

## THE EFFECT OF MIXED ANIMAL-VEGETABLE OILS IN FEED ON EARLY GONADAL MATURATION OF MALE ASIAN REDTAIL CATFISH (*Hemibagrus nemurus*)

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### ABSTRACT

Male broodstock candidates of Asian redtail catfish tend to have aggressive behaviour due to excessive testosterone production, which is exacerbated by poorly-regulated high-cholesterol feeding practices. This study aimed to suppress these conditions by administering the dietary combination of animal and plant-based oils during pre-gonadal maturation of male broodstock candidates (BW:  $215.83 \pm 41.28$  g; BL:  $272.12 \pm 19.89$  mm), reared in a  $2 \times 1 \times 1$  m<sup>3</sup> net cage with the stocking density of two fish per cage. This study was conducted using a completely randomized design with three dietary combination treatments (2% of each oil dosage) with four replicates: base feed with no mixed dietary oil (K), mixed fish oil-maggot oil (IM), and mixed fish oil-palm oil (IS). All parameters, namely reproductive performance (final length/Pt, final weight/Wt, feed intake/FI, gonadosomatic index/GSI, and hepatosomatic index/HSI) and blood chemistry profile (total cholesterol/TC, high-density lipoprotein/HDL, triglycerides/TG, and glucose/GLU), were statistically tested their significance using the analysis of variance and Duncan's multiple range test with 95% confidence level. Significant differences were found in Pt and Wt ( $p < 0.05$ ), where the dietary combination of oils (IM-IS) showed higher Pt and Wt values than the dietary without the mixed oils (K). The IM treatment obtained the highest TG level ( $159.98 \pm 38.44$  mg dL<sup>-1</sup>;  $p < 0.05$ ), yet showed no significant differences from the other treatments in TC, HDL, and GLU levels ( $p > 0.05$ ). This study concludes that the dietary combination of fish oil (2%)-maggot oil (2%) improves lipid deposition during pre-gonadal maturation and regulates energy storage through weight gain, without jeopardizing the somatic growth (length) of male Asian redtail catfish broodstock candidates.

**KEYWORDS:** broodstock; catfish; diets; oil; pre-maturation

**ABSTRAK:** Pengaruh Kombinasi Minyak Hewani dan Nabati dalam Pakan terhadap Pematangan Gonad Awal Ikan Baung (*Hemibagrus nemurus*) Jantan

Calon induk ikan baung jantan memiliki sifat agresif yang salah satunya disebabkan produksi testosteron berlebih akibat pemberian pakan dengan kadar tinggi kolesterol. Penelitian ini ditujukan untuk mengurangi dampak tersebut melalui evaluasi kombinasi

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minyak hewani dan nabati pada pakan selama fase awal kematangan gonad calon induk ikan baung jantan (BW:  $215,83 \pm 41,28$  g; BL:  $272,12 \pm 19,89$  mm), yang dipelihara pada hapa ukuran  $2 \times 1 \times 1$  m<sup>3</sup> dan padat tebar sebesar dua ekor ikan per hapa. Penelitian ini menggunakan rancangan acak lengkap dengan tiga perlakuan pakan kombinasi (dosis tiap minyak 2%) dan empat ulangan, yaitu pakan tanpa kombinasi (K), pakan dengan kombinasi minyak ikan-maggot (IM), dan minyak ikan-sawit (IS). Seluruh parameter, yaitu kinerja reproduksi (panjang akhir/Pt, bobot akhir/Wt, total konsumsi pakan/FI, indeks somatik gonad/GSI, dan indeks somatik hati /HSI) dan profil kimia darah (total kolesterol/TC, high-density lipoprotein/HDL, trigliserida/TG, dan glukosa/GLU), dianalisis menggunakan analisis sidik ragam dan uji jarak berganda Duncan dengan derajat kepercayaan 95%. Perbedaan nyata ditunjukkan pada Pt dan Wt ( $p < 0,05$ ), dimana kombinasi minyak IM-IS menunjukkan nilai Pt dan Wt yang lebih tinggi dibandingkan tanpa pemberian kombinasi minyak (K). Perlakuan IM memperoleh konsentrasi TG tertinggi ( $159,98 \pm 38,44$  mg dL<sup>-1</sup>;  $p < 0,05$ ), namun menunjukkan tidak adanya perbedaan nyata terhadap perlakuan lainnya pada konsentrasi TC, HDL, dan GLU. Penelitian ini menyimpulkan, bahwa kombinasi minyak ikan (2%) dan minyak maggot (2%) mampu memperbaiki penyimpanan lemak untuk fase awal kematangan gonad (trigliserida dalam serum darah) dan mendorong penyimpanan energi melalui peningkatan bobot, tanpa mengganggu pertumbuhan somatik (panjang) dari calon induk ikan baung jantan.

