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Effect of Dietary Prebiotic Supplementation on the Immune Response, Intestinal health and Blood Parameters Following Eimeria Infection of Broiler chicken

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Running title: Dietary Prebiotic and Eimeria Infection

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 antibiotic 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 without 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; 7) salinomycin (0.05 % of diet). At 7 d of age, treatments 2, 3 and 5 were challenged with 20-fold dose of *Eimeria* vaccine via oral gavage. Antibody levels of SRBC were measured aays of age. On days 28 and 42, two birds per replicate were slaughtered to collect ileal digesta for microbial analysis. Samples for blood metabolite parameters, carcass traits and visceral organs weight, intestinal morphology and Interlukin 6 (IL-6) gene expression were collected on day 42.

RESULTS: The results showed that NY supplementation increased 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). Inclusion of NY improved immune system, 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 vs 578.7 µm) and numbers of *lactobacillus* (8.77 vs 8.29 cfu/g) was increased and crypt depth (112.8 vs 144.9 µm) and numbers of *coliforms* (6.19 vs 6.61 cfu/g) was decreased in broilers fed diet containing NY compared to the NC group (P<0.05). Dietary supplementation of NY decreased IL-6 gene expression in challenged and nonchallenged birds compared to the control group (P<0.05).

COCLUSIONS: 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.

1. 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 economic loss of 3 billion US dollars annually 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 modern poultry industry, and eliminating the use of antibiotics during production cycle may cause negative effects on the conversion rate of diets (Salois *et al*., 2016). The wider use of these substances had contributed to the development of resistant bacteria which can infused into the soil, where they can survive and contaminate the environment (Mazhar et al., 2021). For human health, they are a potential risk, so the European Union banned the use of antibiotics as growth promoters in animal feed in 2006. Unfortunately, this ban led to many problems in the production, such as the increase of feed conversion ratio and the increase of animal diseases (Leone and Ferrante, 2023). Thus, feed additives are used as antibiotic alternatives to control the diseases and promote the nutrients utilization (Barberis *et al*., 2015).

Several alternative strategies have proven their effectiveness in coccidiosis control with potential stimulatory effects on performances and immunity (Leone and Ferrante, 2023). They are mainly based on the preservation of the intestinal barrier integrity and the stimulation of the immune response (Kiarie *et al*., 2019). In recent years, prebiotics have been considered as potential alternatives to antibiotics (Teng and 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). It is suggested that the administration of prebiotics not only influences these aspects but also regulates the interaction between the host and the intestinal microbiota comprehensively (Teng and Woo, 2018). Therefore, it was hypothesized that prebiotic supplementation would improve the immune system of broiler chickens challenged with *Eimeria* and modulating specific populations of bacteria in the gut.

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*. The aim of this study was 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.

2. Materials and methods

Birds, diets and management

A total of 420 1-d-old male Ross 308 broiler chicks, with average body weight of 46.8 ± 0.8 g were used in this study. The chicks distributed into 35 homogenous groups of floor pens (1 m × 1 m) according to their initial weight and were allocated to a completely randomized design experiment with 7 treatments and 5 pen replicates (12 birds per pen). All the chicks were vaccinated based on a routine program. Diets formulated according to Ross 308 nutrition

specification booklet (2019). Feed and fresh water 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; challenged with sporulated oocysts of *Eimeria*, (3) basal diet + 0.2 % Nutri Yeast (Persian Kimiazyme Co.,Tehran, Iran) in stsrter, 0.1 % Nutri Yeast in grower and 0.05 % Nutri Yeast in finisher challenged with sporulated oocysts of *Eimeria* (NYC0.2S%0.1%G0.05%F), (4) basal diet + 0.2 % Nutri Yeast in stsrter, 0.1 % Nutri Yeast in grower and 0.05 % Nutri Yeast in finisher without challenge (NY0.2S%0.1%G0.05%F), (5) basal diet + 0.2 % Nutri Yeast in the whole breeding period with sporulated oocysts of *Eimeria* (NY0.2%WPC), (6) basal diet + 0.2 % Nutri Yeast in The whole breeding period without challenge (NY0.2%WP), (7) basal diet + salinomycin (0.05 % of diet). All the dietary treatments were fed continuously for 42 d from 1 d old. At 7 d 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*, *E. maxima*, *E. necatrix and E. tenella* suspended in phosphate buffered saline (PBS). According to the product catalogue of 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 titre of 1.6 x 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. All animal protocols were

approved by the Ethics committee in biomedical research of Gilan University (IR.GUILAN.REC.1402.007).

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

Humoral immune response

To assess the systemic antibody response, chicks were immunized by intramuscular injection of 0.1 ml of 25% sheep red blood cell (SRBC) in phosphate buffered saline (PBS) on days 20 and 34. 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 3000g for 15min, sera were decanted and frozen (-80 °C) until serological examination. Antibody titres for SRBC were determined by microhaemagglutination. Samples were incubated at 56°C for 30 min to inactivate the complement. The titres 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 ºC for 30 min prior to haemagglutination test. The 2-mercaptoethanol- sensitive antibody titres (IgM) were determined by subtracting the 2-ME-resistant antibody titre (total Ig minus IgG titers). The antibody titres 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 (La Sota 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, and sera were stored at -20°C until analysis. Antibody response was measured by the haemagglutination 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 HI test.

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 to be bound to virus during the incubation period, haemagglutination would be considered completely inhibited. The titres were expressed as $\log_2$ of the reciprocal of the highest serum dilution showing haemagglutination inhibition (Salehimanesh *et al*., 2016).

Intestinal morphology

Two bird per pen was 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 Meckel's 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). In the morphometric study, images were captured 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 Eftekhari *et al*. (2015) method, and then villus height: crypt depth ratio was calculated.

Bacterial enumeration of the ileal digesta

At d 28 and 42 two birds per pen were randomly selected, weighed, and slaughtered. Samples of the contents of the proximal ileum collected and stored into 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 incubation period. 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 d 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 the 42 days of age, 4 ml of blood was collected from wing vein from 10 birds in each treatment to measure blood metabolite parameters. The collected blood samples were centrifuged 10 min at 3000 rpm and the serum was separated, then stored at -20°C until assayed to measuring serum biochemical analysis. Serum levels of triglyceride, cholesterol, albumin, total protein (TP), HDL, LDL and 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 the 42 days of age, 10 chickens from each treatment (2 chickens from each replicate), which were taken out randomly from each pen and were killed humanely by knife to study carcass characteristics and organ weight of the broiler. Then, the weight of birds and 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 Meckel's diverticulum to a point 40 mm proximal to the ileocecal junction. The jejunum was defined as the portion of intestine extending from the bile duct entrance to Meckel's 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

