Jurnal Veteriner
pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional, Dirjen Penguatan Riset dan Pengembangan,
Kemenristek Dikti RI S.K. No. 36a/E/KPT/2016

September 2024 Vol. 25 No.3 : 377-383
DOI: 10.19087/jveteriner.2024.25.3.377
online pada http://ojs.unud.ac.id/index.php/jvet

Determining The Hemoglobin Levels of Quail (*Coturnix coturnix japonica*) Using Image Processing from Desktop Scanner

(MENENTUKAN KADAR HEMOGLOBIN BURUNG PUYUH (*COTURNIX COTURNIX JAPONICA*) MENGGUNAKAN PENGOLAH CITRA DARI PEMINDAI *DESKTOP*)

Koekoeh Santoso[1], Sofa Azizah[1], Sus Derthi Widhyari[2], Mukhamad Fakhrul Ulum[3], Kudang Boro Seminar[4], Agik Suprayogi[1]

[1]Veterinary Physiology Division
Dept. Anatomy, Physiology and Pharmacology
[2]Veterinary Pathology Clinic Division,
[3]Veterinary Surgery and Radiology Division
Dept. of Clinic, Reproduction and Radiology
Facultyof Veterinary Medicine , IPB University,
[4]Faculty of Agricultural Technology. IPB University
Jln Agatis, Kampus IPB Dramaga Bogor
Jawa Barat, Indonesia 16680
Email: koekoehsa@apps.ipb.ac.id

ABSTRACT

Various animal diseases require colorimetric-based laboratory diagnose. One of them is the measurement of blood hemoglobin concentration. Anemia needs to be detected as early as possible, because the lack of hemoglobin in the blood could results in a lack of oxygen being carried from the lungs to the tissues. Colorimetry is a quantitative analysis technique for colored samples to determine the concentration of a substance based on the light intensity of the color solution. Colorimetry is commonly used to measure the level of hemoglobin in the blood. Image processing using image J has been widely used for chemical analysis. This study was aimed to determine the potential of image processing from a desktop scanner to measure animal blood hemoglobin. The blood used was taken from five quail. Quail blood was diluted to 100%, 90%, 80%, 70%, 60% and 50% respectively. The results of hemoglobin level readings using the Sahli method were strongly correlated, but significantly different, with a spectrophotometer. The readings of hemoglobin levels using a desktop scanner have a very strong correlation and are not significantly different from the results obtained using a spectrophotometer. The image processing method from a desktop scanner can be used as an alternative to a spectrophotometer to measure blood hemoglobin levels compared to using the Sahli method

Key words: desktop scanner; haemoglobin; image processing; spectrophotometer

ABSTRAK

Berbagai penyakit hewan memerlukan diagnosis laboratorium berbasis kolorimetri. Salah satunya adalah pengukuran konsentrasi hemoglobin darah. Anemia perlu dideteksi sedini mungkin, karena kurangnya hemoglobin dalam darah dapat mengakibatkan kurangnya oksigen yang dibawa dari paru-paru ke jaringan. Kolorimetri adalah teknik analisis kuantitatif sampel berwarna untuk menentukan konsentrasi suatu zat berdasarkan intensitas cahaya larutan warna. Kolorimetri biasanya digunakan untuk mengukur kadar hemoglobin dalam darah. Pengolahan citra menggunakan image J telah banyak digunakan untuk analisis kimia. Tujuan dari penelitian ini adalah untuk mengetahui potensi pengolahan citra dari pemindai *desktop* untuk mengukur kadar hemoglobin darah hewan. Darah yang digunakan diambil dari lima ekor burung puyuh. Darah puyuh diencerkan masing-masing menjadi 100%, 90%, 80%, 70%, 60% dan 50%. Hasil pembacaan kadar hemoglobin dengan metode Sahli berkorelasi kuat namun berbeda nyata dengan spektrofotometer. Pembacaan kadar hemoglobin menggunakan pemindai *desktop* memiliki korelasi yang sangat kuat dan tidak berbeda nyata dengan hasil yang diperoleh dengan menggunakan spektrofotometer. Metode pengolahan citra dari pemindai *desktop* dapat digunakan sebagai alternatif spektrofotometer untuk mengukur kadar hemoglobin darah dibandingkan dengan menggunakan metode Sahli.

