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Research Article

Efficacy of Probiotics on Health Status and Growth Performance of *Eimeria tenella* Infected Broiler Chickens

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Abstract

A probiotic containing *Pediococcus acidilactici* and *Bacillus subtilis* (Smart ProLive) at a $1x10^7$ CFU/mL dose in drinking water were given continuously from the d 14 to the end of the treatment (d 35) in broiler chickens. Experimental infection was produced by oral gavage of sporulated *E. tenella* oocysts at 14th d of age. Feed consumption (FC), live body weight (LBW) and feed consumption rate (FCR) were measured at weekly basis. Villus height and crypt depth in cecum and ileum, and antibody titers in the blood were performed at 28-day-old. Probiotics appeared to be superior to salinomycin on the villus height and crypt depth of cecum and ileum ($P<0.05$). A numerical, but not significant ($P>0.05$) improvement on the LBW was determined at the groups of probiotic and salinomycin+probiotic than that of control and salinomycin groups. Nevertheless, FC and FCR results of the probiotic and salinomycin+probiotic groups were good than that of control and salinomycin groups. Probiotics were effective on the villus heights and crypt depths than that of salinomycin alone. Salinomycin appeared to be good only than control group in all the parameters. Not a significant difference from antibody titers was existed among the groups. Based on these results it can be concluded that a good source of probiotics can be used as natural antimicrobial growth promoters in replacement with forbidden anticoccidials in broiler rearing.

Keywords: *Broiler, Coccidiosis, Eimeria tenella, Probiotics, Salinomycin, Histology, Antibody*

Probiyotiklerin *Eimeria tenella* İle Enfekte Broiler Piliçlerin Sağlık Durumu ve Verim Performansı Üzerine Etkileri

Öz

Pediococcus acidilactici ve *Bacillus subtilis* içeren bir probiyotik (Smart ProLive) 14. günden 35. güne kadar içme suyu ile birlikte sürekli olarak $1x10^7$ KOB/mL dozunda broiler civcivlere verildi. Deneysel enfeksiyon 14 günlük civcivlere sporlu *E. tenella* ookistleri ağız yoluyla verilerek yapıldı. Yem tüketimi, canlı ağırlıklar ve yemden yararlanma oranları haftalık olarak takip edildi. Sekum ve ileumda villus yüksekliği ve kript derinliği ile antikor titre analizi 28 günlük civcivlerde yapıldı. Probiotiklerin sekum ve ileumda villus yüksekliği ve kript derinliği üzerindeki olumlu etkisi salinomisinden daha üstün bulundu ($P<0.05$). İstatistiki önem ortaya çıkmamakla birlikte ($P>0.05$) canlı ağırlık artışı probiyotik ve probiyotik+salinomisin grubunda control ve salinomisin grubuna göre daha yüksek bulundu. Ancak, yem tüketimi ve yemden yararlanma konusunda probiyotik ve probiotik+salinomisin grupları kontrol ve salinomisin gruplarındakinden üstündü. Salinomisin tüm parametrelerde sadece control grubundan üstün olabildi. Antikor titreleri bakımından gruplar arasında fark gözlenmedi. Elde edilen bulgular ışığında iyi bir probiyotiğin kullanımı riskli ve direnç oluşumuna neden olabilecek antikoksidiyaller yerine doğal antimikrobiyal büyütme faktörü olarak kullanılabileceği öne sürülebilir.

Anahtar sözcükler: *Broiler, Koksidiyozis, Eimeria tenella, Probiyotik, Salinomisin, Histoloji, Antikor*

INTRODUCTION

The poultry industry is one of the most important food of animal origin suppliers in the world. The global poultry production has been stated to be 111.000 thousand metric tons in 2015, and world poultry production is projected to increase by 24% over the next decades, reaching 131.255 thousand metric tons in 2025. Poultry meat production will be dominating more than half of the growth of all the additional meat produced by 2025 [1]. The poultry meat

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market is growing fast, with a significant increase in production with time [2-4].

Coccidiosis is a major parasitic disease of poultry with great economic impact, which mainly affects the intestinal tract of birds. The clinical and economic importance of coccidiosis is likely to remain unchanged during the coming decades as long as commercial poultry is reared in large numbers at high densities, which seems necessary to make the poultry industry profitable [5]. Anticoccidial drugs play a major role in combating this disease caused by protozoan parasites of the genus *Eimeria* both therapeutically and prophylactically [6,7]. Nevertheless, extensive use of anticoccidials has led to the development of anticoccidial drug resistance [8]. Even with the shuttle and rotation programs there is no method to fully prevent drug resistance [2,9].

Fortification of feeds of food animals with sub-therapeutic doses of antibiotics to protect against infections and to promote yield performances has recently been an unwanted situation in the point of the view of the public health [10]. Sub-therapeutic uses of drugs are perceived to lead to microbial resistance, as well as consumer concerns regarding residues in food products. The relatively recent ban of sub-therapeutic doses of certain antibiotics as feed additives in the European Union led to a general decline in animal health [11].

Drug resistance and consumer concerns regarding drug usage has been a motivating factor to the practice of live vaccines to control coccidiosis. Vaccines have been stated to provide an alternative for disease protection, capable of limited efficacy as they induce specific protective immunity by exposing the chicken's immune system to *Eimeria* antigens [9,12-14]. However, some drawbacks to live vaccines have been stated to occur. Subunit vaccines may circumvent most shortcomings of live vaccines; however, at present these products has stated to be underperform due to the lack of immunogenicity [5]. Immunity to avian coccidiosis has been stated to be strongly species-specific, therefore the bird will only develop immunity to the species of *Eimeria* present in the vaccine [12,13]. Also, vaccine application to the post-hatch chickens has not found to be so easy to apply [15]. Some secondary infections such as necrotic enteritis may occur after vaccine application [9,14].

