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Sex Detection in Maleo Bird (*MacrocephalonMaleo*Sal.Muller 1846) Nurtured In Ex-Situ Conservation Through Body Morphological and Hormonal Studies

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ABSTRACT

Introduction: Maleo birds (*Macrocephalonmaleo*, SAL MULLER, 1846) are endemic animals of the island of Sulawesi which population continues to decline due to destruction and fragmentation of their habitat, as well as allegedly due to poaching and theft to the mothers and eggs.

Objective: This study aimed to determine the sex of maleo birds through morphological and hormonal studies.

Method: The study was conducted in the maleo bird conservation area of PT. Panca Amara Utama in Uso Village, Batui District, Banggai Regency, Central Sulawesi Province, from August 2016 to December 2018, by using 10 1-day-old maleo birds. Variables observed included cloaca morphology, macrocephalon and estrogen hormone concentration.

Results: The cloaca of Maleo birds has a difference. The difference is in the protrusion on the top of the cloaca. The male Maleo birds have a protrusion in the cloaca while the females do not have it. Organs in the form of protrusion in the cloaca are thought to be a sign of male sex. There are differences in shape and size between the male and female macrocephalon, the males have about 3.96 ± 011 cm width in average with rounded shape while the females have about 3.20 ± 0.10 cm width in average with oval shape. Female maleo birds have a higher concentration of the hormone estrogen than males. Based on the variables mentioned above, it can be concluded that the determination of maleo sex can be done based on macrocephalon size and cloaca shape.

Keywords: Sex, Maleo, Macrocephalon, Cloaca, Hormone

Conclusions: The cloaca morphology, macrocephalon size and homone estrogen concentration can be used to determine the maleo sex in the captivity.

I. Introduction

Maleo conservation activities have led to ex-situ conservation, namely through captivity. Through captivity, the growth, behavior and reproductive processes can be studied. The breeding process also aims to make maleo birds someday domesticated, there will be no extinction. The success of artificial hatching has given hope to the sustainability of maleo birds. The results of artificial hatching have been reported that the hatchability of maleo eggs reaching 67,5% (Tanari et al., 2008), 77% (Hafsah et al., 2008), 77,78%(Manik et al., 2012).however, if habitat destruction and hunting of maleo mothers are not prevented, the maleo population will decline sharply so getting them difficult to lay eggs. To ensure the survival of maleo birds, reproductive aspects, such as sex differences, are very important to study.

Differentiating maleo bird sex is an inseparable part in understanding the animal's reproduction. Maleo is an animal that has the same appearance (monomorphic) both male and female, so that its sex is difficult to distinguish. Maleo birds have the same body size, appearance and feather color in childhood, adolescence and adulthood. Differentiating the sex of animals can be done by taking into account the characteristics of the primary and secondary sex. Primary male sex characteristics are characterized by sperm production in males and ovum production in females, while secondary sex characteristics are from the morphology, morphometry of the body's growth and development of the animal. There have been many sexing methods developed, such as molecular techniques and morphometric analysis (Cerit and Avanus, 2007; Dubiec and Zagalska-Neubauer, 2006; Grant, 2001; Griffiths, 2000; Khaerunnisa et al., 2013; Kocijan et al., 2011; Nugroho and Zein, 2015). However, each of these methods has shortcomings both in terms of time and cost.

Sexing through body morphology combined with hormonal in maleo birds is a method that requires quite good observation accuracy but the results can be used forever without the need for the same observation process. This study aimed to determine the sex of maleo birds through morphological and hormonal studies.

II. Method

Location and Time of Research

The study was conducted in the maleo bird conservation area of PT. Panca Amara Utama, in Uso Village, Batui District, Banggai Regency, Central Sulawesi Province. The study was conducted in January 2017 - December 2018. The faecal analysis of maleo birds was conducted at the Laboratory of Reproduction and Rehabilitation Unit of the Faculty of Veterinary Medicine, Bogor Agricultural University

Research Procedures

Selection of Maleo

There were 10 1-year-old maleo birds used in this study, kept in cages measuring 8 x 8 meters. Each bird is given an identification to distinguish between male and female. The cage is equipped with seed and water feeders.

