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INFLUENCE OF GALACTO-OLIGOSACCHARIDES ON ZOO TECHNICAL PERFORMANCE, CECAL BIOCHEMISTRY AND EXPERIMENTAL COLIBACILLOSIS O103/8+ IN WEANLING RABBITS

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Introduction

In contrast with most other mammalian species, only low levels of *Escherichia coli* are present in the gut of weaned rabbits (Matthes, 1969, Gouet & Fonty, 1973). This is due to the inhibitory influence of the cecal volatile fatty acids (VFA) (Prohaszka, 1980). When cecal pH increases, higher levels of VFA are needed in order to maintain this inhibitory effect. As cecal pH and VFA levels are determined by the composition of the feed and the speed of the intestinal transit, it is quite clear that the food may influence the impact and outcome of colibacillosis. Also concurrent infections and mainly coccidiosis play an important role as they cause a temporary increase of cecal pH. Recently it has been shown that the supplementation of rabbit feeds with 0.2 % of fructo-oligosaccharides increases the cecal levels of volatile fatty acids (VFA) in weanling rabbits and decreases cecal ammonia (Morisse et al., 1990). These modifications might be useful in the prevention of colibacillosis. As galacto-oligosaccharides (GOS) are also being metabolized in the cecum only, they might show a similar action. So we decided to study the influence of GOS on cecal biochemistry before and after weaning (pH and VFA) and its influence on zootechnical performance during fattening. Also the possible preventive effect on experimental infection with an enteropathogenic strain of Escherichia *coli* (sero/biotype O103/8+) was studied.

Materials and methods

Experiment 1

Animals and husbandry. A total of 12 conventional rabbit does, descending from the dam line (Maertens, 1992) of the Institute for Small Stock Husbandry and their offspring were divided at random into 2 groups. They were fed a standard fattening diet supplemented or not with 0.1 % (w/w) galacto-oligosaccharides (GOS) *ad libitum* from 20 to 30 days of age. The pelleted diet contained 16.7 % of crude protein, 15.6 % of crude fibre and a calculated digestible energy content of 9.8 MJ/kg. All kits were weaned the same day at 30 days of age and transferred to the National Institute of Veterinary Research. They were housed individually in heat-sterilized, wire-floored metal cages at 22 °C ambient temperature.

Infective material. Enteropathogenic *E. coli*, recently isolated from experimentally infected rabbits and belonging to serogroup O103:K-:H2/biotype 8+ (strain V2700) were used (Peeters et al., 1988b). The strain was grown on blood agar plates overnight at 37 °C and was used for the experimental infection after 6 hours of incubation at 37 °C in penassay broth. Total number of *E. coli* present in the inoculum was 0.97 x 10^6/ml.

Experimental design. One rabbit of each litter, in total 12 rabbits, was sacrificed at weaning in order to determine cecal numbers of *E. coli*, cecal weight, pH and cecal VFA concentrations. Then the remaining rabbits of the unmedicated or medicated litters were divided into 4 experimental groups of 12 animals with a mean weight of 737 g (coefficient of variation = 13.3 %) respectively. The rabbits received drinking water and the pelleted ration *ad libitum*. The two groups issued from non medicated litters were not medicated, whereas the two other groups issued from medicated litters remained on the supplemented diet. At weaning one unmedicated group (UMI) and one medicated group (MI) was inoculated orally with 0.5 ml of a culture of enteropathogenic *E. coli* O103/8+. The two other groups served as unmedicated uninoculated (UMUI) and as medicated uninoculated (MUI) controls. The four groups were housed in two identical rooms. The individual weight gain and food consumption were recorded at weaning and 7 and 10 days later. Animals were observed daily. Diarrhea was assessed as follows : 0 = no diarrhea; 1 = increased water content of fecal pellets; 2 = pulpy diarrhea; 3 = liquid stools. *E. coli* output was evaluated semi-quantitatively after streaking rectal swabs on G2S, a selective medium for *enterobacteriaceae* (Pohl & Thomas, 1966) : 0 = no colonies; 1 = isolated widely-spaced colonies; 2 = isolated closely-spaced colonies; 3 = confluent growth. Fecal output of *E. coli* belonging to serotype O103/biotype 8+ was checked with specific antisera. Rabbits were also checked on the presence of rotaviruses, *Eimeria spp.* and *Clostridium spiroforme*. Seven and ten days after the experimental infection 6 rabbits of each group were sacrificed in order to determine cecal parameters as outlined above. Moreover, portions of duodenum, jejunum, ileum and cecum were processed for histology.

