



Effects of Dietary Tryptophan Supplementation on Melatonin and Oocyte Maturation in African Catfish, *Clarias Gariepinus*

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Abstract | This study aimed to investigate the potential benefits of tryptophan supplementation in feed and its impact on melatonin, estradiol, and vitellogenin levels and oocyte maturation in African catfish. Thirty out of 90 broodstocks, with an average weight of 700 ± 160 g, were randomly selected for treatment, with a male-female ratio of 1:1. A completely randomized design was used with three levels of tryptophan as treatments (0.33% (X), 0.41% (Y), and 0.49% (Z) in dry feed), replicated individually with a total of five broodstocks for each treatment. Broodstock was maintained in the same environment at a constant temperature of 28 °C, pH between 6-7, dissolved oxygen levels of 4-5 mg L⁻¹, and total darkness (24 hours dark). Microchip tags were used for sample identification and replication. Observations and samples were collected from five individuals every 10 days for 30 days. The results showed that tryptophan had an effect ($p < 0.05$) on melatonin, estradiol, vitellogenin, oocyte diameter, egg diameter, yolk diameter, and hatching rate. However, tryptophan content has a non-linear effect especially on egg and yolk diameter, the result showed at X treatment 1.52 ± 0.05 and 1.27 ± 0.04 mm, and Z treatment 1.53 ± 0.02 and 1.27 ± 0.02 mm, showed a significant difference ($p < 0.05$) compared to the Y treatment 1.35 ± 0.01 mm and 1.11 ± 0.01 mm. Therefore, this study concluded that tryptophan supplementation of 0.49% in dry feed had a positive effect on accelerating the maturation of catfish starting at 20 days of treatment with the following hormone levels, melatonin of 10.69 ± 0.14 ng mL⁻¹, estradiol 1.90 ± 0.03 ng mL⁻¹, vitellogenin 35.84 ± 0.37 ng mL⁻¹, and oocyte diameter of 1.05 ± 0.09 mm.

Keywords | Reproduction, Estradiol, Vitellogenin, Oocyte, Egg diameters, Feed

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INTRODUCTION

African catfish (*Clarias gariepinus*) is an economically important aquaculture species because of its adapt-

ability and production potential. Its popularity stems from several favorable traits, including adaptability to extreme environments, feeding flexibility, and high growth rates (Naorbe, 2021). This species has been introduced into var-

ious countries for aquaculture purposes, with some strains being developed to increase production (Iswanto *et al.*, 2015). Many studies have been conducted to improve cultivation techniques, such as spawning methods (Marimuthu, 2019), growth modeling (Musa *et al.*, 2021), and the potential of monosex populations (Balogh *et al.*, 2023) to improve their commercial feasibility.

Naturally, the reproductive cycle of African catfish is seasonally limited. Control of the reproductive period to allow for flexible ovulation and healthy larval production at any time of the year is essential (Matias *et al.*, 2013; Van Oordt *et al.*, 1987) to allow commercial farmers to obtain catfish larvae at any time (Saadony *et al.*, 2014; Wubie *et al.*, 2023). Various environmental manipulation techniques have been developed to stimulate fish spawning outside of the natural season. These methods include regulating water temperature, photoperiod, and water quality to create optimal conditions for spawning. Temperature and photoperiod are major factors in the seasonal reproductive alignment of fish (Oliveira *et al.*, 2011). By altering these factors, researchers have successfully induced spawning in a variety of species. For example, it can advance the ovulation time of Atlantic salmon 71 days faster than the average ovulation time (King *et al.*, 2007) and three months earlier in turbot fish (Polat *et al.*, 2021). Interestingly, the effectiveness of these techniques can vary depending on the species and specific manipulation of the environment. In some cases, constant light inhibits spawning, whereas shorter light exposure increases the spawning frequency (Bembe *et al.*, 2017). This is due to the influence of the hormone melatonin (Mel) which plays a key role in limiting the reproductive cycle and season in African catfish.

In addition, the nutritional composition of fish feed, particularly lipids, fatty acids, and essential amino acids, significantly affects gonad maturation, steroidogenesis, fertility, fertilization, and egg quality (Andriani *et al.*, 2023; Chemello *et al.*, 2022; Thiruvassagam *et al.*, 2024). The essential amino acid content in feed is known to affect reproductive parameters. However, only tryptophan (Trp) has a direct pathway leading to Mel production. This is because Trp is an essential amino acid that functions as a precursor in Mel synthesis. Other amino acids, such as leucine, are only involved in protein synthesis and energy metabolism, while arginine is involved in nitric oxide production and blood flow regulation (Bagci *et al.*, 2017). Research shows that Mel can improve oocyte quality and maturation by reducing oxidative stress, which adversely affects reproductive parameters (Dominguez *et al.*, 2012). Therefore, the amino acid Trp plays a role in the reproduction and maturation of oocytes.

The synthesis of Mel from Trp involves several enzymatic steps. Initially, Trp is converted to 5-hydroxytryptophan

(5-HTP) by tryptophan hydroxylase, followed by its decarboxylation to serotonin. Then Serotonin is acetylated into N-acetylserotonin by serotonin N-acetyltransferase (AANAT). Finally, N-acetylserotonin is methylated by N-acetylserotonin O-methyltransferase (ASMT) to form Mel (Germann *et al.*, 2016; Zhao *et al.*, 2019). This pathway highlights the essential role of Trp as a precursor for Mel synthesis, which is crucial for maintaining circadian rhythm and reproductive health (Hoseini *et al.*, 2019; Peuhkuri *et al.*, 2012). Mel has been shown to modulate reproductive functions. In teleost fish, Mel influences the hypothalamic-pituitary-gonadal (HPG) axis, which affects the release of gonadotropin-releasing hormone (GnRH) and subsequently luteinizing hormone (LH) and follicle-stimulating hormone (FSH). FSH can regulate the synthesis of estradiol (E₂) in the ovary, and E₂ regulate the synthesis of vitellogenin (VTG) in the liver, thereby promoting oocyte maturation (Azeredo *et al.*, 2017; Hoseini *et al.*, 2019; Sébert *et al.*, 2008). This modulation is particularly important during different reproductive phases, as Mel levels fluctuate in response to environmental light conditions, thereby regulating the reproductive cycles (Acuña-Castroviejo *et al.*, 2014; Sébert *et al.*, 2008). Furthermore, studies have indicated that Mel can potentially enhance oocyte quality and maturation through its antioxidant properties, which protect oocytes from oxidative stress during development (Acuña-Castroviejo *et al.*, 2014). The regulation of oocyte growth and maturation by Trp and Mel in fish is shown in Figure 1.

