

The Review on Structure of Intestinal Flora at Different Growth Stages of Rabbits

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Abstract. Rabbits are both monogastric and herbivore animals, with special digestive and physiological characteristics. The establishment of healthy, stable and diverse digestive tract microflora is of great significance for rabbits to resist intestinal diseases. The purpose of this paper is to summarize the composition and diversity of intestinal microflora in different growth stages of rabbits.

Introduction

Animal digestive tract, especially for the mammals, is very suitable for microbial mass reproduction. These complex microbial communities that survive in the intestine are called microbial flora[1]. Intestinal microflora planted in the small intestine and large intestine plays an important role in maintaining host's function, including energy intake of food, generation of host's key metabolites, development of the immune system, response to gastrointestinal diseases, etc. [2,3].

Rabbits are both monogastric animals, but also herbivores, with special digestive and physiological characteristics. The rabbit farming is an important emerging enterprise in many countries of the world. Studies have found that in a rabbit field which has a mortality rate of 24%, 75% of deaths were caused by diarrhea. A high incidence of digestive diseases in rabbits often related to distortions in microflora composition[4,5]. Any imbalance of microflora can result in alteration of pH, dysbiosis and proliferation of pathogens, with detrimental effects on the animal's health[1]. The establishment of healthy, stable and diverse digestive tract microflora is of great significance for rabbits to resist intestinal diseases.

The digestive system of rabbits is adapted to fermentation of vegetable in colon and cecum. The digestive system and physiology of the rabbit have been described in several reviews[6-8]. Therefore, the present review focuses on gut microflora and its role on health in different growth stages of rabbits.

The Intestinal Microflora and the Animal Health

Berg recalled the definition of autochthonous flora (or indigenous flora) as "resident microorganisms present in all communities of a particular animal species" [9]. They can grow anaerobically in the gastrointestinal tract and are always present in adults. Guts of rabbits are

known to be colonized with balanced gut microflora [10,11]. Through the study on the normal intestinal flora of rabbits fed on different ages including no breast-fed, breast-fed and adult, it was found that there were differences in intestinal microflora in different growth stages, and the predominant microflora of the large and small intestine were different, too. Affected by the growth, age, diet and environment, the intestinal microflora of different digestive tract segments are different in species and quantity [12].

In addition to stable planted intestinal micro-organisms, in the process of feeding, there exist some passing-by flora (Or foreign flora) composted of non-pathogenic or potentially pathogenic bacteria in the host which can only be retained within a few hours and be excreted a few days or weeks later with feces. Under normal circumstances, intestinal flora in the host does not produce pathogenic effect, when the intestinal disorders, immune function decreases, the normal number and proportion of microorganisms are destructed, the passing-by bacteria will quickly multiply, causing the occurrence of related diseases.

Characteristics of Intestinal Microflora During Rabbits' Embryonic Development

Female rabbit uterus is a sterile environment, so the rabbit has been considered sterile in the embryonic period. There have been microbial colonization in gastrointestinal tract during the late embryos, its content reaches 0.8×10^2 CFU/g to 1.2×10^2 CFU/g, mainly including bacteria like *Corynebacterium*, *Micrococcus*, *Bacillus cereus* and *Staphylococcus*[13]. In addition, there exist some bacteria that are not cultural but may play an important role in the intestine in the embryonic stage. The origin of intestinal microflora in the rabbit embryo stage is unclear, which can be derived from the maternal reproductive tract and amniotic fluid. Rabbit embryonic intestinal bacteria can participate in the internal environment nutrient uptake, and involve in regulating embryonic growth and development and intestinal immune defense, but does not cause fetal and maternal infection [14].

Characteristics of Intestinal Microflora in Rabbits During Lactation

Generally speaking, mammalian milk contains antibacterial proteins (such as *immunoglobulin*, *lactoperoxidase*, *lactoferrin* and *lysozyme*) and non-protein compounds (such as *antibacterial lipid* and *oligosaccharides*), which can protect the newborn animal free from pathogens and opportunistic pathogens[15]. But the immunoglobulin of baby rabbits mainly come from the female rabbit's uterus, the antibacterial substance they get from breast milk is lipid rather than immunoglobulin, their lipase in stomach can break the ester bond and release short and medium chain fatty acids, these fatty acids have antibacterial effect, therefore, the newborn rabbits have a similar intestinal flora structure like its embryonic period, the structure is almost sterile. The baby rabbits, however, are born through the female rabbit's vagina and they can be exposed to female rabbit's feces, so they may contact to bacteria, they can also contact bacteria in breeding environment, these bacteria colonize in the rabbits' intestinal tract, then format the intestinal flora [14]. This period is short, with the generation of breast-feeding behavior, intestinal microbes begin colonization, intestinal flora and the surrounding environment are the main sources of intestinal bacteria, and the initial implanted flora is closely related to the final constant flora composition of adult rabbits[16].

