

MANNAN-OLIGOSACCHARIDES CAN MODIFY CAECAL MICROBIAL FERMENTATIONS OF RABBITS IN THE POST WEANING

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ABSTRACT

From 2007 to 2011 a total of 1152 growing rabbits were used in 8 different trials two trials per year were conducted, the first in the winter (January-February), the second in the summer (July-August). For each trial, 144 rabbits were equally divided into three groups (48 rabbits/group) fed the same basal diet and, from weaning (35 days) up to 62 days of age, submitted to the following dietary treatments: a control group (CONT), without additives; a MOS group fed mannan-oligosaccharides (Bio-Mos®; Alltech Biotechnology, Lexington, KY, USA) at 1 g/kg of feed; an antibiotic group (ANT) fed the basal diet supplemented with antibiotics (colistin sulphate, 144 mg/kg; tylosin, 100 mg/kg; and oxytetracyclin, 1000 mg/kg). Feed intake, mortality rate and caecal fermentation characteristics were studied by treatment, season (summer and winter) and their interaction. The mortality rate was significantly ($P<0.05$) lower in rabbits fed MOS (13.6 %) than the control (21.4%) and the ANT group (19.7%). In summer the mortality rate (24.6 %) was higher than in the winter (11.9 %). Rabbits fed antibiotics showed a higher ($P<0.05$) feed intake than the CONT group (133 vs 118 g/d), while no significant differences were recorded for the MOS in comparison to the other groups. MOS group had a higher total volatile fatty acid (tVFA) production than the other two groups (+ 17 and + 35 %, than CONT and ANT groups, respectively, $P<0.01$), due in particular to a higher production of acetate, propionate and valeric acids. Ammonia production was the highest ($P<0.01$) in caecal content from CONT group (6.35 vs. 4.23 and 3.98 Mmol/l, respectively for CONT, ANT and MOS group). During summer feed intake, tVFA and acetate production were significantly reduced (- 10, - 18 and 21 %, respectively) respect to winter. During winter, CONT and ANT groups showed a higher (from 10 to 16 %) production of VFAs (excluding isovaleric acid). On the contrary, rabbits fed MOS had, during winter a lower (from 9 to 13 %) production of VFAs. Mannan-oligosaccharides seemed able to modify caecal fermentations of growing rabbits, particularly when the climatic conditions do not fall in the optimal range of rabbit, when the temperature increases and induces a lower feed intake and a higher mortality rate .

Key words: Mannan-oligosaccharides, antibiotics, rabbit, caecal fermentation, post weaning period.

INTRODUCTION

In the last few years there has been research into alternatives to growth promoter antibiotics in animal production which have been banned by the European Community since January 2006 [EC Reg. 1831/2003; EC Council (2003)]. In rabbit production, antibiotics are still used under veterinary prescription in many farms during the growth period (weaning – 56/60 days) in order to prevent post-weaning enteric disorders and, in particular, epizootic rabbit enteropathy, which is the prime cause of mortality in the European rabbit industry (Dewree *et al.*, 2003).

Prebiotics and in particular mannan-oligosaccharides (MOS), are considered a promising alternative to antibiotics (Kocher, 2006). Several studies (Fonseca *et al.*, 2004; Pinheiro *et al.*, 2004 and 2009; Mourao *et al.*, 2006; Guedes *et al.*, 2009) have shown that the addition of MOS to the diets results in a better intestinal integrity and has a protective effect against common pathogens. MOS are able to bind the mannose receptors on the type 1 fimbriae of some pathogen bacteria (such as *Escherichia coli* and

Salmonella enteritidis in order to prevent their attachment to intestinal mucosa (Firon *et al.*, 1983; Spring *et al.*, 2000). In recent studies, Bovera *et al.* (2010) found that, in comparison with antibiotics, the concentration of 1 g/kg of MOS induced an important reduction of mortality rate in rabbits under critical conditions involving an episode of epizootic rabbit enteropathy.

The paper summarized the results from 4 years of trial in which MOS were used as feed supplementation in growing rabbit. The aim was to check the effect of MOS on rabbit caecal fermentation, in particular during hot and cold climate.