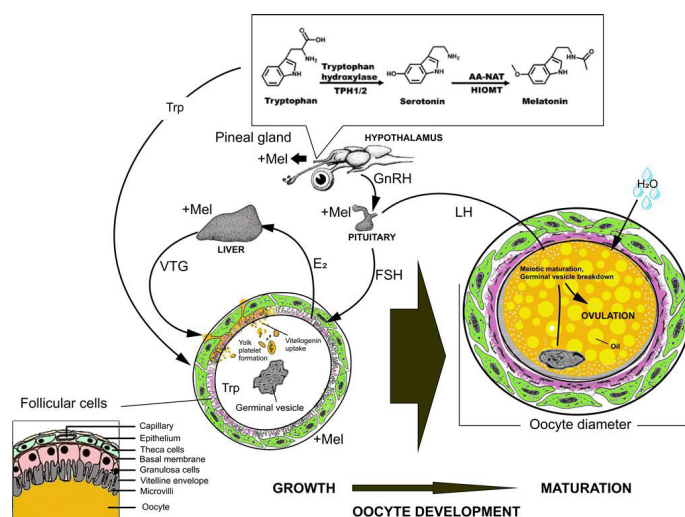


Figure 1: Trp and endocrine regulatory scheme on oocyte growth and maturation in fish (adapted from Babin *et al.*, 2007; Finn and Cerdà, 2024; Pavlov and Emel'yanova, 2016). Abbreviations: GnRH, gonadotropin-releasing hormone; LH, Luteinizing hormone; FSH, follicle-stimulating hormone.

The relationship between Trp, Mel, and reproductive endocrinology is further supported by evidence that Trp supplementation can lead to increased Mel levels in various

animal models (Sánchez-Cárdenas *et al.*, 2011; Sánchez *et al.*, 2008). In livestock, Trp supplementation has been shown to improve reproductive outcomes by enhancing Mel production (Munn *et al.*, 2021). Several studies on Trp supplementation in fish feed formulations have shown that it can affect the reproductive aspects, including gonadal development (Akiyama *et al.*, 1996; Angel-Dapa *et al.*, 2017), hormone regulation (Akhtar *et al.*, 2012; Sahu *et al.*, 2020; Zaminhan *et al.*, 2018), egg quality (Tomaszewska *et al.*, 2021), spawning behavior (Hoseini *et al.*, 2019; Sahu *et al.*, 2020), stress reduction (Sahu *et al.*, 2020; Vieira *et al.*, 2021), and larval development (Thiruvassagam *et al.*, 2024). Therefore, the administration of Trp is necessary because a lack of Trp can cause behavioral changes and affect growth and reproductive performance (Gaye-Siessegger *et al.*, 2007; Narita *et al.*, 2011).

Previous studies showing a clear response of reproductive hormone levels to Trp supplementation have been conducted on Nile tilapia and *Labeo rohita* (Ciji *et al.*, 2013; Zaminhan *et al.*, 2018). However, until now, number of studies have directly examined the effects of Trp supplementation on physiological and reproductive outcomes in African catfish broodstocks have not been found. Meanwhile, Trp needs vary between species following the physiological needs of fish (Moses *et al.*, 2018; Prabu *et al.*, 2022). Therefore, research to ensure sufficient Trp levels in the diet of African catfish is required to support gonadal maturity and reproductive success. This study aimed to investigate the potential benefits of Trp supplementation in feed and its impact on Mel, E₂, and VTG levels and oocyte maturation in African catfish.

Table 1: Trp levels in treated feed.

Treatment (code)	Trp in feed* (%)	Trp level** (g kg ⁻¹ protein)
X (control)	0.33	8.7
Y	0.41	10.8
Z	0.49	12.9

*:Test results of Trp levels in feed after coating (Performed using HPLC-PDA method); **: Trp content after conversion based on feed protein (% Trp in feed ÷ 38% (protein in feed) × 1000 g).

MATERIALS AND METHODS

EXPERIMENTAL MATERIALS

The experimental fish were African catfish broodstock strain “Mutiarā”, *Clarias gariepinus*, with average weight 700 ± 160 g obtained from the Research Institute for Fish Breeding, Sukamandi, Indonesia (certificate number B.810/BRSDM-BRPI/PB.110/VI/2023). Fish were transferred to the laboratory and hatchery on the 4th Building of the Faculty of Fisheries and Marine Sciences, Padjadja-

ran University. Before being transferred and raised at the research location, fish were kept in an outdoor pond with a feeding pattern of 2% body weight, twice a day using commercial rearing feed (30% crude protein, 5% fat, 6% fiber, 13% ash, and 12% moisture). Then in the laboratory and hatchery, fish acclimatized for 10 days indoors with a natural light cycle. During acclimatization, the experimental fish were fed with commercial broodstock feed (38% crude protein, 5% fat, 6% fiber, 12% ash, and 11% moisture) twice a day at 1% body weight.

EXPERIMENTAL DESIGN

This study used a completely randomized design (CRD) consisting of three levels of Trp supplementation in commercial feed. Broodstock for treatment was randomly selected from as many as 30 of the 90 available broodstocks, with a male-female ratio of 1:1. Subsequently, 10 selected broods were reared under each treatment. Rearing was performed using homogenizing factors other than Trp. Trp levels of the feed are shown in Table 1. Repetitive tests were performed individually on five broodstocks using a microchip as a marker. The use of microchip markers for individual identification allowed for proper tracking and analysis of each sample throughout the study.

