Protective role of Multi-strain Probiotic Bacteria against Eimeria in Broilers

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الدور الوقائي للمعزز الحيوي البكتيري متعدد العتر ضد جنس الأًيميريا في فروج اللحم

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المستخلص

تجربتا البحث تشير إلى التحقق في الفعل الوقائي الكامن والمحتمل لبكتريا المعزز الحيوي متعدد العتر PSM في فروج اللحم. تجريبياً تمت إصابة أفراخ فروج اللحم بالأيميريا نوع Eimeria brunntti أو Eimeria mivati ، وفي هذه الدراسة تم فحص البيض الكيسي المطروح مع الزرق، استجابة الأجسام المضادة المناعية والتغيرات النسجية المرضية في فروج اللحم المغذى على المعزز الحيوي PSM. أفراخ فروج اللحم نوع Ross 308 بعمر ١ يوم غذيت على عليقة اعتيادية أو عليقتي المعزز الحيوي بتركيز 0.1% و 0.2% وباستمرار من عمر ١-٣٢ يوم. تجريبياً أصيبت الأفراخ فموياً بالطور الكيسي لبيض E. brunntti بالعدد ٥٠٠٠ أو ١٠٠٠٠بيضة كيسية وبالعدد ٥٠٠٠ لبيض E. mivati بشكل متوالي في اليوم ١٠ و١٢ من العمر.عدد البيض الكيسي المطروحة حصل فيه قلة (P□ 0.05)واضحة في المجاميع المصابة بالنوعين E. brunntti و E. mivati والمغذاة على عليقة 0.1% PSMبالمقارنة مع المجموعتين الأخريين. مجموعة الإصابة E. mivati والمغذاة على عليقة 0.1% PSM شهدت زيادة معنوية (P□ 0.05) في مستوى الأجسام المضادة بالمقارنة مع مجموعة العليقة الاعتيادية، النتائج الحاصلة تبين ان المعزز الحيوي PSMحفز وبفعالية مقاومة الأفراخ المناعية ويحمي ضد النمو السلبي المتصاحب مع داء الأكريات خصوصاً في مجموعة 0.1% PSM. التغيرات النسجية المرضية للفائفي أظهرت قوام نسجي طبيعي مع زيادة طول الزغابات باستخدام PSM بالمقارنة مع مجموعة السيطرة. المعزز الحيوي متعدد العتر يتداخل مع مواقع ارتباط الجرثومة وينتج مضادات جرثومية ببتيدية ويحث الاستجابات المناعية لجسم المضيف خصوصاً مناعة السطوح المصلية وكل ذلك يعزز المقاومة ضد الجراثيم المعوية المصيبة لفروج اللحم مثل أجناس Eimeri spp.

الكلمات المفتاحية: الأيميريا، المعزز الحيوي، الأجسام المضادة، التغيرات النسجية المرضية، فروج اللحم.

Abstract

In the present research, two trials were conducted to investigate the potential protective effects of multi-strain probiotic bacteria in broiler chickens experimentally infected with Eimeria brunetti or Eimeria mivati. In the present study, we examined oocysts shedding; antibody immune responses and histopathology differences of broilers fed the commercial probiotic Poultry Star Me (PSM). Day-old Ross 308 broiler chicks were fed either a regular broiler diet or one of two probiotic diets supplemented with 0.1% PSM or 0.2% PSM. Chicks were orally challenged with 5000 or 10000 sporulated oocysts of Eimeria brunetti or with 5000 Eimeria mivati oocysts on day 10 or 12 of age, respectively. In Eimeria brunette and Eimeria mivati-infected birds, the PSM 0.1% group showed reduction (P□□0.05) in oocyst shedding in birds infected with 5000 Eimeria oocysts as compared with the other two groups. In Eimeria mivati-infected birds, Eimeria-specific antibody levels were higher (P□□0.05) in the PSM fed groups, especially in the PSM 0.1% birds, compared with the regular diet group. These results demonstrate that this PSM probiotic effectively enhances the immune resistance of birds and protects against the negative growth effects associated with coccidiosis (Eimeria infestation), particularly when supplemented at 0.1% PSM of the diet. Histopathology changes revealed normal architecture and greater vilus height was obtained in ileum with the use of PSM in relation to the control group. Multi-strain probiotic bacteria interfere with the pathogen binding sites and produce antimicrobial peptides and induce host immune responses especially mucosal surfaces immunity, thus enhancing resistance against enteric intestinal pathogens like Eimeria spp.

