



Physiological and Behavioral Responses of Dairy Cows which Milked with Portable parlor

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Abstract: In normal use, milking parlor can cause the release of oxytocin through neuroendocrine reflex, but the use of milking parlor sometimes cause stress and lead to a decrease in milk production. In Indonesia at this time, traditional milking by hand is still widely used by dairy farmers. The purpose of this study is to investigate the use of milking parlor to the physiological and behavioral responses of dairy cows through oxytocin levels in serum. This study used 20 multiparous lactating dairy cows aged 3-6 years. The collection of blood samples are carried out at different times: before the use of milking parlor (A), 50 minutes (B) and 100 minutes post-installation milking parlor (C). The behavior of dairy cows observed by kicking or stepping. Serum cortisol levels were measured using ELISA technique. The results showed that there was no significant difference ($p > 0.05$) milking time on serum cortisol levels of three groups treatments. Cortisol levels in the three treatment groups were $7,07 \pm 0,61$ ng/mL; $7,77 \pm 0,81$ ng/mL; and $5,88 \pm 0,74$ ng/mL, respectively. Observation of the behavior of dairy cows using a milking parlor showed that 35% of cows pose a step, 10% raises kicking motion and the rest did not cause stepping or kicking. Pearson correlation test showed that there was no correlation between behavior response and cortisol serum levels ($p > 0.05$). In conclusion, the use of milking parlor portable do not affect serum cortisol levels and the comfort of dairy cows.

Keywords: dairy cow, stress, milking parlor portable, cortisol, behavior.

Introduction

The dairy industry in the world has a purpose to maximizing yield and profit. This case has resulted in a new approach to managing and milking dairy cows, including the implementation of an automatic milking system (AMS) to reduce the labor associated with milking. Central inhibition of milk ejection, ie inhibition of the release of oxytocin from the pituitary gland, often occurs in the milking practice in various types of emotional stress¹. Milking in a foreign environment in cows², fear of people³ and cows primiparous are milked for the first time⁴ as well as the use of milking parlors the first time has been shown to result in inhibition of milk ejection. These events can cause symptoms of chronic or acute stress. Chronic stress can affect the welfare of cows, and acute stress during milking can reduce milk production⁵.

The milking process can seem stressful for dairy cows is seen from the increased levels of cortisol. Typically, the milking parlor can release oxytocin and milk ejection reflex through neuroendocrine, but sometimes it can also be stressful and cause a reduction in milk production. Several studies have been done on

cortisol levels in milking procedures such as the Automatic Milking System (AMS) and Tandem Parlor Milking System (TMS)^{6,7,8,9}. In addition, research also revealed anxiety behaviors such as stepping, lifting and kicking leg in AMS and TMS^{6,7}.

The milking process in Indonesia is largely done manually, although there are now some dairy farms have been using a Tandem or Portable Milking Parlor. Milking parlor has an advantage in improving the effectiveness and efficiency of processes and labor milking¹⁰. But either manual or milking parlor seems to cause stress in dairy cows seen from cortisol increased, while feeding the calf is not the case¹¹. A milk ejection induced by oxytocin as a neuropeptide, which is released from the posterior pituitary in response to tactile stimulation of the nipple by a calf, hand, or a milking parlor¹². How the influence of portable milking parlor on the behavior of dairy cows at the level of cortisol in the blood serum has not been studied. Therefore, the aim of this study was to determine the effect of portable milking parlor on the physiological and behavioral responses of dairy cows.

Experimental

Animals

Twenty Friesian Holstein cows (multiparous), aged of 3-7 years, not estrus, located on Balai Besar Pembibitan Ternak Unggul-Hijauan Pakan Ternak (BBPTU-HPT) Baturraden, Purwokerto, Central Java. Feeding and drinking method, milking method, and blood sampling method, conducted according to the procedure of BBPTU-HPT Baturraden, Purwokerto. The entire method of research has fulfilled requirements of the Ethical Clearance Committee and the Research Laboratory of Integrated Test of Gadjah Mada University with number of 128/KEC-LPPT/XI/2013.