Total RNA from chicken tissues was isolated using the column RNA isolation kit according to the manufacturer's instructions (Dena zist, Iran). A NanoPhotometer spectrophotometer (NanoDrop2000, USA) was used to assess the optical density value (A260/A280) of the total RNA. RNA degradation was monitored with 1% agarose gels. 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 (https://www.ncbi.nlm.nih.gov/genbank/), qRT-PCR primer for the target gene was designed using the Primer 5 software

(PREMIER Biosoft, Palo Alto, CA). Gene expression level of IL-6 was analysed using glyceraldehyde-3-phospate 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 9.

cDNA synthesis

The extracted total RNA was reverse transcribed with Sina Green HS-Qpcr Master Mix,2x (Cinaclon, Tehran Bio Inc, Iran) according to the manufacturer's instructions, and reverse transcription was carried out in a final volume of 20 µL assembled on ice containing RNA template, 1 µL Oligo dT18, 1 µL dNTP mix and DEPC-treated Water. The mixture incubated at 70◦C for 2 min, then 4 µL 5X Buffer M-MuLV and 1.2 µL 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 (Cinaclon, Tehran Bio Inc, Iran) with a total volume of 12.5 µL, which contained 6.25 µL of Sina Green HS-qPCRMix (Cinaclon, Iran), 4.65 Nuclease Free Water, 0.3 µL each of upstream and downstream primer, 1 µL of cDNA template. 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 extention 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 the General Linear Model procedure of SAS (SAS Institute, 2012). The statistical model for data analysis was as below:

$Y_{ij} = \mu + T_i + e_{ij}$,

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

3. Result

Immune response

Data for the immune response against sheep red blood cell (SRBC) and Newcastle disease virus (NDV) are shown in Table 2. Regarding the primary immune response against SRBC, total Ig was lowest in birds fed the PC diet (4.2), and there was significant difference between challenged birds fed NY0.2%WPC diet and PC group ($P<0.05$). The IgM titer was lower in PC group (2.4) than the other groups but there was no significant difference between unchallenged birds and NC group, and also there was no significant difference between challenged birds and PC group. No significant effects were observed on IgG, but the IgG was highest in broilers fed NY0.2%WP diet and lowest in birds fed the PC diet. For the secondary immune response, broilers fed the PC diet showed the lowest total Ig, IgM and IgG titers, whereas broilers fed NY0.2%WP diet had the highest levels of total Ig and IgM. There was no significant difference

in antibody titers against SRBC between birds fed Salinomycin and birds fed NY diets (unchallenged and challenged). For the primary response against NDV at 31 days of age, birds received Salinomycin supplemented diet showed the highest NDV antibody titers but there was no significant difference between birds fed Salinomycin and birds fed NY diet. NDV antibody titers for the secondary response was lowest in birds fed the PC diet (4). Chicks fed NY0.2%S0.1%G0.05%F diet had the highest NDV antibody titers.

Intestinal morphology

The results of the intestinal morphology of broiler chickens at 42 day of age are presented in Table 3. The results showed dietary supplementation of NY in unchallenged birds had significant effect on the villus height, crypt depth, and height:crypt depth ratio in the ileum compared to NC group. Villus height and height:crypt depth ratio was significantly higher and crypt depth was significantly lower for the birds fed NY0.2%WPC diet than the PC group. There was no significant difference between birds fed Salinomycin and birds fed NY in crypt depth. In contrast, the villus height and the ratios of the villus height to crypt depth were significantly different between unchallenged birds fed NY and birds received 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 4), unchallenged birds had lower concentration of *coliforms* and higher *lactobacillus* count in the ileum than the other groups. At 28 and 42 day of age, the birds fed NY0.2%WPC diet, had similar concentration of *lactobacillus*

when compared to birds fed NY0.2%S0.1%G0.05%F diet, and differences between PC group and birds fed NYC0.2%S0.1%G0.05%F diet was not significant. At 28 day of age, salinomycin supplemented group had same concentration of *lactobacillus* as the unchallenged group (NY0.2%S0.1%G0.05%F) and challenged group (NY0.2%WPC). The concentration of *coliforms* of PC group was the highest and significantly different from challenged group fed NY0.2%WPC. At 42 day of age, there was significant difference between unchallenged birds fed NY and NC group. Birds fed NY0.2%WPC had significantly higher concentration of *lactobacillus* and lower *coliforms* when compared to PC group. NY supplementation increased the numbers of *Lactobacillus* and decreased the numbers of coliform in the ileum.

Digestive tract pH

The effect of dietary NY supplementation on the pH of the digestive tract is shown in Table 5. The results showed that there was no significant difference in the pH of the crop among the different treatments (P<0.05). Dietary supplementation of NY had significant effect on the ileum and cecum pH in challenged and unchallenged birds compared to NC group (P<0.05). However, there was no significant difference between birds fed Salinomycin and birds fed NY in the ileum and cecum pH. There was no significant difference between challenged birds fed NY and PC group for the ileum and cecum pH, as well.

Blood metabolites

The effect of dietary treatments on blood metabolites is presented in Table 6. The results showed that concentration of serum total protein and albumin increased (P<0.05), cholesterol and triglycerides concentration decreased (P<0.05) in unchallenged birds fed NY in comparison with

the control group. Also NY supplemented groups in unchallenged birds (NY0.2%WP) had lower cholesterol and higher total protein concentrations (P<0.05) compared with those of control and antibiotic supplemented group. No significant differences were observed in triglycerides, cholesterol, HDL, LDL and VLDL levels between unchallenged birds fed NY (NY0.2%S0.1%G0.05%F) and Salinomycin treatments. There is a numerical decrease in serum VLDL in birds fed NY in comparison with the control. There was no significant difference in serum total cholesterol, triglycerides, total protein, albumin, HDL, LDL and VLDL between unchallenged birds fed NY (NY0.2%S0.1%G0.05%F) and challenged birds fed NY (NY0.2%WPC). This is an indication of that the NY can improve blood metabolite parameters even in condition of *Eimeria* challenge.

Internal organs

The results for the relative weights of internal organs are showed in Table 7. The relative weights of the carcass, gizzard, liver, heart, pancreas, ceca, spleen, tight and abdominal fat were not affected by the dietary treatments. However, there is a numerical increase in tight weight and numerical decrease in abdominal fat in birds fed NY in comparison with the control. There was significant difference in breast weight among treatment (P<0.05). There is also a significant increase in the relative weight of the immune organs (bursa of Fabricius and thymus) and numerical increase in the spleen in NY supplemented broilers.

Different sections of small intestine

The results for the small intestine weight and length are showed in Table 8. There was no significant difference between treatments for duodenum, jejunum and ileum length. The weight of 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 NY and Salinomycin diet (P>0.05). There was no significant difference among treatments groups fed NY and Salinomycin diet for the jejunum weight (P > 0.05).