Key words: Eimeria spp., coccidiosis, probiotic, antibody, histopathology and broilers.

Introduction

Avian coccidiosis is the major protozoa parasitic disease of poultry, and their causative agent called Eimeria causing mortality, mal-absorption, inefficient feed utilization, impaired growth rate in broilers and reduced egg production in layers (1). Drugs and live vaccines are the two main control measures of the disease; however, due to increasing concerns with prophylactic drug use and the high cost of vaccines, alternative control methods are needed. The disease presents tremendous economic significance to the poultry industry, with an estimated worldwide annual loss of more than 3billion$ (2, 3, 4 and 5).

Some commercial probiotic bacteria have been found to enhance development of both the intestinal epithelia and the gastrointestinal lymphoid system (6, 7). A balanced microbial population would support the inherent defense mechanisms of a healthy intestinal tract, resulting in better control of intestinal pathogens (8). Probiotic bacteria exert antagonism against other microorganisms, including most enteric pathogens, primarily through the production of lactic acid and secretion of bacteriocins (9).

Materials and methods

Experimental Designs

Experiment 1: In the initial study, 70 day-old Ross 308 broiler chicks were used to evaluate the protective effects of PSM feeding against E. brunetti. Multi-strain probiotic (Poultry Star Me®:PSM) of Biomin® Company contains four genus of probiotic bacteria (Lactobacillus, Pediococcus, Enterococcus and Bifidobacterium). This product contains a minimum of $2x10^{11}$ CFU/kg was applied in accordance to instructions of manufacturer. Broilers were randomly assigned to 7 pens (n = 10/pen) of an electrically heated battery and were fed a regular non medicated broiler starter diet either without a probiotic (30birds; REG diet) or with the PSM supplemented at the rate of 0.1% (PSM 0.1) or 0.2% (PSM 0.2) of the diet (20 birds each). At 10 day of age, 10 birds from each group were inoculated with either 5000 or 10000 sporulated E. brunetti oocysts (total of 30 birds for each inoculation rate), whereas the remaining 10 birds of the REG diet served as negative controls and were placed at 2birds/cage (5 cages/treatment). Histopathology changes were measured at 0 and 10 day post inoculation (dpi); Specimens were collected from the ileum. They were fixed in 10% buffered neutral formalin. Paraffin sections (thickness: 5 microns) were prepared and were stained by Hematoxylin and Eosin stains (H&E) (20) and examined microscopically. Fecal samples were collected from 5 cages from 6 to 9 dpi.

Experiment 2: In the second trial, 120 day-old Ross 308 broilers were randomly assigned to 12 pens (n =10/pen) of an electrically heated battery and were equally assigned to 1of 3 experimental diets (4 pens/diet group): REG, PSM 0.1%, or PSM 0.2%, as in experiment 1. At 12 day of age, half of the birds from each diet group (n=20) were then orally inoculated with 5,000 E. mivati-sporulated oocysts, and 10 birds from each treatment were placed at 2birds/cage (5 cages/treatment) as in experiment 1.In addition to measuring oocyst shedding (6 to 10 dpi), serum samples were collected 10 dpi, and Eimeria-specific antibody titers were determined by ELISA. Histopathology exam measured as in experiment 1. All diets were formulated to meet or exceed the nutrient requirements for broilers as recommended by the (10). Feed was provided ad libitum, and the animal trials were performed according to the guidelines established by the Beltsville Area Institutional AnimalCare and Use Committee.

Oocyst Shedding

Oocyst shedding was measured as described by (11). Briefly, droppings from 10 birds in 5 cages (2birds/cage) were collected for 4 to 5 day starting on 6 dpi, fecal material was ground and homogenized, and two 35- mL samples were taken, diluted, and the oocysts were counted microscopically using a McMaster counting chamber. The total number of oocysts was calculated using the following formula: total oocysts/bird = oocyst count□ □x dilution factor□ □x

(fecal sample volume/counting chamber volume)/number of birds per cage.

Serum Antibody Levels

Blood samples were obtained 10 dpi from individual birds (n = 10/group), allowed to clot for 4 to 5 hour at 4□C, and the sera were collected. Serum samples were tested for Eimeria-specific antibody Ab levels using ELISA and the optical density was determined with a micro plate reader at 450 nm (12).

