EFFECT OF INULIN SUPPLEMENTATION ON CAECAL MICROFLORA AND FERMENTATION IN RABBITS

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ABSTRACT

Oligosaccharide-based prebiotics, like inulin, are intensively examined as alternatives for antibiotics in rabbits. The aim of the present experiment was to study the effect of dietary supplementation with inulin on the caecal microflora, fibrolytic activity, and production of volatile fatty acids. Pannon White rabbits (n=180) were divided into three groups at weaning (28th day of age) and fed *ad libitum.* Experimental diets were already fed one week before weaning. The control group (C) was fed a standard basal diet with no supplementation; group M received the same but medicated (1 mg/kg diclazuril, 500 mg/kg oxytetracycline and 50 mg/kg tiamulin) diet, while 4% inulin (Frutafit, HD, Brenntag, Budapest) at the expense of barley was added into the diet of the Inulin (I) group. The diets were similar in the level of crude protein, fat and fibre fractions, while diet I differed in starch and sugar content. At 28, 35 and 42 days of age, 6 healthy animals from each group were randomly selected and caecal microflora and fermentation pattern were examined. Body weight (BW) at the age of 28 and 35 days was not significantly different, but at 42 d of age a lower ($P<0.05$) LW was measured in group I. Considering the whole experimental period the weight gain (36±13 g/day) was the smallest and the feed conversion was the greatest (2.46±0.46 g/g) in group I. The incidence of diarrhoea was 1, 2 and 6 in groups C, M and I, respectively. Caecal pH was below 7.0 already at weaning and no significant difference between groups was detected. There was a temporary increase in the number of *E. coli* in each group at 35 days of age, but thereafter it decreased except in group I. The highest cellulase activity was found in the control group, while the lowest in the medicated rabbits. On the 35th d of age the greatest activity of xylanase and cellulase was detected in group M, while on day 42, the highest xylanase was found in the control group. Concentration of total VFA decreased in group C and M at 35 days of age, after that it increased except group I, where the decrease was observed at 42 days of age. The highest acetic acid and lowest butyric acid ratio (7%) were found in group I on the 42nd day. Based on our results, a dietary inclusion with inulin does not improve the zootechnical performances or enhance the caecal environment.

Key words: Rabbit, Caecum, Inulin, Caecal microflora, Fibrolytic activity, VFA production.

INTRODUCTION

Inulin-type fructans are present in significant amounts in various fruits and vegetables, and according to Roberfroid (1998), Roberfroid and Delzennem (2000) can be used as prebiotics. According to the definition of Gibson *et al*. (2004, 2005) a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host well-being and health. Because of the of β-2,1 configuration of the linkages in inulin, it resists digestion in the upper gastrointestinal tract, but is fermented in the hindgut. Adequate caecal fermentation (pH, volatile fatty acids and ammonia level) is very important to prevent digestive disorders in rabbits. One mode of action that has been ascribed to prebiotics is their positive effect on

the production of short-chain fatty acids. However, results in rabbits are controversial (Maertens *et al*., 2004).

The aim of the present experiment was to study the effect of dietary supplementation with inulin on the caecal microflora, fibrolytic activity, and VFA production in rabbits.

MATERIALS AND METHODS

Pannon White growing rabbits (n=180) were randomly allocated into three groups at 21 d of age. Three diets were formulated: the control group (C) was fed a basal diet with no supplementation; the feed of group M was medicated (1 mg/kg diclazuril, 500 mg/kg oxytetracycline and 50 mg/kg tiamulin), while 4% inulin (Frutafit, HD, Brenntag, Budapest) was added into the diet of group I (Table 1).

Table 1: Ingredient composition of experimental diets

Ingredients	Experimental diets		
	Control	Medicated	Inulin
Inulin	-	-	4.00
OTC 50%	-	0.10	-
Tiamutin 10%	-	0.05	-
Barley	12.00	12.00	8.00
Soya 46%	3.00	3.00	3.00
Extacted sunflower meal 37%	15.00	15.00	15.00
Alfalfa meal 19%	34.00	34.00	34.00
Sliced beet, dried	12.00	12.00	12.00
Feeding stuff sugar	1.00	1.00	1.00
Sunflower oil	1.00	1.00	1.00
Wheat bran	18.00	18.00	18.00
Feeding stuff lime	0.10	0.10	0.10
MCP	1.80	1.80	1.80
NaCl	0.40	0.40	0.40
Zeolite universal	1.20	1.05	1.20
Rabbit 0.5% Clinacox	0.50	0.50	0.50

The diets were similar in the level of crude protein (14.33-15.12%), fat (2.13-2.59%) and fibre fractions (NDF: 39.33-40.68%, ADF: 22.01-22.70%, ADL: 3.18-3.55%). Inulin was used to substitute a part of starch in the diet I. So the starch content of C and M diets was 14-16%, while 11% in the diet I. Sugar content of diets C and M was lower (7%) than that of diet I (10%). Experimental diets were given *ad libitum* to rabbits from the 21st day of age. Rabbits were weaned at d 28 of age.

