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The Effect of a Mannanoligosaccharides (Biomos®) on Necrotic Enteritis Infection in Broiler Chickens

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Abstract: This study was conducted to determine the effect of Mannanoligosaccharide (Biomos®, Alltech Inc., IFT, Egypt) added to a broiler ration to reduce or control the prevalence of necrotic enteritis among broiler chicks reared under normal conditions. Seventy five, one-day-old, Cobb chicks were randomly divided to three groups of 25 birds for each. First group consists of nonchallenged negative control group, second group was a positive control group gavaged with *C. perfringens* (CP) (10^7 cfu/mL) daily for 3 consecutive days starting on d 16. The third group was supplemented with 2 g/kg ration from the start to the end of the experiment and orally challenged with *C. perfringens* type A at the the same method for positive control group. On days 1 (19-day-old) and 7 (25-day-old) post-challenge, lesion scores, mortalities and intestinal CP levels were assessed. The log10 *C. perfringens*/g of intestinal contents was significantly reduced in the biomos treated group (MTG) with a log10 value of 1.8 and 2.2 at the 1st and 7th days after challenge respectively compared with 4.3 and 4.7 at the same times respectively in the Positive Control Group (PC). Mortality was reduced to 8% in group supplemented with Biomos in contrast to 20% in positive control group. The present study revealed that the birds given biomos supplementation showed a reduction in the severity of lesion score which reached to 0.57 in contrast to 1.8 in positive control group. In summary, the findings of the present study strengthen the use of biomos as a safe alternative to antibiotics and attractive aid for prevention and control of *Clostridium perferingens* infections in broilers.

Key words: Necrotic enteritis, mannan oligosachrides, biomos, broilers

INTRODUCTION

Enteric pathogens result in huge losses to the poultry industry annually and their control or reduction could potentially save the producers millions of dollars. Recognized as the causative agent of Necrotic Enteritis (NE) (Fukata *et al*., 1991) *Clostridium perfringens* (CP) is such pathogen. Necrotic Enteritis (NE) is an enteric disease of poultry usually noticed between 2-6 weeks after hatching and caused by *Clostridium perfringens* Type A (McDevitt *et al*., 2006). The disease is propagated through horizontal (feed or litter contaminated with CP spores) and vertical (from parents to progeny) modes of transmission (Heier *et al*., 2001; Williams, 2002). Disease onset is accelerated by predisposing factors such as pre-existing damage to the intestinal epithelium by coccidia (*Eimeria* sp.), high dietary levels of certain cereals and fish meal, disturbance to the normal intestinal flora, overcrowding and a variety of environmental (management and climatic) conditions (McDevitt *et al*., 2006).

Depending on the number of CP cells in the intestine, NE can manifest as acute (clinical) or chronic (subclinical). Birds suffering from acute NE typically harbor CP levels of about 10^7-10^9 cfu/g of digesta and demonstrate symptoms characterized by diarrhea, necrotic intestinal lesions, depression in growth rate and feed efficiency and high mortality (up to 40% (McDevitt *et al*., 2006). The chronic form of the disease is subtle in that it affects impaired feed intake and feed conversion at a rate that is difficult to notice until slaughtering and carcass processing in a manner that is difficult to measure until processing when high numbers of carcasses and livers are condemned for low weight and cholangiohepatitis (Lovland and Kaldhusdal, 1999).

Estimates indicate that up to 37% of broilers grown in North America are affected by NE (Annett *et al*., 2002)., it has been estimated that NE cost the global poultry industry two billion dollars in 2000 due to costs of antimicrobial prophylaxis and inefficient feed conversion (Kaldhusdal and Lovland, 2000). Thus there is a need for treatment and preventive measures to control the disease.

Controlling NE disease in poultry is crucial because of the economic loss to poultry producers, the poor health of NE-infected birds and the potential for a food borne illness in humans. Traditionally, NE is controlled by antibiotics such as bacitracin, virginiamycin and lincomycin (Feed Additive Compendium, 2008). The use of antibiotics in livestock feed has been implicated as a

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contributing factor to the development of resistant bacterial strains that sometimes find their way into the food chain and cause human illnesses that do not generally respond to antibiotic therapy (Diarra *et al*., 2007) that has led to the ban of antibiotics growth promotants in the (European Union internet) and a gradual reduction of these antibiotics in the United States.