Milking

Cows are milked with a portable milking parlor twice a day using a vacuum pump M2 parlor brand Masport with \pm 40 kPa suction power. Each session is done for three dairy cows for about 15 minutes each milking session. Before the milking process begins, the stall cleaned by cows handler and followed by feeding the cows to be milked. The udders and teats are dipped into a disinfectant. Nipple immersion into the disinfectant will be performed again after the milking process is completed. Every day cows are milked by the same handler.

Blood samples

Blood sampling performed 3 times, before portable milking parlor installed (\pm 1 minute), 50 minutes and 100 minutes after the milking process begins. Blood sampling performed by authorized personnel. All these activities are done during the afternoon milking. Blood samples were collected into venoject tubes and centrifuged for 15 minutes, 3000 rpm. Serum was collected and stored at 4°C -8 °C prior to analysis of cortisol levels.

Cortisol Assay

Materials used for the assay cortisol is Cortisol ELISA kit products DRG Instruments GmbH, Germany. This procedure is done with assays procedure as follows: microplates characterized by giving appropriate with the desired number in each well; control buffer, standard and serum samples were taken put into wells of 20 μ L /well of according to the numbering; The next step, add 200 μ L of enzyme conjugate into each well and mixed shaker mixing for 10 seconds; then incubated for 60 min at room temperature; after the incubation period is completed, followed by washing well plates 3 times with washing solution (400 μ L per pitting) 3 times; tapping loudly on absorbent paper to remove residual droplets. The 100 μ L substrat were added to each well; then incubated for 15 min at room temperature; the last stage, add 100 μ L stop solution for 10 minutes to each well to stop the enzymatic reaction; and the value of optical density (OD) were read by ELISA reader at 450 nm. Based on data of OD values, cortisol levels were calculated through conversion using the cortisol standard curve.

Animal Behavior

Each individual cow was observed behavioral response of dairy cows during the each session of milking process and recorded the every movement of the cow such as kicking or stepping. Stepping behavior is also known as shuffling, defined as cows shifted his weight from foot to foot behind the other. Kicking behavior is defined as a cow hind leg raised and move strongly and quickly toward the milker or milking parlor⁷.

Data analysis.

Data were analyzed using software statistical Package for Social Sciences (SPSS) 17 for Windows. Results of blood serum cortisol levels will be calculated the mean and standard deviation. Differences between groups were analyzed by one-way test analysis of variance (ANOVA), Duncan test is done if there is a significant difference ($p < 0.05$). The amount of kicking and stepping during the milking process were calculated and analyzed descriptively. Relationship of blood cortisol levels and animal behavior were analyzed with the Pearson correlation test.

Results and Discussion

Cortisol levels in Sera.

Cortisol is the primary glucocorticoid hormone in fish and most mammals, whereas corticosterone is the primary glucocorticoid in amphibians, reptiles, birds and rodents¹³. Under stress conditions will be followed by increased secretion of glucocorticoids in the blood, and can be measured using plasma samples from early going process. Glucocorticoid concentrations began to rise within a few minutes, with the highest concentrations of glucocorticoids duration depending on the nature and duration of the stressor¹⁴.

One way ANOVA test results showed that cortisol levels before milking, 50 minutes and 100 minutes after milking showed that the average difference was not significant ($p > 0.05$) (Table 1).