Table 2: Trp and coating materials in the feed.

Treatment (code)	Trp added (g kg ⁻¹)	Calcium lignosulfonate (g kg ⁻¹ diet)	Water Solvent (mL kg ⁻¹ diet)
X (control)	0	5	125
Y	1.1	5	125
Z	2.2	5	125

EXPERIMENTAL FEED

The experimental feed used was a commercial feed (38% crude protein, 5% fat, 6% fiber, 12% ash, and 11% water) supplemented with L-tryptophan (99.0% purity) using the coating method. The Trp and materials coating were weighed according to treatment (Table 2). For each feed treatment, one kg commercial feed was weighed using a scale (precision 0.01 g). Furthermore, Trp solution (Trp + Calcium lignosulfonate + solvent) was added by evenly spread on the feed. The mixture stirred continuously until it appeared darker and slightly expanded because of the absorption of the Trp solution. The homogeneous feed was dried in an oven at 30°C for at least four hours before being fed to the fish to avoid degradation of Trp. Feed was then stored in an airtight container at room temperature. Trp content in the feed after coating was determined using the HPLC-PDA test method (Table 1).

EXPERIMENTAL PROTOCOL

African catfish broodstocks were acclimatized in each fiber tank (with a diameter of 1.25 m) containing 500 liters of water for seven days. In addition, the maintenance condi-

tions were determined according to the results of preliminary experiments, which showed a temperature of 28°C, pH 6-7, dissolved oxygen concentration of 4-5 mg L⁻¹, and a full dark light period (24 hours dark) with a light intensity of 0 lx. To eliminate the presence of mature oocytes in the female ovaries, ovulation was carried out with Ovaprim (OVP) induction using a dose of 0.5 mL kg⁻¹. The purpose of using OVP is to accelerate ovulation process without disrupting the development and maturation of oocytes (Okomoda *et al.*, 2017; Rahdari *et al.*, 2014). The fish were maintained for 30 days and fed a daily diet of 1% of their total biomass. The amount of feed in each tank was adjusted every 10 days based on the recorded biomass. Furthermore, 20% of the water was replaced daily to maintain the optimal water quality.

SAMPLING

Sampling was performed on Five fish from each treatment group. Serum and egg samples were collected every 10 days. Fish were fasted for 24 hours before sampling. We followed the sampling methods described by Duran *et al.* (2023) and Muntean and Marcus (2016). To reduce stress during sampling, the fish were anesthetized using 0.3-0.5 mL L⁻¹ phenoxy. To obtain a serum sample, as much as 1 ml of blood was collected using a syringe in the caudalis vein, which is located right in the ventral part of the vertebrate bone. Serum was separated from blood cells by centrifugation at 664 ×g for 20 minutes at room temperature. The serum was transferred into a clean microtube using a micropipette. Then, maintained at 2-8°C while handling and stored in freezer at -20°C, then transferred to -80°C before analysis with enzyme-linked immunosorbent assay (ELISA). Egg samples were collected, as described by Szczepkowski and Kolman (2011). An 8FR catheter was inserted through the genital opening to obtain oocyte samples, and 60 oocytes from each treatment were stored in a bottle filled with Bouin solution before measurement. For the measurement of egg, yolk, and perivitelline diameter, 100 eggs from spawning were used for each treatment.

SPAWNING METHOD

Fish spawning was performed after 30 days of rearing. To stimulate ovulation, the broods were injected into the intraperitoneal body using OVP at a dose of 0.5 mL kg⁻¹ body weight. The dose was calculated based on the weight of the fish and physiological NaCl was added as much as the OVP dose for injection. Sixteen hours after the injection, eggs were ovulated from the females by striping. The eggs were then artificially fertilized. The fertilized eggs were incubated in their respective containers according to the treatment until hatching.

MEASUREMENT OF RESPONSE PARAMETERS

The response parameters were the serum content of Mel, E₂, and VTG; oocytes diameter; eggs diameter; yolk di-

ameter; perivitelline diameter; gonadosomatic index (GSI); degree of fertilization (FR); and degree of hatching (HR).

MEL, E₂, AND VTG SERUM LEVELS: The serum contents of Mel, E₂, and VTG were obtained using ELISA kit specifically for fish, obtained from the Bioassay Technology Laboratory (Shanghai, China) with product numbers EA0001FI, EA0016FI, and E0020FI, respectively. Testing was carried out in accordance with the manufacturer's protocol and Buwono *et al.* (2024) as follows: Strips were inserted in the frame as much as the number for use. Prepare the blank well and sample well. Add 50µl standard solution to each well, and 50µl of serum (serum dilution thrice) and 50µl Biotinylated antigen were added to each well. The samples were mixed and covered with a sealer before incubation for 60 min at 37°C. Afterwards, the sealer and liquid were removed, and the wells were washed five times with 300µl wash buffer. The plate was inverted each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. After washing add 50µl Avidin-HRP to the standard well and the sample well, and the plate was covered with a sealer. After incubating the plate for 60 min at 37°C, the plate was washed as described before then add 50µl substrate solution A and 50µl substrate solution B were added to each well, and the plate was covered with a new sealer for 10 min at 37°C in the dark. Add 50µl of stop solution to each well, and the blue color immediately changed to yellow immediately. The optical density (OD) of each well was measured immediately at an absorbance of 450nm within 10 min after the stop solution was added. Serum levels were determined using an Infinite 200 PRO Nano Quant multimode reader (Infinite M200 Pro Nano-quant, TECAN, Austria), and the content of each sample was determined by comparison with a standard curve.

