Global Veterinaria 5 (6): 324-333, 2010
ISSN 1992-6197

Comparative Evaluation on the Effect of Coccidiostate and Synbiotic Preparations on Prevention of *Clostridium perfringens* in Broiler Chickens

Wafaa A. Abd El-Ghany

Department of Poultry Diseases,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: *Clostridium perfringens* (*C. perfringens*) is the organism that causes necrotic enteritis in birds and badly affects the poultry industry. Although Salinomycin is an anticoccidial drug but it may indirectly affects *C. perfringens* infection. Synbiotics preparations are nowadays used as natural products to exclude enteric infections like C. *perfringens*. This study was designed to make a comparative evaluation on the effect of coccidiostate Salinomycin and synbiotic preparation on prevention of *C. perfringens* in broiler chickens. Two hundred day-old meat type chicks were assigned into 5 equal separate groups (1-5) each of 40 birds. Chickens of group (1) and (2) were fed on ration containing the coccidiostate (Salinomycin) and synbiotic preparations, respectively from the first day of old till the end of the study. Birds in group (3) received ration containing both products (Salinomycin and synbiotic) for all entire period of the experiment. Each bird in groups 1, 2, 3 and 4 was orally inoculated with 0.5 ml of *C. perfringens* broth culture (10^9 CFU/ml) at 14 days old. Birds in group (5) were kept as a blank negative control (non-treated and non-challenged). All the groups were kept under complete observation for 3 weeks after challenge (35 days of age). Clinical signs, mortalities, gross lesions, performance variables, intestinal and caecal bacterial count as well as histopathological examination were taken as criteria for evaluation. Results indicated that using of Salinomycin and synbiotic alone or in combination was effective in prevention of *C. perfringens* as expressed by reduction of signs, mortalities, lesions and intestinal count and also improving the performance parameters of broiler chickens. It was concluded that using of some feed additives like Salinomycin was nearly effective as using of natural product like synbiotic in prevention of *C. perfringens*. Moreover, the combination of both treatments was superior in prevention than using of each separately.

Key words: Salinomycin · Synbiotic · *Clostridium perfringens* · Prevention

INTRODUCTION

Enteric pathogens affecting poultry result in great economic losses to the industry and their control could save millions of dollars annually to the producers. *Clostridium perfringens* (*C. perfringens*) type A and C is the causative agent of necrotic enteritis (NE) [1, 2] which is ubiquitous Gram-positive, spore-forming, toxigenic anaerobic bacteria affecting the intestinal tract of birds. There are several predisposing factors which are important in overgrowth of *C. perfringens*, excessive release of α toxin and consequently outbreaks of NE in poultry [3]. The most common contributing factors include defects in management [4, 5], immunosuppression [6], coccidiosis [7-9] and diet composition [10-13]. Outbreaks of NE may result in high mortality, poor feed conversion rate, reduced growth rate, increased condemnation rates and high contamination rates of poultry and risk of transmission to the food chain, posing public health importance [14, 15].

Salinomycin is an ionophorous antimicrobial and also acts as coccidiostatic and therefore decreases the intestinal damage caused by *Eimeria* infections [16], thus reduces the most important predisposing factors for *C. perfringens*-associated necrotic enteritis. Moreover, Salinomycin has very low minimal inhibitory concentrations against *C. perfringens* [17-21].

Corresponding Author: Wafaa A. Abd El-Ghany, Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, Tel: +202-38115446 (Home), Fax: +202-35725240, Mob: +2012-4407992, E-mail: wafaa.ghany@yahoo.com.

Probiotic means "for life" in Geek [22] and it is defined as a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance [23]. Prebiotics are defined as non-digestible food ingredients which beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already present in the intestinal tract. Prebiotics are able to serve as a substrate for one or more bacterial species with a potentially beneficial effect on the host [24]. Combination of prebiotics and prebiotics are known as synbiotics which beneficially affect the host by improving the survival and persistence of living microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria [25]. When probiotics and prebiotics were provided together in the diet, not only the introduction, but also the maintenance of beneficial bacterial populations might be accomplished [26, 27]. The effect of using synbiotics in reducing intestinal colonization by enteric pathogens like *Escherichia coli, Salmonella enteritidis* and *C. perfringens* in the broiler chickens was studied by Kaldhusdal *et al.* [28], Zouelfakar and Elshazly [29], Awaad *et al.* [30] and Abd El-Ghany *et al.* [31] with promising successful results.

The purpose of this study was to compare between the effect of coccidiostate (Salinomycin) and a commercial synbiotic preparation on the prevention of *C. perfringens* in broiler chickens.

MATERIALS AND METHODS

Experimental Chickens: Two hundred day-old mixed sex meat type (Hubbered breed) chicks were obtained from a commercial hatchery and divided into 5 equal groups (1-5) each consists of 40 birds. Each group of birds was kept in separate thoroughly cleaned and disinfected pens and given commercial balanced starter (1 to 14 days old), grower (15to 29 days old) and finisher (30 to 35 days old) rations *ad libitum* consumption. The feed did not contain growth promoters. All the birds in each group were vaccinated against Newcastle disease using live Hitchner B1 vaccine and LaSota vaccine at 6 and 18 days of age, respectively, against Gumboro disease using live intermediate 228E vaccine at 12 days of age and against avian influenza using inactivated (H5 N1 subtype, Re 1 strain) at 7 days old. All living vaccines were given through eye drop instillation, while inactivated one was given subcutaneously at the back of the neck.

Synbiotic Product: A commercial product containing stabilized probiotic (strain of *Entercoccus faecum*), prebiotic (fructo-oligosacharides of inactivated cell wall fragments of non pathogenic microorganisms) and phycophytic substances were used as synbiotic preparation. This synbiotic was added to the bird's ration in a dose of 1 kg/ton feed from day old till the entire period of the experiment.

