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Image Processing Technique to Measure Quail's Meat Blood Content

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Abstract. Blood content in meat was a crucial component of meat from halal and food hygiene perspective since it reveals how the quail were killed. Image processing methods are anticipated to be the affordable, effective, and useful tools for determining the blood content of meat. In this study, 27 quails were divided into three groups according to how they were killed: not slaughtered (group 1), and slaughter withouh hanging (group2), slaughtered while hanging (group 3). Spectrophotometer and image processing were used to measure, based principaly on the malachite green test, how much blood was present in the meat. The images were taken using a cell phone camera then processed with Image J. measurement blood content of group 1, group 2, and group 3 using a spectrophotometer were 0.453 g/dl, 0.421 g/dl, and 0.381 g/dl, while the results using a image processing were 0.688 g/dl, 0.566 g /dl, and 0.247 g/dl, respectively. Using a spectrophotometer or an image processing technique, the measurement revealed that the beef blood contents of each group were significantly different from one another. A paired T-test comparison of the absorbance values measured by spectrophotometer and image processing revealed no statistically significant differences ($p>0.05$) between the two techniques. It may be concluded that using an image processing tool instead of a spectrophotometer is a viable option for determining the meat blood content obtained from, through various killing techniques, quail.

Keywords. Blood Content; Cellphone Camera; Image; Malachite Green; Quail.

INTRODUCTION

Due to the popularity of poultry among consumers, food products made from poultry have significant market potential. The availability of increasingly diverse poultry products, relatively low cost, ease of acquisition, and ease of processing make poultry products popular with consumers as convenience foods. The community enjoys the meat and eggs that quail, a sort of poultry, produces. The Japanese quail is a variety of quail that is frequently farmed in Indonesia (*Cortunix cortunix* Japonica). There are still plenty of chances for quail farming because there is still a shortage of quail eggs and meat. Every year the demand will continue to increase along with the increasing population and high public interest in consuming quail meat (Listyowati & Roospitasari, 1999). Quail meat production for human consumption increased in Indonesia from 1,598.02 tons in 2020 to 1,679.56 tons in 2021, or by 5.1%. (Ditjennak & Keswan, 2012). As a result, there are now more cuts of meat available that are safe, healthful, whole, and halal. An important prerequisite that needs to be addressed during the slaughtering procedure is halal quail meat.

Slaughter is the act of releasing blood from a dead bird by cutting off the oxygen supply to its esophagus, trachea, arteries, and brain (Gregory, 1998). When the blood stops flowing, it means that the heart is no longer able to remove blood from the body since oxygenated blood no longer enters the heart and no blood is being pushed out of the blood vessels that were cut during the slaughter. According to EFSA (2004), the central nervous

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system in the brain stem irreversibly loses function as a result of a lack of oxygen and energy, and as a result, physiological respiration and blood circulation cease. Human error elements that can cause quail deaths include rough handling by buyers or sellers, crowding of birds into small cages, and transportation of birds to slaughterhouses. These variables can create stressful situations that might cause mortality (Vecerek et al., 2016). Physical harm, such as bruising and fractures, can generally be found in poultry deaths caused by transportation or human mistake (Damayanti et al., 2012). Blood is discovered in muscle tissue when fowl die naturally rather than through the blood arteries that are broken during slaughter. This prevents blood from leaving the quail's body. The corneal reflex and/or ceasing blood flow can be used to determine whether someone has truly passed away.

The primary necessity for producing high-quality and halal meat are slaughtering. Meat with improper slaughtering will look unappealing and serve as a favorable environment for bacteria to proliferate (Lawrie, 1995). It is expected that killing animals without following proper protocols may produce traded meat products of poor quality. Because of the blood present in the tissue, the carcass is more susceptible to microbial contamination and decomposition, which lowers its quality (Budiman et al., 2015). According to Azhari (2012), the way of slaughtering poultry by hanging results in more perfect blood loss.

Malachite green and H_2O_2 can be added to the meat extract to check the meat's blood content. Meat from animals that were improperly butchered will include a lot of hemoglobin (Hb). Malachite green is not oxidized because Hb will bond to O_2 (from H_2O_2) in the reaction (remains green). Malachite green will oxidize to blue in the absence of hemoglobin (Hb). Visually, the intensity of the green and blue colors varies (Lukman et al. 2012). Another, more precise, and objective method is required because this one is subjective because it relies on people.

