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Effect of Probiotic on Necrotic Enteritis in Chickens with the Presence of Immunosuppressive Factors

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Abstract: The study included investigation of efficiency of probiotic *Bacillus subtilis* (BP6) in control of necrotic enteritis disease in presence or absence of predisposing factors. In this experiment 100, 1-day old chicks were divided into 4 equal groups; 25 chicks each. Infectious bursal disease (IBD) vaccine was used at 8th day of age via eye drop for groups 1and 2 only. The probiotic (BP6) was given for groups 2 and 3 from first day of age till the end of the experiment (25 days). At 12th days of age chicks of group1 and group 3 were given 1 ml of coccidial vaccine per os (intracrob). Chicks of groups 1, 2 and 3 were infected with10^8cfu/ml *Clostredium perfringens* (*C. perfringens*) per os (intracrob) daily in three successive doses at 14th,15th,16th day of age. Birds of group 4 were left as non treated negative control. Body weight gain (BWG), intestinal/ body weight ratio and liver/ body weight ratio were recorded. The histopathologically changes were recorded in all infected groups except group 3-which treated with probiotic was showed apparently normal histological sections. The histopathologically changes in the liver were some focal area of mononuclear cells infiltration, hepatic necrosis, some congetion and portal tract infiltration with mononuclear cells**.** In the intestine there were focal areas of intestinal mucosal necrosis.In conclusion : Bacillus subtilis BP6 was more effective on coccidia with *C. perfringens* than on IBD with *C. perfringens*.

Key words: *Bacillus subtilis* (BP6) · *C.perfringens* · Coccidial vaccine · IBD vaccine · Chicks

INTRODUCTION

Necrotic Enteritis (NE) is an enterotoxaemic worldwide poultry disease in chickens, caused by the alpha toxin-producing bacterium of *C. perfringens* types A and C [1-3]. NE is a globally important welfare and economic problem in chickens causing economic losses due to mortalities, low growth rate and feed conversion [4, 5] as well as costs associated with disease prevention [6]. *C. perfringens* can cause both clinical and subclinical disease in poultry [7-9]. *C. perfringens* caused damage of intestinal mucosa [3, 6] and the toxigenic strains were isolated from both diseased and healthy chickens [10]. The disease risk factors include concurrent coccidial infection and the dietary use of cereal grains high in non starch polysaccharides such as wheat, barley, rye and oats [8, 11, 12]. Coccidial vaccine had also reported as a cofactor in induction of NE [13, 14]. Immunosuppressive causes played role in induction of NE [15] as infectious bursal disease (IBD) and also mycotoxins increased susceptibility to infectious disease in chickens [16-18]. It is difficult to determine the prevalence of the mild infection in chickens that cause higher condemnation rates in broilers due hepatitis [19].

Competitive exclusion treatment was shown to be effective in lowering numbers of *C. perfringens* in the intestinal tract, reducing the number of gross lesions, mortality and performance losses associated with NE infections. *Bacillus subtilis* (*B. subtilis*) strains have been shown to produce bacteriocins which inhibit the growth of *C. perfringens in vitro*.

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Further experiments are needed to detect the effect of probiotic on NE in chickens.

Our study was planned to induction of NE using toxigenic *C. perfringens* isolate, IBD vaccine and/or coccidial vaccine as predisposing factors with or without probiotic *B. Subtilis* in layer chicks.

MATERIALS AND METHODS

Experimental Chickens: In this experiment one hundred, 1-day old chicks were used. The chicks were floor reared and fed commercial balanced ration without feed additives. The chicks were randomly divided into 4 equal groups; 25 chicks each. Each group was reared in clean separated room and given feed and water adlibitum.

Probiotic: Bacillus subtilis (BP6) lyophilized powder produced by kemin CO. was added to the diet from the first day of age at 450 g\ton till the end of the experiment. Strain:Toxigenic *C.perfringens* type A, isolate was given at dose of 1×10^8 cfu/ml cooked meat broth per os.

Coccivac D: Coccidial vaccine (coccivac D) produced by Schering plough was used and given 1 ml of per os (intracrob).

Infectious bursal disease vaccine (Intermediate plus): Produced by CEVA-PHYLAXIA, Hungary. Batch No 0803V3U1A, given by eye drop.

Histopathological Examination: Tissue specimens from liver and intestine, were fixed in 10% neutral –buffered formalin,sectioned at 3-5 um and stained with hematoxlin andeosin stain (H&E) for histopathological examination by light microscope.

Statistical Analysis: Data are presented as mean± SEM (Standard error of mean). Simple one way ANOVA was processed (performed) for body weight, organs weight ratios using SPSS. Duncan multiple range test was used to differentiate between significant mean.