Today, the poultry industry must focus more attention towards addressing public concern for environmental and food safety. As in many other industries, the global paradigm is shifting from an emphasis on efficiency to one of public security. Consequently, several alternative non-antibiotic strategies are being in a priority for poultry scientists for NE control. There are several non-antibiotic strategies that have shown efficacy in controlling bacterial infections, one of such classes is prebiotic. They can promote competitive exclusion of pathogenic microbes and selective colonization by beneficial microbes (Biggs *et al*., 2007). Of the known prebiotics, Mannan-Oligosaccharide (MOS).

Mannan-oligosaccharides [MOS, Bio-Mos®, (1997) Alltech, Nicholasville, Kentucky, USA] is a non-digestible, complex carbohydrate that is the main component in the outer cell wall of yeast cells (*Saccharomyces cerevisiae* var. *boulardii*) and is thought to act by binding and removing pathogens from the intestinal tract and stimulates the immune system (Delzenne, 2003).

There is a lack of information on the use of MOS as a means of controlling necrotic enteritis in poultry. In the light of the above-mentioned, this study was carried out to evaluate the efficacy of mannan-oilgosacharides supplementation in reducing NE disease associated with broiler chickens.

MATERIALS AND METHODS

Birds: Seventy five, one-day-old broiler chicks (Cobb) were obtained from a commercial farm and raised in naturally ventilated broiler pen with floor covered with wood shavings, using a daily photoperiod of 23h of light and 1 h darken. Birds were randomly assigned to 3 groups separated by wood partitions. Birds were fed standard commercial corn-soy based diets formulated to meet the requirements of broilers chickens A two-phase feeding program was used: starter (0 to 15 d), grower (15 to 42d). Feed and water was provided *ad libitum* throughout the experiment.

The chicks were vaccinated at 6d of age against Newcastle disease (HB1/drinking). At 12d of age, the vaccination against IBD was administered with intermediate strain vaccine via drinking water. At 18d the Newcastle disease vaccine repeated with Clone-30 via drinking water then at 22 day the birds were administered with second dose of IBD vaccine via drinking water.

Table 1: Experimental groups

Treatment[1]	Challenge	Biomos
NC	-	-
PC	+	-
MTG	+	+

[1]Treatment (NC) consisted of unchallenged chicks fed with ration only; Treatment 2 (PC) consisted of challenged chicks with *C. perfringens* on three consecutive days (day 16, 17 and 18) and fed on ration without biomos®; Treatments 3 (MTG) consisted of chicks challenged with *C. perfringens* and fed with ration supplemented with biomos

Experimental treatments: As shown in Table 1 the experiment consitis of three groups; group 1 Negative Control (NC) consisted of chicks not challenged with CP and fed mashed starter diet with no Biomos® added. Group 2 Positive Control (PC) consisted of chicks challenged with CP and fed mashed starter diet with no Biomos® added. Group 3 Biomos treated group (MTG) consisted of chicks that were challenged with of CP inoculum (10^7 cfu/mL) on three consecutive days (days 16, 17 and 18) and fed mashed starter diet with Biomos. Dietary MOS (Biomos®) was included at 2 g/kg in starter, 1 g/kg to the end of experiment as recommended by manufacturer (Alltech Inc., Nicholasville, KY, IFT, Egypt). Duration of experiment was 25 days.

Challenge protocol: One field isolate of *C. perfringens* (type A) was collected from commercial flocks having NE, in different geographical locations in Assiut province and were isolated and cultured characterized by PCR technique as a type A toxin producer (Mohamed *et al*., 2009). For challenge, the organism was cultured anaerobically on Blood Agar Base (Becton, Dickinson and Co., Sparks, MD, USA) containing 5% sheep blood for 18 h at 37°C, then aseptically inoculated into cooked meat medium (Difco Labs, Detroit, MI, USA) and incubated anaerobically overnight at 37°C.