MATERIALS AND METHODS

Animals and experimental design

From 2007 to 2011 a total of 1152 hybrid Hyla growing rabbits were used in 8 different trials, in a specialized rabbit farm in (Benevento, Italy). All the trials were conducted under similar conditions regarding housing, age at weaning and feed. Two trials per year were conducted, the first in winter (January-February), the second in summer (July-August). For each trial, 144 rabbits, housed in bicellular cages (2 rabbits/cage) in the same breeding room (ventilated but not heated) and under the light schedule 16:8 light:dark, were equally divided into three groups (48 rabbits/group). The groups were fed the same basal diet and from weaning (35 days) up to 62 days of age, were submitted to the following dietary treatments: a control group (CONT), without additives; a MOS group fed mannanoligosaccharides (Bio-Mos®; Alltech Biotechnology, Lexington, KY, USA) at 1 g/kg of feed; an antibiotic group (ANT) fed the basal diet supplemented, as normally used in the farm under veterinary prescription, with antibiotics (colistin sulphate, 144 mg/kg; tylosin, 100 mg/kg; and oxytetracyclin, 1000 mg/kg). The basal diet was analysed for dry matter (method number 934.01, Association of Official Analytical Chemists, AOAC, 2004), ether extract, ash, crude protein and crude fibre (method number 945.18, AOAC, 2004), acid detergent fibre and acid detergent lignin (method number 973.18, AOAC, 2004) and amylase-treated neutral detergent fibre (method number 2002.04, AOAC, 2004). The chemical composition of the basal diet used in all the trials is reported in Table 1.

The temperature was measured inside the breeding room every day of the trials.

Table 1: Chemical analysis of the commercial basal diet used in the trials.

Dry matter	Ash	Crude protein	Ether extract	Crude fiber	NDF	ADF	ADL
%				% Dry matter			
88.0	10.6	16.9	3.6	20.9	36.1	21.4	2.7

¹Premix provided per kg diet: vitamin A, 12,000 IU; vitamin D₃, 1,000 IU; vitamin E acetate, 50 mg; vitamin K₃, 2 mg; biotin, 0.1 mg; Fe, 100 mg; Cu, 20 mg; Mn, 50 mg; Co, 2 mg; I, 1 mg; Zn, 100 mg; Se, 0.1 mg; Robenidine, 66 mg. Ingredients: Alfalfa meal, Wheat bran, Barley, Dried beet pulp, Soybean meal, Sunflower meal.

Feed intake was recorded weekly. Mortality rate was recorded daily.

Caecal fermentations

For each trial, at 62 days of age, 20 rabbits per group were slaughtered in a specialized slaughterhouse, after 12 h of fasting. The caeca were tied at both ends, separated by sterile instruments from the rest of the gastrointestinal tract, placed in tightly closed plastic bags, put in pre-warmed thermos and transported within 1 h to the laboratory. In the laboratory, two quotes of caecal content (each about 5 ml) were collected for volatile fatty acids (VFAs) and ammonia (NH₃) measurement. After dilution of the samples with oxalic acid (1:1, v/v), the VFAs were analyzed by a gas chromatography method (Thermo- Electron mod. 8000top, FUSED SILICA Gas chromatograph (Thermo Electron Corporation, Rodano, Milan, Italy) with OMEGAWAX 250 fused silica capillary column 30 m x 0.25 mm x 0.25 mm film thickness; analysis temperature, 125 °C; flame ionisation detector, 185 °C; carrier helium, 1.7 ml/min (Stanco *et al.*, 2003). Branched chain proportion (BCP), a useful index of protein digestion, was determined as the sum of isobutyrate and isovalerate divided by the total VFA production. Ammonia was determined according to the method described by Searle (1984). After centrifugation at 610.5 g for 10 min at room temperature (about 22 °C), the samples were diluted 10 times with water and then 1 ml of the product was deproteinised

using 10% trichloro-acetic acid. NH₃ and phenol were oxidised by sodium hypochlorite in the presence of sodium nitroprusside to form a blue complex. The intensity was measured colorimetrically at a wavelength of 623 nm. The intensity of blue is proportional to the concentration of NH₃ present in the sample.

