THE EFFECT OF LACTATING AND SOME GASTROINTENSTINAL SYMBIOTIC BACTERIA ON THE GROWTH OF YOUNG RABBITS IN PREPUBERTAL PERIOD

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Abstract. The mechanisms of the influence of mothers on the growth and development of young rabbits in the prepubertal period were investigated. It was shown that symbiotic bacteria from the mother's caecotrophes transfer in the gastrointestinal tract of young rabbits by means of contact and stimulate their growth and development in the period from the first to the third months of life. From the caecotrophes of lactating female rabbits, it was identified the symbiotic bacteria strain *Enterococcus faecalis* RB. The dynamics of live weight of young rabbits being kept on the diet with *E. faecalis* RB. or the association of *E. faecalis* RB. and the probiotic strain of bacteria *Bacillus subtilis* B-8130 and the diet without the addition of bacterial preparations was investigated. It was demonstrated that the mother's soft faeces contain microorganisms that have probiotic effect on young rabbits. When the maternal *E. faecalis* RB. was added to the diet, the growth of young rabbits was accelerated.

Keywords: caecotrophes, micro-symbionts, probiotics, breeding animals, prepubertal period.

Rezumat. Efectul alăptării şi a unor bacterii simbiotice gastrointestinale asupra creşterii iepurilor tineri în perioada de prepubertate. Au fost investigate mecanisme de influenţă a mamelor asupra creşterii şi dezvoltării iepurilor tineri în perioada de prepubertate. S-a arătat că bacteriile simbiotice din cecotrofele mamei sunt transferate în tractul gastro-intestinal al iepurilor tineri, prin contact şi acestea stimulează creşterea şi dezvoltarea lor în perioada de la prima până la a treia lună de viaţă. Din cecotrofele femelelor de iepure care alăptează, au fost identificate bacteriile simbiotice din tulpina *Enterococcus faecalis* RB. A fost investigată dinamica de greutate în viu a iepurilor tineri care au urmată o dietă cu *E. faecalis* RB. sau cu asociaţia de *E. faecalis* RB. şi tulpina probiotica de bacterii *Bacillus subtilis* B-8130, precum şi dieta fără adaos de preparate bacteriene. S-a demonstrat că materiile fecale moi ale mamei conţin microorganisme probiotice, care au efect asupra iepurilor tineri. Când *E. faecalis* RB. matern a fost adaugat în dietă, a fost accelerată creşterea iepurilor tineri.

Cuvinte cheie: cecotrofe, microorganisme simbiotice, probiotice, creşterea animalelor, perioada de prepubertate.

INTRODUCTION

Pups of all mammals interact efficiently with the mother during early stages of ontogenesis. Later, depending on species-specific characters, the young also need to progressively reach autonomy from the mother, in both social and alimentary terms. According to Mihailov's method of accelerated upbringing of meat rabbit races offspring should be kept with their mothers up to 3 months. Live weight of these offspring at the age of 3 months was significantly higher than that of offspring which were weaned at the age of 1 month (MIKHAILOV, 1991). According to our previous data this accelerating mother's effect is also true for dwarf rabbits (FEDOSOV & SOKTIN, 2006). However, the mechanisms of the mother's influence on the offspring growth remain unclear.

Rabbits conduct coprophagy, production, and reingestion of soft faeces. During the lactation period in rabbits, the caecal microbiota of pups differs strongly from adults (LAKTIONOV, 2009). This microbiota of young rabbit changed after shift from milk to dry feed, around 3 weeks of age (PADILHA et al., 1995), which also promote the beginning of caecotrophy. During weaning the caecal ecosystem of pups has not yet fully developed and stabilized (GIDENNE, 1997) and thus, it is more susceptible to several enteric infections (LICOIS & GIDENNE, 1999), reaching mortality levels of nearly 0.25 around weaning (KOEHL, 1997). Nursing mother microbiota plays an important role over that of the litter. Caecal colonization that occurs during the lactation process prevailed over that during the partum (ABECIA et al., 2007).

It is hypothesized that intestinal microflora of rabbits at the age of 1 month is not yet fully stable, and during prepubertal period mother microbiota can also influence and support stabilization of microbial ecosystem of intestinal tract of growing rabbits.