IL-6 gene expression

The results of IL-6 gene expression in the ileum of broiler chickens at 42 day of age are presented in the Figure. The results showed that IL-6 gene expression was significantly lower in broilers fed NY0.2%WP and Salinomycin diets. There was no significant difference between birds fed Salinomycin and unchallenged birds fed NY. Broilers fed the PC diet showed the highest IL-6 gene expression. The results showed that there was significant difference between challenged birds received NY and PC diets. Similarly, there was significant difference between unchallenged birds received NY and NC group diets.

4. Discussion

The development of new strategies for control of coccidiosis is essential for the poultry industry. Chickens are highly at risk for coccidial infections due to environmental conditions during production. High animal densities (> 25,000 chickens per building) on floor pens and warm surroundings are favorable for a high transmission, replication and accumulation of *Eimeria* spp. Moreover, the current practices for animal production create a strong selective pressure on coccidia parasites to develop anticoccidial drug resistance. Prebiotics were supplemented in poultry diet to prevent diseases (Elgeddawy *et al*., 2020). The hypothesis that prebiotic supplementation can enhance the immune response is based on this premise that prebiotics alter the gastrointestinal tract microflora by creating favourable conditions for

beneficial bacteria to flourish while discouraging the proliferation of pathogenic bacteria. It has been reported that supplementing broiler diets with prebiotics (mannanoligosaccharide) resulted in a reduction in coccidiosis lesions caused by *Eimeria* species due to improving immune function (Elmusharaf *et al*., 2007).

Yeast cell wall (YCW) as a prebiotic have 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 important 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 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 can also occur directly via stimulating intestinal epithelial cell 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 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 *S. typhimurium*, *C. perfringens*, and *E. 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 GIT increased feed utilization and produce a protective barrier against intestinal infections by improving the integrity of epithelial cells, reducing endotoxin permeability and the risk of pathogen invasion (Teng and 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 prebiotic significantly increased the ileum villus length and the populations of intestinal microbiota of broiler chicks. Since the GIT 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 broiler diet. Prebiotics may reduce the growth of many pathogenic and non-pathogenic intestinal bacteria thereby resulting in reduction in intestinal infectious process and improved villus height (VH) and villus width (VW) (Xu *et al*., 2003).

Sayrafi *et al*. (2011) reported that the prebiotic could be effective alternatives to the antibiotic as the prebiotic caused significant increase in VH and VW than 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 positive effect of prebiotics on the intestinal morphology.

The coccidial challenged group increases crypt depth (CD) and 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 which increases intestinal villi height (Sun *et al*., 2016). Similar to the results of the current study, lower villi height in response to *Eimeria* challenge and the lower rate of villi height: crypt depth 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 (Carrasco *et al*., 2019). The competitive exclusion (CE) 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 GIT but rather used as food source by the beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* in the lower intestine (Adhikari and Kim, 2017; Muthamilselvan *et al*., 2016; Gibson *et al*., 2017). MOS can reduce pathogen colonization by binding to the flagella of the microorganism such as *E. coli* and *Salmonella* (Ricke, 2018). This action would reduce their attachment to the epithelial cells of the intestine and therefore, 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 leads 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 bacterium. Biswas *et al*. (2018) showed that the use of prebiotics in diet, lead to significant ($P<0.05$) reduction coliform count than the control and other dietary supplemented groups at 21 and 42 d. Kim *et al*. (2010) concluded that the addition of prebiotic in the broiler diet caused a significant reduction in the total coliform count than the control and antibiotic received groups, which are consistent with the results of the current study.

The optimum pH of gastrointestinal tract is crucial for the action of digestive enzymes. Both juvenile and adult animals have high GIT pH due to different factors (Gao *et al*., 2021). Juvenile animals have not developed the 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 often makes the gastrointestinal tract pH higher than the suitable range for enzyme activity and beneficial bacteria growth. However, coccidiosis infection has been responsible of causing malabsorption of nutrients which is related with the alteration of pH and morphological alteration including flattened villi and elongated. Increase in intestinal acidity has been reported in chickens infected with either species *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 are in agreement with those of 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 significantly 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 reduces the reabsorption of bile salts and destroys them and thus uses more cholesterol from the blood to produce bile salts and as a result decreases cholesterol in the blood. Synthesis of bile acids from cholesterol in the liver is the most important way of cholesterol excretion. The use of prebiotics and decrease in cholesterol level could be related to de-conjugating of bile salts by means of lactic acid bacteria, as a result they are absorbed less from the intestine and are excreted more in the feces, as well as reduction of the pH in the intestinal tract can be effective in reducing the cholesterol concentration (Shahir *et al*.,2014). Our results related to serum cholesterol and triglycerides 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 about prebiotic supplementation on slaughter performance are seldom and the results of experiments were not quite similar. Our study showed that the prebiotic administration impacted positively the weight of some internal organs such as breast, abdominal fat and immune organs. Results of carcass, thigh, organs (liver, heart, gizzard), 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) showed that birds supplemented with prebiotics had a higher breast muscle weight which is parallel with the results of the current study. Carcass characteristics were

improved by the addition of prebiotic in broiler diet which might be related to inhibition of colonization of intestinal pathogens and improved utilization of nutrients (protein and energy) in diet. Fat deposition in the abdominal area of broilers is regarded as waste in the poultry industry; since it represents a loss in the market and consumer acceptability, and enhances expense during the treatment of effluent produced when processing broilers. Dietary treatments had no significant effect on abdominal fat pad accumulation in the present study but there was numerical decrease in abdominal fat in birds fed NY in comparison with the control. No clear mechanisms have been reported responsible for the reduction of lipid synthesis by prebiotics. It might in part be due to increasing beneficial bacteria such as *Lactobacillus* that decrease the activity of acetyl-CoA carboxylase, which is the rate-limiting enzyme in fatty acids 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 study (Wang *et al*., 2015).

Results for the relative weight of small intestine in this study is in agreement with the results of Awad *et al*. (2009), Hosseini *et al*. (2016). The improvement in the relative weight of small intestine by dietary prebiotic supplementation is correlated to morphometric histological changes, improved surface of absorption and 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 the chicken (Lynagh *et al*., 2000), as well as is important 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 huge 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 production of IL-6 will occur during *Eimeria* infection. Clinical studies have shown that inflammation at 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 important 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 play an important 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 d 21 in the ileum, suggesting an anti-inflammatory effect as well (Lu *et al*., 2014). Reduction of IL-6 expression by NY in this study is in agreement with earlier findings. In this study, reduced ileal IL-6 expression in prebiotics treatment supports a beneficial anti-inflammatory effect of Nutri Yeast 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. In generall, this study shows that NY exerts significant anti-inflammatory effect which may make it a potential antibiotic alternative for broilers.

