Changes in Microbial Flora of Animal Feces after Excretion

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Abstract Excretion of cattle, pigs and chickens were kept at 24°C under high humidity, and changes in microbial flora were observed for 7 days. In the excretion of cattle, the levels of aerobic-cultured bacteria, anaerobic-cultured bacteria, enterobacteriaceae and staphylococci increased 1 day after excretion, and stable until day 7. The dominant aerobic-cultured bacteria was streptococci in fresh feces and was replaced by enterobacteriaceae on and after day 1. Bacteroidaceae was the most abundant anaerobe; however, the level was lower than that of enterobacteriaceae. In the excretion of the pigs, the levels of aerobic-cultured bacteria and streptococci was constant until day 7. The dominant aerobic-cultured bacteria was streptococci in fresh feces and was replaced by enterobacteriaceae on and after day 3. The levels of anaerobes increased until day 3. In the fresh feces, levels of eubacteria and lactobacilli were almost the same as that of enterobacteriaceae; however, enterobacteriaceae was the most abundant on and after day 1. The data from the cattle and pig indicated that enterobacteriaceae became the dominant microbe after excretion, and that the levels of the other microbe, even though anaerobes, were not drastically changed until day 7. Chicken feces showed different tendency that anaerobes was more abundant than aerobes and the levels of either anaerobes examined were larger than that of enterobacteriaceae.


Key words: Cattle, Chicken, Feces, Microbial flora, Pig

Microbial flora in fresh feces of animals have been studied widely. The dominant fecal bacteria in pigs were bacteroidaceae, peptococaceae, eubacteria, lactobacilli, followed by bifidobacteria, spirillaceae, enterobacteriaceae. Those in the rectum of chickens were bacteroidaceae, lactobacilli, followed by enterobacteriaceae and streptococci. In cattle, those bacteria were bacteroidaceae and spirillaceae, followed by enterobacteriaceae and streptococci. Although microbial flora in fresh feces have been investigated, no work has been performed on the changes of microbial flora after excretion. Microbial flora in animal excretion may affect the performance of microbiological treatment systems for animal wastes, i.e., anaerobic digestion, activated sludge and composting. Characteristics of microbial flora could be important for the treatment of wastes. Therefore, as the first step in the study, we planned to investigate whether the microbial flora of animal feces would change without any treatment. In the present paper, we mentioned the change of microbial flora in animal feces which were kept under high humidity free from drying for...
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one week.

Materials and Methods

Animals:

Fecal samples were obtained from Holstein-Friesian cows, sows of Landrace × Large White bred and White Leghorn (Iwaya 505) hens in Miyagi Prefecture, Japan. Cows were 2.5-3 years old and fed on a mixture of one part of hay and two parts of concentrated feed (barley flakes, corn, beet pulp, soybean meal and so on) by a formula of the farm. Their weight were 640-650 kg. Sows were 3-4 years old and fed on a formula diet (Ultra-Hybreed 72, Zen-no) and their weight were 170-180 kg. Hens were 650 days old and fed on a formula diet (New-Mash, Shimizu Shiryo Co., Ltd.) and their weight were 1.8-2.0 kg.

Fecal sampling:

Freshly voided fecal specimens (5-20 g each) were collected from 5 cows, 5 sows and 10 hens. Feces of individuals in each animal species was mixed. The sample at 0 day was obtained from fresh mixed feces, and prepared for microbial investigation 1 hr after collecting from animals. The other part of mixed feces was divided into 3 pieces of 5 g mass and kept aerobically at 24°C under high humidity free from drying before sampling. From each piece, sampling was performed on day 1, 3 and 7. Although there was not much evidence, the size of the mass of the sample was decided by the results of the first experiment concerning pig feces. In the experiment, the numbers of microbes changed gently from 0 to 7 days of storage and we speculated that there might be a small variation between samples of 5 g mass.

