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# PRE-SLAUGHTER STRESS ESTIMATION BY FOURIER TRANSFORM INFRARED SPECTROSCOPY ANALYSIS

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## ABSTRAK

Penelitian ini bertujuan menguji pemanfaatan fourier transform infrared (FTIR) melalui analisis katekolamin (CA) dan kortisol (CO) sebagai indikator stres secara invasif maupun non invasif untuk mengembangkan metode deteksi stress pada sapi. Penelitian ini menggunakan 24 sampel urine yang diambil pada periode pra penyembelihan dan saat penyembelihan dari 12 sapi Peranakan Ongole (PO) di rumah potong hewan. Analisis kadar CA dan CO dilakukan dengan menggunakan metode ELISA dan FTIR. Data konsentrasi CO maupun CA hasil pengukuran dilakukan analisis statistik untuk mengetahui pengaruh proses penyembelihan. Hasil penelitian ini menunjukan rata-rata konsentrasi kortisol urine adalah 2,12 ± 1,68 ng/dl dalam kondisi pra - penyembelihan dan 7,58 ± 3,89 ng/dl pada saat penyembelihan. Konsentrasi katekolamin urine pada saat pra - penyembelihan dan penyembelihan secara berurutan adalah 3,07 ± 2,05 ng/dl dan 4,15 ± 2,68 ng/dl. Hasil analisis CA dan CO menunjukkan adanya korelasi erat antara hasil analisis dengan menggunakan EIA dan FTIR. Pemisahan spektra FTIR antara sampel pra penyembelihan dan saat penyembelihan disebabkan karena perbedaan konsentrasi CA dan CO sample tersebut. Dapat disimpulkan bahwa FTIR dapat untuk menganalisis status stres pada hewan khususnya pada sapi.

*Kata kunci : katekolamin, kortisol, non invasif, FTIR*

## ABSTRACT

The objective of the study was to analyze the potential of fourier transform infrared (FTIR) as stress estimation instrument through measurement of catecholamine (CA) and cortisol (CO) levels by invasive and non-invasive methods. Twelve heads of Ongole grade (PO) cow from slaughterhouse were used in this study. Twenty four urine samples of pre-slaughter and slaughtering were collected to evaluate the CA and CO levels by ELISA method and Fourier Transform Infrared (FTIR). Data of CA and CO levels were statistically analyzed to determine the difference between pre slaughter and slaughter conditions. FTIR spectra were analyzed using chemo-metrics software. The results showed that the levels of urinary cortisol were 2.12±1.68 ng/dl of pre-slaughter and 7.58±3.89 ng/dl of slaughtered and the levels of urinary catecholamine in pre slaughter and slaughter were 3.07±2.05 and 4.15±2.68 ng/dl respectively. The results showed a correlation between the FTIR spectra and the results of ELISA analysis. FTIR spectrums were distributed in different quadrants, this was caused by differences of CA and CO levels between pre-slaughter and slaughtered. It can be concluded that FTIR can be supposed to analyze the status of stress in animals, especially in cattle.

*Keywords: catecholamine, cortisol, non-invasive, FTIR*

## INTRODUCTION

Animal handling, part of animal welfare is a growing issue of concern in many countries around the world. Animal welfare is a major consideration in meat production and is based upon the belief that animals also feel during the sacrifice. Among the things to cope with includes the environmental conditions, other animals, pathogens, and human handling. Their response to these conditions will have effect on their carcass and meat quality. Poor quality animal and meat will have poor processing properties, functional quality, eating quality, and more likely to be unaccepted by consumers.

Pre-slaughter handling involves all the activities and processes animals undergo prior to sticking. These activities and processes take place on the farm, during transportation, marketing and at the slaughter plant. Moreover, the inability to adequately resolve some of these states (e.g. pre slaughter handling) may invoke further psychological distress. Several studies including pre-slaughter stress (Muchenje *et al.*, 2009; Miranda-de la Lama *et al.*, 2010), the quality of the meat (Muchenje *et al.*, 2008a.), consumer perception (Muchenje *et al.*, 2008b; Ngambu *et al.*, 2011.), the behavior of animals in the pasture (Dodzi and Muchenje, 2011) and the effects of supplementation the quality of the meat (Marume *et al.*, 2012; Xazela *et al.*, 2012) have been conducted under experimental conditions. These authors have realized that there are some challenges with research-based or community-based industries such as difficulties with data collection, the small sample size, the reliability of the data collected and in some cases of non-cooperation with stakeholders and the lack of rapid mechanism in the detection of stress conditions in experimental animals. The activation and regulation of the neuroendocrinal stress response-led stimulus has been studied extensively (Chrousos, 1998; Moberg, 2001; Steckler, 2005). Two integrated process includes the center of the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis in response to stress. Sympatho-adrenal component of the autonomic responses mediated by catecholamines (epinephrine and norepinephrine). Activation of the HPA axis is manifested by the release of glucocorticoids (e.g. cortisol) from the adrenal cortex. Concentrations of plasma and urine both of cortisol and catecholamine have been widely used to quantitatively assess the stress response in animals (Tessa and Jeffrey, 1997). Stress rapid detection of animals before slaughter is needed to help the management of animals in order to produce good quality of meat.

