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Drug-Independent Control Strategy of Coccidiosis in Broiler Chickens Using Prebiotic, Probiotic and Symbiotic

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Abbreviations: Preb=Prebiotic, Prob=Probiotic, Symb=Symbiotic, S=Salinomycin, GIT=Gastrointestinal tract FCR=Feed conversion ratio, BW=Body Weight, BWG= Body Weight Gain, PC=Positive control, NC=Negative control, PI=Post infection, BF=Bursa of Fabricus, T1=Chicken group supplemented with microencapsulated phytonutrient, T2=Chicken group supplemented with an acidifiers blend, T3=Chicken group supplemented with microencapsulated phytonutrient and an acidifiers blend, , ND=Newcastle disease, HI=Haemagglutination inhibition, CDS=Coccidial developmental stages, V/C ratio=Villi hight/Crypt depth ratio.

Abstract: *One day-old male Arbor Acres plus broiler chickens (n=700) were used to study the effects of dietary supplementation of Prebiotic (Preb) [inactivated dry yeast derivative containing selected yeast strains including Saccharomyces cerevisiae and Cyberlindnera jadinii], Probiotic (Prob) [Saccharomyces cerevisiae boulardii] and Symbiotic (Symb)[mixture of these preb and prob] in comparison with Salinomycinon (S) on production performance, gut integrity, immune status under coccidian challenge. Seven dietary treatments were used: basal diet for positive and negative controls (PC and NC), basal diet with 2 concentrations of the Preb (400 and 800 ppm), basal diet with S (66 ppm), basal diet with Symb (Preb 200 ppm and Prob100 ppm) and basal diet with the Prob (100 ppm). The used dietary supplements enhanced resistance to experimental Eimeria spp. infection by reducing macroscopic and microscopic lesion scores, improving productive performance variables [body weight (BW), body weight gain, feed conversion ratio (FCR), production number and some of carcass characteristics]. GIT integrity as well as cell mediated and humoral immunity were improved in supplemented groups vs. PC group. Such protection was further reflected by reduced oocyst shedding, particularly in those birds supplemented with Symb. Since Preb, Prob and Symb are generally recognized as safe status, they could be considered as drug-independent control strategy of coccidiosis.*

Keywords: *Probiotics; Prebiotics, Symbiotic, Coccidiosis, Broiler chickens, Performance, Gut integrity.*

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I. Introduction

Chicken GIT provides a means by which the body derives nutrition, furnishes protective mechanisms to safeguard the host and serves as an environment for other living organisms **(Aarestrup,1999)**. Maintaining the balance of good gut health is a key aspect of ensuring the best bird performance and health **(Perry, 2006)**. Coccidiosis is a major and of almost universal importance parasitic disease in poultry production caused by the apicomplexan parasite *Eimeria*. **(Lee *et al.*, 2007; Blake *et al.*, 2015)**. *Eimeria species* are intracellular parasites that must invade the host epithelial cells to replicate. It is a serious intestinal disease in chickens which causes many losses in the poultry industry **(McDonald and Shirley, 2009)**. The protozoan parasites of the genus *Eimeria* causes tissue damage, with resulting interruption of feeding and digestive processes or nutrient absorption; dehydration; blood loss; and increased susceptibility to other disease agents. The financial loss to the poultry industry as a result of coccidiosis worldwide has been estimated annually at US $3 billion (**Williams, 1999, Dalloul and Lillehoj, 2006**). Coccidiosis infection provides a good example of the effect that poor villi development can have where the villi become shortened and their tips are eroded with reducing gut surface area **(Perry, 2006)**. Currently, coccidiosis is controlled mainly with drugs **(Xu *et al.*, 2013)**. However; despite the development of better anticoccidial drugs in the past 50 years the original problem remains unresolved **(Awaad *et al.* , 2003a, Amer *et al.*, 2010)**. Aiming for better prophylactic results against coccidiosis comparison of mixtures of coccidiostates have already evaluated as anticoccidial drugs **(Awaad *et al.*, 2001)**. Due to the increased drug resistance and concern of public health, alternative strategies are required and invented **(Sharman *et al.*, 2010)**. Nevertheless, possible upcoming bans restricting the use of anticoccidials as feed additives, consumer concerns on residues and increasing regulations have prompted the quest for alternative

coccidiosis control strategies **(Peek and Landman; 2011)**. Vaccines played a role in coccidiosis control for a long time, however; their applications still remain very limited with consideration for the safety, costs and demands for high techniques of the farmers or veterinarians **(Peek and Landman, 2011).** Unfortunately, no cross-immunity exists between species of *Eimeria* in birds, and later outbreaks may be the result of different species **(McDougald, 2008)**. Cost-effective vaccines and/or drugs are urgently required to control the diseases caused by the phylum Apicomplexa pathogens, but their complex life cycles and naturally occurring genetic polymorphism makes the development of such vaccines an extremely difficult task **(Blake *et al.,* 2015)**. Another limiting factor for the use of vaccines against coccidioses is that the inclusion of several species of *Eimeria* in one vaccine can cause further depression in BWG, FCR, and a potential vaccine failure **(Dalloul and Lillehoj, 2006)**. A few anticoccidial products have been tested as potential alternatives to drug or vaccine, these include herbal extracts (**Du and Hu, 2004)**, live microbial supplements **(Lee *et al. ;*2007, Lutful- Kabir, 2009, Stringfellow *et al.,* 2011, Awaad *et al.,* 2013, Abdelrahman *et al* 2014, Bozkurt *et al.,* 2014)** and antibodies **(Crane *et al.,* 1988, Smith *et al.,* 1994, Karim *et al.,* 1996)**.

To lay a foundation for alternative strategy to control experimentally induced coccidiosis in broiler chickens; the present investigation was dedicated to evaluate the potential protective effects of a Preb (a synergistic alliance of specific strains of inactivated yeast including *Saccharomyces cerevisiae* and *Cyberlindnera jadinii*), Prob (*Sacharomyces cervicia boulardii)* and Symb (mixture of these Preb and Prob) in comparison with S supplementation.

II. Material And Methods

The prebiotic (Preb): A combination of fractions of inactivated dry yeast derivative of different strains of *Saccharomyces cerevisiae* and *Cyberlindnera jadinii* produced by Lallemand, SAS, France under the name YANG. Batch 510145 E was used.

The probiotic (Prob): A subspecies of *Saccharomyces cerevisiae boulardii* produced by Lallemand, SAS, France under the name LEVUCELL SB 10ME Titan (LSB). Batch 87A1761P312 was used.