Kata-kata kunci: hemoglobin; pengolahan citra; pemindai desktop; spektrofotometer

INTRODUCTION

Colorimetric-based laboratory examination is one of the chemical examinations that is indispensable in establishing a diagnosis in the veterinary world. Various animal disease diagnoses can be confirmed by this test. One of supporting examinations is the measurement of blood hemoglobin levels. Hemoglobin is an oxygen-carrying compound in red blood cells that can be measured chemically. The amount of hemoglobin 100 mL^{-1} of blood can be used as an index of the oxygen-carrying capacity of the blood. Low levels of hemoglobin in the body lead to anemia. Anemia needs to be detected as early as possible, because the lack of hemoglobin in the blood results in a lack of oxygen being carried from the lungs to the tissues. This causes the liver and brain to work not optimally, so the body becomes tired and pale (Herawati 2009). Hemoglobin levels can be determined by various methods, such as the Sahli method, Tallquist, cupersulfate and cyanomethemoglobin (Hidayat and Sunarti, 2015).The colorimetric-based cyanomethemoglobin method is also known as the spectrophotometer method. This method is the gold standard in the laboratory analysis of hemoglobin (Usman and Yuslely, 2003). The price of a spectrophotometer is very expensive and produces quite a lot of reagent waste containing cyanide (De Moreis and De Lima, 2014), so that not all central veterinary laboratories can use a spectrophotometer as a diagnostic support tool. Especially animal health centers located in remote areas.

In remote areas of rural Indonesia, quail are birds that are widely reared because on limited land, quail are able to produce optimally. A research report on the hematology routine of the quail's which is associated with stress has also been reported by Jumadin *et al.* (2018a; 2018b) and Santoso *et al*. (2020). Apart from that, Santoso *et al*. (2019) also reported the profile of immune substances possessed by quail kept in tropical areas. Quail are relatively widely studied as experimental animals in Indonesia.

Along with the very rapid modernization of the era, technological advances spread to remote parts of Indonesia. The use of desktop scanners has become

common in people's lives in cities and areas classified as underdeveloped, frontier and outermost areas.

Modifications of scanners are often used for digital image scanners (Vashist *et al.,* 2015; De Morais 2014). The image, the result of the scan, can be processed using image J software which is commonly used in various branches of science such as Agriculture, Biomedical, and Biometrics. In addition, the use of simple tools that are easy to carry by medical personnel can support health services for animals in remote areas. The study was aimed to determine the potential of image processing from a desktop scanner to measure animal blood hemoglobin.

RESEARCH METHODS

This research was conducted at the Physiology Laboratory of the Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University. The number of quails used in this study were five adult female quails from the Bogor area.

Blood Collection

Blood sampling was carried out on the jugular vein of the quail. Blood was taken using a 1 mL syringe, then put into an *Ethylenediaminetetraacetic acid* (EDTA) tube and stored in a refrigerator at 4 °C.

Measurement of Hemoglobin Level

Quail blood was diluted to 100%, 90%, 80%, 70%, 60%, and 50% respectively. For each dilution, 0.008 mL of blood was taken to be mixed with 2 mL of Drabkin's reagent. The solution was homogenized using a vortex and then allowed to stand for 10 minutes. The absorbance of each solution was measured using a UV-Vis spectrophotometer. Each tube was taken 0.025 mL of the solution using a micropipette to be inserted into the wells on the microplates.

Scan Using a Scanner

The plate that has been filled with the sample is placed into the desktop scanner device. Around the plate is given two layers of dark colored foam so that no light enters or leaves. Then a scan is performed and the scan results are stored in the tagged image file format (TIFF) on the computer for further processing.

Interpretation of Results

The resulting images stored in TIFF and JPEG formats are processed using image processing software called Image J to determine the colors contained in the wells of the plate. The color will be converted into a number based on the red, green, and blue (RGB) color components. The color intensity is then translated into absorbance by Lambert-Beer law, namely:

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

A is the absorbance value, It is the color intensity of each red, green, and blue channel, and I0 is the maximum value of a pixel of 255 (Underwood and Day, 2002). The results of measuring hemoglobin levels from the Sahli method, scanners, and cellphone cameras were compared with hemoglobin levels produced by UV-Vis spectrophotometers. The absorbance of the UV-Vis spectrophotometer was converted to hemoglobin levels with the formula Hb = Absorbance x 36.8.