Identification and counting of microorganisms:

Microorganisms were isolated and classified by the routine methods using non-selective and selective media. After a series of ten-fold dilutions in anaerobic diluents, 0.05 ml aliquots were spread onto following agar plates. For aerobic microbes, one non-selective agar medium and 4 selective agar media were used. Trypticase soy blood (TS) agar (Nissui) was used as a non-selective medium. As selective media, deoxycholate hydrogen sulfide lactose (DHL) agar (Nissui Seiyaku, Tokyo) for enterobacteraeae, triphenyltetrazolium chloride-acridine orange-thallous sulfate aesculin crystal violet (TATAC) agar (Nissui) for enterococci and streptococci, staphylococcus 110 agar (Difco, Detroit, MI, USA) with phenylethylalcohol egg yolk suspension (PEES) for staphylococci and potato dextrose (PD) agar (Difco) with tartaric acid for yeasts and fungi. For anaerobic growth of bacteria, one non-selective agar medium and 7 selective agar media were used. Modified Eggerth-Gagnon (EG) agar (Nissui) was used as a non-selective medium. As selective media, bifidobacteria selective (BS) agar for bifidobacteria, eubacteria selective (ES) agar for eubacteria, neomycin-brilliant green-taufrocholate-blood (NBGT) agar for bacteroides, modified lactobacilli selective (LBS) agar for lactobacilli. Each of TS, DHL agar, TATAC, PEES and PD agar plates were incubated aerobically at 37°C for 24 hr and for 48 hr, respectively. Each of EG, BS, ES, NBGT, and LBS agar plates were incubated at 37°C for 3 days in anaerobic jars filled with an atmosphere of oxygen-free CO2 using Anaero-pack (Mitsubishi Gas Chemical). The identification of 8 bacterial groups, yeasts and fungi was performed with colonial and cellular morphologies, and Gram-reaction. The level of microorganisms were determined by the number of microorganisms which were observed on the culture plates under the incubation condition mentioned above. The number of bacteria on TS agar plate and that on EG agar plate were expressed in this study as the number of aerobic-cultured bacteria and anaerobic-cultured bacteria, respectively. Since numbers of microbes were determined from one sample at each sampling, statistic analysis was not performed in this experiment. We consider from our experiences that there
Results and Discussion

Fecal microbial flora of the cattle is shown in Figs. 1 and 2. The levels of aerobic-cultured bacteria increased on day 1 after excretion, and no detectable changes occurred in the level on day 3 and day 7. The pattern of change of levels of enterobacteriaceae was similar to that of aerobic-cultured bacteria. The levels of streptococci was constant until day 7. The levels of fungi and yeasts decreased on day 1 and increased on day 7. The dominant microbe in aerobic culture was streptococci in fresh feces and was replaced by enterobacteriaceae on and after day 1.

The levels of anaerobic-cultured bacteria increased on day 1 and were constant after day 1. However, the levels of bacteroidaceae, lactobacilli and eubacteria decreased on day 1. Because the number of anaerobic-cultured bacteria in this experiment also contained enterobacteriaceae which is facultatively anaerobic bacteria, the increase in the number of anaerobic-cultured bacteria on day 1 may be caused by the increase in the number of enterobacteriaceae. After that, numbers of these anaerobes slightly increased. There is a possibility that population of anaerobes changed in the course of experiment, since it was mentioned that some anaerobes were killed by oxygen exposure for 10 min although many anaerobes could survive under the presence of oxygen for more than 24 hr3). On day 7, bacteroidaceae was most abundant among these anaerobes. The dominant anaerobic-cultured bacteria, however, may be enterobacteriaceae, because they can grow in the anaerobic culture.

The fecal microbial flora of pigs is shown in Figs. 3 and 4. No detectable changes occurred in the levels of aerobic-cultured bacteria and streptococci until day 7. The levels of enterobacteriaceae, staphylococci, and fungi and

![Fig. 1. Change in the numbers of microbes of aerobic culture in bovine feces after excretion. Number of aerobic-cultured bacteria (○), enterobacteriaceae (●), streptococci (□), staphylococci (■), yeasts and fungi (×) were determined by cultivation on TS, DHL, TATAC, PEES, PD agar plate, respectively.](image)

![Fig. 2. Change in the numbers of microbes of anaerobic culture in bovine feces after excretion. Number of anaerobic-cultured bacteria (△), bifidobacteria (◇), eubacteria (◇), bacteroidaceae (▲), lactobacilli (■) were determined by cultivation on EG, BS, ES, NBGT, and LBS agar plate, respectively.](image)
yeasts increased in the course of experiments. The dominant microbe in aerobic culture was streptococci in fresh feces and was replaced by enterobacteriaceae on and after day 3. The change of dominant bacteria was similar to that observed for microbial flora in cattle feces.

