Original Article

The Indicative Influence of Oxidative Stress on Low Milk Yields in Dairy Cattle

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Abstract

The goal of this study was to identify the correlation between milk yield and milk malondialdehyde (MDA), an oxidative stress marker. The study was conducted using cows from eight small-holder dairy farms during October to December 2004. The data comprised of sample date, milk yield, and the recent calving date were recorded. Morning milk samples were collected monthly for the measurement of somatic cell counts (SCC) and MDA level using the automate counter and the modified Smith's method, respectively. The final data set included 131 milk data from 74 cows. Overall means and SEM of milk yields, somatic cell scores (SCS, the normalize transformation of SCC data), MDA and days in milk were 14.5±0.45 kg/day, 2.97±0.19, 1,643±26 ppb, and 154.8±8.83 day, respectively. The average milk yields ranged from 9.7±2.0 to 17.7±0.9 kg/day. Results from Pearson's correlation coefficients show that MDA, SCS and days in milk were negatively associated with milk yield (p<0.05). In multiple linear regression analysis, only MDA and days in milk were significantly associated with milk yields. The study indicates that the loss of milk yield after intramammary infection may be highly mediated by increased oxidative stress status.

Keywords : days in milk, malondialdehyde, milk yield, oxidative stress, somatic cell count

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บทคัดย่อ

ตัวบ่งชี้ความเครียดออกซิเดชั่นที่มีผลต่อผลผลิตน้ำนมต่ำของโคนม

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วัตถุประสงค์ของการศึกษานี้เพื่อแสดงความสัมพันธ์ระหว่างการให้นมและมาลอนไดอัลดีไฮด์ในน้ำนม (MDA) ซึ่งเป็นตัวบ่งชี้ความเครียดออกซิเดชั่น ทำการศึกษาโดยใช้แม่โคจากฟาร์มโคนม 8 ฟาร์ม ตั้งแต่ ตุลาคม ถึง ธันวาคม 2549 ทำการเก็บข้อมูล วันเก็บตัวอย่าง ปริมาณน้ำนม และวันที่คลอดครั้งสุดท้าย ตัวอย่างน้ำนมในตอนเช้าถูกเก็บทุกเดือนสำหรับการวัดปริมาณเซลล์โซมาติก (SCC) และระดับ MDA โดยใช้เครื่องนับเซลล์อัตโนมัติ และวิธีที่ดัดแปลงจากวิธีของสมิธ ตามลำดับ ข้อมูลสุดท้ายประกอบด้วย 131 ข้อมูลน้ำนมจาก แม่โค 74 ตัว ค่าเฉลี่ยรวมและส่วนเบี่ยงเบนจากค่าเฉลี่ยของน้ำนม คะแนนเซลล์โซมาติก (SCS การแปลงของข้อมูล SCC ให้มีการกระจายตัวปกติ) MDA และ วันให้นม คือ 14.5±0.45 กก./วัน 2.97±0.19 1,643±26 ppb และ 154.8±8.83 วัน ตามลำดับ ค่าเฉลี่ยของน้ำนมมีพิสัยจาก 9.7±2.0 ถึง 17.7±0.9 กก./วัน ผลจากค่าสัมประสิทธิ์ความสัมพันธ์เพียรสัน แสดงให้เห็นว่า MDA SCS และวันให้นมมีความสัมพันธ์ผกผันกับปริมาณน้ำนม (p<0.05) ในการวิเคราะห์สมการสหสัมพันธ์เส้นตรงพบว่า มีเพียง MDA และวันให้นมมีความสัมพันธ์กับปริมาณน้ำนมอย่างมีนัยสำคัญ การศึกษานี้แสดงว่าการสูญเสียน้ำนมจากผลการติดเชื้อเข้าเต้านมอาจมีส่วนมาจากการเพิ่มของสถานะความเครียดออกซิเดชั่น

คำสำคัญ : วันให้นม มาลอนไดออลดีไฮด์ การให้น้ำนม ความเครียดออกซิเดชั่น เซลล์โซมาติก

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Introduction

Mastitis is defined as an inflammation of the parenchyma of mammary glands regardless of its cause. Although mastitis occurs sporadically in all species; it is recognized as the most economic losses in the dairy industry (Barlett et al., 1990, Dijkhuizen et al., 1991). The decrease of milk yield in mastitis manifested cows is due to the damage of milk producing alveolar cells from pathogen invasion (Burvenich et al., 2003). In addition, many studies indicated that the damage is also caused by the inflammatory processes in host factors after intramammary infection (Burvenich et al., 2003). However, most studies were emphasized in the cell damage after the clinical mastitis. On the other hand, the less severe mastitis, subclinical mastitis, and its identification of intramammary infection can be diagnosed by an increase of somatic cell counts (SCC) (Suriyasathaporn et al., 2000[b]). The increase of SCC is also associated with loss in milk yield (Seegers et al., 2003).