Table 1. Ingredients and chemical analysis of the pelleted diet (experiment 2)

Ingredients	%	Chemical composition	% / kg
Alfalfa meal 18	35.0	Dry matter	90.2
Wheat shorts	25.0	Crude protein	18.1
Wheat	16.4	Crude fat	4.1
Full-fat soybeans	8.4	Crude fibre	13.1
Flax chaff	5.0	ADE (MJ/kg)	10.6
Molasses	4.0		
Sunflower meal	2.3		
Soybean meal	1.3		
Vit.- Min. mix	2.5		
DL-methionine	0.06		

Bacteriology. Cecal samples were diluted 1 to 10 in cold PBS-pH 7.2, shaken for 1 minute with a Heidolph shaking device and stored within 10 minutes after sacrificing the animals at 4 °C until tested 2 hours later. Further decimal dilutions were made in cold sterile PBS and 0.1 ml aliquots were streaked on G2S. After 18 hours of aerobic incubation at 37 °C, the number of lactose positive colony forming units was evaluated.

Biochemistry. Cecal VFA concentrations were determined after diluting cecal samples 1:1 in distilled water. Three drops of toluene were added as protection against freezing before storing at - 20 °C. After thawing, aliquots of 5 ml were acidified with one ml of a mixture of 75 ml meta-phosphoric acid (25 %) and 25 ml of formic acid and centrifuged twice at 12,000 r.p.m. for 15 minutes. The concentration of VFA in the resulting supernatant was determined by gas chromatography (Perkin Elmes type 8500 gas chromatograph) using a chrompack WCOT fused silica column type (25 m x 0.22 mm) with a FFAP liquid phase. Statistical analysis of VFA concentrations was done by 2 x 2 x 2 factorial analysis of variance (GOS, challenge, age).

Table 2 : influence of 0.1 % GOS on zootechnical performance, microbiology and cecal parameters after infection with 10^6 *E. coli* O103 (experiment 1)

GROUP	UMUI	MUI	UMI	MI
Zootechnical parameters (mean ± SD)				
- Live weight in g at day 0	723 ± 97a°	762 ± 113a	705 ± 82a	756 ± 100a
- Weight gain in g between d 0-10*	391 ± 18	374 ± 36	375 ± 28	49 ± 278
- Feed conversion ratio**	2.05	2.27	2.35	2.44
Clinical parameters				
- Mortality	0/12	0/12	.4/12	0/12
- Mean diarrhoea-score x 10 (d 5-10 p.i.)	0.08	0.00	7.10	5.71
Microbiological parameters				
- Mean score of C. spiroforme				
- day 0	0.2	0.2	-	-
- day 7	0.2	0.2	0.1	0.1
- day 10	0.0	0.0	0.4	0.3
- Mean score of E. coli				
- day 0	1.0	1.5	0.8	1.3
- day 7	0.9	0.8	2.0	2.4
- day 10	1.3	0.9	2.5	2.4
- Mean score of rotaviruses (d0, 7 and 10)	0	0	0	0
- Mean score of Eimeria spp. (d0, 7 and 10)	0	0	0	0
Caecal parameters (mean ± SD)				
- Mean caecal weight in g				
- day 0	41.3 ± 4.5a	49.7 ± 9.3a	-	-
- day 7	69.8 ± 12.7a	69.4 ± 13.7a	63.7 ± 10.6a	69.9 ± 6.5a
- day 10	82.5 ± 13.9a	77.8 ± 14.9a	76.4 ± 28.9a	72.9 ± 18.8a
- Mean pH				
- day 0	5.80 ± 0.20a	5.78 ± 0.28a	-	-
- day 7	5.97 ± 0.25a	5.96 ± 0.21a	6.25 ± 0.48ac	6.44 ± 0.39bc
- day 10	5.94 ± 0.18a	5.96 ± 0.25a	6.62 ± 0.41b	6.49 ± 0.43b
- Mean E. coli number in log10				
- day 0	2.69 ± 1.49a	3.09 ± 2.52a	-	-
- day 7	1.23 ± 1.92a	2.96 ± 3.25a	7.36 ± 4.30b	8.26 ± 3.00b
- day 10	4.32 ± 2.31a	3.00 ± 0.65a	9.12 ± 1.95b	9.06 ± 2.88b