From the caecotrophes of nursing females it was isolated a microorganism. The strain was identified as *Enterococcus faecalis* RB (SCHLEIFER & KILPPER-BÄLZ, 1984). Enterococci are ordinary inhabitants of the intestine, and normally their numbers were not higher than the total number of coliform bacteria. From the theoretical and practical view point is important to study the effect of combinations of intestinal enterococci with probiotic strains of *Bacillus subtilis* (EHRENBERG, 1835; COHN, 1872) on the growth and development of young rabbits. As a working strain, it was used the strain *B. subtilis* B-8130, which is part of the probiotic feed additives. Intestinal enterococci possess properties indicating ability for their combination with bacillus: they are successfully developing in the contents of the intestine, where conditions are close to the solid phase, and release bacteriocins (EGOROV & BARANOVA, 1999), which can improve the preventive properties of preparation, including against viral infections.

The aim of the study was to examine the mechanisms of the maternal effect on growth and development of young rabbits in the prepubertal period, in particular to study the effect of caecotrophes of female rabbits and strains of enterococci and their combinations with the probiotic strain *Bacillus subtilis* B-8130 on the growth of rabbits.

MATERIAL AND METHODS

The research was conducted at the experimental station "Chernogolovka" of the Institute of Ecology and Evolution of the Russian Academy of Sciences (Russian Federation, Moscow region) in 2007 - 2009. As the object of investigation, there were used dwarf rabbits *Oryctolagus cuniculus* (LINNAEUS 1758).

In all the experiments, young rabbits were maintained in standard cages (50 x 50 x 40 cm) in groups of three individuals (up to 1 month of the age with their mother, then to 3 months of age without a mother). Differences in body weight of young rabbits of the same group were minimal. The offspring were weighed once in 6 days. Before the beginning of the experiments, all rabbits were maintained on a standard diet: grain (oats), hay, vegetables (carrots, cabbage), minerals, water. During the experiment, the diet of young rabbits included also different kinds of rolled oats.

To confirm the hypothesis that symbiotic microorganisms of the mother can transfer to young rabbits in the prepubertal period, there were conducted behavioral observations. Two groups of one-month-old and two groups of two-month-old rabbits were observed continuously for 24 h. A ceramic plate with fresh soft faeces of a lactating female (5 g) was placed before the experiment into each cage. All contacts of the young rabbits with the soft faeces were recorded during the observation.

A pure culture of enterococcus was obtained from freshly collected soft faeces of a lactating female. The morphological, cultural, physiological, and biochemical characteristics of the microorganism were studied by the standard methods of general bacteriology. Phylogenetic analysis based on sequencing 16S rRNA was used to identify the microorganism. The probiotic properties of the strain were assessed by estimating its ability to grow in the presence of 4% NaCl, 40% bile, alkaline pH, and 40% trypsin.

The influence of the isolated microorganism added to the diet on the rabbit growth and development was studied using an original technique. Two-day-old bacterial culture in 0.5% milk was sprayed over the surface of "Hercules" (oat flakes) (100–200 ml of the culture per 1,000 g of flakes), and the mixture was carefully dried. The cells remained viable for 15 days; therefore, a fresh preparation was obtained every two weeks. The "bioflakes-1" was added to the diet of the young rabbits.

To share solid cultivation of *Bacillus subtilis* B-8130 with the selected strain of *E. faecalis* RB. ("biogerkules-2"), it was received two daily liquid cultures of bacilli on glucose-peptone substrate, and enterococci - 0.5% milk. Then, we mixed the sterile substrate (Hercules) with liquid cultures (*B. subtilis* B-8130 and *E. faecalis* RB.) in the ratio 2:1:1, weight / volume. The resulting wet mass was incubated in conditions of limited air access for two days, and then dried by the method described above.

Six groups (offspring per group "n") were observed:

E0 - from 30 to 90 days in the cages of young rabbits, there were added fresh caecotrophes of adult females (5 g per cage) every other day (n = 12), standard diet was used;

K0 - control - from 30 to 90 days young rabbits were maintained on standard diet;

E1 - from 30 to 90 days young rabbits were maintained on the diet "biohercules-1" (n = 6);

K1 - control from 30 to 90 days young rabbits were maintained on the diet of Hercules without bacteria in the same amount as in the experimental group (n = 5).