After intramammary infection, polymorphonuclear cells move rapidly into the secretions of infected quarters causing an increase of SCC in milk (Suriyasathaporn et al., 2000[a]). Subsequently, the cells generate superoxide to kill invading microorganisms (Babior, 1999), causing an increase of oxidative reaction in the udder. Although essential for survival, the undesirable repercussion of inappropriate or excessive oxidative reaction, the so-called oxidative stress, can cause tissue degeneration (Cohen, 1994). Consequently, the loss of milk yield from increased SCC might be stemmed from the increase of oxidative stress level. Therefore, the goal of this study was to identify the correlation between milk yield and malondialdehyde levels (MDA), an oxidative stress marker.

Materials and Methods

Animals and the experimental design: The study was conducted using cows from eight small-holder dairy farms in Chiang Mai province, the Northern part of Thailand, during October to December 2004. Morning milk samples were collected monthly for the measurement of SCC and MDA. After collection, the samples were immediately transported on ice to the laboratory. Milk yield data on sample date and data on calving date were recorded. The SCC measurement was performed within 24 h by the automated counter (Somacount® S150, Bentley, USA).

The measurement of milk MDA: After the SCC measurement, the milk MDA was measured by the modified Smith's method described by Santos and colleagues (1980). Briefly, 100 μl of milk sample was properly mixed with 1 ml of trichloroacetic acid (TCA) using a vortex mixer. Then, 400 μl of thiobarbituric acid (TBA) was added. The mixture was boiled for 30 min and subsequently cooled down by running tap water. The solution was quatduplicate analyzed by UV spectrophotometry at 532 nm against the blank reaction mixture (without TBA).

Statistical analysis: Milk yield (kg/cow/day) was the dependent variable. To normalize the SCC data, they were transformed to somatic cell score (SCS) by taking log2 of (SCC/100,000) and plus 3. The SCS of cow with the SCC below 12,500 cells/cc was treated equally to 0. The day in milk (DIM) was an interval between date of calving and milk test date.

Descriptive statistics, Pearson's correlation coefficients were used to describe the correlation among milk yield, DIM, MDA and SCS. Multiple linear regression analysis with repeated measure analysis or general linear mixed model was used to define the association of the factors to and milk yield. The general linear mixed model structure was,

$$Y = \beta_0 + \Sigma\beta_i x_i + \varepsilon$$

where $\varepsilon = R + e$, R = correlation matrix, e = random error, the fixed part includes the intercept (β_0) and covariates xi and regressors for covariates (β_i)

The stepwise forward regression analysis with free entering method was used, and the maximum restricted log-likelihood test was calculated to identify significant levels. Only significant effects, p-value < 0.05, were included in the final path models.

Results

Descriptive analysis: One hundred and thirty three milk data from 74 cows, in lactation 1 (n = 28), 2 (n = 19) and more than 2 (n = 27), were collected. Numbers of data generated from the same cows were 20, 43, and 9 for 1, 2, and 3 data, respectively. The average milk yields of farms ranged between 9.7 to 17.7 kg/day. Overall means and SEM of milk yields, SCS, MDA and days in milk were 14.5±0.45 kg/day, 2.97±0.19, 1,644±26 ppb and 154.8±8.8 day, respectively. Means and standard error of mean of milk yield were 13.8±0.78 kg/day, 14.6±0.82 kg/day, and 15.7±0.74 kg/day, for lactation 1, 2 and more than 2, respectively. No significant difference among lactation number was observed. Relationships of periods of lactation, SCS, and MDA on milk yields were illustrated in Figure 1. Averages of milk yield from either days 0-60 or days 61-120 were significantly higher than the later periods, and the average of milk yield at day 121 to 180 was also higher than the averages of milk yield at the later periods or after 181 days postpartum (p<0.05). Results from descriptive analysis showed that milk yields were associated with period of lactation, SCS, and MDA. Pearson's correlation coefficients for milk yield and MDA, milk yield and SCS, and MDA and SCS were -0.46, -0.27 and 0.37 respectively.

