EFFECT OF *BACILLUS CEREUS* VAR. *TOYOI* ON CAECAL MICROFLORA AND FERMENTATION IN RABBITS

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ABSTRACT

In commercial rabbit meat production, a major part of the mortality results from diseases of the digestive system that are related mainly (about 25%) around weaning (from days 18 to 50). Besides economic losses, this disease has a negative influence on animal welfare as well. Antibiotics are still widely used to reduce mortality of the growing rabbit, although there is an increasing human health and food safety concern over drug residues in meat products. As an alternative to in-feed antibiotics, the effect of a probiotic, i.e. *Bacillus cereus* var. *toyoi*, was examined in rabbits. One group of Pannon White does (Group T) was fed a diet containing 200 ppm Toyocerin ($2x10^5$ *B. cereus* spores/g feed), while the other group (Group K) received an antibiotic free diet with the same chemical composition. Kits consumed the same diet as their mothers (K and T) till weaning. After weaning at 28 days of age, all litters were divided into two groups, one feeding the same diet as before (KK, n=37 and TT, n=46), while the diet of the other two groups changed (KT, n=38 and TK, n=44). Supplementation of the does diet significantly improved the kit weight gain. The weight of K rabbits (389±8 g) was significantly ($P<0.05$) lower at 3 weeks age compared to treated (T) animals (462±12 g). This could be presumably due to the better milk production of the does. The difference in body weight between the two groups was still marked on week 4. During the whole experimental period (4 to 6 weeks of age) body weight gain (54 g/day) and feed conversion (2.0) proved to be the best in group KT. Morbidity and mortality were lower in animals fed supplemented diet after weaning. The most impressive change was observed in the number of coliform bacteria in the GI tract. Caecal chyme of K rabbits contained significantly higher coliform germ count on day 21 (5.9 CFU $\log_{10}$/g chyme). On week 5 adding Toyocerin into the diet significantly decreased the bacterial count in groups KT and TT (2.0 CFU $\log_{10}$/g chyme). For coliforms the count of 10^3-10^4 bacteria/g chyme in group TK could be considered as physiological, but the count of 10^5 in KK rabbits is considered to be of high risk from the animal health point of view. Total volatile fatty acid concentration was around 66-80 mmol/kg after weaning in all groups, though in the TK and TT groups it was temporarily raised above 100 mmol/kg during the 4th week.

Key words: *Bacillus cereus* var. *toyoi*, Caecal microflora, Volatile fatty acid content, Rabbit.

INTRODUCTION

In commercial rabbit meat production, a major part of the mortality results from diseases of the digestive system that are related mainly (about 25%) around weaning (between days 18 to 50). Beside economic losses, this disease has a negative influence on animal welfare as well. Antibiotics are still widely used to reduce mortality of the growing rabbit, although there is an increasing human health and food safety concern over drug residues in meat products.

Probiotics are possible alternative feed additives for stabilizing the composition of the intestinal microflora and preventing enteral diseases. Results of experiments in which *Bacillus sp*. were tested are quite contradictory (De Blas *et al*., 1991; Zoccarato *et al*., 1995; Bonanno *et al*., 1996). However, according to Hattori *et al*. (1984), Nicodemus *et al*. (2004) and Trocino *et al*. (2005) *Bacillus cereus* var. *toyoi* has positive effect on production of rabbits.

The aim of the study was to examine the effect of *Bacillus cereus* var. *toyoi* on the caecal microflora and fermentation in rabbits, when supplementation was added already to the does after parturition, i.e. during nursing, or only to the growing rabbits.

MATERIALS AND METHODS

One group of Pannon White does (Group T) was fed a diet containing 200 ppm Toyocerin ($2x10^5$ *Bacillus cereus* var. *toyoi* spores/g feed), while the other group (Group K) received an antibiotic-free diet with the same chemical composition. The dose of supplementation was selected considering the results of Trocino *et al*. (2005), who found that the dose of $2x10^5$ spores *B. cereus*/g diet slightly decreased the digestive problems, while no significant effect was observed with a higher inclusion rate.