Data Analysis

Data were analyzed quantitatively using Microsoft Excel 2010 program to create graphs. Statistical analysis by comparing the results of measuring hemoglobin levels from the Sahli method, scanners and cellphone cameras with the results of measuring hemoglobin levels from spectrophotometers using correlation tests, independent T and profiles.

RESULTS AND DISCUSSION

Measurement of Hemoglobin Levels Using the Sahli Method

Hemoglobin is the oxygen-carrying compound in red blood cells. The amount of Hb 100 mL^{-1} of blood can be used as an index of the oxygen-carrying capacity of the blood. Hemoglobin in normal blood is about 15 g per 100 mL. Nationally the percentage of Public Health Centre (Puskesmas) that have Hb Sahli is around 46.3%, the rest do not have or use other Hb measuring devices. When viewed from the location, the percentage of Public Health Centre that have Hb Sahli in urban areas is only 37.6%, while in rural areas it is 49.3%.

Based on the data above, it can be seen that most of the Public Health Centre which tend to have more advanced medical equipment than the Public Health Centre still rely on Hb Sahli as a measuring tool for hemoglobin levels. The use of the visual or Sahli method is no longer recommended, because it has large errors, the tool cannot be standardized and not all types of hemoglobin can be converted into acid-haematin such as keroxy-hemoglobin, met-hemoglobin and sulf-hemoglobin (Faatih *et al*., 2017). The International Committee for Standardization in Haematology (ICSH) recommends the examination of hemoglobin using the cyanomethemoglobin method (spectrophotometer). This method is easy to do because it automatically calculates the hemoglobin level in the erythrocytes. The cyanomethemoglobin method has also been standardized and stable (McPherson and Pincus, 2011). The average results of reading blood hemoglobin levels using a spectrophotometer and the Sahli method were presented in Table 1.

Tabel 1. The average quail blood hemoglobin level calculated using a spectrophotometer and the Sahli method

Blood Concentration	Hemoglobin Level (g dL^{-1})	
	Spektrophotometer	Sahli
100 %	14.919 ± 4.758	12.76 ± 2.512
90 %	14.529 ± 1.256	10.40 ± 2.417
80 %	14.146 ± 2.622	9.76 ± 1.774
70 %	9.671 ± 1.333	7.52 ± 1.622
60 %	10.620 ± 2.191	7.40 ± 1.288
50 %	7.272 ± 1.683	5.04 ± 0.910

The results of the Hb readings in Table 1 were analyzed using the correlation test. Correlation test was conducted to determine the relationship between the pattern of readings of Hb levels from the two tools. Based on the correlation test, the correlation coefficient (R) was positive for the spectrophotometer with Sahli of 0.538. The results of correlation analysis show that the pattern of reading blood hemoglobin levels from a spectrophotometer with the Sahli method has a strong relationship

The deviation of Hb level readings from Sahli and the spectrophotometer is indicated by the mean absolute percentage error (MAPE). The MAPE shows how big the average absolute error of forecasting or alternative tools is compared to the actual value or gold standard. The MAPE value from the spectrophotometer and Sahli's method is 31%. This indicates that the hemoglobin level readings from the Sahli method differ by three digits from the spectrophotometer.

Measurement of Hemoglobin Levels Using a Scanner

Scanners that can be used as absorbance measuring devices are double beam scanners. Figure 1 is a captured image from a scanner saved using the TIFF format (Burger and Burge, 2009; Saunders *et al.*,

2017). Images were analyzed using Image J software to obtain color intensity. This application will divide the pixels in the image into three components, namely red, green, blue channels (Soldat *et al*., 2009).

