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Research Article

Reproductive Performance of Asian Catfish (*Hemibagrus wyckii*, Bleeker, 1858)-Preliminary Study

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Abstract

Background and Objective: Feed level is an important factor that affects oocyte maturation of female *Hemibagrus wyckii* broodstock. The aim of the study was to investigate the effect of different feed levels (700, 1,400 and 2,100 g *lokan* meat per day) on the reproductive performance of female broodfish (initial weight: 2.800 ± 296 g and initial length: 353.2 ± 55.5 mm). **Materials and Methods:** Four experimental and three replicate ponds were used to evaluate the reproductive performance of female broodfish. Broodfish fed formulated feed was used as a control. Five female broodfish were cultured in each pond. Female broodfish were fed *lokan* meat once a day at 17.00 pm during 120 days of the experiments. **Results:** The nutritional content of *lokan* meat (wet base) were 7.08% crude protein, 0.82% crude lipid, 2.44% carbohydrate, 0.29% crude ash and 89.37% moisture content. Feed levels significantly ($p < 0.05$) affected final weight, oocyte maturation, egg size, percentage of ovulation, number of egg per spawn, percentage of egg weight and embryo survival. **Conclusion:** Feed with 2,100 g *lokan* meat per day provided the best result for reproductive performance of female *Hemibagrus wyckii* broodfish.

Key words: Asian catfish, endangered species, cultured stock, lokan meat, reproduction performance

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hemibagrus wyckii, Bagridae (local name is geso), is a carnivorous freshwater finfish native to Indonesia¹⁻³. This species is found in the Riau Province and lives in the Kampar Kiri river⁴, Koto Panjang reservoir⁵ and Kampar Kanan river^{3,6}. Each broodstock can reach a weight of approximately 3,296 g⁷. Due to the high demand and price in the market (13.33 USD kg BW⁻¹), the natural stocks of this species have been overexploited^{3,6}. *Hemibagrus wyckii* has been categorized as "least concern" by the International Union Conservation Nature (IUCN) which lists threatened taxa. However, according to Aryani *et al.*⁷, *Hemibagrus wyckii* in the Kampar Kanan River was categorized as "vulnerable to endangered" due to environmental degradation and overexploitation. Aryani *et al.*⁸ reported that *Hemibagrus wyckii* has a low fecundity with a value of 3,058 ± 144 eggs kg BW⁻¹. However, other studies have found that *Hemibagrus nemurus* has a higher fecundity of 35,950 ± 963 eggs kg BW⁻¹⁹.

Due to the decreasing population of *Hemibagrus wyckii*, it is necessary to culture this species as a source food fish base. Therefore, this species needs to be domesticated. According to Chong *et al.*¹⁰ and Kanyilmaz,¹¹ commercial and formulated feed information is needed for successful domestication. Fresh feed from freshwater seashell (*Pilsbryoconcha exilis*, Unionidae), local name "*lokan*," may be used as feed for *Hemibagrus wyckii* broodfish, since it has been used by local fisherman as a bait to capture *Hemibagrus wyckii* in the Kampar Kanan River. *Lokan* live in the Sungai Paku reservoir in the Kampar Regency in Indonesia. The meat from *lokan* is not utilized as a source of food for local people. *Lokan* is easy to find, has high protein and is valuable for oocyte maturation of broodfish. Understanding nutritional requirements for broodstock are important since it supports biological mechanisms, including oocyte maturation^{12,13}.

Formulated feed for oocyte maturation of broodstock has been evaluated in several species, including *Xiphophorus helleri*¹⁰, *Cherax quadricarinatus*¹⁴, *Ictalurus punctatus*¹⁵, *Rhamdia quelen*¹², *Hemibagrus nemurus*¹⁶, *Osphronemus gourami*¹⁷, *Salmo kettelat*¹ and *Channa marulius*¹³. Although formulated feed has been successfully used to induce oocyte maturation of broodfish, it remains costly. In addition, there is no research regarding *lokan* meat for oocyte maturation of freshwater species and the use of *lokan* meat as fresh feed for oocyte maturation of *Hemibagrus wyckii* broodfish has not yet been evaluated. The aim of this study was to evaluate the effect of fresh *lokan* meat on oocyte maturation in

Hemibagrus wyckii broodfish. The results of this study may increase the number of candidate species available for aquaculture.