All birds were orally challenged with gavage (3 ml) on day 16, 17 and 18 of the experiment with this actively growing culture of *C. perfringens*. Bacterial counts were performed on the culture daily prior to inoculation. The chicks in the control treatment were orally gavaged daily (on days 16, 17 and 18) with 3 mL of freshly prepared sterile fluid thioglycolate broth. The challenge doses, at these concentrations, were chosen based on previous research (McReynolds *et al*., 2009) and have been known to show signs of NE with intestinal lesions.

Pathological examination: Birds were observed on a basis at least once daily for any signs of NE and all birds that died during the course of experiments were necropzied to determine the cause of death. On day 28, the surviving chickens were killed by cervical dislocation, weighed and necropzied immediately. Intestinal tracts were removed and intestinal lesions were scored

according to the method of (Prescott *et al*., 1978). To evaluate gross lesions associated with NE, the jejunum and ileum approximately 10 cm cranial and dorsal to Meckel's diverticulum were examined. Lesion scores were recorded using the following criteria: 0 = no gross lesions, normal intestinal appearance; 1 = thin-walled or friable, gray appearance; 2 = thin-walled, focal necrosis, gray appearance, small amounts of gas production; 3 = thin walled, sizable patches of necrosis, gas-filled intestine, small flecks of blood; 4 = severe extensive necrosis, marked hemorrhage, large amounts of gas in intestine.

Enumeration of *C. perfringens*: Intestinal sampling to determine the concentration of CP in intestinal contents was performed on days 15 (one day pre-challenge), 19 (one day post-challenge) and 25 (7 days post-challenge) of experiment. On each time, four chicks were randomly removed from each treatment then killed.

To quantitatively measure populations of *C. perfringens*, birds were killed by cervical dislocation while feeding normally. The abdominal cavity was opened and all digest contents of ileum were immediately collected under aseptic conditions into sterile plastic bags and put on ice, until they were transported to the laboratory for enumeration of microbial populations. 4 gm of intestinal content was placed in 10 mL of anaerobic thioglycollate, vortex for 30 s and a 0.5-mL aliquot of intestinal digesta was removed and placed into 4.5 mL of thioglycollate medium. Ten-fold serial dilutions were performed, plated on reinforced clostridial agar and incubated (24 h at 37°C). All of the *C. perfringens* culture work was performed in an anaerobic hood. Plates containing colonies exhibiting typical morphology with more than 30 or less than 300 colonies were counted and recorded. Mortality was recorded daily.

Statistical analysis: Bacterial counts were transformed to logarithmic colony forming units (cfu/g) values and all the data were statistically analyzed by with Data were processed by SPSS (1999) 14.0. Tukey test (t-test) was used for comparison of the Latin square with the supplemented diets. Probability values of 0.05 were taken as significant.

RESULTS

The impacts of MOS supplementation on the lesion score, mortality and number of CP in broiler chicks are shown in Table 2. The effects of dietary treatments on are summarized in Table 2. In general, the birds in the control group (NC) were in a very healthy condition and Mortality rate was zero (0%) in NC group which is a clear sign of generally good health conditions of birds. Subsequent to challenge with *C. perfringens* some of the birds in the PC group were dull, depressed and had abnormally wet droppings for the first 3-5 days after challenge. As shown in Fig. 1.

The intestinal gross lesions in most of the birds were conclusive of NE, dilated small intestine with mucoid to roughened mucosa. Also the birds showed liver infarctions (Fig. 2-4). The findings were consistent with clinical NE caused by CP.

Overall the intestinal lesions of NE were reported in 7 birds (28%) in treated group (MTG) in comparison to 9 (36%) in PC group. The data from the experiment showed that Biomos treated group was efficacious ($p<0.05$) in reducing the severity of lesion scores when compared with the positive control, with mean lesion scores of 0.64 compared with 1.6 in the positive control (Table 1).

Five birds died (mortality 20%) during the course of experiment in positive control group. Mortality was reduced to 8% ($p<0.05$) in Biomos treated group (MTG) when compared with the positive control group.