Martinez et al. (2008) assert that a laboratory analysis system that can be used in developing or distant locations should be a straightforward procedure so that it can be used by people with little experience and is affordable. To find blood levels in meat, a quick and practical quantitative approach must be invented. In this study, blood levels in quail meat were compared between those that bled during hanging-style slaughter and those that did not, as well as between those that bled during hanging-style slaughter and those that did not. Using a spectrophotometer and the competitive response of hemoglobin with malachite green, the blood levels in meat were examined. The outcomes were contrasted with mobile image processing methods. A spectrophotometer is highly expensive to use, difficult to get in distant regions, and labor-intensive to operate. The findings of the competitive oxidative reaction of malachite green with meat extract against the addition of H2O2, utilizing a cell phone camera and imageJ software, become an option as a result of the rapid advancements in image processing technology (De Morais, 2014). Many scientific fields, including agriculture, biomedicine, and biometrics, employ ImageJ software (Putra, 2010).

The goal of this study was to determine and compare the blood content in quail meat based on three different lethal methods of killing: hanging, hanging without a hanger, and killing without being slaughtered. This was done in order to demonstrate the potential of image processing techniques to determine the blood content from different lethal methods of quail meat harvesting.

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METHOD

27 quails from smallholder farms in Bogor, West Java, were used in this study. Nine quails were killed without being slaughtered (group 1), nine quails were killed without being hanged (group 2), and nine quails were killed by hanging (group 3). The quail thigh was used as the source of the sample meat. Up to 5 grams of the meat that has been removed from the skin and bones is weighed before it is sliced into little pieces. It was then centrifuged for 10 minutes at 3000 rpm to separate the meat from the meat particles after being soaked in 20 ml of distilled water for 30 minutes (residual). The meat extract was diluted 20 times with 0.1 N HCl solution as the final step.

It is required to create a standard curve that serves as a benchmark for the results before testing the sample. To get the right $FeCl_3$ concentration, 1% $FeCl_3$ was dissolved in 0.1 N HCl to create standard curves. $FeCl_3$ was employed in concentrations of 0, 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.01%, and 0.02% before reacting with malachite green.

Each test tube containing meat extract and the standard solution was added with 0.1 ml Malachite Green 0.1% and 0.1 ml H_2O_2 3%. The solution was then homogenized with a vortex and allowed to stand for 20 minutes. The purpose of allowing the solution to stand still is for the reaction to take place completely. After 20 minutes, each solution was added 0.1 ml of 0.3% KMnO4 then homogenized with a vortex again so that the solution was completely mixed.

Using an image processing technique and a spectrophotometer with a wavelength of 430 nm, the samples were examined. Results from measurements made using a spectrophotometer can be seen right away in the form of absorbance readings. Each meat extract sample was placed into microplates using a micropipette in amounts up to 0.025 ml. The microplate is then set inside a box that has been modified to prevent any light from entering or leaving. A image was then captured using a camera and saved on a computer for further processing.

The image that has been stored on the computer was then processed using ImageJ, an open-source software. ImageJ shows the color components in pixels and converts them into numbers in red-green-blue (RGB) color components or channels. The intensity of the color obtained is translated into absorbance by the Lambert-Beer law, namely:

$$A = -\log T = -\log \frac{I_t}{I_0}$$

A is the absorbance value, I_t is the intensity of each red, green, and blue channel, and I_0 is the maximum value of a pixel, which is 255. Then the absorbance value is entered into the equation of the calibration curve obtained so that the concentration of blood in the meat is known.

Data were analyzed by the one-way ANOVA method using the statistical package for social science (SPSS) 16.0 using. If the test results showed a significant difference ($p < 0.05$), then the data analysis was continued using Duncan's test with a 95% confidence interval.

RESULTS AND DISCUSSION

Spectrophotometer to measurement tool

The spectrophotometer can directly provide results in the form of absorbance values. The absorbance value is the result of the transmitted light and has a relationship with the sample concentration value.

