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Effect of a Mannanoligosaccharide Preparation on *Eimeria tenella* Infection in Broiler Chickens

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Abstract: The hypothesis tested was that the feeding of mannanoligosaccharides (MOS) will suppress the signs of a coccidiosis infection in broilers. Two separate experiments were performed in which part of the broilers used were infected with *Eimeria tenella*. In each experiment there were three treatment groups: a negative control group fed the basal diet and two infected groups fed the basal diet without or with a commercial MOS preparation. The infection of the broiler chickens was successful as based on the caecal lesions, oocyst shedding and schizonts in the lamina propria of the caecum, but did not affect growth performance of the birds. In the infected birds fed the MOS preparation, the number of schizonts was reduced without a decrease in the severity of caecal lesions and without impact on growth performance. It is suggested that the MOS preparation had enhanced the immunity of the infected birds and thereby had decreased the number of schizonts. It is concluded that this study presents evidence for a protective effect of MOS against coccidiosis infection in broilers.

Key words: Mannanoligosaccharides, basal diet, caecal lesions

Introduction

Coccidiosis is a common infectious disease in poultry, causing major economic losses. The protozoan parasite of the genus *Eimeria* multiplies in the intestinal tract of poultry and produces tissue damage, resulting in reduced growth and increased susceptibility to pathogens (McDougald, 2003) such as *Clostridium perfringens*, leading to necrotic enteritis (Helmboldt and Bryant, 1971; Maxy and Page, 1977; Shane *et al*., 1985). In bacteria-free chickens infected with surface-sterilized *Eimeria tenella* oocysts, clinical signs do not develop unlike in chickens with two or more indigenous species of bacteria (Johnson and Reid, 1972; Radhhakrishnan, 1971; Visco and Burns, 1972a; 1972b). Apparently, indigenous bacteria are required for the occurrence of typical caecal coccidiosis in chickens. In the course of development of caecal coccidiosis, the growth of *Clostridium perfringens* and coliforms, especially *Escherichia coli*, is stimulated whereas the growth of *Lactobacillus* spp. is suppressed (Johansson and Sarles, 1948; Rahhakrishnan, 1971). *Lactobacillus* spp have been shown to inhibit the invasion of *Eimeria tenella* in *vitro* (Tierney *et al*., 2004).

It is expected that in the near future the coccidiostatics currently used in animal feeds will be banned. Thus, there is a need for alternative agents to control coccidiosis in poultry. Perhaps, mannanoligosaccharide (MOS) preparations can be useful. These carbohydrate preparations are derived from the cell wall of the yeast *Saccharomyces cerevisiae* and have been reported to suppress pathogens in the intestinal mucosa of chickens and turkeys (Spring, 1999a; 1999b; Sonmez and Eren, 1999; Iji *et al*., 2001; Spring *et al*., 2000). MOS may improve gut health as indicated by increased villi height, uniformity and integrity (Loddi *et al*., 2002) and they modulate gut and systemic immunity (Ferket *et al*., 2002). Possibly even more important in relation to coccidiosis, dietary MOS supplementation increased the levels of *Bifidobacterium* spp. and *Lactobacillus spp* in the intestinal tract and depressed the number of *Enterobacteriaceae* (Fernandez *et al*., 2002). *Lactobacillus* spp. are known to compete with *Clostridium* spp. (Shane, 1985).

In the light of the above-mentioned, an increase in *Lactobacillus* spp. and a decrease in *Clostridium* spp might reduce caecal coccidiosis in broiler chickens. Consequently, it could be hypothesized that the feeding of MOS will suppress coccidiosis infection in broilers. In the present experiments the hypothesis was put to the test by measuring the severity of *E. tenella* infection in broiler chickens fed a diet containing a commercial MOS preparation.

Materials and Methods

Animals and diets: Two separate experiments were performed. One-day old male broiler chicks (Ross 308) were purchased from a local hatchery. On arrival, they were wing-banded and randomly allocated to either the basal or supplemented diet. Two groups received the basal diet and one group received the supplemented diet. The birds were housed in wire-floored, suspended cages that were placed in one room. Ambient

temperature was gradually decreased from 32 °C on day 1 to 25°C on day 21. Throughout the experiments there was continuous lighting. The basal diet did not contain growth promoters or coccidiostatics; the composition was as follows (g/kg diet): wheat (+ xylanase), 250; corn, 321; soyabean meal (46.7% crude protein), 225; peas, 50; sunflower meal (32% crude protein), 40; potato protein, 15; fish meal (72% crude protein), 25; soyabean oil, 40; premix (Research Diet Services, Wijk bij Duurstede, The Netherlands), 5; limestone, 16; monocalcium phosphate, 7; phytase (Natuphos 5000G), 0.1; salt, 1.7; sodium bicarbonate, 1.7; L-lysine HCl, 0.8; D,L-methionine, 1.7. The experimental diet was prepared by supplementation of MOS (Bio-Mos™, Alltech) at a level of 1 g/kg diet at the expense of an identical amount of corn starch. The diets were in pelleted form.