**KATA KUNCI:** *ikan baung; induk; kematangan gonad; minyak; pakan*

## INTRODUCTION

Asian redtail catfish (*Hemibagrus nemurus*) is a freshwater fish species commonly found in Sumatra, Java, and Borneo Islands, Indonesia (Kusdiarti *et al.*, 2020). The fish has been farmed throughout Indonesia, particularly in Central Java, as a viable response to its declining wild population (Mujtahidah *et al.*, 2021; Yuanawati *et al.*, 2022). For example, in Magelang, Central Java, farming activities of this species not only improve its wild stock but also supply local demand for high-quality animal protein. Despite the positive developments, the consumption rate of this fish remains at 3.6% of total fish production, whether from fishing or aquaculture activities (Ikhsanudin *et al.*, 2023).

The low consumption rate of Asian redtail catfish could be attributed to limited production and inconsistent seed supply, due to unsuccessful breeding activities, low fertility (51%) and hatching rate (43%) of the fish eggs (Rasyad *et al.*, 2023). Since they are

mostly sourced from wild-caught populations with no intensive selection, Asian redtail catfish broodstock candidates, specifically male fish, are susceptible to stress and exposure to low-quality diets, which reduces their gonadal maturation capability (Prianto *et al.*, 2015). Male Asian redtail catfish are also more aggressive than female fish, due to high testosterone production, which can lead to cannibalism with an incidence of up to 30% and affect the pre-gonadal maturation process (Heltonika *et al.*, 2023). High testosterone concentrations in broodstock can be caused by exposure to uncontrolled cholesterol levels due to poor feeding practices, namely, feeding the fish with trash fish during the pre-gonadal maturation period (De-Dios *et al.*, 2022). Dietary combined supplementation with animal- and plant-based oils could remediate these issues in male Asian redtail catfish broodstock candidates, particularly during the pre-gonadal maturation period.

Previously, oils, either plant-based or animal-based, have been used to support

gonadal maturation in various broodstock fish. Sattang *et al.* (2021) reported that 1-2% fish oil in diets improved spermatocyte production and accelerated the spawning process in hybrid catfish (*Pangasius larnaudii* × *Pangasianodon hypophthalmus*, Sauvage, 1878). Furthermore, fish oil (60%), combined with plant-based oil, such as linseed oil (40%), could increase the weight gain, gonadosomatic index, and spawning frequency of Gilthead seabream (*Sparus aurata*) (Izquierdo *et al.*, 2015; Turkmen *et al.*, 2017; Ferosekhan *et al.*, 2020). The use of palm oil (50%) + rubber seed oil (50%) had resulted the highest feed intake and final weight, while minimizing stress condition on giant gourami (*Osphronemus goramy*) (Purnamasari *et al.*, 2020) and striped catfish (*Pangasianodon hypophthalmus*) (Fatmawaty *et al.*, 2021), based on elevated high-density lipoprotein and low total cholesterol levels. In Asian redtail catfish, applying fish oil and corn oil (1-2% for each oil) obtained gonadosomatic index value between 16-16.7% (Suhenda *et al.*, 2009).