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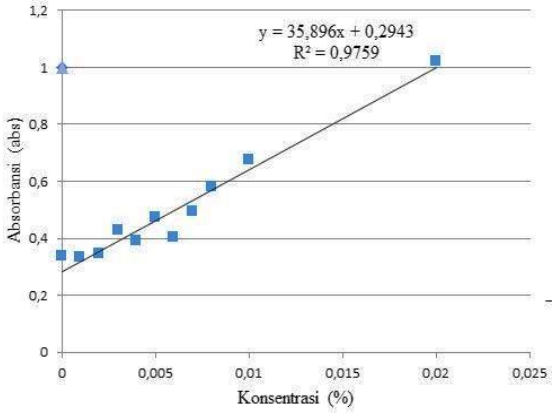
Figure 1: $FeCl_3$ calibration curve at a wavelength of 435 nm using a spectrophotometer.



Color intensity can be measured quantitatively using colorimetric methods. Color specifications for quantitative measurements refer to the International Commission on Illumination (CIE) which states that Red (R), Green (G), and Blue (B) are monochromatic light and can be measured using a spectrophotometer. A spectrophotometer is a tool used to measure the amount of energy that is absorbed or transmitted. If monochromatic light passes through a solution containing a substance that can absorb light, the light will be absorbed by the substance and the rest is transmitted (Harmita 2006). Determination of the maximum wavelength is the most important step in measuring blood levels using a spectrophotometer because the maximum wavelength has maximum sensitivity so it will produce the largest change in absorbance value for each change in concentration value. The absorbance read on the spectrophotometer should be between 0.2 and 0.8 (Ganjar and Rohman 2007).

The resulting absorbance value is then entered into a calibration curve to obtain the blood concentration in meat. The calibration curve is the relationship curve between absorbance (y) and concentration (x). On the calibration curve, the equation y= ax + b will be obtained. The purpose of calibration curve is to determine the linearity of the relationship between the concentration of the solution and the resulting absorbance.

Based on the calibration curve, a linear regression equation is obtained, namely y = 35.896x + 0.2943 with a correlation coefficient R^2 of 0.9759. Lambert Beer's law is fulfilled if the calibration curve is a straight line (Ganjar and Rohman 2007). The acceptance criterion of the correlation coefficient is if r 0.9995 which means that the curve between the absorbance value and the concentration value is linear, that is if there is an increase in the concentration value, it will be followed by an increase in the absorbance value (Ibrahim 2009).

Image processing as a measurement tool

Measurement of color intensity can be done with an alternative method, namely imaging processing. Two main elements make up this method, namely hardware, and software. The main components of hardware in this study are cameras cellphones and personal computers. The camera is used to take an image of meat extract samples that are in a microplate. The software used for image processing is Image J. Image J is used to analyze the image produced by the camera by measuring the color intensity value (RGB color index) in each sample.

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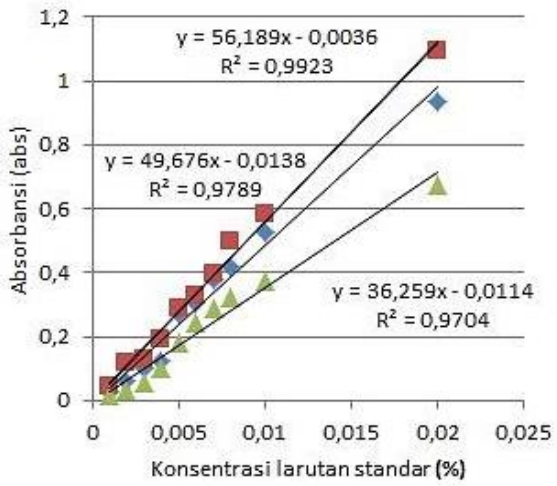
This image is then processed using Image J. The image is further divided into red, green, and blue channels. In some wells, some parts are darker than the original color of the solution. This is because there is an overlap between the top of the well and the bottom of the well (Soldat et al. 2009). The RGB color index obtained is the result of the analysis on the surface of the desired object, not the entire image field. By using Image J software, each well is selected in areas that have relatively the same color using the oval selection function by forming a circle of the same size in each well. The area is then stored in the region of interest and the color intensity is measured in each of the red, green, and blue channels. The intensity of the primary color that has been obtained using Image J is then converted into absorbance values using Microsoft Excel software. Conversion is done using the Lambert-Beer formula, namely:

$$A = -\log T = -\log \frac{I_t}{I_0}$$

where A is the absorbance value, I_t is the intensity of the RGB primary color in each channel, and I_0 is the maximum value of a pixel, which is 255. After the absorbance value is obtained, a standard solution calibration curve is made by connecting the absorbance value to the concentration of the solution.

