DEVELOPMENT OF THE CAECAL MICROFLORA OF NEWBORN RABBITS DURING THE FIRST TEN DAYS AFTER BIRTH

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ABSTRACT

The caecal microflora and the fermentation processes taking place in the caecum play a key role in the digestion of rabbits. Imbalance of the intestinal microflora (dysbiosis) plays a direct or indirect role in the development of digestive disturbances or diseases. The composition of the intestinal microflora is rather simple in rabbits, with the predominance of *Bacteroides*. According to HUDSON *et al*. (1996), the colonisation is triggered by ingestion of the maternal faecal pellets that gets into the nest during nursing. The objective of the experiment was to study the rate of development of intestinal microflora in newborn rabbits after birth and the effect of nursing method and ingestion of maternal faeces on the colonisation of the caecum by bacteria. Pannon White does and their progeny were used in the experiments. The one-day-old pups of average birth weight were distributed into litters of eight, and these litters were randomly divided into the following three groups: Group 'A' (free nursing): freely nursed pups having access to maternal faeces. Group 'B' (controlled nursing): pups nursed once a day and having access to maternal faeces. Group 'C' (controlled nursing): pups nursed once a day and having no access to maternal faeces. Six young rabbits were examined by group on days 2, 4, 6, 8 and 10 after birth. After kindling, swab samples were taken from the vagina of the doe, from the surface of the vulva, from the skin of the nipples and the surrounding hairs. These samples and also samples from the caecal chyme of the pups were subjected to microbiological analysis. The supply of young rabbits with doe's milk (controlled or free nursing) and their access to the doe's faeces have been shown to affect the development of the caecal microflora. In rabbit pups nursed freely and thus having unlimited access to the doe's faecal pellets, colonisation of the caecum by *Bacteroides* microorganisms took place at a faster rate. Once-a-day nursing in the morning (controlled nursing) only slightly decreased the rate of colonisation by *Bacteroides*. Prevention of the ingestion of the doe's faeces only delayed, but did not prevent, the development of the normal intestinal microflora. This indicates that the faeces left behind by the doe has only limited role in the colonisation of the caecum by *Bacteroides* microorganisms.

Key words: Newborn rabbit, caecal microflora, *Bacteroides*, controlled, free nursing.

INTRODUCTION

The caecal microflora and the fermentation processes taking place in the caecum play a key role in the digestion of rabbits. Despite this fact, the development and composition of the intestinal microflora are less well known in rabbits than in other farm or laboratory animals. Imbalance of the intestinal microflora (dysbiosis) often plays a direct or indirect role in the development of digestive disturbances or diseases. The resulting diseases of the alimentary tract may cause even 30 to 50% mortality in a rabbit stock and even the recovered animals show markedly impaired performance.

According to data of the literature (GOUET and FONTY, 1979; FEKETE, 1989), the composition of the intestinal microflora is rather simple in rabbits, unlike the majority of monogastric animals. The concentration of the caecal bacterial flora is $10^{9–11}$/gram, with the predominance of *Bacteroides*, which are non-spore-forming, Gram-negative obligate anaerobes. They colonise the caecum during the first or second week after birth (GOUET and FONTY, 1979). According to HUDSON *et al*. (1996) colonisation is triggered by ingestion of the maternal faecal pellets that get into the nest during nursing.

The objective of the experiment was to study the rate of development of intestinal microflora in newborn rabbits after birth and the effect of nursing method and ingestion of maternal faeces on the colonisation of the caecum by bacteria.

MATERIALS AND METHODS

Pannon White does and their progeny were used in the experiments. The does were kept in hutches of 600×275 mm basic area. At one end of the hutch there was a nest box of 241×550 mm area, with a creep-hole that could be closed. The nest box contained a 160×320 mm nest tray. The rabbits were fed a commercial diet (crude protein: 17%, crude fibre: 15.5%, energy: 11 DE MJ/kg) *ad libitum*. Drinking water was available from flap-valve type automatic waterers *ad libitum*. The temperature of the room was 20–23 °C and the applied lighting cycle was 16L:8D.

Some of the does kindled on day 31 and the others were treated with oxytocin (5 IU/doe). The one-day-old pups of average birth weight were distributed into litters of eight, and these litters were randomly divided into the following three groups:

Group ‘A’ (free nursing): freely nursed pups having access to maternal faeces. In this group the creep-hole of the nest box was open; thus, the doe could enter the nest box freely according to the natural diurnal rhythm, and nothing could disturb the harmony of nursing and excretion of faeces into the nest.