E2 - from 30 to 90 days young rabbits were maintained on the diet "biohercules-2" with the association of *E. faecalis* and *B. subtilis* (n = 5);

K2 - from 30 to 90 days young rabbits were maintained on the diet Hercules mixed with 0.5% milk without bacteria in the same amount as in the experimental group (n = 6).

The results were treated using the Mann-Whitney test by means of Statistika and MS Excel software.

RESULTS AND DISCUSSIONS

Our observations confirmed the contacts of young rabbits with the mother's soft faeces, which could, therefore, be transferred to the gastrointestinal tract of the offspring. The young rabbits were sniffing and stepping on faeces during the day. The one month-old rabbits had contact with female caecotrophes on average 52 times per rabbit and two month-old rabbits had contact 32 times; the number of these contacts represented 15% of the total number of reported behavioral act. Soft faeces were in contact with the food many times. Consequently, the food containing small pieces of faeces was eaten by the young rabbits. In addition, the young rabbits licked their feet on which some of the mother's soft faeces were left. No coprophagy of caecotrophes of the lactating female was observed; the young rabbits ate only its own faeces from the anus.

The results of the experiments regarding the growth of rabbits with the content on different diets are rendered in (Table 1).

In E0, an average increase in body mass of the animals at the end of the experiment compared to the control (K0) was 3.8%. Week weights underwent significant fluctuations; the highest values were registered in control group and the lowest in the group treated with caecotrophes of the lactating females. In the final week of the experiment, the body weight gain in the experimental group exceeded that of the control group with 15.5%. Although the differences were not significant ($P > 0.05$), the trend may, to some extent, serve as a confirmation of a hypothesis about the important positive role of caecotrophes of the mother for the growth and development of young rabbits.

Table 1. Growth rate of rabbits, contained on different diets.
Tabel 1. Ratele de creştere ale iepurilor în funcţie de diferite diete.

Groups / Parameter	K0 Control	E0 With caecotrophes	K1 Control	E1 Ration with *E. faecalis*	K2 Control	E2 Ration with *E. faecalis* and *B. subtilis*
Average weight, g	442 ± 123	459 ± 91	463 ± 85	595 ± 120	522 ± 126	601 ± 175
% of control	100	103.8	100	128.5	100	115.1
The mean weight gain of body weight, g	213 ± 95	246 ± 80	198 ± 72	323 ± 95	239 ± 82	295 ± 75
% of control	100	115.5	100	163.1	100	123.3
The average weekly weight gain, g	21 ± 20	25 ± 16	20 ± 7	32 ± 9	24 ± 15	30 ± 15
% of control	100	115.2	100	160.0	100	123.3

A nonpathogenic bacterial strain identified as *Enterococcus faecalis* was isolated from the soft faeces of a lactating female rabbit. This strain could serve as probiotic, because the cells were resistant to the mixture of 4% NaCl, 40% bile, and 40% trypsin (pH 9.5). This was confirmed by a higher body weight of the young rabbits fed on the diet with *E. faecalis* cells (Table 1). At the end of the experiment, the body weights of the young rabbits were 463 ± 85 g and 595 ± 120 g in the control and experimental groups, respectively; in the experimental group, where the young rabbits received food with the bacterial additive, the body weight was 128.5% of that in the control group ($p = 0.082251 > 0.05$). The average increase in the body weight during the entire period of the experiment was 198 ± 72 g and 323 ± 95 g in the control and experimental groups, respectively (these values significantly differed from each other, $p = 0030303 < 0.05$); an increase in the weekly weight gain of the young rabbits from the control and experimental groups were 20 ± 7 g and 32 ± 9 g, respectively ($p = 0.030303 < 0.05$). Thus, the differences between the two parameters were significant, and at the end of the experiment, a pronounced tendency towards higher values was observed in young rabbits from the experimental group (Fig. 1).