Figure 2: Calibration curve using image processing on the RGB channel



The next calculation was done using the curve with the steepest slope. The sharpest slope of the curve denotes the curve's sensitivity to changes in concentration values. The red channel's calibration curve has the steepest curve according to Figure 3's calibration curve. The linear equation for the calibration curve was y=56.189x-0.0036, and its correlation coefficient was 0.9923. Only the red channel's color intensity would be processed further based on these data.

To calculate the concentration of blood in the sample, the absorbance of the sample solution was plotted on a reference calibration curve. Table shows a comparison of blood content in samples examined using a spectrophotometer and image processing.

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Table 1: Blood content in quail meat after various killing process

Origin of Quail Meats	Blood Content (%)	
	Spectrophotometer	Image Processing
Without slaughtering	0.45±0.035[a]	0.85±0.090[a]
Slaughtering without hanging	0.38±0.031[b]	0.70±0.088[b]
Slaughtering with hanging	0.32±0.028[b]	0.30±0.009[c]

Note: Data is presented in the form of a mean with a standard deviation (x±SD). Different superscript letters in the same column indicate a significant difference ($p<0.05$)

Table 1 demonstrates that quail meat produced through hanging after slaughter tended to have less blood than quail meat produced through slaughter alone. When quails are killed without being hung, the bleeding is frequently insufficient. This is in line with Wulandari's (2000) assertion that chicken slaughter must be performed in a dependent position in order to achieve perfect bleeding results since when poultry is hanged, a lot of blood leaks out due to gravity. The meat with the highest blood concentration is that from corpse quail, which does not bleed out. According to Eriyani (2008), carpse meat contains a significant amount of blood. On each devices, the blood concentration of the bleeding and non-bleeding quail meat varied significantly. There was a substantial difference between the value of blood concentration in meat from slaughtered quail handled with the hanging procedure and without the hanging process in measurements using a spectrophotometer or image processing technique. This demonstrates that there is no difference in sensitivity between utilizing an image processing technique and a spectrophotometer. The data obtained have a low standard deviation value, which indicates a 95% degree of confidence in the data.

Blood concentration, analysis results using a Spectrophotometer and image processing, was further tested by paired T-test statistics. The paired t-test results showed p-values of 0.437, 0.092, and 0.763 for corpse quail, quail slaughtered without hanging, and quail slaughtered with hanging, respectively. In each measurement, there was no significant difference in blood concentration values between the spectrophotometer and the camera at the 95% confidence level based on the results of the paired t-test ($p>0.05$). These results indicate that the camera has the potential to replace the spectrophotometer to measure blood concentration values in telemedicine applications. According to Soldat et al. (2009), measurements made using digital imaging techniques have benefits, including less material being used overall, making them more cost-effective, being much easier to find cameras than spectrophotometers, and being very simple to use because nearly everyone in society today has a camera, including digital and cellphone cameras.

CONCLUSION

There was a significant difference between the blood concentration of meat from unslaughtered and slaughtered quail with or without hanging. In the paired t-test, there was no significant difference between the value of blood concentration in quail meat as measured using a spectrophotometer and image processing tools. Based on this, it can be concluded that the imaging processing can be used as an alternative tools method to measure blood content.

REFERENCES

Damayanti, Y., Winaya, I. B. O., & Anto, M. D. R. (2012). Evaluasi penyakit virus pada

ISSN:2828-4925
DOI: 10.47841/icorad.v1i2.94
Page:41-48

kadaver broiler berdasarkan pengamatan patologi anatomi di rumah pemotongan unggas. J. Indonesia Medicus Veterinus, 1(3), 417-427.

de Morais, C. D. L. M., & de Lima, K. M. G. (2014). A colorimetric microwell method using a desktop scanner for biochemical assays. Talanta, 126, 145-150.