Table 1. Cortisol level

| Treatment Group | Cortisol levels (ng/mL) |
|---------------------------|--------------------------|
| Before milking | 7,07 ± 0,61 ^a |
| 50 minutes after milking | 7,77 ± 0,81 ^a |
| 100 minutes after milking | 5,88 ± 0,74 ^a |

Note : points with same superscripts (a) shows insignificant different ($p > 0,05$)

Cows showed sensitivity during milking, each individual cow is consistent with a certain tendency for milking so that if the behavior consistent routine is disrupted may have implications for the welfare of these animals¹⁵. Cows are milked using portable milking parlor in this study showed the average levels of serum cortisol before milking was 7.07 ng / mL. The results obtained in this study are not much different from that observed in multiparous cows that is 3-6 ng / mL^{2, 16}. Primiparous cows give results slightly higher than multiparous cows about 5-8 ng / mL⁶ and 8-9 ng / ml⁴. So that the average blood serum cortisol levels in this study can be considered within the range of normal basic cortisol concentrations.

Cortisol and prolactin significantly increased responses to suckling¹⁷ while feeding will lower cortisol levels and increase oxytocin¹⁸. Cortisol levels prior to milking in this study may be due to circumstances experienced by cows when cleaning procedure 15 minutes before milking starts, and not because of the effects of milking. This activity can be considered a stressor, stress causes the release of cortisol from the adrenal cortex to the peripheral blood of cow will be delayed for about 5 to 10 minutes after the first exposure to the stressor⁶.

Some researchers found that cortisol levels in AMS cows higher than TMS cows^{6,7,8}. But other researcher got a different result⁹. The use of portable milking parlor can increase cortisol concentrations in multiparous cows from 5 ng / mL to 15 ng / mL within 45 minutes after milking². Cortisol levels within 50 minutes after milking obtained in this study (7.77 ng / mL) were lower than those report. Differences in the

results obtained in this study may be due to differences in blood sampling procedure time, management and milking system that have been used. The milking process does not induce stress directly, but due to some aspects of management in the milking process¹⁹. The welfare of the cows in the milking process, not only concerned about the milking parlor but also how the handling of dairy cattle management itself^{20, 21}.

Overall blood serum cortisol in this study was lower than serum cortisol, which has been reported for the acute stress response after transportation 9-13 ng / mL²², 20-25 ng / mL during milking in a new environment² and 33.33 ng / mL at slaughter²³. It can be concluded that the cortisol levels of the results of this research are still in the range of tolerable level.

Cows Behavior during Milking Process.

The results showed that seven cows (35%) showed stepping motion as much as 1.8 ± 0.41 times, while two cows (10%) showed kicking movement as much as 1.1 ± 0.31 times. Pearson correlation test results showed that the results of significance is 0.1 ($p > 0.05$), so it can be concluded that there is no correlation between cortisol levels and behavior of the cow.

Different types of milking can also influence the response behavior of the animals. AMS cows produced kick and step movement that is more than cows TMS^{7,24}. However other researcher found no difference in the amount of movement during the milking process stage, TMS and AMS cows do not show kicking during milking⁶.

The increased movement made by a cow can be regarded as a sign of anxiety. The cows were milked in a novelty place will be more defecation / urination and vocalizations, as well as a larger amount of stepping movement². But the presence of a person known by a cow can reduce the amount of step movement, vocalization, urination and defecation but not so with a kicking motion. It is also possible in this study where cows showed no behavioral responses redundant because the milking process in this study was accompanied by officers enclosure during the milking process.

Cows with high milk cortisol levels tend to cause excessive behavior patterns to indicate anxiety⁹. However, other research found that cows with higher cortisol concentrations in the blood do not necessarily indicate a behavioral response⁴. This is consistent in this study, that not all cows with high blood cortisol showed a behavioral response. As far as the observations in this study, it is believed that the movement of cow caused by the fear of blood sampling.

Physical Treatment Influence on Level Cortisol

Physical treatments carryout by workers during milking process can affect the behavior and physiological responses based on the level cortisol. Rough handling in the milking process can reduce milk production by causing stress³ which causes cortisol levels to rise. The presence of humans during milking can provide a relaxing effect on the cow so that lower cortisol levels and heart rate, although not significant². Rough handling can lead to cows become frightened conditions that lead to increased residual milk and reduced milk production²⁵. Cows that had negative experiences in humans would be more difficult to handle⁶.

