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授与した学位	博士		
専攻分野の名称	農学		
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学位授与の要件	環境生命科学研究科 農生命科学専攻 (学位規則第4条第1項該当)		
学位論文の題目	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)		
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<b>学位論文内容の概要</b>			
<p>The divergence of gut microbiota in ruminants is affected by many factors including genotype, aging, farm management, geographical locations, and diet formulation. Likewise, 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. Therefore, in this thesis, three experiments were carried out to examine variability, stability, and adaptability of gut microbiota in relation to feeding and nutritional management.</p> <p>In the first experiment, fecal microbiota of seven Holstein heifers was examined during growing stage, wherein weaning and feeding of silage, a lactic acid bacteria-enriched fermented food, were practiced. Populations of total bacteria and <i>Bacteroides-Prevotella-Porphyromonas</i>, <i>Clostridium XIVa</i>, and <i>Clostridium I-II</i> groups were relatively stable during the growing stage, and those of <i>Lactobacillus</i> and <i>Bifidobacterium</i> groups decreased after weaning. Regarding bacterial composition, distinctive changes were shown after weaning in all bacterial taxa examined, whereas no changes were observed thereafter.</p> <p>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. Although fecal microbiota composition was similar in most samples, relative abundances of several families, such as <i>Ruminococcaceae</i>, <i>Christensenellaceae</i>, <i>Turicibacteraceae</i>, and <i>Succinivibrionaceae</i> 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.</p> <p>In the third experiment, digesta- and mucosa-associated microbiota were examined across the gastrointestinal tract using goats. The bacterial populations varied across gut segments and between bacterial taxa. In digesta-associated samples, the populations of total bacteria, <i>Bacteroides-Prevotella-Porphyromonas</i> group, <i>Faecalibacterium prausnitzii</i>, and <i>Ruminococcus albus</i> were low at duodenum, whereas the numbers appeared similar between rumen and large intestine. The population of <i>Lactobacillus</i> 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 <i>Enterobacteriaceae</i>, <i>Lactobacillus</i> group, <i>F. prausnitzii</i>, and <i>R. albus</i> were higher at duodenum than others.</p> <p>It is concluded that 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. Variations with regard to farms, countries, feeding regimes, and individual cows and differences between gut segments and between digesta- and mucosa-associated samples were also clarified. 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.</p>			

## 論文審査結果の要旨

腸内細菌の構成と機能を理解することは、反芻動物の栄養、健康及び生産性を制御するうえで重要である。給与飼料、月齢、遺伝、管理衛生、地理的環境などが腸内細菌叢を変動させると考えられているが、同じ環境及び飼料で飼育されている個体同士でも違いがある。TANG Thuy Minh 氏は、反芻動物の腸内細菌叢がもつ多様性、安定性及び順応性について、飼養管理との関連を中心に調査研究を行った。

最初の実験では、ホルスタイン種育成牛（雌）を対象として、成長過程における腸内細菌叢の変化を調べた。離乳後に *Lactobacillus* spp. 及び *Bifidobacterium* spp. は減少したが、*Bacteroides-Prevotella-Porphyromonas*, *Clostridium XIVa* 及び *Clostridium I-II* の菌数は、離乳前後でも大きく変化しなかった。発酵飼料には *Lactobacillus* spp. が数多く存在したが、その給与で腸内細菌叢が変化することはなかった。

続く実験では、日本と中国で泌乳牛から糞便を採取し、MiSeq による腸内細菌叢解析を行った。*Ruminococcaceae*, *Christensenellaceae*, *Turicibacteraceae* 及び *Succinivibrionaceae* は、農場、調査国及び分離給与と TMR（混合飼料）給与という飼養管理法による違いが認められた。一方、穀類消化に関わる細菌群が同一農場でも個体によって異なる構成を示すなど、腸内細菌叢には一定の適応性が認められた。

最後の実験では、腸内容物と腸粘膜に存在する細菌群を分けて解析した。発酵飼料を含む TMR をシバヤギに給与し、第 1 胃、第 4 胃、小腸、盲腸及び直腸からサンプルを採取して腸内細菌叢を調べた。腸内容物に存在する *Bacteroides-Prevotella-Porphyromonas*, *Faecalibacterium prausnitzii* 及び *Ruminococcus albus* は第 4 胃で菌数が低下したが、第 1 胃と直腸ではほぼ同様の高値を示した。腸粘膜に存在する細菌群は採取部位に関わらず安定した菌数を示し、*Lactobacillus* spp. は給与飼料の *Lactobacillus* spp. にほとんど影響されなかった。

これらの知見は、反芻動物の腸内細菌叢に給与飼料を含めた飼養管理技術が強く影響することを示している。飼料として与えた *Lactobacillus* spp. が腸内細菌叢を改善しないことも意義ある知見であり、TANG Thuy Minh 氏は博士（農学）の学位を授与するに値すると判断した。