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Original Article

Effects of mammae hand massages on oxytocin release, milk yield, and milk quality in dairy cows

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Abstrak

Tujuan: Tujuan dari penelitian ini adalah untuk mengevaluasi pengaruh *Mammae Hand Massages* (MHM) identik dengan 1-1,6 Volt terhadap pelepasan oksitosin, produksi susu, dan kualitas susu pada sapi perah.
Metode: Dua belas ekor sapi perah dengan kriteria sebagai berikut: umur 5 tahun, bulan laktasi ke-1 sampai ke-9, dan berat badan rata-rata 390 ± 5,55 kg. Metode eksperimen menggunakan Rancangan Acak Kelompok (RAK) dengan 4 perlakuan, yaitu T0 (tanpa MHM), T1 (MHM selama 20 detik), T2 (MHM selama 50 detik), dan T3 (MHM selama 80 detik) dengan variable yaitu pelepasan oksitosin, produksi susu, dan kualitas susu.
Hasil: Data yang diperoleh dianalisis menggunakan analysis of variance (ANOVA). Hasil penelitian menunjukkan bahwa MHM berpengaruh sangat nyata terhadap produksi susu (P <0,01) yang dibuktikan dengan perlakuan MHM 50 detik memperoleh rata-rata produksi susu tertinggi (13.08±3.38 liter/ekor/hari) dibandingkan dengan perlakuan MHM 20 detik, MHM 80 detik dan kontrol. MHM (1-1,6 volt) selama 50 detik menghasilkan pelepasan oksitosin tertinggi (0,22955 pcg / 0,1mL) pada 240 detik dibandingkan tanpa perlakuan, MHM 20 detik dan 80 detik. Begitu pula dengan prosentase kadar protein susu (2,96 ± 0,03) dan kadar lemak susu (4.27±0.70) tertinggi pada MHM 50 detik.
Kesimpulan: Dapat disimpulkan bahwa MHM (1-1,6 volt) selama 50 detik meningkatkan pelepasan oksitosin, produksi susu, dan kualitas susu ditinjau dari prosentase kadar protein dan lemak susu.

Kata Kunci: hipofisis posterior; kualitas susu; pelepasan oksitosin; produksi susu; sapi perah FH *crossbreed*

Abstract

Objective: The objective of this study was conducted to evaluate the effects of Mammae Hand Massages (MHM) on oxytocin release, milk yield, and milk quality in dairy cows.
Methods: Twelve dairy cows with the following criterion: 5-yr-old, 1st to 9th month of lactation, and average body weight of 390 ± 5.55 kg were used. Cows were assigned in a randomized block design with 4 treatments, i.e T0 (without massage), T1 (MHM for 20 s), T2 (MHM for 50 s), and T3 (MHM for 80 s). Oxytocin release, milk yield, and milk quality were measured accordingly.
Results: The data obtained were analyzed using analysis of variance (ANOVA). The results showed that MHM had a very significant effect on milk production (P <0.01) as evidenced by the MHM

50 s treatment obtained the highest average milk production (13.08 ± 3.38 liters/head/day) compared to the MHM 20 s, MHM 80 s and control. MHM (1-1.6 volts) for 50 s resulted in the highest release of oxytocin (0.22955 pcg/0.1mL) at 240 seconds compared to control, MHM 20 s and 80 s. Likewise, the percentage of milk protein content (2.96 ± 0.03) and milk fat content (4.27 ± 0.70) was highest at MHM 50 s.

Conclusions: It can be concluded that MHM (1-1.6 volts) for 50 seconds increases the release of oxytocin, milk production, and milk quality in terms of the percentage of milk protein and fat content.