The levels of anaerobic–cultured bacteria increased until day 3, and decreased slightly on day 7. The levels of bifidobacteria increased, but the levels of eubacteria decreased in the course of experiments. There was almost no change in the levels of lactobacilli and bacteroidaceae. In the fresh feces, levels of eubacteria and lactobacilli were almost same as that of enterobacteriaceae. On and after day 1, the levels of enterobacteriaceae were larger than those of bifidobacteria, eubacteria, bacteroidaceae and lactobacilli.

The data from the cattle and pig indicated that enterobacteriaceae became the dominant microbe in feces after excretion, and that the levels of the other microbes were not drastically changed until day 7. It is interesting that even anaerobes could survive in a small mass of feces at 24°C for one week. It is known that the growth of some microbes such as enterobacteriaceae, staphylococci and enterococci are inhibited by the other microbes in the intestine\(^2,9,10,16\). Such bacterial antagonism might also occur in the fresh feces in this experiment. The increase in the number of enterobacteriaceae may suggest the disappearance of such antagonism after excretion.

Chicken fecal flora showed different pattern from those of cattle and pigs (Figs. 5 and 6). The levels of aerobic–cultured bacteria decreased on day 1, and were constant until day 7. The levels of enterobacteriaceae and streptococci showed similar pattern as that of aerobic–cultured bacteria. Staphylococci and fungi and yeasts were lower than the detectable numbers. The dominant microbes in the aerobic culture was enterobacteriaceae and streptococci.

The levels of anaerobic–cultured bacteria slightly increased on day 1, but the level of anaerobic–cultured bacteria in feces on day 7 was almost same as that in fresh feces. The levels of bacteroidaceae and eubacteria in-
creased on day 1 and decreased on day 3. The
level of bifidobacteria increased on day 1 and
were constant until day 7. The levels of
lactobacilli did not change in the course of
experiments. Characteristics in microbial
flora of chicken feces were that anaerobes were
more dominant than aerobes and the levels of
either anaerobes examined were larger than
that of enterobacteriaceae.

The differences of microbial flora among
animal species may result from the differences
in anatomy of the digestive system and compo-
ments of the feed. The chemical and physical
characteristics of feces of animal species may
affect microbial flora after excretion.

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家庭便排泄後における微生物叢の経時変化

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ウシ、ブタ、ニホトリの糞便を24℃で保存し、糞便中の微生物叢の変化を3日間観察した。ウシ糞便では、好気性菌数、嫌気性菌数、enterobacteriaceae 数および staphylococci 数は保存1日目上昇し、以後7日目までほぼ同レベルを維持した。好気性の優占種は新鮮糞便中では streptococci であったが、保存1日およびそれ以降では enterobacteriaceae であった。嫌気性菌では、bacteroidaceae が優占種であったが、その数は enterobacteriaceae 以下であった。ブタ糞便では、好気性菌および streptococci は7日までほぼ一定した数を示した。新鮮糞便中では streptococci が優占好気性菌であったが、3日およびそれ以降では enterobacteriaceae が優占種となった。嫌気性菌数は3日で上昇し、新鮮糞便中では eubacteria および lactobacilli は enterobacteriaceae とほぼ同レベルであったが、1日およびそれ以降では enterobacteriaceae が優占種となった。ウシおよびブタにおいて、enterobacteriaceae が排泄後の糞便中に優占種となること、および嫌気性菌を含めた他の菌種のレベルは7日までほとんど変化しないことが特徴的であった。ニホトリ糞便ではウシ、ブタとは異なる特徴を示し、嫌気性菌数が好気性菌数を上回り、検出した嫌気性菌がいずれも enterobacteriaceae よりも多数認められた。

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