Coccidiostatic Drug: Salinomycin sodium granules (Bio-Cox 60) product of Alpharma Inc. Bridgewater, New Jersey was thoroughly mixed in the ration of the birds at concentrations 60 g/ton (0.0066%) from the day old till the end of the experiment.

The Challenge *C. perfringens* Strain: Toxogenic strain of *C.perferingens* typa (A) was kindly obtained from Microbiology Department, Faculty of Veterinary Medicine, Cairo University. That strain of *C. perfringens* was isolated from broiler chickens flock suffered from NE. The organism was anaerobically cultured on 10% sheep blood agar media containing 200 µg/ml neomycin sulphate incubated in Gaspack anaerobic jar at 37°C for 24 hours, then inoculated in cooked meat medium and incubated overnight at 37°C in Gaspack jar. Culture was centrifuged at 10000 r.p.m. for 10 minutes and the bacterial concentration of the culture was adjusted to a turbidity of opacity tube to 10^9 colony forming units (CFU)/ml. Chickens were orally inoculated with 0.5 ml of *C. perfringens* broth culture at 14 days of age [32].

Experimental Design: Chicks were assigned into 5 equal separate groups (1-5) each consists of 40 birds. Chickens of group (1) and (2) were fed on ration containing the coccidiostate (Salinomycin) and synbiotic preparations, respectively from the day old till the end of the study. Birds in group (3) received ration containing both products (Salinomycin and synbiotic) also for all entire period of the experiment. Each bird in groups 1, 2, 3 and 4 was orally inoculated with 0.5 ml of *C. perfringens* broth culture (10^9 CFU/ml) at 14 days old. Birds in group (5) were kept as a blank negative control (non-treated and non-challenged). All the groups were kept under complete observation for 3 weeks after challenge (35 days of age).

Parameters for Evaluation

Clinical Signs, Mortalities and Gross Lesions: After challenge, all the birds were daily observed for clinical signs of NE and mortalities for 3 weeks observation period

(35 days old). Dead as well as sacrificed birds at 3, 7, 14 and 21 days post challenge were examined for specific NE lesions in the intestinal tracts. Intestinal lesions score on a scale of (0-4) was determined by Prescott *et al.* [33] using the following criteria: 0= Normal, no evidence of gross lesions, 1= Thin, friable small intestine, 2= Focal necrosis and/or ulceration, 3= Patchy necrosis and 4= Severe extensive necrosis.

Birds Performance Variables: Random chickens in each experimental group were weekly weighed and the performance variables including average body weight, feed conversion rate (FCR) and European production efficiency factor (EPEF) after Sainsbury [34] were calculated.

Bacterial Count: The intestinal tracts of sacrificed birds in each group at 3, 7, 14 and 21 days post challenge were collected for bacterial count [35]. About 1-2 grams from the intestinal or caecal contents of 3 birds from each group were pooled. The samples were diluted in buffered peptone water to an initial 10^{-1} dilution. Ten-fold serial dilution was spread in duplicate on blood agar base containing 10% sheep blood with 200 µg/ml neomycin sulphate for enumeration of *C. perfringens*. All the plates were incubated in Gaspack anaerobic jar at 37°C for 48 hours. Hemolytic colonies on blood agar plates were counted and expressed as $\log_{10}$ CFU/g of intestinal or caecal contents. Some colonies were picked, Gram stained and microscopically examined to be confirmed as *C. perfringens*.

Histopathological Examination: Specimens from the intestinal tracts of birds in each group at the end of observation period (35 days old) were collected, fixed in 10% formol saline for 24 hours, washed in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slidge microtome. The obtained tissue sections were collected on glass slides, deparffinized and stained by hematoxylin and eosin stains [36] for histopathological examination through the electric light microscope.

Statistical Analysis: Data were statistically analysed by analysis of variance (ANOVA) after Snedecor and Cochran [37].

RESULTS

No clinical signs were seen in the blank control group all over the observation period post challenge (PC). Three days PC, chickens in *C. perfringens* infected groups (1-4) showed clinical signs in the form of dullness, depression, anorexia, reluctance to move, ruffled feathers and diarrhea. Severe signs were observed in *C. perfringens* infected non treated group, whilst the severity decreased in the treated groups. *C. perfringens* associated mortality was significantly ($P<0.05$) reduced by all treatment levels in comparison to the non treated control. During the course of this study, the mortality rate in the control positive *C. perfringens* infected group was 12.5%. The percentage decreased in the treated groups to reach 2.5, 7.5 and 0% in Salinomycin, synbiotic and Salinomycin with synbiotic treatment, respectively (Table 1). The intestines of dead birds in *C. perfringens* infected groups showed variable degrees of thinning, friability and necrosis. The mean *C. perfringens* intestinal lesion scores at the different intervals (3, 7, 14 and 21 days PC) were shown in Table 2. There was no evidence of *C. perfringens* lesions in birds treated with Salinomycin, synbiotic or their combination at the end of study. Significant ($P<0.05$) reduction was observed in the intestinal lesion scores of the treated chickens as compared with the control birds at the various examination intervals.

The performance variables of chickens in *C. perfringens* infected and treated groups were tabulated (Table 3). The data revealed that there were significant differences ($P<0.05$) in the average body weight between the treated and the untreated groups. The best performance parameters were observed in the birds treated with combination of Salinomycin and synbiotic compounds rather than those treated with individual treatment as indicated by the results of FCR and EPEF.

Considering the results of *C. perfringens* count in the intestine and caecum of sacrificed birds at 3, 7, 14 and 21 days PC, Table 4 revealed that significant ($P<0.05$) reduction in the intestinal and caecal colonization in the treated groups rather than control positive non-treated group on 21 days PC. However, chickens treated with combination of Salinomycin and synbiotic showed statistically the lowest intestinal and caecal count along the course of observation period.

