World Academy of Science, Engineering and Technology
International Journal of Animal and Veterinary Sciences
Vol:8, No:12, 2014

Comparison of Process Slaughtered on Beef Cattle Based on Level of Cortisol and Fourier Transform Infrared Spectroscopy (FTIR)

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***Abstract*—**Stress of slaughter animals starting long before until at the time of process of slaughtering which cause misery and decrease of meat quality. Meanwhile, determination of animal stress using hormonal such as cortisol is expensive and less practical so that portable stress indicator for cows based on Fourier Transform Infrared Spectroscopy (FTIR) must be provided. The aims of this research are to find out the comparison process of slaughter between Rope Casting Local (RCL) and Restraining Box Method (RBM) by measuring of cortisol and wavelength in FTIR methods.

Thirty two of male *Ongole* crossbred cattle were used in this experiment. Blood sampling was taken from jugular vein when they were rested and repeated when slaughtered. All of blood samples were centrifuged at 3000 rpm for 20 minutes to get serum, and then divided into two parts for cortisol assayed using ELISA and for measuring the wavelength using FTIR. The serum then measured at the wavelength between 4000-400 cm^{-1} using MB3000 FTIR. Band data absorption in wavelength of FTIR is analyzed descriptively by using FTIR Horizon MB^{TM}.

For RCL, average of serum cortisol when the animals rested were 11.47 ± 4.88 ng/mL, when the time of slaughter were 23.27 ± 7.84 ng/mL. For RBM, level of cortisol when rested animals were 13.67 ± 3.41 ng/mL and 53.47 ± 20.25 ng/mL during the slaughter. Based on student t-Test, there were significantly different between RBM and RCL methods when beef cattle were slaughtered ($P<0.05$), but no significantly different when animals were rested ($P>0.05$).

Result of FTIR with the various of wavelength such as methyl group (=CH_3) 2986cm^{-1}, methylene (=CH_2) 2827 cm^{-1}, hydroxyl (-OH) 3371 cm^{-1}, carbonyl (ketones) (C=O) 1636 cm^{-1}, carboxyl (COO^{-1}) 1408 cm^{-1}, glucosa 1057 cm^{-1}, urea 1011 cm^{-1}have been obtained. It can be concluded that the RCL slaughtered method is better than the RBM method based on the increase of cortisol as an indicator of stress in beef cattle ($P<0.05$). FTIR is really possible to be used as stub of stress tool due to differentiate of resting and slaughter condition by recognizing the increase of absorption and the separation of component group at the wavelength.

***Keywords*—**Cows, cortisol, FTIR, RBM, RCL, stress indicator.

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I. Introduction

APPLICATION of animal welfare in process of slaughtering is very important, both for animals themselves and also for quality of meat. Stress in animal is a condition of non-specific discomfort which cause of immune defects non-specific, failure of reproduction, and decreased of meat carcass until the death of animals.

Special quality of meat, stress will cause breakdown of muscle glycogen increased significantly [1], increase of the anaerobic glycolysis process until formation of lactic acid [2] decreased of pH, denaturation of protein sarcoplasmic reticulum and finally decreased of water binding capacity on tissue. Thus, this mechanism will cause the meat become pale, soft, and exudative [3]-[5].

Currently, quantitative methods that commonly used to measure stress hormones and their metabolites are EIA (Enzyme-Linked Immunoassay) and RIA (Radioactive Immunoassay). Unfortunately, the materials and equipment are expensive and not practical mainly for a small number of samples even though they produce accurate data. Therefore, it is necessary to find an alternative method that can detect stress with lower cost and more practical as FTIR.

The role of infrared methods is greatly increased in biomedical analysis of hormones. The Fourier Transform Infrared (FTIR) imaging plays an important role in the study of the structure activity relationship for hormones [6], [7]. Therefore, the infrared spectrum was the fingerprint of a molecule [8]. The FTIR would then identify a sample on functional group level. The different bindings such as C-C, C=C, C-C, C-O, C=O, O-H and NH have their own characteristic frequencies as absorption bands in infrared spectrum. These bindings would be identified on different wave numbers according to the absorption bands in infrared spectrums [9].

There are two methods of beef cattle slaughter in Yogyakarta namely Restraining Box Methods (RBM) which modified from Meat Livestock Australia and Rope Casting Local (RCL) where animals walk freely then tightly and reclined. The aim of this study first, are to compare the best method of slaughter beef cattle based on level of cortisol as stress indicator and Second is to find out the alternative tool such as FTIR to detect stress of beef cattle.