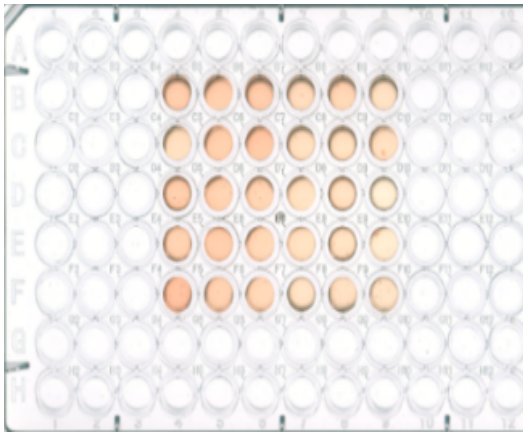

Figure 2. Captured images using a desktop scanner

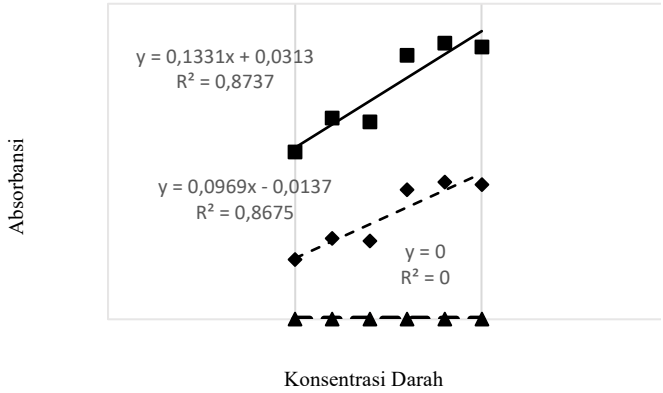

Figure 3. Calibration curve of absorption. - Red channel,-Blue channel,-Green channel of scanner

Each well on the microplate was processed using the oval selection function found in the image J software (Soldat *et al*. 2009). Oval selection was used to obtain uniform color intensity from each microplate well (De Morais and De Lima, 2014). Scanning the object will produce a three-dimensional image. The object has an area of overlap between the wall and the base of the microplate. The extent of the overlapping area is directly proportional to the distance between the scanner and the object, so processing using oval selection must avoid these areas. The use of pixels and the diameter of the oval selection on the same object must be consistent (Soldat *et al*., 2009). In this study, the diameter of the oval selection used is 0.18 x 0.18.

Scanners that can be used as absorbance measuring devices must also have the ability to generate a calibration curve using one of the red, green, or blue channels generated from colorimetric measurements (Soldat *et al*., 2009). The calibration curve is made from the absorbance value at each solution con-centration in each channel. The following is the calibration curve of the three channels.

The equation of the calibration curve is obtained in Figure 3. In the measurement of the hemoglobin level of quail blood there is a calibration curve related to the concentration of the solution. The curve with the steepest slope is selected for further calculations. The steepness of the curve indicates the sensitivity of the channel to changes in blood concentration (De Morais and De Lima, 2014). Based on Figure 3, the blue curve has the steepest slope compared to the other two curves with a line gradient of 0.1331. Then only the color intensity in the blue channel will be further processed to get the quail hemoglobin level.

The color intensity obtained from image processing using image J software is converted into absorbance values using the Lambert-Beer equation (Underwood and Day, 2002). The absorbance is multiplied by a multiplier factor of 89.4 to get the Hb level. The readings from the spectrophotometer and scanner were presented in the Table 2.

Table 2 . The average absorbance and hemoglobin levels of quail blood were calculated using a spectrophotometer and a desktop scanner

Blood Concentration	Absorbance		Hemoglobin (g dL^{-1})	
	Spektrofotometer	*Scanner*	Spektrofotometer	*Scanner*
100 %	0.405 ± 0.129	0.155 ± 0.029	14.919 ± 4.758	11.442 ± 2.627
90 %	0.395 ± 0.034	0.158 ± 0.005	14.529 ± 1.256	11.600 ± 0.453
80 %	0.384 ± 0.071	0.151 ± 0.018	14.146 ± 2.622	11.084 ± 1.618
70 %	0.263 ± 0.036	0.113 ± 0.007	9.671 ± 1.333	8.284 ± 0.585
60 %	0.289 ± 0.060	0.115 ± 0.013	10.620 ± 2.191	8.454 ± 1.119
50 %	0.198 ± 0.046	0.096 ± 0.020	7.272 ± 1.683	7.034 ± 1.748

The results of absorbance readings and quail blood Hb levels calculated using a spectrophotometer and desktop scanner as presented in Table 2 have a positive correlation coefficient (R) of 0.922 with a mean absolute percentage error (MAPE) of 13%. Correlation analysis serves to show how close the relationship between variables, so it can be seen that the patterns of the two tools have very strong similarities (. The MAPE size shows the difference between the spectrophotometer reading results and the scanner reading results reaching one digit.

Further analysis on the three alternative tools was performed using an independent T test. In this analysis, a 95% confidence interval was used. The obtained results indicate that the readings from the Sahli method are significantly different from the spectrophotometer. The readings from the desktop scanner produce readings that are not significantly different from the results of the spectrophotometer readings with a significance of 0.869.