OOCYTE, EGG, YOLK, AND PERIVITELLINE DIAMETER: The diameters of the oocytes, eggs, yolk, and perivitelline were obtained from 60 oocyte and 100 egg for each treatment. This value is the average of the long and short axes of the oocyte or egg. Measurements were performed using a microscope (Olympus CX 21) equipped with an eyepiece micrometer at 40 × magnification. The diameter of the oocyte was measured every 10 days, whereas the egg, yolk, and perivitelline were measured after the egg was ovulated on day 30. Yolk diameter was measured using the same method as that used for the measurement of oocyte or egg diameter, while perivitelline was obtained by subtracting the diameter of the egg by the diameter of the yolk.

GSI, FR, AND HR: GSI was calculated by weighing the body and ovary of each fish with an accuracy of 0.1 g and GSI is calculated using the following formula:

$$GSI = \frac{Gonad\ weight(g)}{Body\ weight\ (g)} \times 100\%$$

FR was observed 10 hours after the fertilization process is carried out. The FR calculation was carried out using the following formula:

$$FR (\%) = \frac{\text{Number of fertilized eggs}}{\text{Number of eggs}} \times 100\%$$

HR was determined by observing the number of eggs hatched after 24 hours of fertilized eggs. The HR was calculated using the following formula:

$$HR (\%) = \frac{\text{Number of egg hatched}}{\text{Number of eggs}} \times 100\%$$

SR observations were carried out after the larvae were 3 days after hatching (dah). The SR calculation was performed using the following formula:

$$SR (\%) = \frac{\text{Final fish count (3 dah)}}{\text{The number of initial fish}} \times 100\%$$

DATA PROCESSING AND ANALYSIS

The data were tested with ANOVA, followed by the Duncan Multiple Range test (DMRT) to find significant differences between treatments. Experimental data were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). Statistical analysis was performed using SPSS v.26 software (IBM, NY, USA), with Trp as an independent variable, whereas the serum levels of Mel, E₂, VTG, GSI, oocyte diameter, egg diameter, yolk diameter, perivitelline diameter, and spawning parameters were dependent variables.

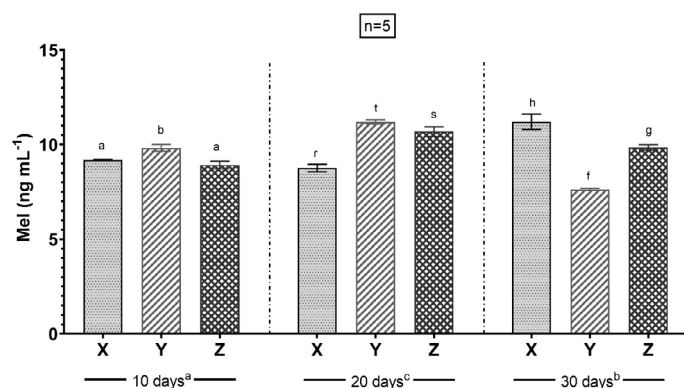


Figure 2: Mel Levels in catfish broodstock after treatment with Trp. Error bars represent the standard deviation (SD) of the mean. The superscript letters above the error bar and observation time show a significant difference between the means using the DMRT multiple range test at $p < 0.05$. Dashed lines separate the DMRT test results for observation times. If there is no significant difference due to the observation time, the difference in hormone levels at that time will use superscript letters above the error bars with the same letter range.

MEL

Statistical analysis was conducted using ANOVA and DMRT tests on the results of the ELISA measurements at a 5% significance level. The results indicated that the effect of Trp treatment on Mel levels was statistically significant ($p < 0.05$). Figure 2 illustrates the Mel levels at 10, 20, and 30 days for each treatment. On day 20 of treatment Mel levels were attained significant higher, were the highest values in the Y and Z treatments (11.20 ± 0.11 , and 10.69 ± 0.26 ng mL⁻¹, respectively); in contrast, the X treatment displayed the lowest value (8.77 ± 0.20 ng mL⁻¹). Thereafter, on day 30 of treatment, Mel levels in the X treatment reached the highest value, 11.21 ± 0.40 ng mL⁻¹, while the Y treatment demonstrated the lowest value, 7.63 ± 0.06 ng mL⁻¹. In treatment X, the lowest Mel levels (9.19 ± 0.04 ng mL⁻¹) were observed on the 10 days of treatment. These results show that an increase in Trp levels in the feed accelerates the increase in Mel levels in the blood serum.

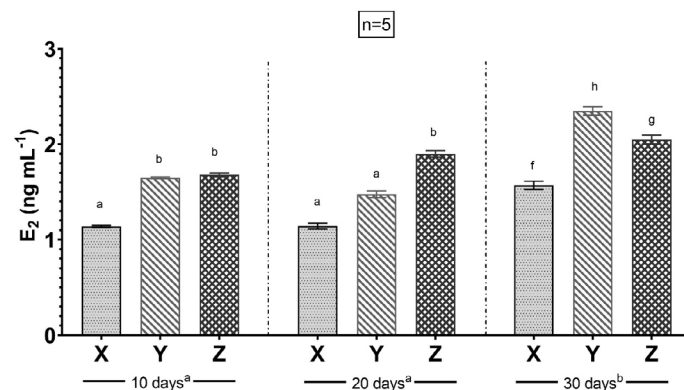


Figure 3: E₂ Levels in catfish broodstock after treatment with Trp. Error bars represent the standard deviation (SD) of the mean. The superscript letters above the error bar and observation time show a significant difference between the means using the DMRT multiple range test at $p < 0.05$. Dashed lines separate the DMRT test results for observation times. If there is no significant difference due to the observation time, the difference in hormone levels at that time will use superscript letters above the error bars with the same letter range.