The organism grew on blood agar plates incubated anaerobically at 37°C and produced a characteristic inner zone of hemolysis. Gram's staining of suspected *C. perfringens* colonies revealed Gram positive bacilli.

Table 1: The effect of Salinomycin and synbiotic treatment on the mortality rate in *C. perfringens* infected and treated broiler chickens

	End of observation period		
Treatment group	No. of sacrificed birds	No. of dead birds	Mortality rate
Infected+ Salinomycin	12	5	1/40 (2.5%)
Infected+ synbioic	12	6	3/40 (7.5%)
Infected+ Salinomycin+ synbiotic	12	3	0/40 (0%)
Infected non treated	12	12	5/40 (12.5%)
Non infected, non treated	12	0	0/40 (0%)

Table 2: The effect of Salinomycin and synbiotic treatment on the intestinal mean lesion scores in *C. perfringens* sacrificed infected and treated broiler chickens

	Mean lesion score			
	Days post challenge			
Treatment group	3	7	14	21
Infected+ Salinomycin	1.20^{a}	0.29^{b}	0.10^{b}	0.00^{b}
Infected+ synbiotic	1.43^{a}	0.50^{b}	0.25^{b}	0.00^{b}
Infected+ Salinomycin+ synbiotic	0.63^{a}	0.06^{a}	0.06^{a}	0.00^{a}
Infected non treated	3.25^{a}	2.66^{b}	1.98^{c}	1.55^{c}
Non infected, non treated	0.00	0.00	0.00	0.00

Intestinal lesions score on a scale of (0-4) was determined after Prescott *et al.* [33] as the following criteria: 0= Normal, no evidence of gross lesions, 1= Thin, friable small intestine, 2= Focal necrosis and/or ulceration, 3= Patchy necrosis and 4= Severe extensive necrosis

The means within a row with no common superscript are considered significantly different (P<0.05)

Table 3: The effect of Salinomycin and synbiotic treatment on the performance variables in *C. perfringens* infected and treated broiler chickens

	Body weight/gm				
Age/Week	Infected+ Salinomycin	Infected+ synbiotic	Infected+ Salinomycin+ synbiotic	Infected non treated	Non infected, non treated
1	124.2±4.20^{a}	118.9±5.61^{a}	130.1±4.21^{a}	99.6±6.01^{b}	132.8±5.4^{a}
2	316.4±5.11^{a}	305.7±4.21^{a}	338.2±5.41^{a}	210.5±6.20^{b}	340±6.10^{a}
3	590.7±7.20^{a}	574.9±9.32^{a}	600.2±9.34^{a}	490.7±9.80^{b}	607.5±7.90^{a}
4	891.8±8.02^{a}	810.6±9.51^{a}	907.5±9.36^{a}	718.5±10.2^{b}	914.3±10.3^{a}
5	1105.2±12.2^{a}	1009.5±11.3^{a}	1210.6±12.4^{a}	991.7±1206^{b}	1212.7±12.7^{a}
Total feed consumption/bird/kg	2.01	2.03	1.92	2.5	1.90
FCR	1.59	1.71	1.43	2.10	1.45
EPEF	199.44	189.67	202.31	169.97	205.50

FCR: Feed conversion rate

EPEF: European production efficiency factor

The means within a row with no common superscript are considered significantly different (P < 0.05)

The values of body weight represent the means±SEM of 40 broiler chickens per group (n = 40)

Table 4: The effect of Salinomycin and synbiotic treatment on *C. perfringens* intestinal and caecal count in infected and treated broiler chickens (log_{10} CFU/g of intestinal or caecal contents)

	Intestinal count Days post challenge				Caecal count Days post challenge			
Treatment group	3	7	14	21	3	7	14	21
Infected+ Salinomycin	0.9±0.12	1.5±0.20	2.3±0.34	3.5±0.11	0.24±0.22	1.5±0.44	1.8±0.22	3.1±0.52
Infected+ synbiotic	0.6±0.14	1.9±0.31	3.4±0.34	4.0±0.11	0.54±0.41	2.0±0.60	3.2±0.10	4.4±0.62
Infected+ Salinomycin+ synbiotic	0.5±0.10	1.2±0.10	2.1±0.34	3.0±0.01	0.16±0.20	1.2±0.21	2.01±0.10	3.2±0.41
Infected non treated	2.4±0.32*	5.7±0.33*	9.4±0.54*	15.4±0.64*	1.7±0.51*	4.5±0.81*	8.9±0.56*	10.1±0.70*
Non infected, non treated	0	0	0	0	0	0	0	0

*: Significant differences (P<0.05)

Table 5: The effect of Salinomycin and synbiotic treatment on the histopathological alterations of the small intestine in *C. perfringens* infected and treated broiler chickens

Histopathological alterations	Treatment groups				
	Infected+ Salinomycin	Infected+ synbiotic	Infected+ Salinomycin+ synbiotic	Infected non treated	Non infected, non treated
Sloughing of the mucosa	+++	-	-	+++	-
Necrosis of the mucosa	+++	-	-	+++	-
Inflammatory cells infiltration in LP	++	+	-	+++	-
Oedema in LP	++	-	-	++	-
Hypertrophy in musculature	-	-	-	++	-

