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Ameliorative Effect of Mannan Oligosaccharide on Pathology of Fowl Cholera in Broiler Birds

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ABSTRACT

The Fowl Cholera (FC) (avian cholera, avian pasteurellosis or avian hemorrhagic septicemia) is a contagious disease affecting domesticated and wild birds. The study was conducted to determine the effect of feeding prebiotic Mannan Oligosaccharide (MOS) on the severity of fowl cholera caused by experimental inoculation of *Pasturella multocida* in broiler birds. A total of 48 healthy, day old, White Leghorn chicks of either sex were used for the experimental studies. The bird were divided into two groups and reared under standard feeding and managerial conditions. Extra supplementation of prebiotic MOS was provided to birds of group II. On 28th day of the experiment birds of both the groups were given bacterial infection orally at the rate of 1 mL kg^{-1} body weight. Thereafter, birds from both the groups were sacrificed after 12, 36 and 48 h of giving bacterial infection and observations were recorded. Mortality was not observed in group II birds given MOS supplementation. At all three intervals the bacterial count in blood were much less in birds given MOS supplementation. Examination of tissues from both the group of birds revealed that salient changes occurred in the lungs, liver, spleen, heart and intestine. However, the lesions were less severe in birds given MOS supplementation at all three intervals. The present study revealed that the birds given supplementation of prebiotic MOS showed a reduced severity of infection at all three intervals. This was reflected in the improved haemato-biochemical parameters, reduced mean blood count of the organism and decreased severity in the gross and microscopic lesions at all stages and all organs studied. It was concluded that MOS supplementation reduces the severity of *Pasturella multocida* in broilers.

Key words: Broilers, fowl cholera, MOS, pasturella, pathology, prebiotic

INTRODUCTION

Pasteurella multocida subspecies *multocida* is the most common cause of fowl cholera, although *P. multocida* subspecies *septica* and *gallicida* may also cause fowl cholera-like disease to some extent (Christensen and Bisgaard, 2000). The incidence of fowl cholera, caused by *Pasteurella multocida*, is reported to be on the increase. Mbuthia *et al.* (2008) documented the occurrence of *P. multocida* among healthy-appearing family poultry in a tropical setting and concluded it to be the most common bacterial disease encountered in village chickens. Once introduced into a flock, *P. multocida* can increase in virulence and spread rapidly, leading to high rates of mortality in the flock (Matsumoto and Strain, 1993). Chronic respiratory infection and an

acute septicemia are the most common hallmarks of the disease (Snipes *et al.*, 1990). Woo and Kim (2006) have opined that to effectively control FC in domestic poultry and minimize damage and economic loss of farms, epidemiological studies on the current FC infection in poultry farms should be conducted in a broader and more systematic manner, including pig and dog hosts infected with PM strains. Apata (2009) has claimed that wide use of antibiotics in poultry has led to an increase in numbers of antibiotic resistant bacterial strains which can also be transmitted from poultry to humans through the food chain.

Mannan-Oligosaccharide (MOS), a prebiotic, is a complex carbohydrate extracted from yeast cell wall and is hypothesized to prevent bacterial infections by blocking the attachment and colonization in the bird's intestine (Panda, 2003). In contrast to the mode of action of most antibiotics and carbohydrate fermentation sources, MOS and possibly other oligosaccharides, serve as alternate attachment sites for Gram-negative pathogens, thereby preventing attachment onto enterocytes and subsequent enteric infection. Adherence of the pathogenic microbe to the enterocyte cell wall is thought to be a prerequisite for the onset of infection (Gibbons and Houte, 1975). Pathogens with the mannose-specific Type-1 fimbriae adsorb to the MOS instead of attaching to intestinal epithelial cells and, therefore, move through the intestine without colonization. Newman (1994) reported that the presence of dietary MOS in the intestinal tract removed pathogenic bacteria that could attach to the lumen of the intestine in this manner. Spring *et al.* (2000) found that 80% of *Salmonella enteritidis* and 67% of *Salmonella typhimurium* freely agglutinated with MOS. However, reports on effect of MOS on Pasteurella are lacking. Thus, studies were conducted to observe the ameliorative effect of feeding MOS on the pathology of induced *P multocida* infection in broilers.

MATERIALS AND METHODS

Freeze dried culture of *Pasteurella multocida* subsp. *multocida*, MTCC No-1161 (Strain designation: D7) was obtained from Institute of Microbial Technology, Chandigarh, India in the month of December, 2009. The bacterial suspension was prepared by inoculating the culture into brain heart infusion broth and incubating at 37°C for 24 h. Turbidity of the broth was adjusted using sterile normal saline to match Brown's opacity tube no.3 which gives a count of 1.1×10^9 organisms per mL (Cruickshank *et al.*, 1980).