The ban on the use of antibiotic growth promoters results in higher feed costs [14]. It has been concluded that future coccidiosis control is unlikely to be achieved solely by using anticoccidial products as feed additives and/or through feed composition and management [5]. Use of anticoccidial drugs and vaccines are generally considered to be successful. Due to the issues related to the, as well as the impending ban on animal feed additives, researchers has recently focused on 'natural' alternatives of drugs to controlling and managing coccidiosis [5].

Alternative controls include nutritionals and probiotics (immunomodulators) or natural feed additives [16-19]. Some of the bacteria used as probiotics are *Lactobacillus, Pediococcus, Bacillus*, *Saccharomyces cerevisiae* and *Enterococcus faecium*. Direct fed microbials (DFM) are include *Aspergillus oryzae* and *Bacillus subtilis* and also found to be useful [20]. In *E. acervulina* infected broilers, lower intestinal development of coccidiosis and lower oocyst production have been explained by enhanced local cell-mediated immunity when a *Lactobacillus*-based probiotic supplemented diet has been used [21]. In a study performed with a *Pediococcus*-based commercial probiotic given to birds infected with an *E. acervulina* or *E. tenella* infection, increased resistance of birds against coccidiosis and a partial protection against growth retardation has been demonstrated [22]. In another study, a *Pediococcus*- and *Saccharomyces*-based probiotic has given to birds infected with 5000 oocysts of either *E. acervulina* or *E. tenella* and less oocyst shedding and a better antibody response has been found in probiotic fed birds compared to non-probiotic controls [23]. Probiotic supplementation is one option currently being explored as a means of reducing the amount and severity of enteric diseases in poultry and subsequent contamination of poultry products for human consumption [24,25].

Numerous efforts to date have been implemented in the control of avian coccidiosis caused by the *Eimeria* parasite. Since the appearance of anticoccidial chemical compounds, the search for new alternatives continues. Today, no product is available to cope with the disease; however, the number of products commercially available is constantly increasing [2]. The objective of this study was to comparatively evaluate the effect of a commercial probiotic product (in the manufacturers' demonstration on the bag, it contains $\geq 1\times10^{11}$ CFU/mL probiotics, *Pediococcus acidilactici* and *Bacillus subtilis)*, alone or in combination with the anticoccidial medicine salinomycin on broiler performance and intestinal health to *E. tenella* infection as evaluated by growth parameters, histological alterations within the intestine, and response to routine vaccines.

MATERIAL and METHODS

Preparation of Sporulated E. tenella Oocyst Suspension (inoculum)

For preparation of artificial infection material of coccidiosis, bloody fecal materials from 15 d old free-range broiler chickens in a local flock were collected and mixed in plastic bags. Then the bags brought to the lab and examined for the presence of presumptive *E. tenella* oocysts in reference to Conway and McKenzie [26]. The positive stool samples were used as the primary oocyst source in our preliminary study. The samples were filtered, centrifuged and sporulated in potassium dichromate at room temperature for seven days. The oocysts were recovered by centrifugation in saturated NaCl solution by washing with distilled water. Then, the material was concentrated by centrifugation and stored

in potassium dichromate solution, quantified in *Neubauer chamber* and stored at 4°C [27,28]. In our preliminary study, sporulated oocyst suspension was passaged in 9 broiler chickens with the age of 7 days for checking pathogenity and cecal localization. Each chicken was placed in one separate plastic pen with plastic mesh bedding. After 10 days of oocyst inoculation, all the birds were euthanized and bloody content and deformations were clearly seen from all the 9 ceca. Not a visual sign of coccidiosis was seen in other parts of the intestines. All the cecal content of the 9 euthanized chickens were collected and sporulated oocysts suspension was prepared as mentioned above and kept in a refrigerator at 4°C until use in the study.

Preparation of Probiotic Drinking Water

A commercial probiotic (Smart ProLive) in the form of 50 g water soluble powder in aluminum bags was purchased from a local Veterinary clinic. It is added to the sterile saline solution (0.9% NaCl, w/v) at recommended dose of 50 mg/L of water, and gently mixed in a sterile Erlenmeyer flask. Total aerobic bacteria were counted from this water. The 10-fold increment serial dilution technique was conducted according to Miller and Wolin [29]. One milliliter of the homogenized suspension was then transferred into 9 mL of 0.9% saline solution (NaCl) and serially diluted from 10^{-1} to 10^{-8} by using the same saline solution tubes. From the last three diluted samples, 0.1 mL each was plated on the Trypticase soy agar (TSA, Merck, Germany) plates and the plates incubated at 37°C for 48 h. All the colonies grown on the plates were counted and results were expressed as $\log_{10}$ colony forming units (CFU) per gram probiotic product. A total of 1.1 x10^{11} CFU/g live bacteria were detected in the probiotic source. After the count of CFU/g of probiotic product, the drinking water of chickens was fortified by addition of 1 g powdered probiotic to 10 L of drinking water to make a probiotic water including 1.1 x10^{7} CFU/mL live probiotic bacteria in it. The probiotic drinking water of the chickens were refreshed 3 d intervals during the experiments. The bags of 50 g probiotic source used in the study kept at room temperature during use as recommended.

