EFFECT OF MANNAN OLIGOSACCHARIDES ON CAECAL MICROBIAL ACTIVITY AND INCIDENCE OF DIARRHOEA IN BROILER RABBITS

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Received - 19.09.2015
Accepted - 22.12.2015

Abstract

A feeding trial was conducted for a period of four months using eighteen weaned White Giant broiler rabbits, belonging to University Rabbit Farm, Mannuthy with three groups of six replicates each, to assess the effect of supplementation of Mannan oligosaccharide (MOS) on caecal microbial count and incidence of diarrhoea. The animals were divided into three groups of six animals as uniformly as possible with regard to sex, age and weight and were randomly alloted to three dietary treatments. The animals were fed with three experimental rations, T1- basal diet (16 per cent CP and 2500 kcal/ kg DE), T2- basal diet + 1.5 g MOS/ kg diet and T3- basal diet + 3 g MOS/ kg diet and fodder adlibitum. Caecal coliform count, total viable count and incidence of diarrhoea during the entire period of experiment were recorded. The results of the study indicated that the addition of MOS at 3 g/ kg diet resulted in significant reduction of coliform count and total viable count was similar in all the three groups. Inclusion of MOS at both levels 1.5 and 3 g / kg diet resulted in reduced incidence of diarrhoea in experimental rabbits when compared to control group without any additive.

Key words: *Prebiotic, Mannan oligosaccharide, Broiler rabbit*

Weaning is one of the most stressful period for rabbits reared under intensive system due to abrupt changes in diet and environment. The instability of digestive system of young rabbits, can increase susceptibility to post weaning digestive disorders. The most common digestive disorders in rabbit production is the occurrence of an enteritis complex. In addition the ban of antibiotic by European Union, necessitate the use of natural alternative feed additive. Oligosaccharides as prebiotics are one of such substance which increases the number of beneficial microflora and also suppresses harmful pathogens (Quigley, 2004). Bio-Mos are phosphorylated mannan oligosaccharides, derived from the outer cell wall of yeast *Saccharomyces cerevisiae*, contain a mannan component which resembles that of carbohydrate on animal gut wall. This serves as alternative attachment for entero-pathogens and can successfully eliminate pathogenic microbes. The aim of this study was to evaluate the effect of dietary supplementation of MOS on caecal microflora and incidence of disease such as enteritis.

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Materials and Methods

Experimental Animals

Eighteen weaned White Giant broiler rabbits of four to six weeks age were selected from University Rabbit farm, Mannuthy. All the animals were housed individually in metallic cages and were divided into three groups of six animals each and randomly alloted to one of the three dietary treatments, (T1,T2,T3) using completely randomised design.

Weighed quantities of respective feed and fodder were offered daily to each rabbit. The left over portion of the feed and green grass were weighed daily and their moisture content was analyzed to calculate the dry matter intake. The animals were maintained on their respective feeding regime for a period of four months.

Experimental diets

The rabbits in the three experimental groups were fed with a diet containing 16 per cent crude protein and 2500 kcal DE/ kg diet for the entire feeding period of 4 months. The three experimental rations were, T_1 – basal diet (control diet, NRC 1977), T_2 - control diet + 1.5g MOS/ kg diet and T_3- control diet + 3g MOS/ kg diet.Concentrate feed was offered in the morning and green grass in the evening. Fresh water was provided *adlibitum* to all animals. Per cent ingredient composition of rations aregiven in Table1.

Caecal microbial count

Caecal pellets were collected from each animal at the time of slaughter in a collection vial with all aseptic precaution. The samples were processed in the laboratory and subjected to microbiological analysis. Nine grams of samples were homogenized in 90 milliliter of normal saline which formed the initial test sample. Further tenfold serial dilution was prepared by transferring one milliliter of inoculum in nine milliliter of the diluents.

Total viable count

Total viable count (TVC) of all samples were estimated by pour plate technique, as described by Morton (2001). From the selected tenfold dilutions, each sample was transferred to the sterile petri-dishes. About 10-15 ml of sterile molten standard plate count agar (Himedia) maintained at 45^{0}C was poured to each inoculated plates and mixed by gentle rotary movement. At room temperature the inoculated plates were allowed to solidify and were incubated at 37^{0}C for 24h. At the end of incubation, plates showing colonies between 30 and 300 were selected and counts were taken with colony counter. The number of colony forming units (cfu) per g/ ml of sample was calculated by multiplying the colony count with dilution factor and expressed as $\log_{10}$ cfu/ g or ml.