The quantitative method that commonly used to measure stress hormones and their metabolites is enzyme-linked immunosorbent assay (ELISA). The method produces accurate data. However, the materials and equipment that they need are expensive and also not practical to be used on small number of samples and have relatively short expired period of their materials. FTIR is one of safe and applicable alternative method to measure compound level of samples both natural and artificial. The method has been used to measure protein level of food and drink, such as milk (Suseno and Firdausi, 2008), protein and glucose in plasma (Petibois *et al.,* 2001), as well as blood analysis of kidney failure patients (Renuga. *et al.,* 2009), urea level of urine (Ohnishi *et al*., 2000; Sjahfirdi *et al*., 2011; Sjahfirdi. *et al.,* 2012). The infrared spectrum is a fingerprint of a molecule (Smith, 1979; Petibios *et al.*, 2000; Syahfirdi *et al.*, 2012), which is the basis of FTIR work, able to identify samples at the level of functional groups. Therefore, this study focuses on developing a method to assess the pre-slaughter stress in cattle based on FTIR.

## 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 number 115/KEC-LPPT/VII/2013, dated July 30, 2013.

### Animals

Twelve heads of Ongole grade (PO) cow with 400-600 kg of body weight were used in this research. All cattle were housed in a cage house with good standard, with 12 hours photoperiod cycle and were given food and water.

### Sample Collections

Twenty four urine samples of pre-slaughter and slaughter were collected from PO cattle slaughterhouse. Pre-slaughter urine samples were collected 6-8 hours before cattle were slaughtered by continuous stroking of the skin just below the vulva to induce urination. Meanwhile, slaughtered cattle's urine collection were conducted during the slaughter process, which were collected from the

bladder directly, for 10 ml each sample, respectively. Samples were collected by using urine tubes and immediately analyzed by fourier transform infrared (FTIR) and enzyme-linked immunosorbent assay (ELISA) or stored at -80$^0$C for further analysis.

**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 for all samples were measured using FTIR equipped with a deuterated triglycine sulfate detector and KBr beam splitter is connected to the computer operating system software. Using micro pipette sample was placed in the contact section of the FTIR instrument with horizontal attenuated total reference (HATR) elements at a controlled ambient temperature (25$^0$C). 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$^{-1}$. These spectra were subtracted from reference spectrum of air acquired by collecting a spectrum from the cleaned blank HATR crystal before the measurement of each sample replication. At the end of every scan, the surface of HATR crystal was cleaned with hexane twice and dried with soft tissue following the collection of each spectrum.

Level of urinary cortisol (CO) and catecholamine (CA) were assayed using commercial KITS products by DRG Instruments GmbH, German with ELISA method. The data of Urine CO and CA level also FTIR spectra were calculated and presented as mean ± SD. The ANOVA was used to determine the reliability of the differences between the tested parameters levels. Differences considered significant at P< 0.05. Duncan's correlation coefficient was used for statistical association between parameters.

## RESULTS AND DISCUSSION

**Level of Cortisol and Catecholamine**

The first step was conducted to find optimal characteristic data of urine CA and CO concentration by EIA method, to create further a model for stress estimation of female cattle during pre-slaughter and slaughter time.

In general, the urine and blood CO in pre slaughter periode, found in this experiment, ranged between 0.44-4.79 ng/dl. While at the time of slaughter, CO urine increased in the range between 2.51 to 13.46 ng/dl. The cortisol data is presented in Table 1. The average urine levels of cortisol were 2.16 ± 1.68 ng/dl in pre-slaughter condition and 6.98 ± 3.26 ng/mg in slaughter time. Concentrations of urinary cortisol were higher in slaughter time than pre slaughter period. The mean percentage increase of urinary cortisol is 222.58%. According to Peter and Bosu (1987), on bovine serum cortisol levels increased 100 times during high stress, based on it can be predicted that the animal handling of slaughterhouse led to a very stressful time.