Approximately 48 day old commercial broilers were reared from day 0 to 4 weeks of age under ideal conditions. The birds were divided into two groups, I (control) and II (experimental), each having 24 birds, respectively. The birds were kept on prestarter ration having 22.5% Crude Protein (CP) and 3000 kcal kg^{-1} metabolizable energy from day 1 to 7 and starter feed having 21% crude protein and 3100 kcal kg^{-1} metabolizable energy from day 8 to 28. Birds of group II (experimental) were fed with extra supplement of prebiotic mannan-oligosaccharide (League Pharmaceutical Pvt. Ltd, Pune) at the rate of 4000 parts per million (ppm) for the entire duration of study. Oral inoculation of *Pasteurella multocida* @ 1 mL kg^{-1} b.wt was given to all birds from both the groups on 28th day of age. The birds were observed for clinical signs and mortality and surviving birds sacrificed by cervical dislocation at 12, 36 and 48 h intervals for haematological, biochemical, microbiological and histopathological studies (Gridley, 1960; Zinkl, 1986).

Statistical analysis: Data gathered from the study was analyzed, using completely randomized design as described by Snedecor and Cochran (1994) using software MSTATC.

RESULTS

Birds exhibited signs of respiratory distress within a few h of giving infection. By 18 h of infection the birds appeared clinically ill and started passing loose droppings. The dyspnoea and diarrhoea increased at 36 h of infection. The present study revealed that the birds given supplementation of prebiotic MOS clinically showed a reduced severity of infection at all three intervals. This was further reflected in the improved haemato-biochemical parameters, reduced mean blood count of the organism and decreased severity in the gross and microscopic lesions at all stages and all organs.

Mortality was not observed in group II birds given MOS supplementation whereas 5 birds from group I died after 24 h of giving infection. The hematological studies revealed that the hemoglobin concentration and total erythrocyte count were significantly decreased at all three intervals in birds given bacterial infection. However, the hemoglobin concentration and total erythrocyte count were significantly more at all three intervals in birds of group II given MOS supplementation. The total leukocyte count of infected chicks from both groups showed a significant increase at all intervals. Heterophil percent increased significantly in group I birds at all three intervals but were within referral range in birds of group II at 36 and 48 h. Although, Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) concentration in serum was significantly high at all intervals in birds of group I they showed non significant changes in birds of group II.

A decrease in blood glucose and plasma protein was observed in both the birds given infection at all three intervals (Table 1).

One milliliter of blood was collected in heparinized vial at 12, 36 and 48 h post inoculation from the birds to ascertain the presence and concentration of the organisms in the blood. Ten fold dilution of blood sample was done and 0.1 mL from each dilution was inoculated in blood agar and incubated at 37°C for 24 h. The calculations were made to count colony forming unit of the organisms in per mL of blood (Table 2).

The concentration of bacteria in both the groups was found to increase with increasing interval. Maximal concentrations of bacteria were observed at 48 h in both the groups. However, at all three intervals the bacterial count were much less in birds given MOS supplementation (Table 2).

Table 1: Mean values of haemato-biochemical parameters at different time intervals (Mean±SE)

		Group I (Time post infection)			Group II (MOS) (Time post infection)		
Parameter	Reference value (n = 6)	12 h	36 h	48 h	12 h	36 h	48 h
Haemoglobin (g%)	10.87±0.59^{a}	9.70±0.71^{b}	9.13±0.65^{b}	9.00±0.51^{c}	9.90±1.06^{a}	9.47±0.62^{ab}	9.68±0.62^{a}
Total erythrocyte count (millions μL^{-1})	2.53±0.16^{a}	2.44±0.32^{b}	2.43±0.46^{b}	2.41±0.38^{b}	2.51±0.15^{a}	2.48±0.20^{ab}	2.49±0.28a^{b}
Total leukocyte count (thousand μL^{-1})	13.09±0.99^{a}	16.96±8.21^{c}	18.16±1.54^{c}	18.37±2.11^{c}	18.32±2.14^{c}	18.83±2.04^{c}	18.77±2.81^{c}
Heterophil (%)	36.83±2.47^{a}	46.33±5.79^{b}	40.83±5.00^{b}	42.50±2.79^{b}	49.17±2.44^{b}	37.33±3.26^{a}	32.33±3.42^{a}
Glucose (mg dL^{-1})	198.38±7.75^{a}	98.35±8.50^{b}	96.38±8.14^{c}	88.03±10.72^{c}	102.77±9.72^{b}	104.78±4.15^{b}	101.08±3.92^{b}
Alanine aminotransferase (U L^{-1})	11.72±0.48^{a}	19.00±0.34^{c}	20.42±0.38^{c}	20.47±0.39^{c}	16.72±0.28^{b}	15.90±0.34^{b}	16.13±0.53^{b}
Aspartate aminotransferase (U L^{-1})	126.20±2.06^{a}	144.67±1.03^{c}	159.32±1.58^{c}	162.78±1.08^{c}	131.38±0.95^{ab}	134.00±3.11^{b}	134.32±3.10^{b}
Plasma protein (g dL^{-1})	3.38±0.08^{a}	2.79±0.37^{ab}	2.24±0.29^{c}	2.08±0.11^{c}	2.72±0.18^{b}	2.88±0.24^{b}	2.68±0.14^{b}