Keywords: dairy cow FH crossbreed; milk quality; milk yield; oxytocin release; posterior pituitary

INTRODUCTION

The main objectives of dairy cattle farming are to provide high-quality milk, increase milk yield and maintain udder health [1]. Automatic Milking System (AMS) method can increase in milking frequency, which should positively affect milk production [2]. However, that equipment is expensive for farmers. Some factors play a significant effect on the variations of milk quality and milk yield, including genetic and non-genetic factors. Milking management is a non-genetic factor that greatly affects milk production. It includes udder preparation (udder massage), milking methods, and teat dipping. Mammae Hand Massage (MHM) is a method of milking that is often used to stimulate milk secretion by signaling the posterior pituitary gland to produce oxytocin [3]. The milking process is allowed to be managed by an exogenous oxytocin (OT). Oxytocin injections cause increased OT blood levels which results in the prolonged myoepithelial and alveolar contractions that ultimately increase milk yield [4]. MHM is effective to stimulate milk-let-down, but there was an interval between the time at stimulation and the time at initial milking. Reimers [5] reported that massage durations (10, 30, and 60 s) had a significant effect on milk production. Meanwhile, Kentjonowaty et al., [6] found that 90 s was the best interval between the time at stimulation and time at milking to produce the best persistence of oxytocin. The oxytocin starts working around 1-2 min after stimulation [7]. Milk usually contains a somatic cell count (SCC), which is an indicator of udder health. The amount of SCC in milk depends on the level of blood oxytocin.

When doing massage to the mammae, the dairy cows should not feel pain but must feel comfortable. Therefore, it is necessary needed to measure the pressure of MHM and the comfortable effect of cows. The pressure of MHM can be measured using a PVDF sensor that is bonded on the beam specimen at one end and the ultrasonic guided waves are launched with a piezoelectric wafer bonded on another end of the beam. Sensitivity of PVDF sensor in terms of voltage is obtained for increasing number of thermal cycles. Piezoelectric-film sensor, which contains piezoelectric materials that can convert mechanical energy into electrical energy [8]. Milk yield and milk quality are also affected by the milking method. Machine milking results in a higher milk yield than hand milking. In addition, the best hand milking method is a combination of whole hands and *strippen* [9]. In Indonesia, the milking methods used in dairy cattle farming vary. Livestock keepers may only use machine milking, another may use a combination of the milking machine and *strippen* or a combination of whole hand and *strippen*. Eventually, it is important to determine the best duration of MHM as a pre-milking stimulation technique and the best milking method to achieve the goals of milking.

Based on the above reasons, this study was conducted to evaluate the effects of Mammae Hand Massages (MHM) on oxytocin release, milk yield, and milk quality in dairy cows.

MATERIALS AND METHODS

Materials design

Twelve dairy cows with the following criterion: 5-yr-old, 1st to 9th month of lactation,

and average body weight of 390 ± 5.55 kg were used in this study. They were treated under similar maintenance management and fed similar types and amounts of diets at UPT PT & HMT Batu, East Java. All animals investigated had good udder health. Experimental method is used by a randomized block design with 4 treatments, i.e. T0 (without massage), T1 (MHM for 20 s), T2 (MHM for 50 s), and T3 (MHM for 80 s).

Milking preparation

Cows were fed concentrate at 04.30 am. The cages and milking equipment were cleaned. Cows were washed and their udders were smeared with vaseline. Cows were rested for 30 min. and treated with mammary hand massage according to the treatments. The udder pressure was measured using a piezoelectric sensor. Before milking using a bucket-type milking machine, the cows were given 90 s. Milk yield and milk quality were measured.

Determination of oxytocin, milk yield, and milk quality

Oxytocin release was determined as per the method described previously [10]. Briefly, oxytocin antibodies were dissolved in 1 mL PBS, diluted in a buffer coating pH 9.6, and incubated overnight at 40°C. They were washed with PBS-T 3 times for 60 s each, blocked with 1% BSA, and left for an hour. Antibodies were washed again with PBS-T 3 times for 60 s each. Blood plasma (primary antibody) was coated with BSA 1% at 37°C for 2 hours and washed with PBS-T 3 times for 60 s each. Anti-rat IgG (secondary antibody) was coated with TBS (1 : 250), incubated at 37°C for an hour, and washed 3 times with PBS-T for 60 s each. The substrate used was PnPP (1 tablet) in 5 ml diethanolamine (pH 9.8) and incubated at 37°C for 30 min. 3 ME NaOH was used to stop the reaction. After 10 and 15 min, the oxytocin levels were determined using the ELISA reader 405 nm OD. Milk yield was determined in the morning and evening during the experiment (15 d) and measured as follows [9] (Hemme, 2010):

$$\sum \text{Milk Production} = \frac{(0.383 \text{ x } \% \text{ fat } + \ 0.242 \text{ x } \% \text{ Protein } + \ 0.7832)}{3.1138}$$

