OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Selenium and Malondialdehyde Levels in Placental Retention Cases in Friesian Holstein (FH) Cows

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Abstract | Long gestation and parturition are capable of triggering oxidative stress leading to placental retention (RP). The production of cows is often affected by RP, resulting in economic losses for the local community in dairy farm. Therefore, this research aimed to measure selenium (Se) and Malondialdehyde (MDA) levels as a candidate for RP parameters in dairy farm. The measurement started with preliminary research of plasma MDA levels in 20 cows comprising 5 RP and 15 non-placental retention (NRP). This was followed by the determination of Se and MDA levels in 46 cows consisting of 21 RP and 25 NRP. The samples were obtained from clinical conditions of dairy cows in the local community in Indonesia which have the recording of RP. The blood samples were taken by 4 groups at different times such as at 3 weeks and 1 week prepartus, 1 day and 3 weeks postpartus through the coccygeal vein. Subsequently, Se and MDA levels were tested with ICP-MS and spectrophotometer, respectively. The results showed that Se levels of RP cows in 3 weeks prepartus (48.45 ng/mL), decreased significantly in 1 week prepartus (34.74 ng/mL) and 1 day postpartus (33.39 ng/mL), followed by an increase at 3 weeks postpartus (34.04 ng/mL). Se levels of NRP cows during 3 weeks prepartus (60.96 ng/mL), decreased significantly at 1 week prepartus (38.84 ng/mL) and 1 day postpartus (37.09 ng/mL) but increased at 3 weeks postpartus (39.53 ng/mL). MDA level of preliminary research showed that RP cows 3 weeks prepartus (2.12 nmol/mL) significantly increased at 1 week prepartus (5.89 nmol/mL) and 1 day postpartus (7.13 nmol/mL). MDA of NRP cows in the period of 3 weeks prepartus (1.92 nmol/mL) increased from (3.04 nmol/mL) to 1 day postpartus (4.14 nmol/mL). Additionally, the evaluation of MDA levels in RP cows in 3 weeks prepartus (2.98 nmol/mL) increased significantly at 1 week prepartus (6.43 nmol/mL). MDA of NRP cows at 3 weeks prepartus (2.01 nmol/mL) also increased at 1 week prepartus (3.15 nmol/mL). Based on these analyses, MDA from blood samples at 1 week prepartus could be used as a proper parameter of RP in dairy farm.

Keywords | Malondialdehyde, Oxidative, Placental retention, Selenium

Received | May 17, 2024; **Accepted** | July 18, 2024; **Published** | September 18, 2024
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Citation | Retnawati DW, Setiadi MA, Supriatna I, Tumbelaka LI (2024). Selenium and malondialdehyde levels in placental retention cases in friesian holstein (fh) cows. Adv. Anim. Vet. Sci. 12(11): 2062-2068.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.11.2062.2068
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Economic losses in dairy farming are attributed to the disruption of cow reproduction influenced by diseases, such as pyometra, metritis, laminitis, and placental retention (RP) (Yasothai, 2014). Specifically, RP affects the economic aspects of the local community dairy farm due to decreased milk production, weak uterine muscles, failure of pregnancy, and lengthening of the calving interval (El-Malky, 2010). This phenomenon could occur due to the conventional management in local community dairy farm and the quality of nutrition that affects the risk of RP. Therefore, the parameters from blood samples are needed to detect the risk of RP before parturition, facilitating the preparation for the medical treatment and prevention of the negative impact of RP on dairy farm.

RP is a condition where the fetal membrane is retained from the mother's body beyond the normal limit of 8-12 hours postpartum (Taylor *et al*., 2010), causing decreased milk production, weak uterine muscles, pregnancy failure, and extended calving interval (El-Malky, 2010). Generally, extended gestation intervals lead to oxidative stress that causes the formation of free radicals, resulting in a decreased immune system and increased incidence of inflammation in subsequent pregnancy (Jovanovic *et al*., 2013). Metabolic demands in prepartus and postpartus periods contribute to increased reactive oxygen species (ROS) which are known to cause lipid peroxides, followed by oxidative stress and tissue damage (Sordillo, 2005).

