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Effects of *Sesamum indicum* Seed Oil on Reproductive Performances, Egg Antioxidant and Biochemical Status in Female African Catfish (*Clarias gariepinus*) Broodstock

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Abstract

Fish, constantly exposed to environmental stressors due to their aquatic habitat and high metabolic rates, are susceptible to oxidative stress. This oxidative stress reduces reproductive performance and leads to economic losses for farmers. The *Sesamum indicum* seed oil, due to its bioactive molecules with various pharmacological properties, could mitigate the effects of these factors and enhance animal performance. This study was conducted to evaluate the effects of *Sesamum indicum* seed oil on the reproductive performances, egg antioxidant and biochemical reproductive parameters in *Clarias gariepinus*. A total of forty-eight (48) catfish broodstock (*Clarias gariepinus*) with an average weight of 150.35 ± 4.19 g were randomly assigned to 4 dietary treatment groups in a completely randomised design. Males were used exclusively for fertility testing. In 90 days, fish in group 1 (control) received diet without *S. indicum* seed oil, while the other diets were incorporated at 0.5%, 1%, and 1.5% (corresponding to 5, 10, and 15 g/kg of diet, respectively). To keep the diets iso-lipidic and iso-energetic, sesame oil was used as a direct substitute for an equal amount of the basal palm oil. At 13 weeks old, blood samples were collected from all fish of each treatment for reproductive hormone analysis. In addition, 8 grams of eggs were collected from each fish per treatment broken and homogenised, and egg samples were collected for oxidative stress markers and biochemical analysis. On the other hand, 16,000 eggs (2000/fish) were also collected per treatment for

egg characteristics, fertility assessment, and hatchability traits. The Fecundity, Fertility, Hatchability and survival rate significantly increased ($P < 0.05$) in fish receiving 15 g of *S. indicum* oil/kg diet compared to the control. The serum levels of LH, FSH and estradiol increased significantly ($P < 0.05$) in fish receiving 15 g of *S. indicum* oil/kg diet compared to both the control and the treatment that received 5 grammes of *Sesamum indicum* seed oil. In addition, the egg level of total Protein, Cholesterol, HDL, LDL, Triglycerides, and the egg level of SOD, CAT, and GPx significantly increased ($P < 0.05$) with a dose of *S. indicum* seed oil. However, the egg level of MDA was significantly decreased ($P < 0.05$) with a dose of *S. indicum seed* oil. It was concluded that administration of 15 g of *S. indicum* seed oil/kg diet enhances reproductive and biochemical parameters in female fish.

Keywords

Sesamum indicum Seed Oil, Egg Stress Markers, Reproduction Parameters, Biochemical Parameters, Females *Clarias gariepinus*

1. Introduction

The aquaculture industry deals with several stressful situations that can compromise the target species well-being, including handling, confinement, fertilization, transport, stocking density, temperature fluctuations and other operations, from the hatchery to the final commercial stage [1]-[5]. Stress induced by such practices has long been suspected to cause mortality, affecting the success in fish production with the consequent economic loss for this sector [6].

Oxidative stress mainly refers to the imbalance between reactive oxygen species production and antioxidant defence systems in organisms [7]. Accumulation of reactive oxygen species (ROS) increases lipid peroxidation and affects reproduction, gamete quality and progeny of a fish [8] [9]. Oxidative stress can also disrupt endocrine pathways related to fish reproduction [10] [11]. To counteract the adverse effects of oxidative stress, the antioxidant capacity of the animal can be reinforced by nutrient supplements of vitamin E [12] and Zinc [13]. Other substances with antioxidant properties, such as herbal medicine: mulberry leaf flavonoids [14], emodin [15], berberine [16], *Sophora flavescens* root extract [17], *Aloe vera* gel extract [18] and tea tree oil [19] are used. This aspect is particularly pertinent in intensively managed aquaculture settings where high stocking densities and suboptimal environmental conditions often give rise to heightened oxidative stress levels in fish populations [20].