Specific gravity, milk fat, and milk protein were measured to determine milk quality using *lactoscan*. Briefly, 40 mL milk samples were put into a beaker glass and the power of *lactoscan* was switched on. The analysis pipe and pH meter probe was inserted into the sample vial. The enter button was pressed and the menu was selected according to the types of milk. The analysis results were displayed on the glass screen. After completing the analysis, the menu button was pressed and the cleaning position was selected. The detector was washed with a daily cleaner solution. The *lactoscan* was switched off.

Data analysis

Oxytocin release, milk yield, and milk quality among treatments were analyzed using analysis of variance (ANOVA).

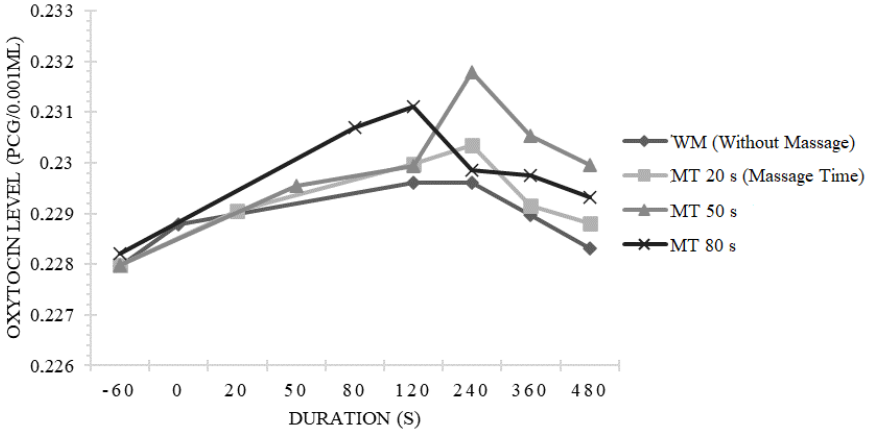

Figure 1. Effect of MHM treatments on oxytocin release

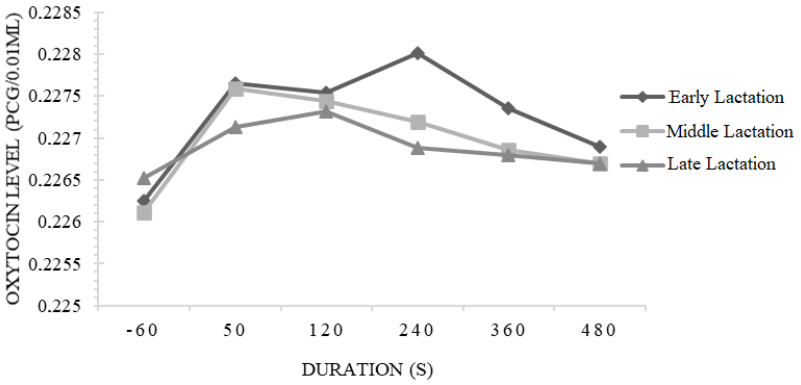

Figure 2. Oxytocin release in each lactation group

RESULTS

The effect of MHM treatments on oxytocin release of dairy cows are showed in Figure 1. Based on Figure 1, MHM 50 s group, oxytocin release increased gradually and reached its highest peak at the 4th (240 s) blood collection, which steadily decreased until 480 s. Differences in oxytocin release among treatments may be attributed to the duration of the massage, which further affects oxytocin release from the posterior pituitary.

Moreover, the application of MHM in Table 1 was significantly affected milk yield (P<0.01). The highest milk yield was observed in MHM 50 s group (13.08 ± 3.38 lt/head/d) as well, followed by MHM 20 s, MHM 80 s, and control (without MHM) groups.

