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Oxidative Stress Status in Heat Shock Sheep Controlled Shearing and Ascorbyl Palmitate Administration

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ABSTRACT. *Indonesia has very hot temperatures and high humidity, making it unfavorable for sheep whose covered with thick fur. Shearing enlarges and accelerates heat dissipation from the sheep's body, giving antioxidants that help reduce the negative effects of excessive free radical production. Ascorbic acid is an antioxidant but is easily decomposed when it enters the digestive system of ruminants. Ascorbyl palmitate (AP) is ascorbic acid in the form of an ester which is insoluble in water. The aim of this study was to analyze the effect of shearing and administration of AP antioxidants on oxidative stress conditions in Padjadjaran sheep subjected to heat shock. The shaving treatment consisted of not shaving (s0) and shearing (s1),and the treatment of giving the antioxidant ascorbyl palmitate consisted of not being given (a0) and being given (a1). Sheep were subjected to shearing, their hair was trimmed until ± 10 mm in length was left, and sheep as object of study were treated with ascorbyl palmitate at a dose of 400 mg head-1 day-1. The combination treatment was not shaved – not given the antioxidant ascorbyl palmitate (s0a0), only shaved (s1a0), only given the antioxidant ascorbyl palmitate (s0a1), and sheared – given the antioxidant ascorbyl palmitate (s1a1). Heat shock is given by exposing the sheep to the sun. The oxidative stress observed and analyzed was the profile of endogenous antioxidants consisting of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA). The results showed that the combination of shearing and Ascorbyl Palmitate had a significant effect ($p<0.05$) on the levels of SOD, CAT and GSH-Px in Padjadjaran sheep receiving heat stress. The conclusion from this study, the shearing treatment and the administration of Ascorbyl palmitate, both independently and in combination, had a beneficial effect on Padjadjaran sheep.*

Keywords: Ascorbyl palmitate, oxidative stress, padjadjaran sheep

INTRODUCTION

In developing countries, including Indonesia, sheep are mostly reared in smallholder farms. Indigenous Indonesian local sheep have good adaptability to tropical climates, disease potential, tick disturbances, and low-quality feed. Padjadjaran sheep are Priangan sheep which are kept by many farmers in Wanaraja District, Garut Regency. They have characteristics of white basic fur, big ears, and triangular tail, with biomolecular character mt-DNA deletion of 75 base pairs at position 1447 base pairs (Prajoga et al., 2015).

Indonesia features a temperature range of 20-30°C, with a temperature amplitude of 1-5°C, rainfall of 200-225 cm per year (Winarno et al., 2019). This condition is unfavourable for sheep because their body covered with thick fur, so the heat dissipation is hampered and has the potential to cause heat stress. Heat stress is an inability condition of livestock to maintain normal body temperature (Rolf, 2015), limiting factor for sheep production (McManus et al., 2009). Environmental change, such as an increase or decrease in temperature, induces in physiological, blood biochemical and behavioural responses (Piccione et al., 2008; Silanikove, 2000). Heat stress increase activity of superoxide dismutase (SOD), catalase (CAT) and

glutathione peroxidase (GPx) as a response to increased levels of free radicals. It can be neutralized by antioxidants such as phenol, polyphenol, flavonoid, vitamin E, tocopherol and vitamin C.

Ruminants are capable of synthesizing vitamin C, but they are prone to deficiency because vitamin C is easily degraded in the rumen. Previous researchers have succeeded in formulating vitamin C not easily degraded in the rumen, namely encapsulation (Madene et al, 2006) and in the form ester (Costa et al.., 2014; Špiclin et al.., 2001; Treichel et al.., 2010). This study combines shearing and administration of antioxidant vitamin C in the form of esters to overcome heat stress in Padjadjaran sheep. The vitamin C used is ascorbyl palmitate (AP), while the variables measured consisted of oxidative stress (SOD, CAT and GPx) enzyme

MATERIALS AND METHODS

Animal. Twenty healthy rams, 12 – 18 months old, 36.2 kg average body weight, breed Padjadjaran sheep from breeding station at Purwakarta regency West Java.