° means with different superscripts are significantly different ($p < 0.05$)

* surviving animals, insufficient data for statistical analysis

**only FCR of surviving animals or of animals with positive FCR was taken into account

Experiment 2

At the same time an experiment was designed at the Institute for Small Stock Husbandry in order to test the effects of increasing dietary levels of GOS on zootechnical performance. GOS were added at 0, 0.1, 0.2, 0.3, 0.4 and 0.5 % concentrations (w/w) to a pelleted diet from weaning till slaughtering age. The composition of the diet is outlined in Table 1. To avoid non-homogeneity of the raw materials used, one batch of the total feed quantity was prepared first and then divided into 6 parts and supplemented with the different drug concentrations. With each of the 6 experimental feeds 15 replicates of 3 animals were tested, this means a total of 270 four-week-old end products

of the selection stock of the Institute for Small Stock Husbandry (Maertens, 1992). They were all weaned on the same day at 27-29 days of age and were fed the experimental diets *ad libitum*. To exclude litter effects, only 6 kits per litter were used and distributed ad random over the 6 experimental groups. Rabbits were ear tagged and kept three by three in flat-deck wire cages at 15-20 °C ambient temperature. The individual weight gain and food consumption were recorded between weaning at 4 weeks and slaughtering at 11 weeks. Recordings, calculations and corrections for mortality were done as reported elsewhere (Maertens & De Groote, 1992). The dose response effects were tested by linear regression analysis using a Statgraphics package.

Experiment 3

This dose response experiment was designed to evaluate the effect of increasing dietary levels of GOS on cecal biochemistry. The experiment was performed simultaneously with experiment 2, rabbits and housing conditions being the same. Six kits were sacrificed at weaning while 18 kits, 6 per treatment, were fed diets containing 0, 0.2 and 0.5 % of GOS. They were sacrificed ten days after weaning in order to determine cecal parameters as outlined above. Dose effects were tested by regression analysis and joined results were subjected to analysis of variance.

Table 3 : influence of 0.1 % GOS on caecal volatile fatty acids (mmol/l) 7 to 10 days after infection with 10^6 *E. coli* O103 (pooled data of experiment 1)

GROUP	UMUI	MUI	UMI	MI
N	12	12	12	12
Acetic acid	57.2 ± 12.7a°	64.6 ± 12.9a	56.8 ± 17.7a	43.9 ± 14.3b
Propionic acid	4.3 ± 0.5a	5.4 ± 1.8a	5.5 ± 3.0a	6.0 ± 2.0a
Butyric acid	9.1 ± 1.5ac	12.0 ± 3.3b	9.8 ± 5.3bc	6.8 ± 5.0a
Isobutyric acid*	0	0	1.5 ± 1.4	0.7 ± 0.2
Valerianic acid*	0	0	3.3 ± 1.1	1.0 ± 0.8
Isovalerianic acid*	0	0	1.4 ± 1.4	0.5 ± 0.4

° means with different superscripts are significantly different ($p < 0.05$)
* insufficient data for statistical analysis

Results and discussion

Influence of 0.1 % of GOS on zootechnical performance and cecal parameters after experimental infection with enteropathogenic E. coli O103/8+ (experiment 1)

Before weaning, all medicated does and sucklings took the GOS-supplemented pellets without problems. No depression nor diarrhea was observed. At weaning no significant differences were observed between cecal weight, pH nor numbers of *E. coli* present (Table 2). No *Eimeria spp.* nor rotaviruses were detected. Three out of 12 animals showed light *Clostridium spiroforme* infection. After weaning, there was no significant influence of 0.1 % GOS on weight gain nor on feed intake. At the level of the cecum no significant differences were observed between cecal weight, pH nor numbers of *E. coli* present 7 and 10 days after weaning (Table 2). No infection by *Eimeria spp.* nor rotaviruses were detected either. Five out of 24 animals showed light *Clostridium spiroforme* infection. Two of them showed discrete signs of diarrhea. Histology showed no morphological changes.