Group ‘B’ (controlled nursing): pups nursed once a day (in the morning) and having access to maternal faeces. In this group the creep-hole of the nest box was closed and the does were allowed to enter the nest box only once a day, at 9:00 a.m. Because of the controlled time of nursing, it is not sure that nursing and defecation were in harmony.

Group ‘C’ (controlled nursing): pups nursed once a day (in the morning) and having no access to maternal faeces. Also in this group, the does were allowed to enter the nest box at 9:00 a.m. only. For the time of nursing the pups were removed from the nest and placed into a nest bowl bedded with clean wood shavings to ensure that no maternal faecal balls remain in the nest and the pups have no opportunity to ingest

them. The pups were placed back into the nest immediately after nursing, then we collected and counted the maternal faecal balls left there.
Six young rabbits were examined by group on days 2, 4, 6, 8 and 10 after birth. The pups were euthanised by an overdose of carbon dioxide and bled. After opening the abdominal cavity and isolation of the caecum, samples were taken from the caecal content and subjected to microbiological analysis. The analyses were always performed between 11:00 a.m. and 12:00.
After kindling, swab samples were taken from the vagina of the doe, from the surface of the vulva, from the skin of the nipples and the surrounding hairs, and then these samples were subjected to microbiological analysis.

For the microbiological analyses, a dilution series was made from 1 g of chyme. The *Bacteroides*, coliform and *Lactobacillus* counts were determined on the basis of parameters summarised in Table 1. After a suitable incubation period the colonies were counted under microscope. The colony count was expressed in log 10 values.
Samples taken from does were used only for the qualitative detection of *Bacteroides*. Culturing was done as specified in Table 1.

Table 1: Culture media and culturing parameters used for the microbiological analyses

Bacterial species	*Culture medium*	*Culturing conditions*
Bacteroides	Schaedler's agar supplemented with esculin, neomycin and Fe-ammonium-citrate	37 °C, anaerobic, 96 h
Lactobacillus	MRS medium	37 °C, anaerobic, 48 h
Streptococcus	Edward's medium	37 °C, aerobic, 48 h
Coliforms, *E. coli*	Chromocult selective medium	37 °C, aerobic, 24 h

The experimental data were evaluated by one-way analysis of variance using SPSS 9.0 programme package.

RESULTS AND DISCUSSION

Changes in the numbers of the main components of the caecal microflora are summarised in Table 2.
In earlier studies (KOVÁCS *et al*., 2002) we found that *Bacteroides* were detectable from the caecal content of already 7-day-old rabbit pups in high concentration (10^9/g chyme). The current studies confirm that their colonisation commences already on the 4th day irrespective of the nursing method and of whether or not the young have had access to their doe's faeces. Although access to maternal faeces had no influence on the appearance of these bacteria, it did affect the rate of their growth in the caecum. Namely, in Group B they were detectable in somewhat lower germ counts between days 4 and 8, while in Group C the difference was well demonstrable up to day 10.
Bacteroides were detectable by the microbiological analysis of samples taken from the rectum of other animal species and of infants as well (SMITH and CRABB, 1961). In calves, lambs, piglets and human infants they were found to appear between days 2

and 4 after birth in a concentration of 10^8–10^{10}, while in rabbits *Bacteroides* were found later, between days 4 and 7, in concentrations similar to those found in the other animal species examined. In farm animal species (other than the rabbit) *Bacteroides* are not dominant components of the large intestinal microflora, and their counts usually range between 0 and 10^6 in adult animals.

GOUET and FONTY (1979) have also found that the intestinal microflora of rabbits starts to develop from the 3rd day of life, until when the alimentary tract is almost sterile.
According to HUDSON *et al*. (1996), young rabbits feed exclusively by suckling in the first two weeks of their life, and they start to ingest the faecal pellets left in the nest by the doe and the straw bedding of the nest in the second week. In the present experiment, the faecal balls left in the nest by the doe after nursing and subsequently removed from there were counted in Group C. On the second day, maternal faeces was found in only two out of the 11 nests examined. However, from the subsequent day onward faecal balls were found in all nest boxes, with the exception of one or two cases. The average number of faecal balls per nest was 3.5 to 4 but it showed high variability: there were does that regularly left behind 0 to 2 (average: 0.7) faecal balls while others 5 to 12 (average: 8.3). The faecal balls disappeared in both Groups A and B: presumably the rabbit pups of a few days of age ate them, and this resulted in the early and fast colonisation of their caecum by *Bacteroides* organisms.