Experimental Design: One hundred day old chicks were divided into four groups 25 in each. All groups were vaccinated against Newcastle disease (ND) at the 5th day of age via eye drop. And IBD at 8th day of age via eye drop for group 1 (gp1) and group 2(gp2) only.

The probiotic (BP6) was given for gp 2 and group 3(gp3) from first day of age till the end of the experiment (25days).

At 12th days of age chicks of gp1 and gp 3 were given 1 ml of coccidian vaccine per os (intracrob).

At 14th,15th and16th day of age chicks of gp 1, gp 2 and gp 3 were given 10^8cfu/ml of *C.perfringens* broth culture (cooked meat broth) per os (intracrob).

Birds of group 4(gp4) were left as non treated negative control.

All groups were subjected to daily observation for clinical signs and/or mortalities with recording of average weekly body weight gain BWG and feed intake(FI) for calculation of feed conversion ratio (FCR) were recorded during the experiment. Three birds /group were randomly sacrificed at 3, 6 and 9 days post infection (dpi) as well as 2 birds from control group for post-mortem with recording of lesions and collection of tissues for histopathological examination. Intestine and liver weights were recorded to calculate organ body weight ratio.

RESULTS AND DISCUSSION

The obtained results are shown in Tables (1, 2a and 2b) and plates (1-3).

Table 1: Average (BWG) and feed intake as well as FCR of layer chicks infected with *C.perfringens* after coccidian vaccine or BP6.

Gr. No.	Treatment	Age / weeks	Av. BWG Mean	Av. FI Mean	FCR
1	Coccivac D + IBD + CP.	1	15.32	55.21	3.6
		2	46.69	111.50	2.39
		3	27.58	139.95	5
2	BP6 + IBD + CP.	1	23.32	63.97	2.74
		2	37.61	120.00	3.19
		3	48.63	164.46	3.38
3	BP6 + CP. + Coccivac D	1	24.92	36.97	1.84
		2	45.42	136.30	3
		3	58	174.20	3
4	Control –ve	1	15.32	55.11	3.6
		2	39.9	109.32	2.74
		3	50.79	140.54	2.77

Table 2 a: Average body weight and liver and intestine weight/g as well as BW ratio of layer chicks infected with *C.perfringens* after coccidian vaccine or BP6

Gr. No.	Treatment	dpi	Mean weight			Liver/ BW. Ratio	Intestine/ BW. ratio
			Body	Liver	Intestine		
1	Coccivac + IBD +C.P.	3	117.80	4.23	16.09	3.70	14.07
		6	125.46	4.08	14.72	3.25	11.76
		9	156.73	6.14	20.64	3.92	13.15
2	BP6+IBD+C.P.	3	134.02	4.37	15.57	3.28	11.72
		6	143.18	5.65	15.81	4.04	11.27
		9	200.72	6.06	21.7	3.02	10.81
3	BP6+ Coccivac D+C.P.	3	128.95	4.54	16.07	3.54	12.53
		6	134.21	4.83	16.61	3.60	12.41
		9	173.65	6.40	22.32	3.70	12.79
4	Control -Ve	3	131.83	4.405	12.965	3.46	10.21
		6	159.87	5.62	13.81	3.64	8.97
		9	157.275	6.11	9.26	3.94	11.95

Table 2b: Statistical analysis of weights

Treatments	No.	Body weight gain*	Liver/body weight ratio	Intestine/body weight ratio	Intestine/body weight ratio
Gp1 ;coccivac+:I.B.D.+C.P	9	124.59^{a}±5.49962	3.7000^{ab}±.59475	14.0667^{ab}±2.39589	12.7711±.85810
GP2:Bp6.+I.B.D. +C.P	10	144.56^{b}±8.35670	3.2767^{ab}±.17324	11.7167^{ab}±.99668	11.7167±..99668
GP3:BP6+coccivacD+C.P.	10	122.29^{a}±6.21463	3.5400^{ab}±.19009	12.5300^{ab}±.82347	12.5300±..82347
GP4:control	17	141.01^{ab}±4.68940	3.5056^{ab}±.19697	12.7711^{ab}±.85810	14.0667±.2.39589

Means with different superscripts are significantly different at p<0.05, *P<0.05

values are expressed as means ± standard errors Body Weights were significantly (P<0.05) between treated groups.

liver/Bw ratio and,Intestinal body weight ratio, :, statistically showed no significance between treated group.