Feeding. Feeding serves a mixture of concentrates (30 %) and forages (70 %) as much as 3.5% dry matter of body weight. The concentrates feed has 13% of protein and 65- 70% Total Digestible Nutrient (TDN). Drinking water serves an ad-libitum.

Chemical. Ascorbyl-6-Palmitate (AP) produce Shandong Zhi Shang Chemical Co Ltd China; Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-PX) and Malondialdehyde (MDA) measure use assay kit produce Elabscience Biotechnology Inc USA.

Experimental procedure. This research was conducted experimental methods designed Factorial Randomized Block Design. There were two level treatments, shearing and AP administration. Shearing treatment consists of not shearing (c_0) and shearing (c_1), while AP administration consist of not given (a_0) and given (a_1) 400 mg AP each head daily. The whole treatments were:

- not shearing – not given AP (c_0a_0)
- not shearing – given AP (c_0a_1)
- shearing – not given AP (c_1a_0)
- shearing – given AP (c_1a_1)

Heat exposure in away animal into chamber made from bamboo and wood, size 150 x 100 x 100 cm in length, width and tall, respectively. Each chamber filled 5 rams appropriate to treatments. Heat exposure is conducted throughout 180 minutes per day, three days a week. for 4 weeks

Blood collection. The blood was collected every week after heat exposure application. Blood was collected by vacutainer tubes with EDTA from vena jugularis as much 5 ml. Furthermore, the blood was centrifuged at 3500 rpm for 10 minutes. The plasm was put into eppendorf tube, and stored at -20°C until analysis.

Parameters. Oxidative stress is determined by measuring the level of SOD, CAT and GSH-PX, and MDA. Spectrophotometer and test kit was used in this assay. Analysis conducted in integrated laboratory UIN Sunan Gunung Djati.

Statistical analysis. The obtained data parameters were analyzed by ANOVA (Steel and Torrie, 1997). Data was calculated using Minitab software. The level of significance was set at $P<0.05$.

RESULT AND DISCUSSION

Environmental situation. In the housing, the temperature minimum range was 20.8^{o}C – 23.4^{o}C, and average of 22.6°C; maximum temperature was 27.4^{o}C – 35.9^{o}C, average of 31.5°C. The relative humidity (RH) maximum range 79% - 99%, average of 83.42%, minimum RH 30% - 89%, average of 53.37%. The range of temperature and humidity was in thermoneutral zone for Padjadjaran sheep. Thermoneutral zone for sheep 12–32^{o}C (Al-Dawood, 2017), 25°C

(Filho et al., 2011), 15°C - 30°C (Gesualdi et al., 2014), 19,5°C - 30°C (Rout et al., 2018). According to that condition, while the sheep was in the cage, they would feel comfortable. Since they were given a heat shock, they will experience heat stress.

Oxidative stress status

Superoxide Dismutase (SOD). SOD level during the study is presented in Fig 1. The average levels of Superoxide Dismutase (SOD) before treatment ranged from 1.52 ± 0.29 to 3.91 ± 2.03 U mL-1. SOD levels in the first and third weeks were affected by the combination of shaving treatment and administration of ascorbyl palmitate ($p<0.05$).

SOD range level in livestock under normal conditions reported by previous researchers, 860 to 1160 U g Hb-1 (Nazifi, Saeb, Ghafari, et al., 2009); 1.62 to 1.69 U mL-1 (Giorgio et al., 2020); 2.46 to 3.01 U mL-1 (Jaguezeski et al., 2018). The difference between the results of present study and previous researchers is most likely due to differences in breeds, age and environmental conditions.