Microbial colonization begins after birth, but the development of the intestinal microflora is a gradual process. At one week old, a large number of *facultative anaerobes* and *obligate anaerobes* will appear in the cecum and large intestine of baby rabbits. At two weeks old, the

content of obligate anaerobes and facultative anaerobes are similar ($10^7\sim 10^{10}$ bact / g) [8]. After birth to 10 weeks of age, the development of intestinal microflora is a stable process of slow development from simple to complex, along with the breast-feeding, feeding and drinking, the intestinal microbial composition will be more complex[4]. Combes *et al.* [17] found that *Archaeobacteria* ($10^5\sim 10^6$ bact/g) which would colonize in intestine could be detected in the cecum of rabbits from the first seventh day. The strictly anaerobic, non-sporulating bacteria, especially Gram-negative bacilli (*Bacteroides*) dominate the digestive flora in every segment of the intestine.

Characteristics of Intestinal Microflora During Rabbit Feeding Period

Feeding period, also known as transitional period, refers to the period from feed intake in the late lactation to complete feed intake after weaning. The structure of intestinal flora is changed by nutritional factors during feeding period. After 15 days old baby rabbits begin to take feed, fiber-containing substances will enter the cecum. At this time, the bacteria associated with fiber decomposition begin to colonize in the intestine, which mainly include cellulose decomposition bacteria, starch decomposition bacteria, pectin and xylose degradation bacteria, etc., in which *Eubacterium cellulosolvens* can decompose fiber, *Bacteroides ruminicola* can decompose pectin and xylan. The bacteria involved in fibrolysis (hydrolysis of cellulose, xylanes, pectins, etc.) only become established after 15 days of age, when intake of solid food begins and a fibrous substrate enters the cecum. Then the fibrolytic flora increases slowly to reach 10^7 bact/g at 25 days of age in conventional rabbits[18]. It should be noted that so long as the rabbits are fed only on milk, the cellulolytic flora does not appear, even in rabbits 35–42 days old [19].

Since baby rabbits begin to take solid feed, bacteria transfer from stomach to small intestine, cecum and colon. At the same time, microbes begin to colonize and ferment in the gastrointestinal tract and form a stable gastrointestinal parasitic system[20]. Bennegadi *et al.* [21] reported that SPF rabbits contain a higher proportion of fiber-decomposing bacteria in intestine than normal rabbits, but there are also opposite results [14], differences in different studies may be related to dietary composition, variety and experimental conditions and other factors of experimental rabbits.

Supplementation also affect other microorganisms' colonization. Padilha *et al.* [14] showed that the number of anaerobic microorganisms in rabbit's cecum was very large (10^{11} bact/g) at 15~22 days of age, and decreased to about 10^{10} bact/g when weaning, after weaning, the number of oxygen bacteria was not significantly changed by age, which is similar to the trend of starch decomposing bacteria.

In short, the intestinal flora structure's change is affected by nutritional factors during feeding period, timely supplement and weaning are necessary to promote the establishment of normal intestinal flora structure.

Characteristics of the Change of Intestinal Flora After Weaning In Rabbits

Rabbits in most farms are weaned between 4 and 5 weeks of age. Because of undeveloped gut immunity system and lack of passive immunity or medium-chain fatty acids (MCFA) protection from doe's milk [15, 22], newly weaned rabbits are susceptible to intestinal infections, which can cause high diarrhea and mortality rate. The normal establishment of intestinal tract and maintenance of dynamic balance in weaned rabbits are beneficial to resist

invasion of pathogens and reduce diarrhea rate. The caecal bacterial diversity increased with age while bacterial structure was modified. The number of bacterial 16S rDNA copies was already high (10^{12}) 1 week after weaning and tended to fall slightly with age. Bacterial community diversity, calculated as the Simpson diversity index, increased slightly with age but was not affected by intake level[23].