The symbiotic (Symb): A combination of the aforementioned Preb and Prob was used.
Diets: Chickens fed *ad libitum* a mash commercial starter diet (23% crude protein and 3000 kcal ME/kg diet) during the first 2 weeks of age, commercial grower diet (21% crude protein and 3100 kcal ME/kg diet) from 2-4 weeks of age, and then commercial finisher diet from 4-6 weeks (19% crude protein and 3200 kcal ME/kg diet). Birds had free access to water. Neither anticoccidial drugs nor antibiotics were added to water supply.

Coccidial oocysts: A mixture of field *Eimeria spp.* oocysts collected from 10 different chicken broiler houses suffering from either intestinal or caecal coccidiosis (or both) were used in experimental infection.

Experimental chickens: One day-old male Arbor Acres plus broiler chickens (n=700) without administration of any coccidian vaccine were assigned into 7 equal groups (1-7) consisting of 100 birds each (4 replicate pens of 25 chickens each) and offered either supplemented or non-supplemented feed. All experimented chickens were floor reared in separate pens at a density of 10 birds/m^2 with fresh wood shavings as bedding with a thickness of approximately 10 cm on a concrete floor and kept in environmentally controlled rooms. Chickens of all groups were vaccinated via intra-ocular route and subcutaneous route with Hitchner B1+H120 vaccine and avian influenza inactivated H5N2 vaccine at 7^{th} and 10^{th} day of age, respectively. La Sota vaccine and 228E IBDV vaccine were given at 14^{th} and 18^{th} day of age respectively via intra-ocular route.

Experimental design: Duration of the trial extended from one day of age up to slaughter (42 days). Chickens of groups 1 and 2 dietary were supplemented with Preb at a dose of 400 and 800 ppm respectively. Those of group 3 received Symb (Preb and Prob at a dose of 200 and 100 ppm respectively). Those of group 4 received S at a dose of 66 ppm. Those of group 5 were supplemented with Prob at a dose of 100 ppm (i.e. $1x10^9$ CFU/kg). While groups 6 and 7 were kept without treatment and served as NC and PC respectively. On day 14 of age, 3 chickens per replicate of groups 1-5 and 7 were tagged and infected by crop gavages with 10^5 of a mixture of sporulated oocysts of *Eimeria spp.* field isolates and mixed with their replicates in infected groups (as seeder birds) for induction of natural exposure. All groups ran contemporaneously.

Measured parameters:
Productive performance: Chicken performance response variables were determined according to **Brady (1968)**, **Sainsbury (1984) and North (1984)**. For BW and BWG; all birds were weighted individually at 1^{st} day and weekly for the entire period of the experiment (6 weeks). Feed consumption measured on the same days of birds weighting. FCR (g feed/g live body wt.), and production number **[**that equals (kilograms of growth per day *

(100 - mortality%)
/ FCR) * 100 after **Timmerman *et al.*, (2006)** was also estimated. Carcass characteristics (dressing%, front part %, hind part %, breast meat %, thigh drumstick %, carcass meat %, heart wt. %, gizzard wt. %, liver wt.%, giblet wt.%,
and intestinal length and diameter) were measured on 10 birds of each group at the end of the experiment (42 days). The mortality rates were also recorded.

Coccidiosis lesion scoring: On d 28 and 42 of age (d 14 and d 28 PI), intestinal lesions of 4 birds (other than seeder birds)/replicate (16 birds/group) were randomly selected, euthanized, and scored for severity of macroscopic coccidian lesions where the upper, middle, and cecal regions of the intestinal tract scored, using the system of **Conway and Mckenzie (1991)** when scoring mixed coccidian infections. Four areas of the intestine were individually examined. The serosal surface was examined first, and the intestine was cut open to see the mucosal surface. A score of 0 to 4 (0 = no lesions.,+1 = mild lesions, +2 = moderate lesions, +3 = severe lesions and +4 = extremely severe lesions or death) was recorded for each chicken for the four following regions: The duodenal and upper intestine, the middle intestine, the lower intestine or ileum and the rectum and the ceca were examined.

Bedding oocyst counting: On day 0, 7, 14 and 21 PI;10 fresh fecal samples of contact birds were collected per pen, pooled, homogenized and oocysts per gram of excreta counted as follows:10 g of litter were soaked in 100 ml of tap water for 24 hours at 4°C in a 200 ml beaker that was tightly covered (either with a lid or Para film).The beaker was shaken vigorously and the litter was filtered through a single thickness of muslin (q.s. filtrate to 100 ml). A 15 ml centrifuge tube was filled with filtrate to 1 cm from the top and centrifuged for 5 minutes at a speed that concentrates the solids. The supernatant was discarded. The pellet was re-suspended in a few milliliters of saturated salt solution (NaCl) with a Vortex, or by gently tapping the tube. More salt solution was added to the original 15 ml volume and the tube was capped and inverted several times. Samples were removed with a Pasteur pipette, and a McMaster counting chamber was filled. The oocysts float to the top of the solution, and the total number was counted with the following calculation: Number of oocysts per gram of litter = n/0.15× volume × 0.1 (Where n = number of oocysts counted,0.15 = volume of the McMaster counting chamber, Volume = 100 ml of water that the litter was in, and 0.1=Correction for 10 g of litter originally taken) (**Hodgson, 1970; Long and Rowell, 1958 and Long *et al.*, 1976).**

Litter condition scoring: On d 7 and 28 PI, litter conditions were graded from 0-5.The following point scale was used to grade the quality of the litter/bedding: 0 = dry, friable material throughout the pen; 1 = predominantly dry material but with some evidence of crusting around drinkers and feeders; 2 = litter material is mostly acceptable but with some areas of wet shavings or capped material; 3 = poor quality litter material with a large proportion of wet areas and capping of the litter; 4 = unacceptable litter quality-wet and capped but with a few areas of dry material remaining; 5 = all litter is wet and soggy, no dry areas left (**Abdelrahman *et al.* , 2014).**

Gut Morphometry and Histopathological assays: At the end of the experiment (42 days), 4 birds of contact birds from groups 1-7 were randomly chosen and sacrificed (one bird/replicate) for gut morphometry. One cm-thick samples were taken from duodenum and jejunum [the intestinal segmentation according to **Samanya and Yamauchi (2002)** as jejunum from the bile duct to Meckel's diverticulum]. Routine histological laboratory methods were adopted and villous histomorphometry for recording the histological indices measured using digital photography and light microscopy. The photos were taken and morphometric analyses was performed. The villous height measured from the apical to the basal region and the crypts from the basis until the region of transition between the crypt and the villous. Five measurements per section had made for each parameter and averaged into one value. For histopathological assay; specimens from duodenum, midgut (from the duodenum past the yolk sac diverticulum), lower small intestine (from the yolk sac diverticulum to the caecal junctures) and caecal regions from all groups were collected and fixed in 10% buffered formalin. Paraffin-embedded sections routinely prepared and stained with Hematoxylin and Eosin **(Bancroft *et al.* 1996)**, and scored for histopathological lesions according to the method described by **Rosales *et al.* (1989)**.