Another animal-based oil, extracted from black soldier fly (BSF) maggot, also has the potential to improve reproductive performance and reduce stress in broodstock candidates. Maggot oil has been applied as a dietary supplement to support fish and shrimp growth at 1.5-1.9% (Herawati *et al.*, 2022; Herawati *et al.*, 2023). Maggot oil also contains arachidonic acid (20:4n-6; 0.1%) and eicosapentaenoic acid (20:5n-3; 0.15%) as main builders of prostaglandin hormone to accelerate gonadal maturation (Sattang *et al.*, 2021; Afriani *et al.*, 2023). However, studies on the application of maggot oil, or its combination with fish oil, in dietary supplementation to support gonadal maturation in broodstock candidates remain limited.

Therefore, this study was conducted to determine the effects of combining plant-based and animal-based oils in the diets of male Asian redtail catfish broodstock candidates during the pre-gonadal maturation period. This study was also aimed at improving reproductive performance and nutrient deposition (lipid/

cholesterol) in male broodstock candidates before broodstock selection for spawning process preparation, by improving body weight, gonadosomatic index, hepatosomatic index, and a stress reduction indicator (cholesterol concentration).

## MATERIALS AND METHODS

### Location and Period

This study was performed in July-September 2025 at the Operational Unit of Freshwater Fish Culture and Hatchery (BPBIAT), Ngrajek, Magelang, for broodstock candidate rearing and the Laboratory of Animal Nutrition, Tidar University, Magelang, for blood chemical profiling of male Asian redtail catfish broodstock candidates.

### Ethical Statement

This study was conducted in accordance with the established guidelines for the treatment of experimental animals and fully complied with the ethical code governing animal experimentation (SNI 8228-4:2022).

### Experimental Design

This study employed an experimental method using a completely randomized design with three diet treatments in four replications: combining fish oil and maggot oil (IM), fish oil and palm oil (IS), and no-oil combination (control/K). The combinations of plant and animal oils were applied at a 1:1 ratio, with each oil dosed at 2% (Purnamasari *et al.*, 2020; Sattang *et al.*, 2021; Afriani *et al.*, 2023). In total, there were 12 experimental units, following the number of treatments and replications.

### Broodstock Candidate and Rearing Media Preparation

The 24 male fish of Asian redtail catfish (body weight/BW:  $215.83 \pm 41.28$  g; body length/BL:  $272.12 \pm 19.89$  mm) were obtained

from BPBIAT, Ngrajek, Magelang. These male fish had been reared for 6-8 months and were validated as broodstock candidates, visually pathogen-free (no red/white spots and no parasite attachments), physically intact, and swam actively. The rearing medium used a  $2 \times 1 \times 1 \text{ m}^3$  net cage, installed in a  $20 \times 10 \times 1.5 \text{ m}^3$  pond. Before stocking into the net cage at 2 fish per cage, the fish were weighed per experimental unit (cage) to determine the initial body weight and ensure weight uniformity. After being fasted for 24 hours, fish were fed with the treatment diets for 30 days.

### Diet Preparation and Feeding

Diets, containing commercial feed (*STP KAE-starter, PT. Suri Tani Pemuka, Japfa Group, Indonesia*; protein 50-52%; lipid 10-11%) and oil combinations (K, IM, IS), were manufactured through a coating method and dried at 60-80°C for 3-4 hours. After drying, all diets were stored in dried and sealed container. Feeding practice was performed until apparent satiation twice a day, namely in the morning (08.00 WIB) and in the afternoon (16.00 WIB). Before and after feeding, all diets were weighed to obtain total feed intake (FI) from the first to the last feeding period (30 days).

### Harvesting (Final Sampling)

All fish were harvested after 30 days of rearing for further gonadal maturation (broodstock selection). Before harvesting, the fish were fasted for 24 hours, then weighed and measured individually to obtain their final weight (Wt) and length (Pt). Blood samples were collected from each fish using a 1-ml syringe. After blood collection, the fish were euthanized to collect and measure the weight of their gonad and liver to determine the gonadosomatic index (GSI) and hepatosomatic index (HSI), respectively.

