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Effect of Mannon Oligosachharide in Control of Avian Pathogenic *E. coil*

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ABSTRACT: Colibacillosis, caused by pathogenic *Escherichia coli*, is one of the main causes of economic losses among the poultry, worldwide. Losses occur at all ages, due to increase in morbidity and mortality, thus directly influencing the management and treatment costs. The characteristics of the disease and its aetiological agent have not yet been well defined. Due to the existence of antibiotic resistance and unavailability of effective vaccine, control of pathogenic *Escherichia coli* is a challenging task, hence an alternative control measure is needed. So, the present study was aimed to characterize the pathogenic *Escherichia coli* isolates and to control pathogenic *Escherichia coli* with prebiotics, as an effective feed additive

It was observed that there was no significant difference between 0.1% and 0.2% levels of D-Mannose, but differed significantly at 0.4% level. However, there was significant difference between D-Mannose, MOS and lactose. Lactose proved to be not much efficient, when compared to the other two prebiotics. Hence, to conclude, D-Mannose would be the prebiotic of choice to be recommended to the poultry farmers to be added as feed additive from day-old to at least up to twenty weeks age of the poultry stock, as a routine, for effective control of the *E. coli* infection, in their farms.

Keywords : Mannon Oligosaccahride, *E.coli*, Prebiotics.

INTRODUCTION

The Indian poultry industry is on the threshold of a major leap with increase in poultry population throughout the country, as a result of newer and better technologies adopted with every passing day.

Management of viral diseases in poultry is much easier and cheaper than that of bacterial diseases, in general, and avian colibacillosis, in particular, in the sense that almost all viral diseases are effectively prevented and controlled by the use of appropriate potent vaccines. Though control of bacterial diseases can be effectively achieved by the use of antibiotics, selection of sensitive antibiotics becomes a hurdle, especially among poultry, because, antibiogram results conducted with a specimen from autopsied birds may not be applicable to the remaining population of the same flock. Such results are almost and always have been proved to be an absolute failure, because of the numerous multipilicity of bacterial serotypes. And again, the ban on poultry products with residual antibiotics, as per the recent regulations of World Trade Organization (WTO) totally restricts the use of antibiotics, either for prevention or for treatment. The same reason has also stood in the way of evolving an effective vaccine to control avian colibacillosis, throughout the world, including India.

Colibacillosis is one of the devastating diseases, which leads to a profound economic loss to the poultry entrepreneurs and causes morbidity and mortality. The mortality rate may range from 5 - 50% in poultry (Wyeth, 1975).

The 'indiscriminate use of antibiotics' as a feed additive or in the phase of outbreak has resulted in the emergence of multiple drug resistant strains, rendering the treatment ineffective and costlier against the infection. Hence, it becomes imperative to seek for alternative methods like sanitation of water, feed and environment. The sanitizers, however effective they may be, have their own limitations either in the form of toxicity or in the form of cost benefit ratio. For example, hydrogen peroxide, though considered as an effective and cheap water, is known for its DNA damaging effect, causing chemical induced tumours on prolonged usage. Environmental sanitation through regular disinfectant sprays is so costly, that it cannot be followed effectively and regularly. There are no known feed sanitizers, except toxin binders.

Mannon Oligaccharide (MOS) supplementation of laying hens under bacterial challenge could improve productive performance probably through modification of intestinal bacterial populations and improving nutrient digestibility (Jahanian and Ashnagar 2015).

MOS supplementation could attenuate *E. coli*-induced intestinal disruption by alleviating intestinal inflammation and barrier dysfunction in broilers (Wang *et al.*, 2016). MOS may constitute a novel and effective plausible alternative that reduces the spread of disease by decreasing virus shedding and contamination of

environment from AIV (H_9N_2) infection in poultry (Akhtar *et al*., 2016).

The application of prebiotic combination of MOS and β-glucan might perform multiple pathways, improving growth performance in broiler chickens (Teng *et al.,* 2021).

It is with this background, that the present study is planned to evolve an effective method for the control of avian pathogenic *E. coli*, with use Mannon Oligo Saccharide as prebiotic.

MATERIALS AND METHODS

Collection of clinical samples. A total of 120 heart blood swabs were collected in sterile method (Ewing, 1986) from diseased and dead birds from poultry farms in and around Namakkal. The collected heart blood swabs were subjected bacteriological analysis.