CA secretion is a primary neural response to stress stimuli through activation of the sympathetic nervous system thoroughly. The hypothalamus will help to prepare the body to fight due to stress stimuli. Stimulation of the sympathetic nerves to the medulla adrenaline causes the release of large amounts of epinephrine and norepinephrine into the blood circulation, and both hormones are then carried in the blood to all tissues of the body (Hughes *et.al*., 2004).

The results of the urinary catecholamine levels are presented in Table 2. Urinary catecholamine level during the pre- slaughter is lower than the level of slaughter time. The levels of urinary catecholamine in pre slaughter and slaughter were 3.07 ± 2.05 ng/dl and 4.15 ± 2.68 ng/dl, respectively. Based on these data showed

Table 1. Urinary Cortisol (CO) Level (ng/dl) of Cow for Pre-slaughter and Slaughter Condition

| Sample Code | Pre-slaughter CO | Slaughtered CO |
|---|---|---|
| 2 | 4.79 ± 1.05 | 5.59 ± 2.21 |
| 12 | 1.36 ± 0.07 | 4.50 ± 0.44 |
| 18 | 0.44 ± 0.21 | 3.00 ± 0.00 |
| 22 | 0.64 ± 0.16 | 10.40 ± 4.64 |
| 30 | 3.03 ± 1.55 | 7.86 ± 1.95 |
| 31 | 4.47 ± 8.66 | 9.39 ± 2.46 |
| 49 | 1.77 ± 1.32 | 2.51 ± 2.04 |
| 50 | 2.13 ± 0.84 | 6.00 ± 0.58 |
| 51 | 0.74 ± 0.01 | 7.49 ± 1.34 |
| 52 | 0.56 ± 0.01 | 9.21 ± 2.33 |
| 53 | 4.79 ± 1.71 | 13.46 ± 3.23 |
| 54 | 1.25 ± 0.21 | 4.31 ± 0.01 |

Table 2. Catecholamine (CA) level (ng/dl) of Cow for Pre-slaughter and Slaughter Condition.

| Sample Code | CA-Pre Slaughter | CA-Slaughter |
|---|---|---|
| 2 | 5.88 ± 0.93 | 7.90 ± 0.41 |
| 12 | 6.64 ± 0.41 | 8.17 ± 0.15 |
| 18 | 3.20 ± 0.09 | 6.08 ± 0.43 |
| 22 | 5.06 ± 0.66 | 5.72 ± 0.28 |
| 30 | 2.49 ± 0.33 | 2.93 ± 0.19 |
| 31 | 3.64 ± 0.57 | 4.79 ± 1.67 |
| 49 | 0.74 ± 0.09 | 0.85 ± 0.41 |
| 50 | 2.93 ± 0.19 | 3.09 ± 0.49 |
| 51 | 3.04 ± 0.53 | 3.15 ± 1.15 |
| 52 | 0.35 ± 0.09 | 0.97 ± 1.33 |
| 53 | 2.71 ± 0.53 | 5.83 ± 0.90 |
| 54 | 0.19 ± 0.19 | 0.31 ± 0.28 |

that an increase in the levels of urine cortisol and catecholamine more than 100% in the slaughter than pre-slaughter condition.

**FTIR Spectra of Urine**

In this step it was conducted to obtain the characteristic urine spectra by FTIR spectroscopy, to create further a model for stress estimation of pre-slaughter and slaughter time using calibration analysis with urine cortisol and catecholamine levels. FTIR spectra Identification cannot determine the concentration of the compound in the sample, but will give an overview of different spectra at each functional group despite the same concentration. Fourier Transform Infrared (FTIR) is a universal instrument that has been used to analyze organic/inorganic samples. The advantages of using FTIR are accurate, safe, rapid, and sensitive (Smith 1979; Rintoul *et al.,* 1998). Based on the principle works FTIR can identify specific functional groups within a component. Special on CA and CO, FTIR can identify the component methyl group ($CH_3$), ketone (=O), methylene ($NH_2$) and OH. Each functional group can be recorded in a specific wavelength.

Figure 1 showed FTIR spectrums of cow urine during pre-slaughter and slaughter time. Similar spectrums were also obtained from serum during pre-slaughter and slaughter time. FTIR spectrum of pre-slaughter and slaughter period both in urine and serum showed separate spectra.

It was shown in Figure 1 that the essential peaks of urine and serum, which were on 500-900, 1500-1650, and 3000-3650 $cm^{-1}$region. In these figure showed different spectra between pre-slaughter and slaughter time. Each represented compound of functional groups, including hormone metabolites and catecholamine in urine and serum.