Fecal Collection

At the age of 16 months, the fecal collection was carried out to analyze maleo's reproductive hormones. Each plot consisting of male and female maleo was separated by making a separator in the cage made of paranet, so that the feces of each maleo could be collected properly. The fecal collection was conducted for two days in a row, so that there were 2 (two) fecal samples collected from each maleo with a total 20 samples from all maleo birds. Fecal collection was conducted in the morning by collecting using plastic spoon and putting the samples into a sample bottle as well as giving each sample a code, then, the bottles were stored in a refrigerator at -40^{0}C. After collecting all maleo fecal samples, the samples were then taken to the laboratory to be analyzed.

Fecal Analysis Procedures

Before analyzing the fecal samples, the samples were firstly dried and mashed using a *freeze dryer*according to the procedures stated by (Heistermann et al., 1993). The fecal powder that has been crushed was taken as much as 0.5 grams and then extracted with 2 ml of methanol in H_2O then shaken using vortex for 10 minutes and centrifuged at 2200 g for minutes then the supernatant is collected and stored at -20^0C. Estrogen and progesterone hormone analyses were conducted using the ELISA kit commercial hormone assay of DRG instruments GmbH Germany (estradiol and progesterone).

Observation on Body Morphology of Maleo Birds

To determine the morphology of the cloaca, the cloaca of 1-day-old maleo was observed. Observation of macrocephalon size was carried out on maleo aged 16, and 24 months old.

Research Variables

There were some variables observed in this study, namely cloaca morphology, macrocephalon height and width, and estrogen hormone concentration

Data Analysis

The data and information collected were analyzed descriptively and quantitatively, and were then presented in the form of regression charts and histograms

Results and Discussion

Maleo is a species of bird that is monomorphic, which has a similar morphological character between males and females. In general, based on the color of feathers, the morphology of the body cannot be distinguished between males and females. Even the dimorphic species have only few observable differences.

The sexing on maleo birds conducted in several research shows different results. The sex of maleo birds can be distinguished by the color of the beak. The male beak is black with red lines while the female beak is black with yellow lines. However, the results of the study (Tanari et al., 2008) showed that the maleo with black beak in yellow stripe turned out male after getting dissected (Saerang, 2010). The nine parameter sizes of *Swinhoe's Storm Petrels*cannotbe used for sex determination even though the color shows that female birds have a color reflection on the feathers in the higher abdomen, and in the crown, the reflection of the color is higher in males stating that. (Choi et al., 2011).

Cloaca Morphology

Cloaca is a posterior orifice that serves as the only opening for the digestive and urinary tracts as well as a copulatory organ. The results of observations on the cloaca of maleo birds showed that, morphologically, the cloaca has differences as shown in Figure 1. The difference is in the protrusion on the top of the cloaca. The male Maleo birds have a protrusion in the cloaca while the females do not have it. Organs in the form of protrusion in the cloaca are thought to be a sign of male sex and it remains until the maleo reaches adulthood while the cloaca which has no protrusion is a sperm-receiving organ like a vagina in a mammal. The copulatory organ in males is generally in the form of small protrusion that can be seen since the age of 1 day in chickens (Biederman and Shiffrar, 1987). The determination of sex in poultry through *vent sexing* (in the form of cloaca) has been used in Japan by Professor Kiyoshi with an accuracy of 95% by opening the DOC cloaca to see the presence or absence of protrusions, which are male reproductive organs that do not develop (rudimentary) (Cerit and Avanus, 2007).

Figure 1.Cloaca Morphology of Male and Female Birds

Macrocephalon Morphology

Macrocepaholon is an organ that has the last growth at the age of 12 months and continues to grow until the age of 3 years. Based on observations on maleo bird's body morphology, especially for macrocephalon, there are differences in the shape and size of male and female macrocephalon (figure 2). The males have about 3.96 ± 011 cm width in average with rounded shape while the females have about 3.20 ± 0.10 cm width in average with oval shape. The method of determining sex in birds can also be done based on body morphology tubuh(Dubiec and Zagalska-Neubauer, 2006).