Bivolarski *et al.* [24]found that the total number of cecal bacteria in 35-day-old weaned rabbits was higher than that in 21-day-old weaned rabbits, at weaning (21 days and 35 days of age), the number of *C. Perfringens* in baby rabbits' cecum was very small (1.656 log₁₀ CFU / g and 1.654 log₁₀ CFU / g, respectively), but more *C. Perfringens* were present in the cecum of the early weaned rabbits at the end of the trial. Early weaning can also increase the number of *E. coli* in the intestinal tract of baby rabbits. These changes may be related to the loss of breast milk's protection after weaning. In addition, cluster analysis showed that young rabbits of the same age could not be clustered in the same cluster, which indicated that even under the strictly same feeding conditions, the intestinal microflora structure of individuals were still different.

Bauerl *et al.* [5]study on cecal microbes in 40-day-old rabbits and Eshar *et al.* [25]study on feces microbes in 35-day-old rabbits showed that the highest abundance of cecal microflora in 30~51-day-old was *Firmicutes*, and among which, rumen bacteria family and Mao spirochete bacteria family had the largest number. By analyzing the *Clostridium's* composition and abundance of rumen bacteria family and *Lachnospiraceae* in baby rabbits after weaning, the results showed that the fiber-decomposing bacteria in the cecum of healthy baby rabbits during weaning to weaning after three weeks was mainly *vibrio vulgaris* and *rumen coccus* belonging to rumen bacteria family, and the relative abundance of *vibrio vulgaris* remained stable, while the relative abundance of rumen coccus tended to decline in the later period. The average proportion of *Lachnospiraceae* accounts for 43.11% in the cecal microflora of baby rabbits, in addition to the weaning day, *Lachnospiraceae* is less than bacteroides division in abundance, *Lachnospiraceae* is the highest bacteria family in abundance in other age groups, among *Lachnospiraceae*, multiple bacteria families are butyric acid producing genus, such as *Clostridium*, *Fusobacterium* and *Eubacterium* genus[26]. The propionic acid content are higher than butyric acid rumen fermented in ruminant animals' rumen and pigs' cecal, which is opposite to rabbits', and this should be associated with high abundance of *Lachnospiraceae* in rabbits' cecum, and it also shows that the animals' gut microbial composition is the main factor affecting the proportion of volatile fatty acid in the fermentation product.

Rhee *et al.* [27]found that the abundances of *Bacteroides* and *Parabacteroides* in the cecum of weaned baby rabbits were significantly decreased, indicating that they are susceptible to weaning stress inhibition, which may also be an important reason leading to weaned baby rabbits' easily diarrhea. Gouet and Fonty (1979) reported that *Lactobacillus* was not detected in the intestine of rabbits by using the pure culture method, however, Zhu *et al.* [28]showed that *Lactobacillus* was present in the cecum of rabbits by real-time quantitative PCR inspection. The cecal microflora of young rabbits has been established at weaning, but the species become more complicated with the increase of age at 3 weeks after weaning. From weaning to three weeks after weaning, the predominant *Eumycotas* in the cecum of baby rabbits are: *Firmicutes*> *Bacteroides*> *Proteobacteria*> *Warts*; the dominant bacteria families are *Helicobacter*> *Ruminococcus*> *Bacteroides*> *Pseudomonas*> *Barnesiella*> *wart microbes*; and the dominant bacterial colonies are four unknown species: *Bacteroides*, *Barnesiella*, *Akkermansia* and *Bacteroides-prevotella*.

Characteristics of the Change of Intestinal Microflora in Adult Rabbits

Adult rabbits intestinal content is about 1000-10000 million micro-organisms per gram, and about 1,000 species, thus it has a very high diversity. At the confluence of the blind colon, the microbial content in feces reaches 10^{10} - 10^{12} bact/g and the abundance of ileum is lower at 10^6 - 10^8 bact/g. Anaerobic bacteria account for the dominance of normal floras in rabbits' intestinal tract, supplemented by aerobic bacteria, bacteria accounted for the dominant position of gut microflora. By using the 16SRNA target gene amplification technique, Combes *et al.* [4] showed that among the number of intestinal content, archaeobacteria is estimated to be 10^7 bact/g, and there also exist archae yeast and protozoa, but no anaerobic fungi have been found so far.