5. **Conclusion**

Dietary supplementation of NY (NY0.2%WP) improved intestinal health and microflora by increasing *Lactobacillus* and decreasing total *coliforms* and pH, by improving the ileum morphology via increasing villus height and decreasing crypt depth. In addition, immune response was improved with a dietary supplementation of NY (NY0.2%WP), by increasing total immonoglubins. There was significant increase in the absolute weight of the immune organs (bursa of Fabricius and thymus), breast and small intestine weight in NY supplemented groups. Addition of prebiotic in the diets reduced the cholesterol as well as triglycerides of blood serum and increased total protein and albumin. IL-6 expression was significantly lower in unchallenged broilers fed NY and Salinomycin.

References

Adhikari, P.A., Kim WK. (2017). Overview of prebiotics and probiotics: focus on performance, gut health and immunity–a review. *Animal Science juornal*, 4, 949–966. https://doi: 10.1515/aoas-2016-0092.

Adhikari, P.A., Cosby, D.E., Cox, N.A., Franca, M.S.S., Williams, M., Gogal RM. (2018). Effect of dietary fructooligosaccharides supplementation on internal organs Salmonella colonization, immune response, ileal morphology, and ileal immunohistochemistry in laying hens challenged with Salmonella Enteritidis. *Poultry Science*, 97, 2525–2533. https://doi: 10.3382/ps/pey101.

Alagbe, EO., Schulze2, H., Adeola, O. (2023). Growth performance, nutrient digestibility, intestinal morphology, cecal mucosal cytokines and serum antioxidant responses of broiler chickens to dietary enzymatically treated yeast and coccidia challenge. Journal of Animal Science and Biotechnology.14:57. https://doi.org/10.1186/s40104-023-00846-z.

Al-Mansour, S., Al-Khalf, A., Al-Homidan, I., Fathi MM. (2011). Feed efficiency and blood hematology of broiler chicks given a diet supplemented with yeast culture. *International Journal of Poultry Science*, 10: 603-607. doi: https:// 10.3923/ijps.2011.603.607.

Angwech, H., Tavaniello, S., Ongwech, A., Kaaya, A.N., Maiorano G. (2019). Efficacy of in ovo delivered prebiotics on growth performance, meat quality and gut health of kuroiler chickens in the face of a natural coccidiosis challenge. *Animals*, 9, 876. https:// doi: 10.3390/ani9110876.

Awad, WA., Ghareeb, K., Abdel-Rahee, M.S., Bohm J. (2009). Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, 84:49–55. doi: 10.3382/ps.2008-00244.

Barberis, A., Nadir Alloui, A., Bennoune, O., Ayachi, A., Agabou A. (2015). Effect of Using an Anticoccidial and a Prebiotic on Production Performances, Immunity Status and Coccidiosis in Broiler Chickens. *Asian Journal of Poultry Science*, 9(3): 133-143. https:// doi: 10.3923/ajpsaj.2015.133.143.

Biswas, A., Messam, R., Kumawat, M., Namit, M., Mandal, A.B., Mir NA. (2018). Effects of prebiotics on intestinal histo-morphometry and gut microflora status of broiler chickens. *Indian Journal of Animal Research*, 53(6) 2019: 785-789. https:// doi: 10.18805/ijar.B-3579.

Bodie, A.R., Micciche, A.C., Atungulu, G.G., Rothrock, M.J., Ricke SC. (2019). Current trends of rice milling byproducts for agricultural applications and alternative food production systems. *Frontiers in Sustainable Food Systems journal*, 3:47. https://doi.org/10.3389/fsufs.2019.00047.

Carrasco, J.M.D., Casanova, N.A., Miyakawa MEF. (2019). Microbiota, gut health and chicken productivity: What is the connection? *Microorganisms*, 7, 374. https://doi: 10.3390/microorganisms7100374.

Celi, P., Cowieson, A.J., FruNji, F., Steinert, R.E., Kluenter, A.M., Verlhac V. (2017). Gastrointestinal functionality in animal nutrition and health: New opportunities for sustainable animal production. *Animal Feed Science and Technology*, 234:88–100. https:// doi. org/ 10. 1016/j. anife edsci. 2017. 09. 012.

Chapman, H.D. (2014). Milestones in avian coccidiosis research: a review. *Poultry Science*, 93 (3):501–511. DOI:10.3382/ps.2013-03634.

Clevers, H. (2013). The intestinal crypt, a prototype stem cell compartment. *Cell Journal*, 154:274–284. https:// doi: 10.1016/j.cell.2013.07.004.

Dankowiakowska, A., Bogucka, J., Sobolewska, A., Tavaniello, S., Maiorano, G., Bednarczyk M. (2019). Effects of in ovo injection of prebiotics and synbiotics on the productive performance and microstructural features of the superficial pectoral muscle in broiler chickens. *Poultry Science*, 98, 5157-5165. doi: 10.3382/ps/pez202.

De Maesschalck, C., Eeckhaut, V., Maertens, L., De Lange, L., Marchal, L., Nezer, C., De Baere, S., Croubels, S., Daube, G., Haesebrouck, J.F., Ducatelle, R., Taminau, B., Van Immerseel Dewulf F. (2015). Effects of xylo-oligosaccharides on broiler chicken performance and microbiota. *Applied and Environmental Microbiology*, 81:5880–5888. https:// doi: 10.1128/AEM.01616-15.

Eftekhari, A., Rezaeipour, V., Abdullahpour R. (2015). Effects of acidified drinking water on performance, carcass, immune response, jejunum morphology, and microbiota activity of broiler chickens fed diets containing graded levels of threonine. *Livestock Science*, 180, 158-163. https://doi.org/10.1016/j.livsci.2015.07.010.

Elgeddawy, S.A., Shaheen, H.M., El-Sayed, Y.S., Abd Elaziz, M., Darwish, A., Samak, D., Alagawany M. (2020). Effects of the dietary inclusion of a probiotic or prebiotic on florfenicol pharmacokinetic profile in broiler chicken. *Journal of Animal Physiology and Animal Nutrition*, 104:549–557. https://doi: 10.1111/jpn.13317.

Elmusharaf, M.A., Peek, H.W., Nollet, L., Beynen AC. (2007). The effect of an in-feed mannanoligosaccharide preparation (MOS) on a coccidiosis infection in broilers. *Animal Feed Science and Technology*, 134, 347–354. https://doi.org/10.1016/j.anifeedsci.2006.11.022.

Fernando, F.S., Okino, C.H., Silva, K.R., Fernandes, C.C., Gonçalves, M.C.M., Montassier, M.F.S., Vasconcelos, R.O., Montassier HJ. (2015). Increased expression of interleukin-6 related to nephritis in chickens challenged with an avian infectious bronchitis virus variant. *Pesquisa Veterinaria Brasileira*, 35:216–222. DOI: 10.1590/S0100-736X2015000300002.

