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

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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 affect 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 (10^7 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 cecal 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].

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Probiotic means "for life" in Greek [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 probiotics are known as symbiotics 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 symbiotics 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 coccidiose (Salinomycin) and a commercial symbiotic 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 (15 to 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.

Symbiotic Product: A commercial product containing stabilized probiotic (strain of Enterococcus faecium), prebiotic (fructo-oligosacharides of inactivated cell wall fragments of non pathogenic microorganisms) and phycophytic substances were used as symbiotic preparation. This symbiotic 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. perfringens type (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 in 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 10,000 r.p.m. for 10 minutes and the bacterial concentration of the culture was adjusted to a turbidity of opacity tube to 10⁶ 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 coccidiose (Salinomycin) and symbiotic preparations, respectively from the day old till the end of the study. Birds in group (3) received ration containing both products (Salinomycin and symbiotic) 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 (10⁶ 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
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, symbiotic and Salinomycin with symbiotic 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 Salinomycine, symbiotic 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 symbiotic 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 symbiotic 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 symbiotic treatment on the mortality rate in *C. perfringens* infected and treated broiler chickens

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>End of observation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of sacrificed birds</td>
</tr>
<tr>
<td>Infected+ Salinomycin</td>
<td>12</td>
</tr>
<tr>
<td>Infected+ symbiotic</td>
<td>12</td>
</tr>
<tr>
<td>Infected+ Salinomycin+ symbiotic</td>
<td>12</td>
</tr>
<tr>
<td>Infected non treated</td>
<td>12</td>
</tr>
<tr>
<td>Non infected, non treated</td>
<td>12</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Days post challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Infected+ Salinomycin</td>
<td>1.20</td>
</tr>
<tr>
<td>Infected+ symbiotic</td>
<td>1.43</td>
</tr>
<tr>
<td>Infected+ Salinomycin+ symbiotic</td>
<td>0.63</td>
</tr>
<tr>
<td>Infected non treated</td>
<td>3.25</td>
</tr>
<tr>
<td>Non infected, non treated</td>
<td>0.00</td>
</tr>
</tbody>
</table>

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 symbiotic treatment on the performance variables in *C. perfringens* infected and treated broiler chickens

<table>
<thead>
<tr>
<th>Age/Week</th>
<th>Body weight/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected+ Salinomycin</td>
</tr>
<tr>
<td>1</td>
<td>124.2±4.20</td>
</tr>
<tr>
<td>2</td>
<td>316.4±5.11</td>
</tr>
<tr>
<td>3</td>
<td>590.7±7.20</td>
</tr>
<tr>
<td>4</td>
<td>891.8±9.02</td>
</tr>
<tr>
<td>5</td>
<td>1105.2±12.70</td>
</tr>
</tbody>
</table>

Total feed consumption/bird/kg: 2.01
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 symbiotic treatment on *C. perfringens* intestinal and caecal count in infected and treated broiler chickens (log$_10$ CFU/g of intestinal or caecal contents)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Intestinal count</th>
<th>Caecal count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days post challenge</td>
<td>Days post challenge</td>
</tr>
<tr>
<td>Infected+ Salinomycin</td>
<td>0.9±0.12</td>
<td>2.3±0.34</td>
</tr>
<tr>
<td>Infected+ symbiotic</td>
<td>0.6±0.14</td>
<td>1.9±0.31</td>
</tr>
<tr>
<td>Infected+ Salinomycin+ symbiotic</td>
<td>0.5±0.10</td>
<td>1.2±0.10</td>
</tr>
<tr>
<td>Infected non treated</td>
<td>2.4±0.32*</td>
<td>5.7±0.33*</td>
</tr>
<tr>
<td>Non infected, non treated</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*: Significant differences (P<0.05)
Table 5: The effect of Salinomycin and symbiotic treatment on the histopathological alterations of the small intestine in C. perfringens infected and treated broiler chickens

<table>
<thead>
<tr>
<th>Histopathological alterations</th>
<th>Treatment groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected+ Salinomycin</td>
</tr>
<tr>
<td>Sloughing of the mucosa</td>
<td>+++</td>
</tr>
<tr>
<td>Neutrosis of the mucosa</td>
<td>+++</td>
</tr>
<tr>
<td>Inflammatory cells infiltration in LP</td>
<td>++</td>
</tr>
<tr>
<td>Oedema in LP</td>
<td>++</td>
</tr>
<tr>
<td>Hypertrophy in musculature</td>
<td>-</td>
</tr>
</tbody>
</table>

+++ = 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. 3: Small intestine of birds infected with C. perfringens and treated with Salinomycin and symbiotic 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 birds 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 symbiotic showed mild focal inflammatory cells infiltration in lamina propria. The small intestines of chickens infected with C. perfringens treated with Salinomycin and symbiotic 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 epithelium with oedema, inflammatory cells infiltration in the lamina propria and hypertrophy in the musculature.
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 permitted *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 bacteriociadally on clostridia [39]. In a study by Emin and Bedná [43], the broiler chickens flocks that treated with coccidiostatic 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-mediated 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, diarrhea and decreased feed consumption were recorded [7, 47-50]. The current results indicated that Salinomycin or symbiotic alone or in combination were able to reduce the signs and mortality rate caused by *C. perfringens*. Similarly, Kulkat 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 probiotic containing FOS or manno-oligosaccharides (MOS) to the ration of broiler

**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.
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 ceca whereas it becomes thin walled, friable, dilated, filled with gas and the mucosal surfaces were covered with a grey-brown to yellow-green diphtheric membrane or pseudomembrane [50, 53]. In this experiment, both of Salinomycin and symbiotic 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 Engberg 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 symbiotic 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 symbiotic 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 oecal 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 oecal colonization. Also, when 1 and 20-day-old chickens were dosed with 10⁶ spores of a Bacillus subtilis strain and challenged 24 hours later with 10⁵ 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 microscopical 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 oedemed 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, Kutkut 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 symbiotic 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