Immune status assessment: To investigate the possible effect of dietary supplements on the humoral immunity; an immunoassay was adopted. For this purpose, blood samples were collected from wing vein from 10 randomly chosen birds (other than seeder birds) at weekly intervals (1-5 weeks of age) from each group. The serum samples were subjected to haemagglutination inhibition (HI) test for determining antibody titers against ND vaccination employing 8 haemagglutinating (HA) units **(Swayne *et al.*, 1998).**To investigate their effect on cell mediated immunity; phagocytic activity of macrophages, lysozyme and nitric oxide activities were applied

on blood samples taken at d 7 and 21 PI on 4 randomly chosen contact birds per group (1 bird/replicate) **(Muller *et al*., 1995)**.

Statistical analyses: One-way analysis of variance using SAS software general liner models procedure **(SAS Institute 1999)** were adopted. The main factors were Preb, Prob, Symb and S treatments. Mean values assessed for significance using Duncan's multiple range test with significance set at $P<0.05$. All percentage values were transferred to arc-sine before the analysis **(Snedecor and Cochran, 1980).**

III. Results And Discussion

All infected chicken groups showed diarrhea that was bloody in some individual cases. Determination of productive performance variables revealed that all dietary supplemented groups resulted in significant higher final BW vs. PC group at d 28, 35 and 42 of age. Among these groups, the highest significant BW at d 42 was observed in Preb 800 supplemented group ($P\leq0.05$). Moreover, all the supplemented groups resulted in numerical higher BW compared to NC group provided that Preb 800 showed a significant increase at 6th week of age. FCR was significantly improved in all supplemented groups vs. PC group at d 35 of age. Nevertheless, Preb 800 and S supplemented groups showed a significant improvement in FCR over other supplemented groups at d 42 ($P\leq0.05$). BWG recorded significant increase at periods 21-28 and for 1-42 days of age in all supplemented groups and NC group vs. PC group ($P\leq0.05$). No significant differences in mortalities were recorded in different treatments (ranged 4 to 9%) **(Table 1)**.The production number was significantly increased in all supplemented groups over PC group ($P\leq0.05$) **(Table 2)**. There were significant increases in some of carcass characteristics including; dressing, front weight, breast meat, carcass meat, liver weight, giblet weight percentages as well as intestinal length and diameter in all treated groups vis. PC group ($P\leq0.05$) **(Table 3)**. The mechanism by which the obtained significant increase in relative weights of some carcass characteristics is not known as the effect of Prob on organ weights in animals is equivocal **(Olnood *et al.*, 2015).** It is already established that the most prominent symptom of avian coccidiosis is growth retardation which is characterized by reduced BW and high mortlity rate causing a major economic effect to the poultry industry **(Dalloul and Lillehoj, 2006**). Obtained productive performance variables (BW, BWG and FCR) in the present study are on line with those reported by other authors **(Huang *et al.;* 2004, Xu *et al.;* 2013, Olnood *et al.;* 2015).** The beneficial effect of Prob supplementation to broiler diet in terms of improved BW and FCR is recorded in studies of several research groups **(Jin *et al*.; 2000, Kalavathy *et al*., 2003, Awaad *et al*., 2003a, O'Dea *et al*.; 2006, Timmerman *et al*., 2006, Onderci *et al*., 2008, Bansal *et al*., 2011** and **Cao *et al*., 2013).**

Significant alleviation of macroscopic lesion score has been observed in Preb 800, and Symb supplemented groups at d 7 PI as well as in Preb 400 and 800, and Symb supplemented groups vs. PC group at d 28 PI. Nevertheless; numerical reduction in lesion score has been recorded in birds treated with Prob and S groups at d 7 and 28 PI vs. PC group. Moreover; it is worthy to mention that at d 28 PI, Symb supplemented group did not show any gross alteration in GIT and gave zero lesion score ($P\leq0.05$) **(Table 4)**. The significant alleviation in macroscopic lesion score recorded in the used dietary supplements in the present study is similar to that reported by **Xu *et al.* (2013)** on studying the protection efficacy of multivalent egg yolk immunoglobulin against *Eimeria tenella* infection in chickens.

Histopathological alterations and microscopic lesion scores are shown in **Figs1-3** and **Table 4**. The microscopic examination of PC group revealed various histopathological alterations involving the different intestinal segments. The lesions were acute and severe in small intestine and became chronic in distal intestinal segment (mainly cecum). The inflammatory reaction was significantly reduced in all supplemented groups in comparison with PC group. The number of CDS were markedly reduced in all treated groups vs. PC group. However, a significant reduction was achieved in Symb supplemented group which in turn affected the inflammatory reaction in intestinal mucosa and reduced it. Caecal lesions in PC group revealed atrophy and hyperplasia of cecal epithelium and crypts with cystic dilatation of cecal glands with formation of crypt abscess associated with mononuclear cell infiltration in cecal stroma while the cecal tonsils showed moderate depletion of lymphoid elements and atrophy **(Fig. 3).** These lesions were markedly reduced in other supplemented groups with reduction in CDS number together with hyperplastic proliferation and increased mitosis of lymphoid elements comprising the cecal tonsils. Amelioration of cecal lesions with stimulation of lymphoid tissue were achieved in Preb 800 supplemented group followed by Prob, Synb, Salinomycin then Preb 400 groups, respectively. The used dietary supplements in the present study not only reduced the establishment of *Eimeria spp.* in GIT of broiler chickens but also reduced CDS with their degeneration. Moreover, addition of both Prob and Preb together (Symb) maximized the anticoccidial effect (resulting in greater efficacy), which might be attributed to their synergistic action. Symbiotic is defined as a mixture of Prob and Preb that beneficially affects the host by improving the survival and the implementation of live microbial dietary supplements in GIT, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health promoting bacteria **(Gibson and Roberfroid,1995, Roberfroid, 1998 , Schrezenmeir and de Vrese, 2001,**

Afify *et al.*, 2003). Similar results have been shown to prevent the establishment of other pathogens in GIT of chickens on using probiotics and prebiotics **(Gustafson and Bowen, 1997, Lee *et al.*,2007). Bozkurt *et al.* (2014)** evaluated Salinomycin, Probiotic and Prebiotic, in *Eimeria spp.* infected broilers and showed a significant improvement in BWG and FCR with a coccidiosis-causing agent and reduced the severity of coccidiosis lesions**. Lee *et al.* (2007)** hypothesized that the Prob (*Pediococcus acidilactici*) interferes with the pathogen infection sites, produce antimicrobial peptides, or induces host immune responses, thus enhancing its resistance to enteric pathogens like *Eimeria.* **Abdelrahman *et al. (*2014)** reported that the Probbacteria may compete for attachment sites and occupy common receptors on the epithelial cells, reduce infiltration by motile *Eimeria* parasitic stages, and consequently, their replication and shedding.

