EFFECT OF MANNAN OLIGOSACCHARIDES ON THE ILEAL MORPHOMETRY AND CECAL FERMENTATION OF GROWING RABBITS

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ABSTRACT

A common problem in rabbits is the occurrence of digestive disorders just after weaning. This problem is usually associated with instability of the cecal microflora and characterized by diarrhea, loss of appetite and increased mortality. In the current study the effects of a mannan oligosaccharide (MOS, Bio-MOS®, Alltech Inc. USA) was compared to a commonly used antibiotic (AGP, Zn-Bacitracin). The current study investigated the effects of MOS and AGP on intestinal morphometry and cecal VFA production. A total of 220 weaned mixed-sex rabbits (32d) were divided into 5 experimental treatments and housed in groups of 4 in 55 flat deck cages located in an experimental rabbit house. The 5 experimental treatments were as follow: Control (no additives);MOS 1 (1 kg MOS/t); MOS 1.5 (1.5 kg MOS/t); MOS 2 (2 kg MOS/t) and AGP (0.1 kg/t). Experimental diets were based on alfalfa, sunflower meal and wheat and beet pulp and were offered *ad libitum* through out the experiment. Intestinal morphology and cecal fermentation was determined in 8 rabbits per treatment slaughtered at day 46. Histologic examination showed significantly longer villi ($P<0.05$) in rabbits fed MOS (503.1, 518.6 and 508.8 for MOS 1, MOS 1.5 and MOS 2, respectively) or AGP (502.5) compared to the control group (403.0). This was also translated into a numerically increased absorption surface. Cecal volatile fatty acid (VFA) concentration differed significantly ($P<0.05$) between treatments. Rabbits fed MOS at 1 kg/t had the highest total VFA concentration (98.02 vs. 51.57 in MOS 1 and Control) and higher butyric acid (14.09 vs. 4.59 in MOS 1 and Control). Furthermore cecal pH was significantly lower in rabbits fed MOS at 1 kg/t and 2 kg/t (5.79 and 5.92) compared to the control (6.33). Results for this study suggest that MOS has a stimulating effect on villi development and production of cecal VFA and reduce cecal pH, which could improve the health status of growing rabbits.

Key words: Bio-Mos, rabbit, mannan oligosaccharide, villi length, VFA.

INTRODUCTION

Digestive disorders are a common problem in weaning rabbits. Specific pathogens such as *E. coli* O103 or *C. spiroforme* can lead to mortalities after weaning in excess of 20%

(PEETERS *et al*. 1995). However, the most common disorder in rabbit production is the occurrence of a enteritis complex which has no actually identified pathogenic agent. Clinical symptoms include increased incidences in diarrhea, poor feed conversion and a general loss of appetite. It has been identified that increased fermentation in the cecum and as a result increased production of volatile fatty acids (VFA) in the cecum could reduce the incidence of digestive disorders as well as contributing substantially to the overall energy supply through cecothrophy (GIDENNE 1996).

The most common prevention for digestive disorders in rabbits is the use of antimicrobial growth promoters (AGP). However, the proposed ban of AGP in the EU in 2006 and the voluntary reduction in the use of AGP in other countries have forced producers to look for alternative growth promoters to be used in rabbit feed. Phosphorylated mannan oligosaccharides (MOS) derived from the outer cell wall of yeast *Saccharomyces cerivisae* have shown to decrease the colonisation of enteric pathogens such as *Salmonella, E. coli* or *Campylobacter* and improve intestinal health in other species (DAWSON and PIRVULESCU 1999; SPRING *et al*. 2000). The growth promoters try to balance the different microbial species within the alimentary tract and to compensate for the adverse effects of the intestinal microflora on the animal efficiency through the following mechanisms: modifying the gut wall structure with considerable changes in ultrastructure, and limiting the turnover of the intestinal epithelial layer with sparing effects on the metabolic energy costs to maintain the gut (ARMSTRONG, 1986). Several reviews have shownthat these changes will result in improved performance for broilers, turkeys or pigs (HOOGE 2004a; HOOGE 2004b; MIGUEL *et al*. 2002). However, little research has been conducted on the effects of MOS on rabbit performance, mortality or changes in the intestinal microflora. BERSENYI and GIPPERT (1995) did report some improvement in weight gain of weaned rabbits when fed MOS over a 6 week period in a smaller scale trial.

The objective of the current experiment was to evaluate different inclusion levels of MOS as a natural growth promoter on intestinal morphometry and cecal VFA production in growing rabbits. These results were compared to a positive control with an antibiotic growth promoter.

