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The Use of Mannan-Oligosaccharides and/or Tannin in Broiler Diets

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Abstract: Mannan-oligosaccharides (MOS) and tannin have been reported to have possible growth promoting properties and to possess properties which may control coccidiosis. A total of 300 broilers were divided into 15 groups of 20 birds (at day 1) and three groups of 20 birds were assigned to each dietary treatment. Treatment 1 was a negative control, basal diet, and MOS (SAF-Mannan, Lesaffre, 0.5 g/kg), tannin (condensed chestnut tannin, 0.5 g/kg) and MOS/tannin (0.5 and 0.5 g/kg respectively) were added to the basal diet to produce Treatments 2, 3 and 4. Treatment 5 was a positive control and contained the antibiotic growth promoter, avilamycin (Maxus®, Elanco). Birds assigned to Treatment 5 also received a commercial vaccine, BayCox 2.5% oral solution, in the drinking water (1 ml/litre) (positive control). Average daily gain, feed intake and feed conversion efficiency were determined weekly for 35 days. The birds were challenged with coccidiosis on day 28 by oral administration of a solution containing sporulated oocysts of Eimeria *acervulina,* Eimeria *tenella* and Eimeria *maxima*. On day 35, birds were slaughtered and dissected and assessed for lesion scores at a number of regions along the intestinal tract. Neither MOS or tannin were effective in improving performance to a level comparable to that achieved with the use of the antibiotic growth promoter. The challenge with coccidiosis was successful, with birds across the treatments showing reduced growth on the subsequent seven days after infection. Supplementation of MOS or tannin either individually or in combination did not reduce the impact of coccidiosis. Chi-squared analysis of the intestinal lesion scores indicated that there was a significant effect of treatment, but it was not clear whether MOS or tannin offered a protective effect against coccidiosis.

Key words: Broilers, mannan-oligosaccharides, tannin and coccidiosis

Introduction

Public concern over the potential of antibiotic resistant bacteria has resulted in the withdrawal of many antibiotic growth promoters within the European Union and the ban on the use of all antibiotic growth promoters, enforced by 2006, means that European poultry producers face a future in which they will be unable to utilize antibiotic substances to promote growth. It is therefore of vital importance, both from an animal and human health perspective, to obtain natural alternatives. Some studies have suggested that oligosaccharides may provide such an alternative (Turner *et al*., 2001). Broilers do not possess the endogenous enzymes necessary to degrade some forms of oligosaccharides and it was therefore considered that their presence in animal feed reduced growth performance (Iji *et al*., 2001). However, it has been reported that the presence of alpha-galactosides in diets for broilers had no effect on live weight gain, feed efficiency, protein digestibility or the digestible energy of the diet (Irish *et al*., 1995). Furthermore, it has been suggested that supplementation with oligosaccharides may have a prebiotic effect by increasing production of lactic acid, thus increasing the proliferation of beneficial bacteria, and boosting the immune system (Savage *et al*., 1996).

Oligosaccharides are also thought to reduce the number of pathogenic bacteria. Bailey *et al*. (1991) reported that supplementing broiler diets with fructo oligosaccharides led to reduced susceptibility to Salmonella colonisation. The mechanism by which oligosaccharides achieve this effect has not been fully elucidated, however, it has been postulated that oligosaccharides block colonisation of pathogens by providing an alternative attachment site for the pathogen rather than the intestinal mucosa. Oligosaccharides are named after the predominant sugar, the most common supplements being fructo oligosaccharide (FOS) and mannan oligosaccharide (MOS). Fructose and mannan are sugars recognized by pathogens but are unavailable for use when bound in the oligosaccharide form. Pathogens are "tricked" into binding to the oligosaccharide and therefore are unable to bind to the intestinal mucosa (CFNP, 2002).