Sesamum indicum seed is an important annual seed crop being cultivated as an ingredient for human food oil, meal, and animal feeds [21] [22]. The *Sesamum indicum* seed oil contains saturated fatty acid [23], 80% unsaturated fatty acids (linoleic acid and linolenic acid) [24], proteins (19% - 25%) [25], various minor nutrients such as vitamins (A, B, C…) and minerals (Zinc, Selenium…), lignans (Ses-

amin, Sesamol, Sesamolin, and Seasaminol) [26]-[29], and polyphenols, phytosterols, phenols, aldehydes, anthraquinones, naphthoquinones, triterpenoids [30], generally have extremely varied properties, including antioxidant properties [31]-[33]. Positive effects of *Sesamum indicum* seed oil on growth performance, feed utilisation, and fillet quality in fish were reported by Mai *et al.* [34], Nguyen *et al.* [35] and Nguyen and Tran [36]. In addition, Dada and Adeparusi [37] reported that *Sesamum indicum* seed powder at the level of 200 g/kg in female catfish broodstock diet increased the growth and reproductive indices compared to the control diet. This study showed some positive effects of *Sesamum indicum* seed powder on reproductive performances, but the effects of *Sesamum indicum* seed oil, which is highly concentrated in active molecules, show limited studies on animal reproduction. However, regarding its chemical composition and particularly its antioxidant properties, it could fight against oxidative stress and improve reproduction. Thus, the objective of the present study was to evaluate the effects of *Sesamum indicum* seed oil on reproductive performances, egg antioxidant and biochemical status in female African catfish (*Clarias gariepinus*) broodstock.

2. Methods and Methods

2.1. Study Area

The study was carried out at the Teaching and Research Farm of the Higher Institute of Agriculture and Management of Obala (LN 04˚10', LE 11˚31'). Obala is located about 1420 m above sea level. The climate is Guinean temperate by altitude, with about 2157 mm of rainfall spread over two seasons from mid-March to mid-June and mid-August and mid-October. The temperature is 20˚C - 32˚C with an average of 26˚C and relative humidity generally exceeds 55%.

2.2. Plan Material and *Sesamum indicum* Seed Oil Extraction

The plant material, *Sesamum indicum* seed, was purchased from a local spice market in Obala, Central Region, Cameroon. The seeds were sun-dried and ground into fine powder and were packed into a nylon bag until required.

Sesamum indicum seeds were purchased from the spice market in Obala. Crude sesame oil was extracted via cold pressing at 37˚C and filtered. To preserve bioactive compounds and prevent oxidation, the oil was sprayed (top-dressed) onto the pellets. Experimental diets were prepared in small batches every week and stored in airtight, light-shielded bags at 4˚C until feeding. The oil was subjected to phytochemical analysis. After extraction, the phytochemical screening was done according to the methods described by Ngbede *et al.* [38] and Banso and Ngbede [39] showed the following results in **Table 1**.

Table 1. Phytochemical constituents of *S. indicum* seed oil.

Constituents	Quantities (mg/100g)
Alkaloids	4.95

Continued

Steraoid	482
Flavonoid	18.50
Phenol	1.09
Lignans	1775

2.3. Animal Material

Forty-eight (48) catfish broodstock, each of *C. garepinus* (CG) (32 females and 16 males), were procured from reputable breeder farms in Minkama, Obala, Central Region of Cameroon. The fish (with an average initial weight of 150.35 ± 4.19 gr) were stocked into the concrete 16 tanks at a density of 3 fish per tank (1 m × 1 m × 1.25 m depth). The basis for selecting the broodstocks was the readiness of the genitals; the gravid female was based on a swollen, reddish genital opening, while the male was based on reddish and pointed genital papillae. The broodstocks were conditioned for two weeks in concrete tanks (100 m^2). During the acclimatization, the broodfish were fed a commercial diet of 40% crude protein twice daily before commencement of the experiment.

N.B: Males were used exclusively for fertility testing.

2.4. Experimental Diet

Feed ingredients, which comprise Fish meal, Soybeans, Yellow Maize, Vitamins, Premix and binder, were procured from Obala Road Fish Market. Forty percent (40%) crude protein diet was formulated using Pearson's square method and sesame oil was incorporated at 0.5%, 1%, and 1.5% (corresponding to 5, 10, and 15 g/kg of diet, respectively). To keep the diets iso-lipidic and iso-energetic, sesame oil was used as a direct substitute for an equal amount of the basal palm oil (Table 2). The soybeans were toasted locally using a frying pan. The Fish meal and Maize were ground into powder using a grinding machine, respectively. The feedstuffs were thoroughly mixed using hand; later, hot water and binder were added to the ingredients to form dough. The feed was pelleted using a hand pelletizer. The pelleted experimental diets were sun-dried and packaged accordingly.