MATERIALS AND METHODS

Broodstock and ponds system: *Hemibagrus wyckii* were collected from upstream areas of the Kampar Kanan River in the Kouk village (0° 19' 23.44"N and 100° 56' 40.05"E), Air Tiris village (0° 21' 24.77"N and 101° 06' 04.90"E) and Tarantang village (0° 21' 05.32"N and 101° 18' 43.96"E). The samples were collected from February-September 2015. The broodfish were caught by local fishermen using traditional fishing gears, local name "*lukah*" (trap nets) and fishing pole using *lokan* as bait. The 80 broodfish were collected from all sites. The samples were transferred by truck to the Research Centre, Faculty of Fisheries and Marine Science, Riau University (Indonesia), Sungai Paku Kampar Regency.

Brood Asian catfish were treated with a prophylactic formalin bath (100 mg L⁻¹) for 1 h in order to remove external parasites and were acclimatized in a pond (6 × 6 × 3 m) for four months prior to the experiments. One unit of pond area was (6 × 6 × 3 m), which was used to culture 20 male broodfish. The broodfish were fed *lokan* meat during acclimatization. In a separate pond, broodfish were fed commercial feed as a control. Four experimental and three replicate ponds were used to evaluate reproductive performance. Five females were selected and stocked in each pond (4 × 4 × 3 m).

Prior to stocking, females were weighed and the length was measured. The average initial weight and length of the female broodfish were 2.800 ± 296 g and 353.2 ± 55.5 mm, respectively. At the beginning of the experiment, all female broodfish had an oocyte nucleus positioned in stage I with germinal vesicles in the central position. The broodfish were fed *lokan* meat once a day at 17:00 h due to their nocturnal behavior during 120 days of the experiments. The depth of water in each pond was 2.0 m. The inlet of the pond arrived from Sungai Paku reservoirs at a rate of 0.2 m³ per sec. The temperature of the pond water ranged from 26-28°C, pH ranged from 6.5-6.8, alkalinity ranged from 42.97-57.33 mg L⁻¹ and hardness ranged from 104.83-110.51 mg L⁻¹.

Feed of broodstock: *Lokan* was collected from local fisherman in the Sungai Paku reservoir and kept in a (2 × 2 × 2 m) area of the pond. Shells of *lokan* were removed and the *lokan* meat was placed in a cold box. The *lokan* meat was collected everyday and put on a plastic basin for 6 h. The meat was then weighed and divided into groups of 700, 1,400 and 2,100 g for each pond of broodfish per day.

Table 1: The amino acid profile, mineral content and gross energy of *lokan* meat (Mean±SD)

Amino Acids (mg kg ⁻¹)	Results
Arginine	0.866±0.0032
Histidine	2.249±0.0083
I-leucine	1.527±0.0031
Leucine	1.822±0.0025
Lysine	0.777±0.0015
Methionine	1.799±0.0020
Phenylalanine	3.030±0.0020
Threonine	3.454±0.0015
Valine	1.904±0.0030
Tryptophan	0.0
ΣEAA	17.435±0.0030
Alanine	1.903±0.0031
Aspartic acid	0.074±0.0020
Cystine	1.455±0.0032
Glutamic acid	1.247±0.0010
Glycine	6.045±0.0025
Serine	3.822±0.0014
Proline	1.612±0.0011
Tyrosine	0.0
ΣNEAA	16.16±0.0021
ΣAA	33.596±0.00051
Mineral (mg 100 g)	
Na	153.28±0.6570
Ca	1306.08±2.9810
P	3040.38±8.5274
K	167.52±0.3362
Mg	6.73±0.1400
Fe	18908.27±15.5193
Mn	6442.96±1.9186
Zn	357.67±1.2006
GE (kJ g ⁻¹)	29.44±0.4801

