Malondialdehyde (MDA) level and protein profile of serum after calving towards the provision of selenium-vitamin E™ on dairy cow frisian holstein (FH)

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Abstract. This study aims to determine effectiveness of Selenium and vitamin E™ intramuscularly to the serum MDA level and protein profile after calving. Twenty pregnant FH cows were used in this experiment, divided into four groups. The control group (K0), Group 1 (K1) : 0.5 mg / ml Selenium + 50 mg vitamin E™, Group 2 (K2) : 1.5 mg / ml Selenium + 50 mg vitamin E™ and Group 3 (K3) : 2 mg / ml Selenium + 100 mg vitamin E™. Selenium-vitamin E™ given intramuscularly at the age of 7 and 8 months of pregnancy, two weeks before birth, 7 and 14 days after calving. Measurement of serum MDA levels and protein profile were carried out on 25 and 45 days after calving. Serum MDA levels on the 25 days after calving very varied results in each treatment group, while serum MDA levels decreased with increasing doses of Selenium and vitamin E™ on the 45 days after calving. There was no difference in serum protein profile in all of treatment groups. This study concludes that administration of selenium-vitamin E™ at a dose of 2 mg/ml Selenium + 100 mg/ml of vitamin E™ can reduce serum MDA levels.

1. Introduction
Pregnancy and lactation periods are conditions that cause physiological changes and induce stress conditions. During pregnancy, all metabolism occurs to support fetal development. Redundant maternal tissues are involved in providing energy for the reproductive process, which causes changes in blood serum biochemical composition [3,16,17] found that during the final period of pregnancy and early lactation there was a very high metabolic change. At the end of pregnancy until the beginning of lactation is a critical transition period, because it determines the condition of fertility in the next period [15]. In this transition period, the physiological and metabolic dairy cows will experience changes due to pressure and changes in the body's immune system [14]. Changes in metabolism and the occurrence of mild inflammation during pregnancy lead to the formation of oxidative stress. During pregnancy, several organs in the body show an increase in basal oxygen consumption resulting in ROS (reactive oxygen species) [20].

ROS is a normal product of an aerobic metabolism. The biggest free radicals found in biological systems are superoxide, hydrogen peroxide, hydroxyl radical and fatty acid radicals [19]. An
imbalance between increased ROS production can increase oxidative pressure, which can cause cell damage through lipid peroxidation [19]. [6] states that Reactive Oxygen Species (ROS) plays an important role in reproductive biology. ROS targets are components of lipids, proteins and DNA [5]. In certain number of ROS plays a role in reproductive function, such as oocyte maturation, folliculogenesis, steroidogenesis, luteolysis, ovulation and endometrial changes [8]. However, high amounts of ROS can cause reproductive pathology conditions, such as placental retention, mastitis, which causes infertility [4]. Malondialdehyde (MDA) is the end product of lipid peroxidation, which can be used to measure the degree of oxidative stress [21]. Antioxidants have a strong influence in maintaining reproduction in females. Antioxidants in the form of vitamin E and vitamin C are combinations that can prevent eclampsia [1]. The aim of this study was to study at MDA levels after parturition after being given Selenium and vitamin E during intramuscular pregnancy.

2. Material And Methods

2.1. Treatment of Animals
This study used 20 healthy FH cows that were pregnant 7 months, with body weights between 400 to 570 kg. The experimental animals were grouped into 4, namely the control group (K0) which was without given Selenium and vitamin E), Group 1 (K1) injected 0.5 mg / ml selenium + 50 mg / ml Vitamin E, Group 2 (K2) injected 1.5 mg / ml of selenium + 50 mg / ml of vitamin E and Group 3 (K3) injected 2 mg / ml of selenium + 100 mg / ml of vitamin E. Vitamin E are given intramuscularly at 7 months, 8 months, two weeks before giving birth, 7 days after calving and 14 days after giving birth. Measurement of MDA levels was carried out through serum on days 25 and 45 after giving birth.

2.2. Serum MDA level analysed
Analysis of MDA levels was preceded by preparing of MDA standard curve using MDA kits with various concentrations. Measurement of MDA levels was carried out using a spectrophotometer at a wavelength of 533 nm [2]. Serum MDA levels were analyzed through TBA testing. A total of 450µL of serum plus 2 ml of TTH reagent (18.8 ml of TCA 40% cold, 28.7 ml of 1% TBA, 0.25N HCl), then heated in a water bath with a temperature of 100°C for 10 minutes. Next the sample was cooled, then centrifuged at 1500x g, 10 minutes. Measurement of MDA levels using a spectrophotometer at a wavelength of 532 nm.

2.3 Protein Profile Analysis using SDS PAGE
The protein profile was analysis by SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis). Electrophoresis was performed on a 12.5% acrylamide separation gel with a 4% stacking gel. Electrophoresis was performed using the Mini-Protean Cell (Bio-Rad) at 200 V for 50 minutes. Gels were stained with Commassie blue. The protein bands were analysed based on the standard proteins (BioRad).

2.4 Data Analysis
The data were analysed using the F test with the significance value of 95 % which were followed by Duncan Test. Protein profile data from SDS-PAGE in the form of protein bands were analysed descriptively

3. Result and Discussion

3.1 Serum MDA level
MDA concentration is one indicator of the occurrence of lipid peroxidation. The results of measurements of MDA levels in serum of cattle injected with Selenium + vitamin E can be seen in Figure 1. The average MDA levels on the 25th and 45th days after calving there was no significant difference. Similar results shown by [3] found that there was no significant difference in MDA levels two weeks after delivery.
MDA levels on the 25th day after delivery showed very varied results in each treatment group. Different things on the 45th day after giving birth, MDA levels decreased along with the dose of Selenium and vitamin E\textsuperscript{TM} given. Administration of a dose of 2 mg / ml of selenium + 100 mg / ml of vitamin E\textsuperscript{TM} reduced MDA levels compared to controls (0.5 mg / ml of selenium + 50 mg / ml of vitamin E\textsuperscript{TM} ml). Ten (10) to 25 days after giving birth, the mother is very susceptible to uterine infection \cite{12}. Bacterial infection in the incidence of sepsis is associated with increased levels of MDA (Rahardjani, 2010). To restore uterine function, after birth there is a process called uterine involution. Uterine involution is a process of reducing the size and regeneration of uterine tissue that occurs 45 days after giving birth \cite{9}. \cite{7} found that MDA levels were not significantly different during the estrus cycle. The results showed a decrease in MDA levels in the group given Selenium and vitamin E\textsuperscript{TM}. Antioxidants are compounds that can control free radicals. vitamin E\textsuperscript{TM} (tocopherol) is one of the antioxidants that can protect cell membranes from ROS attacks \cite{10,11}.

3.2. Protein Profile Analysis

The SDS-PAGE results of the serum shown in Figure 2. The number of serum protein bands and their relative molecular weight were determined. By the comassie staining, the serum contained 11 well stained bands with a molecular weight from 260 kDa to 12.5 kDa. The protein electrophoresis of whole cell yielded about 12 bands (Fig 1) : 240, 140, 130, 100, 90, 70, 50, 32, 25, 17 and 12.5 kDa.

4. Conclusion

Administration of selenium-vitamin E\textsuperscript{TM} at a dose of 2 mg/ml selenium + 100 mg/ ml of vitamin E\textsuperscript{TM} can reduce serum MDA levels. There was no difference in serum protein profile in all of treatment groups.
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