Statistical Analyses

Mean values for fecal oocyst shedding, and Ab titers were compared by the Tukey-Kramer multiple comparisons test following ANOVA, using InStat software (GraphPad, San Diego, CA). Differences between means were considered significant at P □□0.05.

Results:

Oocyst Shedding

Figure 1 shows the mean oocyst shedding per bird of Eimeria infected groups on either regular or probiotic supplemented diets. The uninfected control (REG) groups excreted no oocysts. Feeding 0.1% PSM significantly (P□□0.05) reduced oocyst shedding in birds infected with 5000 E. brunetti oocysts and E. mivati (Figure 1, panel A and B) more than the infected REG birds.

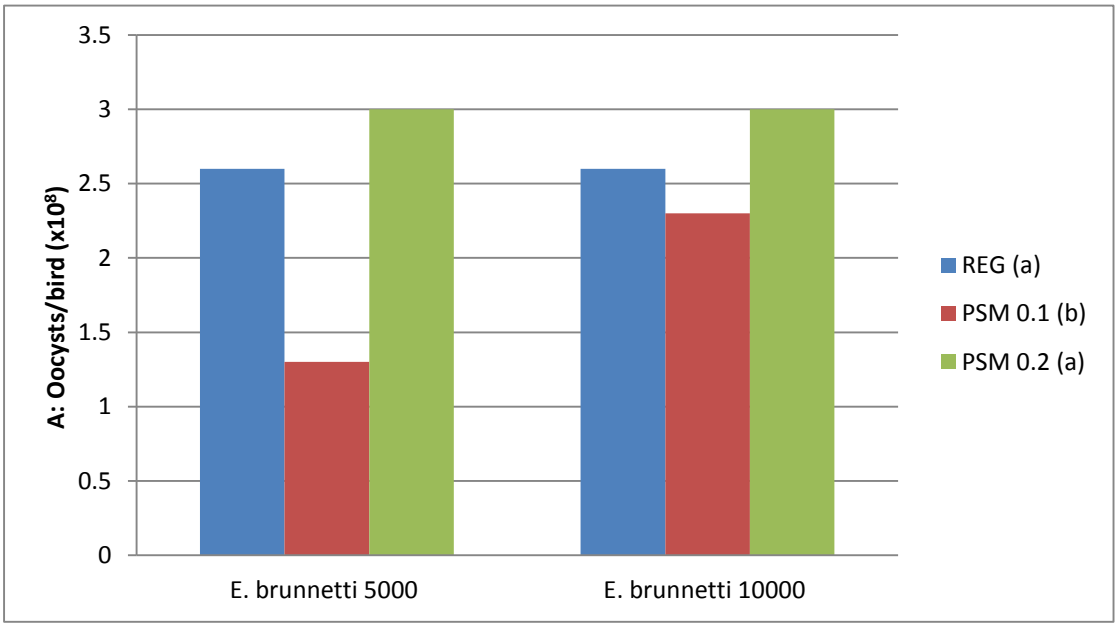

Figure 1 A. Fecal oocyst shedding of broiler chickens fed regular (REG), 0.1 or 0.2% PSM-supplemented diets (PSM 0.1 and PSM 0.2, respectively). Oocysts counted in fecal material collected 6 to 9 day post inoculation with 5000 or 10000 Eimeria brunetti oocysts at d 10 post hatch. Each bar represents the mean ± SD (n = 5). a, b Means lacking common letters differ in uninfected or infected chickens (P< 0.05).

