Chapter 1 General Introduction

Automatic milking systems (AMS) are the latest milking machine utilized in dairy industry. Since 2000, AMS has gradually been accepted by dairy farmers as an advanced technology not only in European countries, but also in North America and Japan. Reducing labor cost and saving work load are major reasons for introduction of AMS by dairy farmers. Cows milked by AMS generally increase milk yield with increased milking frequency. The effects of milking frequency on production performance and nutritional status in cows milked by AMS is important to be studied clearly, because changes in the mammary use of milk precursor components may affect the mobilization of fat stored in the body tissues, especially in an early lactation period. Related to higher milk protein yield, higher milking frequency may also affect mammary amino acid (AA) metabolism in cows milked by AMS. In addition, feeding strategy for close-up period must be applied to minimize the negative effects of negative energy balance on production performance in cows milked with higher frequency by AMS. Therefore, this study aimed to evaluate milk production and nutritional status in early lactating cows managed with AMS, with focusing on milking frequency and prepartum feeding levels.

Chapter 2 Milk production, plasma metabolite profiles and mammary arterial-venous differences of milk precursors in early lactation cows milked at different frequency by an automatic milking system

In Experiment 1, 8 multiparous and 4 primiparous Holstein cows were assigned to two treatment groups of 2 or 4 times milking frequency. After parturition, cows were fed a partial mixed ration (PMR) ad libitum and milked by AMS from 10-18 days postpartum. Measurements were conducted for milk production, plasma metabolite profiles and mammary arterial-venous difference of milk precursors at 20, 35 and 50 days postpartum.

The daily milk yield in AMS period was greater ($P < 0.001$) in 4 times than in 2 times milking cows. Contents of protein, fat, lactose and solids-not-fat (SNF) in milk were not different between treatments. Yields of milk protein, lactose and SNF were greater ($P<0.001$), and fat yield tended to be greater in 4 times milking cows ($P = 0.073$). Average dry matter intake (DMI) and total digestible nutrient (TDN) intake in each period was similar between treatments. Body weight (BW) and body condition score (BCS) were similar between treatments, but BCS decreased with period from 20 to 35 days ($P < 0.05$).
Arterial plasma concentration of glucose was lower ($P = 0.034$) in 4 times milking cows. However, the arterial concentrations of acetate, ketone body, non-esterified fatty acids (NEFA), triglyceride, total cholesterol (T-Cho) and urea-N did not differ between treatments. Arterial concentration of most metabolites, except for triglyceride showed day effects ($P < 0.05$). The arterial-venous difference of plasma metabolites by the mammary tissues were similar between treatments, however estimated mammary plasma flow was higher in 4 times compared with 2 times milking cows ($P < 0.05$) which probably affected milk and milk component yields. Arterial concentrations of hormones (GH, IGF-1 and prolactin) were similar between treatments. In conclusion, increase of milk yield and milk components with 4 times milking frequency by AMS may not affect the extent of fat mobilization from day 20 postpartum onward. Increasing milking frequency from 2 to 4 times daily under AMS increases milk production owing to the increase of mammary blood flow.

Chapter 3 Mammary amino acid metabolism in early lactation cows milked at different frequency by an automatic milking system

In Experiment 2, the blood plasma samples of the coccygeal artery and right subcutaneous abdominal vein obtained in Experiment 1 were analyzed for free AA concentrations. Arterial plasma concentration of glutamate (Glu) was lower ($P = 0.022$) in 4 times milking than in 2 times milking cows. Arterial-venous concentration differences of each essential AA were not significantly different between treatments. Among essential AA except for arginine, the extraction ratio of Lysine (Lys) was highest for both groups at 20 days postpartum. Compared with 2 times milking, 4 times milking cows showed lower extraction ratios of some essential AA ($P < 0.05$ for isoleucine and Lys, and $P < 0.10$ for leucine, threonine and valine), reflecting numerically higher concentrations of these AA in arterial plasma in 4 times milking cows.