Oxidative stress and immune function are caused by vitamin deficiency and lack of minerals such as selenium (Se) in feed (Kendal and Bone, 2006). According to previous research, Se deficiency in the periparturient period decreases the ability of the overall antioxidant system function, (Pilarczyk *et al*., 2012) and glutathione peroxidase (GSH-Px) activity but increases Malondialdehyde (MDA) levels in plasma (Juniper *et al*., 2019). GSH-Px is a selenoprotein playing a role in the antioxidant defense process capable of removing peroxides that damage lipids and protect immune cells from oxidative stress (Ceballos *et al*., 2009). The antioxidant system neutralizes the production of ROS, including GSH-Px which depends on Se as the main component (Ottaviano, 2009). MDA is a dialdehyde compound, an end product of lipid peroxidation that is stable, accurate, and capable of explaining the role of oxidative stress in various diseases (Ayala *et al*., 2014).

During the periparturient period, oxidative stress is an important factor related to susceptibility to infections such as metritis, edema, mastitis, and RP (Kankofer, 2002), including Se and GSH-Px concentrations (Harapin *et al*., 2000; Pavlata *et al*., 2000). Measurement of Se and MDA is expected to be part of preventive diagnosis to provide the necessary data for making appropriate treatment decisions. Therefore, this research aimed to determine the relationship between oxidative stress described by Se and MDA concentrations in blood, serving as an early diagnosis of the occurrence of RP in dairy cows.

MATERIALS AND METHODS

This research received ethical clearance with number 226-2022 IPB issued by the Institute for Research and Community Service (LPPM) IPB on January 25, 2022. The sampling method and sample handling were reviewed under the supervision ethics commission.

Research Animals

Friesian Holstein (FH) cows were located in the farm area of Lembang District, West Bandung Regency, West Java Province. The preliminary research used 20 cows for MDA sampling, while further analysis applied 46 cows for Se and MDA analyses. The continuing sampling was carried out to enhance the quality of sample data based on the clinical results. The cows used were ±8 months pregnant, minimum of 3rd lactation, and 5-10 years old, with Body Condition Score (HCS) of 2.75 - 3.25, a score range of 1-5 (Lowman *et al*., 1976). Selected cows were fed 3 times, namely in the morning with grass and concentrate, in the afternoon only grass, and in the evening with grass and concentrate.

Sampling Blood

This research used a cross-sectional design and the parameters were observed simultaneously during data collection. Blood sampling for Se test was carried out at 3 weeks prepartus, 1 week prepartus, 1 day postpartus, and 3 weeks postpartus. The first MDA test was carried out in 3 weeks prepartus, 1 week prepartus, and 1 day postpartus, while the second MDA was conducted in 3 weeks prepartus and 1 week prepartus. Blood collection was performed through the coccygeal vein or external jugular vein obtained 5 ml using a 10 ml spoit with a 23G needle (Dewi and Durachim, 2014) at 10:00 am with a distance of 4-5 hours from morning feeding.

Blood samples were placed in EDTA vacutainer tubes (Zhejiang, Gongdong, China), which were labeled and put in a transportation bag with a temperature of 4^{O}C. Subsequently, the samples were brought to the laboratory and stored for 1 week in the refrigerator. Blood samples in EDTA vacutainer tubes (Zhejiang, Gongdong, China) were centrifuged (LC - 04B PLUS, Jiangsu, China) at 3000 rpm for 15 minutes. Blood plasma obtained was stored for 1 month in 2 ml Eppendorf in the freezer for Se and MDA analysis.

Analysis of Selenium (Se)

In this research, Se test was carried out using Inductively Coupled Plasma Optical Mass Spectrometry (ICP MS).

Table 1: Se levels of FH cows with RP and NRP cases.

Diagnosis	N	Unit	± 3 Weeks Prepartus	± 1 Week Prepartus	1 Day Postpartus	3 Weeks Postpartus
RP	21	ng/mL	48.45±21.64^{Ab}	34.74±13.71^{Aa}	33.39±15.83^{Aa}	34.04±11.36^{Aa}
NRP	25	ng/mL	60.96±16.79^{Bb}	38.84±16.08^{Aa}	37.09±15.50^{Aa}	39.53±13.75^{Aa}

Different superscript uppercase letters in the same column indicate significant differences ($p<0.05$); Different superscript lowercase letters on the same line indicate significant differences ($p<0.05$).