Bacteriological examination. The collected clinical samples were inoculated in Nutrient broth and incubated for 18 h at 37°C. Then, a loopful from the previously inoculated broth was streaked onto MacConkey agar (Hi media) plates and incubated for 24 h at 37°C. Pink colored colonies were taken up and inoculated onto Eosin Methylene Blue (Hi media) and incubated at 37°C for 24 h. determination of *E. coli* isolates were performed based on the colony and biochemical tests as per Edwards (1986).

Pathogenicity Testing

1. Congo red binding assay

Congo red binding assay of *E. coli* isolates were carried out and interpreted as per Berkhoff and Vinal (1986).

2. Chick lethality test

- **Caudal air sac route.** Pathogenicity of the *E. coli* isolates were ascertained by inoculating 10^8 CFU/ml of *E. coli* through caudal air sac route in day-old chicks as per Panigrahy and Yushen (1990).
- **Intravenous route of inoculation.** Pathogenicity of *E. coli* isolates were ascertained by inoculating 10^8 CFU / ml of *E.coli* organisms through intravenous route among four-week-old chicks as per Arp and Jensen (1980).

Haemagglutination Assay. Haemagglutination assay was carried out, as described by Duguid *et al.* (1979).

Assessment of efficacy of prebiotics. Feeding trials, with male white leghorn chicks was conducted to study the effect of prebiotics *viz*., D-Mannose, lactose, MOS at the dose rate of 0.1%, 0.2%, and 0.4% by individual dosing to control pathogenic *E. coli* infection.

Housing. Chicks from day-old were housed in well-prepared cages, following standard management practices uniformly for all the treatments. Throughout the experimental period, feed and water were provided *ad libidum*.

Prebiotic dosing. The prebiotics *viz*., D-Mannose, lactose, mannon oligosaccharide (MOS) were fed for up to twenty weeks.

The prebiotics *viz*., D-Mannose, lactose, were fed per bird at the dose of 0.1%, 0.2%, and 0.4% through the routes of oral drops, drinking water, and feed respectively, because mannon oligosaccharide was found to be non-soluble in water, it was fed at the dose of 0.4% through feed only.

Challenging with pathogenic *E. coli.* Birds, fed with prebiotics and untreated control birds were challenged with 0.1 ml of 10^8 CFU / ml of pathogenic *E. coli* via intravenous routes at 20th week of age.

Post infection observation. Both prebiotics and untreated control birds were observed for upto ten days for post-infection symptoms.

Evaluation of the efficacy of prebiotics. The effect of prebiotics *viz*., D-Mannose, lactose, and MOS were evolved by counting the number of viable *E. coli* organisms from caecal content of prebiotic- fed and untreated control birds as per Quinn *et al.* (1994).

RESULTS AND DISCUSSION

All the nineteen *E. coli* isolates, which produced characteristic wrinkled red coloured colonies in CR binding assay, caused death of day-old chicks within 2-5 days p.i. inoculated through caudal air sac route.

Pathogenic *E. coli* isolates that caused death of day–old chicks, when administered via caudal air sac route of inoculation, were also administered via intravenous route of inoculation in four-week–old chicks. All the isolates caused death of chicks within 1-4 days of p.i.

In acute cases of deaths, (24 hrs p.i.), colisepticaemic lesions with air sacculitis, and congestion of heart and liver were observed among birds inoculated, intravenously. Chicks died, two to four days of p.i. evinced marked lesions of air sacculitis, pericarditis and perihepatitis during inoculation by caudal air sac and intravenous route of inoculations. The pathognomonic lesions of fibrinous pericarditis and perihepatitis were more severe at about 5th day p.i.

Among nineteen pathogenic *E. coli* isolates, subjected to HA test against RBCs of chicken in the presence of D-Mannose, fifteen isolates evinced MSHA. About three isolates produced wMSHA. None of the isolates produced MRHA. The results of haemagglutination assays are presented in the Table 1.

Efficacy of Prebiotics on Control of *E. coli.* The effect of D-Mannose, lactose and MOS on the colonization of pathogenic *E. coli* was examined. None of the chickens in the groups of T1, T2, T3, and T7 showed any symptoms of *E. coli* infection, but birds in the groups of T4, T5, T6, and T8 showed positive symptoms of *E. coli* infection (Table 3). When the $\log_{10}$ number of *E. coli* cells in the D-Mannose (T1, T2, T3) and MOS (T7) groups were compared with lactose groups (T1, T2, T3) and untreated control groups (T8), a highly significant ($P<0.01$) difference was observed (Table 2 & Fig. 1).