II. Materials and Methods

A. Study Site

Two slaughtering method are compared in this study. The slaughter house with the *Rope Casting Local* (RCL) method which located at CV Restu Bumi Segoroyoso, is compared with house of slaughter with methods of *Restraining Box* (RBM) at the government slaughter house. Both of slaughter house were located in Yogyakarta, Indonesia. The experimental protocol was approved by the Animal Ethics Committee of The Integrated Research and Testing Laboratory, Gadjah Mada University, Yogyakarta Indonesia, according to the number of 115/KEC-LPPT/VII/2013, dated July 30, 2013.

B. Methods of RCL and RBM

In RCL method, all of beef cattle walked freely and enter to large room. Finally, they were traditionally tight (rope casting) while resting and straight cut, whereas in RBM methods, beef cattle pushed by butcher into the restraining box with slippery road. Usually, beef cattle stop at raceway, so that's why the butcher always push them.

C. Animals

Thirty two of male Ongole crossbred cattle (*Bos indicus),* with 400-600 kg of body weight were used in this research. All cattle were maintained with standard feeding such as 40-60 kg of forage, concentrates and water.

D. Blood Collections

Approximately 10 mL of blood samples were collected from the jugular vein then divided into 2 parts for assay of cortisol and measurement of wave length using FTIR. To assay cortisol, all of the whole blood samples were centrifuged at 3000 for 20 minutes. Serum then were frozen at -20^{o}C until assayed using ELISA methods. Collection of blood has been done twice namely when the animals were rested and in the time of slaughter by relocate blood into the tube.

E. Blood Analysis Using FTIR

Spectrum-One ABB Miracle Type MB3000 FTIR Spectrophotometer was used in this research. The spectrum recorded in the mid-infrared region of 4000-650 cm^{-1}. FTIR spectra were collected in the region of 4000 - 650 cm^{-1} from the Min-infrared by adding 32 scans and at a resolution of 4 cm^{-1}. FTIR spectra for all samples were measured using FTIR equipped with a deuterated triglycine sulfate detector and is connected to the computer operating system software. RCL and RBM would be described as the peaks on graphic results. The RCL and RBM levels were obtained from comparing the specific peaks of RCL and RBM.

F. Assay of Cortisol Using Enzyme-Linked Immunosorbent Assay (ELISA)

Serum cortisol levels were assayed using commercial KITS products by DRG Instruments GmbH, Germany. The Cortisol ELISA Kit is a solid phase, based on the principle of competitive binding. This is contains of horseradish peroxidase as conjugate, Tetramethyl benzidine (TMB) as substrate, washing and stop solution.

Basically, endogenous Cortisol of sample competes with a Cortisol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Cortisol in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to he concentration of Cortisol in serum sample. All of data then read using ELISA reader in 450 nm of optical density.

III. Results and Discussion

A. Level of Serum Cortisol

Up to now, level of cortisol is widely used as indicator of stress. The adrenal glands have a key-role in hormonal reactions to stress as they are involved both in the hypothalamic–pituitary–adrenocortical axis and the symphatho-adreno medullary system [10]. Levels of circulating cortisol are maintained under tight regulation of the hypothalamic–pituitary–adrenal axis in a classic negative feedback loop. Prereceptor metabolism of cortisol is regulated by the enzyme 11β hydroxyl steroid dehydrogenase, which interconverts the active hormone cortisol to the inactive metabolite cortisone. Glucocorticoids bind to intracellular receptors, such as the glucocorticoid receptor, 11βhydroxysteroid dehydrogenase (11βHSD); corticotropin releasing hormone (CRH); glucocorticoid receptor (GR), (Fig. 1) [11].

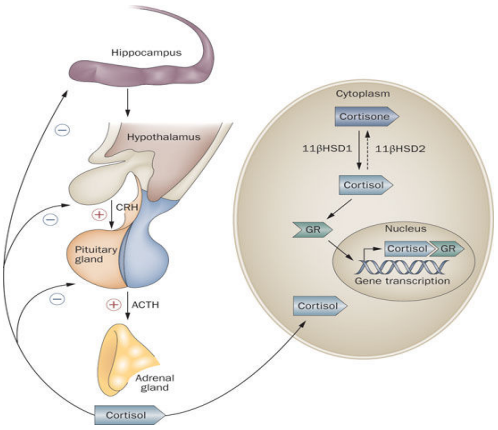

Fig. 1 Circulating cortisol are maintained under tight regulation of the hypothalamic–pituitary–adrenal axis in a classic negative feedback loop [11]

Nevertheless, responses to stressors are complex and context dependent and therefore a combination of different measurements (e.g. physiological and behavioral) for evaluating stress should be considered [12].