Statistical Analysis

The data were analyzed by ANOVA (PROC GLM, SAS, 2000) using a two-way model to test the effect of different additives in the diet and the season as well as their interaction. Differences among means were evaluated by Tukey's test (SAS, 2000). The year effect was not significant and discarded from the model. Differences among mortality rates were tested by Chi-square.

RESULTS AND DISCUSSION

The average maximum temperature recorded in summer was 29 ± 2.3 °C while in winter the maximum average temperature was 8 ± 3.4 °C). Inside the breeding room the average temperature was, respectively 26 ± 2.1 and 18 ± 3.6 °C.

The mortality rate was significantly ($P < 0.05$) lower in rabbits fed MOS (13.6 %) than the control (21.4%) and the ANT groups (19.7%). The hot climate induced a higher mortality rate (24.6 %) than winter (11.9%). Rabbits fed antibiotics showed (Table 2) a higher ($P < 0.05$) feed intake than CONT group (133 vs. 118 g/d), while no significant differences were recorded for MOS in comparison to the other groups. MOS group had a higher tVFA production (Table 2) than the other groups (56.9 vs. 47.4 and 36.7 Mmol/l, respectively for MOS, CONT and ANT groups) due in particular to a higher production of acetate, propionate and valeric acids. The levels of the other VFAs were significantly higher than in ANT group but not different from the control group. The higher level of tVFA in MOS group suggests a more intense fermentative activity of caecal microflora. Looking at the molar proportion of the main VFAs, there is a lower percentage of acetate (74 %) in MOS than in CONT and ANT groups (77 and 78 % respectively) and a lower proportion of propionate (6.8 %) in the CONT

Table 2: Feed intake, volatile fatty acids, branched chain proportion (BCP) and ammonia levels in rabbit caecal content.

	Feed intake*	Acetate	Propionate	Butyrate	tVFA	Isobutyric	Isovaleric	Valerianic	BCP	NH ₃
	<i>g/d</i>	<i>Mmol/l</i>								
	Treatment effect (TE)									
Control	118 ^b	37.2 ^B	3.21 ^B	4.37 ^{AB}	47.4 ^B	0.93 ^{AB}	0.98 ^{AB}	0.70 ^B	0.04 ^{AB}	6.4 ^A
Antibiotic	133 ^a	28.5 ^C	3.06 ^B	3.36 ^B	36.7 ^C	0.58 ^B	0.53 ^B	0.72 ^B	0.03 ^B	4.2 ^B
Mos	125 ^{ab}	42.3 ^A	5.03 ^A	5.89 ^A	56.9 ^A	1.20 ^A	1.38 ^A	1.12 ^A	0.05 ^A	4.0 ^B
	Season effect (SE)									
Summer	119 ^b	31.7 ^B	3.42	4.23	42.1 ^B	0.94	0.95	0.90	0.05	5.2
Winter	132 ^a	40.3 ^A	4.11	4.85	51.9 ^A	0.87	0.98	0.79	0.04	4.56
	P values									
TE	0.012	<0.0001	0.3210	0.004	<0.0001	0.007	0.0011	0.002	0.005	0.001
SE	0.010	<0.0001	0.2812	0.3517	<0.0001	0.527	0.694	0.325	0.097	0.087
TE x SE	0.283	0.0025	0.035	0.028	0.0014	0.042	0.112	0.028	0.2311	0.003
SEM	1.02	2.84	2.99	2.76	4.25	1.12	1.04	0.99	0.20	1.72