Analysis of the proximate composition was based on the method described by the Association of Official Analytical Chemists (AOAC)¹⁸. According to the analysis of the proximate composition (% wet weight base) of the *lokan* meat, moisture was 89.37%, crude protein was 7.08%, fat was 0.82%, crude ash was 0.29% and carbohydrate was 2.44%. The moisture content was analyzed by drying the samples in a hot air oven at 105°C. Crude protein was estimated by the Kjeldahl method. Fat content was estimated by the soxhlet extraction method with petroleum ether. Ash content was determined from the residue remaining after incineration of the samples at 550°C in a muffle furnace. Dietary gross energy was calculated using the conversion factors of 23.7 kJ g for protein, 39.5 kJ g for lipid and 17.2 kJ g for carbohydrate¹⁹.

For amino acid analysis, the methods of Cohen²⁰ were used. Duplicate samples were hydrolyzed with 6 N hydrochloric acid for 24 h at 110°C. Amino acid analysis was determined using a High Performance Liquid Chromatography (HPLC) system, which consisted of a Waters 1525 Binary HPLC Pump, 717 Plus autosampler (Water®) and Water 2475 Multiλ Fluorescence Detector optics (wavelength: 250 nm for excitation and 395 nm for emission). Mineral

compositions (sodium, calcium, phosphorous, potassium, magnesium, iron, manganese and zinc) was analyzed using microwave digestion and an atomic absorption spectrophotometer (Varian SAA 110) air acetylene flame¹⁸. The amino acids profile, mineral content and gross energy (GE) of *lokan* meat (% dry weight base) are presented in (Table 1).

Every month, five female broodfish were removed from each pond. The broodfish were captured with a gillnet forming a net bag with the appropriate mesh size and anesthetized with MS-222 (Sigma-Aldrich St Louis, MO; 40 mg L⁻¹)²¹. Oocyte maturation was assessed in each individual. The fish were returned to their pond after evaluation and no mortality occurred. Fish were fasted 48 h prior to the evaluation.

Assessing oocyte maturation and spawning: All fish were individually marked using floy tags and weighed. Oocytes sampled *in vivo* were taken from females using the method described by Syandri²² and were placed in Serra's solution (6:3:1:70% ethanol, 40% formaldehyde and 99.5% acetic acid) for clarification of the cytoplasm. After 5 min, the position of the oocyte nucleus was determined using a four-stage scale by Krejszef *et al.*²³ as follows.

Stage 1: Germinal vesicle in the central position

Stage 2: Early migration of the germinal vesicle (less than half of radius)

Stage 3: Late migration of the germinal vesicle (more than half of radius)

Stage 4: Germinal vesicle in the periphery or germinal vesicle breakdown (GVBD)

Each group of female broodfish had different oocyte maturation. After 120 days of the experiment, different numbers of fish in each pond demonstrated successful oocyte maturation. Each group of female broodfish received two injections of GnRH analogs with a dopamine antagonist (Ovaprim) applied intraperitoneally under the left pectoral n. The first injection was 0.2 mL kg BW⁻¹ and the second was 0.6 mL kg BW⁻¹ (total 0.8 mL kg BW⁻¹) at 12 h intervals.²⁴ Egg size (50 eggs in each group) was measured using an Olympus.