The diets were fed *ad libitum* from arrival of the birds (day 1) to day 21 in experiment 1 and from days 1 to 14 in experiment 2. Tap water was freely available throughout the experiment. Body weights and feed intake were recorded at day of infection and 6 days post infection (PI).

Experiment 1: 75 one-day old broiler chickens were allocated randomly to three treatment groups of 25 birds each. Each group was then randomly divided into 5 replicates of 5 birds each. Each replicate was housed in a separate cage. On day 12 of the experiment, one group receiving the basal diet and the group receiving the supplemented diet were inoculated with 3500 sporulated oocysts of the *E. tenella* (Houghton) laboratory strain. The oocysts were obtained from the Animal Health Service Ltd. Poultry Health Centre (Deventer, The Netherlands). The oocysts were administered with 1 ml of tap water via an oral gavage directly into the crop. The negative control group fed the basal diet was given water only through gavage. Care was taken to prevent cross contamination throughout the experiment. At the end of the experiment (day 21), the birds were euthanized by cervical dislocation.

Experiment 2: In this experiment the dose of *Eimeria* was raised and administered on day 8. 45 one-day old chickens were randomly allocated to three groups of 15 chickens each, the groups consisting of 5 replicates of 3 birds each. Each replicate was housed in a separate cage. On day 8, one group receiving the basal diet and the group fed the supplemented diet were inoculated with 5000 sporulated oocysts of *Eimeria tenella*. The oocysts were administered as described above. The negative control group was given water through gavage. On day 14, all birds were killed by cervical dislocation.

Analyses and measurements: The numbers of oocysts in faeces were determined in samples collected from each cage during the period of 3 to 6 days post infection in experiment 2. The modified McMaster counting chamber technique of Hodgson (1970) was used. A 10% (w/v) faeces suspension in a salt solution (151 g NaCl mixed into 1 L of water) was prepared. After shaking thoroughly, 1 ml of the suspension was mixed with 9 ml of a salt solution (131 g of NaCl mixed into 1 L of water). Then, the suspension was put into the McMaster chamber using a micropipette and the number of oocysts was counted and expressed per gram of faeces (Peek and Landman, 2003).

In both experiments the severity of coccidial caecal lesions was scored while the investigator was blinded to treatment modality. The 0-4 scoring system described by (Johnson and Reid, 1970) was used.

In experiment 1, tissue sections from caecum (about 4 µm thick) were routinely processed, paraffin embedded and stained with haematoxylin-eosin (HE). Microscopical lesions in the tissues were examined under the microscope (x 400). The presence of *E. tenella* schizonts in the lamina propia of the whole section of the caecum was scored on a 0- 2 scale (0 = no schizonts, 1 = 1 to 3 schizonts, 2 = mass of schizonts).

Fat digestibility was measured using the faeces collected for days 15 to 18 in experiment 1 and for days 8 to 11 in experiment 2. The acid hydrolysis method was applied to extract crude fat from the feed and excreta. Briefly, a 2-g dry sample was weighed in a flask and 2 ml of ethanol was added and the sample was shaken until fully wettest. Subsequently, 10 ml of 8 mol/l HCl was added and after mixing the flasks were placed in a water bath at 80°C for 40 min. Then, 10 ml of ethanol was added and after cooling, 25 ml of diethyl ether was added and the sample was shaken for about 1 min. Then, 25 ml of petroleum ether was added and the sample was shaken for another 1 min. The supernatant consisting of ether-fat mixture was poured into a flask, and the precipitate was extracted further with 15 ml of both ethers. The supernatants were combined and the solvent was evaporated at 50°C. The flask containing the residue was dried in a desiccator over night under vacuum. The residue was weighed and considered to be crude fat.