The working principle of ICP MS is to atomize the element to emit light of a certain wavelength which can be measured simultaneously and at the lowest level of 1- 10 parts per billion or ppb (Hou and Jones, 2000). During the experiment, plasma sample of 1 ml was poured into the tube, added with 10 ml of concentrated HNO3 (sigma, Singapore), and allowed to stand for 15 minutes at room temperature. The tube was closed and deconstructed in a microwave digester at 150^{O}C for 10 minutes. The results of the deconstruction were cooled and put in a 50 ml flask. The tube was rinsed using aquabides quantitatively and combined with the results of the destruction in a 50 ml flask, followed by the adding 0.4 ml of internal standard reagent mixture (Germanium, Indium, Bismuth) 10 mg/L. Subsequently, the solution was filtered with a 0.20 µm RC/GHP filter, placed in volumetric flask diluted with acubides to the mark, and homogenized using a stirrer. Measurement of the intensity of Se sample solution was carried out in the flask absorbed by ICP MS using Germanium internal standard.

Analysis of Malondialdehyde (MDA)

Blood plasma stored for 1 month in 2 ml Eppendorf in the freezer was analyzed for MDA. This MDA assay used a medium of 3 ml of 0.1% orthophosphoric acid, 1 ml of 0.6% thiobarbituric acid, and 0.1 ml of 0.28% hydrated sulfate hydrate solution, added with 0.3 ml plasma. Subsequently, the reaction mixture was heated in water at 1000^{O}C for 60 minutes to form a pink color. The resulting chromogen was cooled at room temperature, added with 1-butanol of 4 ml, and the solution was centrifuged at 2200 xg for 10 minutes. A total of 1 ml supernatant was collected for spectrophotometer measurement at a wavelength of 535 nm and the absorbance value obtained was used to calculate plasma MDA levels.

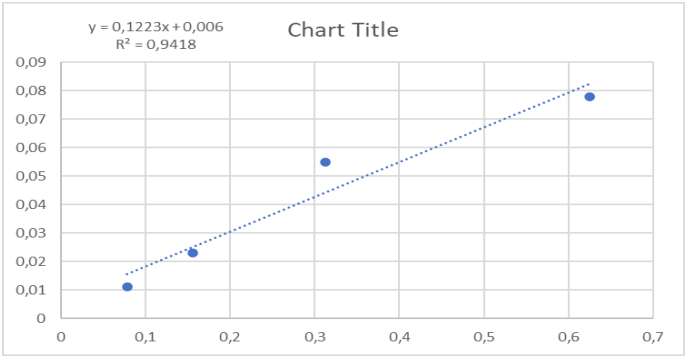

Figure 1: MDA Standard Curve.

Preparation of MDA standard solution was performed by mixing TEP (1,1,3,3-tetraethoxypropane) at a ratio of 1: 80,000 with levels of 0.625, 1.25, 2.5, 5, 10, 20, 40, and 80 µL into 8 different test tubes. Distilled water was added to each test tube of 399.375, 398.75, 397.5, 395, 390, 380, 360, and 320 µL to obtain standard solutions with concentrations of 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5, and 10 nmol/mL. MDA Standard Curve was made by calculating the variable y and x on the absorbance of each concentration to obtain a linear regression equation y=ax+b.

Data Analysis

Se and MDA levels in RP and NRP cows' events were statistically tested using ANOVA test. When the results showed significant differences, the analysis was followed by Duncan's test (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

The results showed that among 46 cows used, 21 experienced RP, and 25 were categorized as NRP. Se level of RP cows in 3 weeks prepartus was 48.45 ±21.64 ng/mL, which decreased significantly in 1 week prepartus to 34.74 ± 13.71 ng/mL. Subsequently, 1 day postpartus decreased to 33.39±15.83 ng/mL but 3 weeks postpartus increased to 34.04±11.36 ng/mL, as shown in Table 1.

Se levels of NRP cows in 3 weeks prepartus was 60.96±16.79 ng/mL but decreased significantly in 1 week prepartus at 38.84±16.08 ng/mL. This was followed by a decrease in 1 day postpartus at 37.09±15.50 ng/mL and increased in 3 weeks postpartus at 39.53±13.75 ng/mL.

The measurement results were made with the linear regression equation of MDA standard absorbance obtained as y = 0.1223x + 0.006, as shown in Figure 1. Variable y served as the absorbance of MDA and variable x was the concentration of MDA standard. Based on the linear regression equation, the value of R^2 = 0.9418 was obtained.

After obtaining the linear regression equation of MDA standard absorbance, the measurement results of cows' plasma MDA absorbance were entered into the equation y = 0.1223x + 0.006 to obtain the value of plasma MDA levels in RP and NRP cows. Based on the results, MDA levels of RP cows in 3 weeks prepartus were 2.12 ± 0.08 nmol/mL, which increased in 1 week prepartus to 5.89 ±

0.09 nmol/mL and 1 day postpartus by 7.13 ± 0.09 nm/mL. Regarding NRP cows, MDA levels in 3 weeks prepartus at 1.93 ± 0.07 nmol/mL increased in 1 week prepartus to 3.04 ± 0.10 nmol/mL, and 1 day postpartus by 4.14 ± 0.12 nmol/mL, as shown in Table 3.