Research has indicated that supplementation with MOS results in an improvement in intestinal morphology and intestinal enzyme activity (Iji *et al*., 2001). These workers supplemented a sorghum/lupin-based diet with four levels (0, 1, 3 and 5 g/kg) of a commercial MOS (Bio-Mos™) and reported a beneficial effect on jejunal villi height and activities of maltase, leucine aminopeptidase and alkaline phosphatase. However, these workers

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reported no effect on performance parameters. Although there is a lack of information on the use of MOS as a means of controlling coccidiosis in poultry, some research has been carried out investigating other non-pharmaceutical alternatives. For example, Ibrir *et al.* (2001) examined the effect of thymol and carvacrol (components of essential oils) on the performance of broiler chicks infected with Eimeria *acervulina* and reported that supplementation significantly improved post-infection performance. One of the aims of the current study was to investigate the role of MOS as an alternative to antibiotic growth promoters and as a possible means of controlling coccidiosis naturally. Coccidiosis is ranked as one of the most serious and costly problems of poultry production as animal performance is severely affected by infection. At present, coccidiosis is controlled by coccidiostats or live attenuated vaccines supplemented in the feed or water. There are a number of problems associated with the use of coccidiostats. First of all, coccidiostats used in the poultry industry have been shown to become less effective or even ineffective due to resistance or tolerance on the part of the parasite. This reduction in efficiency has led to the requirement to change coccidiostats regularly and to employ "shuttle" and "rotation" programmes. However, there is controversy regarding the actual benefit of these programmes (Gard *et al.*, 1978). Despite the problems of resistance and tolerance, the use of coccidiostats is widespread and there is much evidence to support their success in controlling coccidiosis and in promoting growth. Live attenuated vaccines have been shown to be effective in controlling coccidiosis (Williams *et al.*, (1999) but they do not possess any growth promoting properties.

The anti-microbial properties of tannins have been well documented and they have been shown to inhibit a wide range of fungi, yeasts and bacteria (Chung *et al.*, 1998a). Scalbert (1991) put forward three possible mechanisms by which tannins exert their anti-microbial effect. Firstly, tannin may form complexes with microbial enzymes thus inhibiting their activity. Secondly, some component of tannin may act on the membranes of micro-organisms, causing an inhibition of their electron transport system. The third possible mode of action may simply be as a result of iron depletion due to the tannin complexation with metal ions. Chung *et al.* (1998a) suggested that the actual mode of action of tannins on micro-organisms could be a combination of those proposed by Scalbert (1991) and concluded that more research is required to fully elucidate possible mechanisms. Karunakaran and Kadirvel (2001) investigated the use of tannin (extracted from sweet chestnut wood), as a means of promoting growth in broiler chickens. The tannin extract was added at a rate of 0, 0.5 and 1 g/kg and growth performance parameters were determined for the four-week experimental period. There was no advantage in offering tannin in terms of bird performance and inclusion at the highest level (1 g/kg) actually depressed weight gain and feed conversion efficiency. Tannins have been reported to have anti-nutritive properties, as they form complexes with proteins, starches, digestive enzymes and vitamins and minerals, thus reducing intake, digestion and utilization of nutrients (Chung *et al.*, 1998b). Nevertheless, it was postulated by Karunakaran and Kadirvel (2001) that tannin supplementation could have a positive effect on bird health if the birds had been challenged with a gastrointestinal disease such as coccidiosis. The use of tannins as a means of protecting against coccidiosis has not been studied previously.

The main objectives of this study were to examine the effect of MOS and tannin on the performance of broiler chickens and to establish if either product could be used as an alternative to antibiotic growth promoters and to investigate if MOS and tannin can provide a natural means of protecting broiler chickens against coccidiosis.

Materials and Methods

Diet preparation: A starter/grower broiler diet was formulated using a computer-based feed formulation programme (Table 1). Cereals were ground using a hammer mill (5 mm screen) and all raw materials were weighed accurately prior to mixing in a Bentall mixer for 30 minutes. Mannan oligosaccharide (MOS, SAF-Mannan, Lesaffre), condensed chestnut tannin (T), MOS and T or avilamycin (Maxus®, Eli Lilly, Department, Elanco, Germany) were substituted with part of the limestone flour component at the mixing stage, to provide five experimental treatments (Table 2). Diets were cold-pelleted in a Lister 7.5 HP cuber using a 5 mm die, stored in sealed bags (25 kg), labelled and transferred to a cool, dry environment.