C. perfringens were enumerated in the ileum of the birds on day one and seventh post challenge and the baseline CP count determined at one day pre-challenge (day 15 of experiment) and the results indicated that The log10 *C. perfringens*/g of intestinal contents was significantly reduced in the biomos treated group with a log10 value of 1.8 and 2.2 at the 1st and 7th days after challenge respectively compared with 4.3 and 4.7 at the same times respectively in the positive control group.

DISCUSSION

To sustain poultry production to meet global demand, antibiotic replacements are needed. Among the feed additives evaluated to date in poultry, prebiotics are considered favorable alternatives, because they can

Table 2: Effect of the addition of biomos to the diets of experimentally challenged birds on lesion score, mortality and number of CP in the intestine

Treatment	Control		Biomos treated (MTG)
	Negative (NC)	Positive (PC)	
Lesion score[a]	0.21±0.09	1.8[a]±0.12	0.57[ab]
Mortality	0/25	5/25	2/25
% Mortality	0	20[b]	8[ba]
Log10 cfu/g[a] (1st day post challenge)	1.4±0.16	4.3[c]± 0.2	1.8[ca]±0.3
Log10 cfu/g (7th day post challenge)	2.9±0.01	4.7[d]±0.04	2.2[da] ±0.45

Data of lesion scores and number of CP are means of 4 chicks representing each group

Log10 cfu/g is represented by the mean of treatment subset (n = 5)

[a-d]Means of main effects on different sampling days with different supscripts differ significantly at $p<0.05$

Fig. 1: Experimentally infected birds suffered from diarrhea

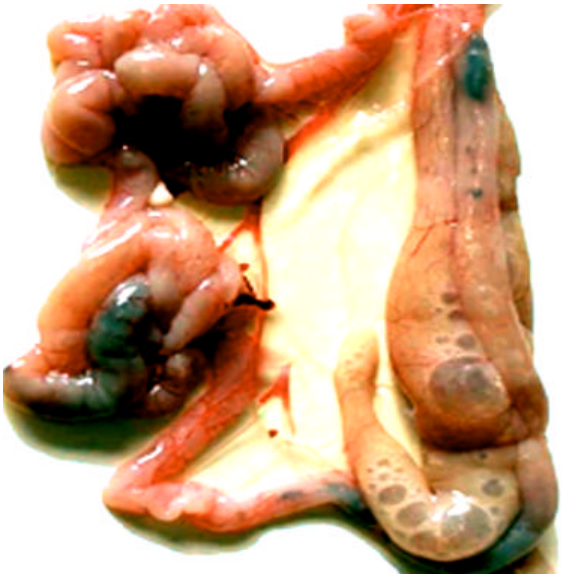

Fig. 2: Dilated intestine with Engorged mesenteric blood vessels of experimentally infected birds

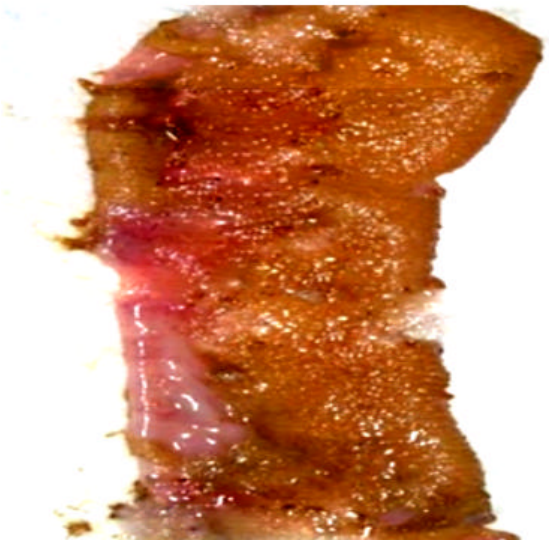

Fig. 3: Roughned necrotized Mucosa of intestine

Fig. 4: White foci representing necrosis on the liver surface

promote competitive exclusion of pathogenic microbes and selective colonization by beneficial microbes (Biggs *et al*., 2007), of the most known prebiotics is Mannan-Oligosaccharide (MOS) (Bornet *et al*., 2002).

