphology of the gastrointestinal tract increased feed utilization. They produced a protective barrier against intestinal infections by improving the integrity of epithelial cells, reducing endotoxin permeability and the risk of pathogen invasion (Teng & Woo, 2018; Swaggerty et al., 2019). Chapman (2014) reported that *Eimeria* spp. infection can result in the malabsorption of nutrients, epithelial inflammation, and villi destruction. De Maesschalck et al. (2015) showed that prebiotics significantly increased broiler chicks' ileum villus length and intestinal microbiota populations. Since the gastrointestinal tract is highly colonized, microbial composition and corresponding microbial physiology are critical. Pelicano et al. (2005) reported that higher villus height and width were recorded when prebiotics were supplemented in a broiler diet. Prebiotics may reduce the growth of many pathogenic and non-pathogenic intestinal bacteria, thereby resulting in decreased intestinal infections and improving villus height and villus width (VW) (Wang et al., 2019).

Sayrafi et al. (2011) reported that the prebiotic could be an effective alternative to the antibiotic as the prebiotic caused a significant increase in VH and VW compared to the antibiotic and control groups due to the ability of prebiotics to modulate the intestinal microbial communities. These results were also confirmed by Ghasemi et al. (2014) and Alagbe et al. (2023), who reported the positive effect of prebiotics on intestinal morphology.

The coccidial-challenged group increases CD. It decreases the VH/CD ratio, which indicates that this parasite can damage the intestinal mucosa and the intestinal absorptive capacity and increase the metabolic cost of intestinal epithelium turnover (Luquetti et al., 2016; Xue et al., 2018; Oikeh et al., 2019).

Acute inflammation caused by *Eimeria* stimulates the proliferation of stem cells at the crypt base, increasing the intestinal villi's height (Sun et al., 2016). Similar to the results of the current study, lower VH in response to the *Eimeria* challenge and the lower rate of VH:CD in challenged birds indicates that infected birds have to spend more energy and nutrients accelerating intestinal epithelial cell turnover to expel parasites from the intestine (Clevers, 2013).

The gut microbiota is a complex ecosystem that influences the physiological response of the host, including their immune development and function, nutrition and metabolism, and pathogen exclusion (Zhao et al., 2013). One of the main functions of the gut microbiota is to prevent the dominance and colonization of pathogenic bacteria by maintaining intestinal homeostasis through the competitive exclusion of pathogenic microbes (Diaz Carrasco et al., 2019). The competitive exclusion mechanism reduces pathogenic bacterial colonization of the intestinal epithelium by preventing bacterial toxins, enhancing the immune system's local activity, and the intestinal epithelium nutrition (Yaqoob et al., 2021). Prebiotics cannot be digested or absorbed by the gastrointestinal tract but instead used as a food source by beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* in the lower intestine (Adhikari & Kim, 2017; Muthamilselvan et al., 2016; Gibson et al., 2017). Mannan oligosaccharide preparation can reduce pathogen colonization by binding to the flagella of microorganisms such as *E. coli* and *Salmonella* (Ricke, 2018). This action would reduce their attachment to the intestine's epithelial cells and promote their elimination through excreta (Adhikari et al., 2018).

Infection with *Eimeria* parasites compromises intestinal integrity and affects nutrient absorption by reducing the function of the intestinal barrier and leading to a bacterial imbalance affecting bacterial-dependent metabolic processes in the gastrointestinal tract. Consequently, an intestinal bacterial imbalance increases the risk of susceptibility to other diseases by disrupting the gut homeostasis of the host (Hessenberger et al., 2016). Wu et al. (2018) demonstrated that *Lactobacillus* enhanced the digestion, absorption, and metabolic functions of the gut by increasing the abundance of beneficial bacteria. Biswas et al. (2018) showed that the use of prebiotics in the diet led to a significant ($P<0.05$) reduction in coliform count compared to the control and other dietary-supplemented groups at 21 and 42 days. Kim et al. (2010) concluded

that adding prebiotics to the broiler diet caused a significant reduction in the total coliform count compared to the control and antibiotic-received groups, which is consistent with the results of the current study.