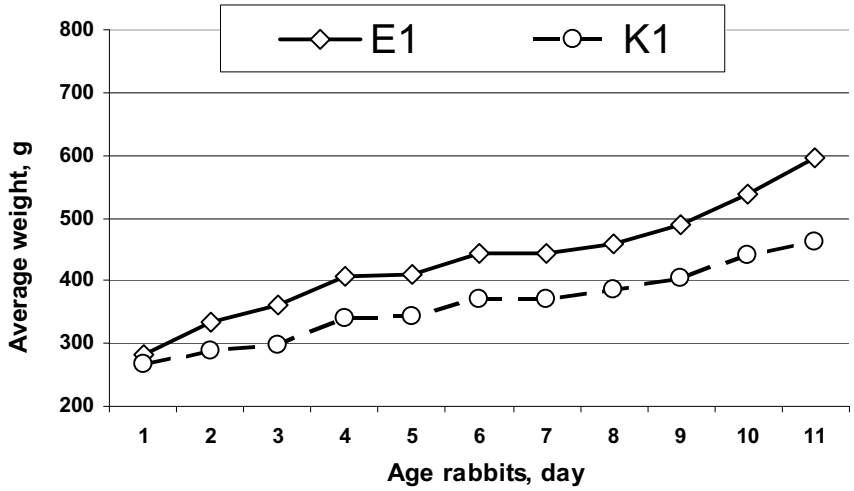

Figure 1. Dynamics of average live weight of rabbits in E1.
Figura 1. Dinamica greutăţii medii în viu a iepurilor în E1.

"Biohercules-2" also had a positive influence on the dynamics of the body weight of the growing rabbits (Fig. 2). Weight of the rabbits (E2) at the end of the experiment exceeded the body weight of the control animals (K2) by 15.5%. The average weight gain over the entire period of the experiment and the average weekly weight gain - by 23.3%.

Formation of the intestinal microbiocenosis in prepubertal rabbits is an important developmental stage, in which the mother actively participates due to the contact transfer of probiotic symbionts of its soft faeces. Our data support the importance of keeping young rabbits together with their mother during this period, because the maternal faeces serve as a source of the necessary symbionts; to a certain degree, an absence of the positive maternal effect may be compensated for by the addition of artificial probiotic preparations to the food. Zoological observations form the basis for efficient isolation of bacteria with probiotic properties.

CONCLUSIONS

The mother's soft faeces contain certain microorganisms, such as *E. faecalis* that have probiotic effect on young rabbits. *E. faecalis* RB. and the association of *E. faecalis* RB. and the probiotic strain of bacteria *Bacillus subtilis* B-8130 included in diet increases the growth of young rabbits in the prepubertal period. Mother actively participates in the formation of the intestinal microbiocenosis in prepubertal rabbits due to contact transfer of probiotic symbionts of its soft faeces to her litter.

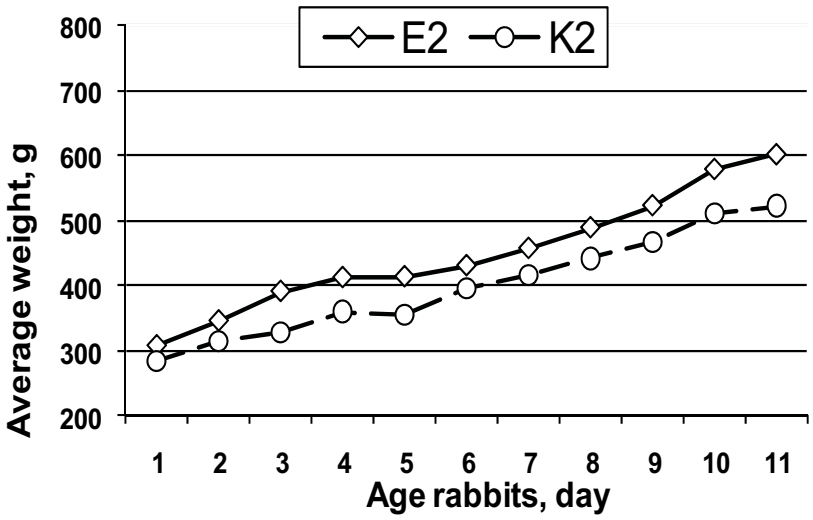

Figure 2. Dynamics of average live weight of rabbits in E2.
Figura 2. Dinamica greutății medii în viu a iepurilor în E2.

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