Statistical analysis: All data for each variable were subjected to univariate analysis of variance using SPSS (SPSS Inc, Chicago, USA). The oocyst values were logarithmically transformed [$\log_{10}$ (X + 1)] to create a normal distribution before being analyzed, and lesion scores were transformed using multinomial transformation. When significant treatment effects were disclosed, differences between the three treatments were evaluated by the post hoc multiple comparison least significant difference (LSD) test. Lesion scores were compared using the non-parametric Mann-Whitney U test. Chi-square analysis was used to compare treatment group values for the semi-quantitative scores

Table 1: Body weight gain (g/day) of the broilers during 6 days post infection

Days post infection	Control		MOS Infected	Pooled SEM	P-value
	Negative	Infected			
12-18 (experiment1)	53.8	51.3	55.8	1.49	0.14
8-14 (experiment 2)	40.8	37.7	38.3	2.69	0.70

Table 2: Feed intake (g/day/bird) of the broilers during 6 days post infection

Days post infection	Control		MOS Infected	Pooled SEM	P-value
	Negative	Infected			
12-18 (experiment 1)	76.7	78.0	82.8	2.12	0.14
8-14 (experiment 2)	45.0[a]	41.5[b]	40.7[b]	0.77	<0.05

[a,b] Mean values within the same row with different superscript letter are significantly different (P<0.05).

Table 3: Feed conversion ratio (feed/gain) of the broilers during 6 days post Infection

Days post infection	Control		MOS Infected	Pooled SEM	P-value
	Negative	Infected			
12-18 (experiment 1)	1.45	1.50	1.49	0.03	0.53
8-14 (experiment 2)	1.30	1.30	1.24	0.04	0.64

Table 4: Faecal oocyst counts [log (X+1)] in experiment 2

Days post infection	Control		MOS Infected	Pooled SEM	P-value
	Negative	Infected			
11-14 (experiment 2)	0.00[a]	4.79630[b]	4.7093[b]	0.233	<0.05

[a,b] Mean values within the same row with different superscripts letter are significantly different (P<0.05).

Table 5: Lesion scores for the infected birds

	Infected controls	Infected, MOS	P-value
Experiment 1 (n=25)	1.1±0.21	1.0±0.17	0.83
Experiment 2 (n=15)	1.70±0.27	1.67±0.23	0.91

Means ± SE

of schizonts found in the lamina propria of the caecum. The level of statistical significance was pre-set at P <0.05.

Results

In both experiments, body-weight gain during the interval of 6 days post infection showed no significant differences between treatments (Table 1). In the infected birds, the MOS preparation had produced a 8.8% increase in group-mean weight gain in experiment 1 and a 1.8% increase in experiment 2.

Post infection feed intake in experiment 1 showed no significant difference between the three treatment groups. In experiment 2, the infected control group and birds given MOS displayed a significantly lower feed intake than the negative control group (Table 2). Group mean feed intake in the infected birds was increased by 6.4% after feeding MOS in experiment 1, but was not increased in experiment 2. Post infection feed conversion was not significantly affected by the treatments (Table 3). In experiment 2 the group-mean feed conversion ratio in the infected chickens was decreased by 4.6% after feeding the mannanoligosaccharide preparation.

Counting of oocysts in faeces was done in experiment 2 only. Table 4 illustrates that there was no significant difference between the infected control group and the infected group given MOS. No oocysts were detected in faeces obtained from the negative control group.

In both experiments the negative control birds showed no caecal lesions (Table 5). The mean lesion score and the frequency distribution of lesions scores did not differ significantly between the infected birds fed the diets without or with MOS (Tables 5 and 6).

The scores for the numbers of *E. tenella* schizonts in the lamina propria of the caecum, as determined in experiment 1, are shown in Table 7. In the infected birds given MOS 13 out of 24 had one or three schizonts whereas only one bird showed a mass of schizonts in the lamina propria. In the infected control group there were 12 out of 25 birds revealing a mass of schizonts and 6 animals displaying one or three schizonts in the lamina propria of the caecum. No schizonts were found in the negative control group. There was a significant difference in schizonts between the infected groups fed the diets without or with MOS.

In both experiments there were no significant differences in fat digestibility between the treatment groups (Table 8). In experiment 1, MOS produced a higher group mean fat digestibility in the infected birds, but this was not seen in experiment 2.

Table 6: Frequency distribution of caecal lesion scores in infected birds and negative control

Treatments	Lesion Score				
	0	1	2	3	4
Experiment 1[1]					
Negative control	25	-	-	-	-
Infected control	8	10	4	3	-
Infected, MOS	9	10	4	2	-
Experiment 2[2]					
Negative control	15	-	-	-	-
Infected control	-	7	2	6	-
Infected, MOS	1	6	5	3	-

[1]n=25. [2] n=15

Table 7: Frequency distribution of number of schizonts in the lamina propria of the caecum in experiment 1

Treatments	Schizonts Score (%)		
	0	1	2
Negative control (n =25)	100	00	00
Infected control (n =25)[a]	28	24	48
Infected, MOS (n= 24)[b]	42	54	04

[a,b]Mean values within the same column with different superscript letter are significantly different (P<0.05).