In the present investigation; although all possible measures to prevent contamination of NC group to keep them free from *coccidian* infection were adopted; *Eimeria species* oocysts has been detected in the fecal material of this group recording macroscopic lesion score (1.00) and some microscopic lesions **(Figs.1-3)**. This might be attributed to the ubiquitous nature of *coccidia* and the possibility of mechanical transmission of *Eimeria* species (**Graat *et al.*, 1994, Abdelrahman *et al.* ,2014)**.

Improvement of intestinal histomorphology shown in **Table 5** (increase villi length, shallow crypt depth and increase V/C ratio) in supplemented groups vs. the PC and NC groups illustrates their positive influence on the microstructure of GIT and its absorptive function. This finding is in complete accordance with those reported by **Olnood *et al.* (2015)**. It is already established that shortening and fusion of villi results in loss of surface area for digestion and absorption of food **(van Dijk *et al.*, 2002)**, whereas the converse is true with longer villi and shallower crypts **(Chiou *et al.*, 1996)**.This might be mediated by a direct increase in dietary energy digestibility or absorption, by a decrease in the energy required for the maintenance of GIT, or a combination of both. Numerous studies have suggested that the effectiveness of a Prob for growth stimulation of birds would be the final result of a positive effect on GIT ecosystem resulting in improved intestinal environment, integrity of the intestinal mucosal barrier, digestive and immune function of intestine and broiler health (**Awaad *et al.*, 2003b, Tellez *et al.*, 2006, Mountzouris *et al.*, 2010**). Regarding our results; the significant reduction in duodenal microscopic lesion score achieved in Symb supplemented group improved intestinal integrity and positively affected the intestinal histomorphometry (the highest villi with the increased V/C ratio). Cosequently; the increased intestinal health performance and intestinal absorption affect in turn bird's health as compared to lower villi height with decreased V/C ratio that reflects the increased epithelial turnover in response to increased number of coocdia.

Eimeria spp. oocyst count on d 7 PI in all supplemented groups was numerically lower than PC group at all examined intervals (d 7, 14, 21 and 28 PI) **(Table 6)**. Litter condition score at d 21 and 42 were significantly lower in different supplemented groups (except Preb 400 supplemented group) vs. PC group **(Table 7)**.

The immune status assessment in the present investigation clarified that the used dietary supplements stimulated different subsets of immune system (Humoral and cell mediated immunity) that in turn played a role in the induction and regulation of the immune response **(Tables 8 and 9)**. The recorded enhancement of phagocytic activity corrected the depression in phagocytosis caused by *Eimeria spp.* infection and played an important role in the control of coccidiosis in the present investigation. Phagocytes (macrophages) are part of the non-specific first line of defense because of their ability to engulf and degrade invading microorganisms **(Sharma and Tizard, 1984)**. Macrophages perform a variety of functions other than phagocytosis; they act as secretor cells, secrete many different proteins such as lysosomal enzymes and cytokines (that play a key role in regulating immunity) **(Tizard; 1996, Stafford *et al.*, 2002)**. Immune modulation of Probiotics and Prebiotics is already well documented in enhancing birds' immune response against *Eimeria spp.* infection, ochratoxicosis and immune dysfunction in chickens **(Dalloul *et al.*, 2003, 2005, Awaad *et al.*, 2005, 2013)**. **Stringfellow *et al.* (2011)** reported that probiotic treated chickens showed an increase in lymphocyte proliferation on day 14th of age with higher levels of heterophil oxidative bursts at day 7 which confirm that probiotic treatments are very useful in modulating the immune response. The Preb used in our study is a product considered as a good source of mannan-oligosaccharides and β-glucans that known for their immunomodulatory effects during an experimental coccidian infection **(Shanmugasundaram *et al.*, 2013, Shanmugasundaram and Selvaraj, 2012)**. β-glucans are known to possess antitumor, antioxidant, and antimicrobial activities by enhancing the host immune functions **(Mowat, 1987, Stokes *et al.*, 1987)**. β-glucans are beneficial for growth performance in broilers and increased CD8 cell **(Chae *et al.*, 2006)**.

The increase in lysozyme activity recorded in the present investigation on usage of dietary supplements is considered as an increase in the number of innate humoral factors that elaborated from the body and showed domestic increase in their concentration **(Weir 1983).** Lysozyme played a definite role in the defense of chickens against *Eimeria* infections which result in decrease of lysozyme levels in chickens **(Khovanskikh;1979, Sotirov and Koinarski; 2003)**. High level of nitric oxide is produced by macrophages in response to *Eimeria spp.* infection and coccidian sporozoites **(Allen and Fetterer, 2000, Lillehoj and Li, 2004,**

PiraliKheirabadi, ***et al.,* 2011)**. In the present study; at d 21 PI, there was a significant reduction of nitric oxide in Preb 400, Preb 800 and S supplemented groups vs. PC group that reflects their valuable role in defense against coccidiosis. Obtained marked hyperplasia of cecal tonsils with mitosis comprising the proliferating lymphoid elements indicates the positive effect of the used supplements in immune modulation.

