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Comparison of Phenotypic and DNA Variation Between Males and Females of Red and Black-Eyed Albino Lovebirds

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Malang and the UNISMA Halal Center Laboratory. This study aimed to measure phenotype variation and DNA of male and female Lovebird Albino with a red and black eyes. The material in this study was 40 Lovebird Albino red eyes and black eyes with male sex (MHJ) and female sex (MHB). The experimental design used descriptive quantitative. The research variables followed wing length, chest size, body length and qPCR DNA with gen TYR. The data were analyzed using a T-test unpaired. The phenotype variation in wing length, chest size, and body length between male and female Lovebird Albino red eye showed no significant effect (P>0,05). The average value on wing length was $13,7 \pm 1,89$ cm (MMJ), $13,2 \pm 1,48$ cm (MHJ), $13,7 \pm 1,31$ cm (MMB), $13,2 \pm 2,16$ cm (MHB). The average value on chest size was 6.5 ± 1.89 cm (MMJ), 6.5 ± 1.48 cm (MHJ), 6.5 ± 0.53 cm (MMB), 6.5 ± 0.53 cm (MHB). The average value for body length was $15,40 \pm 0,97$ cm (MMJ), $15,20 \pm$ 0,79 cm (MHJ), 15,20 \pm 0,79 cm (MMB), 15,20 \pm 0,63 cm (MHB). Average values on qPCR DNA were 37,67 ± 1,79 (MMJ), 35,22 ± 3,66 (MHJ), 37,90 ± 2,12 (MMB) and $35,89 \pm 2,99$ (MHB). The conclusion was phenotype variation and DNA Lovebird Albino red and black eyes on males and females has no significant effect but have propensity Lovebird Albino red eyes both in male and female higher 3,79% until 6,96% compared to black eye seen from wing length and DNA.

ABSTRACT

This research was conducted at the Bambang Iswanto Lovebird house regency

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1. Introduction

Lovebirds are one of the most popular and popular bird species today. Suitable as a hatchery (cultivation) or merely cared for. This is because Lovebirds have reasons to be loved by the wider community, including a variety of feather colours, funny actions and amazing sounds. Lovebirds have great qualities, and can also be used as bird contests that are increasingly popular in various locations in Indonesia. In addition to being a favourite, love birds can also be used as a business, because they have a high price and are simple to cultivate [1]. This is also supported by the opinion of [2] that Lovebirds are animals that can be cultivated and maintained as a passion and favourite, can also be used as a side business after finishing work, because they have great quality. This is also supported by

Masyhuda's research [3] which argues that, due to the speciality of Lovebird, many people are carried away to cultivate this bird.

Lovebirds are popular because they have unique feather colours and unique and smart actions. The bird has a variety of patterns and is very beautiful, from prominent patterns to pastel (soft) colours. The bird is also quite smart like other parrots. One kind of intelligence of this bird is that it has advantages that are different from other birds usually. Based on the advantages contained can be obtained then the Lovebird is a unique type of bird. Because of this uniqueness, it is able to release joy by its owner because it provides a natural atmosphere such as the performance of its attractive appearance, style, and sound [1].

Lovebird farming is an action to breed these birds in order to be able to fulfil orders continuously. Agapornis Fischeri is a monomorpic type of animal such as males or females with an appearance that looks identical, especially the pattern of the feathers [4]. Previous research by Setiadi [5], stated that measurements of body dimensions in Lovebirds carried out included an average femur length in female Lovebirds of 3.17 cm, tibia length of 3.92 cm, tarsometatarus length of 2.69 cm, circumference of tarsometatarus 1.13 cm, third finger 1.74 cm, wing length of 3.22 cm and maxilla length 1.85 cm. Whereas in wild female lovebirds the femur length was 3.59 cm, tibia length was 4.31 cm, tarsometatarus length was 2.98 cm, tarsometatarus circumference was 1.24 cm, third finger was 1.2 cm, wing length was 3.39 and maxilla length was 2.16 cm. Differences in measurement results were seen in domesticated lovebirds. Domesticated male lovebirds have a femur length of 3.83 cm, tibia length of 4.85 cm, tarsometatarus length of 2.25 cm, tarsometatarus circumference of 1.15 cm, third finger of 2.16 cm, wing length of 9.55 cm and maxilla length of 2.50 cm. While the domesticated female Lovebird has a femur length of 4.24 cm, tibia length of 5.09 cm. The difference in body size in Lovebird depends on the type and age of the Lovebird when measurements are taken [5].

One of the genes associated with feather pattern is tyrosine. The patterning of poultry feathers is influenced by the TYR gene [6], [7]. The TYR gene can realise the enzyme, tyrosinase. The enzyme is located in melanocyte cells that realise the pigment called melanin. According to [7], in principle, there are three enzymes that can act as melanin synthesisers, namely tyrosinase, Tyrosinase Related Protein 1 (TYRP-1) and (TYRP-2), but in these three enzymes the maximum function is the tyrosinase enzyme. With high melanin, the absorption of sunlight and vitamin D is thought to cause efficiency in the body's metabolism characterised by higher productivity in chickens and quails [6], [7].