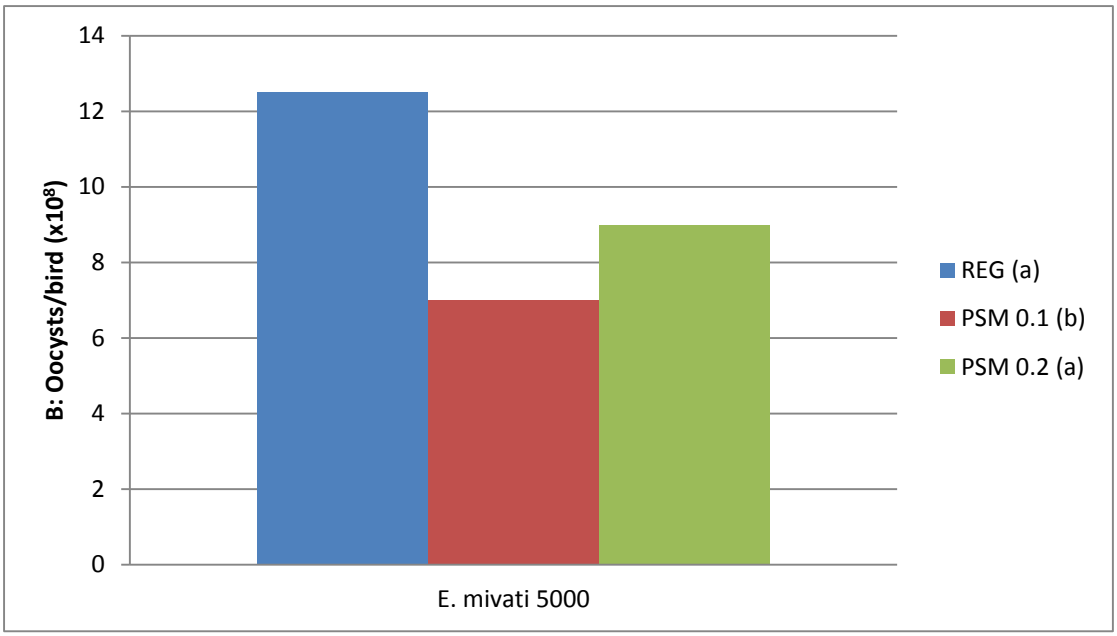

Figure 1 B. Fecal oocyst shedding of broiler chickens fed regular (REG), 0.1 or 0.2% PSM-supplemented diets (PSM 0.1 and PSM 0.2, respectively). Oocysts counted in fecal material collected 6 to 10 day post inoculation by 5000 Eimeria mivati oocysts at d 12 post hatch. Each bar represents the mean ± SD (n = 5). a, b Means lacking common letters differ in uninfected or infected chickens (P< 0.05).

Specific Antibodies Response

To assess antibodies responses to Eimeria antigen, EmMIC2 was used in this study, and the ELISA results are shown in Figure 2. There was no significant difference in Ab_s response to Eimeria antigen among all uninfected groups. However, in the 5000 E. mivati-infected and PSM fed birds, significantly (P□□0.05) higher serum Eimeria-specific Ab levels were detected when compared with those of birds infected with 5000 E. mivati fed a regular diet without probiotic.

