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To cite this article: M T E Purnama *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **441** 012140

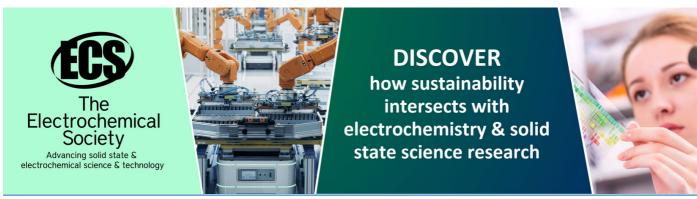View the article online for updates and enhancements.

Oxidative stress parameters in landrace pigs slaughtered by the stunning method

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Abstract. This study aimed to measure oxidative stress parameters during pig slaughter preparation. This study used 60 landrace pigs that were taken the serum and saliva samples under conditions before stunned (T1), after stunned (T2) and after slaughtered (T3). Superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GPx) were also evaluated. The serum was taken through the jugular vein with vacutainer. Saliva was taken with a salivate tube. Serum and saliva were tested using the Cortisol ELISA immunoassay method. The level of SOD, MDA and GPx were analyzed through serum concentration. The results showed significant differences ($p<0.05$) in T2 and increased significantly ($p<0.05$) in T3. The SOD levels showed the opposite results with MDA and GPx. The SOD levels decrease after stunning and increase after slaughter, vice versa. It can be concluded that there were significant differences in serum and salivary cortisol of pigs taken before stunned, after stunned and after slaughtered. The level of SOD, MDA and GPx could be a reference for oxidative stress parameters during handling and restraint in pig slaughter.

1. Introduction

Meat is one of the animal products obtained from livestock. Livestock that is often found in Indonesia is broiler chickens, beef cattle, goats, sheep, horses, and pigs (pork). Pork is a priority, especially in areas that are predominantly non-Muslim. The management of pork cutting methods aims to maintain the quality of pork [1]. The slaughter method plays an important role in determining the quality of meat and can affect post mortem muscle metabolism. Poor slaughter methods can cause preslaughter stress. Pre slaughter stress is one of the factors that can affect the quality of meat such as Dark Firm Dry (DFD) and Pale Soft Oxidative (PSE) [2].

Stunning is a treatment that aims to stun animals before slaughter. Stunning makes it easy to handle animals to be slaughtered and ensures the safety of slaughtering operators. Stunning on pigs is also done by electrical, mechanical or gas methods. Slaughter with stunning and non-stunning is an issue of animal welfare. Stunning can cause stress if done improperly, such as due to poorly trained operators or poor stunning equipment [3,4].

The meat quality is determined by the level of stress during the slaughter process [5]. The dynamics of the levels of Superoxide Dismutase (SOD), Malondialdehyde (MDA) and Glutathione Peroxidase (GPx) can be used as oxidative stress parameters [6]. The slaughter method can be done by stunning before cutting the heart or jugular vein directly. Stunning methods are performed to facilitate handling and reduce stress [7]. This study aimed to measure serum and salivary cortisol levels, serum SOD, MDA, and GPx during pig slaughter preparation.

2.   Material and methods
This study was approved by the Veterinary Public Health Agency and according to the standard operating procedure for pig slaughter in Banyuwangi abattoir. A total of 60 landrace pigs were used in this study under conditions before stunned (T1), after stunned (T2) and after slaughtered (T3). Blood samples were taken with Vacutainer® (BD, USA) through the jugular vein and saliva with salivate tubes through sublingual. Vacutainer from the icebox was transferred to the centrifuge machine Hettich EBA 200® (Hettich, GER). Remove the clot by centrifuging with g-force 800 rcf at a speed of 2500 rpm for 10 minutes. The resulting supernatant was the designated serum.

The sample was maintained at 2-8°C during analysis or kept at -20°C during storage. Serum and saliva were tested using the Cortisol ELISA immunoassay method My-Bio-Source® (San Diego, CA, USA). The remaining serum used to measure serum SOD, MDA and GPx levels according to the method of Ercan and Koçkaya [8]. The data were statistically analyzed by one way ANOVA then followed by the Duncan Test utilizing SPSS v20.

3.   Result and discussion
The result of serum and salivary cortisol showed decreased significantly ($p<0.05$) in T2, then increased significantly ($p<0.05$) in T3 (Table 1.). The result of MDA and GPx showed increased significantly ($p<0.05$) in T2, then decreased significantly ($p<0.05$) in T3 (Table 1.). On the other hand, the SOD level showed the opposite results with MDA and GPx (Table 1.).