E₂

The effect of Trp treatment on serum E₂ levels was significant (Figure 3) based on ANOVA and DMRT tests ($p < 0.05$). E₂ levels in treatments Y and Z were higher than those in treatment X at each observation time point (10, 20, and 30 days). Figure 3 shows that there was an increase in E₂ in treatments X, Y, and Z with the duration of treatment. Meanwhile, at treatment Y there is significant decreased on day 20 which was previously 1.65 ± 0.01 ng mL⁻¹ to 1.48 ± 0.04 ng mL⁻¹, then increased again on 30-day. The highest E₂ levels in X, Y, and Z treatments was obtained

on day 30, 1.57 ± 0.04 , 2.35 ± 0.05 , and 2.05 ± 0.05 ng mL⁻¹, respectively. In treatment X, the lowest E₂ level were detected on day 20 (1.14 ± 0.03 ng mL⁻¹), which did not show statistically significant differences from the values observed on day 10 (1.14 ± 0.01 ng mL⁻¹). However, treatment Z displayed the lowest E₂ level on day 10 (1.68 ± 0.02 ng mL⁻¹). After 20 days experimental duration, E₂ levels in treatment Z (1.90 ± 0.03 ng mL⁻¹) were found to be significantly increased compared to those observed in treatments X and Y. This result shows that the addition of Trp to the feed influences E₂ levels in African catfish.

VTG

Statistical evaluation of the ELISA data was conducted using ANOVA and DMRT tests, with a 5% significance level. The results indicated that Trp treatment had a significant effect on VTG levels ($p < 0.05$). As shown in Figure 4, the highest VTG levels in the serum occurred on day 30 and lowest on day 10 after treatments. Furthermore, for each treatments the higher VTG level in treatment Z (41.35 ± 0.40 ng mL⁻¹) was observed on day 30, then in treatments X (38.68 ± 0.47 ng mL⁻¹) on day 20, lastly in treatments Y (36.39 ± 0.48 ng mL⁻¹) was observed on day 30. Additionally, the lower VTG level in X (32.12 ± 0.48 ng mL⁻¹) and Z (33.81 ± 0.43 ng mL⁻¹) was observed on day 10, whereas in Y (34.36 ± 0.26 ng mL⁻¹) it was observed on day 20. These results show that the VTG content in the X and Z treatments continued to increase on days 10 to 30. Meanwhile, in treatment Y, there was substantial fluctuation in VTG levels. While VTG concentrations in the other treatments showed an increase on day 20, treatment Y demonstrated a decrease on this day, followed by a rise on day 30, with levels exceeding those observed on day 10.

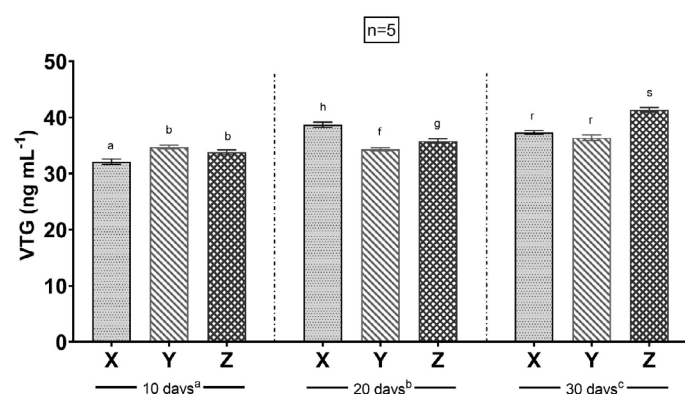


Figure 4: VTG Levels in catfish broodstock after treatment with Trp. Error bars represent the standard deviation (SD) of the mean. The superscript letters above the error bar and observation time show a significant difference between the means using the DMRT multiple range test at $p < 0.05$. Dashed lines separate the DMRT test results for observation times. If there is no significant difference due to the observation time, the difference in hormone levels at that time will use superscript letters above the error bars with the same letter range.

DIAMETER OOCYTE, EGG, YOLK, AND PERIVITELLINE

The results in Table 3 show that Trp had an effect on the diameter of oocytes after 30 days of treatment, based on the results of the ANOVA and DMRT tests ($p < 0.05$). However, on days 10 and 20, there was no significant effect on oocyte diameter ($p > 0.05$). Oocytes in treatment Z reached maturity by day 20. On day 30, based on the DMRT test, oocyte size in treatment X was significantly different ($p < 0.05$) from the other treatments, but no significant difference was observed between treatments Y and Z. The oocyte diameter in treatment X was smaller than in treatment Y. Additionally, the diameter in treatment Z was slightly larger than in treatment Y, with oocyte diameters in treatment Z ranging from 1.11 to 1.25 mm. These results suggest that increasing Trp levels up to 0.49% in the feed positively influenced the acceleration of oocyte maturation after 20 days of treatment.

Table 3: Diameter of oocytes during maintenance.

Parameters	Treatments		
	X	Y	Z
Diameter 10 days (mm)	0.70 ± 0.02	0.73 ± 0.06	0.76 ± 0.04
Diameter 20 days (mm)	0.94 ± 0.03	0.98 ± 0.04	1.05 ± 0.09
Diameter 30 days (mm)*	1.05 ± 0.03^a	1.09 ± 0.03^b	1.18 ± 0.07^b

Note: Values are presented as mean $\pm$ SD. The asterisk indicates that the treatment had a significant impact ($p < 0.05$), and different superscripts indicate a difference in the results after being tested with DMRT at a 5% significance level.

Table 4: Spawning and egg parameters.

Parameters	Treatment		
	X	Y	Z
Egg diameter (mm)*	1.52 ± 0.05^b	1.35 ± 0.01^a	1.53 ± 0.02^b
Yolk diameter (mm)*	1.27 ± 0.04^b	1.11 ± 0.01^a	1.27 ± 0.02^b
Perivitelline (mm)	0.25 ± 0.02	0.25 ± 0.01	0.26 ± 0.01
GSI (%)	22.28 ± 3.64	17.91 ± 1.85	20.53 ± 2.46
FR (%)	90.60 ± 1.69	94.86 ± 0.78	93.76 ± 0.93
HR (%)*	75.99 ± 3.95^a	87.22 ± 1.82^b	91.24 ± 1.12^b
SR 3 dah (%)	91.67 ± 4.41	95.00 ± 3.33	97.22 ± 1.92

Note: Values are presented as mean $\pm$ SD. The asterisk indicates that the treatment had a significant impact ($p < 0.05$), and different superscripts indicate a difference in the results after being tested with DMRT at a 5% significance level.