Experimental Design and Treatments

The study has been permitted by NEU Ethical Board at Meeting No: 2016/2 held at 12th May 2016. The study performed was a 35-day grow-out with 90 Ross 308 mix sexed broilers housed on 4 plastic mesh cages with 3 replicate pens in each. The birds at 10th day of breeding were purchased from a local farm and transferred to the cages after a 30 min journey. A total of 10 birds (5 male and 5 female) were located in each of 9 pens. The 1st cage was received as the two separate groups such that, the upper pen of the 1st cage was received as control that no medication, probiotics and infection were applied (Control group). The chickens of middle and bottom pen of the 1st cage were fed with salinomycin added feed (0.5 g/kg of feed) during the course (Group S). All the chickens in the three pens of 2nd cage were fed with probiotics via drinking water (Group P). All the three flats of 3rd cage were fed with probiotics and salinomycin (Group SP).

Each of ten birds in each pen was marked by using 10 different colors. The male and female birds were recorded. Weight gain of each separate bird and also feed consumption of each separate pen was recorded weekly. At 14 d of age, the birds were infected with sporulated *E. tenella* oocysts by administering them directly into the crop via an oral gavage of the oocysts suspension by a rubber tube adjusted to a plastic syringe [30]. So, except for Control group, four chickens from each pen that marked with the same colors (2 male and 2 female) were artificially infected with 9×10^{4} *Eimeria* oocysts. The oocysts doses were prepared by the section of parasitology. Mortality was recorded during the experiments. Routine vaccination program was applied for immunization. Air conditioner was used to standardize room temperature to meet Ross 308 handbook [31].

All birds had access ad libitum to their particular diets during all the growth period. Both salinomycin via feed and probiotic via drinking water were given to the chickens from 10th day to 35th day of the experiment. The basal diet was a typical mash corn-soybean meal diet that was bought a local commercial broiler feed producer. The formula of the feed is demonstrated in the *Table 1*.

Performance Parameters

All the chickens were individually weighed at 10 (the d of allocation into the pens), 14, 21, 28 and 35th day. The diets were removed and weighed prior to the weighting of the birds. Weighting was completed in one h in each time. Feed consumption (FC) and live body weights (LBW) were recorded weekly. So, Feed Conversion Ratio (FCR) of each separate pen was calculated by dividing weekly FC to LBW.

Oocyst Shedding

The feces of the chickens were checked for oocyst shedding at daily intervals. Feces samples were taken simultaneously from feces trays of each 9 separate pen once a d after 3 day of oocyst inoculation. The feces samples were examined by using the Fulleborn's saturated salt solution method [32].

Histological Examinations

On d 28, a total of the 36 birds, 4 (one infected male, one infected female, one non-infected male and one non-infected female) from each of 9 pens were sacrificed (Ethical Commission report No. 1324/13.06.2017). Then, the ceca incised. After emptying the content the ceca were washed under mild flowing tap water. For micro morphometric examinations, the entire segments of the ceca were fixed in 10% formalin, embedded in paraffin wax, and sectioned to

Table 1. *Ingredients and calculated nutrients, energy of diet*	
Ingredients	**g/kg (as feed basis)**
Maize	580
Soybean meal	310
Soybean oil	42
Monocalcium phosphate	16
Limestone	17
DL-methionine	2
Lysine HCl	0.7
Chromic oxide marker	25
Vitamin-mineral premix*	3
Salt	4
Calculated nutrients and energy	
Protein	201
ME (Mj/kg)	13.4
Ca	10
P	7
Na-phytate	4.5
Ca-tP	1.4
** Supplied following per kg of diet: retinol, 5.400 IU; cholecalciferol, 2.600 IU; α-tochopherol, 11 IU; menadoine sodium bisulphate; 4,4 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44 mg; choline chloride, 770 mg; cyanocobalamin, 13 µg; biotin, 55 µg; thiamine mononitrate, 2.2 mg; folic acid, 1 mg; pyridoxine hydrochloride, 3.3 mg; I, 1 mg; Mn, 66 mg; Cu, 4.4 mg; Fe, 44 mg; Zn, 44 mg; Se, 0.3 mg*	

give 4-µm-thick serial paraffin sections. Then, sections were stained with hematoxylin-eosin to measure the height of intestinal villi and the depth of intestinal crypts under a light microscopy [30]. Histological sections were examined with a Leica DM500 light- microscope coupled with a Leica Microsystem Framework integrated digital imaging analysis system (Leica ICCSO HD, Heerbrugg, Switzerland). The villous height was estimated by measuring the vertical distance from the villous tip to the villous-crypt junction level for 30 villi per section. The crypt depth (the vertical distance from the villous-crypt junction to the lower limit of the crypt) was estimated for 30 corresponding crypts per section [30].

Immunological Examinations

Broiler chickens were vaccinated with live attenuated vaccines against Newcastle Disease Virus (NDV) Avinew© VG/GA strain (Merial-Lyon-France) and Infectious Bronshitis Virus (IBV) Nobilis© IB 4/91 strain (Intervet International BV.-Boxmeer/Netherland). The vaccination was performed at day 1, day 10 with Nobilis and day 1, day 10 and day 18 with Avinew respectively according to the manufacturer's instructions. On day 28, blood samples from the 36 birds that sacrificed for histological analyses were collected and used for analysis of immune response against NDV vaccine and IBV vaccine. The antibody titers were determined by using commercial ELISA test kits against NDV (Biotech, TW4 5PY Hounslow, UK) and IBV (SL5 8BP Ascot, UK).

Statistical Analysis

The results of the study were subjected to one-way analysis of variance (ANOVA) using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences in experimental treatments were tested using Turkey's honestly significant difference following ANOVA with significance reported at $P<0.05$.