Coliform count

Coliform count per milliliter of samples was estimated according to the procedure described by Kornacki and Johnson (2001). From the selected tenfold dilution 0.1 milliliter of sample was inoculated into Violet Red Bile Agar (VRBA) (Himedia) and uniformly distributed with a sterile ' L' shaped glass rod. The plates were allowed to solidify at room temperature and incubated at 37^{0}C for 24h. At the end of incubation, purplish red colonies with a diameter of at least 0.5mm, surrounded by a reddish precipitation zone were counted. The number of organisms was estimated by multiplying the count with dilution factor and expressed as $\log_{10}$ cfu / g or ml.

Statistical analysis

Data obtained on microbial count during the course of experiment was subjected to statistical analysis using analysis of variance (ANOVA) (Snedecor and Cochran, 1994.

Resuls and Discussion

Caecal Microbial Population

The data on caecal microbial count is presented in Table 2. The total viable count in the caecal content of rabbits maintained on three experimental treatments T1, T2 and T3 was 6.95, 6.98 and 6.89 $\log_{10}$ cfu per g, respectively and coliform count was 5.77, 5.66 and 5.15 $\log_{10}$ cfu per g for treatments T1, T2 and T3, respectively. The statistical analysis of the data revealed that there was no significant

difference in total viable count whereas coliform count in T3 was statistically significant from both T1 and T2. The result of the present study confirmed the earlier observation of Rekiel *et al*. (2007) who reported a lowered gut population of *Enterobacteriacea*in 0.1 per cent MOS supplemented group when compared to control. In accordance with the current study Castillo *et al*. (2008) reported that MOS supplementation in pigs diet at 0.2 per cent level resulted in significant reduction in number of *Enterobacteria* but there was no significant difference in Lactobacilli count in jejunal digesta. Whereas Oso *et al* (2013) reported reduced Lactobacillus count in MOS supplemented rabbits than probiotic supplemented.

Incidence of diarrhoea

The total number of diarrhoea cases of rabbits maintained on three experimental treatments T1, T2 and T3 was six, three and three, respectively. Spring *et al*. (2000) reported that the power of MOS to bind the mannose receptors on the type1 fimbriae of pathogenic bacteria in order to prevent their attachment to intestinal mucosa resulted in better disease resistance.In accordance with the current study Grela*et al*. (2006) reported that MOS supplementation in weaned piglets at 0.2 and 0.4 per cent levels noticed a reduction in incidence of diarrhoea and number of diarrhoea days. Similarly Liu *et al*. (2008) noted that the incidence of diarrhoea and diarrhoea scores were significantly decreased ($P < 0.05$) in pigs fed diets with chito oligosaccharides than those fed with control diet. Amber *et al*. (2014) observed that supplementation of Bio Mos, Bio Plus or their mix in diets of growing rabbits decreased pathogenic bacteria (Escherichia coli and Clostridium spp.) in caecum contents (P<0.01 & P<0.001, respectively)

The addition of MOS at 3 g/ kg diet significantly reduced the coliform count whereas the total viable count remains the same. In addition the incidence of diarrhoea during entire period of experiment was reduced in both MOS supplemented group when compared to control.

Table 1. Ingredient Composition of experimental rations, %

Ingredients	Ration		
	T1	T2	T3
Yellow maize	20	20	20
Gingelly oil cake	20	20	20
Soybean meal	17	17	17
Wheat bran	27	27	27
Deoiled rice bran	14	14	14
Mineral mixture	1.5	1.5	1.5
Salt	0.5	0.5	0.5
In addition to 100 kg of above mixture			
Mannan oligosaccharide, g/ kg diet	-	1.5	3
Vitablend AB_2D_3 g/ 100 kg	20	20	20

Each gram of vitablend AB_2D_3contains 40,000 i.u. of vitamin A; 25 mg of vitamin B_2 and 6,000 i.u. of vitamin D_3.

Table 2. Caecal microbial count of rabbits maintained on three dietary treatments, $\log_{10}$ cfu per gram

Parameter	Treatments			
	T1	T2	T3	P value
Total viable count	6.95 ± 0.02	6.98 ± 0.04	6.89 ± 0.13	0.77 ^{NS}
Coliform count	5.77^{b} ± 0.04	5.66^{b} ± 0.04	5.15^{a} ± 0.23	0.02*

Bacterial numbers are expressed as $\log_{10}$ colony forming units per gram
a, b- means with different superscript in the same row differ significantly. (p< 0.05)
NS- nonsignificant, * - significant

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