Experimental infection of unmedicated rabbits with the O103 *E. coli*-strain was followed by severe clinical symptoms : most animals showed liquid diarrhea (Fig. 1) and 4 out of 12 animals died. At necropsy liquid foul smelling cecal content was found and histology established the presence of

attaching effacing *E. coli* and severe villous atrophy from the duodenum to the ileum. Numerous foci of attached *E. coli* were also established in the cecum. This was associated with a steep increase of *E. coli*-numbers in the cecum ($p < 0.01$) and a significant rise of cecal pH ($p < 0.05$) (Table 2). There was also a light increase of cecal *C. spiroforme* 10 days p.i. Supplementation of the feed with 0.1 % GOS exerted only little effect. Although no mortality was noted, medicated animals showed severe anorexy and growth depression (Table 2). Semi-quantitative evaluation of fecal *E. coli*-numbers showed no significant influence of 0.1 % GOS (Fig. 2). Perhaps, enumeration of fecal *E. coli*-numbers should be more appropriate in order to establish differences more clearly. Yet, supplementation with 0.1 % GOS was associated with some improvement of the diarrhea-score 7 to 10 days post-infection (Fig. 1).

Fig 1 : influence of 0.1 % GOS on diarrea score after infection with 10^6 *E. coli* O103 (experiment 1)

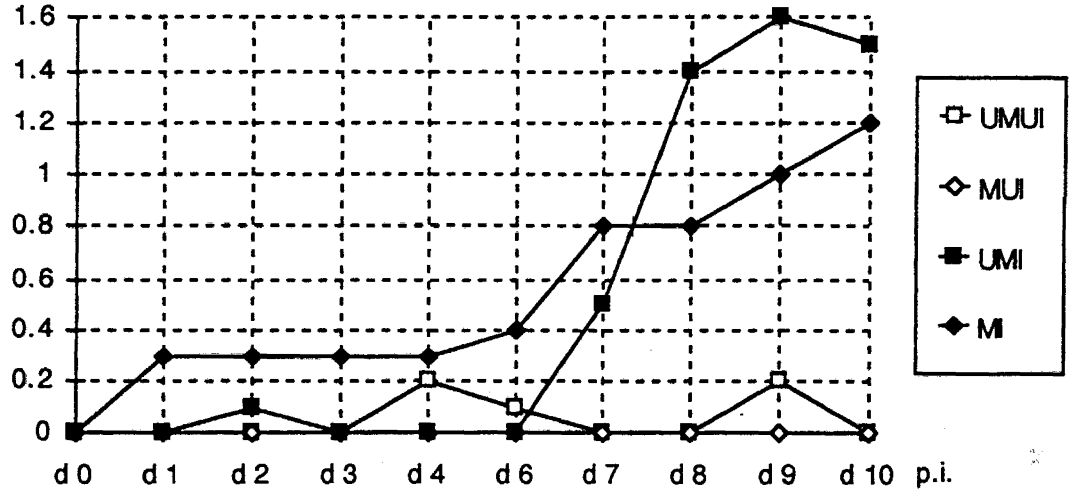
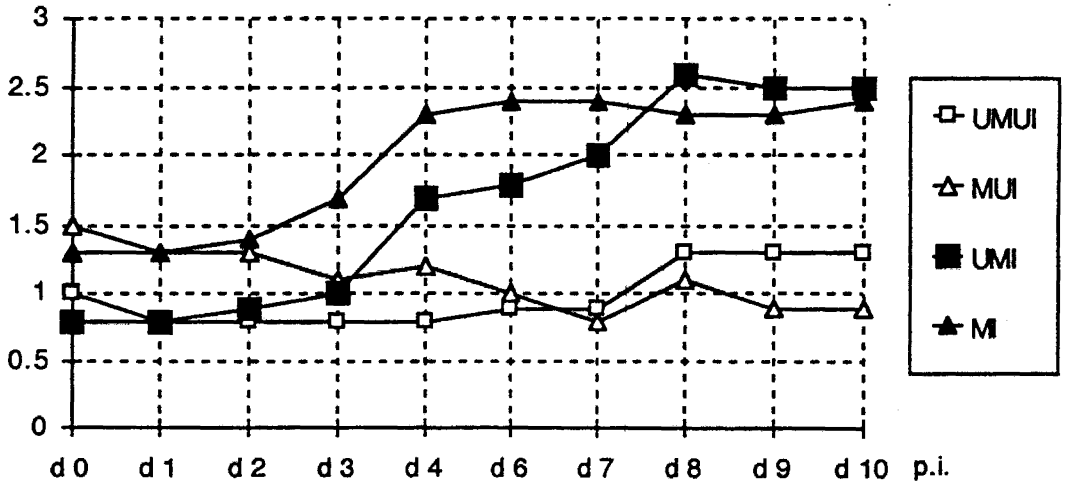


