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Sex Determination in Racing Pigeons (*Columba livia*) Molecularly Using Young Feather Samples

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Abstract. The racing pigeon (*Columba livia*) has an aesthetic value and is in high demand. Pigeon is monomorphic, difficult to distinguish between male and female individuals, especially at the age of offspring. Knowing the sex of birds from an early age is necessary to prepare male bird individual as racing bird. Accurate determination of the sex of racing pigeons from an early age was carried out to have scientific confirmation of the molecular method of determining the sex in racing pigeon. Under Lampung Disease Investigation Center program, molecular methods of PCR techniques using primers 2550F and 2718R were applied to determine the sex of bird based on the presence of Z and W chromosomes from young feathers of racing pigeons. Sampling young feathers was done in individual breeders in Kota Metro and East Lampung. Molecular analysis was carried out at the Biotechnology Laboratory of the Lampung Disease Investigation Center, consisted of extraction, amplification, electrophoresis, and data analysis. Fifteen young feather samples showed five male individuals with electrophoresis visualization formed one band measuring 600 bp and ten female individuals showed two bands measuring 600 bp and 400 bp.

Keywords: *Columba livia*, PCR, pigeons, sex, young feathers

INTRODUCTION

Indonesia has a variety of birds, including pigeons (*Columba livia*). Pigeons are seed-eating birds with a small head morphology and a large body measuring 32 cm that can be found active on the ground level or on the canopy (upper stratum of trees such as twigs, branches) (Wahyuni, 2021). Pigeons are divided into four types based on their maintenance purposes, racing pigeons, consumption pigeons, ornamental pigeons, and post pigeons. Racing pigeons are a species that is widely kept by the community (Aji *et al.*, 2015).

Pigeons used as racing birds are male individuals, trained from early ages. Nurkarimah (2019) stated that bird breeders visually distinguish the sexes through morphological characteristics. This method is considered ineffective as Columbidae birds are monomorphic, so it is quite difficult to determine the sex especially in early ages. Disastra (2021) stated that there was a mistake in the bird breeders in determining the sex of the Columbidae. An effective method is needed to determine the sex of birds as it is important to improve maintenance management, population development, suppression of breeding costs, and conservation efforts (Maciej *et al*., 2017).

The molecular method is considered to be able to provide accurate results and can be applied to various ages including young birds. Pigeons have only a small amount of feathers in the hatching stage, which is called young feathers. These young feathers will fall out as the pigeon grows and be replaced with new feathers (Sari *et al*., 2023). Under Lampung Disease Investigation Center program, determination of sex on pigeons using young feather with molecular techniques was conducted. The molecular technique used is Polymerase Chain Reaction (PCR) using specific primary markers 2550F and 2718R. The primer used encodes the CHD (Chromodomain Helicase DNA-binding) gene. Birds have a Z and W chromosome system. Male birds have ZZ chromosomes and females have ZW chromosomes (Pambuko *et al*., 2023). The results of this study are to scientifically confirm the method of determining sex in young pigeon molecularly.

RESEARCH METHODS

Sampling for young feathers was carried out in February – March 2025 in Kota Metro and East Lampung. Each young feather sample obtained was placed in a tube containing 1 mL of physiological NaCl solution and labeled. The collected samples are stored in the freezer to prevent DNA damage and transported to the Lampung Disease Investigation Center. In the

Laboratory of Biotechnology, samples are extracted using a Viral RNA/DNA Mini Kit in a biosafety cabinet based on the available built-in protocols. The extracted DNA was measured in quantity using Invitrogen™Purelink™Qubit™ the daDNABR Assay (consisting of dsDNA HS buffer and reagent) on the Qubit Fluorometer device. The next stage is the creation Qubit™Qubit™ of a Master mix which is carried out in the PCR work station by mixing four reagents, including 12.5 µl HS Red Mix, 1 µl forward primer, 1 µl reverse primer and 5.5 NFW in a 0.2 ml PCR tube. The primer used is a primer that is specific to the CHD gene (Table 1).