+++ = Severe ++ = Moderate + = Mild - = Nil LP = Lamina propria

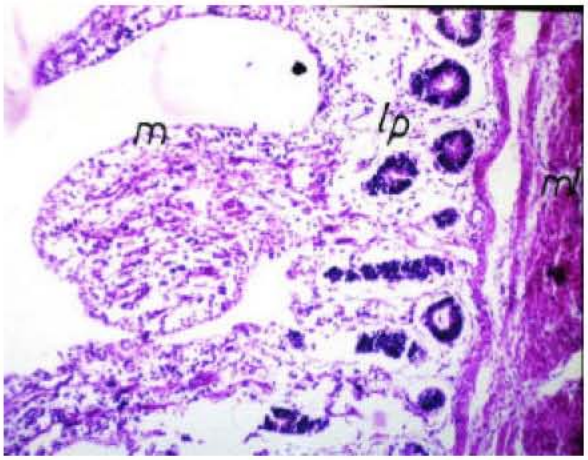

Fig. 1: Small intestine of birds infected with *C. perfringens* and treated with Salinomycin showed sloughing and necrosis in the mucosal lining epithelium (m) with oedema and inflammatory cells infiltration in lamina propria (lp) and hypertrophy in the musculature (ml) (H&E X40)

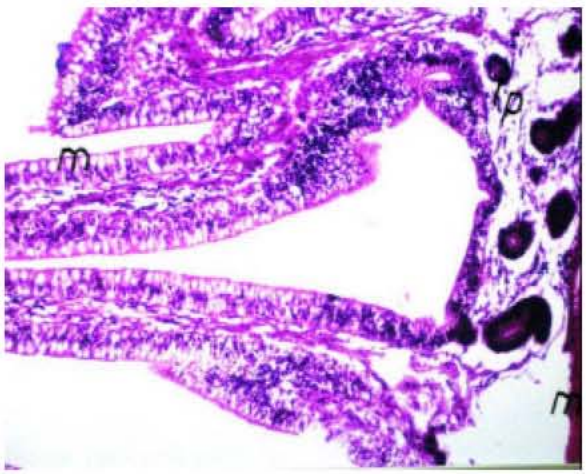

Fig. 2: Small intestine of birds infected with *C. perfringens* and treated with synbiotic showed mild focal inflammatory cells infiltration in lamina propria (lp) (H&E X40)

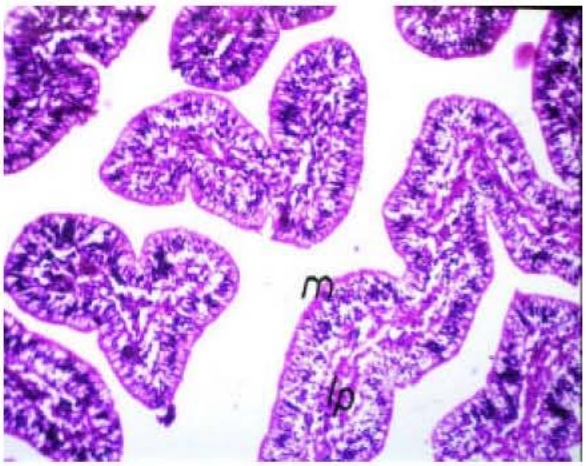

Fig. 3: Small intestine of birds infected with *C. perfringens* and treated with Salinomycin and synbiotic showed normal histological structure of the mucosal lining epithelium (m), lamina propria (lp) and muscularis (ml) (H&E X40)

Results of the histopathological changes in the small intestines that collected from the bids in different groups were seen in Table 5 and Figures (1-5). Birds that challenged with *C. perfringens* and treated with Salinomycin showed sloughing and necrosis in the mucosal lining epithelium with oedema and inflammatory cells infiltration in lamina propria and hypertrophy in the musculature while those treated with the synbiotic showed mild focal inflammatory cells infiltration in lamina propria. The small intestines of chickens infected with *C. perfringens* treated with Salinomycin and synbiotic revealed normal histological structure of the mucosal lining epithelium, lamina propria and muscularis which were similar to non infected non treated birds. Infected non treated group showed sloughing and necrosis in the mucosal lining epihelium with oedema, inflammaory cells infiltration in the lamina propia and hypertrophy in the musculature.

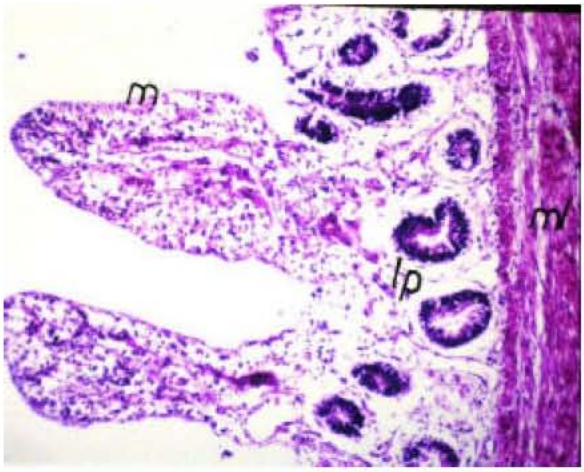

Fig. 4: Small intestine of birds infected with *C. perfringens* showed sloughing and necrosis in the mucosal lining epihelium (m) with oedema, inflammaory cells infiltration in the lamina propia (lp) and hypertrophy in the musculature (ml) (H&E X40)

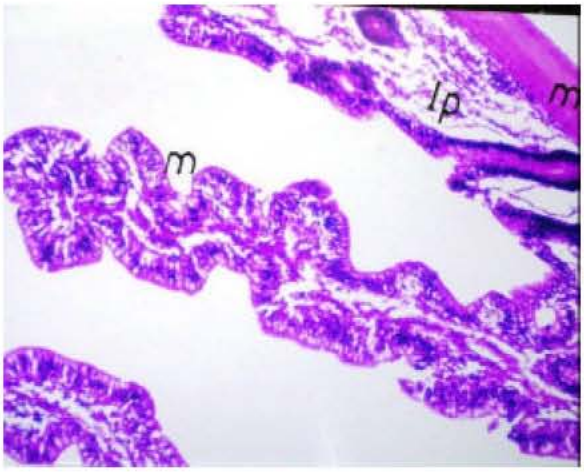

Fig. 5: Small intestine of birds infected with *C. perfringens* showed normal histological structure of the mucosal lining epithelium (m), lamina propria (lp) and muscularis (ml) (H&E X40)

DISCUSSION

C. perfringens is an obligatory anaerobic bacterium of the intestinal tract of chickens [38]. Predisposing factors, such as intestinal damage due to coccidiosis, should be taken into account when designing a control program of *C. perfringens*. A combination of measures that include avoiding predisposing factors and combating the pathogen itself seems to be the strategy of choice.