Kits consumed the same diet as their mothers (K and T) till weaning. After weaning at age 28 days, all litters were divided into two groups, one feeding with the same diet as before (KK, n = 37 and TT, n = 46), while the diet of the other two groups changed (KT, n = 38 and TK, n = 44) as shown in Table 1.

Table 1: Experimental groups

Groups	Diet of the doe and the kits		Diet of the growing rabbits	
	Basic diet	Toyocerin	Basic diet	Toyocerin
KK	+	-	+	-
KT	+	-	+	200 ppm
TK	+	200 ppm	+	-
TT	+	200 ppm	+	200 ppm

Data of morbidity and mortality were registered and "health risk index" (HRI) was calculated as the sum of morbidity and mortality (Bennegadiet *et al.*, 2000). Feed consumption (g/litter/week) and body weight (g/rabbit) were measured from the 3rd week of age.

At the age of 3, 4 and 5 weeks six healthy animals from each group were euthanised by CO_2 gas at 14:00 h. Body weight was measured after exsanguination. 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 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 the caecal contents, strictly anaerobic bacteria were cultured on Schaedler's agar (Sharlan Chemie, Barcelona, Spain), the selectivity of which was increased by the addition of esculin (Merck, Darmstadt, Germany), neomycin (Merck, Darmstadt, Germany) and Fe-ammonium citrate (Sharlan Chemie, Barcelona, Spain). Subsequently the samples were incubated in anaerobic conditions at 37°C for 96 hours. Coliforms were cultured on a Chromocult differentiation medium (Merck, Darmstadt, Germany). The samples were incubated at 37°C, under aerobic conditions, for 24 hours. Total aerobe germ count was determined on blood agar after incubation at 37°C, under aerobic conditions, for 48 hours. After the incubation time had elapsed, the colonies were counted with a Titriplaque colony counter (LMIM, Esztergom, Hungary). The colony counts were expressed in $\log_{10}$ colony forming units (CFU) related to 1 g of sample.

About 3 g of caecal chyme were homogenized with 4.5 ml metaphosphoric acid (4.16%), then centrifuged at 10,000 x g for 10 min and filtered. The concentration of volatile fatty acids was determined by gas chromatography (Shimadzu GC 2010, Japan). 2-ethyl-butyrate (FLUKA Chemie GmbH, Buchs, Switzerland) was used as the internal standard. Parameters: Nukol 30 m x 0.25 mm x 0.25 µm capillar column (Supelco, Bellefonte, PA, USA), FID detector, 1:50 Split ratio, 1 µl injected volume, helium 0.84 ml/min. Parameters of the detector: air 400 ml/min, hydrogen 47 ml/min, temperature: injector 250°C, detector 250°C, column 150°C.

Statistical analysis of the data obtained was carried out by the SPSS statistical software package using the version 10.0. Effect of treatment, age and their interaction was analyzed by the analysis of variance. The significance of differences between groups was tested by the Tukey post-hoc test.

RESULTS AND DISCUSSION

When investigating the results it has to be taken into consideration that till the 4th week (till weaning) we had only two groups (K and T), so parameters of the kits reflect the supplementation of their mothers diet. Results of the 4th week (1 day after weaning) were influenced by the fact that the kits consumed an increasing amount of their mothers diet from the age of about 3 weeks.

Supplementation of the does diet increased significantly the growth of the kits. On the 3rd week BW of the T rabbits was significantly (P<0.05) higher (462±12 g) than BW of the K rabbits (389±8 g). It was presumably due to the higher milk production of the doe and ,consequently, better nutrient supply of the kit. Examining the whole experimental period, KT rabbits reached the highest BW (Table 2) and their feed conversion was the best as well (2.0 g/g compared to 2.1, 2.3 and 2.5 g/g, respectively).

Table 2: Body weight of the groups (mean± S.D.)

	Age (weeks)	Group			
		KK	KT	TK	TT
Body weight (g)	4	610 ± 53[ab]	547 ± 73[a]	717 ± 42[b]	653 ± 62[ab]
	5	993 ± 48	1077 ±104	935 ± 68	1020 ± 86
	6	1269 ± 85	1301 ± 98	1250 ± 92	1239 ± 103

[a,b]significant difference between groups (P<0.05)

Rabbits consuming supplemented diet after weaning had significantly better health condition (Table 3). Health problems were mainly due to diarrhoea. In the caecal content of dead rabbits high numbers (10^6-10^7 CFU/g) of *E. coli* were detected. The high HRI of the group KK was similar to that experienced under farm conditions (Trocino *et al*., 2005).