Table 1: Diet Composition

Component	Amount (g/kg)
Wheat	580.3
Maize	50
Prairie meal	50
Soya (HP)	200
Soya (Full fat)	50
Soya Oil	23.2
Lysine	3.31
Methionine	0.03
Threonine	1
NSP powder	0.4
Phytase	0.4
Limestone	19.5
Monodicalcium	12
Salt	1.61
Sodium bicarbonate	2
Minerals and vitamins	6.25

Birds and experimental procedures: This work was conducted under the Animals (Scientific Procedures) Act,

Table 2: Experimental treatments

	Mannan oligosaccharide (MOS) (g/kg)	Condensed Chestnut tannin (T) (g/kg)	Avilamycin (g/kg)[#]
Negative Control	0	0	0
MOS	0.5	0	0
T	0	0.5	0
MOS + T	0.5	0.5	0
Positive control	0	0	1.0

0Component not included. [#]The drinking water of the birds offered the positive control treatment was supplemented with a commercial coccidiosis vaccine (BayCox 2.5% oral solution) at an inclusion rate of 1 ml/litre to provide protection against coccidiosis

Table 3: *In vitro* viscosity (cPs) and energy contents (MJ/kg DM) of dietary treatments

	In vitro viscosity (cPs)	Energy (MJ/kg DM)
Negative control	4.90	19.1
Mannan oligosaccharide (MOS)	5.29	19.3
Condensed Tannin (T)	*	*
MOS + T	5.67	19.2
Positive control	5.70	19.4

*Due to technical problems, analysis was not conducted on the condensed tannin treatment

1986, Project Number 2520 b entitled "Alternatives to Pharmaceuticals in broilers" and approved by the Newforge Ethical Committee. A total of 320 male Cobb broiler chicks were obtained on the day of hatch from O'Kane Poultry Ltd, Ballymena, Northern Ireland. All 320 were weighed, and those at the top and bottom of the weight ranges were removed. The 300 which composed the middle weight range were then assigned to a total of 15 pens (1.2 m x 0.9 m), giving a total of 20 chicks per pen. Each pen was assigned to one of five experimental treatments, giving three pen replicates per treatment. Diets were sampled for *in vitro* viscosity determination using a Brookfield digital viscometer according to the method of Bedford and Classen (1992) and for gross energy determination using an adiabatic bomb calorimeter (Parr, Model 1271).

Feed and water were offered *ad libitum* from simple bell troughs for a total of 35 days. Four birds from each pen were removed at 14 days and six were removed at 28 days to avoid overstocking. Birds to be removed were weighed in order to maintain as far as possible a relatively uniform mean weight across the pens. Three of the birds removed on day 28 were used to obtain *in vivo* viscosity values for ileal digesta. Starting temperature was set at 35°C and reduced by 1°C at 48 hr intervals until it reached 24°C. Light was provided for 18 hr with the dark cycle being between midnight and 0600 hr.

Feed consumption and bodyweight gain were recorded on a weekly basis for each pen of birds and feed efficiency (feed:gain) was calculated. Mortality was recorded on a daily basis.

On day 28, birds were orally challenged with coccidiosis. A solution containing oocysts of E. *acervulina*, E. *tenella* and E. *maxima* (50,000, 15,000 and 15,000 sporulated oocysts per ml was deposited in the crop of each bird using a dropper pipette (1 ml)). Inoculation with this level of sporulated oocysts has been previously reported to result in sub-clinical coccidiosis and in a reduction in performance of 20% (Ralph Marshal, Personal Communication). At the end of the experimental period (35 days), eight birds from each pen were dissected to score for the presence of intestinal lesions arising as a result of coccidiosis infection. The intestine was opened at the infection sites corresponding to each strain of Eimeria (i.e. duodenum to posterior intestine for E. *acervulina*, caecum for E. *tenella* and main intestine to ileum for E. *maxima*) and the degree of damage to the epithelium was determined. A score of between one and three was assigned, with three representing the severest level of infection (Johnson and Reid, 1970). Two of the birds on day 35 were used to obtain *in vivo* viscosity values for ileal digesta.