In conclusion, regardless of obtained disparate results of the used Prebiotic, Probiotic and Symbiotic, generally speaking all of them enhanced resistance to experimental *Eimeria species* infection by decreasing macroscopic and microscopic lesion score, improving both performance productive variables and GIT integrity as well as immunity vs. Positive Control group. Such protection was further reflected by reduced oocyst shedding (particularly in birds supplemented with Symbiotic) that completely accords with results reported by **Dalloul *et al.* (2003)** who mentioned that administration of a commercially Lactobacillus-based preparation to chickens from hatch to 3 weeks significantly decreased the number of *E. acervulina* oocysts in the treated chickens. Eventually, the advantages of using such natural products in the control of coccidiosis is the lower risk of developing resistance, such as that observed with chemical drugs. This could be attributed to the fact that balanced microbial population would support the inherent defense mechanisms of a healthy GIT, resulting in better control of intestinal pathogens **(Pollmann *et al.*, 2005)**. Moreover these alternatives are friendly to the environment, producers, and consumers **(Quiroz- Castañeda and Dantán-González; 2015)**. Since Prebiotics, Probiotics and Symbiotics are generally recognized as safe status, they could be considered as valuable drug-independent control strategy for chicken coccidiosis.

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Conflict Of Interest

The authors declare that they have no conflict of interests.

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Table 1. The Effect of diet supplementation on productive performance.

Body Weight (g)							
Groups	Age						
	1 day	7 days	14 days	21 days	28 days	35 days	42 days
Preb (400)	43.1±0.35	192.9±2.79^{a*}	457.3±5.04^{a}	952.2±9.42^{a}	1395.5±19.31^{a}	1819.5±30.32^{a}	2339.4±33.66^{b}
Preb (800)	43.7±0.29	173.7±2.56^{c}	419.1±6.24^{cd}	903.2±10.90^{b}	1310.3±19.86^{b}	1738.5±22.37^{b}	2430.2±27.54^{a}
Synb	43.0±0.26	176.9±2.31^{c}	438.2±4.83^{b}	925.7±8.71^{ab}	1303.4±17.72^{b}	1748.6±21.37^{ab}	2304.8±32.77^{b}
S (66)	42.9±0.27	172.1±2.16^{c}	413.6±5.39^{d}	872.9±9.63^{c}	1354.8±13.99^{ab}	1754.1±25.33^{ab}	2374.2±24.78^{ab}
Prob (100)	43.3±0.31	184.1±2.75^{b}	437.2±5.90^{b}	930.7±11.00^{ab}	1391.6±17.43^{a}	1783.5±24.71^{ab}	2370.8±29.45^{ab}
NC	43.4±0.27	176.4±2.16^{c}	435.2±5.53^{b}	908.7±10.21^{b}	1307.9±15.37^{b}	1731.1±19.50^{b}	2301.4±22.80^{b}
P C	43.1±0.26	179.3±2.24^{bc}	430.2±4.92^{bc}	903.6±10.10^{b}	1115.3±16.56^{c}	1521.5±23.88^{c}	2116.9±28.45^{c}
Probability	0.4760	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Body weight gain (g)							
	0–7 days	7–14 days	14–21 days	21–28 days	28–35 days	35–42 days	1–42 days
Preb (400)	149.9±2.83^{a*}	264.2±5.30^{a}	494.9±9.48	443.4±20.92^{abc}	419.1±33.21	513.7±45.99^{b}	2296.2±33.70^{b}
Preb (800)	129.9±2.61^{c}	245.4±6.51^{bc}	483.2±11.24	407.1±21.30^{bcd}	428.4±31.35	693.5±37.93^{a}	2386.3±27.56^{a}
Synb	133.9±2.31^{bc}	261.4±5.24^{ab}	487.2±10.06	380.5±18.04^{d}	434.5±28.26	558.4±39.60^{b}	2261.8±32.79^{b}
S (66)	129.1±2.18^{c}	241.5±5.78^{c}	459.3±10.19	481.4±15.95^{a}	402.0±29.84	622.7±37.22^{ab}	2331.2±24.82^{ab}
Prob (100)	133.0±2.18^{c}	258.9±5.54^{abc}	473.7±10.09	397.8±17.85^{cd}	420.4±23.02	566.1±29.40^{b}	2258.0±22.81^{b}
NC	140.9±2.76^{a}	253.0±5.77^{abc}	493.5±10.88	461.5±20.35^{ab}	380.9±29.06	585.3±38.44^{ab}	2327.7±29.48^{ab}
P C	136.2±2.24^{bc}	251.1±5.25^{abc}	473.8±10.67	211.7±17.82^{e}	404.4±27.67	604.6±35.57^{ab}	2073.8±28.46^{c}
Probability	0.0001	0.0416	0.1691	0.0001	0.8837	0.0431	0.0001
Feed Consumption (g)							
	0-7 days	8-14 days	15-21 days	22-28 days	29-35 days	36-42 days	1-42 days
Preb (400)	20.7±0.42	49.0±0.98	91.8±2.55	110.0±3.89	124.6±6.51	159.5±4.71	3889.2±110.35
Preb (800)	19.3±0.69	48.9±0.83	94.4±2.56	108.7±0.56	126.5±3.87	165.2±9.56	3940.6±59.49
Synb	20.8±0.28	51.8±1.77	92.7±3.31	102.0±4.45	126.2±4.08	158.2±6.86	3861.9±100.83
S (66)	20.4±0.23	51.0±2.47	94.0±1.39	112.6±0.68	122.4±2.00	156.2±1.47	3890.8±37.25
Prob (100)	21.1±0.19	50.6±1.25	92.7±2.68	104.1±5.11	128.3±4.24	162.5±7.61	3914.3±65.00
NC	20.1±0.53	47.1±1.36	90.5±3.45	110.9±3.06	123.9±3.73	166.7±14.15	3913.4±94.70
P C	21.1±0.21	49.4±1.23	95.3±3.53	102.0±2.66	115.6±1.60	161.5±8.57	3813.0±99.58
Probability	0.0674	0.4609	0.9120	0.1627	0.4138	0.9727	0.9577

FCR (g feed/g live BW)						
	1-7 days	1-14 days	1-21 days	1-28 days	1-35 days	1-42 days
Preb (400)	0.969±0.034^{c*}	1.068±0.019	1.187±0.024^{b}	1.360±0.016^{c}	1.520±0.027^{c}	1.663±0.047^{ab}
Preb (800)	1.043±0.016^{abc}	1.142±0.027	1.263±0.037^{a}	1.452±0.030^{b}	1.602±0.015^{b}	1.624±0.036^{b}
Synb	1.095±0.038^{a}	1.159±0.017	1.249±0.026^{ab}	1.432±0.008^{b}	1.574±0.020^{bc}	1.690±0.073^{ab}
S (66)	1.109±0.03^{a}	1.199±0.047	1.321±0.023^{a}	1.433±0.018^{b}	1.596±0.028^{b}	1.639±0.011^{b}
Prob (100)	1.113±0.040^{a}	1.156±0.037	1.268±0.021^{a}	1.437±0.025^{b}	1.605±0.023^{b}	1.701±0.019^{ab}
NC	0.998±0.027^{bc}	1.076±0.035	1.184±0.018^{b}	1.350±0.017^{c}	1.539±0.017^{bc}	1.648±0.029^{b}
P C	1.082±0.012^{ab}	1.145±0.024	1.282±0.012^{a}	1.679±0.019^{a}	1.762±0.016^{a}	1.802±0.062^{a}
Probability	0.0140	0.0726	0.0062	0.0001	0.0001	0.0453