Automation seems to affect physiological and behavioral responses of dairy cows during milking. The average concentrations of cortisol were slightly lower at TMS the cows from the cows AMS⁶. While other study, states that AMS can be a problem for the cows because of the lack of Human-Animal Interaction soothing effect and increased social disruption in the waiting room AMS⁷. The result of cortisol level in this study is different with the other research² may be due to differences in milking management, or maintenance of the cows as recorded¹⁹

Physical Treatment Influence The Ability Of The Adaptation Process And Behavior

Physical treatments performed by workers in animal will lead to physiological changes and eventually shown in their behavior. Animals adapt to the treatment performed by workers has led to the acceptance of animals and is considered the activities of their daily routine. Milking in early lactation can cause stress on primiparous the cows, then the cows from time to time will adapt to the milking parlor⁴. Multiparous cows have lower stress levels than primiparous cows because they were adapted to the technology²⁶.

Conclusion

Based on the results, it can be concluded that the dairy portable milking parlor does not interfere dairy cows comfortable.

References

1. Bruckmaier RM, Normal and Disturbed Milk Ejection in Dairy Cows, *J. Dom Anim End.*, 2005, 29; 268–273.
2. Rushen J, Munksgaard L, Marnet PG, and De Passille AM. Human Contact and the Effects of Acute Stress on Cows at Milking, *J of App An Behav Sci*, 2001, 73(1). Pp 1–14.
3. Munksgaard L, and Rushen, J. Fear of People by Cows and Effects on Milk Yield , Behavior , and Heart Rate at Milking , *Jurnal Dairy Sciences* , 1999, 82, 720–727.
4. Reenen CGV, Van der Werf JT, Bruckmaier RM, Hopster H, Engerl B, Noordhuizen JPT, and Blokhuis HJ. Individual Differences in Behavioral and Physiological Responsiveness of Primiparous Dairy Cows to Parlor Milking, *J of Dairy Sci*, 2002, 85(10). Pp 2551–2561.
5. Jacobs JA and Siegford JM, Invited review: The impact of automatic milking systems on dairy cow management, behavior, health, and welfare. *J Dairy Sci*. 2012 May;95(5):2227-47.
6. Hopster H, Bruckmaier RM, Van der Werf JTN, Korte SM, Macuhova J, Korte-Bouws G, and Van Reenen CG. Stress Responses During Milking; Comparing Conventional and Automatic Milking in Primiparous Dairy Cows, *J Dairy Sci*, 2002, 85(12), Pp 3206–16.
7. Wenzel C, Schonreiter-Fischer S, and Unshelm J. Studies on Step–Kick Behavior and Stress of Cows During Milking in an Automatic Milking Sistem, *J Livestock Prod Sci*, 2003, 83, Pp 237–246.
8. Hagen K, Lexer D, Palme R, Troxler J, and Waiblinger S. Milking of Brown Swiss and Austrian Simmental Cows in a Herringbone Parlour or an Automatic Milking Unit, *J Appl Anim Behav Sci*, 2004, 88(3-4), Pp 209–225.
9. Gygax L, Neuffer I, Kaufmann C, Hauser R, and Wechsler B. Milk Cortisol Concentration in Automatic Milking Sistem Compared with Auto-Tandem Milking Parlors, 2006, *J Dairy Sci*, 89(9), Pp 3447–54.
10. White J, O'Callaghan EJ, Corcoran B, and Esmonde H. Design of a Robotic Manipulator for Automatic Application of Milking Cups, In Summary of Papers Presented at the Agricultural Research Forum; 71. Agricultural Research Forum. Ofah, Ireland, March 15- 16 2006, Pp 71.
11. Lupoli B., Johansson B, Uvnas-Moberg K, and Svennersten-Sjaunja K. Effect of Suckling in Release of Oxytocin, Prolactin, Cortisol, Gastrin, Cholecystokinin, Somatostatin and Insulin in Dairy Cow dan Their Calves, *J Dairy Res*, 2001, 68, Pp 175-187.
12. Bruckmaier RM, and Wellnitz O. Induction of milk ejection and milk removal in different production systems, *Journal of Animal Science*, 2008, 86(13 Suppl), 15–20.
13. Squires EJ. *Applied Animal Endocrinology*, 2003, CABI Publishing. Ontario, Canada, Pp 204-206.
14. Cockrem JF. Individual Variation in Glucocorticoid Stress Responses in Animals, *J General and Comp Endocrinology*, 2013, 181, Pp 45–58.
15. Hopster H, Van der Werf JTN, and Blokhuis HJ. Side Preference of Dairy Cows in the Milking Parlour and Its Effects on Behaviour and Heart-Rate During Milking, *J Appl Anim Behav Sci*, 1998, 55, Pp 213–229.
16. Hopster H, Van der Werf JTN, Erkens JHF, and Blokhuis HJ. Effects of Repeated Jugular Puncture on Plasma Cortisol Concentration in Loose-Housed Dairy Cows, *J Anim Sci*, 1999, 77, Pp 708–714.
17. Tancin V, Kraetzl WD, Schams D, and Bruckmaier RM. The effect of conditioning to suckling, milking and of calf presence on the release of oxytocin in dairy cows, *J Appl. Anim. Beh. Sci*, 2001, 72:235–246.
18. Linstrom T, Redbo I, and Uvnas-Moberg K. Plasma oxytocin and cortisol concentrations in dairy cows in relation to feeding duration and rumen fill. *Journal of physiology and behavior*, 2001, volume 72, issues 1-2, pages 73-81
19. Hagen K, Langbein J, Schmied C, Lexer D, and Waiblinger S. Heart Rate Variability in Dairy Cows- Influences of Breed and Milking System, *J Phy dan Behav*, 2005, 85(2). Pp 195–204
20. Wiktorsson H, Pettersson G, Olofsson J, Svennersten-Sjaunja K, and Melin M. Welfare Status of Dairy Cows in Barns with Automatic Milking, Relations Between the Environment and Cow Behavior, Physiologic, Metabolic and Performance Parameters. Swedish Agricultural University: 2003, Project