Urine contains many compounds each having their own absorption spectrum in the mid-IR region. Thus, the IR spectrum of urine is a superposition of all these individual spectra and the intensities of the absorption bands in this spectrum are proportional to concentrations of the components (functional group level) (the Bouguer- Lambert-Beer law). The urine spectrum contains several absorption bands in the mid-IR region (4000–550 $cm^{-1}$) which are typical for biological samples (Figure 1). The peaks with wave numbers 1300-900 $cm^{-1}$, 1630-1530 $cm^{-1}$, 1720-1600 $cm^{-1}$ and 2880-2850 $cm^{-1}$ are usually identified in functional group materials and characterize C-O (lactate, glycerol, saccharide), $NH_2$ (amine), C-H (amide) and $CH_3$ bending vibrations, respectively (Petibios *et al*., 2000).

The obtained spectra were used to demonstrate the stress condition during pre-slaughter and slaughter time via chemo-metrical analysis (Spectrum-One ABB MIRAcle Software). This software is used to view the differences between the spectra of the sample groups (pre-slaughter and slaughter time). Based on the results of chemo-metrics analysis, spectra were classified using principle component analysis software (Spectrum-One ABB MIRAcle Software).

Figure 2 showed the result of FTIR spectra analyses using chemo-metric software in 2880-2850, 1720-1600, 1630-1530 and 900-1300 $CM^{-1}$ which shows that the spectra of pre-slaughter and slaughter urine were separated on different quadrant.

**Correlation**

In recent years there are an increased number of studies on the relationships between the dynamics of change in CA and CO as indicators under different stress conditions (Linares *et al.*, 2008; Khaustova *et al.*, 2010; Astuti *et al*., 2010). Most of these studies were done by measuring

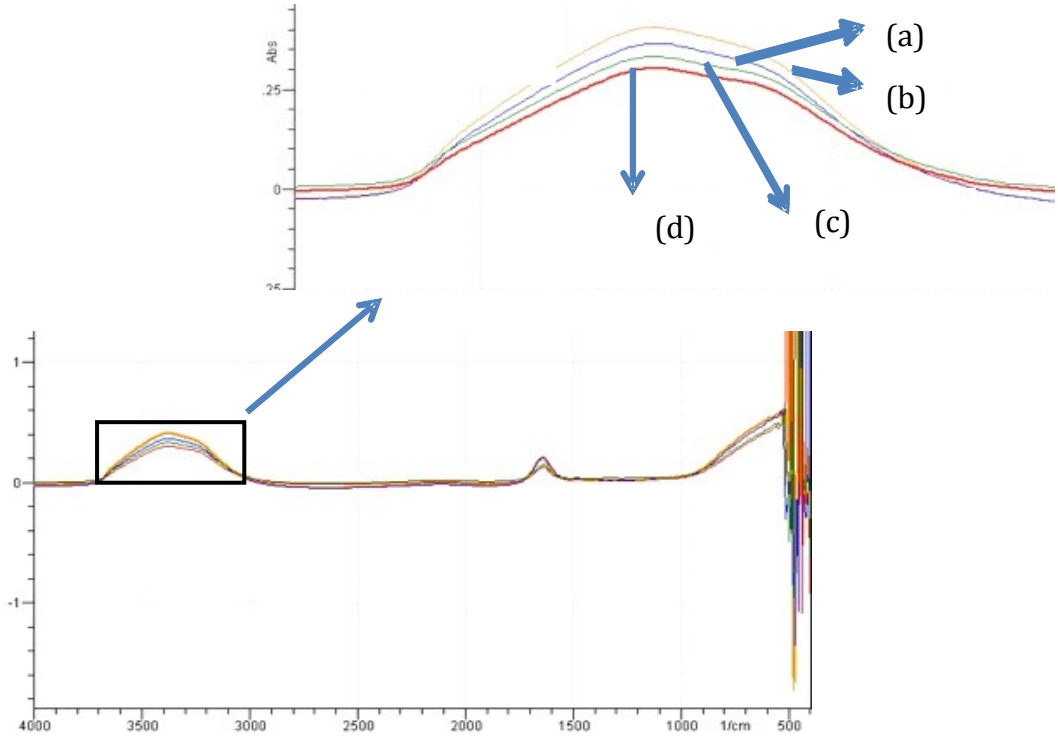


Figure 1. FTIR Spectra Pattern of Cow Urine and Serum for Pre-slaughter and Slaughter Time in Region 4000 – 500 CM-[1]. The symbols represent slaughter urine spectra whey slaughter urine spectra (a), slaughter serum spectra (b), pre-slaughter serum spectra (c) and pre-slaughter urine spectra (d).

multiple parameters with the invasive method. Infrared spectra analyses of pre-slaughter and slaughter urine cow to stress estimation by FTIR instrument for the first time.