Prevention of coccidiosis may indirectly prevent infection and losses from *C. perfringens*, since the presence of *Eimeria* species was a predisposing factor for the development of necrotic enteritis [15]. That explanation might be due to that coccidial infection induced severe intestinal mucosal damage that permited *C. perfringens* to induce necrotic enteritis [39]. Removal of coccidiostats from poultry feed was therefore also a predisposing factor for the development of necrotic enteritis [40]. It was documented in numerous studies that coccidiostatic drugs were able to prevent *C. perfringens*-associated necrotic enteritis [9, 41, 42]. The use of anticoccidial drugs, of which some (i.e. the ionophores) also acted bactericidally on clostridia [39]. In a study by Emik and Bednik [43], the broiler chickens flocks that treated with coccidicidal drugs showed disappearance of necrotic enteritis. Williams *et al.* [44] found that the severity of necrotic enteritis was reduced in birds immunized with an attenuated coccidial vaccine, subsequently infected with *E. maxima* and then challenged with *C. perfringens,* while the lesions were severe in non-vaccinated birds. Controversial result was obtained by the work of Waldenstedt *et al.* [45] who indicated that vaccinated un-medicated birds had a higher caecal count of *C. perfringens* than unvaccinated medicated bird and a lower slaughter weight. The vaccinated groups had higher numbers of *C. perfringens* in the intestinal tract and experienced an outbreak of necrotic enteritis. It was not clear whether this was due to the mucosal damage caused by the attenuated coccidial vaccine or to the lack of coccidiostatic antibacterial drugs in the feed, or else to the damage caused by coccidial field infections in vaccinated groups. Probably also the level of attenuation of the vaccines could play a role.

The predominant feature of necrotic enteritis was acute death with mortality rates that could reach 50% [10, 46]. Clinical signs include depression, dehydration, ruffled feathers, diarrhoea and decreased feed consumption were recorded [7, 47-50]. The current results indicated that Salinomycin or synbiotic alone or in combination were able to reduce the signs and mortality rate caused by *C. perfringens*. Similarly, Kutkat *et al.* [51] found that chickens that received a probiotic containing *Lactobacillus acidophilus* in the feed for 10 days before experimental infection with *C. perfringens* were protected by a percentage of 70%. Also, Hofacre *et al.* [52] demonstrated that addition of prebiotic containing FOS or manno-oligosaccharides (MOS) to the ration of broiler

chickens induced significant decrease in mortality from 60 to 30% due to necrotic enteritis in an experimental challenge trial.

Most evident *C. perfringens* macroscopical lesions can be seen in the small intestine and sometimes caeca whereas it becomes thin walled, friable, dilated, filled with gas and the mucosal surfaces were covered with a grey-brown to yellow-green diphteric membrane or pseudomembrane [50, 53]. In this experiment, both of Salinomycin and synbiotic were effective in decreasing significantly the lesion scores due to *C. perfringens* infection. Parallel results were obtained by Martel *et al.* [20] who recorded that Salinomycin decreased the severity of lesions due to *C. perfringens* associated-necrotic enteritis. In addition, Hofacre *et al.* [54] showed that a commercial probiotic preparation reduced gross lesions of necrotic enteritis in chickens.

The results of this trial also demonstrated improvement in the performance parameters (body weight and feed conversion rate) in the treatments groups than controls. The depression of growth rate and feed efficiency of birds in the control positive group was may be due to the damage of the intestine and the subsequent reduction in digestion and absorption of food. Concur results were also obtained by Johansen *et al.* [21], Hofacre *et al.* [52] and Radu *et al.* [55] who showed that Salinomycin increased the mean body weight and improved feed conversion ratio weight in the treated chickens as compared to un-treated *C. perfringens* infected ones. The results of Engburg *et al.* [56] also indicated that Salinomycin was superior to plant extract in improving the production rate in broiler chickens after infection with *C. perfringens*. However, Madian and Abd El-Ghany [57] demonstrated significant effect of a synbiotic preparation in improving chickens performance when compared with virginiamycin growth promoter antibiotic.

Considering the results of *C. perfringens* colonization in the treated and control groups, it was shown that either Salinomycin or synbiotic significantly reduced both intestinal and caecal colonization. The role of Salinomycin was evaluated comprehensively by Martel *et al.* [20], Johansen *et al.* [21], Vissienon *et al.* [42], Engberg *et al.* [56] and Elwinger *et al.* [58] who demonstrated that Salinomycin reduced the counts of *C. perfringens* in the intestinal tract of broilers. In addition, a decrease in faecal shedding of *C. perfringens* in broilers at slaughter age after treatment with Salinomycin was reported by Bolder *et al.* [59]. Engberg *et al.* [41] concluded that Salinomycin decreased both *C. perfringens* and cecal lactic acid bacteria in birds. Knarreborg *et al.* [60] determined that when combined avilamycin and Salinomycin caused shift in the species of Lactobacillus found within the broiler ileum, reduced plate counts of *C. perfringens* as well as caused a shift in the nucleotide sequence of the alpha-toxin gene produced by *C. perfringens*. On the other side the role of probiotics in reducing colonization of *C. perfringens* was studied by Takeda *et al.* [61] and Pascual *et al.* [62] who estimated that dietary lactose was effective in reducing the *C. perfringens* cecal colonization. Also, when 1 and 20-day-old chickens were dosed with 10^9 spores of a *Bacillus subtilis* strain and challenged 24 hours later with 10^5 CFU of *C. perfringens*, colonization and persistence of *C. perfringens* were suppressed [63].