Gao, C.Q., Shi, H.Q., Xie, W.Y., Zhao, L.H., Zhang, J.Y., Ji, C., Ma QG. (2021). Dietary supplementation with acidifiers improves the growth performance, meat quality and intestinal health of broiler chickens. *Animal Nutrition*, 7: 762-769. https://doi.org/10.1016/j.aninu.2021.01.005.

Ghasemi, H.A., Kasani, N., Taherpour K. (2014). Effects of black cumin seed (Nigella sativa L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers. *Livestock Science*, 164:128-34. https:// doi:10.1016/j.livsci.2014.03.014.

Gibson, G.R., Hutkins, R., Sanders, M.E., Precott, S.L., Reimer, R.A., Salminen, S., JScott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K., Reid G. (2017). Expert consensus document: The international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterol. Hepatology*, 14:491–502. https:// doi: 10.1038/nrgastro.2017.75.

Hassanpour, H., Zamani, Moghaddam, A.K., Khosravi, M., Mayahi M. (2013). Effects of synbiotic on the intestinal morphology and humoral immune response in broiler chickens. *Livestock Science*, 153, 116–122. https://doi.org/10.1016/j.livsci.2013.02.004.

Hessenberger, S., Schatzmayr, G., Teichmann K. (2016). In vitro inhibition of Eimeria tenella sporozoite invasion into host cells by probiotics. *Veterinary Parasitology*, 229, 93–98. https://doi: 10.1016/j.vetpar.2016.10.001.

Hosseini, S.M., Nazarizadeh, H., Ahani, S., Azghandi MV. (2016). Effects of mannan oligosaccharide and Curcuma xanthorrhiza essential oil on the intestinal morphology and stress indicators of broilers subjected to cyclic heat stress. *Archives Animal Breeding*, 59, 285-291. doi:10.5194/aab-59-285-2016.

Kiarie, E.G., Leung, H., Akbari Moghaddam Kakhki, R., Patterson, R., Barta, JR. (2019). Utility of feed enzymes and yeast derivatives in ameliorating deleterious effects of coccidiosis on intestinal health and function in broiler chickens. *Front Veterinary Science*, 6:473. https:// doi. org/ 10. 3389/ fvets. 2019. 00473.

Kim, D.K., Lillehoj, H.S., Hong, Y. H., Park, D.W., Lamont, S.J., Han, J.Y., Lillehoj EP. (2008). Immune-related gene expression in two B-complex disparate genetically inbred Fayoumi chicken lines following Eimeria maxima infection. *Poultry Science*, 87:433–443. doi: 10.3382/ps.2007-00383.

Kim, G.B., Seo, Y.M., Kim, C.H., Paik IK. (2010). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science*, 90(1):75-82. https://doi: 10.3382/ps.2010-00732.

Kim, S.A., Jang, M.J., Kim, S.Y., Yang, Y., Pavlidis, H.O., Ricke SC. (2019). Potential for prebiotics as feed additives to limit foodborne Campylobacter establishment in the poultry gastrointestinal tract. *Frontiers in Microbiology*, 10:91. https://doi.org/10.3389/fmicb.2019.00091.

Kumar, S., Shang, Y., Kim WK. (2019). Insight into dynamics of gut microbial community of broilers fed ith fructooligosaccharides supplemented low calcium and phosphorus diets. *Frontiers in Veterinary Science*, 6:95. https://doi.org/10.3389/fvets.2019.00095.

Leone, F., Ferrante V. (2023). Effects of prebiotics and precision biotics on performance, animal welfare and environmental impact. A review, *Science of the Total Environment,* 901, 165951. https://doi.org/10.1016/j.scitotenv.2023.165951.

Lepine, A., de Vos P. (2018). Synbiotic effects of the dietary fiber long-chain inulin and probiotic Lactobacillus acidophilus W37 can be caused by direct, synergistic stimulation of immune Toll-like receptors and dendritic cells. *Molecular Nutrition & Food Research*, 62:e1800251. https:// doi:10.1002/mnfr.201800251.

Leung, H., Yitbarek, A., Snyder, R., Patterson, R., Barta, J.R., Karrow, N., Kiarie E. (2019). Responses of broiler chickens to Eimeria challenge when fed a nucleotide-rich yeast extract. *Poultry Science*, 98:1622–1633. http://dx.doi.org/10.3382/ps/pey533.

Lu, H., Adedokun, S.A., Adeola, L., Ajuwon KM. (2014). Anti-Inflammatory Effects of Non-Antibiotic Alternatives in Coccidia Challenged Broiler Chickens. *Journal of Poultry Science*, 51: 14-21. DOI:10.2141/jpsa.0120176.

Luquetti, B.C., Alarcon, M.F.F., Lunedo, R., Campos, D.M.B., Furlan, R.L., Macari M. (2016). Effects of glutamine on performance and intestinal mucosa morphometry of broiler chickens vaccinated against coccidiosis. *Scientia Agricola*, 73:322–327. https:// doi. org/ 10. 1590/ 0103- 9016- 2015- 0114.

Lynagh, G.R., Bailey, M., Kaiser P. (2000). Interleukin-6 is produced during both murine and avian Eimeria infections. *Veterinary Immunology and Immunopathology*, 76 :89-102. doi: 10.1016/s0165-2427(00)00203-8.

Maiorano, G., Stadnicka, K., Tavaniello, S., Abiuso, C., Bogucka, J., Bednarczyk M. (2017). In ovo validation model to assess the efficacy of commercial prebiotics on broiler performance and oxidative stability of meat. *Poultry Science*, 96, 511-518. doi: 10.3382/ps/pew311. Epub 2016 Sep 23.

Mazhar, S.H., Li, X., Rashid, A., Su, J.M., Xu, J., Brejnrod, A.D., Su, J.Q., Wu, Y., Zhu, Y. G., Zhou, S.G., Feng, R., Rensing C. (2021). Co-selection of antibiotic resistance genes, and mobile genetic elements in the presence of heavy metals in poultry farm environments. *Science Total Environment*. 755. https://doi.org/10.1016/j. scitotenv.2020.142702.

Micciche, A.C., Foley, S.L., Pavlidis, H.O., McIntyre, D.R., Ricke SC. (2018). A review of prebiotics against Salmonella in poultry: current and future potential for microbiome research application. *Frontiers in Veterinary Science*, 5:191. https://doi.org/10.3389/fvets.2018.00191.

Moss, A.F., Chrystal, P.V., Dersjant-Li, Y., Selle, P.H., Liu, SY. (2018). Responses in digestibilities of macro-minerals, trace minerals and amino acids generated by exogenous phytase and xylanase in canola meal diets offered to broiler chickens. *Animal Feed Science and Technology*, 240, 22–30. 10.1016/j.anifeedsci.2018.03.011.