[Dirjen PKH] Direktorat Jenderal Peternakan dan Kesehatan Hewan. (2016). Populasi Puyuh Berdasarkan Provinsi 2012-2016. [Internet]. [diunduh 2017 Februari 5]. Tersedia pada : http://www.pertanian.go.id/NAK2016fix/Pop _PuyuhProp2016.

European Food Safety Authority. (2004). Welfare aspects of animal stunning and killing methods. Scientific report of the scientific panel for animal health and welfare on a request from the commission related to welfare aspects of animal stunning and killing methods (Question No EFSAQ-2003-093). http://www.efsa.europa. eu/en/science/ahaw/ahaw_opinions/495.html. Diunduh:21 November 2022

Eriyani, C. (2008). Laporan tahunan uji daging bangkai. Padang (ID): Dinas Peternakan Sumatera Barat.

Ganjar, I.G., & Rohman A. (2007). Kimia Farmasi Analisis. Yogyakarta (ID): Pustaka Pelajar.

Gregory, N.G. (1998). Animal Welfare and Meat Science. Oxon: CABI.

Harmita. (2006). Buku Ajar Analisis Fisikokimia. Depok (ID): Departemen Farmasi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Indonesia.

Ibrahim, S., Sriwulan S. (2007). Farmakokimia Metode Volumetri. Bandung (ID): Institut Teknologi Bandung Press.

[KEMENTAN RI] Kementrian Pertanian Republik Indonesia. (2015). Outlook Komoditas Pertanian Sebsektor Peternakan Daging Ayam. Jakarta (ID): Pusat Data dan Sistem Informasi Pertanian Kementrian Pertanian Republik Indonesia.

Lawrie, R.A. (1995). Ilmu Daging. Edisi kelima. Terjemahan: Parakkasi, A dan Y.Amwila. Universitas Indonesia Press, Jakarta.

Listiyowati, E, Roospitasari, K. (1999). Puyuh Tata Laksana Budidaya Secara Komersial. Jakarta (ID): Penebar Swadaya.

Lukman, D.W., Sudarwanto, M., Sanjaya, A.W, Purnawarman, T., Latif, H., Soejoedono, R,R. (2012). Penuntun Praktikum Higiene Pangan Asal Hewan. Pisestyani H, editor. Bogor (ID): Institut Pertanian Bogor.

Martinez, A.W, Phillips, S.T., Carrilho, E., Thomas, S.W., Sindi, H., & Whitesides, G.M. (2008). Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. Analytical Chimestry 80:3699-3707.

Rasyaf, M. (1995). Pengelolaan Usaha Peternakan Ayam Pedaging. Jakarta (ID): Gramedia Pustaka Utama.

Soldat, D.J., Borak, P., Lepore, B.J. (2009). Microscale calorimetric analysis using a desktop scanner and automated digital image processing software for the quantification of absorbed substance. Jurnal Analalytical Chemestry 59(2): 102-106.

Sucipto, S., Wardani, R. I., Kamal, M. A., & Setiyawan, D. T. (2020). Analisis teknoekonomi alat penyembelihan ayam untuk mendukung implementasi sistem jaminan halal. Jurnal Teknologi Industri Pertanian, 30(1).

Sugiharto, R. E. (2005). Meningkatkan keuntungan beternak puyuh. Agromedia.

Hidayat, N., & Sunarti, S. (2015). Validitas Pemeriksaan Kadar Hemoglobin Menggunakan Metode Hb Meter pada Remaja Putri di MAN Wonosari. Kes Mas: Jurnal Fakultas Kesehatan Masyarakat Universitas Ahmad Daulan, 9(1), 25016.

Vecerek, V., Voslarova, E., Conte, F., Vecerkova, L., & Bedanova, I. (2016). Negative trends in

ISSN:2828-4925
DOI: 10.47841/icorad.v1i2.94
Page:41-48

transport-related mortality rates in broiler chickens. Asian-Australasian journal of animal sciences, 29(12), 1796.

Wulandari, W.A., Wiwit, E., Gunawan. (1997). Pengembangan Konsep Model Sistem Halal Produk Daging Ayam di Rumah Potong Hewan. Prosiding. Lokakarya Nasional Keamanan Pangan Produk Peternakan.