E-510 digital camera mounted on a binocular microscope Olympus BX51. Ripe gamete donors were anesthetized with MS-222 (40 mg L⁻¹).²¹ At 24±4, 23±4 and 23±2 h after injection, eggs were stripped into a plastic vessel. Eggs were fertilized using the "dry method" as described²⁵. Males were stimulated with a half dose of the same hormonal preparations used to stimulate the females. Sperm was collected using plastic syringes. Spermatozoa motility was not

estimated. One egg sample (75 eggs each) from each female was mixed with 0.3 mL of pooled milt sample. Eggs samples were incubated in a small aquarium [water temperature: $29 \pm 1^\circ\text{C}$; level of Dissolved Oxygen (DO $6.32 \pm 0.05 \text{ mg L}^{-1}$)]. During the eyed-egg stage (7-day-old embryos), the survival of embryos was recorded. Statistical differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey tests ($p < 0.05$) for significant differences.

RESULTS AND DISCUSSION

In the present study, female broodfish fed *lokani* meat had successful oocyte maturation. The percentage of oocyte maturation increased with increasing levels of feed weight with values of 46.67 ± 11.54 , 73.33 ± 11.54 and

$93.33 \pm 11.34\%$, after receiving 700, 1,400 and 2,100 g of food, respectively (Table 2). The highest amount of *lokani* meat (2,100 g) fed to the female broodfish resulted in the highest nutrition. During the domestication process in a new habitat, changes in behavior, physiology and morphology may occur,^{11,23,26} including the food habits of fish^{10,27,28}. Nutrition has a significant effect on fecundity and fish maturation¹². According to Sink *et al.*,¹⁵ the Channel catfish (*Ictalurus punctatus*) can exhibit increased spawning and individual egg weight when fed a single animal protein source compared to a plant source.

The feed levels also had a significant effect on egg size (2.79 ± 0.02 , 2.82 ± 0.01 and 2.85 ± 0.01 mm, respectively; ($p < 0.05$). Different egg sizes of *Channa marulius* broodstock have also been observed with different crude protein levels in the diet¹³. According to Chambers *et al.*²⁹ the egg size, yolk

Table 2: Reproduction performance of female *Hemibagrus wyckii* broodfish (Mean \pm SD)

Parameters	Domesticated fish
Group 1 (Control)	157 g formulated feed/day
Final weight (g)	2.850 ± 160^a
Oocyte maturation (%)*	0
Eggs size (mm)	-
Ovulation (%)**	-
Latency time (h)	-
Fecundity (number of eggs per <i>spawn</i>)	-
Percentage of eggs weight (%)***	-
Embryo survival (%)	-
Group 2	Feed level 700 g of <i>lokani</i> meat
Final weight (g)	$2.900.60 \pm 73.47^a$
Oocyte maturation (%)*	46.67 ± 11.54^a
Eggs size (mm)	2.79 ± 0.01^a
Ovulation (%)**	80.89 ± 19.24^a
Latency time (h)	24 ± 4.0
Fecundity (number of eggs per <i>spawn</i>)	1.523 ± 68^a
Percentage of eggs weight (%)***	0.50 ± 0.02^a
Embryo survival (%)	37.33 ± 4.0^a
Group 3	Feed level 1,400 g of <i>lokani</i> meat
Final weight (g)	3022.00 ± 64.08^b
Oocyte maturation (%)	73.33 ± 11.54^b
Eggs size (mm)	2.82 ± 0.01^b
Ovulation (%)	80.55 ± 17.34^a
Latin time (h)	23.0 ± 4.0
Fecundity (number of eggs per <i>spawn</i>)	1704 ± 43^b
Percentage of eggs weight (%)*	0.54 ± 0.02^b
Embryo survival (%)	48.44 ± 4.68^b
Group 4	Feed level 2,100 g of <i>lokani</i> meat
Final body weight (g)	$3.166.60 \pm 102.62^c$
Oocyte maturation (%)	93.33 ± 11.34^c
Eggs size (mm)	2.85 ± 0.01^c
Ovulation (%)	100^c
Latin time (h)	23.0 ± 2.0
Fecundity (number of eggs per <i>spawn</i>)	1.775 ± 20^c
Percentage of eggs weight (%)*	0.62 ± 0.02^c
Embryo survival (%)	55.10 ± 6.84^c