The results of ANOVA and DMRT for spawning and egg parameters are shown in Table 4. The treatment effects observed on egg diameter and yolk were significantly different ($p < 0.05$) but had a non-linear effect. This was evident from the fact that the diameter in the Y treatment is smaller than that in the X treatment, whereas the diameter in the Z treatment is larger than that in the X treatment. In addi-

tion, the observation results also showed significant differences in HR ($p < 0.05$), with the highest value in treatment Z and the lowest in treatment X. Meanwhile, perivitelline, GSI, FR, and SR did not show any significant differences in the results ($p > 0.05$). This shows that Trp treatment in feed only has an impact on egg diameter and yolk, but not on spawning parameters, except HR.

To visually determine the effects of Trp treatment on egg quality, fish gonads were histologically examined on day 30. The histological results showed differences in yolk cavities for each treatment (Figure 5). This is related to the density of the yolk, which is an important indicator of egg quality; the fewer the cavities in the yolk, the higher the density. The appearance of yolk cavities consecutively started from the lowest to the highest starting from treatments Z, X, and Y. These histological findings provide useful information on the potential effects of different Trp treatments on oocyte development and maturation. Therefore, it can be concluded that administration of the right dose of Trp in the diet can improve egg quality by increasing yolk density.

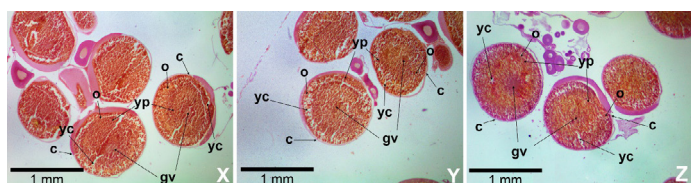


Figure 5: Gonadal histology after 30 days of treatment. Germinal vesicle, gv; yolk platelet, yp; yolk cavities, yc; oil, o; chorion, c. Bar scale = 1 mm, magnification 40 $\times$.

The amino acid requirements of Trp varies depending on its size and species. In this study, an increase in Trp levels in the feed was performed to determine the effects on hormones and reproductive outcomes in African catfish. Trp serves as a precursor to the production of the hormones serotonin and Mel, which are essential for regulating stress responses and behavioral patterns in fish (Teixeira *et al.*, 2023). Several studies have shown that Trp levels in different feeds vary depending on the type and size of fish. For example, Nile tilapia *Oreochromis niloticus* measuring 38.02 $\pm$ 0.1 g requires a Trp of 10.4 g kg⁻¹ protein (Zaminhan *et al.*, 2018), while those measuring 185.6 $\pm$ 27.8 g are 4.7–6.4 g kg⁻¹ protein (Cardoso *et al.*, 2022), and Indian carp *Cirrhinus mrigala* which is 0.62 $\pm$ 0.02 g requires a Trp of 9.5 g kg⁻¹ protein (Ahmed and Khan, 2005). Trp is influenced by many biological and environmental factors that can affect its absorption and metabolism of Trp (Prabu *et al.*, 2020). This statement was reinforced by FEEDAP (2014) and Hoseini *et al.* (2019), who suggested that Trp requirements in fish can range from 0.1% to 1.3% in the diet, depending on the species and its specific physiological needs. Therefore, the amount of Trp used in this study was divided into three levels: 0.33% as the control (Trp content in commercial feed), then periodically added as much as

0.08% for the next two treatments, so that it became 0.41% and 0.49%, in order to be able to determine the impact on hormones and reproductive outcomes in African catfish.

Based on the observation results, the addition of Trp levels to feed significantly affected Mel levels in the blood serum of African catfish ($p < 0.05$). The highest levels of Mel in the blood occurred in treatments Y and Z (11.20 $\pm$ 0.11 and 10.69 $\pm$ 0.26 ng mL⁻¹) after 20 days of treatment, faster when compared to treatment X (11.21 $\pm$ 0.40 ng mL⁻¹) where the highest values appeared at 30 days after treatment (Figure 2). This shows that the higher the Trp level in the feed given to African catfish broodstock, the faster the Mel level will increase. This result is in line with those of Ciji *et al.* (2013) and Devassykutty *et al.* (2022), where the Trp supplementation showed a positive correlation between Trp intake and Mel production. Trp content has been shown to increase Mel synthesis and reduce aggression in feed intake in African catfish broodstock. Similarly, Trp food supplementation has been shown to modulate aggression and stress responses in species such as rainbow trout (*Oncorhynchus mykiss*) and Senegal sole (*Solea senegalensis*) (Azeredo *et al.*, 2019; Winberg *et al.*, 2001). Under stressful conditions, proper Trp levels can enhance the immune response and reduce the incidence of stress-related disorders, thereby improving health, growth performance, and overall survival rates (González-Silvera *et al.*, 2018; Peixoto *et al.*, 2024; Prabu *et al.*, 2020). In addition, Trp can help maintain the Mel rhythm by synchronizing the reproductive cycle and optimizing spawning success (Peuhkuri *et al.*, 2012). This is strengthened by the presence of Mel receptors in the ovaries of fish (Sutradhar *et al.*, 2023; Zhao *et al.*, 2021), which indicates the presence of direct reproductive physiological effects on the regulation of steroidogenesis and folliculogenesis (Drag-Kozak *et al.*, 2018). Therefore, the best outcome for increasing Mel hormone synthesis in African catfish is Trp intake from Z treatment.

