氏 名 GATHINJI Peter Kiiru 授与した学位 博 士: 学 専攻分野の名称 農 学位授与番号 博甲第 6 7 3 3 号 学位授与の日付 2022年 22日 9月 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) Lactation-long changes in milk microbiota, milk composition, and metabolic profiles of Holstein and Jersey cows and the environmental microbiota of their barns 学位論文の題目 (ホルスタインおよびジャージーの泌乳期間における乳汁細菌叢,乳成分,代謝プロファ イルと牛舎環境細菌叢の変化に関する研究) 論文審査委員 教授 森田 英利 准教授 荒川 健佑 准教授 鶴田 副日 教授 西野 直樹

## 学位論文内容の要旨

The global demand for high-quality milk is soaring and this demand galvanizes farmers to boost high-quality milk production while considering improving the breed of dairy cows. Jerseys produce milk with high protein and fat contents compared to Holsteins, while Holsteins promise a much higher milk yield than Jerseys. Lactating cows experience variable metabolic changes, especially during the periparturient period, which may impact milk composition and microbiota. Two experiments were conducted in this study using Holstein and Jersey cows. Milk and blood samples were collected every other month from eight Jersey and Holstein cows reared in Okayama, Japan, throughout a lactation period. Airborne dust and bedding samples were also collected to investigate the influence of environmental microbiota on milk microbiota.

Experiment 1 examined a Holstein herd at the Okayama Prefecture Livestock Research Institute. Milk fat and protein contents increased steadily as the lactation period advanced. The SCC level remained unchanged and below the threshold (2.0×10<sup>5</sup> cells/mL), indicating that the cows were healthy across the lactation. Although plasma total cholesterol (T-Cho) and non-esterified fatty acids (NEFA) levels remained stable across the lactation, the GOT and GPT levels were unexpectedly higher towards the end of the lactation period. Milk microbiota was dominated by Lactobacillaceae, Lachnospiraceae, Muribaculaceae, Micrococcaceae, and Eubacteriaceae. This finding was in contrast to other studies that often reported changes across seasons and lactation periods. Airborne dust microbiota was predominated by S24-7, Lactobacillaceae, Ruminococcaceae, and Lachnospiraceae, and the principal coordinate analysis (PCoA) clustered milk and airborne microbiota together. Hence, the airborne dust microbiota may significantly influence the milk microbiota.

Experiment 2 examined a Jersey herd at the Chugoku-Shikoku Dairy College. Milk fat and protein contents increased as the lactation period advanced. The SNF and MUN levels were higher at the advanced stages of the lactation period, possibly due to the decline in milk yield. The levels of plasma total protein were higher in the late than in the early lactation. The plasma NEFA was the highest in the first month after parturition and decreased progressively towards the last month of the lactation. The T-Cho was lowest in the first month, peaked in the second month, and progressively declined. Plasma GOT and GPT levels also fluctuated across the lactation and peaked in the fourth month after parturition. The most abundant families of the milk microbiota across the lactation period were Burkholderiaceae, Enterobacteriaceae, Oxalobacteriaceae, Ruminococcaceae, and Erysipelotrichaceae. Burkholderiaceae and Oxalobacteriaceae were also detected in airborne dust and the abundances changed comparably to the milk microbiota. However, the PCoA clustered milk and airborne microbiota separately, except for the samples collected in the first month after parturition. The susceptibility of milk microbiota to the contaminants of cowshed may vary between seasons and be affected by the nutritional and metabolic statuses. Indeed, plasma GOT levels were positively and negatively related to the abundance of Burkholderiaceae and Enterobacteriaceae in milk.

The two studies in this thesis indicated that the milk microbiota of healthy cows could be contaminated with opportunistic bacteria in the cowshed, without showing mastitic symptoms and elevated SCC levels. The contamination of cowshed microbiota would increase when the cows are at a depression of metabolic function like increased GOT levels. Subtle disorders do not necessarily induce mastitis and other periparturient problems.

## 論文審査結果の要旨

本研究では、ホルスタイン種とジャージー種を対象として、泌乳期間を通した血液性状と乳汁細菌叢の変化および乳汁細菌叢と牛舎環境細菌叢の関係について調査した。世界的に高品質な牛乳の需要が高まっており、牛乳タンパク質や脂肪の割合が高いジャージー種に興味を抱く生産者は多い。

実験1では、岡山県畜産研究所のホルスタイン種8頭を調査対象とした。泌乳期間が進むにつれて乳脂肪および乳タンパク質含量は増加した。体細胞数は横ばいで基準値以下であり、泌乳期を通じて健康な牛であった。血漿中の総コレステロール(T-Cho)および遊離脂肪酸(NEFA)濃度は泌乳期を通して安定していたが、GOTおよびGPT値は泌乳期終盤に予想外の高値を示した。乳汁細菌叢は、Lactobacillaceae、Lachnospiraceae、Muribaculaceae等が優勢で非常に安定しており季節や泌乳期で変化するという他の報告とは異なっていた。主座標分析(PCoA)では乳汁細菌叢と空気粉塵の細菌叢が同じクラスターに分類された。

実験2では、中国四国酪農大学校のジャージー種8頭を調査対象とした。実験1と同様に泌乳期が進むにつれて乳脂肪および乳タンパク質含量は増加した。血漿中のNEFA濃度は分娩後1ヶ月目に最も高く、泌乳初期は著しい負のエネルギーバランスにあると判断された。泌乳期間中最も割合が高かった乳汁細菌叢は、Burkholderiaceae、Enterobacteriaceae およびOxalobacteriaceaeであった。BurkholderiaceaeとOxalobacteriaceaeは空気粉塵からも検出され、その割合は乳汁細菌叢と同じように変化した。血漿中のGOT濃度は、乳中のBurkholderiaceaeおよびEnterobacteriaceaeの存在量と正および負の関係があり。乳汁細菌叢が空気粉塵に汚染される程度は栄養状態に影響されると考えられた。

本研究から、乳房炎の予防を含む高品質牛乳の生産には、飼養および衛生管理が重要であることが確認された。16SrRNAアンプリコン解析でこれらを示したことは新規性も高く、Gathinji氏は環境生命科学研究科の博士(農学)の学位を受ける資格があるものと判断した。