There were 5 female broodfish in each pond. The mean values in columns indicated by a letter are significantly different ($p < 0.05$). *Number of matured females $\times 100\%$ /number of female broodfish, **Number of ovulating females $\times 100\%$ /number of mature female broodfish, *** Weight of eggs $\times 100\%$ /weight of female broodfish

reserves and hatch age are affected by yolk volume. Although each individual female broodfish was injected with the same dosage of GnRH analogues (Ovaprim) (0.8 mL BW⁻¹), differences in the percentage of ovulation were obtained (Table 2), suggesting a possible relationship between egg size and oocyte maturation. However, another study found that there was no relationship between oocyte diameter and female ovulation in the Common tech (*Tinca tinca*, *Cyprinidae*) species³⁰. Furthermore, we found that feed levels had a significant effect ($p < 0.05$) on the number of eggs per spawn (Table 2), which may have also affected the quality of the eggs. Egg quality is an important factor for the success of fish spawning³¹. The female broodfish can easily spawn after hormonal injection under controlled conditions. In several studies, *Leuciscus idus*^{23,24} and *Platichthys stellatus*³¹ were also shown to easily spawn after hormonal treatment and other studies have shown that spawning induction using hormonal injections can synchronize maturation of gametes (sperm or eggs) in different fish species^{32,33}. However, in a study by Krist'an *et al.*³⁴, showed that the ovulation of pikeperch (*Sander lucioperca* L) was not influenced by HCG and mGRHa hormones.

At a temperature of $28 \pm 2^\circ\text{C}$, the latency time of the female broodfish were 24 ± 4.0 , 23 ± 4.0 and 23 ± 2.0 h, respectively. The different latency times were due to the biological quality of the gametes. According to Krejszeff *et al.*,²³ the biological quality of gametes can affect the latency time of ide (*Leuciscus idus*). Moreover, latency time in each fish species is affected by the dosage of the hormone, type of hormone, water temperature and fotoperiod^{23,24,31}. The latency time of pikeperch (*Sander lucioperca* L) was not significantly affected by different dosages of HCG hormones³⁴. The percentage of egg weight differed among the treated groups and ranged from 0.50 ± 0.02 - $0.62 \pm 0.02\%$ (Table 2). These values were relatively low because the female broodfish has smaller gonads. Numerous studies have shown that the percentage of egg weights of ide (*Leuciscus idus*) ranges from 14.3 ± 2.1 - $16.7 \pm 2.0\%$ ²³, 6.51 ± 2.6 - $9.21 \pm 5.3\%$ for pikeperch (*Sander lucioperca*)³⁴ and 4.53 - 10.32% for green catfish (*Hemibagrus nemurus*)⁹.

Feed level has a significant effect ($p < 0.05$) on embryo survival. The mean embryo survival rates were 37.33 ± 4.0 , 48.44 ± 4.68 and $55.10 \pm 6.84\%$ in fish fed 700, 1,400 and 2,100 g of lokan meat, respectively (Table 2). Embryo survival differences were positively correlated with the size of the eggs. According to Brooks *et al.*³⁵ egg size and the composition of the egg may be used as indicators of hatchability and fry quality. Other studies have shown that larger egg size results in better hatching rates^{36,37}.

In conclusion, 2,100 g of lokan meat was sufficient to maintain the broodstock of *Hemibagrus wyckii* in good conditions with the best reproduction performance. Despite positive effects on oocyte maturation, egg size and number of eggs per spawn, the effects on hatching rate and survival embryo remain to be investigated.

SIGNIFICANCE STATEMENTS

- *Hemibagrus wyckii* was categorized as a "vulnerable to endangered" species, therefore requiring domestication
- Lokan meat is a good source of protein for oocyte maturation, especially for carnivorous fish
- *Hemibagrus wyckii* is a carnivorous fish; however, these fish cannot eat commercial feed, so pellets should be provided during the fingerling and juvenile stages

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