The increase in the hormone Mel due to the administration of Trp to African catfish also resulted in an increase in E₂. The results showed a significant difference ($p < 0.05$) in E₂ levels among the three treatments. E₂ levels are shown in Figure 3, the Y and Z treatments were higher than those in the X, 2.35 $\pm$ 0.05, 2.05 $\pm$ 0.05, dan 1.57 $\pm$ 0.04 ng mL⁻¹, respectively. Increased levels of dietary Trp have been correlated with increased E₂ concentrations in various teleost species (Akhtar *et al.*, 2012; Zaminhan *et al.*, 2018). This is because of the existence of a mechanism involving the HPG axis, where Mel acts as a neurotransmitter that modulates the release of GnRH, leading to increased secretion of LH and FSH. These hormones are essential for the regulation of E₂ synthesis in the ovaries, thus promoting oocyte maturation and vitellogenesis (Akhtar *et al.*, 2012; Azeredo *et al.*, 2017). Due to the increase in E₂, the process of growth and maturation of oocytes occurs (Table

3). Elevated concentrations of E_2 resulting from increased Trp administration play a pivotal role in accelerating oocyte development. This process is mediated by increased vitellogenesis due to increased E_2 levels, which is an important phase in oocyte maturation. This is evident from the results of this study, which showed an increase in E_2 levels in blood serum in African catfish on day 20 due to 0.49% Trp treatment.

In addition to E_2 , VTG hormone profile is a crucial indicator of gonadal maturity in various fish species. This relationship is primarily due to the close association between E_2 and VTG levels and the stages of ovarian oocyte development. E_2 plays a pivotal role in stimulating vitellogenesis, a process that involves synthesis and accumulation of VTG, which is essential for oocyte growth and maturation. The presence of E_2 triggers the liver to produce VTG, which is then transported to the ovaries, where it is incorporated into developing oocytes, thereby facilitating their growth and maturation (Chatakondi and Kelly, 2013; Finn and Cerdà, 2024). Empirical studies have demonstrated this relationship across various fish species, including the Channel catfish (*Ictalurus punctatus*) (Chatakondi and Kelly, 2013), Striped catfish (*Pangasianodon hypophthalmus*) (Pamungkas *et al.*, 2019), and Largemouth Bass (*Micropterus salmoides*) (Dominguez *et al.*, 2012). Additionally, research on Neotropical catfish (*Rhamdia quelen*) has shown similar patterns, indicating that VTG levels correlate with oocyte development stages (Fernandes *et al.*, 2021). Similarly, in the Chilean flounder (*Paralichthys adspersus*), VTG has been identified as a reliable biomarker for assessing reproductive status (Leonardi *et al.*, 2010). In Nile tilapia (*Oreochromis niloticus*), elevated VTG levels have been linked to enhanced reproductive performance and oocyte quality (Tirado *et al.*, 2017). Furthermore, studies on Fathead minnows (*Pimephales promelas*) have reinforced the significance of VTG as an indicator of reproductive health and maturity (Leese *et al.*, 2021). In the current study, the VTG values in treatments X, Y, and Z continued to increase significantly at each observation time, with the highest value on day 30. These results suggest that Trp supplementation influences the hormonal dynamics of E_2 and VTG, thereby accelerating the increase in oocyte size in African catfish, which serves as a key indicator of gonadal maturity (Table 3). The image presented in Figure 5 further illustrates this relationship between E_2 and VTG accumulation, which was significantly higher in treatment Z than in treatments X and Y. This trend underscores the role of Trp in enhancing reproductive parameters, particularly its effects on E_2 and VTG levels, ultimately leading to improved oocyte development and maturation.

The perivitelline diameter did not show significant differences among the three treatments ($p > 0.05$), suggesting that environmental factors had a dominant effect on this pa-

rameter. The perivitelline diameter is influenced by a combination of environmental factors, biochemical composition, and physiological processes during fertilization, with environmental factors being particularly important. Several studies have demonstrated that environmental conditions, such as temperature and salinity, significantly impact the perivitelline in fish eggs. Diameter of perivitelline are varied significantly in response to environmental stressors and the buoyancy (Chen *et al.*, 2021; Luo and Yang, 2023). Additionally, water quality and habitat conditions also affect the growth and biochemical profiles of fish, which in turn influence the perivitelline diameter (Habib *et al.*, 2023). These studies reinforce the conclusion that environmental factors are the key determinants of the perivitelline in fish eggs. Therefore, since the environmental conditions were similar across all treatments, no significant differences in perivitelline diameter were observed.

Trp levels, egg size, and yolk have a complex relationship with non-linear response patterns. The egg diameter and yolk after ovulation in the X and Z treatments (1.52 ± 0.05 and 1.53 ± 0.02 mm, respectively) showed a significant difference ($p < 0.05$) compared to the Y treatment (1.35 ± 0.01 mm) (Table 4), but not in the perivitelline cavity. The diameters of the eggs and yolk in the Y treatment were smaller than those in the X and Z treatments. Trp can increase the production of Mel, which is essential in modulate reproductive synthesis (Hoseini *et al.*, 2019; Maitra and Pal, 2017; Tirado *et al.*, 2017). However, when the Trp content in feed was increased, adverse results were obtained as observed in the treatment Y. The Trp content in the Y treatment negatively affected the diameter of the egg or yolk and VTG produced (Table 4). The decrease in diameter is due to a decrease in VTG levels on the 20 day (Figure 4). This can be caused by potential toxicity, metabolic disorders, and essential amino acid imbalances (Sun *et al.*, 2011). Excessive Trp can lead to increased levels of kynurenine, a metabolite that has neurotoxic effects and potentially interferes with normal hormonal signaling (Clua *et al.*, 2012). This is also interfere with the absorption of other amino acids and synthesis of VTG, and potentially cause a decrease in the size of the yolk because of the insufficient nutritional requirements for proper yolk formation (Ogawa *et al.*, 2023; Teixeira *et al.*, 2023). However, if Trp levels continue to increase, metabolic balance can be restored. At higher concentrations, the resulting negative effects could be reversed, leading to better outcomes, as observed in the Z treatment. This phase can be associated with increased resistance to stressors because Trp is known to modulate stress responses through its metabolites (Wolkers *et al.*, 2011). Several studies have shown that higher Trp levels can lead to increased synthesis of serotonin and Mel, which are neurotransmitters that play a role in various metabolic processes, potentially increasing yolk size and quality of the yolk (Hu *et al.*, 2018; Di Pizio and Nicoli, 2020). At opti-

