

## 学位論文の要旨

### Abstract of Thesis

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学位論文題目 Title of Thesis (学位論文題目が英語の場合は和訳を付記)

Robustness and resilience of gut microbiota in ruminants in relation to feeding and nutritional management  
(給与飼料及び飼養管理との関連からみた反芻動物腸内細菌叢の堅牢性及び順応性)

学位論文の要旨 Abstract of Thesis

Gastrointestinal tract of ruminant is the natural habitat for a dense and dynamic microbial community. The symbiotic microbiota is in charge of digesting and fermenting plant materials into nutrient sources that are acquired for body maintenance, growth, and production by the host. The divergence of gut microbiota is affected by many factors including genotype, aging, farm management, geographical locations, and diet formulation. Food-borne microbiota, i.e. lactic acid bacteria in silage, may also confer transient changes in the gut microbiota. Meanwhile, differences can be seen even when animals consume the same diet and show similar physiological profiles. Because microbial activity and metabolism in the gut is a key to secure health and productivity of ruminants, understanding robustness and resilience of the gut microbiota is of great importance. In this thesis, three experiments were carried out to examine variability, stability, and adaptability of gut microbiota in relation to feeding and nutritional management. Feces were used to determine the gut microbiota in heifer and dairy cow experiments, and digesta and mucosa samples collected from various gut segments were used in goat experiment. Silage was given to animals in the entire or a part of test period; hence, the effect of food-borne bacteria on the gut microbiota was also examined.

In the first experiment, fecal microbiota of seven Holstein heifers was examined during growing stage, wherein weaning and commencement of silage feeding were practiced. Denaturing gradient gel electrophoresis (DGGE) and quantitative PCR (qPCR) were employed for microbiota analyses. Populations of total bacteria and *Bacteroides-Prevotella-Porphyromonas*, *Clostridium* XIVa, and *Clostridium* I-II groups were relatively stable in abundance during the growing stage, and those of *Lactobacillus* and *Bifidobacterium* groups decreased after weaning. Regarding bacterial composition, distinctive changes were shown after weaning in all bacterial taxa examined, whereas no changes were observed thereafter. The composition of *Lactobacillus* group changed over the growing stage, while maintaining high similarity between individual heifers. These findings indicated that weaning had a marked influence on the gut microbiota especially in the composition. Management during this transition period may be critical in shaping gut microbiota, whereas silage microbiota would have no effects.

In the second experiment, the microbiota of whole crop corn silage and feces of 18 silage-fed lactating cows were examined in Japan and China, using high-throughput Illumina MiSeq sequencing. Corn silage was provided at 20-40% ratio in diet on a dry matter basis. *Lactobacillaceae* were dominant in all silages, followed by *Acetobacteraceae*, *Bacillaceae*, and *Enterobacteriaceae*. In feces, the predominant families were *Ruminococcaceae*, *Bacteroidaceae*, *Clostridiaceae*, *Lachnospiraceae*, *Rikenellaceae*, and *Paraprevotellaceae*. Therefore, *Lactobacillaceae* of corn silage

appeared to be eliminated in the gut. Although fecal microbiota composition was similar in most samples, relative abundances of several families, such as *Ruminococcaceae*, *Christensenellaceae*, *Turicibacteraceae*, and *Succinivibrionaceae* varied between farms and countries. In addition to the geographical location, differences in feeding management between total mixed ration feeding and separate feeding appeared to be involved in the variations. Moreover, a cow-to-cow variation for concentrate-associated families was demonstrated at the same farm; two cows showed high abundance of *Succinivibrionaceae* and *Prevotellaceae*, whereas another had a high abundance of *Porphyromonadaceae*. These results indicated adaptive responses of gut microbiota to feeding management, with individual variations observed in the degree of responses even in the same farm.

In the third experiment, digesta- and mucosa-associated microbiota were examined across the gastrointestinal tract using goats. Corn silage was given at 40% ratio in diet on a dry matter basis. In addition to feed (silage) and rectum (feces) samples, rumen, abomasum, duodenum, and cecum samples were collected and subjected to DGGE and qPCR analyses. Regardless of digesta- and mucosa-associated samples, the bacterial populations varied across gut segments and between bacterial taxa. In digesta-associated samples, the populations of total bacteria, *Bacteroides-Prevotella-Porphyromonas* group, *Faecalibacterium prausnitzii*, and *Ruminococcus albus* were low at duodenum, whereas the numbers appeared similar between rumen and large intestine. The population of *Fibrobacter succinogenes* was greater in the rumen than in the large intestine, whereas the opposite trend was seen for the number of *Enterobacteriaceae*. The population of *Lactobacillus* group was highly stable across the gut segments. In mucosa-associated samples, variations of bacterial numbers between gut segments were small regardless of bacterial taxa, whereas the numbers of *Enterobacteriaceae*, *Lactobacillus* group, *F. prausnitzii*, and *R. albus* were higher at duodenum than others. *Lactobacillus* spp. in silage had no influence on gut-associated *Lactobacillus* spp. Difference of *Lactobacillus* composition between digesta- and mucosa-associated samples was shown regardless of gut segments. These results indicated dynamics of bacterial populations in the gastrointestinal tract and concrete differences between digesta- and mucosa-associated microbiota.

In conclusion, diet formulation and feeding management showed substantial influence on shaping the gut microbiota in ruminants, whereas food-borne bacteria like silage lactic acid bacteria had little ability of modulation. Weaning had a great impact regardless of bacterial taxa and difference between total mixed ration feeding and separate feeding had effects on the proportions of concentrate- and fiber-associated bacteria. Variations with regard to farms, countries, and individual cows were also seen in relative abundances of several bacterial families. Differences between gut segments and between digesta- and mucosa-associated samples were distinctive. Because most of the findings to date are derived from rectum samples, there is still a large room for improvement on understanding the roles and functions of gut microbiota in ruminants.