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GENERAL BIOLOGY

# Stimulation of Development of Rabbit Offspring by Probiotic Bacteria of the Mother's Soft Faeces

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The study of mother–offspring interactions at various stages of ontogeny is important for understanding physiological characteristics, both species- and individual-specific, and the formation of adult behavior [1]. These processes during the lactation period have been studied well in rodents and lagomorphs. The prepubertal period is less investigated. In lactating female *Rattus norvegicus* and *Acomys cahirinus* (rats and spiny mice, respectively), a maternal pheromone is synthesized in the caecum and released in faeces to serve as an attractant for the young [2–4]. The pheromone appears in females when the purely milk diet of their young is replaced by mixed food; the pheromone synthesis ceases by the moment of weaning. Eating the mother's excrements containing the pheromone is assumed to prevent necrotic enterocolitis in the young [5]. The active pheromone fraction includes deoxycholic acid formed via cholic acid dehydroxylation and deconjugation by the intestinal bacteria *Lactobacillus* spp. and *Bifidobacterium* spp.

The presence of the mother stimulates the development of weanlings [6, 7]. When kept together with the mother until the age of three months, young dwarf rabbits gain weight more rapidly than control young rabbits (in the absence of the mother) [8]. However, the mechanisms of the mother's influence on the offsping growth remain unclear. We assumed that the mother's soft faeces containing useful microsymbionts that are transferred to the offspring's gastrointestinal tract have a stimulating effect. Adult rabbits eat their own soft faeces containing intestinal bacterial biomass, proteins, and organic acids [9–11]. Deprivation of coprophagy has proved to have a negative effect on their physiological state [12].

We studied the maternal influence on the growth and development of prepubertal rabbits, including the possibility that bacteria of the mother's soft faeces are transferred to the offspring's body. Symbiont microorganisms were isolated from soft faeces of the lactating female rabbits, and their influence on the growth of young rabbits was studied.

Dwarf colored rabbits were used in our experiments. Young rabbits were kept in standard cages ($50 \times 50 \times 40$ cm$^3$), three individuals matched for the body weight per cage. They received the standard diet. The following food composition was used: hay (mostly cereal), fodder grain (oats), flakes (rolled oats), vegetables (carrots, cabbage, apples, etc.), bread, and mineral additives (calcium phosphate and sodium chloride). 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 [13]. 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 (GOST 21149-93; 100–200 ml of the culture per 1000 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 resultant "bioflakes" was added to the diet of the young rabbits. The experimental group ($n = 6$) included young rabbits under one month of age and their mother; afterwards, the same young rabbits were

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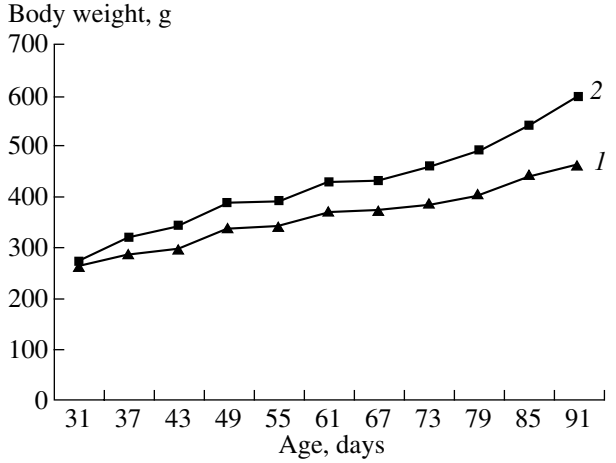


Changes in the body weight of young rabbits: *1*, the control group; *2*, experimental young rabbits fed on *E. faecalis*-containing diet.

kept until the age of three months in the absence of the mother and fed on a "bioflakes"-containing diet. The control group ($n = 5$) included rabbits under one month of age together with their mother; afterwards, the same young rabbits were kept until the age of three months in the absence of the mother and fed on the same amount of out flakes containing no bacteria. The other conditions were identical. The young rabbits were weighed every six days to trace their body weight gain.

The results were treated using the Mann–Whitney test by means of the Statistika and MS Excel software. The chemical parameters of thigh muscles were determined by standard methods [14].

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. In total, 192 contacts with the soft faeces that were put to the cage were recorded. Soft faeces were in contact with the food (grain and bread) many times before 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 was left. No coprophagy of the lactating female was observed; the female ate only its own faeces from the anus. Maternal soft faeces were not gustatorily attractive for young rabbits. Coprophagy seems to be a behavioral act acquired by young rabbits due to imprinting and imitation of the maternal behavior.

Chemical composition of thigh muscle tissue of young rabbits fed on *E. faecalis*-containing diet and control rabbits, in percent

| Parameter | Control group | Experimental group |
|---|---|---|
| Moisture | 76.80 | 76.44 |
| Protein | 81.13 | 83.13 |
| Lipids | 4.98 | 6.01 |
| Ash | 5.27 | 5.03 |

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 supplemented with *E. faecalis* cells (figure). At the end of the experiment, the body weights of the young rabbits were $463 \pm 85$ g and $595 \pm 120$ 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 $198 \pm 72$ g and $323 \pm 95$ g in the control and experimental groups, respectively (these values significantly differed from each other, $p = 0.030303 < 0.05$); an increase in the weekly weight gain of young rabbits from the control and experimental groups were $20 \pm 7$ g and $39 \pm 9$ g, respectively ($p = 0.030303 < 0.05$). Thus, the differences between 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.

Thus, we have demonstrated that the mother's soft faeces contain microorganisms that have probiotic effect on young rabbits. When the maternal *E. faecalis* was added to the diet, the growth of young rabbits was accelerated. The probiotic stimulated both protein and lipid metabolisms in the experimental rabbits, which was confirmed by chemical analysis of thigh muscles (Table).

Formation of the intenstinal microbiocenosis in prepubertal rabbits is an important developmental stage, in which the mother actively participates due to 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 addition of artificial probiotic preparations to the food. Zoological observations form the basis for efficient isolation of bacteria with probiotic properties.

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SPELL: 1. caecum, 2. faeces, 3. weanlings, 4. offsping