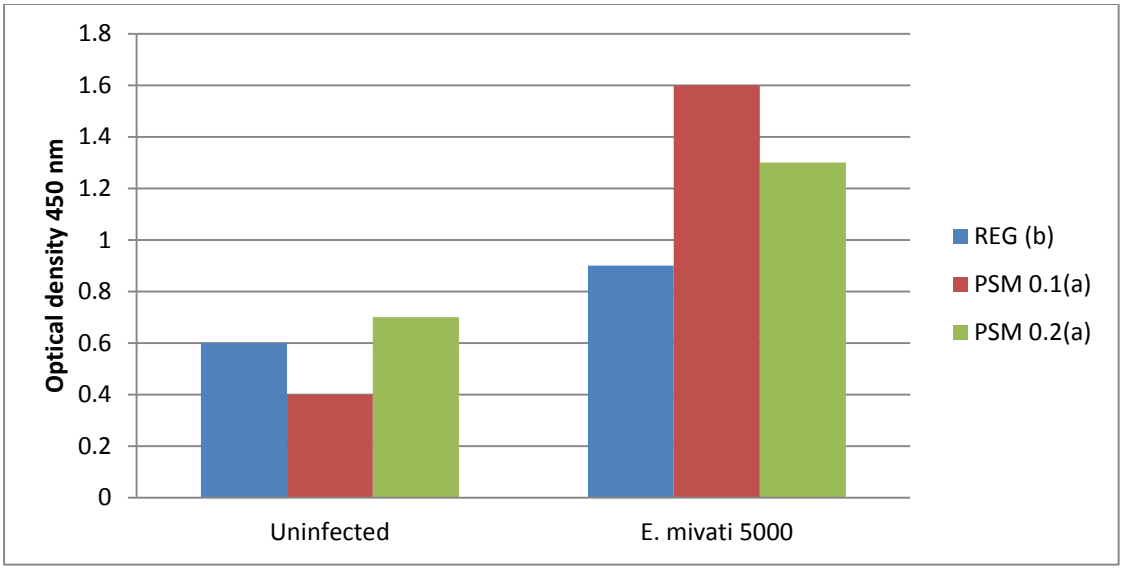

Figure 2. Anti-EmMIC2 antibody response of broilers fed non probiotic (REG), 0.1 or 0.2% PSM-supplemented diets (PSM 0.1 and PSM 0.2, respectively) for 21 d. Birds were either uninfected or infected with 5000 Eimeria mivati oocysts at d 12 post hatch, and sera were sampled 10 d post inoculation. Each bar represents the mean ± SD (n = 10). a,b Means lacking common letters differ in uninfected or infected chickens ($P < 0.05$).

Histopathology Findings

Histopathological changes showed necrosis in crypts and villi of intestine (ileum) although there is invasion of oocysts in Emeria infected non PSM supplemented birds (figure 3).

Ileum architecture reveals severe destruction of crypts and villi, also shortening the height of iliac villi, in addition to coagulative necrosis in Emeria infected non PSM supplemented birds (figure4).

Histopathological changes in ileum of PSM supplemented birds showed normal architecture, no Eimeria oocysts invasion in intestine, prominent height of ileac villi and there is no intestinal necrosis as in figure 5.

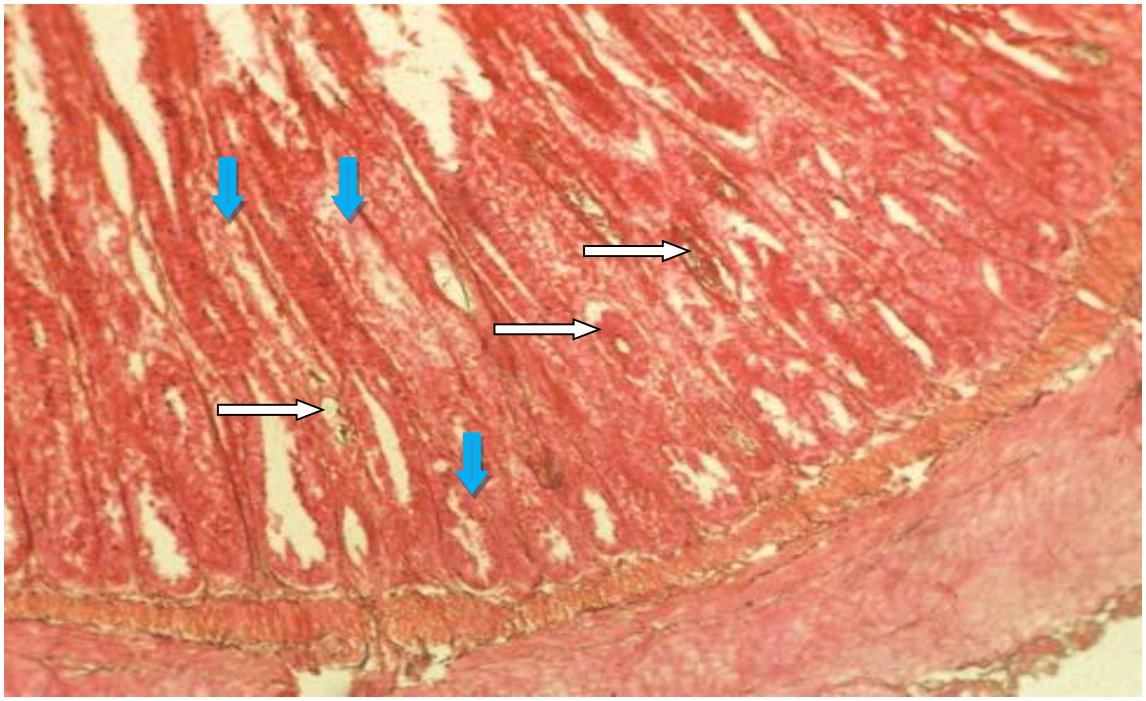

Figure 3. Shows Eimeria oocyst invasion in villi, crypts of ileum (white horizontal arrows), also prominent coagulative necrosis (blue vertical arrows) in non-supplemented PSM infected Eimeria birds (H&E stain X125).

