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Liver histopathology of broiler chicken (*Gallus domesticus*): A veterinary forensic model 48 h postmortem

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ABSTRACT: Veterinary forensic science has not received adequate attention in Indonesia; therefore, its development needs to increase. This research aimed to study veterinary forensic science through histopathology of broiler chicken livers 48 h after death. Seventy-five broiler chickens aged 7 days were euthanized and divided into 25 groups based on post-euthanasia necropsy (n=3). Chicken cadavers were necropsied every 2 h postmortem, and livers were collected to prepare histopathological sections and stained with haematoxylin-eosin (HE). Liver histopathology evaluation and the results obtained were analysed using ImageJ software version 1.53a. The relationship between histopathological variables and postmortem time was analysed using the Pearson's method. The results showed that decay began at the 18th h postmortem which was marked by the number of hepatocyte cell nuclei. At the 20th hour, putrefactive bacteria were found, and at the 22nd hour, there was an increase in the distance between the hepatocytes. Based on the Pearson correlation value, the number of hepatocyte cell nuclei, the distance between hepatocytes, and the presence of putrefactive bacteria have a strong to very strong relationship with postmortem time; therefore, these results can be used to determine the time of death (postmortem interval).

Keywords:
autolysis, broiler chicken, liver, histopathology, postmortem interval

■ INTRODUCTION

Illegal trade in rare birds can cause many deaths, owing to poor handling of the transportation process. Illegal trade is related to law, so it is necessary to carry out further investigations into the discovery of dead animals. The cause, time, and manner of death in animals can be determined through the application of veterinary forensic science (Byard & Boardman 2011). Microscopic examination of tissues can be used to estimate the time of death and increase the accuracy of investigating the cause of death (Brooks 2016, Dettmeyer 2018) and even the process of decomposition (Moraleda *et al*. 2022). This research aimed to study veterinary forensics on the death of rare birds using an animal model in the form of broiler chickens after 48 h of death.

■ MATERIALS AND METHODS

All research procedures were approved by the Animal Ethics Commission, Institute for Research and Community Service, IPB University (Number 162/KEH/SKE/XII/2019). 75 DOC broilers were kept for 7 days, euthanised using the cervical dislocation method, and placed in the SKHB paddock. The chickens were divided into 25 groups, each consisting of 3 chickens. Each group of chickens was placed in an open area without a base and covered with a wire. Every 2 h, namely the 0th, 2nd, 4th, 6th, 8th, and 48th hour after death, a necropsy was carried out to remove the chicken liver, and histopathological preparations were made and HE stained. Humidity and environmental temperature were measured during observations. The variables observed were the number of hepatocyte nuclei, colour of the hepatocyte cytoplasm, and distance between hepatocytes and bacterial colonies. The histopathological variables were calculated and measured using ImageJ version 1.53a software in three microscopic fields of view at 400 × magnification. The close relationship between variables and observation time was analysed based on the Pearson method and presented in the form of a scatter graph using the R Software. Pearson correlation coefficient (r) values include 0.00-0.10 for a very weak relationship, 0.10-0.39 for a weak relationship, 0.40-0.69 for a moderate relationship, 0.70-0.89 for a strong relationship, and 0.90-1.00 for a very strong (very strong) relationship.

■ RESULTS AND DISCUSSIION

The environmental temperature at the time of observation was 23.4-35.1 °C with a humidity of 62.4-99.9%. The optimum temperature for enzyme and bacterial activity in the decomposition process is 21-37 ºC with humidity 45-60%

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(Paczkowski & Schütz 2011). Microscopic observations of model chicken livers revealed changes in the number of nuclei, cytoplasmic colour, distance between hepatocytes, and the presence of putrefactive bacteria. The coefficient value r for the number of hepatocyte nuclei is -0.84 (Figure 1A). The number of hepatocyte nuclei at the 48th hour was much lower than at the 0th hour, and decreased as the time of death increased. This is caused by hepatocyte lysis due to the activity of hydrolytic enzymes, thereby damaging cell structures and connections between cells. Autolysis causes cell nuclear chromatin to condense, chromatin boundaries shift and causes cells to disintegrate into debris (Huang *et al*. 2021). The number of hepatocyte nuclei was strongly correlated with the postmortem time. Calculation of the number of hepatocyte nuclei as a reference for postmortem interval.