RESULTS

The infection dose determined in our preliminary study, a total of 5000-6000 sporulated presumptive *E. tenella* oocysts were given per chicken to cause the signs of an apparent infection. Oocyst suspensions were given to the each separate 14 day-old chickens via intra-crop tube inoculation to the experiment groups except for the Control Group. In these studies, it was seen that the ceca were highly infected 6th day after inoculation *(Fig. 1)*. The first oocyst shedding in the feces was seen after 6 d of the oocyst inoculation made at 14th day. Except for the Control group, all the groups shed oocysts in the faces from 20th day to 35th day. No oocyst contamination from other groups to the Control group was detected (data has not been shown). Both at the preliminary study and at the experimental study, visual signs of coccidiosis have been determined in the parts of intestines except for the ceca. During the 1st and 2nd weeks of the experiment, feed consumption of Control group was higher than that of other groups. In the other 3 groups, not a significant difference was appeared during this time period. Nevertheless, at the 3th week of the experiment, FC of P and SP groups were significantly lower than that of Control and S groups *(Fig. 2a)*.

At the start d of experiment (d 14), the average LBWs of the chickens were ranged from 396 to 417 and there were no statistically significance between the groups ($P>0.05$). After that week of infection, LBW of the groups differed slightly from each other during experiment. Since LBW of group P and SP were higher than that of Control and S groups, there were no statistical significance during all the time periods of the experiment ($P>0.05$). The Mean ±SD values of LBW of the groups were such that Control (1207±121 g), S (1309±87 g), P (1393±63 g) and SP (1372±126 g) at the d 35 *(Fig. 2b)*.

The FCR of Control group appeared to be higher than that of the other groups during the experiment period. Since the FCR of all groups were high at the 1st week of the experiment, it decreased gradually after this time period. The best result was seen in the P group, and 1.56±0.30 FCR has recorded in the last week of breeding. The second FCR was recorded in the SP group (1.79±0.36) at the same week. The FCR result of salinomycin applied group (Group S) was 2.08±0.49. The results of S group was not better than that of group P or SP. Since the positive effect of probiotics

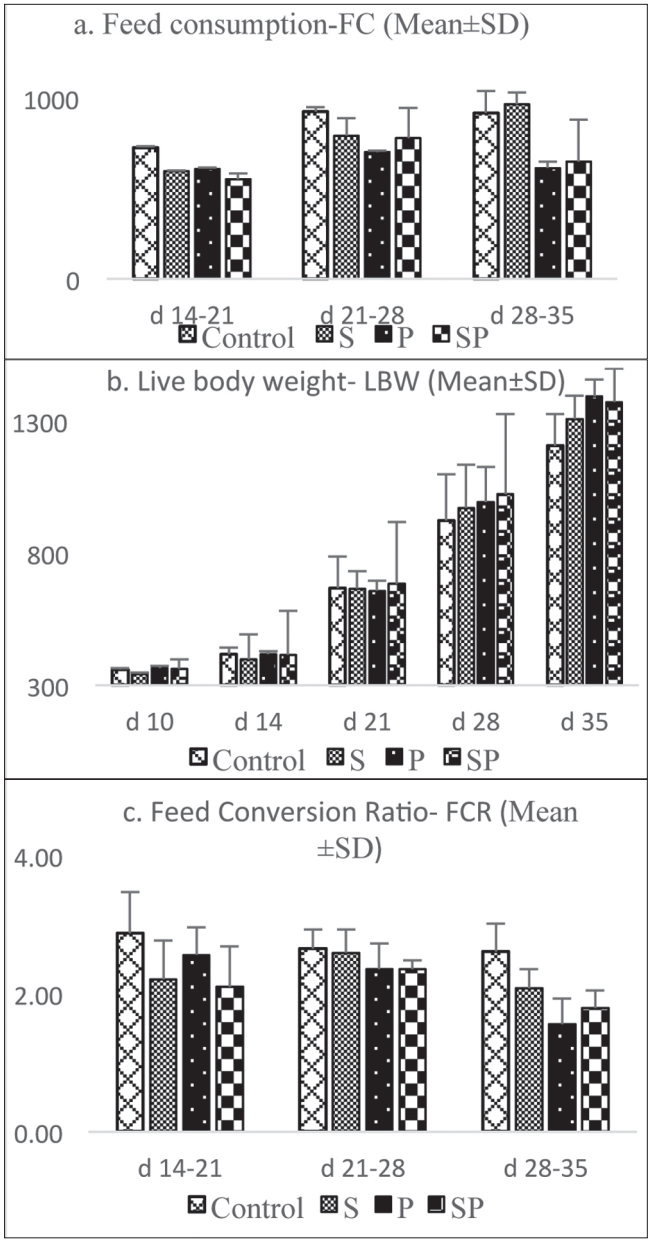

Fig 1. Feed consumption (a), live weight gain (b) and feed conversion ratio (c) results from the study of anticoccidial efficacy of some probiotics in comparison with the anticoccidial medicine salinomycin. Control: control group chickens that neither oocysts nor salinomycin and probiotics were given, S: salinomycin by adding to the feed at a 2.5 g/kg dose, P: probiotics added to drinking water at a dose $1.1x10^7$ CFU/mL, SP; both S and P at the same doses of the groups of S and P were given

alone (the group P) on the FCR was better than that of S and SP groups at the week of oocyst gavage, the positive effect of this group on the FCR was good than that of S and SP groups at last 2 weeks of the experiment. The Control group (FCR 2.61±0.72) and the group S (FCR 2.08±0.49) represented higher results of FCR than that of groups P and SP at the last week of the experiment *(Fig. 2c)*.

