学 位 論 文 の 要 旨

論文題目　**Studies on Mastitis Caused by Translocated-Bacterial Components in Ruminants（反芻動物における細菌成分の移行による乳房炎に関する研究）**

広島大学大学院生物圏科学研究科
生物資源科学専攻
学生番号　D170512
氏　　名　Fika Yuliza Purba

Mastitis is one of the most important diseases in ruminants regarding the economic, animal health, and food hygiene perspectives. Knowledge of the causative pathogens is important to effectively control intramammary infections. Lipopolysaccharide (LPS), a Gram-negative bacterial component can translocate within the body and its presence can induce the inflammatory response, whereas the mechanism of its translocation to the mammary gland is poorly understood. This study aimed to determine whether mastitis can be caused by bacterial components (LPS) through the endogenous pathway.

1. Association of endometritis with the mammary gland inflammatory condition in dairy cows

Multiple metabolic and inflammatory diseases such as endometritis and mastitis occur during the periparturient period in dairy cows. If the bacterial component can be transferred, there must be a close association between endometritis and mastitis. Thus, this study aimed to examine the possible association of endometritis with mammary gland inflammatory conditions in post-partum dairy cows. Sixty-one Holstein dairy cows were clinically examined for endometritis at 30-50 days after parturition. Blood and milk samples were collected for the measurement of Haptoglobin (Hp), and lipopolysaccharide-binding protein (LBP) in plasma, and somatic cell count (SCC), IL-1β, and TNFα, and IL-8 concentrations in milk. Of the 61 dairy cows, 49 cows were determined as healthy cows, while 12 cows were diagnosed with endometritis. The average and maximum SCCs were not significantly different between the healthy cows and those with endometritis. However,

when the maximum SCC was classified as <300, 300-1,000, or >1,000 × 10^3 cells/ml, the percentage of cows with the maximum SCC < 300 × 10^3 cells/ml was significantly lower in the endometritis group than in the healthy group. These results suggested that high SCC might be related to the occurrence of endometritis in dairy cows, indicating that some bacterial components can be transferred between the uterus and mammary gland.

2. **Effects of intrauterine infusion of bacterial lipopolysaccharides on the mammary gland inflammatory response in goats**

This experiment aimed to determine the effects of LPS infusion on the mammary gland inflammatory response to clarify the possibility of bacterial components translocation from the uterus to the mammary gland. Sixteen goats were divided into two groups, subjected to intrauterine infusion of LPS or saline (control). The concentrations of IL-1β and IL-6 in milk were higher in the LPS group compared to that in the control group, whereas there were no changes in milk yield or SCC. In the immunohistochemistry, LPS was detected in the connective tissues and interepithelial spaces of the alveoli of the mammary glands at 24 h after intrauterine infusion of LPS. These results suggest that intrauterine-infused LPS can be translocated from the uterus to the mammary glands and caused some inflammatory responses.

3. **Effects of intrauterine infusion of bacterial lipopolysaccharides on the mammary gland inflammatory response in dexamethasone-treated goats**

The combined effects of multiple stressors, including high and low temperature, nutritional deficiency, parturition, the transition to the milking herd, and herd management during the periparturient period, can increase and prolong the magnitude of immunosuppression and increase animal susceptibility to disease. The previous study revealed that LPS can translocate from the uterus to the mammary gland and induce a weak inflammation, thus, this experiment aimed to determine if dexamethasone (immunosuppressive agent) treatment enhances the translocation of LPS from the uterus to the mammary gland to induce a heavy inflammatory response. Sixteen goats were divided into control and LPS infusion groups after daily dexamethasone administration for 5 days. Milk and blood samples were collected before and after LPS infusion. The mean SCC in the LPS group was significantly higher, whereas the milk yield was significantly lower than that in the control group after LPS infusion. Plasma lipopolysaccharide-binding protein (LBP) and serum amyloid A (SAA) concentrations were significantly higher in the

LPS group than in the control group after LPS infusion. Furthermore, the LPS group has higher milk concentrations of IL-1β, S100A8, and lactoferrin than the control group after LPS infusion. In the immunohistochemistry, LPS was detected in the connective tissues and inner alveolar spaces of the mammary glands at 24 h after LPS infusion. These results suggest that dexamethasone administration enhanced the translocation of intrauterine-infused LPS to the mammary gland, where it induced a heavier inflammatory response than in normal physiological conditions.

4. Translocation of carbon black particles from the uterus to mammary gland

In the previous experiment, LPS was detected in the mammary gland, whereas this cannot prove that LPS observed in the mammary gland comes from the uterus. Therefore, this study aimed to confirm LPS translocation from the uterus to the mammary gland using carbon black ink. Four goats were assigned to 4 different treatments of carbon black ink intrauterine infusion. Two goats were intrauterine infused with carbon black ink and sacrificed at 6 and 24 h after carbon black ink infusion. Two other goats were induced with mastitis by LPS intramammary infusion, 16 h before infused with carbon black ink into the uterus and sacrificed at 6 and 24 h after carbon black ink infusion. Carbon particles were found in the mammary tissue of goat with mastitis and sacrificed 6 h after carbon black ink infusion. These results suggested that carbon particles can translocate from the uterus to the mammary gland through the bloodstream. However, the translocation of carbon particles to the mammary gland might be influenced by the inflammatory condition of the mammary gland.

5. Conclusion

The high SCC in milk may be associated with the occurrence of endometritis in dairy cows. Besides live bacteria, a bacterial component such as LPS in the uterus can also cause the inflammation of the mammary gland through the bloodstream. The inflammatory response of the mammary gland caused by LPS translocated from the uterus was heavier in the immunosuppressive condition compared to normal physiological conditions in the animal. This knowledge is expected to be useful to understand the mechanism of LPS translocation in dairy cows and establish the control mechanism of mastitis.