Statistical analysis was performed using Analysis of Variance (ANOVA) and Chi-squared tests.

Results and Discussion

The dietary treatments were similar in terms of *in vitro* viscosity and energy content (Table 3). *In vitro* viscosity ranged from 4.90 to 5.70 cPs and energy content ranged from 19.1 to 19.4 MJ/kg DM.

The use of mannan-oligosaccharides and tannin as growth promoters: The birds on the positive control treatment consumed significantly more ($P < 0.05$) feed between 7 and 14 days than those on the other treatments (Table 4). These birds had a higher average daily gain (53.1 vs 39.7 g/day) and more efficient feed conversion (1.42 vs 1.75). Overall, the birds offered the positive control treatment had significantly higher average daily gains and better feed conversions than those offered the other treatments. There were no significant differences between the performance of birds offered the other treatments. After inoculation with the sporulated oocytes, average daily gain and feed efficiency was reduced (when compared with the performance of birds from 21-28 days) for all treatments, except for those birds offered the positive control. Feed intake was not affected by coccidiosis challenge.

Supplementation with MOS, tannin or MOS/tannin in combination did not significantly improve the performance of birds compared with those offered a

Table 4: Effect of treatment on dry matter intake, average daily gain and feed efficiency

	Growth stage of birds (days)	Negative control	Mannan oligosa-ccharide (MOS)	Condensed Tannin (T)	MOS+T control	Positive	SEM	P
Dry matter intake (g/d)	0-7 d	13.6	14.5	14.8	14.4	16.8	0.97	NS
	7-14 d	36.7^{a}	37.6^{a}	35.2^{a}	33.1^{a}	41.5^{b}	1.51	<0.05
	14-21 d	69.6	70.5	67.9	69.7	71.3	2.14	NS
	21-28 d	86.6	91.5	86.0	89.2	99.5	3.80	NS
	0-28 d	51.6	53.5	51.0	51.6	57.3	1.55	NS
	28-35 d	137.4	135.6	136.2	137.8	143.1	6.66	NS
	0-35 d	68.8	69.9	68.0	68.8	74.4	1.72	NS
Average daily gain (g/d)	0-7 d	14.0	14.4	14.9	14.1	17.8	0.98	NS
	7-14 d	26.9^{a}	25.7^{a}	24.0^{a}	23.3^{a}	34.8^{b}	1.23	<0.001
	14-21 d	43.6^{a}	44.5^{a}	42.2^{a}	43.1^{a}	57.7^{b}	2.18	<0.01
	21-28 d	60.0^{a}	61.2^{a}	58.6^{a}	59.9^{a}	75.9^{b}	3.20	<0.05
	0-28 d	36.9^{a}	37.1^{a}	35.7^{a}	35.9^{a}	46.0^{b}	1.50	<0.001
	28-35 d	53.0^{a}	55.9^{a}	54.9^{a}	47.8^{a}	81.7^{b}	5.78	<0.05
	0-35 d	40.1^{a}	40.8^{a}	39.6^{a}	38.3^{a}	53.1^{b}	1.86	<0.01
Feed:Gain	0-7 d	0.97	1.02	0.99	1.02	0.95	0.030	NS
	7-14 d	1.37	1.47	1.48	1.42	1.20	0.068	NS
	14-21 d	1.60^{b}	1.59^{b}	1.62^{b}	1.62^{b}	1.24^{a}	0.073	<0.05
	21-28 d	1.45	1.51	1.47	1.49	1.31	0.064	NS
	0-28 d	1.40^{b}	1.45^{b}	1.43^{b}	1.44^{b}	1.25^{a}	0.035	<0.05
	28-35 d	2.92	2.60	2.54	3.12	1.85	0.336	NS
	0-35 d	1.73^{b}	1.73^{b}	1.72^{b}	1.82^{b}	1.42^{b}	0.071	<0.05