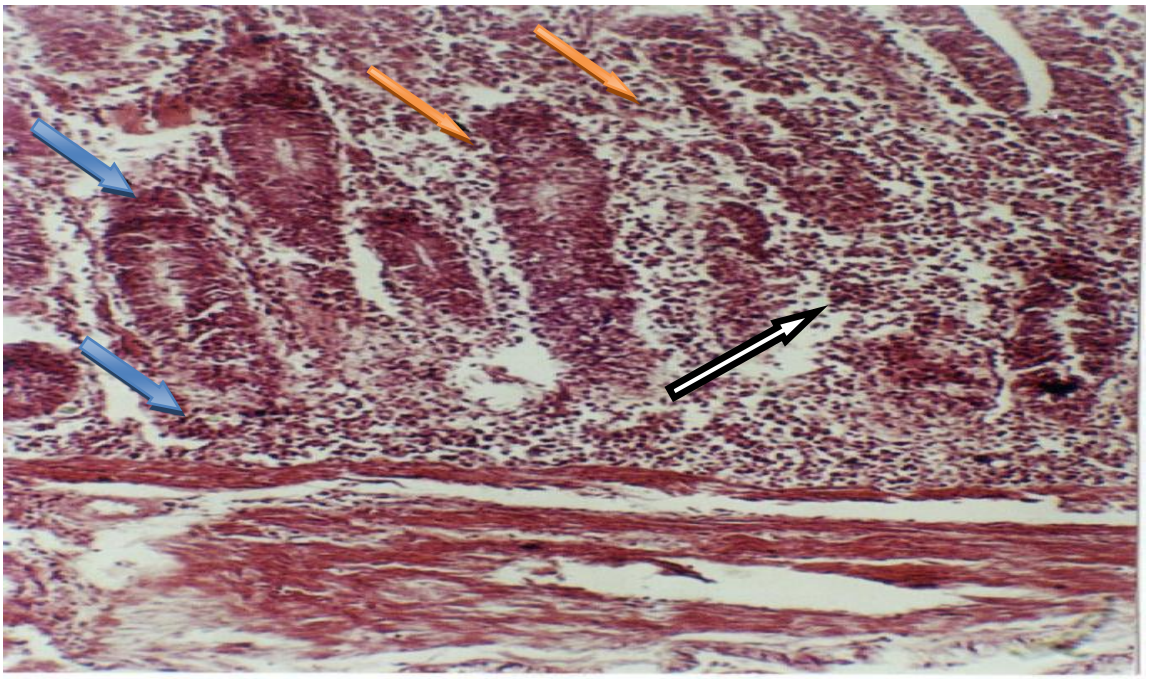

Figure 4. Shows shortness in villi of ileum (blue arrows), destruction of intestinal architecture (white arrow), and prominent coagulative necrosis (yellow arrows) in infected Eimeria- non supplement PSM birds (H&E stain X125).