- Report D24 from The EU Project on Implications of The Introduction of Automatic Milking on Dairy Farms
21. Sorensen JT, Hindhede J, Rousing T, and Fossing C. Milking System. In : Proceedings from the First North American Conference on Robotic Milking, Wageningen Pers. Wageningen Toronto, 2002, Pp VI-54 [<http://www.automaticmilking.nl>]
 22. Hulbert LE, Csarroll JA, Burdick NC, Randel RD, Brown MS, and Ballou MA. Innate Immune Responses of Temperamental and Calm Cow After Transportation, *J Vet Immun and Immunopath*, 2011, 143(1-2). Pp 66–74.
 23. Probst JK, Hillmann E, Leiber F, Kreuzer M, and Spengler NA. Influence of Gentle Touching Applied Few Weeks Before Slaughter on Avoidance Distance and Slaughter Stress in Finishing Cow, *J Appl Anim Behav Sci*, 2013, 144(1-2). Pp 14–21.
 24. Gyax L, Neuffer I, Kaufmann C, Hauser R., and Wechsler B. Restlessness Behavior, Heart Rate and Heart-Rate Variability of Dairy Cows Milked in Two Types of Automatic Milking Systems and Auto-Tandem Milking parlours, *J Appl Anim Behav Sci*, 2008, 109(2-4). Pp 167–179
 25. Rushen J, De Passille AMB, and Munksgaard L. Fear of People by Cows and Effects on Milk Yield, Behavior and Heart Rate at Milking, *J. Dairy Sci*, 1999, 82, Pp 720-727
 26. Kovács L, Kézér L, and János T. Measuring Stress Level of Dairy Cows during Milking Using by Geometric Indices of Heart Rate Variability, *J Anim Sci and Biotech*, 2013, 46(1), Pp 213–217.