Efficacy of D-mannose. The effect of D-Mannose on control of *E. coli* infection showed significant results, when compared to other prebiotics (Table 1). There was significant reduction ($P<0.01$) in viable *E. coli* cells / gm of caecal content in the D-Mannose-fed-groups of birds. No symptoms of *E. coli* infection were observed in the D-Mannose-fed-groups of birds. Birds in these groups evinced no lesions of *E. coli* infection (Table 2). Within the D-Mannose-fed-birds, 0.1 and 0.2% D-Mannose fed groups produced better results than the 0.4% D-Mannose-fed-birds. It was observed that there was no significant difference between 0.1 and 0.2%

levels of D-Mannose, but differed significantly at 0.4%. No mortality could be observed among the D-Mannose-fed-groups of birds.

Efficacy of Lactose. Lactose produced no significant reduction ($P<0.01$) in viable *E. coli* cells / gm caecal content, when compared to D-Mannose, and MOS-fed-birds. But, when compared to untreated control birds, there was significant reduction ($P<0.01$) in viable *E. coli* cells / gm caecal content. The symptoms of *E. coli* infection were observed within the lactose-fed-groups (Table 2). In the T4 trial group (0.1% lactose), one bird showed airsacculitis on necropsy. Two birds with severe airsacculitis and one bird with mild airsacculitis could be observed in T5 trial group (0.2% lactose) group of birds. Among T6 trial group (0.4% lactose) group, four birds with severe airsacculitis and two birds with pericarditis, perihepatitis lesions could be observed.

Efficacy of MOS. MOS-fed-groups evinced significant reduction ($P< 0.01$) in viable *E. coli* cells / gm caecal content, when compared to lactose fed groups, but slightly less significant, when compared with D-mannose-fed-groups (Table 1). MOS-fed- groups of birds exhibited no symptoms of *E. coli* infection. The mortality rate and percentage of mortality are presented in the Table 4.

Table 1: Direct Haemagglutination of Pathogenic *E. coli* Isolates Against Chicken Erythrocytes.

Sr. No.	Isolate No.	Direct haemagglutination		
		MSHA	MRHA	wMSHA
1.	**1**	+ + + +	-	-
2.	**2**	+ + + +	-	-
3.	**3**	+ + + +	-	-
4.	**5**	+ + + +	-	-
5.	**6**	+ + + +	-	-
6.	**7**	+ + + +	-	-
7.	**8**	-	-	+
8.	**9**	-	-	+
9.	**10**	+ + + +	-	-
10.	**12**	+ + + +	-	-
11.	**13**	-	-	+
12.	**14**	+ + + +	-	-
13.	**15**	+ + + +	-	-
14.	**18**	+ + + +	-	-
15.	**23**	+ + + +	-	-
16.	**25**	+ + + +	-	-
17.	**29**	+ + + +	-	-
18.	**32**	+ + + +	-	-
19.	**36**	+ + + +	-	-

MSHA – Mannose sensitive haemagglutination
MRHA – Mannose resistant haemagglutination
wMSHA – Weak mannose sensitive haemagglutination
'+ + + +' – Indicates the quickest haemagglutination (100%)

Table 2: Effect of Prebiotics on Control of *E. coli* Infection on 20-week-old Chicks Challenged Intravenously with 10^8 CFU of *E. Coli.*

Sr. No.	Treatment groups	Routes of administration of prebiotics[1]	*E. coli* / gm of caecal content[2]
1.	**Control (T8)**	Nil	8.73 ± 0.04^{a}
2.	**D-Mannose - 0.1% (T1)**	Oral drops	2.33 ± 0.06^{e}
3.	**D-Mannose - 0.2% (T2)**	Dinking water	2.35 ± 0.07^{e}
4.	**D-Mannose - 0.4% (T3)**	Feed	2.56 ± 0.04^{d}
5.	**Lactose - 0.1% (T4)**	Oral drops	4.69 ± 0.02^{b}
6.	**Lactose - 0.2% (T5)**	Dinking water	4.72 ± 0.02^{b}
7.	**Lactose - 0.4% (T6)**	Feed	4.75 ± 0.02^{b}
8.	**Mannose oligosaccharide (T7)**	Feed	2.83 ± 0.02^{c}

[a-e]Means with different superscripts differ significantly.
[1] Prebiotics were provided from day-old to twenty weeks of age
[2] Log $_{10}$ mean numbers of viable organisms per gram of caecal contents ± standard of sample mean (SEM) of 10 chicks /group.