The effect of combination between the competitive exclusion (CE) compound and the ionophorous anticoccidial agent narasin for controlling field infection with *C. perfringens* was studied by Kaldhusdal *et al.* [28] who stated that treatment was associated with positive but statistically non-significant effects on gut health. Delayed intestinal proliferation of *C. perfringens* and delayed appearance of the organism in the gut lesions were found in CE-treated flocks. This delay was associated with improved production performance at slaughter.

The results of the histopathological examination of the small intestine in birds in different groups were accord with those reported by Al-Sheikly and Truscott [48, 49, 64] who described macroscopical and micrscopical intestinal lesions of experimental *C. perfringens* infection in broiler chickens as slight oedema and dilation of vessels, with some sloughed epithelium visible in the intestinal lumen at one hour post inoculation (PI). At 3 hour PI, the intestine had a grayish and thickened mucosa with marked oedema resulting in detachment of the epithelial layer from the lamina propria, especially at the apex of the villi. Early stages of degeneration of the villi tips and increases in the amount of sloughed epithelium and fibrinous exudates were apparent at this stage of the infection. Five hours PI, coagulation necrosis of the epithelial layer and lamina propria at the villus tip was detected. Blood vessels were congested and/or occluded with hyaline thrombi. Large numbers of Gram-positive bacteria colonize the necrotic tissues and villi were shortened. Mononuclear cells and heterophils infiltrate the lamina propria. Histological lesions at 8 and 12 hours PI were characterized by massive necrosis of the villi,

with necrotic zones reaching the crypts. Fibrin and cellular debris were present in the intestinal lumen at this stage. Histopathological alterations in field cases of necrotic enteritis were similar as in the afore-described experiment [47, 50, 53]. In addition, Kutkat *et al.* [51] observed hyperplasia in the epithelial cells lining the mucosal layer of chickens that received feed *Lactobacillus acidophilus* before *C. perfringens* inoculation.

In conclusion, using of some feed additives like Salinomycin was nearly effective as using of natural product like synbiotic in prevention of *C. perfringens* as expressed by reduction of signs, mortalities, lesions and intestinal count and also improving the performance parameters of broiler chickens. Moreover, the combination of both treatments was superior in prevention than using of each separately.

REFERENCES

1. Fukata, T., Y. Hadate, E. Baba and A. Arakawa, 1991. Influence of bacteria on *Clostridium perfringens* infections in young chickens. Avian Dis., 35: 224-227.
2. Ficken, M.D. and D.P. Wages, 1997. Necrotic enteritis. in Diseases of Poultry. 10th ed. Calnek, B. W., ed. Iowa State Univ. Press, Ames., pp: 261-264.
3. Dahiya, J.P., D.C. Wilkie, A.G. Van Kessel and M.D. Drew, 2006. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim Feed Sci. Technol., 129: 60-88.
4. Cowen, B.S., L.D. Schwartz, R.A. Wilson and S.I. Ambrus, 1987. Experimentally induced necrotic enteritis in chickens. Avian Dis., 31: 904-906.
5. Kaldhusdal, M., M. Hofshagen, A. Lovland, H. Langstrand and K. Redhead, 1999. Necrotic enteritis challenge models with broiler chickens raised on litter: evaluation of preconditions, *Clostridium perfringens* strains and outcome variables. FEMS Immunol Med Microbiol., 24: 337-343.
6. McReynolds, J.L., J.A. Byrd, R.C. Anderson, R.W. Moore, T.S. Edrington, K.J. Genovese, T.L. Poole, L.F. Kubena and D.J. Nisbet, 2004. Evaluation of immunosuppressants and dietary mechanisms in an experimental disease model for necrotic enteritis. Poult Sci., 83: 1948-1952.
7. Al-Sheikhly, F. and A. Al-Saieg, 1980. Role of Coccidia in the occurrence of necrotic enteritis of chickens. Avian Dis., 24: 324-333.
8. Shane, S.M., J.E. Gyimah, K.S. Harrington and T.G. Snider, 1985. Etiology and pathogenesis of necrotic enteritis. Vet. Res. Commun., 9: 269-287.
9. Jackson, M.E., D.M. Anderson, H.Y. Hsiao, G.F. Mathis and D.W. Fodge, 2003. Beneficial effect of β-mannanase feed enzyme on performance of chicks challenged with *Eimeria sp.* and *Clostridium perfringens*. Avian Dis., 47: 759-763.
10. Riddell, C. and X.M. Kong, 1992. The influence of diet on necrotic enteritis in broiler chickens. Avian Dis., 36: 499-503.
11. Drew, M.D., N.A. Syed, B.G. Goldade, B. Laarveld and A.G. Van Kessel, 2004. Effects of dietary protein source and level on intestinal populations of *Clostridium perfringens* in broiler chickens. Poult Sci., 83: 414-420.
12. Wilkie, D.C., A.G. Van Kessel, L. White, B. Laarveld and M.D. Drew, 2005. Dietary amino acids affect intestinal *Clostridium perfringens* populations in broiler chickens. Canadian J. Anim Sci., 85: 185-193.
13. Palliyeguru, M.W.C.D., S.P. Rose and A.M. Mackenzie, 2010. Effect of dietary protein concentrates on the incidence of subclinical necrotic enteritis and growth performance of broiler chickens. Poult Sci., 89: 34-43.
14. Lovland, A. and M. Kaldhusdal, 2001. Severely impaired production performance in broiler flocks with high incidence of *Clostridium perfringens*-associated hepatitis. Avian Pathol., 30: 73-81.
15. Van Immerseel, F., J. De Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck and R. Ducatelle, 2004. *Clostridium perfringens* in poultry: an emerging threat for animal and public health. Avian Pathol., 33: 53-549.
16. McDougald, L.R., G.F. Mathis and D.P. Conway, 1996. Effects on semudaramicin, salinomycin and monensin on performance, shank pigmentation and coccidial lesions in broiler chickens in floor pens. Avian Dis., 40: 68-71.
17. Devriese, L.A., G. Daube, J. Hommez and F. SssHaesebrouck, 1993. *In vitro* susceptibility of *Clostridium perfringens* isolated from farm animals to growth-enhancing antibiotics. J. Appl. Bacteriol., 75: 55-57.
18. Watkins, K.L., T.R. Shryock, R.N. Dearth and Y.M. Saif, 1997. *In vitro* antimicrobial susceptibility of *Clostridium perfringens* from commercial turkey and broiler chicken origin. Vet Microbiol., 54: 195-200.