Discussion

In both experiments there was a successful infection with *Eimeria tenella* as indicated by the caecal lesions, the shedding of oocysts and the presence of schizonts in the lamina propria. The birds had been inoculated with either 3500 or 5000 sporulated oocysts. McDougald (2003) reported that inoculation with 1000 to 3000 sporulated oocysts would be sufficient to cause bloody droppings, and other signs of infection. Conway *et al.* (1993) were only able to produce a significant reduction in body weight at a dose of 10000 sporulated *E. tenella* oocysts. In the present experiments no significant effect of infection on weight gain was seen. The lack of susceptibility of growth performance to the infection with *E. tenella* must relate to the birds, the pathogenicity and the dose of the *E. tenella* strain used, and/or the experimental conditions. It is generally accepted that weight gain is the variable most sensitive to coccidiosis and anticoccidial efficacy of treatments (Barwick *et al.,* 1970; Johnson and Reid, 1970; Long, 1970).

In experiment 1, the inoculation was done when the birds were aged 12 days and in experiment 2 the age was 8 days while the dose of sporulated oocysts was increased. Lesion scoring was performed at 9 days post infection in experiment 1 and at 6 days post infection in experiment 2. It would be expected that the infection would be more severe in experiment 2 than in experiment 1 (Rose, 1967; Johnson and Reid, 1970). Indeed, the lesions scores were higher in experiment 2 than in experiment 1. There was no effect of MOS on the caecal lesions. However, in experiment 1 feeding of the MOS preparation had caused a significant decrease in the number of schizonts found in the lamina propria of caecum of the infected birds. Only one out of 24 birds fed the diet supplemented with MOS had a mass of schizonts whereas large clusters of schizonts were seen in 12 out of 25 infected control chickens. The protective effect of MOS might be related to an improvement of intestinal function (Loddi *et al.,* 2002) or immunity modulation (Ferket *et al.,* 2002). Jeurissen *et al.* (1996) found in immune chickens that significantly fewer sporozoites reached the crypt epithelium and that the formation of schizonts was inhibited. Sporozoites that had failed to reach the crypt epithelium within 48 hours after infection were detected within macrophages or were surrounded by them, pointing at control of the intensity of a primary infection.

A reduction in schizonts as seen in the infected birds fed MOS should be associated with lower caecal lesion scores mediated by the *E. tenella* infection (McDougald, 2003). It is not known why feeding of MOS reduced the number of schizonts without affecting the caecal lesion scores. The most pathogenic stage is the second-generation of schizonts, which matures after 4 days of the production of clusters of large schizonts, which may contain hundreds of merozoites. The schizonts develop deep in the lamina propria so that the mucosa is damaged seriously when the schizonts mature and the merozoites are released. Clearly, further investigations will have to be done to check the reproducibility of the effect of the mannanoligosaccharide on the number of schizonts.

In both experiments the apparent fat digestibility was not influenced by the infection with *E. tenella*. In contrast, Adams *et al.* (1996) showed that fat digestion was reduced from 86 to 21% in chickens infected with *Eimeria acervulina*. The discrepancy between the two studies probably relates to the different species of *Eimeria* and the type of lesions that they induce. *E. tenella* induces lesions in the caecum whereas *E. acervulina* induces lesions in the small intestine. Fat digestion depends on the integrity of the small intestine rather than on that of the caecum.

In conclusion, these experiments describe a successful infection of broiler chickens with *E. tenella* as based on caecal lesions, oocyst shedding and schizonts in the lamina propria of the caecum. The infection did not affect growth performance of the birds. In infected birds fed the MOS preparation, the number of schizonts was reduced without a decrease in the severity of caecal lesions. Perhaps MOS had enhanced the immunity of the infected birds and thereby decreased the number of schizonts. The hypothesis tested in this study was that

Table 8: Apparent fat digestibility (% of intake) in the three treatment groups

Days post infection	Control		MOS Infected	Pooled SEM	P-value
	Negative	Infected			
12-18 (experiment 1)	80.8	80.1	85.1	2.11	0.23
8-14 (experiment 2)	81.2	81.7	81.4	0.38	0.69

the feeding of MOS would suppress coccidiosis. It can be concluded that evidence was found for a protective effect of the MOS preparation used, but this effect was not associated with a decrease in caecal lesions and improved growth performance.

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