Muthamilselvan, T., Kuo, T.F., Wu, Y.C., Yang WC. (2016). Herbal remedies for coccidiosis control: A review of plants, compounds, and anticoccidial actions. Evidence-based Complement. *Alternative. Medicine,* 2657981. https://doi.org/10.1155/2016/2657981.

Oikeh, I., Sakkas, P., Blake, D.P., Kyriazakis I. (2019). Interactions between dietary calcium and phosphorus level, and vitamin D source on bone mineralization, performance, and intestinal morphology of coccidia-infected broilers1. *Poultry Science*, 98:5679–5690. https:// doi. org/ 10. 3382/ ps/ pez350.

Pelicano, E.R.L., Souza, P.A., Souza, H.B.A., Figueiredo, D.F., Boiago, M.M., Carvalho, S.R., Bordon VF. (2005). Intestinal mucosa development in broiler chickens fed natural growth promoters. *Revista brasileira de ciencia avicola*, 7(4):221-229. https://doi.org/10.1590/S1516-635X2005000400005.

Ricke, SC. (2018). Impact of prebiotics on poultry production and food safety. *Yale Journal of Biology Medicine*. 91:151–159. PMID: 29955220.
Ross Nutrition Supplement, 2009. Availablefrom: (http://www.aviagen. com/).
Ruff, M.D., Reid WM. (1974). Coccidiosis and Intestinal pH in chickens. *Avian Diseases*, 19(1): 52-58. PMID: 235255.
Salehimanesh, A., Mohammadi, M., Roostaei-Ali Mehr M. (2016). Effect of dietary probiotic, prebiotic and symbiotic supplementation on performance, immune responses, intestinal morphology and bacterial populations in broilers. *Journal of Animal Physiology and Animal Nutrition* (Berl), 100(4):694-700. https://doi: 10.1111/jpn.12431.
Salois, M.J., Cady, R.A., Heskett EA. (2016). The environmental and economic impact of withdrawing antibiotics from US broiler production [abstract]. *Journal of Food Distribution Research*, 47:79–80. https:// doi: 10.22004/ag.econ.232315.
SAS (2012). Statistical Analysis System: User's Guide; Version 9.3 Edn. Cary, NC: SAS Institute.
Sayrafi, R., Soltanalinejad, F., Shahrooz, R., Rahimi S. (2011). Comparative study of the effect of alternative and antibiotic feed additives on the performance and intestinal histo morphometrical parameters of broiler chickens. *African Journal of Agriculture Research*, 6:2794-2799. DOI: 10.5897/AJAR10.1031.
Shahir, M.H., Afsarian, O., Ghasemi, S., Tellez G. (2014). Effects of Dietary Inclusion of Probiotic or Prebiotic on Growth Performance, Organ Weight, Blood Parameters and Antibody Titers Against Influenza and Newcastle in Broiler Chickens. *International Journal of Poultry Science*, 13 (2): 70-75. http://doi: 10.3923/ijps.2014.70.75.
Sun, L.L., Dong, H.B., Zhang, Z.C., Liu, J., Hu, Y., Ni, Y.D., Grossmann, R., Zhao RQ. (2016). Activation of epithelial proliferation induced by Eimeria acervulina infection in the duodenum may be associated with cholesterol metabolism. *Oncotarget journal*, 7:27627–27640. http://doi: 10.18632/oncotarget.8490.
Swaggerty, C.L., Callaway, T.R., Kogut, M.H., Piva, A., Grilli E. (2019). Modulation of the immune response to improve health and reduce foodborne pathogens in poultry. *Microorganisms*, 7:65. http://doi: 10.3390/microorganisms7030065.
Swaggerty, C.L., Pevzner, I.Y., Kogut MH. (2015). Selection for pro-inflammatory mediators produces chickens more resistant to Eimeria tenella. *Poultry Science*, 94:37–42. doi: 10.3382/ps/peu053.
Tavaniello, G., Maiorano, K., Stadnicka, R., Mucci, J., Bogucka, Bednarczyk M. (2018). Prebiotics offered to broiler chicken exert a positive effect on meat quality traits irrespective of delivery routes. *Poultry Science*, 97, 2979-2987. doi: 10.3382/ps/pey149.
Tellez, G., Nava, G.M., Vicente, J.L., Franceschi, D.M., Morales, E.J., Prado, O., Terraes, J.C., Hargis BM. (2010). Evaluation of Dietary Aspergillus Meal on Intestinal Morphometry in Turkey Poults. *Intrtnational Journal of Poultry Science*, 9: 875-878. DOI: 10.3923/ijps.2010.875.878.
Teng, P.Y., Woo KK. (2018). Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers. Frontiers in Veterinary Science. *Frontiers in Veterinary Science*, 5:245. doi: 10.3389/fvets.2018.00245. https://doi.org/10.3389/fvets.2018.00245.
Teng, P.Y., Yadav, S ., Souza Castro, F.L., Tompkins, Y.H., Fuller, A.L., Woo KK. (2020). Graded Eimeria challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight

junctions of broiler chickens. *Poultry Science*, 99:4203–4216. https://doi.org/10.1016/j.psj.2020.04.031.

Wang, W., Yang, H., Wang, Z., Han, J., Zhang, D., Sun, H., Zhang F. (2015). Effects of prebiotic supplementation on growth performance, slaughter performance, growth of internal organs and small intestine and serum biochemical parameters of broilers. *Journal of Applied Poultry Research*, 43, 33-38. https://doi.org/10.1080/09712119.2014.887010.

Wu, S., Liu, Y., Duan, Y., Wang, F., Guo, F., Yan, F., Yang, X., Yang X. (2018). Intestinal toxicity of deoxynivalenol is limited by supplementation with Lactobacillus plantarum JM113 and consequentially altered gut microbiota in broiler chickens. *Journal of Animal Science & Biotechnology*, 9:74. https://doi: 10.1186/s40104-018-0286-5.

Wu, X.Z., Wen, Z. G., Hua, J. L. (2019). Effects of dietary inclusion of Lactobacillus and inulin on growth performance, gut microbiota, nutrient utilization, and immune parameters in broilers. *Poultry Science*, 98:4656–4663. http://dx.doi.org/10.3382/ps/pez166.

Xu, Z.R., Hu Xia, C.H., Zhan, M.S., Wang MQ. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science,* 82(6):1030-1036. http:// doi: 10.1093/ps/82.6.1030.

Xue, G.D., Barekatain, R., Wu, S.B., Choct, M., Swick RA. (2018). Dietary L-glutamine supplementation improves growth performance, gut morphology, and serum biochemical indices of broiler chickens during necrotic enteritis challenge. *Poultry Science*, 97:1334–1341. http:// doi: 10.3382/ps/pex444.