There are an estimated 400 different species of bacteria inhabiting the rabbit caecum, of which 25 are considered the most abundant[29]. In adult rabbits, strict anaerobes are the most prevalent bacteria and of these, *Bacteroides* species are considered dominant[30]. The overall composition of the microbial community, as well as the presence or absence of specific bacteria, can be either beneficial or detrimental for the health of pet rabbits and either contribute to, or prevent, digestive disturbance and disease. The four bacteria investigated were *Bacteroides*, *E. faecium*, *Fibrobacter succinogenes* and *Clostridium spiroforme*. *Bacteroides* species, *E. faecium* and *F. succinogenes* are well-recognised as belonging to the caecal flora of healthy adult rabbits[31,32]. He *et al.* [2]found that the composition of intestinal microflora in healthy rabbits remained stable, and the total number of ERIC-PCR bands in intestinal microflora was least in rectum and largest in cecum.

Bauerl *et al.* [5]studied genomic DNA of rabbit cecal bacteria by high-throughput sequencing, depicted a comprehensive picture of the cecum microbial species distribution for the first time. In healthy rabbits' cecum microflora, the *Firmicutes* accounts for the absolute advantage, which is about 78.25%, followed by *Bacteroidetes* of 15.75%. *Verrucomicrobia* and *Tenericutes* are less, which are only 2.40% and 2.39% respectively. In *Firmicutes* are mainly *Clostridium Ruminococcus* and *Lachnospiraceae* of *Clostridium*, in *Bacteroidetes* is *Rikenellaceae*. From the view of oxygen preference, most strains of rabbit cecum have special anaerobic characteristics. According to these characteristics, model of rabbit cecum microbial species is very similar to its related species -- mouse[33].

Massip *et al.* [34]suggested that the in vivo dominant bacterial community in 63-day rabbits were *Firmicutes* (90%), *Bacteroides* (4.6%), *Actinobacteria* (0.9%) and *Proteobacteria* (0.7%), respectively via 16S rDNA high-throughput sequencing. Zhu *et al.* [26]showed that *Firmicutes*, *Verrucomicrobia*, *Proteobacteria* and *Bacteroidetes* played an important role in all of the libraries.

Huyben *et al.* [33]found that 83.3%-99.7% of DNA sequences belong to *Firmicutes* and *Bacteroidetes* via testing the bacterial compositions of caecal contents in epidemic diarrhea rabbits and healthy rabbits. Combes *et al.* [4]studied the intestinal microbial composition of rabbits from 2 to 70 days of age by using the CE-SSCP method, they found that age had a significant effect on bacterial population structure and diversity. Blind microbial composition of weaned rabbits was more complex with age. The result of 16SrDNA cloning showed that *Firmicutes* accounted for more than 90% of amplified sequences, while *Bacteroides* accounted for only 4%.

Wang *et al.* [35]found that *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Verrucomicrobia* (90.5%-97.7%) played an important role in the cecum, duodenum and jejunum of growing rabbits. The results were consistent with the findings of Monteils *et*

al.[36] . Results of the phylogenetic analysis indicated that rabbit caecum is inhabited by members of the genus *Methanobrevibacter* and is possibly one-species dominated, because all the retrieved sequences exhibited similarity values of 99% or higher[37].

Meanwhile, they observed that *Ruminococcus* and unclassified *Clostridiales* were the leading member of all samples at the genus level, Eshar *et al.* [25] studied the fecal microorganisms of new zealand white rabbits at the age of 66 days, 74 days and 81 days by high throughput sequencing, and also found that there was no significant difference in the structure of cecal flora between the age of 66 and 81 days. After weaning, intestinal flora structure of rabbits is tending to be stable, without other factors disturbing this situation, the microbial community in adult rabbits' cecum (including diversity, structure, total number of bacteria, abundance of *Firmicutes* and *Bacteroides* genus *Prevotella*) remained stable[1]. Using the high-throughput sequencing technique to detect the distribution of bacteria in the cecum of 63-day-old rabbits, it was found that *Firmicutes* predominated (about 90%, bacteroidetes, 4.6%), followed by actinomyces (0.9%) and proteus (0.7%). The study showed that the structure of flora was found to be remarkably changed compared with that of healthy rabbits, the normal dominant bacteria such as *Ruminococcus* and *Alistipes* decreased significantly, while the number of potential pathogens such as *Clostridium* and *Bacteroides* increased significantly[38]. Meanwhile, the bacterial abundance of cecal microbes in the infected rabbits was also significantly lower than that in healthy rabbits[5], these changes all confirm the close relationship between the number of microbial flora in cecum and the intestinal health of rabbits.

In conclusion, the composition and diversity of intestinal flora are different in different growth stages of rabbits. Healthy, stable and diverse flora of digestive tract is of great significance to anti intestinal diseases in rabbits.

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