Mortality rate (%)							
	1-7 days	8-14 days	15-21 days	22-28 days	29-35 days	36-42 days	1-42 days
Preb (400)	2.00±1.15	2.00±2.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	4.00±1.63
Preb (800)	3.00±1.00	1.00±1.00	0.00±0.00	0.00±0.00	1.00±1.00	0.00±0.00	5.00±1.00
Synb	3.00±1.00	0.00±0.00	0.00±0.00	0.00±0.00	1.00±1.00	1.00±1.00	5.00±2.52
S (66)	3.00±1.00	0.00±0.00	0.00±0.00	1.00±1.00	0.00±0.00	0.00±0.00	4.00±0.00
Prob (100)	2.00±1.15	0.00±0.00	1.00±1.00	1.00±1.00	1.00±1.00	0.00±0.00	5.00±1.00
NC	3.00±1.00	1.00±1.00	0.00±0.00	1.00±0.00	1.00±1.00	0.00±0.00	6.00±3.46
P C	2.00±1.15	1.00±1.00	2.00±1.15	1.00±1.00	1.00±1.00	2.00±1.15	9.00±4.12
Probability	0.9539	0.7485	0.1362	0.8012	0.9117	0.1362	0.7891

* Means with different, superscripts, within age, are significantly different ($P \leq 0.05$).

Table 2. The effect of diet supplementation on Production number and

Coefficient of Variation of body weight.

Treatment	Production number	C.V. (%) at 42 days	um weight (g) at 42 days	um weight (g) at 42 days
Preb (400)	317.0±16.38^{a*}	12.9	3070.00	1645.00
Preb (800)	332.8±7.60^{a}	10.3	2960.00	1840.00
Synb	306.3±30.60^{a}	13.0	2900.00	1235.00
S (66)	325.3±6.02^{a}	9.8	2915.00	1680.00
Prob (100)	300.5±2.75^{a}	9.2	2780.00	1710.00
NC	317.3±18.27^{a}	11.5	2960.00	1600.00
P C	250.5±14.78^{b}	12.9	2800.00	1400.00
Probability	0.0375			

* Means with different, superscripts, within trait, are significantly different ($P \leq 0.05$).

Table 3 . Effects of diet supplementation on Carcass quality.

Organ Treatment	Dressing %	Front part %	Hind %	Breast meat %	Thigh Drumstick%	Carcass meat %
Preb (400 g/T)	67.24±0.39^{a*}	37.79±0.31^{a}	29.45±0.29 ^{d}	19.49±0.20 ^{a}	14.60±0.64	34.09±0.68 ^{a}
Preb (800 g/T)	67.39±0.38 ^{a}	37.08±0.31^{a}	30.32±0.16 ^{b}	19.58±0.34 ^{a}	14.36±0.09	33.94±0.36 ^{a}
Synb	67.30±0.06 ^{a}	37.20±0.14 ^{a}	30.11±0.10^{bc}	19.10±0.40 ^{a}	14.14±0.23	33.24±0.58 ^{a}
S (66 g/T)	67.13±0.09 ^{a}	37.07±0.11 ^{a}	30.06±0.19^{bcd}	19.12±0.31 ^{a}	14.05±0.14	33.17±0.37 ^{a}
Prob (100 g/T)	67.27±0.48 ^{a}	37.13±0.43 ^{a}	30.14±0.19^{bc}	19.14±0.22 ^{a}	14.30±0.15	33.44±0.28 ^{a}
NC	67.03±0.24 ^{a}	37.43±0.19 ^{a}	29.53±0.27 ^{cd}	19.37±0.27 ^{a}	14.06±0.51	33.43±0.38 ^{a}
P C	64.70±0.46 ^{b}	33.22±0.40 ^{b}	31.47±0.22 ^{a}	15.32±0.29 ^{b}	13.98±0.07	29.30±0.31 ^{b}
Probability	0.0001	0.0001	0.0001	0.0001	0.8544	0.0001
Organ Treatment	Heart %	Gizzard %	Liver %	Giblet %	Intestinal Length cm	Intestinal Diameter cm
Preb (400 g/T)	0.427±0.020	2.23±0.11	3.10±0.12 ^{a*}	5.76±0.17 ^{a}	202.9±1.00 ^{a}	1.05±0.017^{a}
Preb (800 g/T)	0.433±0.012	2.41±0.08	3.08±0.10 ^{a}	5.92±0.11 ^{a}	201.4±2.79 ^{a}	1.04±0.022^{a}
Synb	0.452±0.033	2.45±0.14	3.24±0.16 ^{a}	6.14±0.28 ^{a}	202.8±2.45 ^{a}	1.02±0.029^{a}
S (66 g/T)	0.441±0.019	2.36±0.10	3.21±0.09 ^{a}	6.02±0.18 ^{a}	200.6±1.89 ^{a}	1.05±0.017^{a}
Prob (100 g/T)	0.449±0.024	2.29±0.12	3.20±0.11 ^{a}	5.94±0.13 ^{a}	202.8±1.61 ^{a}	1.07±0.015^{a}
NC	0.442±0.024	2.14±0.06	3.18±0.14 ^{a}	5.76±0.21 ^{a}	201.2±1.36 ^{a}	1.02±0.025^{a}
P C	0.476±0.027	2.08±0.09	2.61±0.13 ^{b}	5.17±0.17 ^{b}	190.9±1.95 ^{b}	0.93±0.030^{b}
Probability	0.8448	0.1278	0.0086	0.0137	0.0002	0.0018

* Means with different, superscripts, within trait, are significantly different ($P \leq 0.05$).

Table 4. The effect of diet supplementation on Intestinal macroscopic and microscopic lesion score.

