## Studies on the occurrence, pathological conditions, and reduction method for subacute ruminal acidosis (SARA) in dairy cows

Summary of Doctoral Thesis

Marika Maeda

Graduate School of Veterinary Medicine and life Science

Nippon Veterinary and Life Science University

## Summary

Recently, subacute ruminal acidosis (SARA) has become a problem associated with the management of lactating cows. Acute ruminal acidosis is defined as a condition in which ruminal pH decreases because of lactate accumulation when an animal consumes a large excess of grain. In SARA, ruminal pH is depressed for several hours per day due to accumulation of volatile fatty acids (VFA) and insufficient rumen buffering. Causes of SARA include feeding excessive amounts of non-structural carbohydrates, insufficient dietary fiber, highly fermentable forages, short adaptation time of dry cows to lactational concentrate diets, heat stress, over-crowding, and poor stall comfort. SARA has severely damaged the livestock industry economically. However, there is still a lack of field data and knowledge about the relationship between SARA and metabolic disease, because it is difficult to monitor ruminal pH in the field.

The objectives of this study were to reveal the impacts of SARA and to establish preventive measures against SARA. In this study, a field survey of SARA, using a wireless radio transmission pH measurement system (pH sensor), was conducted, and blood levels of various hormones and compounds related to energy metabolism in cows with SARA were evaluated. Additionally, the preventive effect of wood kraft pulp (KP) was investigated in cows with SARA.

The first chapter describes the SARA field study in the Mogami region, Yamagata prefecture, using pH sensors and investigates the influence of differences in rearing methods on ruminal and reticular pH. The results of the field survey suggested that ruminal pH values in herds are influenced by the feeding system [total mixed ration (TMR), or separated], starch concentrations in feed, diet changes of cows during transition, and other factors. We demonstrated that the prevalence of SARA 45 days before and after parturition differed among 5 farms, as it ranged from 29.0 to 77.4%. The prevalence at Farm S, with separate feeding, was highest. In contrast, the prevalence at Farm I, with lower starch concentrations than the other 3 TMR farms, was the lowest. Daily changes in pH were significantly different among the 5 farms during the 4 days after parturition. Cows in Farm H, with rapidly increased concentrated feeding before delivery, showed significantly greater daily changes than cows in Farm I, with no change in feeding during the peripartum period. Thus, we suggest that feeding changes during peripartum might influence the dynamics of ruminal and reticular pH. Additionally, the mean pH calculated during days 11 to 20 after parturition were significantly different among the 5 farms. Cows in Farm S, with separate feeding, showed the lowest mean pH. Cows tend to preferentially sort against long particles, such as roughage, in favor of shorter particles such as concentrated feed. In separate feeding, sorting is considered to be easier. Excessive sorting can result in refusal of physically

effective fiber (peNDF) and a difference between the assumed and the real intake of peNDF. The low mean pH of Farm S may have been due to sorting behavior, and it was suggested that differences in feeding methods might influence the dynamics of ruminal and reticular pH. Collectively, these results suggest that differences in feeding methods, starch concentration of the food, changing feeding material during peripartum, and the rate of increase in concentration of feed may influence the dynamics of ruminal and reticular pH. Furthermore, in Farms S and I, there were individual variations in pH, even when the same diet was fed. The cause of susceptibility to SARA may include genetic factors, the inherent ruminal microbial population, and instability of ruminal microflora due to previous exposure to SARA.

The second chapter describes the investigation of 11 lactating cows, during 2015-2016 at Farm S, presumed to have SARA based on the field survey described in Chapter 1. We continuously monitored ruminal and reticular pH and measured blood concentrations of hormones and metabolites related to energy metabolism. To manage longitudinal pH data and control for the effect of time on the measured variables, we calculated the following two pH parameters: low pH time, which was the total amount of time that ruminal pH was <5.6 or reticulum pH was <6.3 at 1, 4, and 8 weeks after parturition (1w-low pH time, 4w-low pH time, and 8w-low pH time); and SARA-positive days, which was the number of days that SARA was detectable based on the criteria described above, in the 30 days after parturition. Adiponectin (ADN) concentrations at 4 weeks after parturition correlated with 1w-low pH time. Additionally, there was a strong correlation between the number of SARA-positive days and ADN concentrations at 4 weeks, and average ADN concentrations at 1 and 4 weeks. These results suggest that ADN may serve as an index to assess SARA.

SARA induces ruminal lipopolysaccharide (LPS) release and triggers an acute phase inflammatory response. Tumor necrosis factor-alpha secreted by macrophages induces insulin resistance. ADN is an adipocytokine secreted by adipose tissue and is involved in the regulation of glucose and fatty acid metabolism. ADN improves insulin resistance by reducing gluconeogenesis in the liver and promoting sugar uptake in skeletal muscles. ADN also affects bovine monocyte inflammatory responses by reducing their responsiveness to LPS during metabolic stress. Thus, ADN concentrations may increase as a compensatory mechanism to ameliorate insulin resistance by reducing inflammatory responses to LPS and promoting insulin sensitivity, with more frequent SARA.

