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Breeding of *Philemon buceroides* by Synchronization of Pregnant Mare Serum Gonadotropine (PMSG) Reproductive Hormone and Estrogen as Conservation for Preventing Its Extinction

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Abstract: The research aims to figure out how to breed *Philemon buceroides* in captivity and make recommendations for conservation activities in-situ and ex-situ. *Philemon buceroides* are bred by giving the Pregnant Mare Serum Godadotrophin (PMSG) and Estrogen hormones and artificial insemination to birds who are sexually mature and physically healthy at least one year old. The birds in this study were *Philemon buceroides* that had been reared for at least a year to lessen stress. According to the researchers, the *Philemon buceroides* sample was acclimatized for 7 days in each birdcage. The treatment group was superovulated the next day to produce more eggs than normal, using PMSG hormone at a dose of 150 I μ in 10 milliliters of ferologic NaCl / Kg BW once a day for three days. Only 10 milliliters of ferologic NaCl were given orally to the control group. The following day, lust/Estrus synchronization was performed utilizing the Estrogen hormone at a dose of 150 I μ in 10 milliliters of ferologic NaCl orally once a day for three days. Sperm was taken from male *Philemon buceroides*. As a result, synchronizing the reproductive hormones Pregnant Mare Serum Gonadotrophin (PMSG) and Estrogen in male and female *Philemon buceroides* considerably influenced the increase in ovulation, however the eggs generated by the mother *Philemon buceroides* have yet to produce chicks. Furthermore, obtaining male sperm is challenging. Just one sperm pulled out one drop from the three guys, while the other two did not.

Keywords: Conservation, Breeding, *Philemon buceroides*

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Introduction

The koa¹kao (*Philemon buceroides*) is a protected bird species based on the Republic of Indonesia Law No. 5 of 1990 and the Decree of the Minister of Forestry No. 301/Kpts-II/1991. Protection of the bird was carried out because of its limited distribution, high economic value, important ecological role, very little information on its biological characteristics, its population is almost extinct, and it is difficult to breed

in captivity (Prana et al., 1997., Yamin & Khairuddin, 2018).

On the islands of Lombok and Sumbawa, the population of *Philemon buceroides* is thought to be nearly extinct (Yamin, 2018). According to the report of the Sub-Balai for Natural Resources Conservation of West Nusa Tenggara (1995), uncontrolled hunting has destroyed the natural populations of several bird species on the islands of Lombok and Sumbawa, which have economic value. In West Nusa Tenggara,

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Philemon buceroides is a bird with high economic value, because it is favored by many people to be kept in cages. The price is hundreds of thousands to millions of rupiah per head. It is not surprising that hunting for this bird species has increased, thus triggering a drastic decline in its population. If this action is allowed and there are no studies for its breeding and conservation efforts, then Philemon buceroides will become extinct in the not-too-distant future (Iswandi et al., 2017).

The threat of extinction for Philemon buceroides is getting more significant because it is a bird that is difficult to breed in captivity. His captive efforts have so far been unsuccessful. It was reported that Philemon buceroides is one of six bird species that are very difficult to breed in captivity. The bird has only reproduced once in nine years at the Tapan Mini Indonesia Indah bird sanctuary. The failure of breeding efforts can be caused by various factors, including incompatibility of the content and chemical composition of the nutritional value of food in captivity with natural habitats, nesting materials, cage size that is not sufficient to perform certain behaviors before and during mating. It's like Indian horses who have to run all day before mating (Suana et al., 2002).

For successful captive breeding of Philemon buceroides, which is becoming increasingly scarce, various biological aspects are needed, especially: behavioral aspects, use of resources, nests, content and chemical composition of the nutritional value of food, reproductive biology in natural habitats, and conservation efforts. These factors greatly affect vitality, body resistance, and survival. With the background as described above, this research specifically studies breeding efforts by synchronizing the hormones Pregnant Mare Serum Gonadotrophin (PMSG) and Estrogen Hormones on the reproduction of Philemon buceroides in captivity (Yamin, 2021). The results are expected to be useful for: Managers as input and basic data for determining management policies and conservation efforts for Philemon buceroides both in-situ and ex-situ. Communities, the success of conserving biological resources, especially bird fauna, ensures the continuity of providing the needed resources, increasing income, and improving the welfare of the community, especially those around the area, both from tourist visits and direct use of Philemon buceroides as a source of protein or from its sales.

Method

This research is the third stage; the first stage is the identification of the type of food; the second analysis of the nutritional value of food; the third, breeding experiments of Philemon buceroides. Identification and analysis of the nutritional value of food Philemon buceroides have been carried out at the Laboratory of

Taxonomy and Analytical Chemistry, University of Mataram. Breeding experiments with hormone synchronization and artificial insemination were carried out in the Gunungsari birdcage, West Lombok. The third stage is carried out from March to October.