The current study demonstrated the beneficial effect of the Biomos® which revealed its supplementation favored the reduction of NE lesions, mortality and number of bacteria CP in the gut of broiler chickens. This lowering trend in the previously mentioned results is important because the intestinal integrity became more than that not medicated, so if intestinal lesion scores can be reduced, the infected birds have a much greater chance of recovering from the disease and weigh better when recover than infected not medicated one.

The mode of action by which biomos interacts with Clostridia to lower counts is not fully clear, but may be due to:

1) Dietary MOS has been shown to improve intestinal function or gut health (for example, increased villi height, uniformity and integrity (Loddi *et al*., 2002).

2) MOS may modulate humoral immunity against the colonization of specific pathogens, allowing them to be presented to immune cells as attenuated antigens due the production of Short Chain Fatty Acid (SCFA) derived from MOS fermentation that decreases intestinal pH and

reduces the presence of pathogenic bacteria. Butyrate is one of the SCFA produced and its deficiency is associated with intestinal atrophy and functional fails, including decreased immune response (Brouns *et al*., 2002). However, an increased butyrate supply leads to the development of intestinal epithelium, cell differentiation and increased immune response, probably due to the higher number of T-cells in the intestine (Peuranen *et al*., 2006).

3) MOS may also serve as an alternate attachment site in the gut for gram-negative pathogenic organisms with mannose-specific type-1 fimbriae, which adhere to intestinal epithelial cells to initiate the colonization. These bacteria bind to MOS present in the intestinal tract and pass through the gut, instead of attaching to host epithelial cells stimulating a strong immune response and elevating the strength of the intestinal mucosa in studies with poultry (Ferket *et al*., 2002).

Dietary MOS supplementation increased the levels of *Bifidobacterium* sp. and *Lactobacillus* sp. in the intestinal tract and depressed the number of *Enterobacteriaceae* (Fernandez *et al*., 2002). The normal gut flora serves as the first line of defense against pathogens. The normal microbial flora employs various mechanisms that aid in the defense of the gastrointestinal tract of the host. One member of the normal bacterial microflora is *Lactobacillus*, which is capable of producing several compounds that aid in pathogen reduction. Reuterin, a bacteriocin produced by *Lactobacilli*, has been shown in vitro to be inhibitory against *Salmonella*, *Shigella*, *Clostridium* and *Listeria* (Lee *et al*., 2000). Many of the commensal bacteria produce compounds known as bacteriocins that affect both gram-positive and gram-negative bacteria. Other products such as hydrogen peroxide also are produced by commensal bacteria. Hydrogen peroxide results in the peroxidation of lipid membranes and increased bacterial membrane permeability. Other protective products include short-chain fatty acids, which are created by the commensal bacteria as an end product of microbial fermentation. These compounds are predominantly the volatile fatty acids, acetic, propionic and butyric and have been shown to be biological indicators of a healthy microbial ecosystem, as well as having inhibitory effects on pathogenic bacterial colonization in chickens (Nisbet *et al*., 1996).

Abbas *et al*. (2000) and Gao *et al*. (2008) suggested that MOS can increase the concentration of immunoglobulins IgA in the intestines, which may limit the number of pathogens such as *Clostridium perfringen* through its binding to antigens (perhaps such as CP α-toxin) and prevents them from passing through the mucosal membrane and establishing infection and lesions (Kulkarni *et al*., 2010). To confirm this, further studies need to be performed to investigate the mechanism(s) of the immunomodulatory activity of MOS in poultry.

Total mortality in an untreated NE-infected flock group was 16% which run in parallel with that mentioned before which could range from 10 to 40% (Ross Tech, 1999). However we noticed, there was an increase in the CP population among the challenged treated group from the 1st day to the 7th day after challenge. This could be due to fact that the litter on floor serves as continues source of CP challenge due to the coprophagic activity of birds (Line *et al*., 1998)

In conclusion, given the current interest in use of safe alternatives, Biomos is attractive alternative to antibiotics for the control of NE in broiler chickens. These additives inhibit the count of CP in the broiler digestive tract, reduce the chicken mortality and reduce the necrotic lesions in comparison with the positive control group. Further investigation is required to understand the exact mechanism of action of Biomos®.

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