In our preliminary studies, ceca of the artificially infected chickens by using *E. tenella* oocysts demonstrated highly infected and were full of blood after 6 days post-inoculation

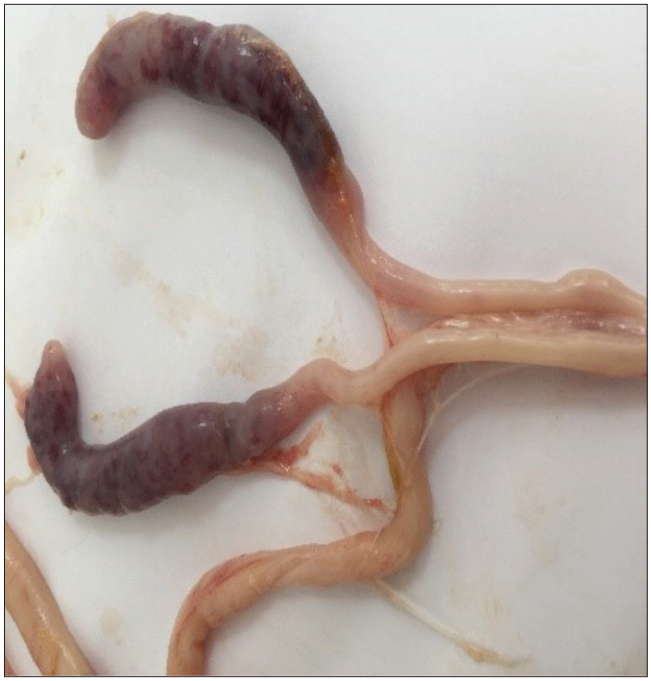

Fig 2. The cecum of a 20 d old broiler chicken infected with *E. tenella* oocysts at the 14th d of its life

(Fig. 1). At the 6th day of post-inoculation of chickens with *E. tenella*, gametocytes and numerous intracellular schizonts containing merozoites were observed between crypt epithelial cells of the cecum. Severe bleeding and erosions from luminal epithelial tissues were seen *(Fig. 3a)*.

The chickens were sacrificed at the day of 28 of the breeding and histological examinations were made. Severe inflammation, infiltrating neutrophils, eosinophil and mononuclear leukocytes were observed in the lamina propria of the ceca of the infected animals *(Fig. 3a)*. In these animals, the villi were partially lost their surface epithelial cells and became atrophic. Some of intestinal glands have also became atrophic and turned to vacuoles *(Fig. 3b)*.

In all the infected groups, the epithelial cells of the villi were mostly prismatic and some flattened. Nevertheless, in the non-infected animals in same group, fully prismatic epithelial layers were seen *(Fig. 3c)*. These results have demonstrated that epithelial tissue disposition and damage have seen clearly in the infected animals. Histological results have demonstrated no shedding of the infection in each pen from infected to non-infected chickens.

Neither villus heights nor crypt depths were different between the cecum of control and S group chickens ($P>0.05$). Also, neither villus heights nor cd were different between the cecum of P and PS group chickens ($P>0.05$). No statistically significant difference have existed between the groups in the point of view of crypt depths of ceca of infected chickens ($P>0.05$). Both P and SP have demonstrated a positive effect on the villus heights of ceca of infected and non-infected chickens ($P<0.05$). P