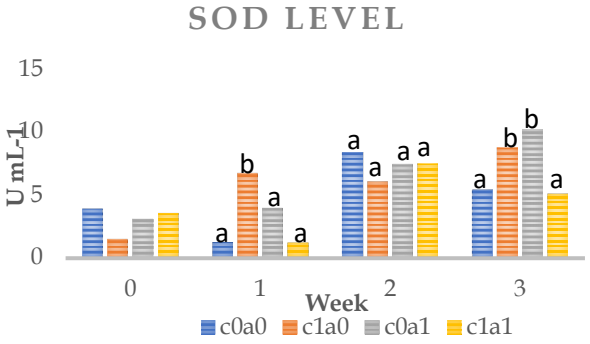

Figure 1. SOD Levels of Padjadjaran Sheep

Changes in SOD level between weeks did not show a specific pattern. SOD converts superoxide radicals (O2.-) into hydrogen peroxide (H2O2) and molecular oxygen (O2) (Weydert & Cullen, 2010). Free radicals in the form of both ROS and NOS are generated in normal aerobic metabolism and will have a biological effect on cells if their concentration increases (Weydert & Cullen, 2010). Heat stress is caused by an increase in free radicals due to oxidative stress (S. Ghosh et al., 2014). SOD, lipid peroxidase (LPO) and GPx are used as markers of oxidative stress (Rathwa et al., 2017). The SOD level in zebu cattle during summer 5.91 ± 0.06 units mg-1 was higher compared to winter 3.28 ± 0.08 units mg-1 (Chetia et al., 2017). The high concentrations of SOD, LPO and GPx during summer are evidence of heat stress conditions (Chaudhary et al., 2015; M. Ghosh et al., 2015; Maan et al., 2013). Normal cells able to detoxify superoxide radicals using enzymatic antioxidants, SOD, GPx and CAT (Sunil et al., 2011).Shearing or AP administration only insufficient effect to control of SOD, combination of both shearing and AP administration able to control of SOD.

Supplementation of antioxidants such as vitamins E and C protects the body's defense system from excessive free radical production during heat stress and maintains health stability (Menéndez-Buxadera et al., 2014; Silanikove et al., 2010), decreased rectal temperature and respiration rate (Sivakumar et al., 2010) and reduces heat stress in goats. The concentrations of SOD (Megahed et al., 2008), and catalase (Lallawmkimi, 2009) are lower in summer than in winter.

In goats (Nwunuji et al., 2014) and horses (Onmaz et al., 2011) there were decrease in SOD activity after pick up treatment. Pick up or transport led to an increase in ROS production that exceeded the ability of endogenous antioxidants (Nwunuji et al., 2014). Supplementation of α-tocopherol acetate 100 mg kg-1 ration or Se 1.2 mg kg-1 ration did notnot affect on SOD activity in sheep although increasing temperature from 28°C to 40°C (Chauhan et al., 2014).

Catalase (CAT). Catalase concentrations during the study are presented in Fig 2. Catalase levels (CAT) before treatment ranged from 0.0467 ± 0.03 to 0.1136 ± 0.073 U mL-1. Shearing treatment and administration of ascorbyl palmitate had no significant effect ($p>0.05$) on weekly CAT concentrations.