Mammary uptakes of each essential AA except for tryptophan were higher ($P < 0.05$) in 4 times than in 2 times milking cows. In contrast, mammary uptakes of each non-essential AA excluding alanine, aspartate, serine and tyrosine were not different between treatments. Ratios of mammary uptake to milk output of branched-chain AA were lower ($P < 0.05$) and uptake to output ratios of glutamine, Glu and glycine were tended to be lower ($P = 0.055$) in 4 times than in 2 times milking cows. In conclusion, the increase of milk protein yield with frequent milking by AMS is probably caused by the increase of mammary blood flow and efficient conversion of essential AA to non-essential AA or reduction of AA oxidation in the mammary tissues.

Chapter 4 Effects of prepartum feeding levels on milk production and plasma metabolites in postpartum cows milked by an automatic milking system

In Experiment 3, 7 multiparous Holstein dry cows were divided into two treatment groups supplying diets at 125% (H) or 100% (M) of TDN requirements, respectively, during last 3 weeks of dry period (close-up period). After parturition, cows in both treatments were fed PMR ad libitum and started milking by AMS from 3 weeks postpartum. Total DMI before calving was actually higher ($P < 0.01$) for cows in the H treatment compared with the M treatment. Dry matter intake at week 3-6 postpartum was similar between treatments, although DMI at week 1-2 was numerically higher for cows in the M treatment. Although postpartum BW change showed similar results between treatments, cows in the M treatment had numerically higher energy balance at week 1-2. Milk yield at week 1-2 was tended to be higher ($P = 0.09$) for cows in the M treatment compared with the H treatment. Compositions of fat and protein in milk at week 1-2 were
similar between treatments, but lactose and SNF contents were higher ($P < 0.05$) for cows in the H treatment than those in the M treatment. At week 3-6 postpartum, protein and SNF contents were lower ($P < 0.05$) for cows in the H treatment. Milk fat yield was higher ($P < 0.05$) for cows in the H treatment compared with the M treatment at week 3-6, while milk yield did not differ between treatments.

Arterial plasma concentration of glucose was tended to be lower ($P = 0.06$) for cows in the H treatment compared with the M treatment at week 1-2 postpartum but similar between treatments at week 3-6. At week 1-2 and 3-6 postpartum, arterial plasma concentrations of ketone body and NEFA were not significantly different between treatments. Plasma triglyceride and T-Cho concentrations at week 1-2 postpartum were similar between treatments, but T-Cho was higher ($P < 0.01$) for cows in the H treatment compared with the M treatment at week 3-6 postpartum. Arterial concentrations of hormones were similar between treatments at all weeks postpartum. In conclusion, high feeding level at prepartum period showed no benefit for milk production and nutritional status based on plasma profile in postpartum cows milked by AMS, except for slight increase of milk fat yield.

Chapter 5 Udder health and reproductive performance of dairy cows milked by an automatic milking system

In this chapter, somatic cell counts (SCC) and some reproduction data for cows in Experiment 1 and 3 were analyzed. In Experiment 1, average SCC were similar between 4 times and 2 times milking cows. Overall, the probability for occurrence of high SCC ($> 100,000$) was lower for 4 times milking than 2 times milking cows. Calving intervals, days to first service and service per conception mean values were similar between 4 times and 2 times milking cows. In Experiment 3, calving interval was 653 and 526 days for cows in the M and H treatment, respectively. Although days to first service were similar between treatments, service per conception was higher for cows in the M treatment compared with the H treatment.

Increasing milking frequency may positively affect SCC and not affect the reproductive performance in this experiment. In addition, increasing feeding levels at close-up period may provide benefit for overall milk production by AMS through improving reproductive performance.

Chapter 6 General Discussion

In summary, the results of this study indicate that increasing milk yield with increasing milking frequency does not enhance fat mobilization till 3 weeks of lactation. The increase of milk yield with frequent milking by AMS is mainly owing to the increase of mammary plasma flow which affects AA uptake for milk protein synthesis. Efficient conversion of essential AA to milk protein by mammary gland may also affect higher milk protein yield in cows with higher milking frequency. On the other hand, feeding levels in prepartum period may not affect milk yield but increase milk fat production in early lactation by AMS.