The optimum pH of the gastrointestinal tract is crucial for the action of digestive enzymes. Both juvenile and adult animals have high gastrointestinal tract pH due to different factors (Gao et al., 2021). Juvenile animals have not developed a digestive tract system, and gastric acid secretion in the digestive tract is insufficient, while for adult animals, it is due to physiology, feed, environment, and other factors. This condition often makes the pH of the gastrointestinal tract higher than the suitable range for enzyme activity and beneficial bacteria growth. However, coccidiosis infection has been responsible for causing malabsorption of nutrients, which is related to the alteration of pH and morphological alteration, including flattened and elongated villi. An increase in intestinal acidity has been reported in chickens infected with either species of *Eimeria*. Ruff and Reid (1974) showed that the intestinal content was significantly lower in pH in birds after 5–9 days post-infection of *E. acervulina*, *E. mivati*, *E. maxima*, *E. necatrix* and *E. brunetti* than in uninoculated control birds causing the impairment in absorption of nutrients. Consequently, *Eimeria*-induced pH reductions likely impact nutrient digestion and absorption in the intestinal lumen.

Our results agree with Leung et al. (2019), who found that yeast extract increased SCFA in the absence of *Eimeria* but reduced SCFA and increased pH in the presence of *Eimeria*. Supplemental yeast extract significantly increased pH to more basic and decreased total SCFA compared with non-supplemented birds in *Eimeria*-challenged birds.

Feeding prebiotics has the priority probably due to the increasing population of bacteria that produces esterase enzyme, which can reduce the reabsorption of bile salts and destroy them and thus uses more cholesterol from the blood to make bile salts and, as a result, decreases cholesterol in the blood. The synthesis of bile acids from cholesterol in the liver is the most important way of releasing cholesterol. The use of prebiotics and decrease in cholesterol levels could be related to the de-conjugating of bile salts using lactic acid bacteria. As a result, they are absorbed less from the intestine. They are excreted more in the feces, and reduction of the pH in the intestinal tract can effectively reduce the cholesterol concentration (Shahir et al., 2014). Our results related to serum cholesterol and triglyceride concentrations are consistent with previous studies. These studies have shown that prebiotics exhibited lipid-lowering properties, which might be related to the changes in the intestinal bacterial flora composition, which ferments prebiotics to produce SCFAs in the gut and then causes a decrease in the systemic levels of blood lipids and cholesterol (Swaggerty et al., 2019).

The experiments on prebiotic supplementation on slaughter performance are seldom conducted, and the results of the experiments were not entirely similar. Our study showed that prebiotic administration positively impacted the weight of some internal organs, such as breasts, abdominal fat, and immune organs. Results of carcass, thigh, organs (liver, heart, gizzard), and weights were in line with (Yalcinkaya et al., 2008), who reported no effect of prebiotics on thigh, liver, heart, carcass, and gizzard weights. Studies conducted by Maiorano et al. (2017), Dankowiakowska et al. (2019) and Tavaniello et al. (2018) show that birds supplemented with prebiotics have a higher breast muscle weight, which is parallel with the results of the current study. Carcass characteristics were improved by adding prebiotics to the broiler diet, which might be related to the inhibition of colonization of intestinal pathogens and the improved utilization of nutrients (protein and energy) in the diet. Fat deposition in broilers' abdominal area is considered waste in the poultry industry since it represents a loss in the market and consumer acceptability. It also enhances expenses during the treatment of effluent produced when processing broilers. In the present study, dietary treatments did not significantly affect abdominal fat pad accumulation. Still, there was a numerical decrease in abdominal fat in birds fed with NY compared to the control. No clear mechanisms have been reported for lowering lipid synthesis by prebiotics. This gap might partly be due to the increasing number of beneficial bacteria, such as *Lactobacillus*, which decreases the activity of acetyl-CoA carboxylase, the rate-limiting enzyme in fatty acid synthesis. The significant increases in the absolute weight of the immune organs (thymus and bursa) in this study were in harmony with the results of previous research (Wang et al., 2015).