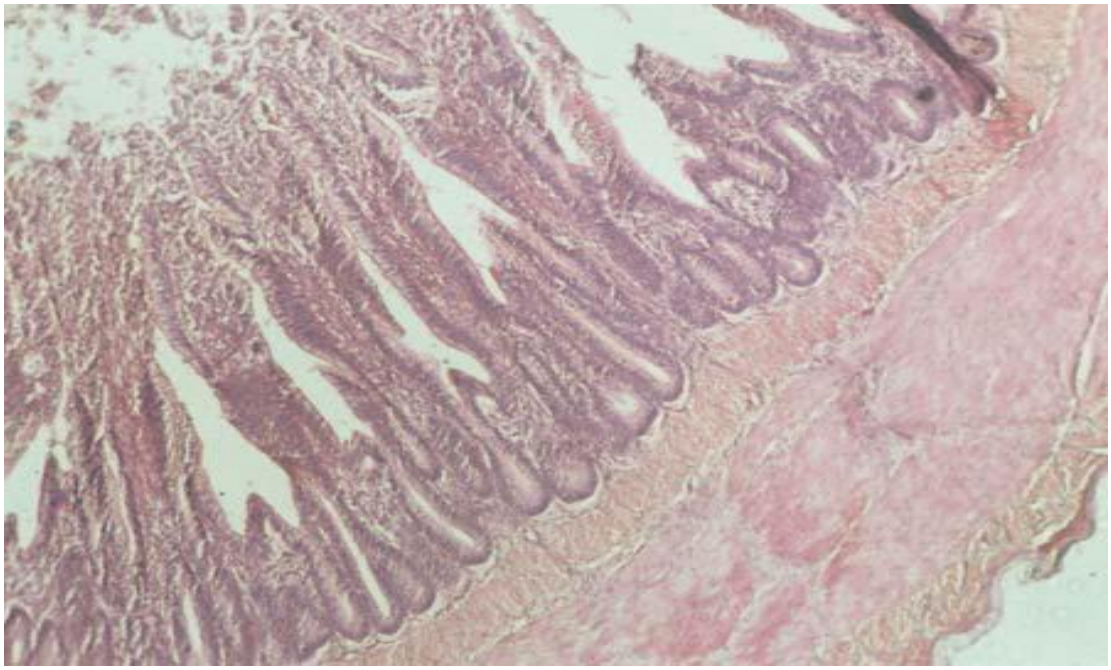

Figure5. Shows normal ileum architecture and prominent normal villi and crypts in challenged Eimeria- supplement PSM birds (H&E stain X125).

Discussion

The present study was conducted to investigate the beneficial effects of multi strain probiotic bacteria PSM on susceptibility of chickens to (Eimeria infestation) coccidiosis.

PSM enhanced resistance to experimental E. brunetti infection was best exemplified by reduced oocyst shedding, particularly in those birds infected with 5000 Eimeria brunetti oocysts and Eimeria mivati oocyst. Although, it is desirable to see positive effects with both parameters, direct correlation between histopathology observation and reduction in Eimeria oocyst shedding has always been the case correlated together with probiotic studies (13). The differential effect with the two Eimeria species tested could be attributed to the species-specific infection sites, where probiotic organisms may favor colonizing one site over the other (14). Administration of PSM induce protective immunity against E. brunetti and E. mivati infection, some strains of probiotic bacteria species produce antimicrobial peptides (bacteriocins) that inhibit gram-positive spoilage and pathogenic bacteria (15;16). These bacteriocins they have been shown to exert high antimicrobial activity against infectious agents (17). In the current study, we showed that multi-strain probiotic bacteria provided some degree of defense against E. brunetii and E. mivati infections in broiler chickens.

The microneme protein EmMIC2 was cloned from E. mivati (18, 19, 13). Additionally, EmMIC2 represents 1 of nearly 30 Eimeria genes that have been cloned and characterized at the molecular level (20). Eimeria specific antibodies to EmMIC2 antigen were significantly (P□□0.05) higher in chicken fed the PSM diet in E. mivati-

infected birds. The role of parasite-specific antibodies has been extensively studied in coccidiosis (21, 22, and 13). Although, humoral immunity to coccidiosis seems to play a minor function (23, 3). Eimeria infections trigger a significant specific antibody immune response in serum (13), and immune globulins could have a contributory function in the defense of the host against Eimeria (24, 25). Eimeria mivati-infected birds fed PSM produced more parasite-specific antibodies in the blood circulation, and these Ab mediated responses may play a more protective role against a secondary E. mivati infection than a single inoculation, as was the case in the present work. Histopathology findings refer to localizing of Eimeria oocysts and coagulative necrosis in villi crypts of ileum that reflect on the destruction of ileac architecture and shortness of villi in ileum of Eimeria infected birds that un supplemented with PSM (figure 3, 4) this agree with (4, 13 and 26) they refer to moderate to heavy infections of Eimerias, the tips of villi are broken off, while there is normal and healthy ileac architecture and either no Eimeria oocyst localization, no necrosis and also no villi destruction in Eimeria infected birds that supplemented with PSM (figure 5).

Lactobacillus acidophilus is reported as a nonpathogenic and nontoxic bacterium inducing healthy intestinal conditions in sheep (27).The probiotic bacteria competes pathogen on microbial adhesion and binding sites to prevent its invasion and in other hand produce organic acids that alter intestine medium from alkaline to acidic medium who undesired and harmful for infective pathogens like Eimeria spp., also induce and modulate mucosal surfaces immunity reflect on general immune response both of them enhance body immune defense (28, 29 and 30).

In conclusion, these results demonstrate that a PSM enhances the resistance of birds and creates protection against coccidiosis infestation.

However, the mechanistic details mediating such protection are not fully understood and remain to be clarified, especially in light of the wide array of immune cells activated by probiotic bacteria. In particular, analysis of the different cytokines and chemokines induced by feeding PSM will provide valuable new information on its protective immunity to coccidiosis.

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