Oxytocin release on difference lactation stages

By doing Mammae Hand Massage (MHM) for 80 s, the posterior pituitary could optimally secrete oxytocin (Figure 2). However, the oxytocin level in this treatment reached its highest peak before milking. This is because 80 s was the longest time for the massage and there was interval time (90 s) between final massage and initial milking (Figure 1 and Figure 2).

Effects of mammae hand massage (MHM) in each lactation stages on the milk yield

The effects of Mammae Hand Massage (MHM) in each lactation group on the milk yield showed in Table 2. The results showed that the MHM and lactation stage had no significant effects (P> 0.05) on the milk yield. It is noted that the total dry matter content in milk was relatively similar among treatments.

Effect of mammae hand massage (MHM) on average milk content

MHM had no significant effect (P>0.05) on milk fat (Table 4), but the effect of MHM treatments on milk fat contents tends to more high percentage on late lactation. The higher fat contents showed on the MHM for 50 s

Table 1. Average of Daily Milk Yield in Each MHM Treatment

Treatment	Milk Yield (liter/head/day)
Without Massage (Control)	9.84±2.94[a]
Massage Time (80 Second)	11.01±3.60[a]
Massage Time (20 Second)	11.65±3.40[ab]
Massage Time (50 Second)	13.08±3.38[bc]

[a,b,c] Superscript which is showed significant different (P<0.01)

Table 2. Average of Daily Milk Yield in Each Lactation Group

Group	Milk Yield (liter/head/day)
Early Lactation (1-3 months)	13.75±1.45[a]
Middle Lactation (4-6 months)	12.83±1.52[a]
Late Lactation (7-9 month)	7.60±1.21[b]

[a-b] Superscript which is showed significant different (P<0.01)

Table 3. Effect of lactation stages and MHM treatments on average density of milk (gr/cm^3)

Treatment	Group			∑	X
	Early lactation	Middle lactation	Late lactation		
T0	1.03	1.03	1.03	3.08	1.03±0.00074
T1	1.03	1.03	1.03	3.08	1.03±0.00057
T2	1.03	1.03	1.03	3.09	1.03±0.00035
T3	1.03	1.03	1.03	3.08	
∑	4.11	4.11	4.12		
Average	1.03±0.00091	1.03±0.00109	1.03±0.0005		

T0 = Without Massage; T1 = MHM for 20 s; T2 = MHM for 50 s; T3 = MHM for 80 s

(5.08) in late lactation than other treatments in the same group.

Application of MHM and stages of lactation had no significant effect (P>0.05) on milk protein (Table 5). The interesting part showed the total milk protein content in the MHM for 50 s (T2) which had a higher value than other treatments. It was indicated that treatment of MHM for 50 s has a high percentage on each group.

DISCUSSIONS

Based on Figure 1, MHM 50 s may be able to stimulate the posterior pituitary to produce oxytocin, which in turn induces the construction of myoepithelic cells surrounding each alveolus, causing milk ejection. External stimuli can produce impulses, which are transmitted by afferent sensory nerves through the segmental Central Nervous System (CNS) to The Paraventricular Nuclei (PVN) and Supra Optic Nuclei (SON) of the hypothalamus, then transported to the posterior pituitary to promote oxytocin release and secreted from the bloodstream into the udder [11]. It stimulates the construction of myoepithelic cells surrounding alveoli, and milk was released [3]. Bruckmair and Wellnitz [7] concluded that milk ejection occurs about 40-120 s after udder stimulation. Similarly, Maciel [12] reported that oxytocin begins working about 60-120 s after stimulation. Around these times, the oxytocin reaches the udder and milk ejection is released; milk production reaches its highest peak at about 2 min after installation of the milking machine.