Body weight and feed consumption were measured daily and weekly, respectively. Data of mortality and morbidity were registered daily during the whole experimental period. At 28, 35 and 42 days of age 6 healthy animals from each group were randomly selected and slaughtered. The digestive tract was removed immediately and the caecum was separated. The pH value of the fresh caecal content was determined by a manual automatic pH meter (OP-110, Radelkis, Hungary). According to the older literature (Gouet and Fonty, 1973) the caecal microflora in rabbits was found to consist of simple, non sporulated, strictly anaerobic gram-negative *Bacteroides*. For the microbiological examinations of these strictly anaerobic bacteria, they were cultured on Schaedler's agar, the selectivity of which was increased by the addition of esculin, neomycin and Fe-ammonium citrate. Subsequently the samples were incubated in anaerobic conditions at 37°C for 96 hours. *E coli* was cultured on a Chromocult differentiation medium, incubated for 24 hours at 37°C, under aerobic conditions,. The colony counts were expressed in $\log_{10}$ colony forming units (CFU) related to 1 g of a sample. The fibrolytic activity of the caecal bacteria was analysed by measuring the concentration of cellulase, xylanase and pectinase. The method described by Gidenne et al. (2002) was used with minor modifications. The reducing sugars were quantified spectrofotometrically at 540 nm using dinitrosalicylic acid instead of p-hydroxybenzoic acid hydrazide. The quantity of released sugars was expressed as micromoles (μmol) of reducing sugars per gram (g) of dry matter (DM) of caecal content and per hour (h). The

concentration of volatile fatty acids was measured by gas chromatography (Shimadzu GC 2010 instrument, Japan), using 2-ethyl-butyrate (FLUKA Chemie GmbH, Buchs, Switzerland) as the internal standard.

Data were analysed by using the GLM procedure of SPSS (10.0). The significance of differences between groups was tested by the LSD post hoc test.

RESULTS AND DISCUSSION

There was no significant difference between groups in body weight (BW) at the age of 28 and 35 days. At 42 d of age the smallest live weight was measured in group I (Table 2). Considering the whole experimental period the weight gain (36±13 g/day) was the smallest and the feed conversion was the worst (2.46±0.46 g/g) in group I, compared to the weight gain (46±16 g/day and 44±14 g/day) and feed conversion (2.12±0.33 g/g and 2.41±0.39 g/g) of rabbits C and M, respectively.

Table 2: Body weights (mean±S.D.)

Groups	Age (days)		
	28	35	42
n=60		Body weight (g)	
C	570±82.0[a]	953±134[b]	1275±178[a]
M	563±80.9[a]	946±119[b]	1250±180[ab]
I	566±68.9[a]	942±111[b]	1195±146[b]

Means with the same superscript in a column are not significant (P>0.05)

No significant differences among groups in morbidity were detected, though the incidence of diarrhoea was 1, 2 and 6 in groups C, M and I, respectively. All these cases were observed between 35 and 42 days of age. No animals died in groups C and M, while the mortality was 2 in group I.

The dry matter content of the caecal digesta was around 23, 20 and 18% at 28, 35 and 42 days of age, respectively. The pH value of the caecum was below 7.0 already at weaning (Table 3). No significant differences between groups in the weight, dry matter content and pH of the digesta were observed. By the 28th day, anaerobic bacteria were present in large quantities in the caecum. Their number slightly decreased in groups C and M by 42 d of age (Table 3). There was a temporary increase in the number of *E. coli* in every group at 35 days of age, but thereafter it decreased except in group I. The count of 10^5 per g in rabbits fed with inulin is considered to be of high risk from the animal's health point of view.

Table 3: The caecal pH value and the germ counts of anaerobes and *E. coli* expressed in $\log_{10}$CFU /g chymus (mean±S.D.)