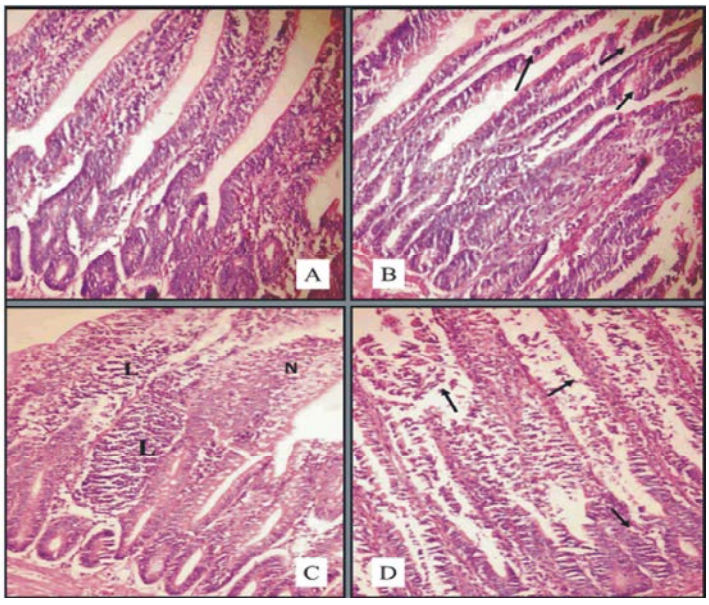

Plate 1: Intestinal sections of control negative and chicken of group 1 infected with IBD vaccine Coccivac D and *C. perfringens* (H. & E. x200):

A: A:Control negative showing healthy histological layers of small intestine.

B: 3 dpi showing necrosed mucosa (arrows)

C: 6 dpi showing necrosed mucosal epithelium (n) with submucosal leucocytic infiltration.

D: 9 dpi showing necrotic enteritis (arrows).

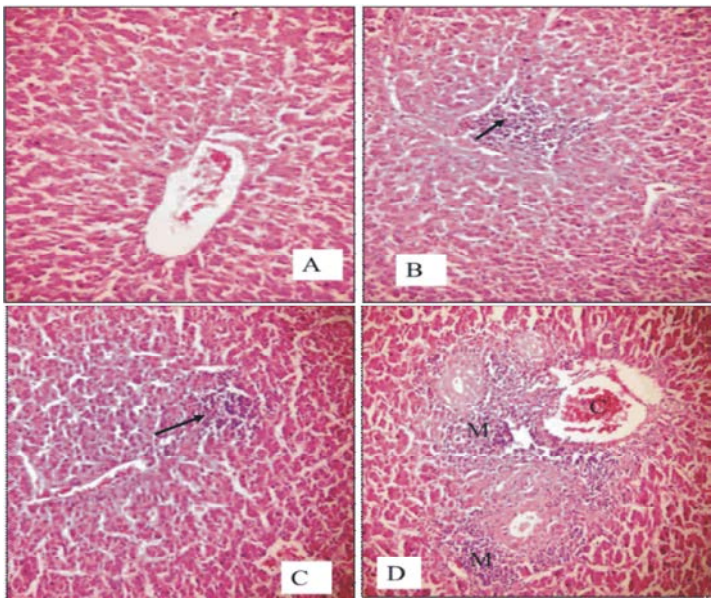

Plate 2: Liver sections of control negative and chicken of group 1 infected with IBD vaccine Coccivac D and *C. perfringens* (H. & E. x200):
A: Liver control negative showing hepatic parenchyma.
B: 3 dpi showing focal area of mononuclear cells infiltration (arrow).
C: 6 dpi showing focal area of necrosed hepatocytes (arrow).
D: 9 dpi showing portal tract infiltration with mononuclear cells (m) together with congestion (c

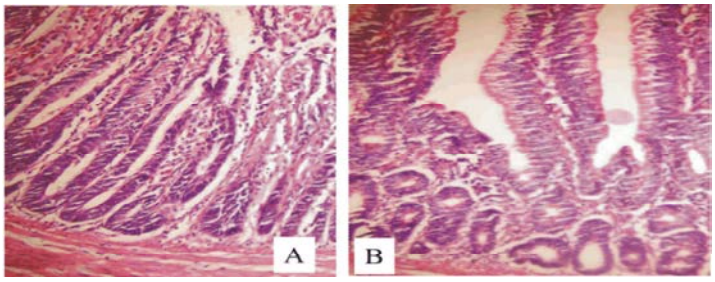

Plate 3: Intestinal sections of chicken of group 3 given BP6 in ration, Coccivac D and infected *with C. perfringens* (H. & E. x200):
A: 3 dpi showing apparently normal histological section.
B: 6 dpi showing slight mucosal reaction.

There were no marked clinical signs or mortality could be detected during this experiment but there is subclinical signs expressed by FI,BWG, FCR.

