



Cortisol Determination in Blood Serum as Stress Indicator in Beef Cattle that are Slaughtered With or Without Stunning

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Abstract

Cortisol concentration in blood serum can be made as an indirect indicator to measure the level of stress in cattle. Cattle can experience stress when faced with something frightening and uncomfortable, such as rough handling before slaughter. The aim of this research is to assess the stress level of beef cattle by measuring the cortisol concentration in blood serum of beef cattle with various methods of slaughter, either preceded or not preceded with stunning. To determine cortisol in blood serum of beef cattle, radioimmunoassay (RIA) was used in this research. This research was conducted in 8 (eight) slaughterhouses in West Java Province and Banten with a sample size of 82 samples.

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The research was carried out by 4 methods of treatment before slaughter, (1) slaughter that begins with stunning by percussive captive bolt stunning gun (2) slaughter that begins with stunning by pneumatic captive bolt stunning gun (3) slaughter without stunning by using restraining box Mark IV, and (4) slaughter without stunning by using rope to forcefully throw down cattle / conventional method. Average cortisol hormone concentrations in blood serum of cattle for each method of slaughter respectively are 22.4, 19.3, 24.9, and 44.9 ng/mL. High level of cortisol in blood serum of cattle slaughtered by conventional method shows high level of stress in cattle due to poor handling before slaughter. High stress level may indicate that the beef cattle are handled without regard to animal welfare aspects.

Keywords: cortisol; slaughter; beef cattle; stress.

1. Introduction

Meat demand in Indonesia today is fulfilled by slaughter of local cattle, imported cattle, and also imported meat from several countries, namely Australia, New Zealand, United States of America, and Canada. In early June 2011 cattle imported from Australia was stopped temporary due to an issue regarding torture of cattle from Australia in some slaughterhouses before slaughter. Animal handling without regards to animal welfare aspects in the slaughterhouse is not in accordance with the World Organization for Animal Health (OIE) guidelines, particularly those that are relevant to the principles of animal welfare [1] and contrary to the Republic of Indonesia Legislation Number 18 Year 2009 regarding Animal Husbandry and Health.

Along with the development of technology and in order to fulfill animal welfare aspect, various methods of animal slaughter have been applied in slaughterhouse, either slaughter not preceded with stunning, such as animal slaughter using restraining box Mark IV, or with stunning before slaughter. Animal slaughter by stunning method has been introduced and applied in a number of slaughterhouses in Indonesia. Stunning before slaughter is done by various ways among others by using the captive bolt stunning (percussive and pneumatic) method that aims to minimize stress and pain before and after slaughter. However stunning results of these animals in some slaughterhouse are not fully in accordance to the requirements, including when viewed from the aspect of *halal*.

Different treatment towards animal before slaughter will have impacts on the difference in stress level of the animal. Stress in animal will increase the cortisol hormone in the blood serum; therefore cortisol concentration in blood serum can be used as an indirect indicator to measure stress in animal [2]. Cortisol is a steroid hormone (glucocorticoid) that is produced by the adrenal gland. The secretion of this hormone is stimulated by the adrenocorticotrophic hormone (ACTH) [3]. This hormone acts in stimulating gluconeogenesis in the liver, helps glucagon and epinephrine hormones in the glycogenesis process, prevent glucose uptake and protein synthesis as well as being anti inflammation [4].

Publication that compares stress level in beef cattle through measuring cortisol in blood serum by several slaughter methods is not yet available. It is expected that the data from this research can become one of the references in choosing which slaughter method is best in order to implement the principles of animal welfare in

slaughterhouse as well as an effort to produce good quality meat.

2. Materials and Methods

2.1. Sample Collecting and Preparation

Blood samples from all slaughter methods (n=82 samples) were obtained at night during the slaughtering of male castrated beef cattle (steer) from Brahman cross breed. Blood samples were collected from 8 slaughterhouses both from beef cattle slaughtered preceded by stunning (using percussive captive bolt stunning gun and pneumatic captive bolt stunning gun) as well as slaughter without stunning (using restraining box Mark IV and using rope to forcefully throw down cattle / conventional method). As much as 5 ml of blood per cattle from each treatment/ slaughter methods were contained from the gush of blood through the common carotid artery after slaughter. After letting the blood settle for 1 hour in room temperature, blood was then placed in a cooling box and transported to the laboratory. After separation of serum and centrifuged at a speed of 2500 rpm for 10 minutes, serum was frozen at -18 °C before testing.

2.2. Sample Testing

Radioimmunoassay method (Kit RIA- RK-240T, Izotop, Budapest) was used to test the cortisol concentration in blood serum. Reagent and sample were conditioned at room temperature before using. Every two (duplo) coated tube was marked/ labeled for each standard (standard 1 until standard 6/ S1-S6), serum control and serum sample. All reagent and sample were homogenized by using a tube shaker.

Respectively as much as 10 µL standard (S1-S6), serum control, and serum sample were placed in coated tube that has been marked/ labeled. As much as 500 µL tracer was inserted to each tube then as much as 500 µL antiserum was inserted to each tube. The tubes entire surface were closed tightly and shaken consistently throughout incubation (2 hours) in room temperature. Liquid from the tube were discarded, then were placed upside down on top of tissue paper for 2 minutes to absorb any residual liquid in the tube. Cortisol concentration in each tube was calculated by using Automatic Gamma Counter A 6.24.

3. Results and Discussion

Immunoassay methods like radioimmunoassay (RIA) have often been used to detect cortisol hormone levels in animal blood serum, including cattle blood serum. Limit detection of commercial kit RIA that is used in this research for cortisol is 1.05ng/ml. A typical standard cortisol test work using RIA method can be seen in Figure 1.