Yalcinkaya, H., Gungori, T., Bafialani, M., Erdem E. (2008). Mannan oligosaccharides (MOS) from Saccharomyces cerevisiae in broilers: effects on performance and blood biochemistry. *Turkish Journal of Veterinary & Animal Sciences*, 32(1): 43-48. https://journals.tubitak.gov.tr/veterinary/vol32/iss1/8.

Yaqoob, M.U., Abd El-Hack, M.E., Hassan, F., El-Saadony, M.T., Khafaga, A.F., Batiha, G.E., Yehia, N., Elnesr, S.S., Alagawany, M., El-Tarabily, K.A., Wang, M. (2021). The potential mechanistic insights and future implications for the effect of prebiotics on poultry performance, gut microbiome, and intestinal morphology. *Poultry Science,* 100:101143. https://doi.org/10.1016/j.psj.2021.101143.

Yu, H., Zou, W., Wang, X., Dai, G., Zhang, T., Zhang, G., Xie, K., Wang, J., Shi H. (2019). Research Note: Correlation analysis of interleukin-6, interleukin- 8, and C-C motif chemokine ligand 2 gene expression in chicken spleen and cecal tissues after Eimeria tenella infection in vivo. *Poultry Science*, .https://doi.org/10.1016/j.psj.2019.10.071

Zhao, Y., Bao, Y., Zhang, L., Chang, L., Jiang, L., Liu, Y., Zhang, L., Qin J. (2013). Biosafety of the plasmid pcDNA3-1E of Eimeria acervulina in chicken. *Experimental Parasitology*, 2013, 133, 231–236. https://doi: 10.1016/j.exppara.2012.11.026.

أثیر مکمل جیره ای پری بیوتیک بر پاسخ ایمنی، سلامت روده و پارامترهای خونی به دنبال عفونت آیمریا در جوجه های نر گوشتی

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چکیده

زمینه مطالعه: آنتی بیوتیک ها داروهای ارجح برای کنترل این بیماری کوکسیدیوز هستند. با این حال استفاده طولانی مدت از یونوفر ها منجر به مقاومت در برابر این داروها می‌شود.

هدف: مطالعه حاضر به منظور ارزیابی اثر پری بیوتیک نوتری ییست بر ریخت شناسی و جمعیت میکروبی روده و سیستم ایمنی در جوجه های گوشتی تحت چالش خفیف آیمریا انجام شد. تعداد 420 جوجه نر یک روزه سویه راس در یک طرح کاملا تصادفی با 7 تیمار و 5 تکرار استفاده شد. تیمارهای آزمایشی شامل: 1)کنترل منفی (بدون پری بیوتیک و بدون چالش) 2)کنترل مثبت (بدون پری بیوتیک و چالش داده شده با اوسیست های اسپوریله شده آیمریا) 3) 0/2 درصد نوتری ییست در دوره آغازین، 0/1 درصد در دوره رشد، 0/05 درصد در دوره پایانی، چالش داده شده با اوسیست های اسپوریله شده آیمریا، 4) 0/2 درصد نوتری ییست در دوره آغازین، 0/1 درصد در دوره رشد، 0/05 درصد در دوره پایانی، بدون چالش آیمریا، 5) 0/2 درصد نوتری ییست در کل دوره پرورش، چالش داده شده با اوسیست های اسپوریله شده آیمریا، 6) 0/2 درصد نوتری ییست در کل دوره پرورش، بدون چالش آیمریا، 7) سالینومایسین، 0/05 درصد جیره به عنوان کوکسیدیواستات در کل دوره پرورش. در سن 7 روزگی تیمارهای 2، 3 و 5 با 20 برابر دوز واکسن آیمریا چالش داده شدند. در سن 31 و 41 روزگی دو پرنده در هر قلم برای خونگیری انتخاب و تیتر آنتی بادی سرم اندازه گیری شد. در روز 28 و 42، پرندگان برای جمع آوری هضم روده برای تجزیه و تحلیل میکروبی ذبح شدند. ارزیابی پارامترهای خون، ویژگی‌های لاشه و وزن اندام‌ها، مورفولوژی روده و بیان ژن اینترلوکین 6 (IL-6) در روز 42 انجام شد.

نتایج: نتایج نشان داد که مکمل نوتری ییست باعث افزایش معنی دار غلظت پروتئین کل سرم (3/10 در مقابل 2/57 گرم بر دسی‌لیتر) و کاهش تری‌گلیسیرید سرم (50/6 در مقابل 57/3 میلی‌گرم بر دسی‌لیتر) و کلسترول (108/6 در مقابل 133/9 میلی‌گرم بر دسی‌لیتر) نسبت به گروه شاهد منفی شد. مشاهده شد که گنجاندن نوتری ییست باعث بهبود سیستم ایمنی، اسیدیته روده و وزن نسبی اندام های ایمنی، ماهیچه سینه و روده کوچک در مقایسه با گروه شاهد منفی شد ($P<0.05$). ارتفاع پرز (806/6 در مقابل 578/7 میکرومتر) و تعداد لاکتوباسیلوس (8/77 در مقابل 8/29 پرگنه بر گرم) افزایش یافته و عمق کریپت (112/8 در مقابل 144/9 میکرومتر) و تعداد کلی‌فرم ها (6/19 در مقابل 6/61 پرگنه بر گرم) در جوجه های گوشتی تغذیه شده با جیره نوتری ییست نسبت به گروه شاهد منفی کاهش یافته است ($P<0.05$). مکمل نوتری ییست باعث کاهش بیان ژن IL-6 در پرندگان چالشی و غیرچالشی نسبت به گروه شاهد شد ($P<0.05$).

نتیجه‌گیری کلی: نتایج این مطالعه فرضیه ما مبنی بر اینکه استفاده از پری بیوتیک نوتری ییست دارای خواص محافظتی در برابر کوکسیدیوز در جیره جوجه های گوشتی است را تایید کرد.

کلمات کلیدی: آیمریا، پاسخ ایمنی، سلامت روده، پری بیوتیک.

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

Item	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[1]	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

[1] Supplied per kg diet: vitamin A, 11 000 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.

Table 2
Effect of dietary treatments on primary (d 31) and secondary (d 41) antibody response against sheep red blood cell and Newcastle disease virus (NDV).

Item	31 d				41 d			
	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]
SEM[8] (n = 10)	0.159	0.131	0.109	0.155	0.193	0.092	0.15	0.202
P value	0.0001	0.013	0.147	0.0007	0.0002	0.0454	0.0001	0.0013

[a–d] Means within a column with different superscripts are significantly different (P < 0.05)
[1] negative control; unchallenged
[2] positive control; challenged with sporulated oocysts of Eimeria
[3]0.2 percent Nutri Yeast in stsrter,0.1 percent Nutri Yeast in grower and 0.05 percent Nutri Yeast in finisher challenged with sporulated oocysts of Eimeria
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[6]0.2 percent Nutri Yeast in the whole breeding period without Eimeria challenge
[7]salinomycin (0.05 percent of diet)
[8] Standard error of mean

Table 3
Effect of Nutri Yeast on the morphology of the ileum sample in broilers on d 42.