Lesion Treatment	Microscopic lesion score			Macroscopic lesion score		
	Epithelial sloufing & necrosis	Inflammatory reaction	Dilated blood vessels	CDS*	7 days PI	14 days PI
Preb (400 g/T)	2.44+0.24^{bc}	2.44+0.24^{bc}	1.55+0.29 ^{ab}	3.77+0.57 ^{b}	2.20±0.74^{ab}	0.20±0.13^{bc}
Preb (800 g/T)	1.50+0.42^{de}	1.63+0.38 ^{cd}	0.63+0.26 ^{c}	1.13+0.34 ^{c}	0.80±0.39^{b}	1.00±0.52^{bc}
Synb	1.00+0.17 ^{e}	1.22+0.15 ^{d}	1.11+0.11 ^{abc}	1.00+0.33 ^{c}	0.60±0.40^{b}	0.00±0.00^{c}
S (66 g/T)	2.00+0.22^{cd}	2.00+0.22^{bcd}	0.86+0.26^{bc}	1.30+0.28 ^{c}	2.40±0.45^{ab}	1.80±0.53^{abc}
Prob (100 g/T)	2.83+0.17 ^{b}	2.83+0.31 ^{b}	1.33+0.33 ^{abc}	1.21+0.39 ^{c}	3.80±1.00^{ab}	2.00±1.03^{ab}
NC	1.43+0.37 ^{de}	1.57+0.30 ^{d}	0.86+0.26^{bc}	2.77+0.96^{bc}	0.00±0.00^{b}	1.00±0.52^{bc}

P C	3.88+0.13[a]	3.75+0.25 [a]	1.88+0.35 [a]	5.57+0.64 [a]	6.40±0.27[a]	3.00±0.89[a]
Probability	0.0001	0.0001	0.0251	0.0001	0.0413	0.0148

Means with different, superscripts, within trait, are significantly different ($P \leq 0.05$).
*CDS=Coccidia developed stages/5 random high power field.

Table 5. Effects of diet supplementation on histomorphology of Dudenum andJujenum.

Groups	Deodenum			Jejunum		
	Villi height	Crypt depth	V/C ratio	Villi height	Crypt depth	V/C ratio
Preb (400	1001.32±11.51[d]	129.38±3.36 [d]	7.94±0.21 [b]	746.42±17.44 [c*]	116.66±5.78[bc]	6.59±0.37[bc]
Preb (800	1305.75±14.17 [b]	122.15±3.79 [d]	11.17±0.40 [a]	1148.01±11.40 [a]	143.76±7.15 [a]	8.45±0.38 [a]
Synb	1537.07±21.79[a]	143.81±3.36 [c]	10.95±0.26 [a]	975.20±9.79 [b]	144.94±8.41 [a]	7.41±0.45 [ab]
S	1225.34±15.13 [c]	119.52±4.98 [d]	10.93±0.37 [a]	967.80±10.13 [b]	127.41±5.63 [ab]	8.02±0.42 [a]
Prob	957.87±16.39 [d]	160.45±5.53 [b]	6.22±0.17 [c]	613.37±9.89 [d]	127.46±6.55 [ab]	5.13±0.28 [d]
NC	991.99±25.56 [d]	177.11±6.57 [a]	5.84±0.19 [c]	626.00±10.37 [d]	103.82±3.60 [c]	6.22±0.25 [c]
P C	822.79±11.44 [e]	149.94±6.43[bc]	5.98±0.29 [c]	522.14±11.27 [e]	113.97±5.06[bc]	4.82±0.23[d]
Probability	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Means with different, superscripts, within trait, are significantly different ($P \leq 0.05$).

Table 6. The effect of diet supplementation on *Eimeria spp.* oocyst count.

Age Treatment	7 days	14 days	21 days	28 days
Preb (400 g/T)	2458.25±2159.41	10883.25±4296.23[a*]	2333.50±1037.29	796.00±331.15
Preb (800 g/T)	1945.75±1131.57	1154.00±521.56[b]	91.75±34.99	187.50±61.19
Synb	687.50±577.55	1308.25±476.02[b]	1979.25±621.77	492.00±208.67
S (66 g/T)	3254.25±2715.01	1412.50±88.67[b]	1045.75±524.33	333.50±128.97
Prob (100 g/T)	1575.00±570.38	7025.00±4399.44[ab]	991.75±132.60	329.00±121.15
NC	50.00±34.01	279.00±153.08[b]	766.75±160.74	129.00±40.53
P C	8441.75±8163.85	5845.50±2176.62[ab]	1537.25±719.48	529.25±215.82
Probability	0.6759	0.0485	0.1562	0.2172

* Means with different, superscripts, within age, are significantly different ($P \leq 0.05$).

Table 7. The effect of diet supplementation on litter condition score.

Age Treatment	21 days-old	42 days-old
Preb (400 g/T)	2.25±0.16[ab*]	2.75±0.16[ab]
Preb (800 g/T)	1.75±0.16[bc]	2.25±0.31[b]
Synb	1.75±0.31[bc]	2.25±0.16[b]
S (66 g/T)	1.50±0.19[c]	2.25±0.31[b]
Prob (100 g/T)	1.75±0.16[bc]	3.25±0.16[a]
NC	1.50±0.19[c]	2.00±0.27[b]
P C	2.75±0.16[a]	3.50±0.42[a]
Probability	0.0003	0.0013

* Means with different, superscripts, within age, are significantly different ($P \leq 0.05$).

Table 8. Effects of diet supplementation HI test against ND vaccination.

Chicken groups						
biotic 400	biotic 800	Synbiotic	Salinomycin	Probiotic	Blank Ctrl	Positive ctrl
5.70[bc]	6.80 [a]	5.50[bc]	6.00 [ab]	5.88 [abc]	5.40[bc]	4.89 [c]
Probability	0.0086					

* Means with different, superscripts, within trait, are significantly different ($P \leq 0.05$).

