CHAPTER III

MATERIALS AND METHODS

1. Research scope and methods

1.1 Farms selection

The 11 small holder dairy farms with an average herd size less than 20 milking cows in Chiang Mai and Lamphun provinces. The selected farms have used the bucket type of milking machine.

1.2 Cows selection

Holstein-Friesian >75% cross breed milking cows were randomly selected five cows from each farms. Therefore, there were totally used 55 cows from selected farms. They were in their first to sixth lactation numbers and had completely healthy teats indicated by no clinical sign and history of clinical mastitis for at least 3 months.

1.3 Data collection

1.3.1 Milking machine data

The milking machine performances were tested by the pulsator tester model Pt-V, Tetra pak (Thai) Ltd., DeLaval, 1999. The milking machine performances included vacuum level (Kpa); phase A, B, C and D (%); pulsation ratio (phase A+B: phase C+D); pulsation rate (cycles/min.) and limping (%), respectively. The milking machine performances were tested from the first vacuum tap adjacent the vacuum

pump. The data were collected during the machine turn on but not during milking period.

1.3.2 Teat structure data

The teat structure factors were evaluated using the ultrasound machine model ALOKA SSD 500 with 5 MHz linear probe. The ultrasound probe approaches at lateral aspect of the teats in vertical line, as modified from Neijenhuis, et al., 2001 and Gleeson, et al., 2002. The teat structure factors consisted of the teat-canal length (TCL), the teat-width (TW) at the top of the teat canal, the teat-wall thickness (TWT) at 1 cm above the end of the teat canal and the teat-cistern width (TCW) at 1 cm above the end of the teat canal. (Figure 4 and 5)

The teat structures were scanned in each teat of the udder separately at before and after the evening milking period. In addition, each sample was duplicated for accuracy. The teat ultrasonography was the only inside investigation therefore the teat end scoring system was used for outside investigation. (Figure 6)

The teat end scoring system in this study was categorized in 3 scores;

Score 1 = normal teat end, smooth teat end and not protrusion

Score 2 = moderate teat end, rough teat end and protrusion

Score 3 = severe teat end, very rough teat end and/or lesions

The teat end score was scored at before attach and after detach the teat cup.

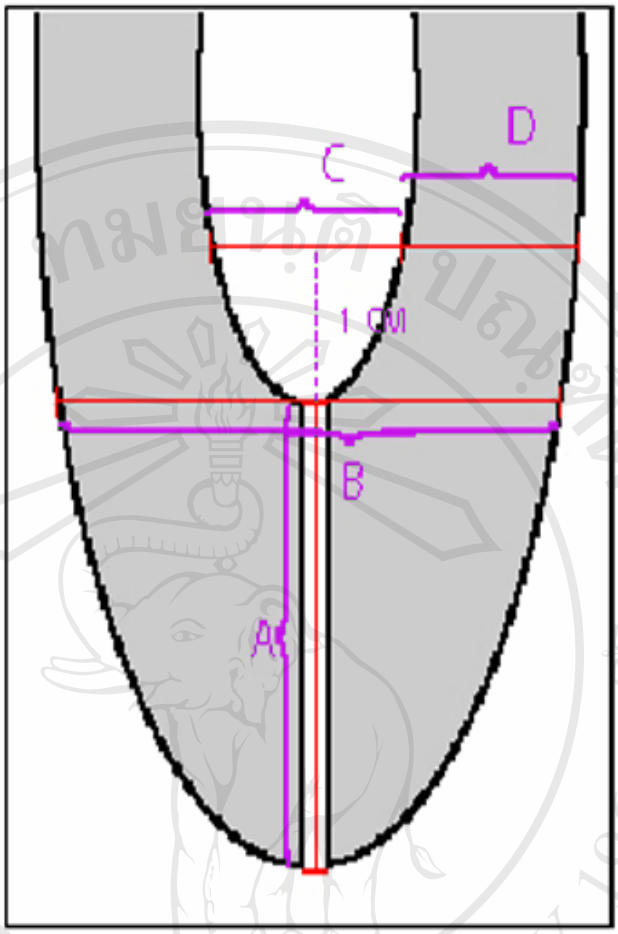

Figure 4 Measurement of teat structures, the teat-canal length (A), the teat-width (B), the teat-cistern width (C) and the teat-wall thickness (D).