A, B, C: $P < 0.01$; a, b: $P < 0.05$; SEM: standard error of means.; tVFA: total volatile fatty acid; BCP: branched chain proportion. * for the entire experimental period (35 - 62 days)

than in ANT and MOS groups (8.3 and 8.8 % respectively), indicating a different activity of microflora in fermentation of carbohydrate sources. BCP value was higher (0.05 vs 0.03, $P < 0.01$) in MOS than in ANT group but was not different from MOS and CONT group; ammonia production was the highest (6.35 Mmol/l, $P < 0.01$) in caecal content from CONT group. Since isobutyric and isovaleric acids (used in BCP calculation) as well as valeric acid are produced, respectively, from the degradation of the amino acids valine, leucine and proline (Van Soest, 1994), their higher production suggests higher protein degradation (Bovera et al., 2007). In addition, NH₃ is an end

product of protein degradation, but it is used by bacteria, in combination with carbon chains produced from carbohydrate fermentation, to synthesise new amino acids for bacterial growth (Van Soest, 1994). In our trial, the rabbits fed MOS and antibiotics did not show differences in caecal ammonia production, while the control group had a significantly higher value. The lower or similar ammonia level in combination with a higher protein degradation, as shown by the MOS vs. the CONT and ANT groups, can be explained by a better synchronism in carbohydrate and protein degradation.

The hot climate in summer reduced the feed intake (119 vs. 132 g/d, $P < 0.01$) and total VFA production (42.1 vs. 51.9 Mmol/l) mainly due to the lower acetate level than in winter. The molar proportion of the acetate, butyrate and propionate were unaffected, while the proportions of branched fatty acids was higher in the summer (2.23, 2.25, 2.14 and 1.68, 1.89, 1.52 %, respectively during summer and winter). This suggests that during summer the caecal microflora was more able in protein fermentation.

The interaction between the tested factors was almost always significant for the VFAs. Figure 1, as an example, represents the interaction between treatment and season for total VFA production and the same trend was for the other VFAs. In general, during winter, CONT and ANT groups showed a higher production of VFAs (from 9 to 13 %) (excluding isovaleric acid). On the contrary, rabbits fed MOS had, during winter a lower production of VFAs. However, no significant interaction was recorded for BCP. In the other hand, for ammonia production the levels during Summer were higher (5.80 vs. 4.85 and 5.23 vs. 4.62 Mmol/l) than in the winter for CONT and ANT groups, while the opposite occurred for MOS group.

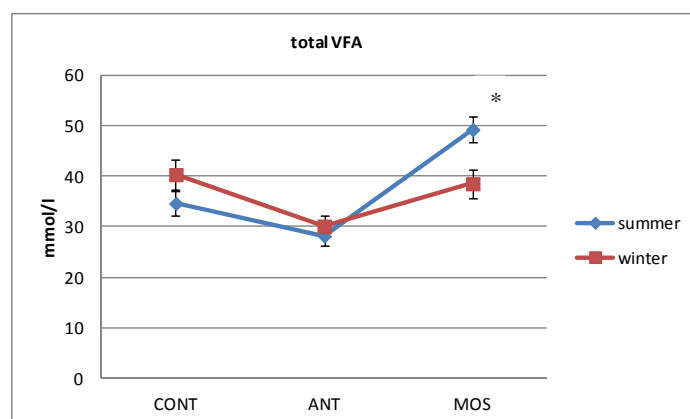


Figure 1: Treatment x season effect (mean \pm standard deviation) for total VFA production (*: $P < 0.01$).

Interaction suggests that MOS had a high efficacy in the modification of caecal fermentation parameters during the hot climate period, in which the high temperature reached inside the breeding room strongly affects the rabbit performance. Under this condition MOS seemed useful to increase the caecal fermentations and induce a better synchronism in the fermentation of carbohydrate and protein sources, reducing the level of ammonia. However, when there are no particular stress condition (hot climate or pathogenic events) the effect of MOS was not different from that of antibiotic but, also, there are no differences in respect of the untreated group.

CONCLUSIONS

A heat stress (summer vs winter climate) induced a lower feed intake and a higher mortality rate in growing rabbits. Under a high temperature conditions (summer), mannan-oligosaccharides seemed to be able to modify caecal fermentations of the growing rabbits, with an increase in total volatile fatty acids production and a potentially better synchronism in carbohydrate and protein fermentations.

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