### Blood Chemistry Profiles

Blood samples from three randomly selected fish were centrifuged at 5,000 rpm for 10 minutes to obtain plasma. These samples were determined to have their chemistry profiles, namely total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TG), and glucose (GLU). The blood chemistry profiling was determined using *Human GmbH blood test kit (Human Diagnostics Asia Pacific, Pte., Ltd., Singapore)*.

### Water Quality

During the rearing period, water quality parameters were maintained at 29.25-29.71°C, pH 8.36-8.53, dissolved oxygen 7.24-7.77 mg L<sup>-1</sup>, and ammonia 0.01-0.02 mg L<sup>-1</sup>, following Setiadi *et al.* (2022).

### Data Analysis

Data analysis was performed using *Microsoft Excel 2024 (Microsoft Inc., USA)* and *SPSS 16.0 (IBM Inc., USA)*. All measured parameters, namely FI, Wt, Pt, GSI, HSI, TC, TG, HDL, and GLU, were tested for homogeneity and normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Data that met the criteria for homogeneity and normality were further analyzed using one-way analysis of variance (One-way ANOVA) at a 95% confidence level. If significant differences were found ( $p < 0.05$ ), analysis was continued using Duncan's multiple range test (DMRT) to determine the most effective diet to improve reproductive performance and blood chemistry profiles of male Asian redtail catfish broodstock candidates.

## RESULTS AND DISCUSSION

### Reproductive Performance

The reproductive performance in this study comprises several parameters: feed intake (FI), final length (Pt), final weight (Wt),

gonadosomatic index (GSI), and hepatosomatic index (HSI). The reproductive performance results of male Asian redtail catfish broodstock candidates are presented in Table 1.

In reproductive performance, all parameters indicate non-significant differences ( $p>0.05$ ), except in Pt (final length) and Wt (final weight). The final length and weight of male broodstock candidates fed different supplemented diets (IM and IS) were higher than those in the control diet (K), yet no significant difference was found in the FI parameter ( $p>0.05$ ). This condition means that supplying animal-based oils (fish and maggot oil) or animal- and plant-based oils provides more efficient growth (somatic and tissue improvements) and more energy deposit, rather than energy for catabolism or physiological maintenance, resulting in low nutrient intake (e.g. feed intake/FI) to support the reproduction process (Villamarín *et al.*, 2016; Liu *et al.*, 2022), specifically the spermatogenesis process in male fish. Similar results were also reported by Ayisi *et al.* (2018) in Nile tilapia (*Oreochromis niloticus*), who applied palm oil and fish oil, and Kimou *et al.* (2023) in African catfish (*Heterobranchus longifilis*), who combined fish oil and maggot oil.

Despite showing a promising association among Wt, Pt, and FI, the supplemented-diet treatments had no effect on GSI and HSI values ( $p>0.05$ ). However, this result confirms

that male Asian redtail catfish broodstock candidates, as in spotted scat (*Scatophagus argus*), start to allocate extra metabolizable energy to reproduction (gamete production), while the gonad and liver remain at constant weight during pre-gonadal maturation (Wang *et al.*, 2022). Furthermore, liver and gonad are main organs in supplying energy for the reproduction process (spermatogenesis) (Zhou *et al.*, 2022), thus both organs in male Asian redtail catfish broodstock candidates fed with animal- and plant-based oils (IM and IS diets) may only change their nutrient component deposition, like lipids and glycogens, during pre-gonadal maturation. Another study reported by Suhenda *et al.* (2009) also showed no significant difference in GSI, when female Asian redtail catfish broodstocks were fed with fish oil and corn oil-supplemented diets under 1-4% dosage of each oil. In addition, other fish, such as mahseer (*Tor tambroides*) broodstock fed with fish oil and corn oil at a 1:1 ratio (Abduh *et al.*, 2021) and hybrid catfish (*Pangasius larnaudii*  $\times$  *Pangasianodon hypophthalmus*, Sauvage, 1878) broodstock fed with 1-2% fish oil-supplemented diets (Sattang *et al.*, 2021), obtained no significant difference in GSI and HSI.