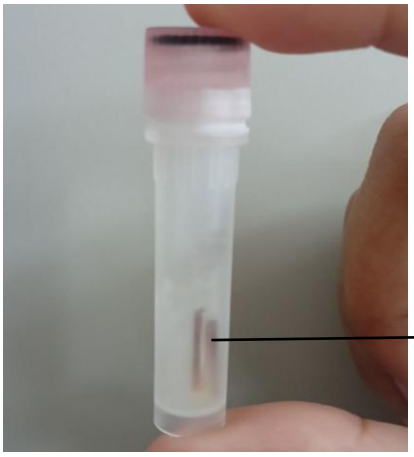
Table 1. DNA amplification primary sequence (Fridolfsson and Ellegren, 1999)

Primary	**Sequence (5'-3')**
2550F	GTT ACT GAT TCG TCT ACG AGA
2718R	ATT GAA ATG ATC CAG TGC ABOUT

The addition of the DNA template is carried out by adding 5 µl of extracted DNA to the prepared master mix. Running PCR was carried out using a thermos=cycler machine which was set with a pre-denaturation stage of 95°C for 5 minutes, 94°C for 30 seconds, 50°C for 45 seconds, 72°C for 1 minute for 35 cycles and a post-extension stage of 72°C for 5 minutes (Fitriana *et al*., 2023). The amplified DNA (amplicon) was proceeded electrophoresis using agarose gel. Amplicon is inserted as much as 6 µl in the gel drain. The electrophoresis process was carried out for 35 minutes with a voltage of 100 V and a strong current of 300 A. The results of electrophoresis were visualized under blue light and analyzed based on the visible band luminescence.

RESULTS AND DISCUSSION

Samples (N=15) were obtained in 2 sub-districts in Kota Metro and 5 villages in East Lampung, Lampung, placed in a tube containing 1 mL of physiological NaCl and labeled with a sample code (Figure 1).



Picture 1. Young feather samples

Confirmation of sex and age morphologically was obtained through interviews with each racing pigeon breeders. Some bird breeders were unsure with sex determination of racing pigeons at early ages. The age of the racing pigeons obtained ranges from 1 – 3 weeks, some of the breeders are unable to confirm the exact age of the racing pigeon hatchlings obtained (Table 2).

Table 2. Location of the sampling of young feathers of racing pigeons

No.	Sampling Location	Coordinate Points	Sample Code	Sample Type	Bird Age	Sex (EO)	Time
1.	Labuhan Ratu Induk, Labuhan Ratu, East Lampung	5° 6'50.38"S, 105°40'25.22"E	1	Young feather	3 weeks	♀	February 28, 2025
2.	Rajabasa Lama Induk, Labuhan Ratu, East Lampung	5° 6'22.50"S, 105°39'4.80"E	2	Young feather	3 weeks	♂	March 1, 2025
3.	Braja Asri, Way Jepara, East Lampung	5° 9'25.34"S, 105°42'26.83"E	3 4	Young feather Young feather	3 weeks	♀ ♀	March 12, 2025
4.	Labuhan Ratu 4, Labuhan Ratu, East Lampung	5° 9'47.90"S, 105°36'59.73"E	5	Young feather	2 weeks	♂/♀	March 13, 2025
5.	16 A, West Metro, Kota Metro	5°8'47.28"S, 105°17'16.68"E	6	Young feather	2 weeks	♀	March 14, 2025
6.	26 Plains, South Metro, Kota Metro	5° 9'43.15"S, 105°18'1.97"E and 5° 9'44.06"S, 105°18'1.81"E	8 9 12 13 14 15	Young feather Young feather Young feather Young feather Young feather Young feather	2 weeks	♂ ♂ ♂/♀ ♂/♀ ♂/♀ ♂/♀	March 14 and 16, 2025
7.	Raman Daya 4, North Raman, East Lampung	4°58'10.55"S, 105°27′41.77"E	7 10 11	Young feather Young feather Young feather	2 weeks	♂/♀ ♂/♀ ♂/♀ ♂/♀	March 15, 2025

Information: ♂ = male, ♀ = female, ♂/♀= uncertained between male and female individuals, EO= *Expert opinion*

The results of the extraction of young feathers' DNA were measured in concentration to determine the quantity of DNA obtained. The extraction DNA concentration was measured using a Qubit Fluorometer with ng/µl units (Table 3).