Changes in the colour of the hepatocyte cytoplasm were measured using the red value level in RV units using the ImageJ software. The average hepatocyte cytoplasm color at the 0th to the 20th hour was 125.89 RV units, then became 136.29 RV units at the 22nd to the 30th hour, then decreased to 133.81 RV units until the 48th hour. A higher red value was indicated by an increasingly bright red colour of the cytoplasm. The hepatocyte cytoplasmic colour r coefficient value of 0.36 (Figure 1B) shows a weak correlation; therefore, it is not recommended as a reference for postmortem interval. Changes in the colour of hepatocyte cytoplasm are caused by a decrease in pH due to reduced cell oxygen levels after death. The decrease in pH is also caused by glycolysis, which produces carbon dioxide and lactate via autolysis (Brooks 2018). A more acidic cytoplasm absorbs more eosin, and the colour of hepatocytes gradually becomes redder (Figure 1E) as the postmortem time increases.

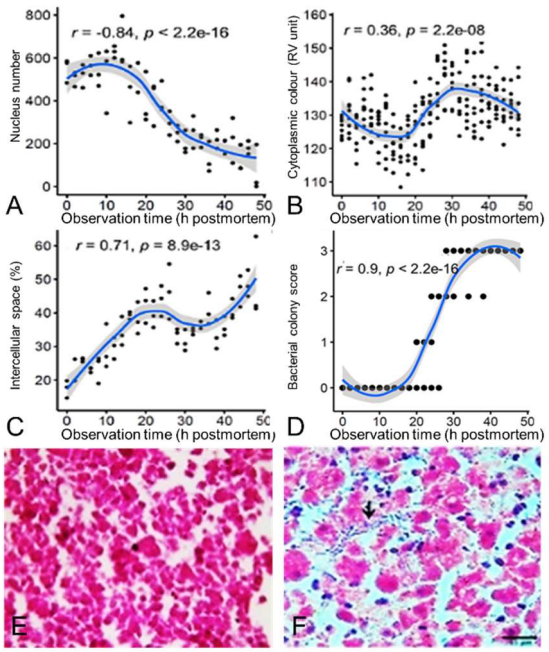

Figure 1. Pearson's histopathology test graph for broiler chicken liver observation time 48 h postmortem. (A) Number of hepatocyte nuclei, (B) cytoplasmic colour, (C) distance between hepatocytes, (D) bacterial colony scoring, (E) hepatocytes that lost nuclei and (F) bacterial colonies (black arrow) at 42 h postmortem.

The r value of the distance between hepatocytes was 0.71 (Figure 1C). This shows a strong correlation between hepatocyte distance and postmortem time, and that the distance between hepatocytes widens as postmortem time increases. The distance between hepatocytes is caused by the accumulation of gas resulting from the activity of putrefactive bacteria, and a reduction in the number of hepatocytes due to autolysis. Intrinsic bacteria in the putrefaction process begin to digest the intestines and surrounding tissue, and enzymes from lysed cells also begin to destroy the tissue. Decomposition involves autolysis and putrefaction. Autolysis is caused by enzyme activity while putrefaction is caused by bacterial activity from the digestive tract or from the gas which spreads throughout the body (DiMaio & DiMaio 2001; MacKenzie & Path 2014). Putrefactive bacteria were detected starting at the 20th h postmortem, and the number of bacteria increased with time (Figure 1F). A bacterial r value 0.9 shows a very strong relationship between bacterial colonies and observation time (Figure 1D). The presence of bacteria is an accurate method for determining the postmortem interval (Guo *et al*. 2016).

■ CONCLUSION

Histopathological variables, such as the number of hepatocyte nuclei, the distance between cells, and the presence of putrefactive bacteria, can be used as references for the postmortem interval.

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