The non-significant differences found in almost all parameters (FI, GSI, and HSI) indicate a longer rearing time for feeding the supplemented diets, namely 107-117 days

Table 1. Reproductive performance of male Asian redtail catfish broodstock candidates, fed with combined oil-supplemented diets

Parameters	K	IM	IS
FI (g)	173.25 $\pm$ 32.12	132 $\pm$ 72.75	104.5 $\pm$ 17.90
Pt (mm)	263.68 $\pm$ 2.45 <sup>a</sup>	290.25 $\pm$ 7.76 <sup>ab</sup>	305.75 $\pm$ 31.34 <sup>b</sup>
Wt (g)	156 $\pm$ 0.00 <sup>a</sup>	230.5 $\pm$ 32.28 <sup>b</sup>	264.75 $\pm$ 68.63 <sup>b</sup>
GSI (%)	0.0045 $\pm$ 0.00	0.0037 $\pm$ 0.0023	0.0049 $\pm$ 0.0027
HSI (%)	0.0097 $\pm$ 0.00	0.0095 $\pm$ 0.0015	0.0085 $\pm$ 0.0005

Note: All values are presented in average  $\pm$  standard deviation (SD). Different superscript letters in the same line indicate significantly different values ( $p<0.05$ ). K = Supplementation of oil combination absence (0%/control), IM = Dietary supplementation of fish oil (2%) and maggot oil (2%), IS = Dietary supplementation of fish oil (2%) and palm oil (2%). FI = feed intake; Pt = Final length; Wt = Final weight; GSI = Gonadosomatic index; HSI = Hepatosomatic index.

(Utiah *et al.*, 2007), and much older broodstock candidates that should have been reared for 12 months (Aryani & Suharman, 2015). In this study, the broodstock candidates were only 6-8 months old and reared for only 30 days.

### Blood Chemical Profiles

Blood chemical profiles in this study include plasma cholesterol levels (total cholesterol/TC and high-density lipoprotein/HDL), plasma triglycerides (TG), and plasma glucose (GLU), which are presented in mg/dL. Blood chemical profiles of male Asian redtail catfish broodstock candidates on pre-gonadal maturation are depicted in Figure 1.

Blood chemical profiles of male Asian redtail catfish broodstock candidates showed no significant difference in all parameters ( $p>0.05$ ), except the triglyceride level (TG). The dietary combination of fish oil (2%) and maggot oil (2%) yielded the highest TG level ( $p<0.05$ ), yet showed no significant differences among the other treatments in GLU, HDL, and TC levels ( $p>0.05$ ). This condition indicates that fish fed with fish and maggot oil-supplemented diet offer a higher energy supply for steroidogenesis and testicular membrane formation (Kumar *et al.*, 2018), specifically during

pre-gonadal maturation and deposition, than other treatments. Steroidogenesis occurs when cholesterol, including triglycerides, is converted into steroid hormones, like testosterone and progestogen ( $17\alpha, 20\beta$ -dihydroxy-4-pregn-3-one/DHP) (Soranganba & Singh, 2019), while maintaining the aggressiveness level from the male fish that may coexist with chemical signalling and social cues in rearing media (Saraiva *et al.*, 2017). Similarly, Egessa *et al.* (2025) reported that dietary supplementation with fish oil and maggot oil (ratio 1:1) did not result in a significant difference in total cholesterol levels in African catfish hybrids (*Clarias gariepinus*  $\times$  *Heterobranchus longifilis*), except for glucose levels. Moreover, He *et al.* (2024) reported that a dietary combination of fish and maggot oil (25-75%) showed no significant differences in total cholesterol and triglyceride levels in white shrimp (*Litopenaeus vannamei*), except for HDL levels.