Table 2: Mean MDA Standard Absorbance.

	MDA levels (nmol/mL)	Mean absorbance
S1	0.078	0.011
S2	0.156	0.023
S3	0.312	0.055
S4	0.625	0.078
S5	1.25	0.187
S6	2.5	0.395
S7	5	0.525
S8	10	0.891

Table 3: MDA levels of FH cows with RP and NRP cases in preliminary research.

Diagnosis	N	Unit	± 3 Weeks Prepartus	± 1 Week Prepartus	1 Day Postpartus
RP	5	nmol/mL	2.12 ±0.08^{Ba}	5.89±0.09^{Bb}	7.13±0.09^{Bc}
NRP	15	nmol/mL	1.93±0.07^{Aa}	3.04±0.10^{Ab}	4.14±0.12^{Ac}

Different superscript uppercase letters in the same column indicate significant differences ($p<0.05$); Different superscript lowercase letters on the same line indicate significant differences ($p<0.05$).

In the continuing part of this research, 46 dairy cows were obtained, consisting of 21 RP and 25 NRP. The results of MDA levels in RP dairy cows in the period of 3 weeks prepartus showed 2.98 ± 0.18 nmol/ mL, which increased in 1 week of prepartus by 6.43 ± 0.12 nmol/ mL. In NRP samples, MDA levels in 3 weeks prepartus were 2.01 ± 0.12 nmol/ mL and increased in 1 week prepartus, as shown in Table 4.

Table 4: MDA levels of FH cows with RP and NRP on continuing research.

Diagnosis	N	Unit	± 3 Weeks Prepartus	± 1 Week Prepartus
RP	21	nmol/mL	2.98±0.18^{Aa}	6.43±0.12^{Bc}
NRP	25	nmol/mL	2.01±0.12^{Ab}	3.15±0.13^{Bd}

Different superscript uppercase letters in the same column indicate significant differences ($p<0.05$); Different superscript lowercase letters on the same line indicate significant differences ($p<0.05$).

A significant difference was observed in all values of MDA levels of both RP and NRP cows in the preliminary and continuing research. The highest MDA levels occurred in RP cows at 1 week prepartus, which could be the candidate parameters to detect RP risks before the parturition of dairy cows. Deficiency in minerals such as Se can affect the transition period, thereby causing postpartus reproductive disorders including endometritis and RP. The metabolic demands associated with late pregnancy, parturition, and early lactation contribute to the increase in ROS (Sordillo, 2005). Moreover, Se is an essential element that protects organisms from oxidative damage (Gresavoka *et al*., 2013). It is also selenoprotein that plays a role in maintaining redox status and detoxifying ROS (Zhang *et al*., 2020). Based on this research, Se concentration in RP group was lower than NRP. The decrease observed in RP group could be attributed to high levels of ROS, which produced more lipid peroxides, leading to a reduction in glutathione peroxidase (GSH-Px) (Mikulková *et al*., 2020). Jovanović *et al*. (2013) stated that GSH-Px activity was dependent on Se in bovine blood. In this research, blood GSH-Px activity was significantly lower in RP cows compared to NRP. However, Kankofer *et al*. (2001) observed an increase in GSH-Px metabolites in RP group of cows compared to NRP in peripartum period. Generally, a multicomponent antioxidant system will balance ROS, including Se-dependent GSH-Px (Ottaviano *et al*., 2009).

In this research, Se in RP and NRP cow groups showed a decrease until parturition and increased again at 3 weeks postpartus. Joksimović-Todorović and Davidović (2013) stated that Se and vitamin E were natural antioxidants playing a significant role in preventing RP. These nutrients increase neutrophil activity, enhance the chemotactic effect, and phagocytosis of opsonized pathogenic microorganisms. The quality of nutrition for feeding in conventional management will give different clinical results in blood samples.