Table 4. Average milk fat contents (%) in each lactation and MHM treatment

Treatment	Group			∑	X
	Early lactation	Middle lactation	Late lactation		
T0	3.49	3.81	4.16	11.46	3.82±0.34
T1	3.71	3.78	4.21	11.70	3.90±0.27
T2	3.84	3.89	5.08	12.81	4.27±0.70
T3	3.77	4.02	4.23	12.02	4.01±0.23
∑	14.81	15.50	17.68		
Average	3.70±0.15**	3.88±0.11**	4.42±0.44*		

T0 = Without Massage; T1 = MHM for 20 s; T2 = MHM for 50 s; T3 = MHM for 80 s. *p<0.05 **p<0.01

Table 5. Average milk protein content (%) in each lactation and MHM treatment

Treatment	Group			∑	X
	Early lactation	Middle lactation	Late lactation		
T0	2.68	2.83	2.89	8.40	2.80±0.11
T1	2.80	2.92	2.89	8.61	2.87±0.06
T2	2.93	2.99	2.94	8.86	2.96±0.03
T3	2.87	2.73	2.89	8.50	2.83±0.09
∑	11.28	11.47	11.60	34.37	
Average	2.82±0.11	2.87±0.11	2.90±0.03		

T0 = Without Massage; T1 = MHM for 20 s; T2 = MHM for 50 s; T3 = MHM for 80 s

In addition, the effect of oxytocin hormone on milk ejection occurs only during 7 min [5]. Similarly, Arora [13] stated that the lack of response to normal stimuli for milk ejection and addiction in animals are the main disadvantages of continuous usage of oxytocin injections. Bruckmaeir and Blum [14] concluded that milk ejection is released about 1-2 min after stimulation, and the effectiveness of oxytocin occurs about 6-8 min.

The highest milk yield was observed in MHM 50 s group (13.08 ± 3.38 lt/head/day) rather than the control group which lower milk yield (9.84 ± 2.94 lt/head/day) because the posterior pituitary did not secrete oxytocin optimally. Similar to the results of this study, Williams [15] and Reimers [5] reported that udder stimulation before milking increases milk yield in dairy sheep and oxytocin release in dairy cows, respectively. Udder stimulation also affects two-minute milk yield and time in low milk flow rate in dairy cows [15]. Furthermore, udder stimulation has been reported to have a significant effect on average daily yields of actual and 7% FCM in buffaloes [14]. However, udder stimulation for less than 10 s is not able to induce milk-let-down [15].

The posterior pituitary had optimally secreted oxytocin in the treatment of MHM 80 s (Figure 2). Besides, the levels of oxytocin at the 4th minute started to decline, which in turn decrease the rate of milk ejection. This indicated that the effectiveness of oxytocin in this MHM 80 s group was the lowest. Wieland et al. [16] concluded that the effectiveness of oxytocin is only 5 min, and the milk ejection occurs at 20-60 s after udder stimulation [5]. In this period, the oxytocin reaches the udder, and milk is released optimally, starting from 60 to 90 s after stimulation. Milk ejection reaches the highest peak about 2 min from the time of milking machine installation. It was notable that the effect of oxytocin is only for 7 min [5]. Therefore, milking must be completed within 7 min.

Following the results in (Table 1), the previous studies by Weiland et al. [16] and Sannino et al. [17], who reported that the quality of milk is influenced by feed consumption. The effect of milk production due to whether energy and protein are balanced in the diet, the urea concentration is outside the physiological range of values as well [18].

The MHM for 50 s has a high percentage (Table 4) because the milk fat has lower gravity than milk. The milk fat is always at the top layer of the milk. Thus, although the oxytocin release in the blood among treatments differed, milk fat contents were relatively similar among treatments [17]. The total protein content was high on T2 (Table 5) It is because milk protein may be emulsified homogeneously with milk. Although milk yield among treatments differed, milk protein content was relatively similar. Previous studies reported that milk protein did not differ among different maintenance management [16, 19]. Another study showed that stimulation of the vaginocervical area in the bovine was a more efficient stimulus for oxytocin release than was mammary stimulation during lactation [20].

CONCLUSIONS

Oxytocin release increased gradually and reached its highest peak at the 4th (240 s) blood collection because MHM 50 s may be able to stimulate the posterior pituitary to produce oxytocin. Dairy cows treated with Mammae Hand Massage (MHM) for 50 s (equal to 1 - 1.6 volts), and rested 90 s before milking showed the best oxytocin profile, milk yield, and milk quality.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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