The breeding efforts of the birds were carried out by administering the hormone Pregnant Mare Serum Godadotrophin (PMSG) and the hormone Estrogen and artificial insemination to birds that are sexually mature at least one year old and physically healthy. The experiment used 10 mother birds, 5 males and 5 females each. Philemon buceroides, which were sampled in this study, were prioritized from fan-owned birds kept for at least 1 year to reduce stress.

According to their group, Philemon buceroides sampled were acclimatized for 7 days in their respective cages. The next day, the treatment group was superovulated to obtain more eggs than normal by using PMSG hormone at a dose of 150 μ in 10 milliliters of ferological NaCl/Kg BW solution once a day for three consecutive days. The control group was only given 10 milliliters of ferologic NaCl solution orally. The next day, the synchronization of estrus/estrus was carried out using the hormone Estrogen at a dose of 150 μ in 10 milliliters of ferological NaCl solution orally once a day for three consecutive days.

Synchronization of lust was carried out on all groups of birds, both male and female. Then the male and female birds are mated. Each cage is filled with one pair of Philemon buceroides (male and female). After the eggs are produced and incubated by the mother, the male birds are separated. If the results are obtained (Philemon buceroides chicks), then the chicks are nurtured to be released back into their natural habitat in nature.

Result and Discussion

Efforts to breed Philemon buceroides in this research activity include 5 (five) activities, namely the provision of cages or cages, food sources, playgrounds, nesting materials, and matchmaking.

Provision of Breeding Cages

Breeding cages are cages where Philemon buceroides mate. In this study, Philemon buceroides was carried out with independent partners, namely the ten birds, each 5 females and 5 males, who were given the freedom to choose their mate. In this study, the breeding cage for Philemon buceroides was 4 meters x 15 meters x 5.5 meters in a coconut plantation area. The cage is equipped with plants and trees that are favored in the bird's natural habitat, such as: mango (*Mangifera indica*), jackfruit (*Arthocarpus integra*), sweet star fruit (*Averhoa carambola*), water guava (*Eugenia sp*), Turi

(*Sesbania grandiflora*), papaya (*Carica papaya*), banana (*Musa paradica*), orange (*Citrus sp*), sweet potato (*Manihot utilissima*), boar (*Thladiantha punctata*) and winged bean (*Psophocarpus tetragonolobus*). The size of the cage is 5.5 meters high, 4 meters wide and 15 meters long. In addition, there are also various vines and grass in the cage that are useful for nesting materials. Vegetation is the most important element, especially in bird habitat, because it functions as a source of food, protection, and breeding place. In addition to the elements of vegetation, the cage is also equipped with a water source for drinking and bathing (Alikodra, 1997; Sulandart & Zein, 2012).

Procurement of Parent Candidates for Philemon buceroides

Table 1. Initial Physical Condition of Parent Koak kao (*Philemon buceroides*) in captivity

Sample/Parent	Origin	Age (Month)	Sex	Weight (Gram)	Cage captivity (month)
1	Sumbawa	24	Female F	345	12
2	West Lombok	18	Female F	315	8
3	West Lombok	26	Male M	370	12
4	Central Lombok	24	Male M	355	16
5	Central Lombok	24	Male M	340	10
6	Lobar	9	Female F	298	8
7	Bima	26	Female F	356	10
8	Sumbawa	48	Male M	367	36
9	West Lombok	60	Female F	363	48
10	West Lombok	24	Male M	340	18
Everage		28.3		345	17.8

The Table 1, shows that the age, length of maintenance, owner status, health level, and origin of each bird are not the same. The selection of prospective broodstock should be carried out, because it will affect the desire to marry, find a mate, and the ability to reproduce. However, this cannot be done because of the limited availability of the bird (*Philemon buceroides*) in the market and in the community. A good candidate for parent *Philemon buceroides* has the following characteristics. The feathers of the entire body look clean, because dull feathers indicate an unhealthy bird. Sharp eyes, sturdy body, fresh, and energetic. The koak kao, whose droopy eyes look lazy, shows that the bird is not healthy. Parent age ranges from 2 (two) to 3 (three) years. The koak kao, which is more than five years old, has a decreased desire to mate (desire), as well as the koak kao, which is less than two years old, has not yet fully developed sex cells (Jepson & Ladle, 2005; Sulandart & Zein, 2012).