mal doses, Trp is known to improve growth, feed efficiency, disease resistance, and reproductive system health; facilitate better absorption and utilization of nutrients during critical stages of egg development; and improve reproductive performance in various fish species (Abidi and Khan, 2010; Mi *et al.*, 2018; Wei *et al.*, 2024; Youssr *et al.*, 2016; Youssr and Howell, 2015). This non-linear relationship emphasizes that the right dose in the food formulation of African catfish can increase egg diameter, however, if the dose used is not appropriate, it may cause a decrease in egg diameter.

The impact of inappropriate Trp dosage on egg quality was also demonstrated by the histological analysis result that presented in Figure 5. Treatment Z showed the highest yolk density, followed by treatment X. In the treatment Y, despite having higher Trp levels than treatment X, it had the lowest yolk density and the worst egg quality among the three treatments. This observation is in line with the existing understanding that yolk density is an important determinant of egg quality, with denser yolk indicating a superior quality (Fouad *et al.*, 2021). Previous studies have shown that Trp can cause metabolic imbalances and significantly affect the yolk composition and egg quality (Fouad *et al.*, 2018; Reading *et al.*, 2018). Furthermore, a relationship between diet composition and yolk density has been documented, indicating that changes in diet can lead to significant changes in egg quality metrics (Swain *et al.*, 2023). These findings suggest that Trp dosage is not always linear with egg quality but must be appropriate and able to meet the physiological needs of fish. Therefore, it can be concluded that the use of Trp doses in treatment Z (0.49%) optimized reproductive performance and egg quality in African catfish.

The effect of Trp treatment on reproductive parameters (GSI, FR, and SR 3 dah) in African catfish was not significant ($p > 0.05$) but not on HR (Table 4). Similar results were obtained in European bass, Persian sturgeon, Black sea turbot, and juvenile Silver pompano, where Trp supplementation did not cause significant changes in GSI, FR, or SR (Aydin *et al.*, 2020; Devassykutty *et al.*, 2022; Machado *et al.*, 2019). According to (Li *et al.*, 2017) GSI and FR are influenced more by age, size, and health status factors. In addition, the absence of Trp on GSI, and FR may be due to the fact that these three indicators are more influenced by factors other than Trp. Results from other fish studies suggest that Trp supplements may provide benefits but are not separate from the interaction with environmental and genetic conditions to form better reproductive outcomes (Morbey and Mema, 2018; Bazin *et al.*, 2023; Gavary and Roberts, 2017). Trp can also affect embryonic development. Adequate Trp can optimize egg quality, maintain better embryonic development, and increase hatching success in various fish species (Hoseini *et al.*, 2019; Rezaei Aminloo *et al.*, 2019; Seifi Berenjestanaki *et al.*, 2014). Therefore, this

study observed a linear relationship between increasing Trp content in broodstock feed and HR, suggesting that higher Trp levels may enhance reproductive performance by positively influencing hatching success in African catfish.

Meanwhile, the SR 3 dah was not significantly affected because of the availability of yolk reserves that can be sufficient for the needs of larvae. Sufficient yolk reserves ensure that larvae can meet their metabolic needs from the beginning until they begin eating food. The utilization of the yolk is carried out fully from the post-hatching period to maintain the larvae during their development (Elfidasari *et al.*, 2017; Asiah *et al.*, 2022). The absorption efficiency of the yolk affects the allocation of energy to critical developmental processes, which in turn affects survival (Vlahos *et al.*, 2015). This suggests that the ability of larvae to utilize yolk reserves may affect their SR. However, as larvae transition to fingerlings, their nutritional requirements increase, making them more susceptible to the quality of the broodstock diet. This finding aligns with previous studies that have shown that the nutritional quality of broodstock can influence the growth and survival of offspring in later developmental stages (Izquierdo *et al.*, 2001; Langi *et al.*, 2024).

CONCLUSIONS AND RECOMMENDATIONS

Trp supplementation of 0.49% in dry feed had a positive impact on the acceleration of maturation of African catfish oocytes after 20 day treatment, where reproductive hormone regulates such as Mel (10.69 ± 0.14 ng mL⁻¹), E₂ (1.90 ± 0.03 ng mL⁻¹), and VTG (35.84 ± 0.37 ng mL⁻¹), resulting in an oocyte diameter of 1.18 ± 0.07 mm.

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NOVELTY STATEMENTS

This study is the first to investigate the effects of dietary Trp supplementation on melatonin, estradiol, and vitellogenin hormones, and their role in supporting gonadal maturation and reproductive success in African catfish broodstock. This research addresses a knowledge gap, as this topic has not been extensively explored in this species.

All the authors contributed to this study. Conceptualization, experimental performance, methodology, sample collection, formal analysis, and initial draft writing were conducted by Epro Barades. Iskandar, Ibnu Dwi Buwono, and Yuli Andriani supervised and provided critical feedback and discussed the writing results. Ayi Yustiati, Roffi Grandiosa, and Ratu Siti Aliah supervised the study. All authors have read and agreed to the final version of the manuscript. All authors acknowledge and agree with the submission and publication of this manuscript.

ETHICAL STATEMENT

This study was conducted in accordance with the guidelines and ethical standards of national health research and development in Indonesia and the Eighth Edition Guidelines for the Care and Use of Laboratory Animals. This study was approved by the research ethics committee of Universitas Padjadjaran with letter number 309/UN6. KEP/EC/2024.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest regarding the publication of this article.

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