Both CA and CO levels are indices of stress hormonal systems, but the extent to which these 2 systems are correlated is unclear. Using EIA and FTIR, we examine how the relationship between the concentration of CA and CO in urine and serum of cattle for pre slaughter and slaughter time. CO is a relatively long-term effector: it has a half-life measured in hours, and its effects last even longer. CA is an immediate-response hormone: it has a short half-life, and its effects disappear rapidly if the hormone is no longer present. This difference is a consequence of the mechanism by which the hormones act. CO increases (or decreases) the amount of a given enzyme. Epinephrine acts by modulating the activity of existing enzymes (Elizabeth and Naomi, 2004).

CA and CO concentration measurements using EIA method in urine samples showed the difference between the pre- slaughter and slaughter time. There was an increase in the concentration of CA and CO at the time of slaughter in urine samples. In FTIR analysis of the wave numbers 1300-900 $cm^{-1}$ , 1630-1530 $cm^{-1}$, 1720-1600 $cm^{-1}$ and 2880-2850 $cm^{-1}$ are illustrates the spectral separation occurs at different quadrant between pre slaughter and slaughter time. The results demonstrate compatibility between the analysis of CA and CO using EIA and FTIR. It is assumed that the separation of the FTIR spectra in line with the increased levels of CA and CO from the sample.

The urine cortisol in pre slaughter condition found in this experiment, ranged between 0.5 - 4.7 ng/dl. Whereas at the time of slaughter in slaughterhouse, CO level in urine increased in range between 3 - 14.1 ng/dl. The mean levels of urinary CA in pre slaughter and slaughter time were 3:07 ± 2:05 ng/dl and 2.68 ± 4.15 ng/dl, respectively. Based on these results it can be concluded that FTIR can be developed as an instrument that can be used in the determination of the physiological status of particular stress in cattle.

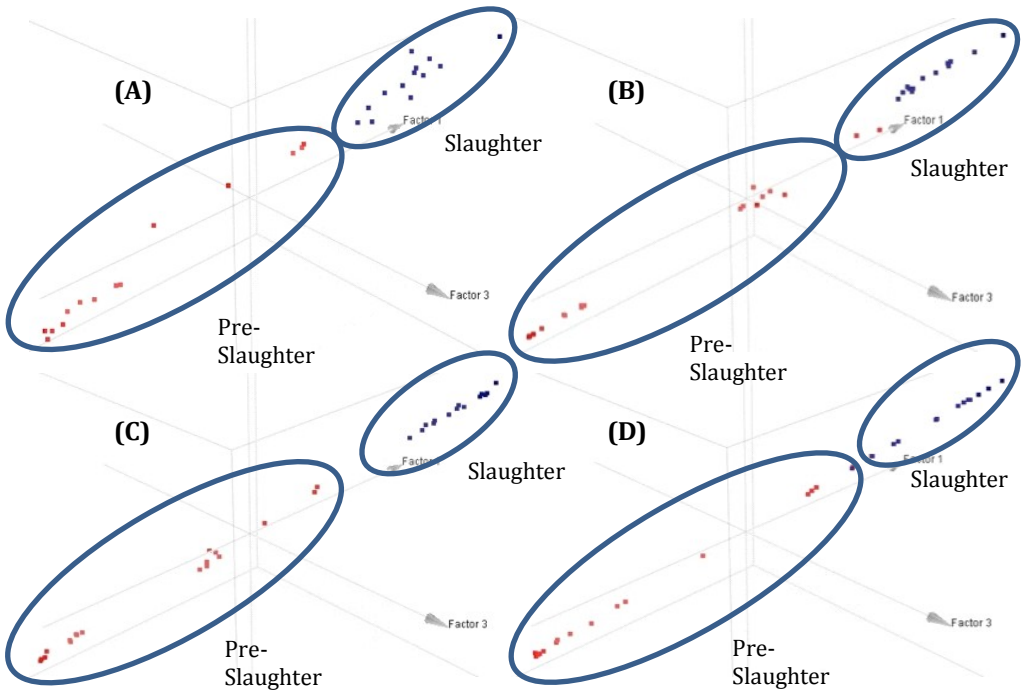


Figure 2. FTIR Spectra of Cow Urine. (a) 900-1300 $CM^{-1}$, (b) 1630-1530 $CM^{-1}$, (c) 1720-1600 $CM^{-1}$, and (d) 2880-2850 $CM^{-1}$.

## CONCLUSION

FTIR can be developed as an instrument that can be used in the determination of the physiological status of a particular stress in cattle.

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