Table 3: The Health Risk Index (HRI)

	Period examined	Group			
		KK	KT	TK	TT
HRI (%)	Between weeks 4-5	2.7	0	6.8	0
	Between weeks 5-6	35.5	6.2	8.4	2.9

The pH value of the caecum is usually about 7.0 around weaning, then decreases to around 6.0 depending on microbial activity and feeding pattern (Cheeke, 1987; Fekete, 1990.). Before weaning T rabbits had higher pH, while at the end of the experiment TT rabbits had the lowest values (Table 4).

Table 4: The pH value and the number of the coliform bacteria in the caecal chyme (mean± S.D.)

Groups	Age (weeks)		
	3.	4.	5.
		pH	
KK (n=6)	6.7 ± 0.2[a]	6.1 ± 0.3	6.3 ± 0.2[ab]
KT (n=6)		6.3 ± 0.3	6.3 ± 0.2[ab]
TK (n=6)		6.4 ± 0.4	6.6 ± 0.4[a]
TT (n=6)	7.2 ± 0.2[b]	6.3 ± 0.2	6.0 ± 0.1[b]
		Coliforms (CFU log10/g chyme)	
KK (n=6)	5.9 ± 1.1[a]	3.0 ± 0.1	5.0 ± 0.0[a]
KT (n=6)		3.0 ± 0.3	2.0 ± 0.1[b]
TK (n=6)		3.0 ± 0.2	3.3 ± 0.3[c]
TT (n=6)	4.3 ± 1.5[b]	3.0 ± 0.1	2.0 ± 0.0[b]

[a,b,c]significant difference between groups (P<0.05)

The germ counts of anaerobic bacteria growing on the Schaedler agar in the caecal content (data not shown) were similar to those experienced in previous experiments (Kovács *et al*., 2002, 2003;

Zomborszky-Kovács, 2002). Significant difference was found in the number of coliforms, being significantly higher in K rabbits (Table 4). For coliforms the count of 10^3-10^4 bacteria/g chyme in group TK could be considered as physiological, but the count of 10^5 in KK rabbits is considered to be of high risk from the animal health point of view.

There was no significant difference between groups in the total volatile fatty acid (tVFA) content or the individual volatile fatty acid contents (acetic acid, propionic acid and butyric acid) except at 4 weeks of age, when tVFA concentration temporary increased in KK and KT groups (Table 5).

Table 5: Total volatile fatty acid production (mmol/kg) (mean± S.D.)

Groups	Age (weeks)		
	3.	4.	5.
KK (n=6)	74.0 ± 11.8	107.8 ± 2.3[b]	73.2 ± 8.1
KT (n=6)		103.6 ± 4.2[b]	80.7 ± 3.3
TK (n=6)		80.5 ± 8.2[a]	66.4 ± 4.4
TT (n=6)	65.6 ± 4.5	79.9 ± 2.7[a]	74.3 ± 9.3

[a,b]significant difference between groups (P<0.05)

With correspondence to the literature (Gidenne, 1996; Kovács *et al*., 2002) the ratio of the acetic acid (within the tVFA content) was between 75-75%. The ratio of the propionic acid was above 10% before weaning, but thereafter it decreased to 5.5-7.0%. In contrast, the percentage of butyric acid increased from 5.5-6.5% to 14.0-17.0%, so the ratio of the two fatty acids (C3/C4) decreased from 2.0 to 0.4-0.5. No differences between groups were detected.

CONCLUSIONS

The supplementation of the diet with *Bacillus cereus* var. *toyoi* had a positive effect on production before weaning. The amount of anaerobic bacteria did not change but the coliform count significantly decreased. This was presumably the main reason of the better production and health status. More research is needed to underline the exact mode of action.

ACKNOWLEDGEMENTS

The research was funded by the OTKA foundation (project No.: T046999).

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