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）

Milk, udder skin, and fecal microbiota and their relationships with blood metabolites and milk composition in dairy herds

（乳牛の乳汁、乳房皮膚および糞便細菌叢と血液性状および乳成分の関係）

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Owing to improvements in milking devices and procedures, milk is now less likely to be contaminated with high numbers of pathogens that cause contagious mastitis and foodborne illnesses. However, controlling the pathogens that cause environmental mastitis remains insufficient and requires further improvement. Once mastitis occurs, it is difficult to compensate for losses during treatment because milk production after recovery is often lower than that expected without mastitis. Thus, improved hygiene management and diagnosis methods that prevent mastitis should be further explored.

Although the cowshed microbiota affects the milk microbiota, transmission cannot be straightforward. Udder skin that comes into contact with the barn and bedding may have defense and elimination functions against pathogens and contaminants. Many studies have reported the relationship between udder skin, teat skin, and milk microbiota; however, little is known about whether the skin microbiota varies with nutritional status and milk composition and how bedding, udder skin, and milk microbiota are related.

This study examined the milk, udder skin, and fecal microbiota in healthy cows managed using automatic milking systems in a free-stall barn. Samples of bedding microbiota and blood metabolites were also collected and analyzed. The first experiment was conducted in September 2018, and the same set of samples were collected in the second experiment in August 2020.

In Exp. 1, samples were collected from 10 non-mastitic cows with an average of 95 days in milk (DIM), 33.4 kg/d milk yield, 3.01% milk protein, 3.28% milk fat, 8.62% milk solid-not-fat (SNF), 18.4 g/L milk urea nitrogen (MUN), and 119×10^3 cells/mL somatic cell count (SCC). Two cows had high SCC ($>10^6$ cells/mL), but no distinctive differences were found in milk composition and blood metabolites compared with the other eight cows. The principal component analysis (PCA) plots indicated cows with high SCC appeared to produce milk with high fat content and low plasma non-esterified fatty acid (NEFA) concentration. Plasma haptoglobin (HP) concentration appeared to be low in cows with high MUN but did not have a relationship with SCC.

The five most abundant families of milk microbiota were Moraxellaceae (35.3%), Bacillaceae (13.1%), Lactobacillaceae (8.5%), Streptococcaceae (4.7%), and Lachnospiraceae (3.7%), and those of udder skin microbiota were Peptostreptococcaceae (10.2%), Moraxellaceae (7.9%), Carnobacteriaceae (7.8%), Corynebacteriaceae (7.3%), and Lachnospiraceae (7.1%). The five most abundant taxa of fecal microbiota were Oscillospiraceae (15.5%), Lachnospiraceae (9.0%), Rikenellaceae (8.4%), RF39 (7.3%), and Christensenellaceae (5.9%), and those of bedding

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microbiota were Corynebaceriaceae (16.1%), Carnobacteriaceae (13.6%), Peptostreptococcales-Tissierellales (7.7%), Staphylococcaceae (7.1%), and Oscillospiraceae (5.5%). The principal coordinate analysis (PCoA) demonstrated that milk, udder skin, and fecal microbiota were separate groups, and the bedding microbiota was grouped with the udder skin microbiota.

In Exp. 2, samples were collected from another 10 non-mastitic cows with an average of 145 DIM, 35.7 kg/d milk yield, 3.22% milk protein, 4.03% milk fat, 9.02% milk SNF, 13.0 g/L MUN, and 36×10^3 cells/mL SCC. All cows had acceptably low SCC ($<10^6$ cells/mL). The PCA plots indicated cows with high SCC appeared to have high plasma cholesterol and urea nitrogen concentrations and low NEFA concentrations. Similar to the results of Exp. 1, plasma HP concentration appeared to be low in cows with high MUN without a clear relationship with SCC.

The five most abundant families of milk microbiota were Muribaculaceae (17.9%), Lactobacillaceae (15.9%), Lachnospiraceae (14.1%), Eubacterium coprostanoligenes group (9.5%), and Rhizobiaceae (8.6%), and those of udder skin microbiota were Lachnospiraceae (9.2%), Lactobacillaceae (8.1%), Muribaculaceae (7.3%), Rhizobiaceae (7.2%), and Sphingomonadaceae (6.1%). The five most abundant taxa in fecal microbiota were Oscillospiraceae (19.0%), Rikenellaceae (16.2%), Lachnospiraceae (9.2%), Bacteroidaceae (6.0%) and Prevotellaceae (5.7%), and those of bedding microbiota were Carnobacteriaceae (15.5%), Corynebaceriaceae (9.7%), Oscillospiraceae (8.8%), Planococcaceae (5.5%), and Aerococcaceae (5.4%). The PCoA demonstrated that milk, udder skin, fecal, and bedding microbiota were separate groups. In both Exp. 1 and 2, the abundances of typical environmental pathogens, i.e., Enterobacteriaceae, Staphylococcaceae, and Streptococcaceae, were low (<0.5%) in fecal microbiota and did not exceed 5% in udder skin and milk microbiota.

Analyzing the 2018 and 2020 samples revealed that Ruminococcaceae, Christensenellaceae, and Oscillospiraceae characterized the fecal microbiota. Moraxellaceae, Bacillaceae, and Enterococcaceae characterized the milk microbiota in the 2018 samples and Oxalobacteraceae and Muribacuraceae in the 2020 samples. Erysipelotrichaceae characterized the udder skin microbiota in the 2018 samples, and Rhizobiaceae, Sphingomonadaceae, and Lactobacillaceae in the 2020 samples. Through network analysis, many bacterial taxa in the milk, udder skin, and fecal microbiota were shown to be related to blood metabolites and milk composition. However, only a few have shown a positive or negative relationship across the 2018 and 2020 samples. None of the milk composition was related to milk, udder skin, or fecal microbiota. Except for the relationships between BUN and udder skin Erysipelotrichaceae and between BUN and udder skin Peptostreptococcaceae, blood metabolites were unrelated to milk, udder skin, or fecal microbiota. No blood metabolites were associated with typical mastitis pathogens, regardless of the milk, udder skin, fecal, or bedding microbiota.

These findings indicated that milk and udder skin microbiota varied at the herd level and were unrelated to the nutritional status and milk composition in cows without clinical mastitis. Although several prevalent taxa were shared in the bedding and udder skin microbiota and the udder skin and milk microbiota, selection and elimination occurred during transmission, regardless of pathogens and non-pathogens. The udder skin and milk microbiota were not related to the milk composition and blood metabolites, indicating that the nutritional status of the cows may not dictate milk contamination with environmental microbiota.