SUGGESTION

This research needs to be continued to increase the number of experimental animals in order to obtain additional data to strengthen the results that have been obtained.

ACKNOWLEDGEMENT

We thank IPB University for its support through the Penelitian Terapan Unggulan [Perguruan Tinggi (PTUPT) program Master Contract Number 1/E1/KP-.PTNBH/2021 on the date March 8th 2021 with Derivative Contract Number 2075/IT3.L1/PN/2021 on the date March 15th 2021. Also, we would like to thank Ragunan Zoo and its staff for letting us carry out our research with the COVID-19 protocols. We also thank the zookeepers for their assistance in this research.

REFERENCES

Burger W, Burge MJ. 2009. *Principles of Digital Image Processing Fundamental Technique.* London. Springer.

De Morais C, De Lima K. 2014. A colorimetric microwell method using a dekstop scanner for biochemical assays. *Talanta* 136(1) :145-150

Dany F, Nikmah UA. 2017. Use of Hemoglobin measuring devices in community health centers, village maternity cottage and health center in the village. *Journal of Health Services Research and Development* 1(1): 32- 39.

Hansun S. 2012. The IHSG data forecasting uses fuzzy time series. *Indonesian Journal of Computing and Cybernetics Systems.* 6(2) : 79- 88

Herawati N. 2009. Anemia and the role of erythropoietin. *Biotrends* 4(1): 35-39

Hidayat N, Sunarti. 2015. Validity of checking hemoglobin levels using the Hb meter method in young women at State Madrasah Aliyah Wonosobo. *Public Health* 9(1) : 11-18

Long KD, Yu H, Cunningham B. 2014. Smartphone instrument for portable Enzyme-Linked Immunosorbent Assay. *Biomedical Optics Express* 5(11): 1- 15

Jumadin L, Satyaningtijas AS, Santoso K. 2018a. Extract Of Cassava Leaves Is A Good

Antioxidant For Mature Quail Which Exposed To Heat In Short Time. *J Veteriner* 18(1): 135-143

Jumadin L, Satyaningtijas AS, Maika Z, Darlian L , Ummah W, Santoso K. 2018b. The Total Erythrocyte Count, Hematocrit Value, Hemoglobin Concentration and Erythrocyte Index In Quail Exposed Heat Exposure And Cassava Leaves Extract. *J Veteriner* 19(3): 335-341

Mc Pherson R, Pincus M. 2011. *Henry's Clinical Diagnosis and Management by Laboratory Methods.* 22nd ed. New York (US): Saunder Elseveir.

Santoso S, Widyadhari AS, Poetri ON , Jumadin L. 2019. Immune Response of Adult

Quails (*Coturnix coturnix japonica*) Treated With Cassava Leaf Extract To Overcome Heat Stress). *J Veteriner* 20(4): 519-525.

Santoso S, Widyadhari AS, Poetri ON , Jumadin L. 2020. Leukocyte Profile Of

Adult Quails (*Coturnix coturnix japonica*) Treated With Cassava Leaf Extract To Overcome Heat Stress. *J Veteriner* 21(3): 367-373

Saunders D, Collmann R, Borda A. 2017. Reflectance Transformation Imaging and Imagej: Comparing Imaging Methodologies for Cultural Heritage Artefacts. London (UK): BCS Learning and Development.

Soldat D, Barak P, Lepor B. 2009. Microscale colorimetric analysis using a dekstop scanner and automated digital image analysis. *Journal of Chemical Education*. 86(5): 617- 620.

Subanar, Suhartono. 2009. Wavelet Neural Networks untuk Peramalan Data Time Series Finansial. Yogyakarta (ID): Universitas Gajah Mada.

Underwood A, Day R. 2002. *Quantitative Chemical Analysis*. 6th Ed. Jakarta. Erlangga.

Usman, Yuslely. 2003. Validation of the classification of anemia in children under five years by looking at the paleness of the palms. *Media Litbang Kesehatan*. 13(1) : 14- 19

Vashist S, Oordt T, Schneider E, Zengerle R, Stetten F, Luong. 2015. A smartphone based colorimetric reader for bioanalytical applications using the screen based bottom illumination provided by gadgets. *Biosensors and Bioelectronics* 67: 248-255