Item	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]	772.43[ab]	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]
SEM[8] (n = 10)	26.31	3.21	0.27
P value	0.0001	0.0001	0.0144

[a–d] Means within a column with different superscripts are significantly different ($P < 0.05$)
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[6]0.2 percent Nutri Yeast in the whole breeding period without Eimeria challenge
[7]salinomycin (0.05 percent of diet)
[8] Standard error of mean

Table 4
Effect of Nutri Yeast on intestinal microflora of broilers (log10 cfu/g).

Treatment	28 d		42 d	
	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]
SEM[8] (n = 10)	0.051	0.037	0.052	0.042
P value	0.0001	0.0001	0.0001	0.0001

[a–d] Means within a column with different superscripts are significantly different (P < 0.05)
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[6]0.2 percent Nutri Yeast in the whole breeding period without Eimeria challenge
[7]salinomycin (0.05 percent of diet)
[8] Standard error of mean

Table 5
Effect of Nutri Yeast on the digestive tract pH of broilers.

Treatment	pH at 28 d			pH at 42 d		
	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]
SEM[8] (n = 10)	0.057	0.032	0.030	0.056	0.031	0.030
P value	0.0635	0.0001	0.0001	0.0371	0.0001	0.0001

[a–d] Means within a column with different superscripts are significantly different ($P < 0.05$)
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[6]0.2 percent Nutri Yeast in the whole breeding period without Eimeria challenge
[7]salinomycin (0.05 percent of diet)
[8] Standard error of mean

Table 6

Effect of Nutri Yeast on blood parameters of broilers.

Treatment	Blood parameters						
	Triglyceride	Cholesterol	Total protein	HDL	LDL	VLDL	Albumin
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]	1.502 [a]
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]
SEM[8] (n = 10)	0.84	1.72	0.048	0.53	1.302	0.17	0.023
P value	0.0001	0.0001	0.0001	0.0003	0.0001	0.0001	0.0001

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[7]salinomycin (0.05 percent of diet)
[8] Standard error of mean

Table 7

Effect of Nutri Yeast on organ weights of broilers (gr).

Organ	Dietary treatment								
	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]n	SEM[8] (n = 10)	P value
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	0.037
Cecum[9]	8.97	8.66	8.29	10.17	9.86	9.67	9.38	0.243	0.366

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[6]0.2 percent Nutri Yeast in the whole breeding period without Eimeria challenge
[7]salinomycin (0.05 percent of diet)
[8] Standard error of mean
[9]% of live weight
[10]% of carcass weight

Table 8

Effect of Nutri Yeast on weight and Length of small intestine of broilers.

Organ	Dietary treatment								
	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]n	SEM[8] (n = 10)	P value
Duodenum weight*	12.74^{de}	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

$^{a–d}$ Means within a column with different superscripts are significantly different ($P < 0.05$)
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[6]0.2 percent Nutri Yeast in the whole breeding period without Eimeria challenge
[7]salinomycin (0.05 percent of diet)
[8] Standard error of mean
*gr
**cm

Table 9
Quantitative real-time PCR primers used in this study.

Gene amplified	Primer sequence (5'- 3')	Amplification size (bp)	GenBank accession number
IL-6	F: CTTCGACGAGGAGAAATGCC R: TGACTTCAGATTGGCGAGGA	229	NM_204628.2
GAPDH	F: GGAGTCCACTGGTGTCTTCA R: GACCCTCCACAATGCCAAAG	233	NM_204305.2

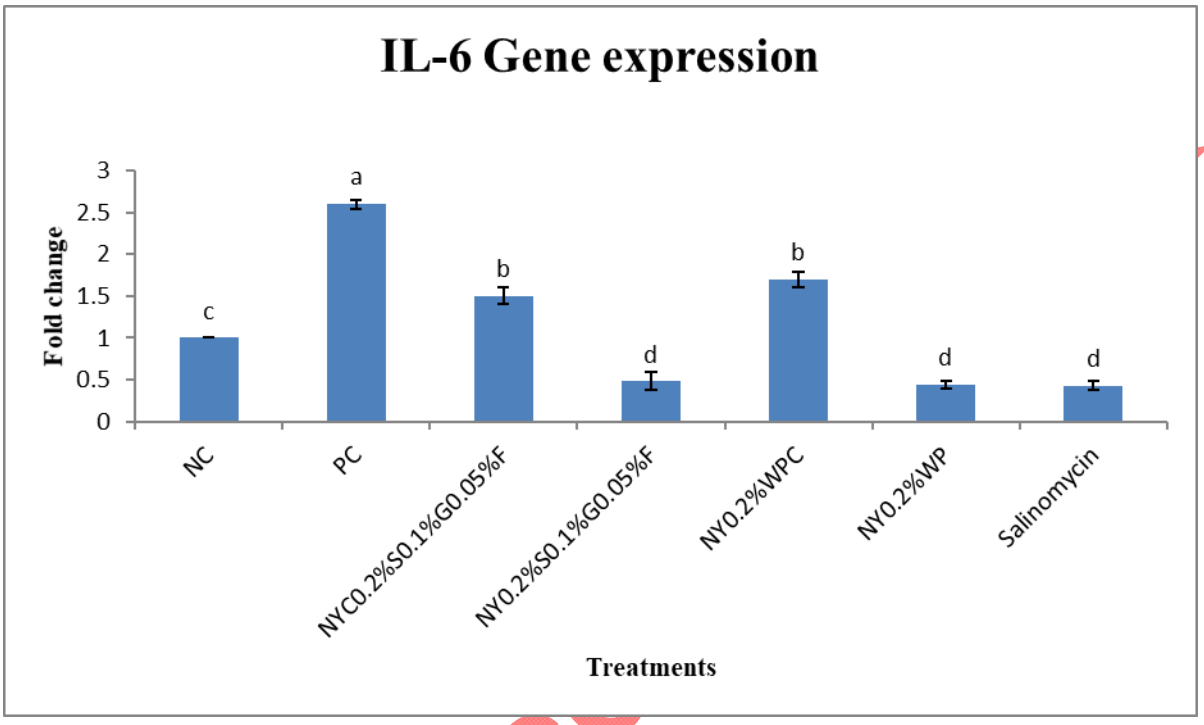

Figure: Relative IL-6 expression in the ileum on d 42. Data are presented as means±SE, n＝10,