Figure 2.Macrocephalonof Male and Female Maleo

To prove that the morphometry of maleo birds including macrocepalon size and cloaca shape that can be used to differentiate the sex of maleo, there was a surgery performed to a female maleo bird. Based on the results of surgery, the maleo which has a macrocephalon size of 3 cm and the shape of the cloaca split symmetrically was identified as female, as evidenced by the presence of yolk that has grown perfectly. The difficulty found determining sex through surgery was the status of maleo bird as protected endemic animal, so that the surgery was very limited.

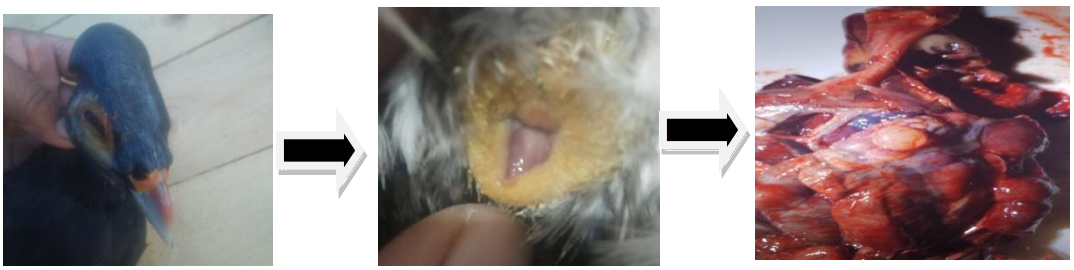

Figure3.Results of Surgery on Maleo Bird to Prove the Sexing

Estrogen Hormone Concentration

The measurement of reproductive hormone concentration can be used as a guide to determine the sex of wild animals that do not show a clear sex difference. The results of the analysis of faecal samples on 10 maleo bird showed that the concentration of the hormone estrogen was ranging between 189,8 – 1531.05 ng/gr. There were 3 Maleo birds having estrogen concentration ranging between 1364,90 – 1531,05 ng/gr which could be identified as female since the females have higher concentration of estrogen hormone than males. 7 male maleo birds have a low estrogen concentration ranging between 189,8 – 961,4 ng/gr.

Table 1.Estrogen Hormone Concentration (ng/gr) and Macrocephalon Width ofMaleoBirds (n= 10)

Code	Hormone Concentration (ng/gr)		Macrocephalon Width (cm)	
	Male	Female	Male	Female
M 1	189.8	-	3.8	-
M 2	206.1	-	4.0	-
M 3	296.8	-	4.1	-
M 4	-	1531.05	-	3.3
M 5	584.5		3.8	-
M 6	-	1464.25	-	3.2
M 7	961.4	-	4.0	-
M 8	-	1364.90	-	3.1
M 9	617.6	-	4.0	-
M 10	287.1	-	4.0	-
Average	449.0	1453.4	3.96 ± 011	3.20 ± 0.10

There is a relationship between the concentration of estrogen hormone and the shape and width of the macrocephalon to determine sex. Maleo bird with low estrogen concentration of ± 449.0 ng/gr has a large rounded shape and average width of macrocephalon of 3.96 ± 0.11 cm, while maleo with high estrogen concentration of ± 1453.4 ng/gr has a width of 3.20 ± 0.10 cm with oval shape.

The determination of sex in short-beaked echidna was conducted by comparing the estradiol hormone and androgen. Estradiol hormone concentrations in males have higher androgen concentrations than females, while female short-beaked echidna have much higher estradiol versus androgen ratios than males (Oates et al., 2002). The fecal samples of females have higher concentrations of estrogen compared to males (Cerit and Avanus, 2007).

Most aves are monomorphic organisms (Dubiec and Zagalska-Neubauer, 2006). The obstacle that is often found in determining the sex of birds is the number of monomorphic bird species, that is, they have similar morphological characteristics between males and females, even dimorphic species have little difference. Sex identification is based on morphological differences such as body size and color of feathers, but due to the diverse morphological characteristics of birds due to geographical differences and differences between species of birds, this method faces many obstacles (Cerit and Avanus, 2007). Maleo birds in captivity have morphological characteristics such as the same feather color (black) with white mixed with orange chest and orange beak, so it cannot be used to distinguish male and female sex.

III. Conclusions

Based on observations on body size there were only cloaca morphology and macrocephalon size could be used to determine the sex of maleo birds in captivity. The concentration of estrogen hormone can also be used to determine the sex of maleo birds.