Table 3. Results of DNA concentration measurements of young feathers of racing pigeons

Sample Code	Concentration (ng/µl)
1	29,80
2	17,90
3	42,80
4	6,86
5	5,94
6	15,20
7	9,54
8	120,00
9	7,52
10	4,04
11	13,10
12	34,00
13	21,40
14	28,40
15	19,70

Electrophoresis was successfully performed, the success of amplification can be seen based on the presence of a band luminescence (Figure 2).

Picture 2. Visualization of the results of DNA electrophoresis of young pigeon feathers

Measurement of DNA quality using electrophoresis is one of the simple molecular methods. If the electrophoresis results show a clear band, it means that the DNA is of good quality (Triani, 2020) (Table 4).

Table 4. The visualization of young feather DNA electrophoresis

Sample Code	Result	
	Male (♂)	**Female (♀)**
1		+
2		+
3		+
4		+
5	+	
6		+
7	+	
8	+	
9	+	
10		+
11		+
12		+
13		+
14	+	
15		+
Total 15	**5**	**10**

Caption: + = detected as ♂ = male ♀ individual = female individual

Based on the results of the analysis in of 15 samples, 5 samples were male and 10 samples were female. Individuals with male sex were shown with sample codes 5,7,8, 9, and 14 with the formation of one band on the electrophoresis result with a size of 600 bp representing the ZZ chromosome and individuals with female sex were shown with sample codes 1,2,3,4,6,10,11,12,13, and 15 with the formation of two bands with a size of 600 bp and 400 bp representing the ZW chromosome. This gender confirmation is in accordance with the findings of Fridolfsson and Ellegren (1999).

Validation of the sex of the young pigeons is carried out by matching through information received from the expert opinion of the pigeon owner with the molecular test results that have been carried out. Expert opinion is the opinion of pigeon owners who are considered experts in knowing the bird sex based on morphological characteristics. The results of molecular determination of bird sex that have been carried out are stated to match the conjecture of

pigeon owners. However, in sample Code 2 there is a difference between the opinion of the pigeon owner and the results of the molecular test carried out. The owner of the pigeon suspected that sample Code 2 was male, but the results of molecular analysis yielded two bands measuring 600 and 400 bp indicating that the sample was a female individual (ZW chromosome).

The difference in the conjecture of the pigeon owner and the results of this molecular analysis may be due to the morphological characteristics of pigeons at the age of 1-3 weeks and the difference between male and female individuals is not clearly visible. Determination of sex in birds based on morphological characteristics is generally carried out at the age of 3-4 weeks. At the age of 3-4 weeks, young pigeon begin to learn to fly (Bachelor *et al.*, 2022).

Molecular determination of bird sex has various advantages. Molecular methods are effectively used to determine the sex of birds with monomorphic properties that are difficult to distinguish physically and can be applied to different ages of birds including the age of juveniles. Thus, the time required is faster with accurate examination results compared to conventional techniques that are carried out by waiting for birds to reach sexual maturity to be morphologically differentiated. Knowing the sex of birds early can improve the breeding process to get superior offspring and can increase the economic value or selling price of birds. The PCR technique of determining the sex of birds can also be used as a conservation effort, on the breeding process, especially for endangered bird species (Nugroho and Zein, 2015).

CONCLUSION

A total of 15 samples were obtained with 5 samples confirmed as male individuals and 10 samples confirmed as female individuals. Young feathers can be used as samples for sex confirmation using PCR methods.

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