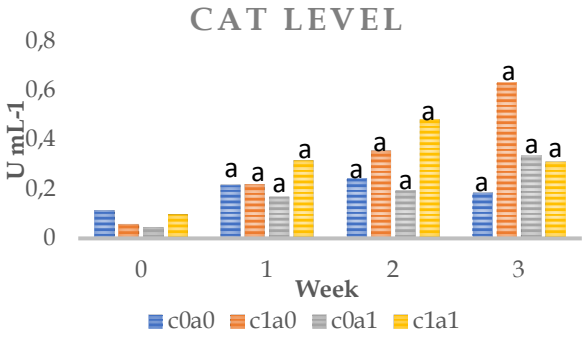

Figure 2. CAT Levels of Padjadjaran Sheep

Previous studies reported the value of the catalase range in cattle sincer normal conditions, male fat tail sheep from Iran 1587 to 2067 U g Hb-1 (Nazifi, Saeb, Ghafari, et al., 2009), Lacaune sheep 5.01 to 5.99 U mL-1 (Jaguezeski et al., 2018) healthy goats 28.88 ± 14.02 µmol L-1 (Kizil et al., 2007), male goats from Iran 1587 to 2067 U g Hb-1 (Nazifi, Saeb, Baghshani, et al., 2009). Compared to before heat shock treatment, CAT levels after heat shock increased several times. The concentration of CAT in zebu cattle during summer was higher (44.86 ± 0.52 units mg-1) compared to winter (23.60 ± 0.40 units mg-1) (Chetia et al., 2017). Catalase and peroxidase convert hydrogen peroxide to water. If the conversion of H_2O_2 is disturbed, the cell will toxication (Weydert & Cullen, 2010).

Administration of 500 mg L-1 in poultry drinking water , decrease of catalase level compare to no administration (Adenkola & Angani, 2017). Catalase is an important enzymatic antioxidant in breaking down hydrogen peroxide and maintaining oxidation-reduction reactions in homeostatic cells (Nandi et al., 2019). The role ofcatalase is as a catalysator of dismutase of hydrogen peroxide into water and oxygen (Ganaie et al., 2013). SOD and catalase protect cell damage against free radicals by protecting the hydroxyl groups from superoxide ions to hydrogen peroxide (Miyazaki et al., 1991). Activity of catalase during summer is higher than in winter (Lallawmkimi, 2009). If catalase is hampered, H_2O_2 cannot be removed (Weydert & Cullen, 2010).

Heat exposure increases ROS production and triggers oxidative stress which can cause cell poisoning (Bernabucci et al., 2002). Heat shock increases the activity of enzymatic antioxidants such as SOD, CAT and GPX as a cell response to increased levels of Reactive Oxygen Species (ROS) (Pandey et al., 2012), disruption of the stability of ROS concentrations causes mitochondrial damage, histological abnormalities and changes in mitochondrial morphology (Lewandowska et al., 2006). In the rainy season and winter, catalase levels in the blood of buck are higher than in the summer (S. Ghosh et al., 2014). CAT levels from the first day to the 5th day after shearing decreased compared to before shearing (Hefnawy et al., 2018).

The lack of effect of ascorbyl palmitate and shearing on catalase levels in heat shock sheep was most likely due to the fact that the dose of AP 400 mg per head was insufficient for antioxidants needed to overcome heat stress in Padjadjaran sheep. Another cause was that Padjadjaran sheep had good heat resistance, even without ascorbyl palmitate and without shearing (c0a0), the CAT levels detected were the same as in other treatments.

Glutathione Peroxidase (GSH-PX). The average of GSH-Px levels before treatment ranged from 0.0354 ± 0.02 to 0.2969 ± 0.11 U mL-1. In the first week of heat shock treatment, there was a combination effect of shearing and ascorbyl palmitate administration on the GSH-Px levels of Padjadjaran sheep. GSH-Px concentrations during the study are presented in Figure 3. Under normal conditions, the range of GSH-PX in male fat tail sheep from Iran is 180 to 205 U g Hb-1 (Nazifi, Saeb, Ghafari, et al., 2009) while in male goats from Iran 280 to 305 U g Hb-1 (Nazifi, Ghafari, Jahromi, et al., 2009) and in dairy goats 2.27 to 2.33 U L-1 (Giorgio et al., 2020).