The recorded average (BWG) of gp 1 given IBD, Coccivac D and infected by *C.perfringens* culture (control +ve) was significantly variant than gp 2. The average BWG of chicken at gp 3 (probiotic BP6, Coccivac D and *C.perfringens)* was significantly variant than gp 2 (Tables 2a and 2b) and the results agreed with Pedersen *et al* [14] who reported that chickens in the groups receiving both coccidia vaccine and C. perfringens developed the subclinical form of NE. demonstrated by low growth rate than groups receiving either coccidia vaccine or C. perfringens alone. Also Wyatt *et al*. [34] reported that the depression weight was more severe in chickens receiving both IBD and coccidia than receiving either IBD or coccidia alone. It was reported that groups received BP6 showed milder lesions and higher body weights than non medicated groups where probiotics were used to control of NE in chickens.

In addition, all treated groups showed an increase in total FCR compared to the control negative group, this result agreed with Hofacre *et al*. [21].

Average feed intake of group 2(probiotic Bp6, I.B.D. *and C.perfringens*) was higher than the control negative group at all intervals.

Recorded FCR of groups 2 and 3 at the 2nd week was higher than that of groups 4 and 1. The FCR in gp 2 at the 1st week was lower than that in groups 1, 3 and 4 (Table 1). The group 4(control negative) was the best at the 3rd week of age. Wyatt *et al*. [34] was reported that groups received BP6 showed milder lesions and better FCR than non treated groups where probiotics were used to decrease NE in chickens.

PM lesions in sacrificed chickens proved that gp4 (control negative) showed no detectable lesions. Chickens of gp 1 those given (IBD vaccine + coccidia vaccine and infected with C. *perfringens* culture) showed liver necrosis, serosal intestinal hemorrhages and mucosal thickness with hemorrhages at 3 dpi, necrotic foci and intestinal thickness with haemorrhage at 6th dpi, while Turkish towel appearance was seen at 9 dpi. these results due to groups received BP6 showed milder pathological lesions than non treated groups the results agreed with Wyatt *et al*. [34].

Body Weights were significantly differences between treated groups.

There is no significance variance statistically between treated groups in Liver /body weight ratio and Intestinal body weight ratio.

The disease can be divided into 2 categories, clinical and subclinical. Clinical signs of NE include depression, decreased appetite, diarrhea and severe necrosis of the intestinal tract, while subclinical form lead to decreased body weight gain and increased feed conversion rate, will have severe consequences for the poultry industry [20, 21]. NE disease has caused potential losses among chickens in Egypt [22].

From our results indicated that the predisposing factors (coccidial vaccine, I.B.D. vaccine) of NE disease affected on FI,B.W.G. and F.C.R. in chickens.

Understanding the progression of NE is very difficult due to its complexity and incrimination of several predisposing factors such as dietary components, immunosuppression, mechanical irritation of the gut and sudden gut microflora changes appear to contribute to this syndrome [23-25] IBD virus infection or vaccination can be also incriminated.

Many attempts were carried out experimentally and field to prevent and or control of NE including drugs, probiotics and vaccination.

The most important known predisposing factor is intestinal damage caused by coccidial pathogens, especially Eimeria species [11, 26-30]. This intestinal damage will result in release of plasma proteins into the lumen of the intestinal tract. Since the minimal requirements for growth of C. perfringens include more than 11 amino acides besides many factors and vitamins [31, 32], leaking of plasma to the intestinal lumen can provide a necessary growth substrate for extensive proliferation of these bacteria.

There were no marked clinical signs or mortality detected during this experiment, this finding agreed with Cowen *et al*. [33] who reported only a small incidence of NE in some chickens challenged with C. perfringens but failed to induce signs of NE in others. Pedersen *et al*. [14] who carried out an experiment to establish an infection and disease model for C. perfringens using coccidia vaccine at 10 times the prescribed dosage and found that no mortality was detected in any of the groups, however, chickens developed the subclinical form of NE.

The histopathological lesions were recorded that group 3 showed apparently normal histological sections in the intestine with slight mucosal reaction (plate 3) than other groups which were showing focal areas of intestinal mucosal necrosis and hepatic necrosis.

Intestinal and cecal lesion were recorded by Long *et al*. [27]. The detected thickened mucosa with necrosis at the 3rd dpi with whole broth culture was also reported by Al-Sheikhly and Truscott [3]. Liver lesions characterized by swollen, discolored livers with necrotic foci [35].

Regarding the histopathologicaly changes with the detected lesions in form of focal areas of intestinal mucosal necrosis, hepatic necrosis and impaired performance (poor BWG and FCR) without clinical signs indicated the induction of mild form of the disease as described by Cooper. and Songer[4] and Lovland and Kaldhusdal, [19] The more clear lesions in groups previously given coccidial vaccine cleared the possible role of coccida vaccine in induction of subclinical NE [14].

This Study Proved That:

- Experimentally subclinical NE was induced in presence of IBD and/or coccidian vaccine as predisposing factor by repeated dose of C.perfringens broth culture orally.
- Efficiency of probiotic Bacillus subtilis (BP6) in control of the disease in presence or absence of predisposing factors.

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