Table 1. The mean and standard deviation of the dynamics of oxidative stress parameters

Activity	Serum cortisol (µg/mL)	Salivary cortisol (µg/mL)	Serum SOD (/mg protein)	Serum MDA (/mg protein)	Serum GPx (/mg protein)
T1	80.23[b]±0.45	31.03[c]±0.56	39.11[b]±0.42	36.11[a]±0.70	0.11[a]±0.01
T2	82.67[a]±0.43	42.31[a]±0.58	34.46[c]±0.41	58.17[c]±0.51	0.21[c]±0.01
T3	73.11[c]±0.75	36.31[b]±0.32	38.35[b]±0.61	39.21[a]±0.46	0.11[a]±0.01

[a,b,c] *Different superscripts in the same column indicate significant differences among activity ($p<0.05$)*

The ideal animal slaughter is the cutting of the esophagus, trachea and the main blood vessels in the neck [9]. The slaughter tool used must be sharp to reduce the pain. The method before pig slaughtered can use electrically stunning and also directly slaughtered without being stunned. Handling and animal restraint before slaughter has a role to reduce animal stress [10].

The pig can be restrained into a pinched cage and tie the Naso-premaxillary portion to be slaughtered in the neck area. Stunning using electricity is done to reduce stress due to the extensive slaughter process in the heart of the pig. Stress can increase during the process before slaughter [11]. In the previous study, stress was found in cattle with an indicator of an increase in plasma cortisol after being slaughtered [12].

The stunning method on pig has been shown to reduce serum and salivary cortisol levels. However, it showed an increase in serum and salivary cortisol levels after being slaughtered (Table 1.). Cortisol is a hormone marker to indicate the stress oxidative. The Hypothalamic – pituitary – adrenal axis pathway is related to the stress response. The hypothalamus will stimulate the pituitary to produce adrenocorticotropic hormone (ACTH). Furthermore, ACTH plays a role in triggering the adrenal cortex to release cortisol during stress [13]. Salivary cortisol responds to every animal handling, restraint, and transportation [14].

The level of MDA and GPx are the best markers of oxidative stress because they lead specifically against lipid peroxidation. Inflammatory reactions due to oxidative stress are expected to be reflected by high levels of MDA [15]. Superoxide anions have selective reactivity and are formed by a number of enzyme systems through auto-oxidation reactions and enzymatic transfer electrons. Hydroxyl radicals are released due to water radiolysis in biological systems. Decreasing SOD levels can be an indicator that the cell has carried out the oxidation process to reduce Hydroxyl radicals [16].

4. Conclusion
In conclusion, there were significant differences in serum and salivary cortisol of pigs taken before stunned, after stunned and after slaughtered. The level of SOD, MDA and GPx could be a reference for oxidative stress parameters during handling and restraint in pig slaughter.

5. References
[1] Soesilowati E S 2011 *J Indones Soc Sci Hum.* **3,** 151-160
[2] Sabow A B, Sazili A Q, Zulkifli I, Goh Y M, Ab Kadir M Z A, Abdulla N R, Nakyinsige K, Kaka U and Adeyemi K D 2015 *Meat Sci.* **104,** 78-84
[3] Adzitey F 2011 *Int Food Res J.* **18,** 485-491
[4] Kim G D, Lee H S, Jung E Y, Lim H J, Seo H W, Lee Y H, Jang S H, Baek S B, Joo S T and Yang H S 2013 *Livestock Sci.* **157,** 312–316
[5] Grunert K G, Bredahl L and Brunsø K 2004 *Meat Sci.* **66,** 259-272
[6] Todorova I, Simeonova G, Kyuchukova D, Dinev D and Gadjeva V 2005 *Comp Clin Pathol.* **13,** 190-194
[7] Becerril-Herrera M, Alonso-Spilsbury M, Lemus-Flores C, Guerrero-Legarreta I, Olmos-Hernández A, Ramírez-Necoechea R and Mota-Rojas D 2009 *Meat Sci.* **81,** 233-237
[8] Ercan N and Koçkaya M 2017 *Turk J Agric Food Sci Technol.* **5,** 1493-1496
[9] Morzel M, Sohier D and Van de Vis H 2003 *J Sci Food Agric.* **83,** 19-28
[10] Gregory N G 2005 *Meat Sci.* **70,** 481-491
[11] Hartung J, Nowak B, Waldmann K H and Ellerbrock S 2002 *Dtsch Tierarztl Wochenschr.* **109,** 135-139
[12] Önenç A and Kaya A 2004 *Meat Sci.* **66,** 809-815
[13] Tsigos C and Chrousos G P 2002 *J Psychosom Res.* **53,** 865-871
[14] de Jong I C, Prelle I T, van de Burgwal J A, Lambooij E, Korte S M, Blokhuis H J and Koolhaas J M 2000 *Physiol Behav.* **68,** 571-578
[15] Tsikas D 2017 *Anal Biochem.* **524,** 13-30
[16] In S, Hong C W, Choi B, Jang B G and Kim M J 2016 *Mol Neurobiol.* **53,** 777-791