Groups	Age (days)		
	28	35	42
		pH value	
C	6.1±0.3[a]	6.9±0.3[b]	6.5±0.5[ab]
M	6.2±0.5	6.5±0.4	6.5±0.7
I	6.4±0.3	6.7±0.3	6.6±0.3
		E. coli	
C	4.0±0.7[a]	5.6±1.5[b]	4.3±1.2[ab]
M	3.6±0.1[a]	4.8±1.3[b]	3.9±0.4[ab]
I	4.1±0.7	4.2±1.0	5.0±1.3
		anaerobic bacteria growing on the Schaedler agar	
C	9.3±0.8[ab]	9.8±0.5[b]	8.8±0.3[a]
M	10.0±0.3[b]	9.9±0.3[b]	8.8±0.3[a]
I	9.3±0.7[ab]	9.9±0.7[b]	9.1±0.7[a]

[a,b] Significant differences (P<0.05) between ages. Differences between groups were not significant

The highest cellulase activity was found in the control group, while the lowest in the medicated rabbits at 42 days of age (Table 4). The activity of xylanase enzyme increased except in the control group, where there was a temporary decrease at 35 d. On the 35th d of age the greatest activity of xylanase and cellulase was detected in group I, while on day 42, the highest xylanase was found in the control group. No significant differences between groups were detected except at the age of 35 d. The activity of pectinase was two times higher than the activity of cellulase (data not shown).

Table 4: Caecal microbial enzyme activity (means±S.D.)

Groups	Age (days)		
	28	35	42
		Cellulase (μmol/g DM/h)	
C	79.1±8.2^{ab}	66.7±2.7^{aAB}	88.8±10.1^{b}
M	65.9±7.0^{a}	72.5±7.3^{abB}	78.9±14.6^{b}
I	64.8±9.5^{a}	62.3±4.1^{aA}	83.5±10.2^{b}
		Xylanase (μmol/g DM/h)	
C	136±80.8	118±30.3^{A}	171±31.1
M	97.7±11.2^{a}	157±19.9^{bB}	162±16.0^{b}
I	87.8±23.7^{a}	102±17.8^{aA}	157±16.0^{b}

Means with different superscripts ^{a,b} or ^{A,B} were significantly different (P<0.05), for age and groups, respectively

In the present study, the concentration of total VFA decreased in group C and M at 35 days of age, after that it increased except in group I, where the decrease could be observed at 42 days of age (Table 5). There was an increase in the proportion of acetic acid and decrease in butyric acid in group I on the 42nd day. This could presumably be due to a relatively higher (18.0 and 18.5%) hemicellulose (NDF-ADF) and cellulose (ADF-ADL) content of the diet.

Table 5: Concentration of volatile fatty acids (means±S.D.)

Groups	Age (days)		
	28	28	28
		Total VFA (mmol/kg)	
C	48.1±8.5^{b}	48.1±8.5^{b}	48.1±8.5^{b}
M	54.9±3.3	54.9±3.3	54.9±3.3
I	49.9±7.2	49.9±7.2	49.9±7.2
		Acetic acid (mol.%)	
C	79.8±2.7^{B}	79.8±2.7^{B}	79.8±2.7^{B}
M	75.1±2.0^{A}	75.1±2.0^{A}	75.1±2.0^{A}
I	81.8±2.7^{B}	81.8±2.7^{B}	81.8±2.7^{B}
		Propionic acid (mol.%)	
C	8.7±2.5^{AB}	8.7±2.5^{AB}	8.7±2.5^{AB}
M	11.5±2.8^{bB}	11.5±2.8^{bB}	11.5±2.8^{bB}
I	7.2±2.4^{A}	7.2±2.4^{A}	7.2±2.4^{A}
		Butyric acid (mol.%)	
C	10.3±1.8	10.3±1.8	10.3±1.8
M	11.1±0.9	11.1±0.9	11.1±0.9
I	9.8±0.7^{b}	9.8±0.7^{b}	9.8±0.7^{b}

Means with different superscripts ^{a,b} or ^{A,B} were significantly different (P<0.05), for age and group, respectively

Having examined several scientific papers related to the effect of oligosaccharides in different species (incl. humans), results seem to be very much inconsistent (considering age, dose, composition of the diet, farm or experimental conditions etc.) (Falcao-e-Cunha *et al*., 2007). Information related to rabbits are limited, but also not unequivocal (Gidenne, 1995; Lebas, 1993; Luick *et al*., 1992; Maertens *et al*., 2004; Volek *et al*., 2007). Nevertheless, our results with inulin fed rabbits were unexpected and do not fit with the increased VFA production and lower pH found in other comparable works (Maertens *et al*., 2004; Volek *et al*., 2007).

CONCLUSIONS

The inclusion of 4% of inulin in the diet of weanling rabbits did not result in increased production parameters and also no positive effects on caecal microflora or fermentation pattern was observed. The medicated diet had also a very limited effect on the production parameters and fermentation pattern although a higher total caecal VFA concentration and xylanase activity was determined at 35 d of age.

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