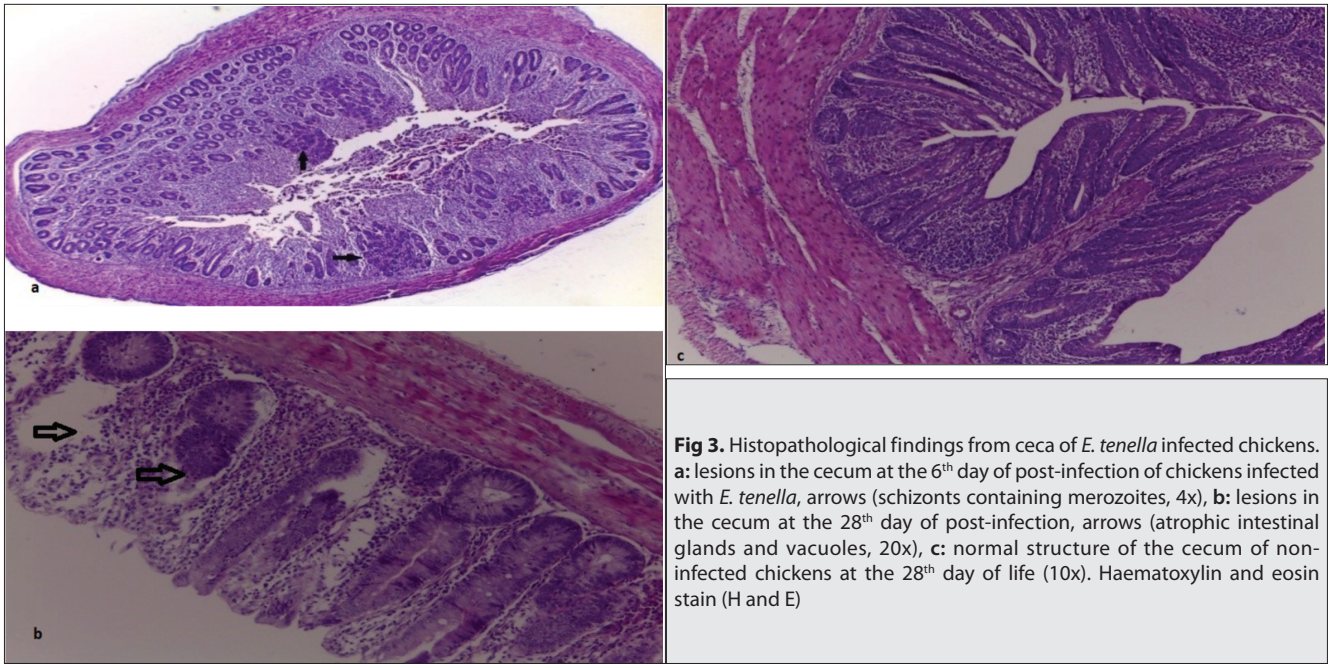

Fig 3. Histopathological findings from ceca of *E. tenella* infected chickens. **a:** lesions in the cecum at the 6th day of post-infection of chickens infected with *E. tenella*, arrows (schizonts containing merozoites, 4x), **b:** lesions in the cecum at the 28th day of post-infection, arrows (atrophic intestinal glands and vacuoles, 20x), **c:** normal structure of the cecum of non-infected chickens at the 28th day of life (10x). Haematoxylin and eosin stain (H and E)

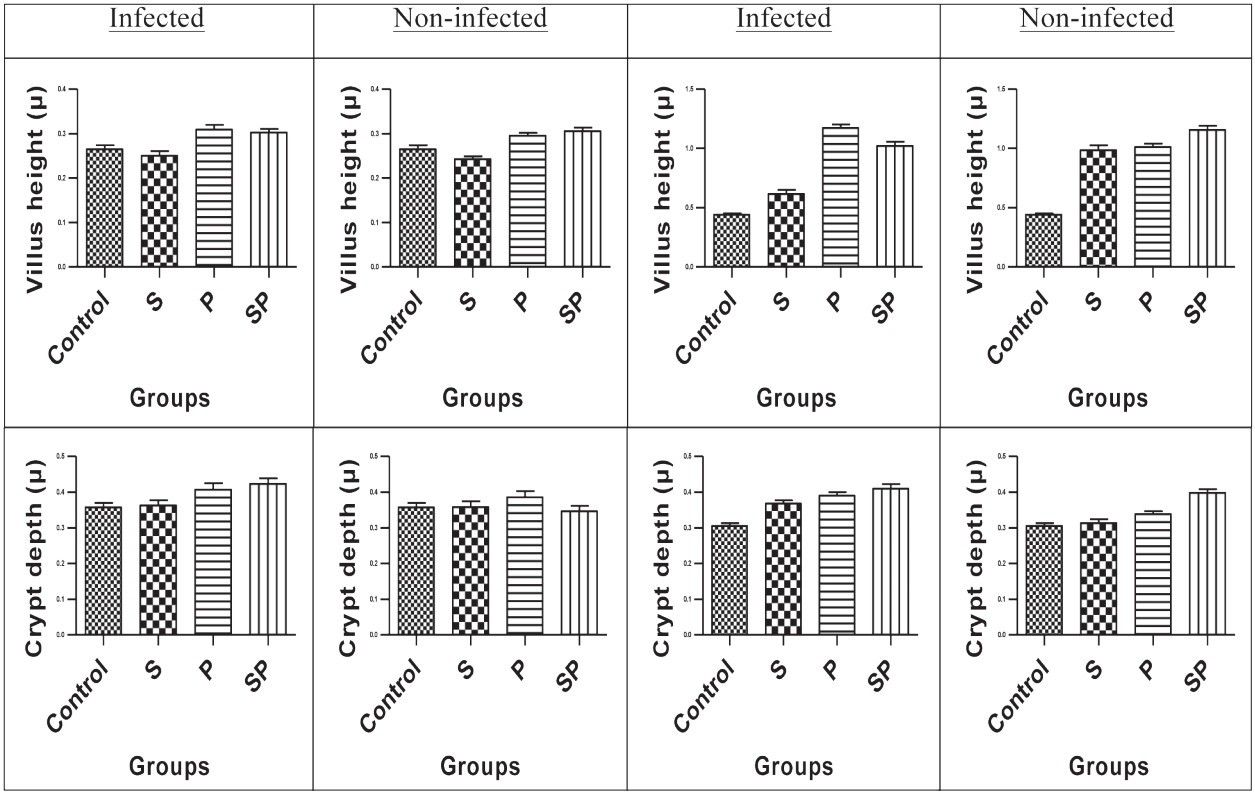

Fig 4. Villus heights and crypt depths (Mean±SEM) of ceca and ileum of infected and non-infected (infected by using *E. tenella* oocysts at the 14th day of life) chickens of 28th day age. Control: control group chickens that neither oocysts nor salinomycin and probiotics were given, S: salinomycin by adding to the feed at a 2.5 g/kg dose, P: probiotics added to drinking water at a dose $1.1x10^7$ CFU/mL, SP: both S and P at the same doses of the groups of S and P were given

appeared to be more effective on the villus heights of ceca of chickens *(Table 2)*.

All the three of S, P and SP appeared to be effective on the villus heights of ileum of infected and non-infected chickens *(Fig. 4)*. Except for a result of no statistical difference between S and P on the villus height of ileum of infected chickens, SP and P appeared to be more effective than S and control samples on the villus heights of ileum of infected and non-infected chickens *(Table 2)*.