19. Knarreborg, A.M.A., R.M. Simon, B. Engburg, B. Jensen and G.W. Tannock, 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol., 68: 5918-5924.
20. Martel, A., L.A. Devriese, K. Cauwerts, K. De Gussem, A. Decostere and F. Haesebrouck, 2004. Susceptibility of *Clostridium perfringens* strains from broiler chickens to antibiotics and anticoccidials. Avian Pathol., 33: 3-7.
21. Johansen, C.H., L. Bjerrum and K. Pedersen, 2007. Impact of salinomycin on the intestinal microflora of broiler chickens. Acta Vet Scand., 49: 30.
22. Gibson, G.R. and R. Fuller, 2000. Aspects of *In vitro* and *In vivo* research approaches directed towards identifying probiotics and prebiotics for human use. J. Nutr., 130: 391S-395S.
23. Fuller, R., 1999. Probiotics for farm animals. Probiotics. A Critical Review, 15-22. Wymondham, UK - In: G.W. Tannock, (Ed.), pp: 49.
24. Gibson, G.R. and M.B. Roberfroid, 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr., 125: 1401.
25. Patterson, J.A. and K.M. Burkholder, 2003. Application of prebiotics and probiotics in poultry production. Poult Sci., 82: 627-631.
26. Le Leu, R.K., I.L. Brown, Y.A. Hu, R. Bird, M. Jackson, A. Esterman and G.P. Young, 2005. A synbiotic combination of resistant starch and bifidobacterium lactis facilitates apoptotic deletion of carcinogen-damaged cells in rat colon. J. Nutr., 135: 996-1001.
27. Macfarlane, S., E. Furrie, A. Kennedy, J.H. Cummings and G.T. Macfarlane, 2005. Mucosal bacteria in ulcerative colitis. Br. J. Nutr., 93: 67-72.
28. Kaldhusdal, M., C. Schneitz, M. Hofshagen and E. Skjerve, 2001. Reduced incidence of *Clostridium perfringens*-associated lesions and improved performance in broiler chickens treated with normal intestinal bacteria from adult fowl. Avian. Dis., 45: 149-156.
29. Zouelfakar, Sahar, A. and M. Elshazly, 2002. Effect of probiotics and prebiotics combination (Biomin IMBO and Biomin C-EX) on *E.coli* infection in broiler chickens. J. Egypt Med. Associ., 62: 113-119.
30. Awaad, M.H., A. Manal, A. Sahar and S. Basma, 2004. Effect of *Pediococcus acidilactici* and *Saccharomyces boulardii* on intestinal and caecal colonization on *Salmonella typhimurium* and *Clostridium perfringens* in broiler chickens. J. Egypt Med. Associ., 72: 114-120.
31. Abd El-Ghany, Wafaa, A., K. Madian, A. Ebtehal and M. Gehan, 2006. The effect of combined competitive exclusion culture with mannan-oligosaccharides and ciprofloxacin on *Salmonella enteritidis* colonization in broiler chickens. 12th Scient. Cong., Fac. Vet. Med. Assiut Univ. Egypt, pp: 493-525.
32. Dahiya, J.P., D. Hoehler, D.C. Wilkie, A.G. Van Kesse and M.D. Drew, 2005. Dietary glycine concentration affects intestinal *Clostridium perfringens* and lactobacilli populations in broiler chickens. Poult Sci., 84: 1875-1885.
33. Prescott, J., R. Sivendra and D.A. Barnum, 1978. The use of bacitracin in the prevention and treatment of experimentally-induced necrotic enteritis in the chicken. Canadian Vet J., 19: 181-183.
34. Sainsbury, D., 1984. Systems of management. Ch.9 P. 102. In "Poultry Health and Management". 2nd ed. By Sainsbury, D. Granada Publishing Ltd. 8 Grafton Street, London WIX3 LA.
35. Cruickshank, R., J.R. Duguid, B.P. Marmion and R.H.A. Swain, 1975. Text book of Medical Microbiology, 12th ed. Churchill, Livingstone, Edinburgh and New York.
36. Banchroft, J.D., A. Stevens and D.R. Turner, 1996. Theory and practice of histological techniques. 4th ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.
37. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Ed., (Iowa State College Press, Ames, IA), pp: 39-63.
38. Shaprio, S.K. and W.S. Sarles, 1949. Microorganisms of the intestinal tract of chickens. J. Bacteriol., 58: 531-544.
39. Williams, R.B., 2002. Anticoccidial vaccines for broiler chickens: pathways to success. Avian Pathol., 31: 317-353.
40. Elwinger, K., C. Schneitz, E. Berndtson, O. Fossum, B. Teglof and B. Engstrom, 1992. Factors affecting the incidence of necrotic enteritis, caecal carriage of *Clostridium perfringens* and bird performance in broiler chicks. Acta Vet Scand., 33: 369-378.
41. Engberg, R.M., M.S. Hedemann, T.D. Leser and B.B. Jensen, 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult Sci., 79: 1311-1319.