Fig 2 : influence of 0.1 % GOS on fecal *E. coli* output after infection with 10^6 *E. coli* O103 (experiment 1)



Medication of the rabbits with 0.1 % GOS was followed by a rise of VFA : in unmedicated uninfected rabbits the VFA concentrations at weaning and 7 to 10 days later were similar to those reported earlier (Prohaszka, 1980, Peeters et al., 1988a, Morisse et al., 1990). Supplementation of

the feed with 0.1 % of GOS from 10 days before weaning resulted in a 9 % rise of acetic acid concentrations at weaning, whereas the other VFA remained unchanged. Seven to 10 days after weaning, acetic acid levels rose by 9-17 %, propionic acid by 14-39 % and butyric acid by 28-36 % in the GOS medicated rabbits. Multifactorial analysis did not reveal any significant effect due to the age of the rabbits; therefore data were pooled. GOS supplementation was associated with a significant increase of the butyric acid level ($p < 0.05$), whereas a similar tendency ($p < 0.10$) was observed for acetic acid. Other VFA were hardly detected (only one animal showed presence of isovalerianic acid) (Table 3). Experimental infection with *E. coli* O103 resulted in a significant decrease of acetic and butyric acid levels in GOS-medicated animals ($p < 0.05$), whereas the level of propionic acid remained unchanged. This may be ascribed to depressed feed intake following infection, as reported before (Peeters et al., 1988a). Moreover, in most unmedicated infected animals there was a rise of isobutyric acid, valerianic acid and isovalerianic acid. This indicates a change of the normal cecal fermentation pattern, probably induced by the experimental *E. coli* O103/8+ infection. In the infected GOS medicated group only one rabbit showed an increase of isobutyric acid, valerianic acid and isovalerianic acid p.i., suggesting that GOS gave some protection against the altered cecal fermentation pattern by enteropathogenic *E. coli.* Variability of VFA concentrations was much more pronounced in infected rabbits than in uninfected controls.

Influence of 0.1 to 0.5 % of GOS on zootechnical performance during fattening (experiment 2)

No significant influence of any of the supplementation levels of GOS on weight gain, feed intake or feed conversion ratio has been established (Table 4). Animals were in excellent shape; zootechnical performance was high and overall mortality was limited to 4 %. Mortality was mainly due to repiratory problems. Regression analysis did not reveal any dose response effect on zootechnical performance.

Table 4 : influence of GOS supplemented diets on zootechnical performance (mean ± SEM) (exp. 2)

% GOS	0.0	0.1	0.2	0.3	0.4	0.5
N	45	45	45	45	45	45
Initial weight (g)	638 ± 10	637 ± 9.6	645 ± 10	639 ± 7.5	638 ± 4.7	643 ± 4.7
Finishing weight (g)	2416 ± 22	2469 ± 25	2455 ± 28	2424 ± 22	2436 ± 21	2394 ± 25
Daily weight gain (g)	42.3 ± 0.4	43.5 ± 0.6	43.0 ± 0.7	42.5 ± 0.5	42.9 ± 0.5	41.7 ± 0.6
Daily feed intake (g)	125 ± 1.9	126.5 ± 2.3	125.7 ± 2.2	126.0 ± 2.1	124.7 ± 1.3	122.0 ± 2.2
Feed conversion	2.95 ± 0.02	2.91 ± 0.04	2.92 ± 0.02	2.96 ± 0.03	2.91 ± 0.02	2.93 ± 0.02
Mortality (N)	1	3	1	2	1	3

Influence of 0.2 and 0.5 % of GOS on cecal biochemistry (experiment 3)

The data of both treatment groups (0.2 and 0.5 % of GOS) were pooled, as no dose effects were established by statistical analysis. The data confirmed the results of experiment 1 : supplementation of rabbit feed with GOS induced a significant ($p < 0.05$) rise of cecal concentrations of acetic and butyric acid (Table 5). This rise was more pronounced than the one observed during experiment 1 with 0.1 % of GOS. Cecal weights of GOS supplemented rabbits tended to decrease ($p < 0.10$) suggesting reduced cecal retention time of digesta (Carabaño *et al.*, 1988). In contrast with the observations of experiment 1, GOS supplementation was also associated with a drop of cecal pH with 0.09-0.26 pH units ($p < 0.05$), although pH was already low in control rabbits in comparison with other experiments (Morisse *et al.*, 1990). The results did not indicate any enhanced effect on cecal biochemistry at a 0.5 % inclusion level.