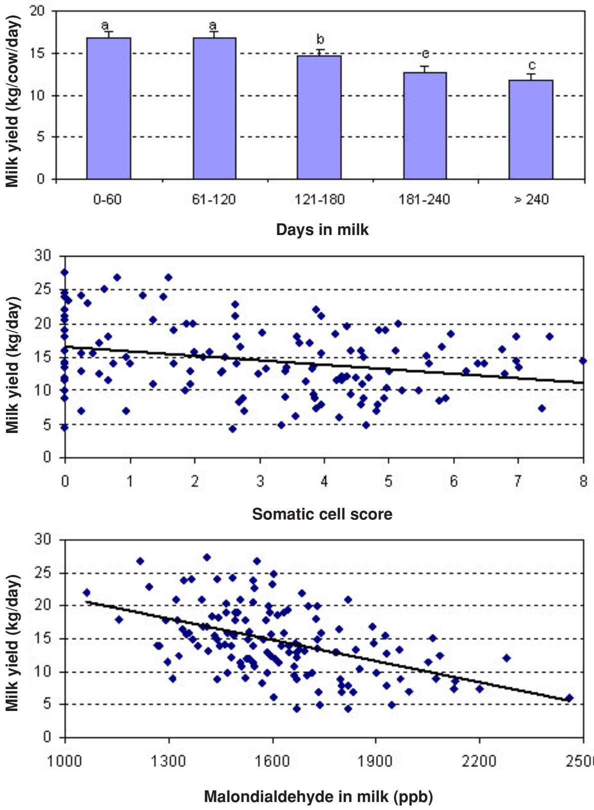

Figure 1. The relationship of milk yield to days in milk, somatic cell score, and malondialdehyde, as shown in 1A, 1B, and 1C, respectively. Data were collected during October to December 2004 (n=133) from small holder dairy farms in Thailand. Superscript letters denote that the differences along the variable were significant.

Path Models: Milk Yield and MDA: Figure 2 shows significant relationships resulting from the models for MDA and milk yield. The periods of lactation and MDA were negatively associated with milk yields (p<0.01), but not for SCS. Similar to the results of descriptive statistics, averages of milk yield from either days 0-60 or days 61-120 were significantly higher than the later periods, and the average of milk yield at day 121 to 180 was also higher than the averages of milk yield at the later periods or after 180 days postpartum (p<0.05). The value of β-estimate for MDA (x 100 ppb) was -0.35, meaning that milk yield decreased 0.35 kg when MDA increased 100 ppb. For the path model of MDA, MDA was associated with either SCS or period of lactation (p<0.05). A mean of MDA in the last period (more than 240 days postpartum) was significantly higher than the means of MDA during

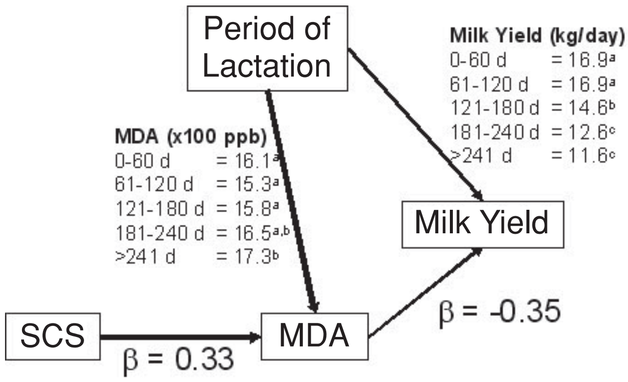

Figure 2. Path diagram of events associated with malondialdehyde (MDA), milk yield, and somatic cells scores (SCS). β indicates a risk independent variable to dependent variable. Data were collected during October to December 2004 (n = 133) from small holder dairy farms in Thailand. Superscript letters denote that the differences along the variable were significant.

0-60, 61-120, and 121-180 days postpartum. The value of β-estimate for SCS was 0.33, meaning that MDA increased 33 ppb when SCS increased 1 score.

Discussion

In general, milk yield in the first lactation of dairy cows is lower than older cows. There was no differences between lactation number observed in this study because the data used in this study were originated from single lactation. The genetic improvement of the first lactation cows might be reduced the difference of milk yield among lactation. In this study, the peak of milk yield was during 60 to 120 days postpartum (Figure 1A), which coincided with a previous report for Thai dairy cows (Suriyasathaporn et al., 2003), and gradually declined until the end of lactation. The decline in milk production is due, in part, to the reduction of mammary cells in udders (Knight and Peaker, 1984; Wilde and Knight, 1989). The cell loss during the declining phase of lactation in goats and cows is apparently the result of programmed cell death, also called apoptosis (Quarrie et al., 1994; Wilde et al., 1997). Apoptosis is mediated by oxidative stress (Best et al., 1999; De Nigris et al., 2001). In support of our study, the MDA, as an oxidative stress marker, was highest during late lactation (> 240 d postpartum).