Table 3: Macroscopic Lesion Score in Experimental Birds Inoculated with 10^8 CFU of *E. coli* Cells.

Sr. No.	Treatment groups	No.of birds inoculated/ No. of trial birds	Airsacculitis				Pericarditis				Perihepatitis			
			+ + +	+ +	+	0	+ + +	+ +	+	0	+ + +	+ +	+	0
1.	**T1**	10 / 10	-	-	-	-	-	-	-	-	-	-	-	-
2.	**T2**	10 / 10	-	-	-	-	-	-	-	-	-	-	-	-
3.	**T3**	10 / 10	-	-		-	-	-	-	-	-	-	-	-
4.	**T4**	10 / 10	-	1	-	-	-	-	-	-	-	-	-	-
5.	**T5**	10 / 10	-	2	1	-	-	-	-	-	-	-	-	-
6.	**T6**	10 / 10	-	2	2	-	-	2	-	-		2	-	-
7.	**T7**	10 / 10	-	-	-		-	-	-	-	-	-	-	-
8.	**T8**	10 / 10	7	3	-	-	8	2	-	-	6	4	-	-

Macroscopic lesion score (+) indicates the intensity of post mortem lesions considering '+ + + +' as the maximum.

Table 4: Mortality Percentages of Experimental Birds Challenged with 10^8 CFU of *E. coli* Cells.

Sr. No.	Treatment groups	No. of birds challenged / No. of trial birds	No. of chicks died	Percentage of mortality
1.	**T1**	10 / 10	-	-
2.	**T2**	10 / 10	-	-
3.	**T3**	10 / 10	-	-
4.	**T4**	10 / 10	-	-
5.	**T5**	10 / 10	-	-
6.	**T6**	10 / 10	-	-
7.	**T7**	10 / 10	-	-
8.	**T8**	10 / 10	10	100

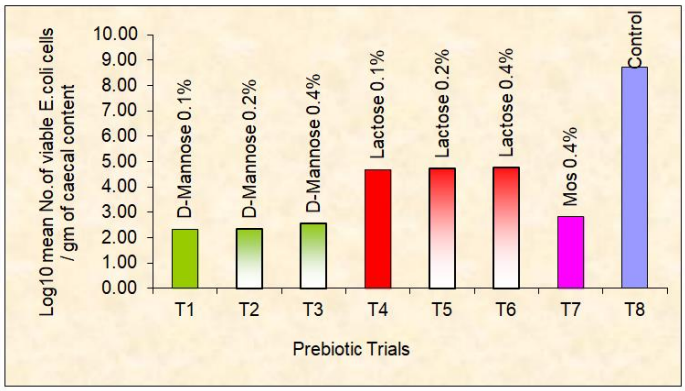

Fig. 1. Efficacy comparison of prebiotics on control of pathogenic *E. coli* infection in chicken.

The mannose sensitive haemagglutination is mediated by 'type 1' fimbriae of *E. coli* isolates (Eisenstein, 1988). Arp and Jensen (1980); Ghanbarpoor and Pourbakhsh (2003). Leclerc *et al*. (2003) also observed that the HA assay by *E.coli* isolates was inhibited by the presence of mannose. None of the isolates produced MRHA, which correlated well with the results of Raja Swaminathan (1997).

Efficacy of Prebiotics on Control of *E. coli.* Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and / or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health. Microbes are able to attach themselves to mucosa by recognition of oligosaccharide binding site on the epithelial cells of intestinal wall. Dietary oligosaccharides attract microbes away from the intestinal binding site, thus reducing colonization and disease occurrences, releasing the mucosa to perform its function of secretion, digestion and nutrient absorption (Chesson, 1993).

In the present study, the prebiotics were fed to birds from day-one onwards upto twenty weeks of age, so as to enable them to get attached to receptors of enterocytes and epithelial cells, before the attachment of the *E. coli* organism to them.

The $\log_{10}$ number of *E. coli* cells in the D-mannose-treated (T1, T2, T3), and MOS-treated (T7) groups were compared with lactose-treated (T1, T2, T3) and untreated control groups (T8). A highly significant ($p<0.01$) difference was observed in the present study.

Efficacy of D-mannose. Bacterial attachment is mediated through binding of bacterial lectins to receptors containing D-Mannose moiety. It may be possible to block the lectins with D-Mannose or similar sugars and to inhibit bacterial attachment (Eshdat *et al*., 1978). Based on this concept, the present study was taken up to alleviate infection by *E. coli* among poultry stock.