a, b means without common superscript are significantly different

Table 5: Effect of treatment on *in vivo* viscosity (cPs) of ileal digesta

	In vivo viscosity (28 d)	*In vivo* viscosity (35 d)
Negative control	2.97	4.46
Mannan oligosaccharide (MOS)	5.88	5.14
Condensed Tannin (T)	6.83	5.28
MOS + T	6.33	5.80
Positive control	7.78	7.46
SEM	0.978	1.394
P	NS	NS

negative control. Furthermore, performance was significantly lower than the birds offered a positive control, which contained avilamycin as a growth promoter. Therefore, it can be concluded that neither MOS, tannin or MOS/tannin in combination was effective in promoting growth to a level equivalent to that achieved by the antibiotic growth promoter. This is in contrast to results reported by Bolduan *et al.* (1997) who stated that MOS could improve performance to a level comparable to that produced by antibiotic growth promoters. However, Oyofo *et al.* (1989) reported that whilst MOS supplemented drinking water resulted in reduced colonisation of S. *typhimurium* within the gastrointestinal tract, there was no significant effect on weight gains. The results of the current study are also in agreement with those of LeMieux *et al.* (2003). These workers reported that in order for MOS to exert a growth promoting effect, it must be used in combination with an antibiotic. In theory, the MOS/tannin combination treatment should have simulated this MOS/antibiotic combination effect but this effect was not observed in the present study. However, this may be attributed to the lack of response to tannin supplementation in terms of performance and not as a result of MOS inefficacy in the presence of antibiotics.

The *in vivo* viscosity of ileal digesta was not significantly affected by dietary treatment (Table 5) on either day 28 or day 35. Values ranged from 2.97 to 7.78 cPs for day 28 and 4.46 to 7.46 for day 35. MOS is derived from the cell walls of certain strains of *Saccharomyces cerevisiae* yeast and therefore the product contains a high level of β-glucan. β-glucan consists of a linear chain of glucose units joined by both β-(1-3) and β-(1-4) linkages and is known to increase gut viscosity and affect nutrient digestion and absorption in poultry (Choct and Annison, 1992). However, the *in vitro* viscosity values of the MOS supplemented diet were similar to those of the other diets indicating that the level of β-glucan present in MOS was not sufficient to affect *in vitro* viscosity. This is supported by the values for *in vivo* viscosity of the ileal digesta, as there was no significant difference at either day 28 or day 35. It can therefore be concluded that the β-glucan present in MOS did not exert an anti-nutritive influence, as no negative effect was observed on performance or *in vivo* viscosity.

Tannins have also been reported to exert an anti-nutritive effect as they form complexes with proteins, starches, digestive enzymes and vitamins and minerals, thus reducing intake, digestion and utilization of nutrients Chung *et al.* (1998b). However, in this study the addition of tannin (0.5 g/kg) did not result in any reduction in

Table 6: Effect of treatment on average lesion scores (analysed by Chi-squared test)

	Lesion Score	Negative control	Mannan oligosaccharide (MOS)	Condensed Tannin (T)	MOS+T	Positive control	Total
E. *acervulina*	< = 0.05	5	10	9	6	13	43
$Chi^2 = 17.55$	1	6	11	11	12	7	47
d.f. = 8	>1	13	3	4	6	4	30
P < 0.05	Total	24	24	24	24	24	120
E. *maxima*	< = 0.05	14	10	17	16	17	74
$Chi^2 = 17.80$	1	2	10	6	5	2	25
d.f. = 8	>1	8	4	1	3	4	20
P < 0.05	Total	24	24	24	24	23	119
E. *tenella*	< = 0.05	13	14	17	22	23	89
$Chi^2 = 25.98$	1	5	3	2	2	0	12
d.f. = 8	>1	6	7	5	0	1	19
P < 0.01	Total	24	24	24	24	24	120

performance. Karunakaran and Kadirvel (2001) investigated the effect of supplementing broiler diets with 0, 0.5 or 1 g/kg sweet chestnut tannin and reported that tannin at an inclusion rate of 0.5 g/kg did not impact on performance. However, when tannin was included at 1 g/kg, live weight gain and feed efficiency were reduced. Therefore, the level of tannin used in the present study should not have elicited an anti-nutritive effect.

