

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)

Microbiological studies on constraints and opportunities of dairy cattle production in Vietnam
(ベトナム酪農の制限要因及び発展性に関する微生物学的研究)

学位論文の要旨 Abstract of Thesis

Vietnam is a tropical country and the southern regions like Ho Chi Minh City and surrounding Mekong Delta have distinct wet and dry seasons with air temperature ranging from 25°C to 35°C and humidity at about 80%. Hot and humid climates have negative impacts on productivity of dairy cows, especially for high-yielding and improved breeds. Nevertheless, the population of dairy cows in Vietnam has increased at an annual rate of about 10% in the last decade, and nearly half of them are reared in and around Ho Chi Minh City. Average milk yield per cow is as low as 4,600 kg per year; hence, dairy industry of Vietnam still has a large room for improvement in productivity. Regardless, the fact that biomass production is rapid and enormous in tropical environments should be a great advantage in regard to feeding and nutrition. If proper preservation is undertaken with forages and local feed resources including by-products, feed supply for ruminant livestock can be stabilized. Meanwhile, farmers and industries have yet suffered from production-related diseases such as mastitis and infertility, because dairy production is not a traditional practice in Vietnam. The prevalence of subclinical mastitis (>200,000 somatic cell count per milliliter of milk) was reported to be 88% at cow-level, and 50% of cows were shown undetected in estrus on day 60 postpartum at dairy smallholders in Ho Chi Minh City. Although these diseases are considered multifactorial, infectious agents are involved and the traits can be exacerbated by nutrition and management. In this thesis, four experiments were carried out to examine potentials, limitations, and prospects of dairy cattle production in Vietnam. Topics include forages and by-products utilization, feeding and nutrition, and farm management in relation to mastitis and endometritis prevention.

In the first experiment, fermentation characteristics and aerobic stability of total mixed ration (TMR) silage was examined. Rice straw (Rs) and corn stover (Cst) were mixed with molasses (M) and other feeds as TMRs, which were then preserved as silage in Hue, Vietnam. The bacterial community was assessed by denaturing gradient gel electrophoresis (DGGE). All the silages were well preserved, with lactic acid serving as the major preservative. However, the lactic acid content was low in the Rs-M silage, while the ethanol content was substantial in the Cst-M silage. Heating due to spoilage was observed at 115 h and 81 h after silo opening in the Cst-M and Cst-TMR silages, respectively, whereas no heating was observed for 7 d after the opening of the Rs-M and Rs-TMR silages. Among the 14 bacterial strains identified in the silages by the DGGE analysis, only three were lactic acid bacteria (LAB). In the aerobically unstable Cst-TMR silage, bands indicative of

Acetobacter pasteurianus, *Staphylococcus* sp., and *Streptomyces* sp. were specifically observed. These results indicate that although desirable lactic acid fermentation can be expected in a TMR silage in a tropical environment, aerobic stability is lowered if Cst instead of Rs is used as the ingredient crop.

In the second experiment, direct-cut and wilted elephant grass silages were prepared in Hue, Vietnam, with and without a mixture of *Lactobacillus paracasei* and *Lactococcus lactis* (LP+LC) or *Lactobacillus buchneri* (LB). The microbial community was assessed using DGGE and quantitative PCR (qPCR). Acetate was predominantly produced during the fermentation of control and LB-inoculated silages, whereas the content was not sufficient to inhibit aerobic spoilage for 7 days in control silage. LP+LC inoculation greatly enhanced lactate content, suppressed the alcohol content, and did not improve aerobic stability or decrease the total fungal population. The bacterial community and *L. buchneri* populations were similar between control and LB-inoculated silages; however, *Kluyveromyces delphensis* was undetectable and the total fungal population was lowered in LB-inoculated silage.

In the third experiment, a practical survey of fecal microbiota was performed by visiting 12 small-scale dairy farms at three regions (Tay Ninh, Tien Giang, and Lam Dong) in southern Vietnam. Fifty-four fecal samples were collected from 12 farms and qPCR was employed to examine the microbiota. The populations of total bacteria and *Bacteroides-Prevotella-Polyphyromonas* group were stable regardless of the regions. Cows in Lam Dong, where climate is similar to temperate and cows show good milk performance, had higher *Ruminococcus albus* and *Lactobacillus* populations than cows in Tay Ninh and Tien Giang. The methanogen population were not different in individual cows and regions.

In the fourth experiment, the relationship between the fecal, milk, uterine, airborne dust, and water microbiota in dairy farms in southern Vietnam was evaluated. Two sets of airborne dust and water samples from the cowshed and six sets of fecal, milk, and uterine samples from dairy cows were collected from two farms in Ho Chi Minh City at 20-40 days postpartum. The microbiota was characterized using Illumina MiSeq sequencing. The predominant species in the fecal microbiota was not as predominant in the milk microbiota, but occasionally appeared in the uterine microbiota; this result indicated that the fecal microbiota might be more likely to contaminate the uterus than the milk microbiota. The microbiota from the airborne dust and water samples did not show a relation with either the milk or the uterine microbiota, whereas *Moraxellaceae*, the most abundant family in the airborne dust and water microbiota, was detected at low proportions in the milk microbiota. The farm-to-farm difference was more apparent for the milk microbiota than for the fecal and uterine microbiota, indicating a significant influence of milking management on the microbiological quality of milk. It is noteworthy that *Methylobacteriaceae*, *Dermacoccaceae*, and *Bacillaceae* were the prevalent families in the microbiota of several milk and uterine samples; however, these findings did not help us understand the role of the microbiota in fertility and subsequent conception.

In conclusion, although acetic or alcoholic fermentation can take place in grass and by-products ensiling, desirable lactic acid fermentation may be obtained if LAB are inoculated and stored as TMRs. To secure aerobic stability, ensiling as TMRs is recommended rather than use of homo-fermentative LAB. Based on practical survey of fecal microbiota, a high productivity in Lam Dong farms could be due to increased populations of *Ruminococcus albus* and *Lactobacillus* group. The fact that methanogen populations were similar between three regions indicates that relative energy loss through methane may be greater in Tay Ninh and Tien Giang than Lam Dong. Because the predominant species in the fecal microbiota was not as predominant in the milk microbiota and occasionally appeared in the uterine microbiota, further cares need to be provided to avoid fecal microbiota contamination with the uterus microbiota.