Feeding

Philemon buceroides is a honey-sucking bird, which is a group of honeysucking birds from the Meliphagidae tribe. Birds are nectivores, frugivores, and insectivores because their diet consists of nectar, fruits from certain plant species, and various insects. *Philemon buceroides* differs from other members of the Meliphagidae tribe in that it requires honey and nectar

Philemon buceroides is one of the rare bird species. Its existence in natural habitats, communities, and bird markets is very rare. Initially, the prospective broodstock was planned as a sample of 5 pairs (10 individuals) in this study. Of these, there were five female parents and five male parents. The description of each prospective parent obtained as a sample of this study can be seen in Table 1.

every day throughout its life. The bird sucks nectar only occasionally, especially before the breeding season. The types of plants whose flower nectar is favored by *Philemon buceroides* are, *Sterculia oblongata*, *Thespesia populnea*, *Eugenia sp.*, *Cocos nucifera*, *Ceiba petandra*, *Bombax buonopozense*. and *Bombax. ceiba*. The preferred fruits are berries, *Saripellus asper*, *Thladiantha punctata*, *Ficus septica* and *Ficus superba*. Preferred insects are *Cryptotympanus accuta*, *Oecophylla samaradigna*, *Kampi neurobasis*, *Hymenoptera species*, *Petanga sp.*, and *Lepidoptera P. buceroides* feed twice a day, in the morning from 06.00 to 10.00 in the afternoon from 15 to 18.30. They are often paired or solitary in their activities, rarely found in large groups (Yamin, 2021).



Figure 1. *P. buceroides* feed plants in captivity

In this study, the feeding of the birds in the experimental breeding cage was adjusted to the type of food mentioned above, namely food in the form of fruits and insects. The fruits given are papaya (*Carica papaya*), banana (*Musa paradica*), and boar (*Thladiantha punctata*) alternately. Fresh flowers were given after PMSG hormone was administered to fulfill *P. buceroides* nectar. In addition, there are several types of nectar-producing plants that are flowering and fruiting in the cage. such as: Turi/flower koak kao (*Sesbania grandiflora*), sweet star fruit (*Averhoa carambola*), guava (*Eugenia sp*), Turi papaya (*Carica papaya*), banana (*Musa paradica*), boar (*Thladiantha punctata*), and winged winged (*Psophocarpus tetragonolobus*).

As for the fulfillment of insects, insects purchased from the market are given, such as: crickets, Hong Kong caterpillars, and banana caterpillars once a week. In addition, the attack can be obtained/caught yourself from insects that enter the captive cage. As for maintaining the health and vitality of *Philemon buceroides*, vitamin B complex is given through drinking water obtained from the market once every two weeks.

Nest Preparation

Philemon buceroides nests in Natural Habitats were found on *Alstonia spectabilis*, *Alstonia*



Figure 2. Nest preparation of *Philemon buceroides* in breeding cages

The nest is made in a round cage with 25 cm x 35 cm from liana material, grass stems, banana stem fibers, and coconut fiber. Then the nest is placed in the upper corner of the cage and tree branches found in the cage as in its natural habitat. Of the 5 nests provided, only 3 were used to lay eggs. Each parent uses the same nest in each spawning period (until now it has been 4 periods), does not want to move to another nest. The nest used is a nest placed in the upper corner of the cage. This is because the bird in nature makes a nest at

angisteloba, and *Bombax buonopozense* trees. At a height of between 25 meters to 35 meters from ground level. The nest is placed in a sub-canopy of trees whose tops do not blend with the canopy of other trees (Yamin, 2018; Yamin, 2021).

The nest material for *Philemon buceroides* is about 90% made of woven lianas with an average diameter of 0.30 mm and a length of between 15 cm and 100 cm. In addition, the nest material is the roots of tree nails, fibers, tree moss, and some twigs with a diameter of 0.20 mm - 0.40 mm. Seeing the type of nest material, it is certain that the bird never took it from the ground [10]. The nest is oval in shape, 13 cm high, 12 cm wide, hole diameter 10 cm, hole depth 9 cm, and wall thickness between 2.50 cm to 5 cm. Weight (mass) between 314 grams to 330 grams, hanging (tied) with 11 to 17 lianas stems on 5 to 7 twigs with a diameter of 5.45 cm to 23.26 cm.

The Koak Kao's nest in this study was originally provided from its nest taken from its natural habitat. Still, after being placed in the cage the nest was dismantled and the *P. buceroides* attempted to rearrange it. The rearrangement of the nest from the natural habitat by the bird was unsuccessful because the material was stiff, unable to be tied to twigs or perch wood in the cage. To meet the bird's need for a nest, the researchers made modifications to the nest's material, structure, and shape as shown in Figure 2.

the top of a tree canopy whose canopy does not merge with the canopy of other trees (Yamin, 2018; Iswandi et al., 2017; Yamin, 2021). Another thing to note is that the bird completes its nest material from various materials found in the cage such as banana leaves, wool thread, and burlap sacks.