Values with different superscripts indicate significant differences within the same row ($p \leq 0.05$)

Table 2: Mean bacterial concentration in the blood at different time intervals (cfu mL^{-1})

Time interval (h)	Group-1 (Pasteurella infection)	Group-2 (MOS + Pasteurella infection)
12	2.18×10^{3}	1.98×10^{3}
36	3.50×10^{4}	2.45×10^{4}
48	5.70×10^{5}	3.34×10^{5}

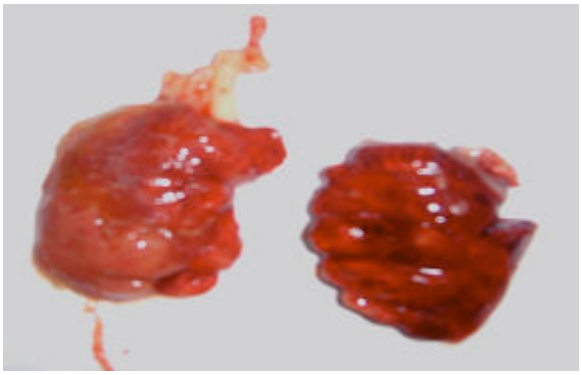

Fig. 1: Lungs of bird from group 2 bird 36 h post bacterial inoculation showing large areas of hemorrhages more prominently on left lung

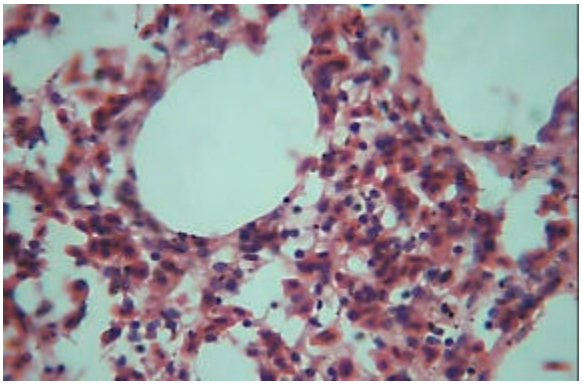

Fig. 2: Section of above lung showing infiltration of leukocytes in the parabronchial region. H and EX 400

The sequential gross and microscopic changes were noted in chickens sacrificed at different intervals. Examination of tissues from different group of chickens revealed that salient changes occurred in the lung, liver, spleen, heart and intestine. Gross lesions included fully haemorrhagic soft lungs (Fig. 1), areas of leukocyte infiltration were observed at 36 h in the lung tissue (Fig. 2) haemorrhages on epicardium, discolored enlarged liver with ecchymotic haemorrhages (Fig. 3), spleenomegaly and thickened intestine with prominent mesentery vasculature. Microscopic examination of lung tissue revealed presence of fibrinous oedematous fluid in the air vesicles with greatly dilated and broken alveolar septa and large areas of emphysema. Liver changes comprised dilated sinusoids that compressed the hepatic cords with early degenerative changes and vacuolation of hepatocytes (Fig. 4). Myocardial degeneration with swollen granular myocytes was observed at 36 h. Microscopic examination of intestine revealed villous atrophy, mucosal crypts were dilated and contained abundant number of inflammatory cells. The changes were more marked in chickens given bacterial infection without MOS supplementation at all three intervals.

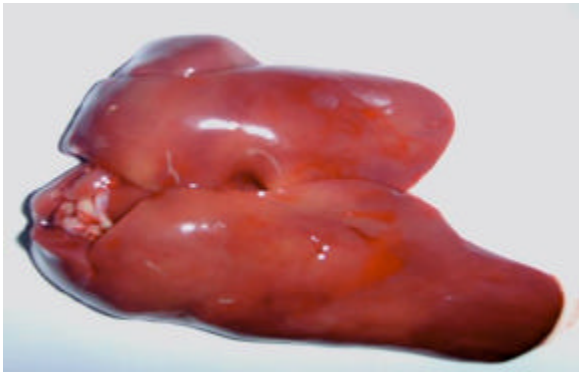

Fig. 3: Swollen liver from group 2 bird with small necrotic foci and hemorrhages 36 h post bacterial inoculation