According to Masruroh et. al [7] that there is still no statement or limited information about the features of the various phenotypes associated with Lovebirds. Setiadi [5] states that Lovebirds have main characteristics including the colour of the feathers and the shape of the hooked beak. While the main characteristics of Lovebird birds lie in a variety of diverse feather colours, while the feather colour and body shape of males and females have differences, namely the shape of the body size, beak, and character traits of Lovebird birds, so based on this it is very important to conduct research on Comparison of Phenotypic Variations and DNA of Red and Black-Eyed Albino Lovebirds between Males and Females.

2. Method

This research was conducted from 09 June 2022 to 07 September 2022 at the house of Mr Bambang Iswanto, SIdorahayu Housing, Block C, RT 31 RW 07, Wagir District, Malang Regency, and at the halal center (PHC) laboratory of the Islamic University of Malang, Malang, East Java. The materials used in this study included red and black-eyed albino lovebirds with a total of 40 birds for phenotype variation testing as many as 40 birds and for DNA testing as many as 12 birds. The equipment used in this research are measuring tape for phenotype testing and measuring pipette, spin down, vortex, column strip, and CB3 column for DNA testing. The method used in this research is descriptive method. With techniques on purposive sampling with criteria from: Lovebird breeders and Male / female Red-eyed Lovebird broods and studs with male and female Black-eyed Lovebirds.

Variables observed included wing length, chest circumference, body length, and DNA. The results of the study were analysed using the unpaired t test. Wing length measurements were taken from 10 male and female red-eyed albino lovebirds and male and female black-eyed albino lovebirds. Then measured using a measuring tape as many as 10 tails of each type, then record the data in the book. Measurement of chest circumference of 10 male and female red-eyed Albino Lovebirds and male and female black-eyed Albino Lovebirds, respectively. Then measured using a measuring tape as many as 10 birds of each type, then recorded the data in the book. Body length measurements were taken for 10 male and female Red-eyed Albino Lovebirds and male and female Black-eyed Albino Lovebirds, respectively. Then measurements were taken for 10 male and female Red-eyed Albino Lovebirds and male and female Black-eyed Albino Lovebirds, respectively. Then measurements were taken for 10 male and female Red-eyed Albino Lovebirds and male and female Black-eyed Albino Lovebirds, respectively. Then measurements were taken for 10 male and female Red-eyed Albino Lovebirds and male and female Black-eyed Albino Lovebirds, respectively. Then measurements were taken for 10 male and female Red-eyed Albino Lovebirds and male and female Black-eyed Albino Lovebirds, respectively. Then measurements were taken for 10 male and female Red-eyed Albino Lovebirds and male and female Black-eyed Albino Lovebirds, respectively. Then measured using a measuring tape as many as 10 birds of each species, then recorded the data in the book.

DNA sampling is carried out in several steps as follows:

- a. DNA research was carried out at the Halal Centre laboratory at the Islamic University of Malang.
- b. Taken 3 samples of male and female Albino Lovebird feathers totalling 12 feathers for DNA isolation.
- c. Take Lovebird feathers as needed and place in a 1.5 ml microtube container and add 0.5 ml of Aquabides (ddH2O).
- d. Add GA buffer solution to a volume of 200 ul, then centrifuge 12,000 rpm for 1 minute.
- e. Add 20 ul of proteinase K buffer and vortex until homogeneous.
- f. Incubate at 56°C for 1 hour.
- g. Add 200 ul of GB buffer into the sample, vortex to homogeneous then spin down and incubate at 70°C for 10 minutes.
- h. Add 200 ul Ethanol (96-100%) into the sample, vortex to be homogeneous then spin down.
- i. Pipette and put the mixture on Tianamp spin colom CB3. Then centrifuge for 30 seconds, 12,000 rpm. Discard the solution in the microtube column and place the spin colom in the collection tube.
- j. Added 500 ul of bufferGD to the spin colom. Then centrifuge 30 seconds, 12,000 rpm. Discard the solution in the microtube column and place the spin colom in the collection tube.
- k. Add 700 ul of PW buffer to the spin colom. Then centrifuge 30 seconds, 12,000 rpm. Discard the solution in the microtube column and place the spin colom in the collection tube.
- 1. Added 500 ul of PW buffer to the spin colom. Then centrifuge 30 seconds, 12,000 rpm. Discard the solution in the microtube column and place the spin colom in the collection tube.
- m. Centrifuge 2 minutes, 12,000 rpm, to dry the membrane.
- n. Place the CB3 column into a new 1.5 ml tube. Then add 50-200 ul of TE buffer at the centre of the membrane.
- o. Incubate at room temperature for 2-5 minutes. Then centrifuge 2 minutes, 12,000 rpm.
- p. Total DNA, then store at -20°C to -80°C.
- q. Running using TYR primers (R-reverst and forword).
- r. Prepare TYR (R) and (F) primer materials that have been diluted 1 ul per 1000 ul with ddH2O or aquabides in a 1.5 ml tube and homogenise.
- s. Prepare the stripcup.
- t. Add 3 ul of isolated sample.
- u. Add 10 ul of greenSYBR.
- v. Add 7 ul ddH2O.
- w. Add TYR (R) and (F) primers 1 ul each per sample, then homogenise with a spindown. Set the computer running to determine the results of DNA data. Wait approximately 1 to 2 hours until data is obtained.

