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

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ABSTRACT

Application of animal welfare in process of slaughtering is very important, both for animals themselves and also for quality of meat. 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 methods by measuring of cortisol and wavelength in FTIR methods.

Thirty two of male Ongole cross bred 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 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 HorizonMB\(^\text{TM}\).

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 at slaughtered. 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 (\(=\text{CH}_3\)) 2986 cm\(^{-1}\), methylene (\(=\text{CH}_2\)) 2827 cm\(^{-1}\), hydroxyl (-OH) 3371cm\(^{-1}\), carbonyl (ketones) (C=O) 1636 cm\(^{-1}\), carboxyl (COO\(^-\)) 1408 cm\(^{-1}\), glucose 1057 cm\(^{-1}\), urea 1011 cm\(^{-1}\) have been obtained. It can be concluded that the RCL slaughtered method is better than the RMB 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 differentiation of resting and slaughter condition by recognizing the increase of absorption and the separation of component group at the wavelength.

Keywords: FTIR, Cows, stress indicator
I. INTRODUCTION

Application of animal welfare in process of slaughtering is very important, both for animals themselves and also for quality of meat. Stress of animals will affect quality of meat since muscle glycogen breakdown will increased significantly [1], increase of the anaerobic glycolysis process until formation of lactic acid [2] decreased of pH, denaturation of protein sarcoplastic reticulum and finally decreased of water binding capacity on tissue. Because of this mechanism, meat to be pale, soft and exudative [3][4][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 sample even they produce the 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 infra red methods is greatly increased in biomedical analysis of hormones. The fourier transform infrared (FTIR) imaging play 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=O, 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

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.

A. Study Site

For comparison of slaughter, it would be used the slaughter house with the Rope Casting Local (RCL) method which located at CV Restu Bumi Segoroyoso, versus slaughter house with The Restraining Box (RBM) methods at The government slaughter house. Both of slaughter housed were located in Yogyakarta.

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 race way, so that why the butcher always push them.

C. Animals

Thirty two of male beef 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 King grass and concentrates and water.

D. Blood Collections

Approximately 10 mL of blood samples were withdrawn from the coccygeal 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 were centrifuged for 3000 rpm with duration of 20 minutes. Serum then were frozen at -20°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**

*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\(^{-1}\).

Level of serum cortisol were assayed using commercial KITS products by DRG Instruments GmbH, German with enzyme-linked immunosorbent assay (ELISA) method.

**III. RESULTS AND DISCUSSION**

**A. Level of Serum Cortisol**

Level of serum cortisol with different slaughter method can be explained as below: using RCL method, average of serum cortisol when the animals were rested 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 at slaughtered (Table 1). 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) (Table 1).

Table 1. Comparison level of Cortisol (ng/mL) between RCL and RBM methods (p<0.05), when cows were slaughtered.

<table>
<thead>
<tr>
<th>Method of slaughtered</th>
<th>Resting (ng/mL)</th>
<th>The time of slaughter (ng/mL)</th>
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<tbody>
<tr>
<td>RCL (conventional)</td>
<td>11.47 ± 4.88*)</td>
<td>23.27 ± 7.84*)</td>
</tr>
<tr>
<td>RBM 1</td>
<td>13.67 ± 3.41*)</td>
<td>53.47 ± 20.25**)</td>
</tr>
</tbody>
</table>

Different superscript indicate significantly different (P<0.05)

It would be reported, there is very close relationship between level of cortisol and
handling problems in stunning box [10]. The use of the poorly box designed head restrained device which greatly increased behavioral agitation and the time required to restrain 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. In this experiment, RBM method made animals more got stress 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 [11]. This opinion supported by Whittington and Hewitt [12] that RCL method is more successful because of animal's behavior is more natural compared to the other methods.

C.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 was consist of some different absorbance range from 4000-600 cm⁻¹. This value was the similar as biological sampels [13]. Value of calibration indicated spread of RCL (red) and RBM method (green) at the different coordinate (Figure 1 and Figure 2).
Figure 1. Wavelength and the rate absorption of component groups in cortisol, serum at the time of slaughter between RBM and RCL method.

Figure 3. Comparison absorbance of wave length of serum beef cattle which slaughtered using RCL (red) and RBM methods (green).

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 [14]. Salivary cortisol can be detected using this material based on absorbance of primary and secondary amine group (NH, NHR), νN-H and or ν O-H, methyl (–CH₃) and methylene (–CH₂), group of CO(ester), carboxyl groups –COO, protein glycosylation and phosphor [15].
Almost the same as serum, absorbance wavelength of urinary sample were grouped regularly (Figure 3). After circulating in body, cortisol still were excreted in urine as a free cortisol or total cortisol [16] [17] so that it can be detected using FTIR. 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.

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 (Figure 2 and Figure 3). It could be thought that Spectroscopy Fourier Transform Infrared (FTIR) is expected to provide results more quickly and accurately than hormonal.

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

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References