In dairy cattle, the SCC is commonly used as a proxy to define the state of mammary gland infection (Suriyasathaporn et al., 2000[b]). An increase of SCC ≥ 200x10^3 cells/ml or SCS ≥ 4 is optimal for the prediction of intramammary infection (Schepers et al., 1997). In support of a previous study (Hortet and Seegers, 1998), we also found that milk yield was negatively associated with SCS ($p<0.05$). For our descriptive analysis, loss in milk yield was approximately 323 g/day per one SCS increased. This loss was in the same range of previous studies that the estimated milk loss due to the increase of each unit in SCS was approximately 91 and 181 kg/lactation (Raubertas and Shook, 1982; Fetrow et al., 1991), or 346 to 366 g/day (Miller et al., 2004). In other words, the effect of SCC on milk yield in this study was not different from others.

Lipid peroxidation is a biochemical oxidative degradation of unsaturated fatty acids that causes irreversible denaturation of essential proteins. With respect to the widespread distribution of unsaturated fatty acid in the cellular membranes, the peroxidative damage has the potential to affect many cellular functions (Stark, 2005) and interfere with the regulation of several metabolic pathways (Miller et al., 1993). Antioxidants limit this damage, yet peroxidative events occur when oxidant stress increases. Cytotoxic aldehydes, such as MDA that is the end product of lipid peroxidation, remain after termination of lipid peroxidation (Halliwell and Gutteridge, 1990). In this study, MDA was used as an oxidative stress marker in the udder. An increase of somatic cell count was related to the increase of MDA in milk (Figure 2). Increased MDA in milk may be due to increased radical formation or decreased antioxidative defenses. Oxidative stress has been proposed as etiological in numerous pathologies causing the increased radical formation (De Haan et al., 2003). Intramammary infection, indicated by increased SCC in milk, and mastitis are the important pathological condition in dairy cow udders. Milk with higher SCC has shown to have more infiltrated neutrophils, and this caused an increase of oxidative reactions (Su et al., 2002) and apoptosis (Tian et al., 2005). Some of the oxidants produced by the respiratory burst of neutrophils and other immune cells (Laurent et al., 1991) could oxidize plasma ascorbic acid, thereby reducing its concentration. In dairy cows, lipid peroxidation levels were increased and the levels of blood glutathione peroxidase, an antioxidant, were decreased in mastitic cows in comparisons to healthy cows (Atroshi et al., 1996). Evidences of using antioxidants in mastitic cows showed that the ascorbic acid concentration in the serum of cows was decreased in mastitic cows (Kleczkowski et al., 2005). In addition, increased severity of clinical mastitis signs were associated with large decreases in concentration of vitamin C, as an antioxidant, in milk from the challenged quarter (Weiss et al., 2004).

In Figure 2, increased MDA resulted in decreased milk yield. In addition, the exclusion of SCS from the final model indicated that the effect of SCS on loss of milk yield might be mediated by increase of MDA. It is possible that the increase of SCC itself might not affect milk producing process, but the process might be impaired by the state of oxidative stress. The peroxidative damage has the potential to affect many cellular functions and finally lead to cell death (Stark, 2005; Esper et al., 2006; Munoz-Casares et al., 2006). Giving the importance to biology of oxidative stress, a variety of mechanisms evolved to deal with free radicals from antioxidants, such as vitamins C and E, passing by enzymes that detoxify free radicals to a number of enzymes that catalyze the repair of damage caused by free radicals. The mere existence of enzymes to prevent and repair damage by free radicals is a strong indicator that free radicals are biologically important, potentially dangerous molecules (Beckman and Ames, 1998). In an experimental *E. coli* mastitis study, loss in milk yield, as a parameter for severity of mastitis, was negatively associated with a change in concentration of vitamin C, an antioxidant, in milk from the challenged quarter (Weiss et al., 2004). From the recent study, the results suggested that antioxidants may be effective tools for protecting the mammary tissue against neutrophil-induced oxidative stress during bovine mastitis (Lauzon et al., 2005).

In conclusion, concentrations of MDA in milk, as an oxidative stress marker, are negatively associated with milk yields in dairy cattle. To reduce the milk yield loss during mastitis, the attempt to decrease the severity of oxidative stress in mastitis cows; by either decrease of free radical or increase of antioxidants, should be investigated.

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