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Abstract of Thesis/Dissertation

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Title : Monitor and modulate immune responses in bovine mastitis to improve antibiotic stewardship and efficiency in milk production

(抗菌性物質の適正使用と乳生産効率の改善に向けた牛乳房炎における免疫応答の監視と調節)

Abstract

Mastitis, one of the most common diseases in dairy cows, is the inflammation of mammary glands, mainly triggered by bacteria invading mammary glands. Mastitis impacts animal health and milk production significantly, thus one major reason for giving dairy cows antibiotics is to treat or prevent mastitis. However, huge antibiotic use in dairy farming has raised public concerns over the emergence of antimicrobial resistance, and antibiotic therapies for mastitis control are occasionally unnecessary or inefficient. This thesis aimed to improve antibiotic stewardship in mastitis control without sacrificing animal health and the efficiency of milk production, by monitoring or modulating the immune responses of cows.

Selective dry cow therapy (**SDCT**) allocates antibiotics selectively to cows with higher intramammary infection (**IMI**) risks at dry-off. In Chapter 1, we investigated the usefulness of SDCT. In the trial, we used milk culture, California mastitis test (**CMT**), and mastitis records to identify cows supposed to receive antibiotic treatment. We evaluated herd hygiene and the duration of teat sealant adherence to elucidate their influences on SDCT. SDCT reduced antibiotic use by around 33%, but poor herd hygiene and shortened duration of external teat sealant adherence led to increased risks of IMI in quarters that had not received antibiotics.

The CMT is currently the most popular and accurate cow-side test to detect mastitis, working through lysing and precipitating somatic cells. In Chapter 2, we investigated the dynamics of somatic cell score (**SCS**), differential somatic cell count (**DSCC**; the combined proportion of polymorphonuclear leukocytes and lymphocytes in somatic cells), and macrophage proportion (**MAC**; equaling 100 – DSCC) during mastitis and how they cause variations in the CMT results. We performed the CMT on d 0, 3, 5, 7, 14, and 21 after identifying mastitis, and simultaneously measured SCS, DSCC, and MAC. Data were analyzed using a cumulative logit mixed model. Both SCS and MAC are positively related to CMT scores. During the healing and chronic stages of mastitis, MAC tends to increase, thus in these stages of mastitis milk with low SCS may show positive CMT reactions.

Immune responses in mastitis have been known to differ depending on the causative pathogen and disease severity, but the effects of these factors on DSCC have yet to be investigated. In Chapter 3, we investigated how the severity and causative pathogen of mastitis influence immune indicators, namely SCS, white blood cell count (**WBC**), the albumin/globulin ratio (**A/G ratio**), and DSCC. We collected blood and milk samples on 0, 3, 5, 7, 14, and 21 days after mastitis occurred, and grouped the cases by disease severity and causative pathogen. Data were analyzed using a linear mixed model and estimated marginal means for levels of indicators were calculated at each time point and compared between groups. We found WBC changed drastically in coliform or severe mastitis, slightly increased in streptococcal mastitis but remained stable in staphylococcal mastitis. The A/G ratio dropped sharply only in severe mastitis. A non-linear relationship was found between DSCC and SCS, and this relationship was affected by the pathogen. When cows recovering from *Streptococcus dysgalatiae* mastitis, DSCC decreased while SCS remained high, suggesting a recovery process requiring more macrophages.

The impact of mastitis on milk production can be estimated using SCS and DSCC, but previous investigations were mostly based on predefined thresholds of these two traits or neglected their interaction, possibly causing bias. In Chapter 4, we used generalized additive models to estimate the nonlinear interaction of SCS and DSCC and its effect on milk yield and composition by different parity of cows. Results show that the effects of SCS and DSCC were nonlinear, and they interacted with each other to affect milk yield and composition. Milk yield was negatively related to SCS but positively related to DSCC. When SCS was high or when

DSCC was low, the effect of the other somatic cell trait was more pronounced. Cows with high SCS and low DSCC may suffer from chronic mastitis, and milk loss and composition change were most severe in such cows. Especially for cows with high parity, their milk yields can be reduced from 40 kg/d to 20 kg/d.

Chitosan is a biomaterial with immunomodulatory properties, which has been proposed as an alternative to antibiotics for controlling infectious diseases. In Chapter 5, we conducted two experiments to evaluate whether we can apply chitosan to prevent dry period IMI and recurrent clinical mastitis. In the first experiment, we enrolled cows without IMI before dry-off, and we orally administrated low-molecular-weight chitosan 10 g for 3 days to half of these cows at dry-off. We compared the A/G ratio, cytokine levels, and dry-period IMI risks between cows that had received and not received chitosan. In the second experiment, we enrolled cows with clinical mastitis, and from the next day, we orally administrated low-molecular-weight chitosan 10 g for 5 days to half of these cows. We compared SCS, DSCC, WBC, A/G ratio, cytokine levels, bacteriological cure rate, and time until recurrence between cows that had received and not received chitosan. Results showed that the oral administration of chitosan had no effects on cows' immune systems, also unable to prevent dry-period IMI and recurrent mastitis.

Although we failed to control mastitis through the oral administration of chitosan, we believe antibiotic stewardship in mastitis control can benefit from our other findings. We suggest that when implementing SDCT use (internal) teat sealants concurrently, also better to evaluate herd hygiene conditions in advance. Whenever possible, evaluate the causative pathogen and the severity of mastitis immediately after its occurrence. Combined with this information, levels of WBC, DSCC, and the A/G ratio can be used to facilitate treatment planning and prognosis evaluation. Avoid extending antibiotic treatment solely based on the CMT result. Bacterial culture of milk may be a better aid. For subclinical mastitis, we can rapidly screen quarters with high SCS and low DSCC (possibly chronic IMI) using the CMT. The negative effect of high SCS and low DSCC on productivity was most pronounced in high-parity cows. Since antibiotic therapy is ineffective for chronic IMI in these cows, early dry-off and culling should also be considered. Antibiotic stewardship in mastitis control can be further improved with continuing research efforts, and a society that ensures animal and human health is promising.