MATERIAL AND METHODS

A total 220 mixed-sex rabbits with 32 days old (weaning) were divided into 5 experimental treatments and housed in groups of 4 in 55 flat deck cages located in an experimental rabbit house. The 5 experimental treatments were as follow: Control (no additives); MOS 1 (1 kg MOS/t); MOS 1.5 (1.5 kg MOS/t); MOS 2 (2 kg MOS/t) and AGP (0.1 kg Zn-Bacitracin/t). Experimental diets were based on alfalfa, sunflower meal and wheat and beet pulp and were offered *ad libitum*. Rabbits were kept in darkness, with one hour a day light for management work. On d46, eight rabbits per treatment were randomly selected and killed by sudden cervical dislocation. Intestinal samples (ileum) were collected for histologic examination and a sample of the content of the cecum was collected immediately after slaughter and stored at -20°C for VFA analysis. The villi measurements were done according to a modification of the method by JAEGER *et al.*

(1990) and IJI *et al.* (2001). VFA analysis was conducted according to CZERKAWSKI (1976). The data was analyzed as a completely randomized design with 2 treatments. Analysis of variance was performed using the general linear models procedure of SYSTAT 5.0 (1992).

RESULTS AND DISCUSSION

A profound way to analyse the effect of a feed additive on gastrointestinal health is the histopathological examination of the small intestine. Histological examination of the ileum showed significantly increase (P<0.05) of villi height in rabbits fed MOS or AGP compared to the unsupplemented control (Table 1). Crypt depth and the ratio between villus height and crypt depth was not affected by dietary treatments and, as a result, rabbits receiving MOS or AGP showed greater values for the absorption area compared to the control group (P=0.17). These findings were confirmed upon examination of tissue samples on scanning electron microscopy (SEM). SEM scanned showed that rabbits fed MOS appeared to have longer villi as well as a more regular villi structure compared to rabbits fed the control diet or the AGP supplemented diet (Figures 1-3).

Table 1. Villi measurements (µm) and absorption area (10^3 µm^2) on day 46.

	Treatment						
	Control	MOS 1	MOS 1.5	MOS 2	AGP	SE [1]	P value
n	8	8	8	8	8		
Villi height µm	403.0 [b]	503.1 [a]	518.6 [a]	508.8 [a]	502.5 [a]	13.45	0.03
Crept depth	140.3	142.2	151.2	162.1	153.9	4.25	0.50
Villus height/crept depth	2.9	3.6	3.5	3.3	3.5	0.13	0.50
Absorption area	135	159	166	167	169	5.02	0.17

[1] Standard error of mean. Values within rows without a same superscript are significantly different (P<0.05).

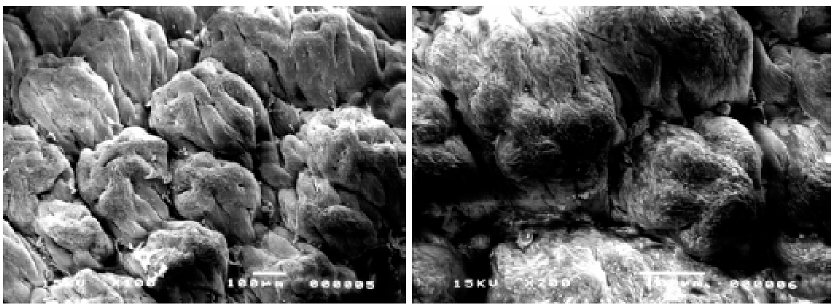


Figure 1. Ileum of rabbits (d46) fed unsupplemented control diet.

As a result of the increase in villi length, the overall absorptive surface area was increased, which could result in reduced risk for disease (MONSAN and PAUL 1995). Several studies have demonstrated that MOS has the ability to bind pathogenic bacteria expressing type-1 fimbrae such as *Salmonella* species, *E.coli* (SPRING *et al*. 2000). In addition, it has been shown that MOS has an indirect influence on the composition of the intestinal microflora. In a study with turkeys the intestinal concentration of *C. perfringens* was significantly reduced when MOS was added to the diet (FINUCANE *et al*. 1999). *Clostridia* do not express type 1 fimbrae hence these effects are believed to be indirectly associated with the shift in growth of beneficial bacteria that could reduce the risk of digestive disorders.