Table 2. *Statisitical analysis summary of the villus heights and crypt depths of ceca and ileum of infected and non-infected chickens*

Compared Groups	Cecum				Ileum			
	Villus Heights		Crypt Depths		Villus Heights		Crypt depths	
	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non-infected
Control vs S	No	No	No	No	Yes***	Yes***	No	Yes***
Control vs P	Yes*	Yes**	No	No	Yes***	Yes***	Yes*	Yes***
Control vs SP	Yes**	Yes**	No	Yes**	Yes***	Yes***	Yes***	Yes***
S vs P	Yes***	Yes***	No	No	No	Yes***	No	No
S vs SP	Yes***	Yes**	No	Yes**	Yes***	Yes***	Yes***	Yes***
P vs SP	No	No	No	No	Yes***	Yes***	Yes*	No

Tukey's Multiple Comparison Test; Significant, P<0.05

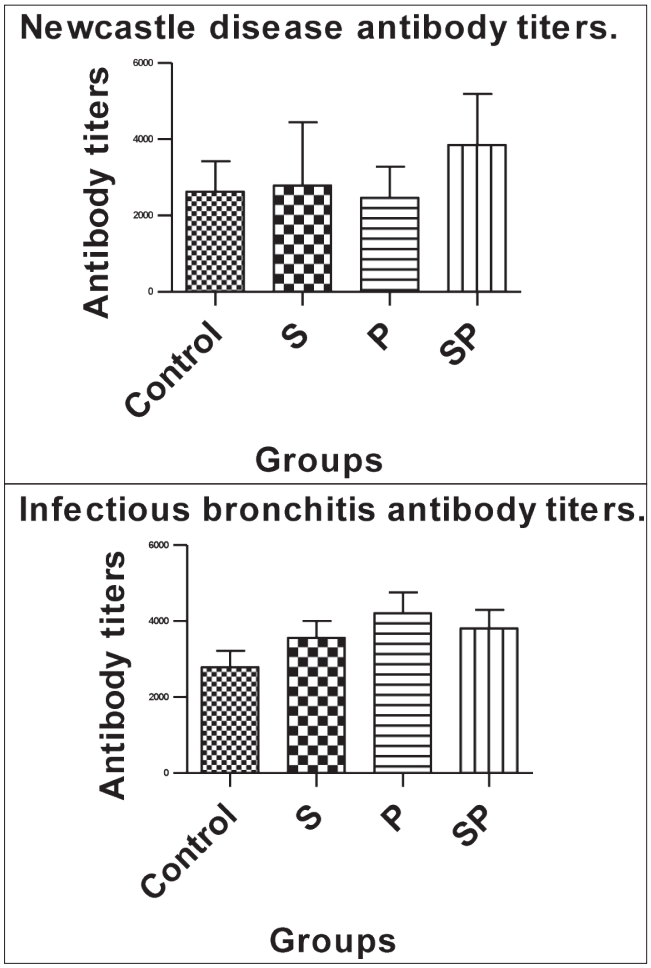

Fig 5. Antibody titers (Mean±SEM) of chickens of 28th day age. Control: control group chickens that neither oocysts nor salinomycin and probiotics were given, S: salinomycin by adding to the feed at a 2.5 g/kg dose, P: probiotics added to drinking water at a dose $1.1x10^7$ CFU/mL, SP: both S and P at the same doses of the groups of S and P were given

Combined effect of S and P appeared to be more effective than that of S or P alone *(Table 2)*. The S or P alone has not demonstrated a good action on the crypt depths of ileum of infected chickens. All the three of S, P and SP appeared to be effective on the crypt depths of ileum of non-infected chickens *(Table 2)*. No statistical difference existed between S and P groups, also between P and SP groups in the crypt depths of ileum of non-infected chickens (P>0.05). The *Fig. 4* is represented to check out the numerical results as figures.

No statistically significant difference has existed among the groups in the point of view of Newcastle or infectious bronchitis disease antibodies *(Fig. 5)*.

DISCUSSION

Poultry meat industry is one of the leading meat producers almost all over the world. Both economical and feasible impacts of the industry are forcing it to grow fast. One cause for this is also fast growing World's human population and accordingly growing the demand of protein of animal origin [4]. The future challenges of the poultry-meat industry regarding sustainability, social acceptance of intensive animal production, and the introduction and dissemination risk of highly infective poultry diseases. Breeding of meat poultry has many problems to solve. One leading problem is disease and accordingly economical loses [33]. Although it is differed from country to country, the most occasionally prevailed diseases are respiratory and digestive system diseases. These diseases include necrotic enteritis, coli-septicemia, infectious bronchitis, chronic respiratory disease, infectious bursal disease (IBD) and Newcastle disease [34]. One of the most important digestive system diseases of broiler chickens is coccidiosis. *E. tenella* is one of the most prevailed causative agents of coccidiosis of the broiler chickens [35]. This protozoon is located basically to the ceca of the chickens. The disease causes to death or sub-latent chronic disease. Even though chicken is not died, the chronic form and sub-clinic form of the disease may cause poor LBW, high FCR and secondary diseases [20,36]. In this study, the visible signs of coccidiosis infection in the ceca of chickens at 6th day of post-infection has clearly demonstrated *(Fig. 2)*.

After the ban of the most of anticoccidial drugs and antibiotic growth promoters, the industry came to face with breeding performance problems and also disease control problems. From the date of 2006, when such restrictions on the use of anticoccidials and AGP's took place, an

emergence for research on new friendly anticoccidials and replacer for AGP have occurred. After the rest of 12 year of this new period many researches have conducted on the subject. Some plant based extracts, live beneficial microorganisms (probiotics) and vaccine applications have been recommended by researchers [2,37,38].

Not a significant difference from antibody titers was existed among the groups *(Fig. 5)*.