The minimum Se concentration during the transition period in cows' blood plasma is 30 ng/mL. However, when the value is below this concentration, the placenta will retain significantly from 5-6% to 20-22% (Jaskowski, 1987; Wentink *et al*., 1988). In this research, Se levels in cows were still in the normal range without difference between RP and NRP, due to the sufficient intake of feed containing Se. According to El-Shahat and Monem (2011), Se deficiency can cause reproductive diseases including fertility disorders, abortus, RP, and neonate weakness. The presence of cell or tissue damage during parturition requires vitamin E and Se to improve neutrophil function and accelerate postpartus uterine contractions. However, there are some changes in the placental antioxidant defense system in the case of RP as variations in lipid peroxidation production.

The most frequently used biomarker of oxidative stress as a laboratory parameter is MDA. This biomarker is a dialdehyde compound that is the end product of oxidative decomposition of low molecular weight polyunsaturated fatty acids. MDA reacts with thiobarbituric acid, producing a red pigment that can be measured spectrophotometrically

(Erisir *et al*., 2006). Oxidative stress causes mitochondrial dysfunction leading to cellular energy deficiency, accumulation of cytotoxic mediators, and cellular damage. The brain is particularly susceptible to damage caused by oxidative stress through ROS (Butterfield, 2009). In this research, MDA levels in RP cows were higher compared to NRP, which could be attributed to increased oxidative stress due to parturition caused by PGF2α (prostaglandin F2α). Furthermore, RP cows had significantly higher plasma MDA ($P < 0.01$) compared to NRP cows (Jovanović *et al*., 2013). The imbalance of ROS production is followed by oxidative stress which results in easy exposure to diseases in the transition period. Oxidative stress is often indicated by the production of lipid peroxides, showing that applicable biomarkers are lipid peroxide intermediates, namely MDA. In this research, the absorbance value of each MDA standard level was measured and read with a spectrophotometer to determine the level of oxidative stress in RP cows. According to Bernabucci *et al*. (2005), MDA increases due to the presence of macrophages infiltrating the adipose tissue. TNF-α (tumor necrosis factor-alpha) produced by these macrophages and adipocytes can also increase MDA production.

The high increase in MDA is attributed to metabolic and endocrine changes related to RP, particularly antioxidative enzymes that can protect against oxidative stress. Khudhair *et al*. (2021) reported that the decrease in glutathione peroxidase was significantly lower in RP cows compared to NRP. Ahmed *et al*. (2019) stated that the decrease in catalase was significantly lower in RP cows compared to NRP. Furthermore, RP cows showed significantly higher plasma MDA at 5.32 nmol/mL compared to NRP at 4.68 nmol/mL (Jovanovic *et al*., 2013). This was supported by Yuqiong Li *et al*. (2021), where FH cows experiencing RP had MDA levels of 6.00 nm/mL while healthy cows were at 3.66 nmol/mL. High MDA concentration shows a low amount of antioxidants and increased ROS including elevated lipid peroxides in line with the high risk of oxidative stress (Castillo, 2005; Gong and Xiao, 2016). Research on MDA during inflammation is divided into two statements, where some described an increase (Atroshi *et al*., 2006; Taysi *et al*., 2002), and others showed no significant changes during inflammation (Friedman *et al*., 2002; Uslu *et al*., 2003).

High MDA concentrations show that the presence of oxidation processes in the cell membrane of the animal body has an organized antioxidant system, both enzymatic and nonenzymatic working synergistically (Ponnampalam *et al*., 2022). Additionally, it serves as an indication of oxidative stress in cows (Wang *et al*., 2021). In this research, MDA levels were the major factor that affected the increasing value observed in 1 week prepartus.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this research showed that Se levels as an antioxidant did not experience significant changes and were still in the normal range. MDA levels as a marker of oxidative stress at 1 week prepartus experienced a significant increase due to high protein metabolism, showing potential application as a candidate of risk parameter in RP case.

ACKNOWLEDGMENTS

The authors are grateful to the Agricultural Extension and Human Resource Development Agency, Ministry of Agriculture, for providing educational scholarships. Furthermore, the authors are grateful to Koprasi Peternakan Sapi Bandung Utara (KPSBU) Lembang for sampling and the National Research and Innovation Agency (BRIN) for testing samples in this research.

NOVELTY STATEMENT

Malondialdehyde (MDA) as a biomarker of oxidative stress which can be used as an indicator of placental retention.

AUTHOR'S CONTRIBUTIONS

DWR, MAS, IS, LGY are equal authors. The authors conducted the study, conceptualized the study, analyzed the data, and finalized the manuscript. The authors have read, reviewed, and approved the final content of the manuscript and agree to the conditions outlined in the copyright assignment form.

Conflict of interest

The authors have declared no conflict of interest.

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