Table 5 : influence of GOS supplemented diets on cecal parameters (mean ± SD) (experiment 3)

% GOS	At weaning	10 days post weaning			
	0.0	0.0	0.2	0.5	0.2 + 0.5 pooled data
Cecal weight (g)	37.8 ± 6.7	79.2 ± 9.2a°	70.0 ± 10.8	70.8 ± 11.4	70.4 ± 11.6a
pH	5.96 ± 0.10	5.97 ± 0.16a	5.71 ± 0.13	5.88 ± 0.18	5.79 ± 0.18b
Acetic acid (mmol/l)	59.8 ± 11.7	57.4 ± 12.4a	71.5 ± 19.3	70.0 ± 14.0	70.7 ± 17.6b
Propionic acid (mmol/l)	5.2 ± 1.3	4.1 ± 0.9a	4.7 ± 0.8	5.4 ± 1.1	5.0 ± 1.1a
Butyric acid (mmol/l)	8.8 ± 2.6	9.7 ± 3.4a	13.9 ± 2.7	11.4 ± 1.8	12.7 ± 2.7b

° means with different superscripts are significantly different ($p < 0.05$)

As in vitro and in vivo experiments showed that high cecal VFA and low cecal pH have a protective effect against cecal *E. coli* proliferation (Prohaszka, 1980, Peeters et al., 1988a), it is quite possible that a dietary level of 0.2 % of GOS will show a stronger inhibitory effect on *E. coli* proliferation than did the 0.1 % level of experiment 1. This should be confirmed by further experiments.

Conclusions

Both experiments 1 and 3 show clearly that incorporation of galacto-oligosaccharides in the feed may increase cecal VFA levels, which confirms the observations of Morisse *et al.* (1990) with fructo-oligosaccharides. GOS also decrease cecal pH. According to evidence from the literature these levels may inhibit cecal *E. coli* proliferation and may prevent colibacillosis in the field. Yet, a dietary level of 0.1 % showed insufficient action against experimental colibacillosis, although mortality was reduced and although some preventive effect on diarrea-score and some inhibitory effect on production of isobutyric and (iso)valerianic acid became evident. Further experiments should confirm if higher levels show a more distinct preventive effect on colibacillosis. As 0.5 % did not show any enhanced activity on cecal VFA and pH in comparison with 0.2 % GOS, the latter concentration should be assayed. Coccidiosis induced *E. coli* proliferation could be used as experimental model to mimic polyfactorial induced colibacillosis.

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Summary

The influence of feed supplementation with 0 to 0.5 % of galacto-oligosaccharides (GOS) on zootechnical performance and cecal parameters was studied in weanling rabbits during three experiments. When 0.1 % GOS was administered from 10 days before weaning, only a 9 % increase of cecal acetic acid was established at weaning age. Seven to 10 days after weaning an increase of the cecal levels of acetic acid (+ 9-25 %) and of butyric acid (+ 28-43 %) was noted when GOS was administered from weaning age at dietary levels of 0.1 and 0.2 %. This rise was more pronounced at a dietary level of 0.2 % of GOS than at a level of 0.1 %. Butyric acid showed a significant rise at both 0.1 and 0.2 % concentrations ($p < 0.05$), whereas a significant rise of acetic acid became evident from 0.2 % onwards ($p < 0.05$). Higher dietary levels did not further increase the level of cecal volatile fatty acids. GOS-supplementation was also associated with a significant drop of cecal pH (- 0.18), while cecal weight tended to decrease (- 12 %) from an incorporation level of 0.2 % onwards. When 0.1 to 0.5 % of GOS was administered during the whole fattening period no significant changes of weight gain, feed intake or feed conversion were noted in healthy rabbits. At a dietary level of 0.1 %, GOS reduced the clinical effects only partially after experimental infection with enteropathogenic *E. coli* O103/8+. The possible preventive effect of higher dietary levels on experimental colibacillosis will be further investigated.