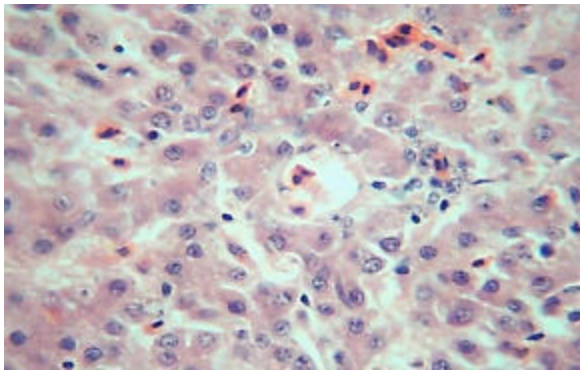

Fig. 4: Section of liver of birds from group II showing hyperemia and hemorrhages 36 h post bacterial inoculation. H and EX 100

DISCUSSION

In the present study, mortality in experimental birds was observed only in birds without supplementation of MOS at 24 h. Islam *et al.* (2004) reported incubation period of *Pasteurella multocida* varied from 12 to 48 h with 100% mortality rate between 24 to 72 h of infection. Although, a number of studies have been conducted to study the pathogenesis of pasteurellosis in birds there is limited information available on clinical pathology. Haematological changes are commonly used to determine the body status and to assess the impact of environmental, nutritional and or pathological stress (Elagib and Ahmed, 2011). Although, packed cell volume, haemoglobin concentration and red blood cell count had been reported to increase with age in chickens (Islam *et al.*, 2004).

Reduction in haemoglobin and erythrocyte count is a sign of anaemia. The occurrence of anaemia in fowl cholera has been noted by earlier workers although whether haemolytic or otherwise was not stated. Probably, the cause of anaemia in the present studies is due to the

bacterial septicaemia. The total leukocyte count of infected chicks showed a significant increase at all intervals. Similar to our findings, increase in leukocyte count in *Pasturella multocida* infections has been reported earlier by Mahmood *et al.* (2004). A similar observation was made by Islam *et al.* (2004) during their studies on fowl cholera in ducks. The endotoxin, a cell wall component of this organism might be responsible for the alterations of circulating leukocyte counts and its structure. It is well documented, that elevation in concentration of plasma enzymes occur as a result of their escape from disrupted hepatic parenchyma cells or altered membrane permeability. The acute hepatopathy caused by Pasteurella might thus be responsible for elevation in ALT and AST in present experiment. Similar to present observations, Shivachandra *et al.* (2005) reported mild to moderate congestion of blood vessels and haemorrhages in the intermycium of the heart, lung and liver in cases of fowl cholera. The sequential pathology of fowl cholera in broiler birds observed in the present study is in agreement with that reported by earlier workers in different avian species (Hunter and Wobeser, 1980; Islam *et al.*, 2003). Christensen and Bisgaard (2000) have stated that the possible virulence factors include the following: The capsule, endotoxin, outer membrane proteins, iron binding systems, heat shock proteins, neuraminidase production and antibody cleaving enzymes. The present study revealed that the birds given supplementation of prebiotic MOS showed a reduced severity of infection at all three intervals. This was reflected in the improved haemato-biochemical parameters, reduced mean blood count of the organism and decreased severity in the gross and microscopic lesions at all stages and all organs studied. Hajati and Rezaei (2010) have stated that prebiotics alter the immune system, prevent colon cancer, reduce pathogen invasion including *Salmonella enteritidis* and *E. coli*. Shashidhara and Devegowda (2003) and Markovic *et al.* (2009) have also observed the protective effect of prebiotic MOS against infections particularly the Gram negative bacteria like *Salmonella* and *E. coli*. Spring *et al.* (2000) have opined that MOS, do not selectively enrich for beneficial bacterial population instead, they are thought to act by binding and removing pathogens from the intestinal tract and stimulation of the immune system. A large number of reports have suggested that MOS may influence the physical properties of the epithelial lining itself (Hajati and Rezaei, 2010). Savage *et al.* (1997) observed an increase in the number of goblet cells in the duodenum and jejunum with an inclusion of 0.33% Bio-MOs in the diet of birds. Ghosh *et al.* (2007) concluded from their studies on Japanese Quail that MOS supplementation improved villus height in duodenum, jejunum and ileum leading to better function of secretion, digestion and absorption of nutrients.

Although, the ameliorative effect of MOS was observed in the present study it was not complete and the infected birds had appreciable pathological lesions in the target organs. Nevertheless, as pointed out by Ogueke *et al.* (2010) it is important to manipulate the content of gut flora with the view to increasing the numbers and activities of the presumed probiotics and reducing those of the pathogens by use of the prebiotics.

Thus, it can be concluded that although MOS supplementation may reduce the severity of bacterial infection in birds it is unable to give complete protection.

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