Testing of cortisol hormone in beef cattle blood serum using RIA method can illustrate the level of stress in cattle, both from cattle slaughtered by stunning (pre-slaughter stunning) using percussive captive bolt stunning gun and pneumatic captive bolt stunning, as well as slaughter without stunning using restraining box Mark IV and using rope to forcefully throw down cattle (conventional method).

Complete concentration of cortisol in blood serum of cattle for each slaughter method is presented in Table 1.

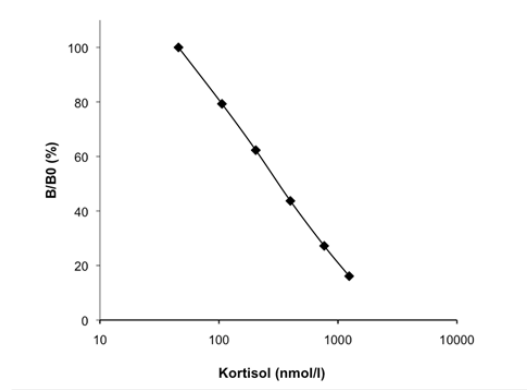


Figure 1: Typical standard RIA work for cortisol testing (1 nmol/L = 0.362 ng/mL)

Table 1: Cortisol concentration in blood serum of cattle slaughtered with and without stunning

Method	n (head)	Cortisol level (ng/mL)			
		average	min	max	\pm sd
Percussive captive bolt stunning	28	22.4	4.9	50.7	16.50
Pneumatic captive bolt stunning	20	19.3	5.9	45.8	12.45
Restraining box Mark IV	19	24.9	6.4	61.7	18.82
conventional	15	44.9	11.2	99.6	26.59

Highest cortisol concentrations obtain from blood serum of cattle slaughter conventionally is with an average 44.9 ng/mL. This result indicates that the method causes the highest stress effect compared to other methods. Rough handling before slaughter by conventional method without stunning can hardly be avoided because with this method the animal will undergo various handling which is a critical stage that can cause the animal stress, such as being tide in various parts of the body, like the head, nose, and feet, which is then followed by restraint and overthrow of the animal to the floor. Generally after collapsing, the cattle is not slaughtered strait away because the officers need time to bind and keep the animal in place to minimize movement so that this stage is the next critical stage that makes the animals more stress. Cortisol levels in slaughter with percussive captive bolt stunning, pneumatic captive bolt stunning, restraining box Mark IV methods dose not show a significant difference ($p>0.5$). The results of this study also show that cortisol concentration in blood serum of cattle in each method of slaughter is highly variable (minimum and maximum concentration are vary different), this illustrates that level of cortisol in each method are highly influenced by the implementation of animal welfare aspects in each individual cattle before slaughter, that is handling during transport and the condition/ handling while cattle is in the restraining box or the slaughter area. Variation between cortisol levels in individual cattle in the same slaughter method, can also be caused by the various handling and different ability of each individual cattle in response to handling. This is in compliance to what is mentioned by [5] that individual from the same species/animal breed with the same environmental condition can have various different behaviors. The results of [6] research shows that blood cortisol concentration during slaughter with good handling in average are 21 ng/mL, Stress in animals can stimulate hypothalamic-pituitary-adrenal (HPA) that will cause an increase in cortisol level in the blood. High levels of cortisol in blood can cause the absorbment of glucose to not maximize,

muscle and bone weakness as the glycogen in the muscle convert into glucose and lactic acid, imbalance of nitrogen due to changes of amino acid to glucose in the process of gluconeogenesis in the liver, and increases water excretion [5]. Cattle that experiences stress before slaughter will cause a decrease production of postmortem lactate acid and the pH of meat remains high. This will result in a reduction in meat quality produced that generates dark-cutting meat [7, 6].

4. Conclusion

Cattle that are slaughter by conventional method without prior stunning causes higher stress compared to slaughter with percussive captive bolt stunning, pneumatic captive bolt stunning, and retraining box Mark IV method.

References

- [1]. [OIE] Office International des Epizooties. Slaughter of Animals (Chapter 7.5), Terrestrial Animal Health Code. Paris, France: Organization for Animal Health. 2008.
- [2]. J. Peeters, J. Sulon, J.F. Beckers, D. Ledoux, M. Vandenneede. Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotropic hormone challenge. *Equine Veterinary Journal*. vol. 43 (4), pp. 487-493. 2011.
- [3]. J. Blahova, R. Dobsikova, Z. Svobodova, P. Kalab P. Simultaneous determination of plasma cortisol by high performance liquid chromatography and radioimmunoassay methods in fish. *Acta. Veterinaria Brno*. vol.76, pp. 59-64. 2007.
- [4]. J.G. Cunningham, B.G. Klein. *Textbook of Veterinary Physiology*. Missouri USA: St. Louis, 2007, pp 410-427.
- [5]. G.C. Miranda-de la Lama, W.S. Sepiulveda, H.H. Montaldo, G.A. Maria, F. Galindo. "Social strategies associated with identify profiles in dairy goats". *Applied Animal Behavior Science*, vol. 134, pp. 45-55. 2011.
- [6]. L. Mounier, H. Dubroeuq, S. Andanson, I. Veissier. 2006. Variations in meat pH of beef bulls in relation to conditions of transfer to slaughter and previous history of the animals. *Journal Animal Science*. vol.84, pp.1567-1576.
- [7]. N.G. Gregory, T. Grandin. *Animal Welfare and Meat Science*. New York, USA: CABI Publishing, 1998, pp 4-145.
- [8]. J. Rushen, A.M. de Passile, M.A.G. von Keyserlingk, D.M. Weary. *The Welfare of Cattle*. The Netherlands: Springer Dordrecht, 2008, pp 43-69.