Matching Koak kao (*Philemon buceroides*)

After giving hormones to both mothers (male and female) koak kao birds bred in this study, then each

mother bird was mated with a permanent partner, namely male and female mothers were put into one cage. The first marriage was on March 20, 2005. Of the five couples (ten tails) none of them wanted to marry that way (permanent couples). They were reluctant to marry, quarreled and fought all day.

To overcome this, then the coca kao bird is mated freely, that is, the two types of parent birds are given the freedom to choose their life partner. First, the parent koak kao (male and female) were each placed in

a cage measuring 60 cm x 60 cm x 90 cm and placed nearby for one week (March 21 - 27, 2005), then placed in a cage measuring 4 meters x 15 meters x 5.5 meters to choose your own partner. Koak kao that was put in and released in the cage for a week did not seem to be strong enough to fly, reluctant to use the trees found in the cage, depending on the walls and roof of the cage. The description of each parent bird in the cage is as follows in Table 2.

Table 2. A depiction of a mother koak bird mating freely in a captivity cage

Sample	I	II	III	IV	V	VI	VII	VIII	IX	X
Sex	F	F	M	M	M	M	F	M	F	F
Age	24	18	26	24	24	9	26	48	60	60
Captivity Period	12	8	12	16	10	8	10	36	48	48
Mating	March			June	-	-	Mei	-	Sep	Sep
				March						
				June						
Nesting	March	June	-	-	-	-	June	-	-	-
Egg	6,7,8,9	8,9	-	-	-	-	7,8,9	-	-	-
Σegg/period	2,2,2,1,2	2,2	-	-	-	-	2,2,2	-	-	-
Incubation	13- 21	14	-	-	-	-	15	-	-	-

Of the five pairs of *Philemon buceroides* birds that were put into the breeding cage, in early April 2005 three females were seen looking for and lifting nesting materials until early June 2005. On 16 and 17 June 2005 one female laid eggs, followed by another female on July 3, and August 15, 2005. Of the 5 females, 3 had successfully laid eggs, four, six, and eight times (period). The number of eggs each period is 2 eggs except in the 3rd period, one egg. Incubation of eggs in the first period by each parent is between 14–15 days, the second period is between 19 and 21 days, the third and fourth periods are 17 days. The incubated eggs do not yet contain emberio (zygote) and produce piyek (children), because they are not fertilized. The eggs produced are oval with a length of 28 mm to 33.5 mm, a weight of 5.35 grams to 7.82 grams, a diameter of 22.00 mm to 24.3 mm, and a shell thickness of 0.5 mm shown in Figure 3.



Figure 3. *Philemon buceroides* eggs are produced by synchronization of PMSG and estrogen hormones.

The remaining two female parents have not laid eggs, but they have started looking for and lifting nest materials. From the four males, it was seen that two were still not strong enough to fly, presumably because they had been kept in a cage measuring 60 cm x 60 cm x 90 cm, they were too old, about seven years, while the others were too young, about eight months, and poor nutrition. The failure of the *Philemon buceroides* breeding efforts in this study was thought to be due to 2 (two) things, namely: (1) fertilization of the egg by sperm cannot yet occur, because ejaculation occurs outside the female parent's body during copulation. It is presumably because the trees are too close together and the captive cage is not high enough (5.5 meters). What is worth pointing out here is that the bird copulates in the air while flying. In connection with this, to fertilize female sex cells by male sex cells to occur naturally, there must be sufficient space (height and length) to carry out copulation movements. In nature, the bird copulates by flying more than 15 meters high (Yamin, 2021); (2) Disruption of incubation by the parent. As mentioned above, all of the male and female *Philemon buceroides* bred in this study were placed in a cage measuring 4 meters x 15 meters x 5.5 meters together, so that brooders who were incubating often received interference from the broodstock. other. The incubating parent of *Philemon buceroides* is seen frequently leaving the eggs in the nest because it is disturbed by the non-incubating mother bird, so that the incubated eggs are thought to have not received optimal incubation. As a result, the incubated eggs cannot produce *Philemon buceroides* chicks.

Conclusion

From the breeding efforts and the discussion above, it can be concluded that the synchronization of the reproductive hormones of Pregnant Mare Serum Gonadotrophin (PMSG) and Estrogen to male and female parents of *Philemon buceroides* greatly affects the increase in ovulation of *Philemon buceroides*. The eggs produced by the parent *Philemon buceroides* in this study did not produce chicks. Its presumably because the eggs were not fertilized due to the limited space for the parent *Philemon buceroides* to perform the necessary movements in mating. The disturbance to the parent *Philemon buceroides* which was incubating by the partner. Another *Philemon buceroides* in the breeding cage. In this connection and considering the existence of *Philemon buceroides* in nature and society which is increasingly rare and difficult to breed, efforts to breed and conserve these birds need to be given attention and continued so that we do not lose the unique fauna of the area.

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