The use of mannan-oligosaccharides and tannin as a means of controlling coccidiosis: Table 6 presents the average lesion scores of representative intestinal regions affected by E. *acervulina*, E. *tenella* and E. *maxima*. There were significant treatment effects indicating that there was an association between lesion score and treatment.

Birds were orally challenged with coccidiosis at 28 days and were given sufficient inoculant to reduce overall performance by 20% (Ralph Marshal, Personal Communication). Given the average daily gain of the birds offered the negative control treatment between 21 and 28 days (60 g/day) and that of the potential gain between 28 and 35 (72.9 g/day Cobb 500, 1998) it can be concluded that the challenge with coccidiosis was successful in reducing performance by 27%. The average daily gain of birds offered the positive control treatment containing live vaccine, between 28 and 35 days was slightly lower (7%) than the predicted performance indicating that the vaccine was effective and conferred some protection against coccidiosis. This is a key finding as it indicates that live vaccine may not be fully effective in controlling coccidiosis and further highlights the need for an effective alternative to coccidiostats. This is in keeping with the conclusions of Waldenstedt *et al.* (1999) who reported that the performance of vaccinated broilers was slightly impaired in comparison with those under antibiotic coccidial control.

In terms of performance, none of the supplements were effective in reducing the impact of the disease. It was expected that lesion scores would be lower for the birds offered the MOS, tannin and MOS/tannin treatments due to the anti microbial effect of MOS and tannin (LeMieux *et al.*, 2003; Karunakaran and Kadirvel, 2001; Allen *et al.*, 1997 and Oyofo *et al.*, 1989) and it did appear that the tannin treatment numerically reduced the combined lesion score figure when compared to the negative control. The results from the Chi-squared test indicated that there were significant effects of treatment on lesion score. Further research is required to fully investigate the effect of MOS and tannin on lesion scores.

The actual values for the lesion scores associated with the individual strains of Eimeria are lower than those reported in the literature. For example, Hooge *et al.* (1999) inoculated 14-day old broilers with E. *acervulina*, E. *maxima* and E. *tenella* (100,000, 50,000 and 10,000 sporulated oocysts per bird respectively) and reported composite coccidial lesion score values of 3.656 for birds offered a control diet (-41% higher than that observed in the current study). Similarly, Alviar *et al.* (1981) inoculated 14-day old broilers with approximately 75,000 sporulated oocysts consisting mainly of E. *tenella* and reported a lesion score of 3.30 for the control group. It is possible that inoculation with coccidiosis was administrated at too late a stage in the life of birds in the present study (28 days) as it has been reported that the period between 21 and 28 days is the time of the highest potential for clinical and subclinical coccidiosis to occur (Reyna *et al.,* 1983). However, as the performance values indicate, the challenge with coccidiosis was successful. It is interesting to note that the birds in the positive control group exhibited lesions. This has been reported previously (Bushell *et al.*, 1992; Shirley *et al.*, 1995 and Williams, 1994) and it has been suggested that use of lesion scores as a means of assessing coccidiosis may be misleading.

Conclusions

1. MOS and tannin supplementation of broiler diets did not improve performance to a level equivalent to that achieved by the inclusion of an antibiotic growth promoter.

2. Further studies are required to investigate the interactive effect between MOS, tannins and antibiotic growth promoter.
3. The challenge with coccidiosis was successful and performance was reduced in the negative control group (unprotected) by over 20%. Performance in the vaccinated group was slightly reduced, indicating that the vaccine was not fully effective in protecting against coccidiosis.
4. In order to maximize the effectiveness of lesion scoring as a method to assess the severity of coccidiosis, inoculation should take place earlier (between 21 and 28 days).
5. MOS or tannin supplementation was not effective in protecting against coccidiosis.
6. Tannin supplementation appeared to reduce the detrimental impact of coccidiosis on the intestinal tract as lower lesion scores were obtained from the tannin supplemented groups. This effect requires further investigation.

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