Figure 5 The teat ultrasonoghaphic method, modified from Neijenhuis, et al., 2001 and Gleeson, et al., 2002.

score 1

score 2

score 3

Figure 6 The teat end scoring system, modified from Wilson, D.J. et al. 2000

Score 1 = normal teat end, smooth teat end and not protrusion

Score 2 = moderately teat end, rough teat end and protrusion

Score 3 = severe teat end, very rough teat end and/or lesions

1.3.3 Milk samples data

The 30 ml. of milk samples were collected from each quarter separately for quarter somatic cell count (QSCC) data. After that, the milk samples were kept in cooling box immediately and sent to the laboratory. The somatic cell count were measured by the automatic count machine model Somacount® S150 using the automated electronic fluorescent dye method.

2. Experimental design

In this study, cross sectional experimental design was used. The data were collected only one time at evening milking in each farm.

3. Sample size

The sample size calculated by WinEpiscope program version 2.0. According to Snedecor and Cochran, two-tailed tests were used when one was interested whether or not there was any difference between the means. The formula for the needed sample size were 112 quarters.

where: $$n = \left(\frac{Z(a) + Z(b) \times SD}{m1-m2} \right)^2$$

- Z(a) = the value of Student's t at the specified confidence level
- Z(b) = the value of Student's t (2-tailed) at the specified power
- SD = the (expected) standard deviation
- m1 = mean in population 1 (SCC < 200,000 cells/ml)
- m2 = mean in population 2 (SCC $\geq$ 200,000 cells/ml)
- m1-m2 = the (expected) difference between the treatments

4. Statistical analyses

4.1 Somatic cell count data

An udder inflammation free state was a state in which a cow had a somatic cell < 200,000 cells/ml. An udder inflammation state was defined as somatic cell $\geq$ 200,000 cells/ml. The SCC was categorized in to two groups by using SCC at 200,000 cells/ml. indicated the cut point (Schepers, 1997). The descriptive data of SCC will be described by geometric means.

4.2 Definition of variables

The dependent variable was SCC and divided in to two groups, first group as SCC < 200,000 cells/ml. and second group as SCC $\geq$ 200,000 cells/ml.

The independent variables mainly were milking machine performances and teat structures. The milking machine performances consisted of the vacuum levels, the phase A-D, the pulsation ratio, the pulsation rate and the limping, respectively. The teat structures included the teat-canal length (TCL), the teat-width (TW), the teat-wall thickness (TWT) and the teat-cistern width (TCW), respectively (Figure 4).

4.3 Data analysis

The descriptive statistic and student's T-tests were developed for each variable. P-value less than 0.01 is highly significant, P-values less than 0.05 is considered significant and P-values less than 0.10 is a tendency of significance.

For calculated student's T-tests, the variables were grouping by somatic cell count in to two groups as SCC < 200,000 cells/ml and SCC $\geq$ 200,000 cells/ml. The

variables of milking machine performances and teat structures were entering at each pairs of the high and the low somatic cell count.

The comparison among the teat canal length, the teat diameter, the teat cistern width and the teat wall thickness between before and after milking were calculated by student's T-test.

The descriptive statistic of the mean difference between before milking and after milking of the teat canal length, the teat diameter, the teat cistern width and the teat wall thickness with the teat end scores.

The teat end score changing between before attachment and after detachment of the teat cup were calculated by the correlation test.

The descriptive statistic and significant difference between log of quarter somatic cell count and teat end score after detach teat cup were calculated by ANOVA test.

In additional study, the individual cow management data such as the lactation number, the day in milk, the cleaning time, the milking time, the milk yield, the milking rate and the order to milking were also calculated by student's T-test. Furthermore, the categorical farm management data such as the cleaning cloth, the disinfectant, the dry cloth, the strip milk test, the slipping teat cup, the teat cup fall off, the dry vacuum pump, the spring regulator type, the close pipeline, the 1 ½ " pipe diameter, the replace liner, the water pulsator type, the NaOH cleaning, the acid cleaning, the pipeline cleaning and pulsator cleaning were calculated by Chi-square test and odds ratio for each pairs of data.

The log of quarter somatic cell count and the typing barns were comparison by ANOVA test.

5. Location of research operation and data collection

Samples and data collection were studied in the small holder dairy farms in Mae-On sub-district and Mae wang district in Chiang Mai province and Ban-Thi district in Lamphun province, respectively.

In the part of laboratory procedure, the study of somatic cell count was performed at the microbiological laboratory, Faculty of Veterinary Medicine, Chiang Mai University.