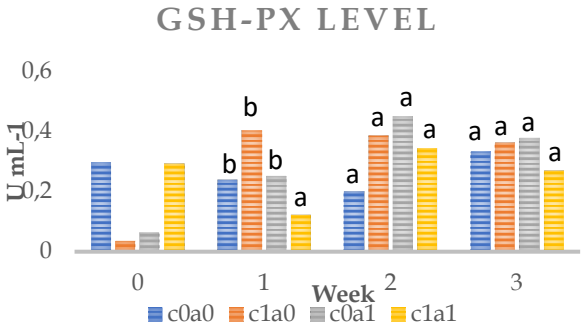

Figure 3. GSH-PX Levels of Padjadjaran Sheep

Statistical analysis showed that in the first week of heat shock, there was a significant effect ($p<0.05$) of interaction of ascorbyl palmitate and shearing, while in the second and third weeks of ascorbyl palmitate and shearing the effect was not significant ($p>0.05$) on GSH-Px levels. Glutathione peroxidase is a selenium-dependent enzymatic antioxidant, GSH-Px converts hydrogen peroxide to water (Weydert & Cullen, 2010). In the first week, there were a heat shock ram to the heat exposure, shearing, and AP administration sufficient to repress GSH level in the blood. The increased production of hydrogen peroxide due to SOD activity during heat stress is generated simultaneously with an increase in GSH-Px (Ganaie et al., 2013). GSH-PX concentrations will increase when animals have

stress such as during birth process (Bernabucci et al., 2002; Ganaie et al., 2013), and during summer (Chetia et al., 2017).

Malondialdehyde (MDA). The mean MDA levels before treatment ranged from 0.0936 ± 0.08 to 0.2731 ± 0.08 nMol mL-1. In the first week of heat shock, there was an interaction effect of shearing and ascorbyl palmitate administration on the MDA levels of Padjadjaran sheep. MDA concentration during the study is presented in Figure 4.

The statistical analysis showed that in the first week, there was an interaction between the treatment of ascorbyl palmitate and shearing on MDA levels, while in the second and third weeks, the administration of ascorbyl palmitate and shearing had no significant effect on MDA levels. In the first week, the MDA levels in sheep that were only sheared treatment (c1a0) were significantly higher ($p<0.05$) compared to other treatments; this is a sign that the sheep are suffering stress after being sheared, in the second and third weeks the sheep are able to adapt.

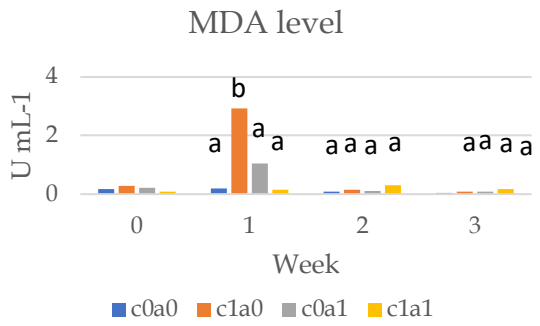

Figure 4. MDA Levels of Padjadjaran Sheep

MDA levels describe the lipid peroxidation in tissues. Fatty acids in cell membranes release hydrogen molecules resulting in an increase in MDA (Celi, 2011). Increasing temperatures in the comfort zone cause the formation of malondialdehyde (MDA) due to lipid peroxidation (Horváth & Babinszky, 2019). In cattle MDA concentration during summer, 133.4% versus autumn (Guo et al., 2018).

The MDA levels of sheep c0a0 were not significantly different from c1a1, this implies that when sheep are sheared, they need to be given ascorbyl palmitate so that they do not heat stress. The MDA levels increased from the first to the 10th day post shearing compared to before shearing (Hefnawy et al., 2018). Increased MDA concentration is an indicator of excessive free radical production due to shearing in sheep (Aktas et al., 2017). Stressful conditions, including heat shock, induce excessive production of free radicals so that in cells, the ratio of oxidants and antioxidants becomes unbalanced (Piccione et al., 2011).

CONCLUSION

The combination of shearing and Ascorbyl palmitate administration treatment had an effect on increasing SOD and GSH-Px levels; as well as reducing MDA levels of Padjadjaran sheep that received heat shock. AP dosage requires reassessment in order to provide optimal effect in controlling heat stress in sheep.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript. Conflicts of Interest should be stated in the manuscript.

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