AGPs are used as growth enhancers and health promoter of digestive system of meat chickens [8,38,39]. Probiotics also have been recommended as natural grow promoting agents in replacing with AGPs [37,40]. Until now, there is no officially recommended probiotic formula or application method [30,41]. Thus, research results and recommendations have been different from one researcher to another. In this study we have used a combination of two live probiotic strains *(B. subtilis* and *P. acidilactici)* by adding drinking water of broiler chickens at a dose of $1.1x10^7$ CFU/mL from 14 d to 35 d. The results have demonstrated that probiotic use may help problems caused by *E. tenella* infection in broiler chickens. All the results determined in that study have demonstrated superiority of probiotics over salinomycin use. FC, LBW, FCR, villus height and crypt depth values appeared to be good in P and SP groups when compared with the Control and S groups *(Fig. 2, Fig. 4)*.

Health promotion and growth enhancing effect of probiotics have been well documented [36]. Nevertheless, there are some researchers that have not confirmed positive probiotic effect on the broiler chicken growth performance or health status [42-44]. Bino Sunder *et al*.[45] have reviewed that anticoccidial resistance is a big problem for broiler chicken breeders all over the world and probiotic use is one of the promising solutions. The researchers have summarized that probiotics modify receptors on enterocytes and this impairs or destroys sporozites and/or merozoites from pathogenity on enterocytes. Chen *et al*.[38] have also demonstrated that probiotics have been effective on the growth rate and the inflammation of broiler chickens caused by *E. tenella* infection. Health promotion *(Table 2, Fig. 3, Fig. 4)* and growth enhancing effect *(Fig. 2)* of probiotics used in this study were determined clearly and the results have confirmed many other researchers' results [2,36,37,41,46]. At the 3rd week of the experiment, FC and FCR of P and SP groups were significantly lower than that of Control and S groups *(Fig. 2a,c)*. A good source of probiotics applied continuously during all the breeding time period may be a good alternative to AGPs and anticoccidial health promoters in broilers.

Broiler chickens are fast growing animals and bred intensively. The conditions leads the stress and thus immunity of the body and especially the digestive system is of importance. Also, consistency of mucosal layer of intestines, villus heights and also the crypt depths in that absorptive layer are so important both on health and growth performances. Heak *et al*.[20] have evaluated the results of 49 different studies made on the effect of probiotics on the epithelial tissue of the small intestines of chickens and only 32 of them have favored the DFM over control on villus heights. Nevertheless, the researchers has not been determined the positive effect of DFM on the crypt depth when checked the 96 studies made before. Our results are in agreement with that 32 studies, and we also demonstrated the positive effect of probiotics on villus heights of cecum and ileum *(Table 2, Fig. 4)*. Taheri *et al*.[42] have also determined positive effect of probiotics on villus height. Ştef *et al*.[47] have also demonstrated the positive effect of probiotics on the growth performances, gut health and disease prevention. Heak *et al*.[20] have demonstrated that there have not been a significant positive effects of probiotics on the crypt depths bot in cecum and ileum (n=96 comparisons in research studies). We determined in this study that probiotics were more effective on the villus heights and crypt depths both in the ceca and ileum of chickens both infected and not. Differences among the results of the studies may be due to difference from analysis days, or any other factor such as breeding strategy, difference between probiotic strains, etc.

Almost all the researchers have chosen about 7th d after oocyst gavage in their hispathological studies. These researchers have occasionally chosen that day for scoring the gross lesions of intestines visually [38]. In this study, we chosen the day of histological examination day as the 28th day of broiler life. At the 14 d post-infection, examination of ceca might be more valuable since gross lesions would be recovered and health of the ceca and ileum after recover from infection would be more efficiently determined by histological examinations. Neither infected nor control chicken have demonstrated visible lesion from their intestines (data has not shown). In our preliminary studies, we demonstrated the difficulty of analysis of the ceca at the week of infection due to gross lesions and bleedings. So, we think that not the week of infection but 1 week after oocyst shedding would be chosen for histological examinations of intestines to check out the effect of probiotics on the health status of intestines of broiler chickens.

In this study, we determined the positive effect of daily water based feeding with a mix strain probiotic source at a $1.1x10^7$ CFU/mL in the drinking water during whole feeding period can enhance the natural resistance to the *E. tenella* infection. Giannenas *et al*.[41] have also demonstrated such results in their study. The researchers recommended a multi-strain probiotic use for a natural protection against coccidiosis in broiler chickens. Ritzi *et al*.[18] have also suggested in their study that probiotic supplementation via drinking water can be alternative to AGPs and can enhance performance and help alleviate the negative effects of a mixed *Eimeria* infection. Ariyadi and Harimurti [48] have also suggested that probiotics may

stimulate proliferation of intestinal epithelium regulate mucosal barrier formed by mucin in the intestine of broiler chickens. Giannenas *et al*.[30] have also suggested that a mixture of probiotic substances has given considerable improvement in both growth performance and intestinal health in comparison with infected control birds an fairly similar improvement to an approved anticoccidial during a mixed *Eimeria* infection. Contrary to these findings, Lu *et al*.[49] have demonstrated the superiority of salinomycin to a commercial probiotic and some other natural DFM alternatives.

In conclusion, the results of the present study suggest that in the absence of in-feed anticoccidial drugs, treatment with probiotics could alleviate impact of coccidiosis infection on broiler chickens. Beneficial effects of probiotics on the intestinal health could minimize the side effects of coccidiosis. Economical loses due to the infection and also public health concerns due to use of DFMs could be minimized. Future researches on the use of probiotic sources as alternative to AGPs and anticoccidial drugs can support growing regimes that include no AGPs and such medicines.

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