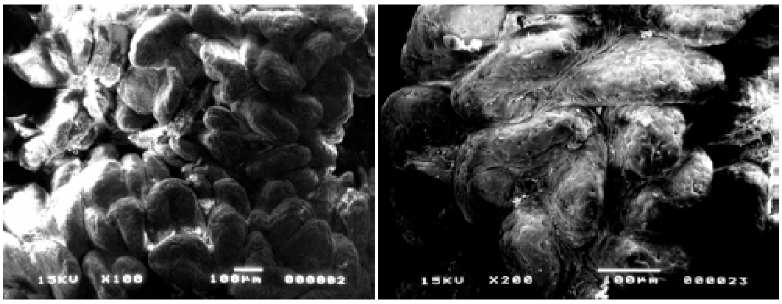
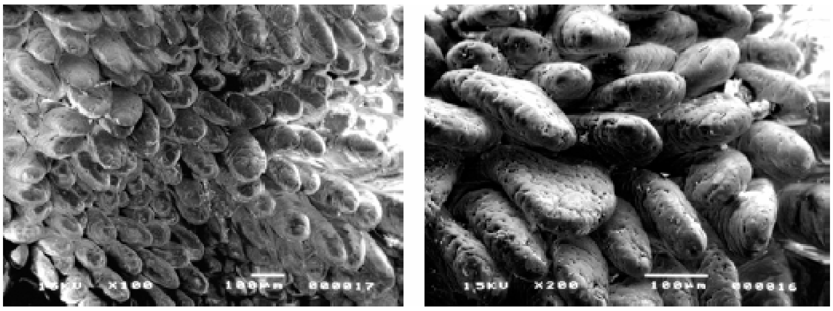


Figure 2. Ileum of rabbits (d46) fed diets with AGP.



Figures 3. Ileum of rabbits (d46) fed diets with MOS 2.

Table 2. Volatile fatty acids concentration (mmol.1000 ml^{-1}) and pH of cecal samples on d46.

	Treatment						
	Control	**MOS 1**	**MOS 1.5**	**MOS 2**	**AGP**	**SE [1]**	**P value**
N	8	8	8	8	8		
Acetic acid	43.11^{b}	79.01^{a}	63.53^{ab}	68.16^{a}	65.24^{ab}	7.79	0.043
Propionic acid	3.87	4.92	4.92	3.84	4.04	0.67	0.609
Butyric acid	4.59^{c}	14.09^{a}	9.32^{bc}	11.39^{ab}	10.72^{b}	1.64	0.006
Total VFA	51.57^{b}	98.02^{a}	77.76^{ab}	83.39^{a}	80.00^{a}	9.28	0.024
pH	$6{,}33^{c}$	$5{,}79^{a}$	$6{,}19^{bc}$	$5{,}92^{ab}$	$5{,}87^{ab}$	0,07	0,035

[1] Standard error of mean. Values within rows without a same superscript are significantly different (P<0.05).

An important tool to qualitatively evaluate the microbial activity in the intestine is to measure the VFA concentration as end-products of microbial fermentation (BELLIER and GIDENNE 1996). Acetic acid was the predominant VFA, followed by propionic and butyric acid. MOS 1 and MOS 2 significantly increased (*P*<0.05) the concentration of acetic acid, butyric acid and total VFA in the cecum compared to the control group (Table 2). Rabbits fed MOS 1 to have increased butiric acid production in the cecum compared to the AGP group (P<0.05) and control group had the lower production. No significant differences between the MOS and AGP treatments were observed on VFA production, however rabbits fed MOS 1 tended to have increased VFA production compared to the AGP group. As expected, diets with higher concentration of VFA presented lower pH value in the cecum. Rabbits fed diets with MOS 1 and MOS 2 had significantly (*P*<0.05) lower pH compared to the unsupplemented control. There were no pH differences between the AGP group and MOS 1 or MOS 2 groups.

The VFA concentration in the cecum change slowly with the age of the rabbit and acetic acid is typically the predominant VFA in the ceca of rabbits (GIDENNE *et al*. 1998). GIDENNE *et al*. (2002) found that the microflora in the cecum of rabbits is established at weaning, and only minor changes occur with ageing. In the current study significant differences in the concentration as well as the molar proportion of VFA were found fourteen days after weaning as consequence of the inclusion of MOS or AGP in the diet. Rabbits fed MOS or AGP had significantly higher concentration of total VFA and butyric acid in the cecum compared to the control. Furthermore rabbits fed MOS 1 or MOS 2 tended to have higher concentrations of acetic and butyric acid compared to all other treatments. The increase in VFA concentration in these treatments was related with a decrease in the cecal pH. *In vitro* studies by PROHASZKA (1980) found that high VFA concentration and a low cecal pH have a negative effect on the proliferation of *E. coli*. These findings were later confirmed by PEETERS *et al.* (1995) who found that high VFA levels and a reduced cecal pH in rabbits resulted in a protective effect against experimental enteropathogentic *E. coli* infection. It can therefore be concluded that the addition of MOS could have a similar effect on gastrointestinal morphology and could provide some protection against enteric diseases.

CONCLUSIONS

The results of this study suggest the addition of MOS could improve the intestinal integrity and reduced the cecal pH, which could improve the protection of weaned rabbits against common pathogens.

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