In this experiment, level of serum cortisol in rested animals, both in RCL and RBM were measured respectively. For RCL, average of serum cortisol concentration was 11.47 ± 4.88 ng/mL when the time of slaughter was 23.27 ± 7.84 ng/mL. For RBM, level of cortisol when rested animals were 13.67 ±

3.41 ng/mL and 53.47 ± 20.25 ng/mL during the slaughtering (Table I). Using t Test, there were a significant interaction between the slaughter namely RBM and RCL methods (P<0.05). However, there was no significant difference for the rested animals (P>0.05) (Table I).

It would be reported, there is very close relationship between level of cortisol and handling problems in stunning box [13]. The use of the poorly box designed head restrained device which greatly increased behavioral agitation and the time required to restraint the animal resulted in cortisol level jumping from 24 ng/mL to 51 ng/mL. In the worst case, the level increase to 96 ng/mL

TABLE I
COMPARISON LEVEL OF CORTISOL (NG/ML) BETWEEN RCL AND RBM METHODS (P< 0.05), WHEN CATTLE WERE SLAUGHTERED

Slaughtering method	Resting (ng/mL)	The time of slaughter (ng/mL)
RCL (conventional)	11.47 ± 4.88*)	23.27 ± 7.84*)
RBM 1	13.67 ± 3.41*)	53.47 ± 20.25**)

Different superscript indicate significantly different (P<0.05)

In this experiment, RBM method made animals got stress more than RCL method due to force of beef cattle by butchers and slippery race way so that level of Cortisol in RBM method is higher than RCL. In addition to prior reasons, the doors-designed is like dead end; different floor texture made cattle did not want to follow the track and worried. Waiting for a long time at the restraining box or in track made animals got extraordinary stress [14]. This opinion supported by earlier researcher [15] that RCL method is more successful because of animal's behavior is more natural compared to the other methods.

B. Measurement of Wave Length Using Fourier Transform Infrared Spectroscopy (FTIR)

In this experiment, FTIR was used to measure of serum specimen with the range of wave length from 4000 – 400 cm^{-1}. The spectrum consists of some different absorbance range from 4000-600 cm^{-1}. This value was the similar as biological samples [16]. Based on wavelength and rate absorption, there was a significantly different (Fig. 2).Value of calibration indicated spread of RCL (right circle) and RBM method (left circle) at the different coordinate (Fig. 3).

FTIR Spectroscopy is a universal tool that has been used to analyze and identify chemical compounds, such as carbohydrates and esters, as well as the chemical bonds between atoms [17]. Structure of Cortisol is consist of ketone (=O), methyl group and OH (Fig. 4).

Since cortisol excreted in saliva, fecal and urine after circulating in blood, salivary cortisol can be detected using this material based on absorbance of primary and secondary amine group (NH,NHR), *v*N-H and or*v* O-H, methyl, (=CH_3) and methylene (=CH_2), group of CO(ester), carboxyl groups *v*COO, protein glycosylation and phosphor [18].This condition as similar as urinary cortisol of cattle where absorbance was methyl (=CH3), methylene (=CH2) and ketone (=O) [19]

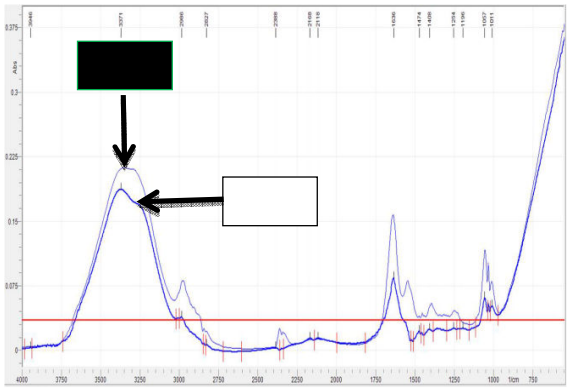

Fig. 2 Wavelength and the rate absorption of component groups in cortisol, serumat the time of slaughterbetween RBM (■) and RCL(□) method