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References

[1.] Biederman, I., Shiffrar, M.M., 1987. Sexing day-old chicks: A case study and expert systems analysis of a difficult perceptual-learning task. J. Exp. Psychol. Learn. Mem. Cogn. 13, 640–645. https://doi.org/10.1037/0278-7393.13.4.640

[2.] Cerit, H., Avanus, K., 2007. Sex identification in avian species using DNA typing methods. Worlds Poult. Sci. J. 63, 91–100. https://doi.org/10.1017/S0043933907001316

[3.] Choi, C.-Y., Nam, H.-Y., Park, J.-G., Lee, K.-G., 2011. Swinhoe's Storm Petrels (Oceanodroma monohris) Show No Apparent Sexual Dimorphism in Size and Color. Ornithol. Sci. 10, 145–149. https://doi.org/10.2326/osj.10.145

[4.] Dubiec, A., Zagalska-Neubauer, M., 2006. Molecular techniques for sex identification in birds. Biol. Lett 43, 1–12.

[5.] Grant, A., 2001. DNA sexing of brown kiwi (Apteryx mantelli) from feather samples, DOC Science Internal Series. ed, 13. Department of Conservation, Wellington.

[6.] Griffiths, R., 2000. Sex Identification Using Dna Markers, in: Molecular Methods In Ecology (Backer A. J., Ed.). Blackwell Science, London, pp. 295–321.

[7.] Hafsah, Yuwanta, T., Kustono, Djuwantoko, 2008. KARAKTERISTIK HABITAT MIKRO SEBAGAI DASAR POLA PENETASAN TELUR MALEO DI TAMAN NASIONAL LORE LINDU SULAWESI TENGAH. J Agroland 15, 223–228.

[8.] Heistermann, M., Tari, S., Hodges, J.K., 1993. Measurement of faecal steroids for monitoring ovarian function in New World primates, Callitrichidae. Reproduction 99, 243–251. https://doi.org/10.1530/jrf.0.0990243

[9.] Khaerunnisa, I., Sari, E., Ulfah, M., Jakaria, J., Sumantri, C., 2013. Avian Sex Determination Based on Chromo Helicase DNA-binding (CHD) Genes Using Polymerase Chain Reaction (PCR). Media Peternak. 36, 85–90. https://doi.org/10.5398/medpet.2013.36.2.85

[10.] Kocijan, I., Dolenec, P., Tanja, S., D. Nenadic, D., Pavokocik, G., Dolenec, Z., 2011. Sex-typing bird species with little or no sexual dimorphism: an evaluation of molecular and morphological sexing. J. Biol. Res.-Thessalon. 15, 145–150.

[11.] Manik, H., Yuwanta, T., (Kustono), K., 2012. MODIFIKASI PENETASAN TELUR BURUNG MALEO GUNUNG (Aepypodius arfakianus) UNTUK MENINGKATKAN DAYA TETAS. Bul. Peternak. 35, 24. https://doi.org/10.21059/buletinpeternak.v35i1.587

[12.] Nugroho, H.A., Zein, Moch.S.A., 2015. EVALUASI METODE PENENTUAN JENIS KELAMIN PADA NURI KEPALA HITAM (Lorius lory, Linnaeus 1758). Zoo Indones. 24, 24–29.

[13.] Oates, J., Bradshaw, D., Bradshaw, F., Lonsdale, R.A., 2002. Sex identification and evidence of gonadal activity in the short-beaked echidna (Tachyglossus aculeatus) (Monotremata: Tachyglossidae): Noninvasive analysis of faecal sex steroids. Aust. J. Zool. 50, 395–406.

[14.] Saerang, J.L.P., 2010. KAJIAN BIOLOGIS MALEO (Macrochepalon maleo) YANG DIPELIHARA SECARA EX SITU (Disertasi). Institut Pertanian Bogor, Bogor.

[15.] Tanari, M., Rusiyantono, Y., Hafsah, 2008. TEKNOLOGI PENETASAN TELUR BURUNG MALEO (Macrocephalon maleo Sal. Muller 1846) SEBAGAI UPAYA KONSERVASI. J Agroland 15, 336–342.