In the third chapter, we describe calculations of Pearson's correlation coefficient for the association of ADN concentration and ruminal characteristics. Additionally, we compared ruminal characteristics, milk production, and reproduction performance in high-value versus low-

value groups based on low pH time and frequency of SARA. There was a negative correlation between mole fraction of acetic acid in rumen and ADN concentrations at 4 weeks post-parturition. The result suggests that ADN reflects the ruminal condition, supporting the conclusion described in the second chapter. There were no significant differences in ruminal characteristics, milk production, or delivery intervals between high-value and low-value groups. The low-value group with less-frequent SARA tended to exhibit higher milk urea nitrogen (MUN) than the high-value group, with more frequent SARA at a single point between 50-80 days in milk (DIM). This result is similar to that of a previous study which suggested that MUN might be potentially used as an indicator to identify cows that are tolerant to high-grain diets at the farm.

In the fourth chapter, we describe the investigation of the effect of wood kraft pulp (KP) feed on reducing SARA and improving lipid metabolism in cows with SARA. Our study involved 11 Holstein-Friesian transition cows that were suspected to be affected by SARA at Farm S during 2015-2016. Of these cows, some were randomly chosen to receive feed supplemented with KP. We provided them with KP in place of oat hay. Containing nutritious fiber, KP is thought to increase the neutral detergent fiber (NDF) content of feed and stabilize the ruminal pH. The energy value of KP is considered equal to that of corn and it has a slow digestion rate. The two previously mentioned pH parameters, blood parameters, milk production, and delivery intervals were compared between cows supplemented with KP and cows without KP. Our results showed that administration of KP during 2015 improved pH parameters, and circadian changes at 7 days pre-parturient, 7 days post-parturient, and 14 days post-parturient, when dry cows were given feeds with low levels of NDF, high levels of NFC, and high levels of starch (feeds that can decrease ruminal pH during dry periods). In this study, we replaced roughage with KP. Thus, cows supplemented with KP received slightly more NDF content than cows without KP. These results suggest that a slow digestion rate of KP as well as an increase in the NDF content of feed may contribute to an improvement in pH parameters. In contrast, administration of KP during 2016 led to less improvement in ruminal or reticular pH when cows were given feeds with an increased gap in NFC levels and starch concentrations between pre-parturient and postparturient periods (feeds that can cause disruption of ruminal pH). Cows fed with KP tended to exhibit slightly higher total VFA concentrations in the rumen at 4 weeks post-parturition, higher blood total cholesterol (TC) concentrations, higher MUN, lower blood ADN concentrations, and shorter delivery intervals than cows not fed KP. The causes for this result might include the change of organic matter fermentation and/or increased dry matter intake (DMI). Higher ADN in cows not fed with KP and lower ADN in cows fed with KP are consistent with the conclusion described in the second chapter, which suggests that ADN may reflect ruminal conditions, and ADN concentrations may increase as a compensatory action to ameliorate insulin resistance by

reducing inflammatory responses to LPS and promoting insulin sensitivity with more frequent SARA. However, cows fed with KP tended to exhibit higher LPS concentrations in the rumen than cows not fed with KP. Considering a previous study which suggested that the translocation of LPS and inflammatory response might not only be due to increased LPS in the rumen but also fermentation and LPS production in the large intestine, a further, more detailed, study is required. Collectively, these results suggest that supplementation of KP could improve ruminal and reticular pH and lipid metabolism in cows with SARA, although the effects of KP may depend on the constituent concentrations of feeds.

This study demonstrated the effectiveness of ADN as an estimative index for SARA as an inflammatory and a metabolic disease. In contrast, previous studies have suggested that inflammatory responses vary among methods of SARA induction, but depend on the particular diet fed for induction. Additionally, the starch content of digesta, fermentation, and LPS production in the large intestine have been described as factors affecting translocation and acute phase responses in cows with SARA. Therefore, further studies measuring inflammatory markers, cytokines, and LPS concentrations in the large intestine may assist in clarifying the relationships among SARA, inflammatory response, and ADN. Further studies of the impacts of body weight variation and other inflammatory diseases are also required. This study also demonstrated the effectiveness of KP supplementation as a method of decreasing SARA. Amounts and types of diet replaced with KP are well worth considering for maximal effectiveness. Additionally, understanding the relationship between SARA and ADN may permit the effects of KP on ruminal or reticular pH and metabolism to be evaluated by measuring ADN levels.

The productive lifespan of lactating cows in Japan is getting shorter year by year. Concentrate diets have been largely fed to cows for the purpose of increasing milk yield and improving milk quality. However, abnormal ruminal fermentation causes metabolic and hoof diseases, resulting in a shortened productive lifespan. Therefore, restoring ruminal fermentation is key to enhancing the long-lasting productivity of the animals. In this study, we conducted a field survey of SARA and demonstrated that the concentration of ADN, which is an adipocytokine involved in the regulation of glucose and fatty acid metabolism, is associated with ruminal fermentation. Additionally, we demonstrated the possibility of decreasing SARA by feeding KP. This report is expected to lead to future studies to determine the effectiveness of ADN as a biomarker for SARA, and the impacts of SARA as a metabolic disease, as well as effective preventive measures against SARA. This study may also contribute to solving the pathophysiology and prophylaxis of SARA.