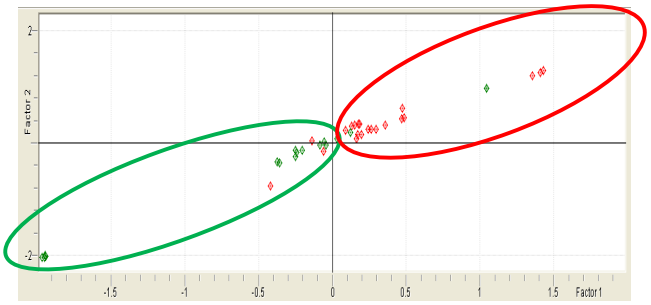

Fig. 3 Comparison absorbance of wave length of serum beef cattle which slaughtered using RCL (right circle) and RBM methods (left circle)

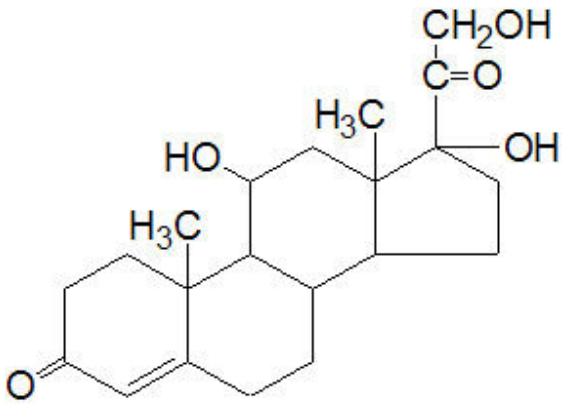

Fig. 4 Structure of Cortisol

Progesterone as one of reproductive hormone can be detected also using FTIR. Fourier Transform Infrared Spectroscopy itself can be used to determine the estrus cycle, in which progesterone could be recognized by ketone (1724 cm^{-1}), methyl (1375 cm^{-1}), and methyl-ketone (1354 cm^{-1}) [20]. Almost the same as serum, absorbance wavelength of urinary sample were grouped regularly (Fig. 5). After circulating in body, cortisol still were excreted in urine as a free cortisol or total cortisol [21], [22] so that it can be detected using FTIR. Based on its function, FTIR has been used also in biomedical research to analyze component of gallstone and renal failure patients [23] until and civil

engineering to analyze thermal sensitivity and determine index penetration of asphalt pavement [24]. This study indicated that FTIR can identify the component of methyl group (CH3), ketone (=O), methylene (NH2) and OH. Each functional group can be recorded in a specific wavelength.

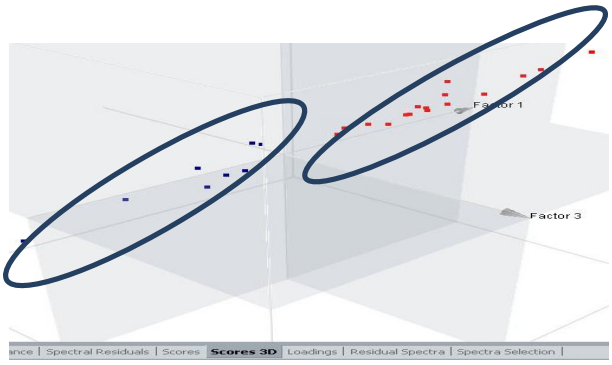

Fig. 5 Comparison absorbance of wavelength of urinary beef cattle which slaughtered using RCL (right circle) and RBM methods (left circle)

In this experiment, FTIR can be used to distinguish the slaughtering process namely RBM and RCL based on the increase of absorption and separation groups in cortisol and serum components due to stress (Figs. 2 and 3). It could be thought that Spectroscopy Fourier Transform Infrared (FTIR) is expected to provide results more quickly and accurately than hormonal assays.

IV. Conclusion

From results and discussions, it can be concluded that the RCL is better than the RMB method based on the increase of cortisol as an indicator of stress in beef cattle ($P<0.05$).

Based on absorbance of wave length, beef cattle which slaughtered using RCL and RBM methods can be distinguished, so that FTIR is one of alternative tool to determine stress in beef cattle both using serum or urine for sample.

Acknowledgment

It is our duty to appreciate the Directorate General of Higher Education, Ministry of National Education Indonesia for supporting budget, CV Restu Bumi and "Giwangan" Slaughter house, for giving a permission to take a blood sample and to attend the slaughtering process.

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