Table 9. Effects of diet supplementation on on cell mediated immunity

Treatment	Phagocytic%		Phagocytic index	
	Day 7 PI	Day 21 PI	Day 7 PI	Day 21 PI
Preb (400 g/T)	55.75±1.80[a*]	58.00±2.55 [b]	0.150±0.029 [ab]	0.140±0.015[b]
Preb (800 g/T)	55.50±3.75 [a]	75.00±1.08[a]	0.118±0.009 [abc]	0.253±0.023 [a]
Synb	46.00±1.29 [b]	62.75±2.06 [b]	0.163±0.055 [a]	0.130±0.015[b]
S (66 g/T)	49.25±3.30 [ab]	62.00±3.74 [b]	0.040±0.007 [c]	0.150±0.027[b]
Prob (100 g/T)	57.50±0.96 [a]	63.50±3.66 [b]	0.083±0.024 [abc]	0.160±0.026[b]
NC	55.25±0.85 [a]	67.00±4.88 [ab]	0.120±0.027 [abc]	0.210±0.050 [ab]
P C	34.25±3.99 [c]	57.50±2.25 [b]	0.060±0.018[bc]	0.155±0.017[b]
Probability	0.0001	0.0122	0.0499	0.0444
Treatment	Lyzozyme (µg/ml)		Nitric oxide (µmol/ml)	
	Day 7 PI	Day 21 PI	Day 7 PI	Day 21 PI
Preb (400 g/T)	7.00±0.70	14.35±1.53[b]	5.96±0.66	4.67±0.52[cd]
Preb (800 g/T)	12.35±2.68	52.63±10.99[a]	4.71±0.18	5.42±0.40[bcd]
Synb	6.47±1.23	20.75±3.75[b]	6.51±0.28	6.89±0.53[ab]

S (66 g/T)	12.32±6.89	17.10±5.32 ^{b}	5.07±0.87	4.41±0.04^{d}
Prob (100 g/T)	15.78±5.49	25.68±6.93 ^{b}	5.93±0.55	6.19±0.90^{abc}
NC	12.03±1.91	13.35±2.26 ^{b}	6.90±0.70	5.31±0.42^{bcd}
P C	6.25±0.84	17.36±2.46 ^{b}	5.85±1.08	7.51±0.49^{a}
Probability	0.4140	0.0010	0.3236	0.0033

* Means with different, superscripts, within trait and age, are significantly different ($P \leq 0.05$).

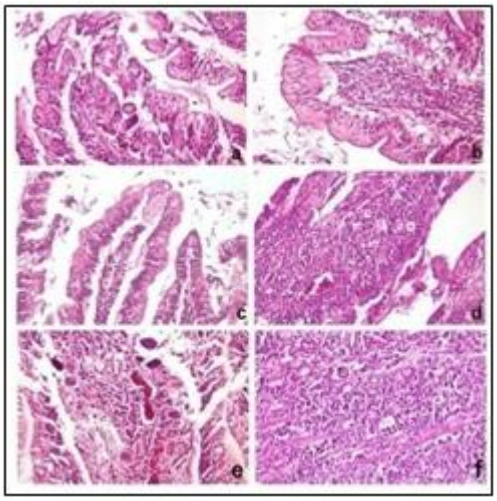

Fig.1. Duodenum: a) Preb (400) group is showing mild focal epithelial necrosis with edema, dilated blood vessels and inflammatory cell infiltration with presence of coccidial stage. **b)** Preb (800) group is showing maintenance of epithelial integrity with mild edema and inflammation. **c)** Symb group showing minimal inflammatory cells infiltration with mild edema. **d)** S group is showing moderate inflammatory reaction with sloughing of epithelial mucosa note the presence of coccdial stages. **e)** PC group is showing severe disruption of intestinal epithelium with moderate dilatation of blood vessels note the massive number of different coccidial stages disrupting the intestinal epithelium. **f)** Prob group is showing severe inflammatory reaction of intestinal mucosa with presence more of coccidial stages compared with other treated groups. (X400).

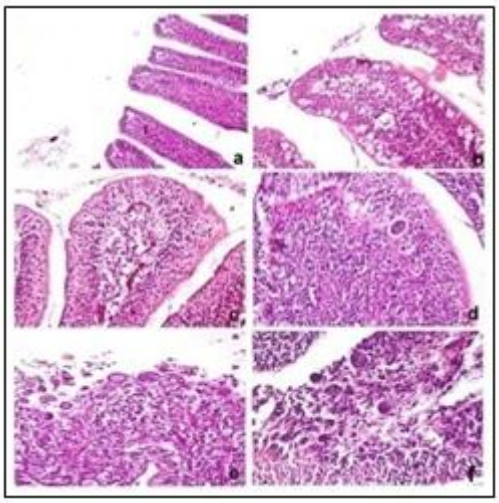

Fig.2: Jejunum: a) Preb (400) group is showing mild inflammation of intestinal mucosa with solitary individual coccidial stage(X200). **b)** Preb (800) group is showing goblet cell hyperplasia of intestinal epithelium with mild inflammatory cell infiltration. Note the degenerated coccdial stage in the lamina propria (X400). **c)** Symb group showing hyperemia of lamina proprial blood capillaries with inflammatory cell infiltration (X400). **d)** S group showing moderate inflammatory reaction with sloughing of epithelial mucosa. Note the presence of coccdial stages (X400). **e)** PC group shows severe disruption of intestinal epithelium. Note the massive number of different coccidial stages disrupting the intestinal epithelium. **f)** Prob group shows moderate inflammatory reaction of intestinal mucosa with presence of more coccidial stages compared with other treated groups. (X400).

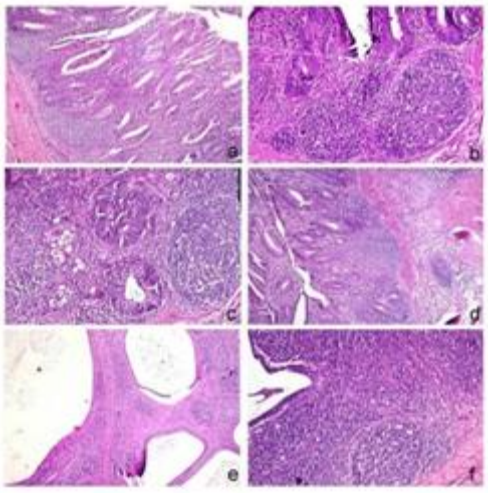

Fig.3. Cecum: a) Preb (400) group shows hyperplasia of cecal crypts with formation of crypt abscess with mononuclear cell infiltration. Note the presence of coccdial stages within crypt epithelium with focal depletion of cecal tonsils (X200). **b)** Preb (800) group shows marked hyperplasia of cecal tonsils with mitosis comprising the proliferating lymphoid elements with reduction of coccidial stages (X200). **c)** Symb group shows moderate number of coccidial stages in the interstium with inflammatory cell infiltration. Note the moderate hyperplasic cercal tonsils (X400). **d)** S group shows moderate reduction in density of coccidial stages with moderate inflammatory reaction extending into the underlying muscular layer and mild hyperplasia of cecal tonsils (X200). **e)** PC group shows severe cystic dilatation of cecal glands with atrophy of cecal tonsils (X100). **f)** Prob group is showing moderate inflammatory reaction of interstium with mild hyperplasia of cecal tonsils with reduction of coccidial stages (X200).

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