In the present study, there was significant reduction ($P<0.01$) in viable *E. coli* cells / gm of caecal content in the D-Mannose-fed-group of birds. Hence, no symptoms of *E. coli* infection could be observed among them, even after challenge with pathogenic *E. coli*, thus correlating well with results of Naughton *et al.* (2001). The same result was recorded by Oyofo *et al*. (1989)

also against *Salmonella typhimurium* with mannose. The adherence of *E. coli* to urinary tract mucosa can be blocked with antitype 1 fimbrial monoclonal antibody, or with mannose containing receptor analogue; such interference inhibits the development of urinary tract infection (Abraham *et al*., 1985). No mortality was noticed between differently dosed-D-Mannose-fed groups of birds.

The present study indicated that, the trial birds fed with 0.1% D-mannose as oral drops (T1) and addition of 0.2% D-mannose in drinking water (T2) significantly reduced the colonization of pathogenic *E. coli*, when compared with 0.4% D-mannose in feed (T3). The less response in case T3 may be, most probably due to wastage of the D-Mannose, along with left over feed, as against the T1 and T2, wherein wastage of the D-Mannose can be avoided, by forcibly dispensing the D-Mannose via oral drops (T1) and by limiting the provision of drinking water to the concerned trial group (T2), just sufficient for the corresponding trial birds to consume. These results indicated that final dose per bird was the influencing factor in the prebiotic dosing, against *E. coli* infection. This was highly supported by the findings of Ofek *et al.* (1977). Results of the *in vivo* data reported herein are consistent with the *in vitro* model system reported by Oyofo *et al*. (1989).

Efficacy of lactose. In the present study, lactose produced no significant reduction in viable *E. coli* cells / gm caecal content, when compared to D-Mannose, and MOS-fed birds. The symptoms of *E. coli* infection were observed within the lactose-fed-groups of birds after experimental challenge with pathogenic *E. coli*. The results of the present study suggest feeding of birds with lactose is not a viable means in itself of reducing or eliminating the *E. coli* infection, which accorded with findings of Waldroup *et al*. (1995); Johannsen *et al*. (2004) suggests that the addition of lactose and *L. acidophilus* did not reduce the *S. typhimurium.* But the reports of Ziprin *et al*. (1990); Hinton *et al*. (1991); Corrier *et al*. (1993) revealed that the lactose at 7.0% in combination with caecal anaerobic culture significantly reduced Salmonella colonization.

Efficacy of MOS. In the present study, MOS-fed groups evinced significant reduction in viable *E. coli* cells / gm caecal content, when compared to lactose-fed-groups, but slightly less significant, when compared with D-mannose-fed-groups. MOS-fed-groups of birds did not evince any symptoms of *E. coli* infection after experimental challenge with pathogenic *E. coli*. These results are in agreement with findings of Sims *et al*. (2004).

In the present study, no mortality was observed among MOS-fed-groups, these results being supported by those of Hooge (2004). Turkey-fed-MOS, during a specific challenge from *S. typhimurium* had a decreased incidence of faecal contamination, whereas broilers fed with MOS had reduced faecal counts of *S. dublin* and *E. coli* (Spring *et al.*, 2000) organisms. It could be concluded, Mannan-oligosaccharide supplementation in layers and broiler feed improved the immune response of broilers and layers mitigated pathological lesion resulted from *E. coli* infection (Fadl *et al*., 2020).

Biswas *et al.* (2021) reported that mannan oligosaccharides (MOS) may be incorporated at 0.2% level in diet for improved physico-chemical indices, antioxidant and oxidative stability and carcass characteristics of broiler chickens meat and it may be suitable replacer of antibiotic growth promoter.

SUMMARY

The superiority of mannose is not only on its drastic reduction of coliform count/ gm of caecal content, but also its convenience to be used in feed water or as oral dosing, because of its clear water-soluble nature. And it has been well documented that its action through competitive exclusion of *E. coli* might be the major contribution in the control of *E. coli*. As such, it assumes that it is acting both as prebiotic and probiotic. Also, all the possible drawbacks of any probiotic in the form of directly fed microbes (DFM) have been found to be absent in mannose, because it is an abiotic substance. Owing to multiple advantages of D-Mannose, it may be recommended to the poultry farmers to employ it as feed additive from day-old onwards at least up to twenty weeks age of the poultry stock (probable age of start of production), for possibly effective control of the *E. coli* infection, in their farms, as a routine.

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Conflicts of Interest. None.

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