Results for the relative weight of the small intestine in this study agreed with the results of Awad et al. (2009) and Hosseini et al. (2016). The improvement in the relative weight of the small intestine by dietary prebiotic supplementation is correlated to morphometric histological changes, improved surface absorption, and a decrease in pathogenic bacteria (Tellez et al., 2010).

Cytokines are essential effector molecules of innate and adaptive immunity against pathogenic microorganisms. *IL-6* is important in the induction of immune effector responses to *Eimeria* infections in chicken (Lynagh et al., 2000), as well as is an essential factor in inflammatory and immune responses and is a multifunctional cytokine that plays a vital role in many acute-phase reactions, autoimmune diseases, and hematopoietic mechanisms, particularly inflammatory bowel disease (Yu et al., 2019). *Eimeria* infection causes a tremendous mucosal inflammatory response in the gut through invasion of, and subsequent damage to, epithelial cells. Inflammation is a component of the acute phase response, which is orchestrated by cytokines, including *IL-6* and *IL-6* is produced during immune responses to parasite infection (Lynagh et al., 2000). It is, therefore, reasonable to assume that the production of *IL-6* will occur during *Eimeria* infection. Clinical studies have shown that inflammation in the intestinal mucosa is accompanied by enhanced secretion of *IL-6* (Lynagh et al., 2000). Since infection with *Eimeria*, through the invasion of gut epithelial cells, is known to produce local inflammation, it seems likely that *IL-6* will play an essential role in the mucosal response to this parasite. Consistent with our results, many studies have shown that the relative expression levels of *IL-6* are higher in infectious conditions than in noninfectious conditions after challenge with different pathogens (Kim et al., 2008; Fernando et al., 2015). Results indicate that *IL-6* was correlated and played an essential role in coccidiosis infection of chicken (yu et al., 2019). The results showed that the expression levels of *IL-6* in the ileum of the infected group were all higher than those of the uninfected group ($P<0.05$).

Consistent with our results, researchers showed that salinomycin significantly reduced *IL-6* expression at day 21 in the ileum, suggesting an anti-inflammatory effect as well (Lu et al., 2014). NY's reduction of *IL-6* expression in this study agrees with earlier findings. In this study, reduced ileal *IL-6* expression in prebiotics treatment supports a beneficial anti-inflammatory effect of NY, and our results are in line with Lu et al. (2014) that showed, prebiotic significantly reduced *IL-6* expression in the ileum both on d 21 and 42 compared with the negative control group. This study indicates that NY exerts a significant anti-inflammatory effect, which may make it a potential antibiotic alternative for broilers.

Conclusion

Dietary supplementation of NY (NY0.2%WP) improved intestinal health and microflora by increasing *Lactobacillus*, decreasing total *coliforms* and pH, and improving the ileum morphology via increasing VH and decreasing CD. In addition, the immune response was enhanced with dietary supplementation of NY (NY0.2%WP) by increasing total immunoglobins. There was a significant increase in the absolute weight of the immune organs (bursa of Fabricius and thymus) and breast and small intestine weight in NY-supplemented groups. Adding prebiotics to diets reduces cholesterol and triglycerides in blood serum and increases TP and albumin. *IL-6* expression was significantly lower in unchallenged broilers fed with NY and salinomycin.

Ethical Considerations

Compliance with ethical guidelines

All animal protocols were approved by the Ethics Committee in Biomedical Research of University of Guilan, Rasht, Iran (IR.GUILAN.REC.1402.007).

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This study was conducted as part of the PhD dissertation of Mahnaz Bayat, approved by the Department of Animal Science, Faculty of Agriculture Science, University of Guilan, Rasht, Iran.

Authors' contributions

Project administration, and writing the original draft: Mahnaz Bayat; Conceptualization, investigation, methodology, data curation, supervision, review and editing: Hassan Darmani Kuhi, Mohammad Roostaei-Ali Mehr; Formal analysis and software: Navid Ghavi Hossein-Zadeh.

Conflict of interest

The authors declared no conflict of interest.

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