Table 2: Composition of the caecal microflora expressed in log germ count/g chyme (average and range)

Group	Age (day)				
	2	4	6	8	10
			Bacteroides		
A	<2	4.8^{a} (<2-5.0)	7.2^{a} (6.5-7.7)	8.2 (7.8-8.5)	8.5 (8.1-9.0)
B	<2	4.2^{a} (3-5.2)	6.0^{ab} (4.8-7.1)	7.8 (6.4-9.2)	8.6 (8.3-8.9)
C	<2	2.4^{b} (<2-2.6)	4.3^{b} (4.0-5.0)	6.4 (2.0-8.0)	7.4 (6.0-8.5)
			Lactobacilli		
A	6.0 (4.2-7.7)	4.0 (3.3-4.3)	4.8^{a} (3.2-6.2)	4.1 (3.4-5.5)	3.5 (<2-4.7)
B	4.6 (3.6-5.7)	3.9 (2.7-5.3)	3.1^{ab} (2.0-3.7)	3.7 (2.3-6.1)	2.8 (<2-4.3)
C	5.1 (4.5-5.4)	3.6 (3.1-4.1)	2.7^{b} (2.5-2.9)	3.1 (2.0-4.3)	3.1 (<2-4.1)
			Coliforms		
A	5.6 (3.5-7.7)	5.6 (4.8-6.0)	4.1^{ab} (3.4-6.8)	6.4 (5.2-7.0)	4.3 (3.0-6.4)
B	<2	<2	4.0^{a} (<2-5.9)	4.2 (3.1-6.1)	3.8 (<2-5.3)
C	<2	3.6 (<2-6.7)	6.7^{b} (6.5-7.0)	6.4 (5.2-7.0)	5.4 (<2-7.7)

^{a,b} significant difference between groups ($P<0.05$)

The somewhat slower colonisation of the caecum by *Bacteroides* organisms in Group B may have been due to the fact that does having access to the nest only at a given nursing time excreted less faecal balls than does having free access to the nest (Group A). The young rabbits of Group C were prevented from ingesting maternal faeces. We assumed that in these animals bacteria in a number sufficient to initiate colonisation by *Bacteroides* could get into the digestive tract from the genital organs, the fur or the nipples. The microbiological analysis of swab samples taken from the does showed that young rabbits can become “infected” by *Bacteroides* already in the doe’s vagina. These bacteria could be detected in much higher numbers from the

surface of the vulva than in the vagina. In some cases *Bacteroides* could be cultured also from the surface of the fur.

While lactobacilli, coliforms and streptococci constitute the main components of the intestinal microflora in the majority of farm animals, in the rabbit these bacteria can be found in traces only. In the first week of life their number is usually between 10^2 and 10^8, but subsequently they "disappear" and their count falls below 100. In the present experiment, the free nursing method (Group A) resulted in higher lactobacillus counts throughout and also higher coliform counts in the first four days of life. In Group C, a major rise was found in the coliform count by day 6, and on day 10 the coliform count was still higher than in the other two groups. This was presumably due to the slower establishment of the main microflora (i.e. the slower rate of colonisation of the caecum by *Bacteroides*). Although it could be established that the *Lactobacillus* and coliform counts were in inverse ratio to the *Bacteroides* count, a clear antagonism between the normal bacterial components of the microflora could still not be demonstrated.

The composition of the intestinal microflora is not decisively determined by nutrition (SMITH, 1965). Thus, e.g. the alimentary tract of rabbits is not colonised by bacteria that establish themselves in other rodents (mouse, hamster) given the same nutrition. However, the count of bacteria constituting the intestinal microflora can be influenced by nutrition, as shown also by our results. According to the studies of SMITH (1965), in rabbits fed dried milk powder the concentration of lactobacilli in the caecum increased and reached levels of $10^{8–9}$.

CONCLUSIONS

The supply of young rabbits with doe's milk (controlled or free nursing) and their access to the doe's faeces have been shown to affect the development of the caecal microflora. In rabbit pups nursed freely and thus having unlimited access to the doe's faecal balls, colonisation of the caecum by *Bacteroides* microorganisms took place at a faster rate. Once-a-day nursing in the morning (controlled nursing) only slightly decreased the rate of colonisation by *Bacteroides*. Prevention of the ingestion of the doe's faeces only delayed, but did not prevent, the development of the normal intestinal microflora. This indicates that the faeces left behind by the doe has only limited role in the colonisation of the caecum by *Bacteroides* microorganisms.

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