Although no significant differences in other blood profile parameters were observed, the following condition is reasonable, as triglycerides (TG) are more sensitive to changes in dietary lipid supply than HDL and CHO levels (Mohamed *et al.*, 2019). As also reported by Shen *et al.* (2022) in juvenile black seabream *Acanthopagrus schlegelii* after being

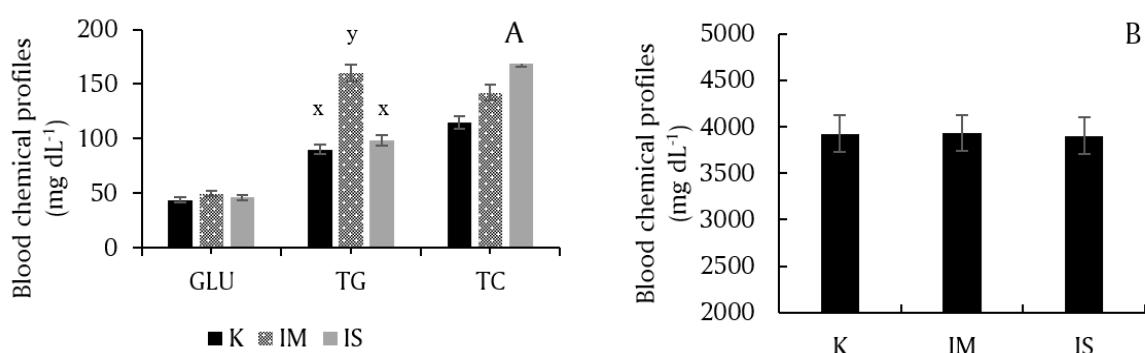


Figure 1. Blood chemical profiles (GLU, TG, TC = A; HDL = B) of male Asian redtail catfish broodstock candidates fed with oil-supplemented diets. All values are presented in average  $\pm$  SD. Different letters (x,y) above the bar indicate significantly different values ( $p<0.05$ ). K = Supplementation of oil combination absence (0%/control); IM = Dietary supplementation of fish oil (2%) and maggot oil (2%); IS = Dietary supplementation of fish oil (2%) and palm oil (2%). GLU = Plasma glucose (mg dL<sup>-1</sup>); TG = Triglycerides (mg dL<sup>-1</sup>); TC = Total cholesterol (mg dL<sup>-1</sup>); HDL = High-density lipoprotein (mg dL<sup>-1</sup>).

fed with fish oil, palm oil, and soybean oil at high concentration level in diets (13.5%) and Gong *et al.* (2024) in largemouth bass (*Micropterus salmoides*) fed with 7% soybean oil-supplemented diet, unchanged HDL, CHO, and GLU may indicate an optimal condition during pre-gonadal maturation, where broodstock candidates were more prone to perform reproduction process, that required more TG level, while still regulating optimal nutrient transport and nutrient deposition, as parts of body maintenance components. In addition, according to Sattang *et al.* (2021), maggot oil contains saturated fatty acid (lauric acid C12:0) and polyunsaturated fatty acids (PUFA), like arachidonic acid (20:4n-6), that may be associated with high TG level in male broodstock candidates for further gonadal development, namely spermatogenesis. Therefore, for further optimal application, a dietary combination of fish oil (2%) and maggot oil (2%) ensures the lipid deposit improvement and energy supply for further gonadal maturation in male Asian redtail catfish broodstock candidates.

## CONCLUSIONS

This study concludes that dietary supplementation of animal-based and plant-based oils had no effect on pre-gonadal maturation of male Asian redtail catfish. However, in blood chemical profiles, the highest triglyceride level found in animal-based oil dietary supplementation (fish oil and maggot oil) may provide a ready-to-use energy for further gonadal maturation in male Asian redtail catfish broodstock candidates, before the broodstock selection program begins.

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## AUTHOR CONTRIBUTION

TM: Conceptualization, formal analysis, supervision and writing – review and editing; SSQT: Conceptualization, methodology, and writing original draft preparation; MA: Methodology, formal analysis, and visualization; SJR, MASO, RTA, and AF: Investigation, project administration, and writing original draft preparation.

## DECLARATION OF COMPETING INTEREST AND USE OF GENERATIVE AI

The authors declare that there are no competing interests. During the preparation of this work, the authors used *Copilot* (Microsoft Inc., USA) to proofread the manuscript. The authors reviewed and edited the material as needed and take full responsibility for the content of the article.

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