42. Vissiennon, T., H. Kroger, T. Kohler and R. Kliche, 2000. Effect of avilamycin tylosin and ionophore anticoccidials on *Clostridium perfringens* enterotoxaemia in chickens. Berlin Munch Wochen., 113: 9-13.
43. Emik, F. and P. Bedrnik, 2001. Controlling coccidiosis in broiler growing. Poult Inter., 40: 36-42.
44. Williams, R.B., R.N. Marshall, R.M. La Ragione and J. Catchpole, 2003. A new method for the experimental production of necrotic enteritis and its use for studies on the relationships between necrotic enteritis, coccidiosis and anticoccidial vaccination of chickens. Parasitol Res., 90: 19-26.
45. Waldenstedt, L., K. Elwinger, P. Thebo and A. Uggla, 1999. Effect of betaine on broiler performance during an experimental coccidial infection. Poult Sci., 78: 182-189.
46. Wijewanta, E.A. and P. Seneviratna, 1971. Bacteriological studies of fatal type A *Clostridium perfringens* infection in chickens. Avian Dis., 15: 654-661.
47. Helmboldt, C.F. and E.S. Bryant, 1971. The pathology of necrotic enteritis in domestic fowl. Avian Dis., 15: 775-780.
48. Al-Sheikly, F. and R.B. Truscott, 1976. The pathology of necrotic enteritis of chickens following infusion of broth cultures of *Clostridium perfringens* into the duodenum. Avian Dis., 21: 230-240.
49. Al-Sheikly, F. and R.B. Truscott, 1976. The pathology of necrotic enteritis of chickens following infusion of crude toxins of *Clostridium perfringens* into the duodenum. Avian Dis., 21: 241-255.
50. Gadzinski, P. and R.J. Julian, 1992. Necrotic enteritis in turkeys. Avian Dis., 36: 792-798.
51. Kutkat, M.A., S.R. Nagwa and A. Ebtehal, 2002. Effect of *Lactobacillus acidophilus* on controlling of *Clostridium perfringens* and *E. coli* infections in native breed chickens. J. Egypt Med. Associ., 62: 89-101.
52. Hofacre, C.L., T. Beacorn, S. Collett and G. Mathis, 2003. Using competitive exclusion, mannan-oligosaccharide and other intestinal products to control necrotic enteritis. J. Appl. Poult Res., 12: 60-64.
53. Broussard, C.T., C.L. Hofacre, R.K. Page and O.J. Fletcher, 1986. Necrotic enteritis in cage-reared commercial layer pullets. Avian Dis., 30: 617-619.
54. Hofacre, C.L., R. Froyman, B. Gautrias, B. George, M.A. Goodwin and J. Brown, 1998. Use of Aviguard and other intestinal bioproducts in experimental *Clostridium perfringens* -associated necrotizing enteritis in broiler chickens. Avian Dis., 42: 579-584.
55. Radu, J., C. Van Dijk, R.K. Wheelhouse, C.A. Hummant and P. Gadbois, 1987. Feed and water consumption and performance of male and female broilers fed salinomycin and maduramicin followed by a withdrawal ration. Poult Sci., 66: 1878-1881.
56. Engberg, R.M., B.B. Jensen and O. Hojberg, 2008. Plant of the *Juglandaceae* family as alternative to antibiotic growth promoters in broiler production. 16th European Symp Poult Nutr., pp: 293-296.
57. Madian, K. and A.Wafaa, 2006. Study on the effects of prebiotic, synbiotic and probiotic, as alternatives to antibiotic growth promoter on the performance, intestinal colonization, humoral immune response and clinical serum chemistry of the broiler chickens. Proc 2nd Inter Conf, Vet Res Division. National Res Center, Cairo, Egypt, pp: 145-172.
58. Elwinger, K., E. Berndtson, B. Engstrom, O. Fossum and L. Waldenstedt, 1998. Effect of antibiotic growth promoters and anticoccidials on growth of *Clostridium perfringens* in the caeca and on performance of broiler chickens. Acta Vet. Scand., 39: 433-441.
59. Bolder, N.M., J.A. Wagenaar, F.F. Putirulan, K.T. Veldman and M. Sommer, 1999. The effect of Flavophospholipol (Flavomycin) and Salinomycin sodium (Sacox) on the excretion of *Clostridium perfringens*, *Salmonella enteritidis* and *Campylobacter jejuni* in broilers after experimental infection. Poult Sci., 78: 1681-1689.
60. Knarreborg, A.M.A., C. Lauridesen, R.M. Engberg and S.K. Jensen, 2004. Dietary antibiotic growth promoters enhance the bioavailability of a-tocopheryl acetate in broilers through medications of the lipid adsorption processes. J. Nutr., 134: 1487-1492.
61. Takeda, T., T. Fukata, T. Miyamoto, K. Sasai, E. Baba and A. Arakawa, 1995. The effects of dietary lactose and rye on cecal colonization of *Clostridium perfringens* in chicks. Avian Dis., 39: 375-381.
62. Pascual, M., M. Hugas, J. I. Badiola, J.M. Monfort and M. Garriga, 1999. CTC2197 prevents *Salmonella enteritidis* and *Clostridium perfringens* colonization in chickens. Applied Environ Microbiol., 65: 4981-4986.
63. La Ragione, R.M. and M.J. Woodward, 2003. Competitive exclusion by spores of *Salmonella enterica* serotype *Enteritidis* and *Clostridium perfringens* in young chickens. Vet Microbiol., 94: 245-256.
64. Al-Sheikly, F. and R.B. Truscott, 1976. The interaction of *Clostridium perfringens* and its toxins in the production of necrotic enteritis of chickens. Avian Dis., 21: 256-263.