Data analysis using unpaired t test.

The formula is as follows:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\left(\frac{\sum X_1^2 + \sum X_2^2}{n_1 + n_2}\right)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$
X₁ = male
X₂ = female
n₁ = number of male record
n₂ = number of female record

3. Results and Discussion

Body Length

Based on the results of the unpaired t test, it shows that the wing length of red-eyed and blackeyed Albino Lovebirds is not significantly different (P> 0.05). But there is a tendency that the wing length of red-eyed Albino Lovebird tends to be 3.79% higher than that of black-eyed Albino Lovebird in both males and females. (Figure 1).



Figure 1. Average wing length of albino lovebirds

The average wing length of male red-eyed Albino Lovebirds is 13.7 cm and that of female redeyed is 13.7 cm as well as the wing length of male black-eyed Albino Lovebirds is 13.2 cm and female black-eyed is 13.2 cm. Both in males and females, the wingspan of Albino Lovebirds is lower than the wingspan of Talaud parrots which ranges from 18 cm to 22 cm with an average of 20.41 cm [8]. However, when compared to the size of the wings on Nuri birds, Lovebirds are much shorter. According to Aeni [9] Nuri birds have a wing length of 15 cm and a tail length of 5.5 cm.

Chest Circumference

Based on the results of the unpaired t test, the average chest circumference of red-eyed and black-eyed Albino Lovebirds in males and females is not significantly different (P>0.05), with an average value of 6.5 cm. The same chest circumference between the two types of Lovebird is in the thickness of the meat attached to the breastbone. The sternum is a member of the body's regulatory skeleton where the existence of the bone is really useful as the most meat attachment area and is used as a marker that becomes the standard when measuring [10], [10a], [10b]11, 12.

Body Length

Based on the results of the unpaired t test, it shows that the body length of Red-eyed and Black-eyed Albino Lovebirds in males and females has no significant effect (P> 0.05). But from the average there is a tendency for male Red-eyed Albino Lovebirds to be 1.32% higher than black-eyed Albino Lovebirds. (Figure 2).



Figure 2. Body length of an Albino Lovebird

The results showed that the body length measurement of male Red-Eyed Albino Lovebird was 15.4 cm and the female was 15.2 cm. While the male Black-Eyed Albino is 15.2 cm and the female is the same at 15.2 cm. The body length of the Albino Lovebird is greater than the Malay Serindit bird according to the opinion of Riefani et al [13], the Malay Serindit bird is one of the bent-beaked birds with a small size as well as a single part of the genus Loriculus (Serindit) which occupies the island in Sumatra and Kalimantan having a body length of between 12 cm and weighing 28 grams.

DNA

Based on the results of the unpaired t test, it shows that the DNA of Red-eyed and Black-eyed Albino Lovebirds in both males and females has no significant effect (P> 0.05). But there is a tendency that the DNA of female Red-eyed Albino Lovebirds is 6.96% higher than Black-eyed Albino Lovebirds, while males tend to have 5.60% compared to females. (Figure 3).



Figure 2. Albino Lovebird DNA Average

The DNA primer used in this study is the TYR (Tyrosinase) gene, which shows the TYR content in red-eyed albino lovebirds in both males and females is higher at 5.60% to 6.96%, so it has a higher comparison with black-eyed lovebirds. The TYR gene is a gene that has a function as a patterning of body parts including eyes. Tyrosinase has a function in the manufacture of melanin, the enzyme can switch a protein (amino acid) called tyrosine to another compound known as dopaquinone. A series of additional chemical results can divert dopaquinone as melanin in the skin, hair follicles and fur [14]. This is also reinforced by [6] which states that protein plays a role in fur development as well as body shape which has an impact on appearance. Supported by the opinion of [7], [15], [16] tyrosine content has an influence on melanin production, anti-stress genes, and the immune system. With high melanin, the absorption of sunlight and vitamin D which is thought to cause efficiency in body metabolism is characterised by higher productivity in chickens and quails [6], [7].

4. Conclusions

Phenotypic variation (wing length, chest circumference, body length) is not significantly different between males and females but there is a tendency for the wing length of Red-eyed Albino Lovebirds to be 3.79% higher than that of Black-eyed Albino Lovebirds. DNA in Red-eyed Albino Lovebirds is not significantly different from Black-eyed Albino Lovebirds in both males and females but there is a tendency for higher red-eyed Lovebirds ranging from 5.60% to 6.96% than black-eyed Lovebirds.

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