Table 2. Ingredient composition (kg) and proximate composition (% DM) of basal diet.

Ingredients (gr/kg diet)	T1	T2	T3	T4
Fish meal	250	250	250	250
Corn meal	170	170	170	170
Soyabean meal	300	300	300	300
Blood meal	100	100	100	100
Palm oil	**100**	**95**	**90**	**85**
Sesam oil	**00**	**5**	**10**	**15**
Vitamin-mineral premix	30	30	30	30

Continued

Proximate composition				
Crude protein	40.4	40.4	40.4	40.4
Crude lipid	13.5	13.5	13.5	13.5
ASh	12.1	12.1	12.1	12.1
Gross energy (mL/kg)	16.9	16.9	16.9	16.9

Vitamin-premix-A Pfizer livestock product containing the following per kg of feed: A = 4500 I.U, D = 11,252 I.U, E = 71 I.U, K3 = 2 m, B12 = 0.015 mg, panthothenic acid = 5 mg, nicotinic acid = 14 mg, folic acid = 0.4 mg, biotin = 0.04 mg, choline = 150 mg, colbalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20 mg, iodine = 0.6 mg, selenium = 2.2 mg, zinc = 20 mg, antioxidant = 2 mg.

2.5. Experimental Design

The broodfish were randomly assigned to four dietary treatments, each performed in quadruplicate (4 replicates per treatment). A total of 48 fish were stocked into 16 concrete experimental tanks (1 m × 1 m × 1.25 m) at a density of three fish per tank, respecting a sex ratio of 1:2 (one male and two females per replicate). **N.B**: Males were used exclusively for fertility testing. All fish were fed their respective experimental diets throughout the study period. The control diet was without *S. indicum* seed oil (T1); while the other diets were included with 5, 10 and 15 g/kg as treatment in T2, T3 and T4 respectively (Table 2). The fish were fed twice daily (8.00 AM and 6.00 PM) at 5% of their body weight for the period of 90 days. After the 90 days of feeding, they were removed and bred artificially to test for their reproductive performance.

- **Milt Extraction**

Sixteen (16) males were sacrificed for milt extraction without hormonal inducement. Milt from males within the same treatment was pooled, thoroughly homogenized, and subdivided into eight equal portions. The collected milt was stored on ice (4˚C) to maintain viability. Fertilization was subsequently activated using a 9 g/L NaCl solution, maintaining a constant sperm-to-egg ratio of 1 mL per 2 g of eggs.

- **Hormonal inducement**

Female broodstock from each group were transferred to the hatchery and induced with a single dose of Ovaprim (0.5 mL/kg body weight). Following a 12-hour latency period, from each individual, 16 g of eggs were collected to be used for both fertilization trials and biochemical analysis.

3. Data Collection

3.1. Blood and Egg Sampling Preparation

Eight grams (8 g) of egg samples were collected from each fish and crushed in a mortar placed on ice containing 9 g/L NaCl solution to obtain 15% homogenates. The mixture was centrifuged at 3000 rpm for 30 min; the supernatant was then

removed and stored at −20˚C for the determination of biochemical and oxidative stress markers. Simultaneously, blood samples were collected from the caudal vein of each fish into non-heparinized tubes and centrifuged at 3000 rpm for 15 min. The resulting serum was stored in 1.5 mL Eppendorf tubes at −20˚C for reproductive hormone analysis.

3.2. Biochemical Analysis

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E_2) were determined using a commercial ELISA kit (Diagnosis Automation, Inc., Calabasas, USA). The supernatant of egg was assayed for protein, cholesterol, high-density lipoprotein (HDL), triglycerides, and low-density lipoprotein (LDL) using Calbiotech, Inc., Biochemical Kit and its designated protocol. While the oxidative markers such as lipid peroxidation (MDA), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were assayed as described in Jimoh [40].

3.3. Reproductive Parameters

3.3.1. Gonadosomatic Index and Fecundity Calculation

After 90 days of trial, each female broodstock was weighed using an electronic scale (SF-400, China) to the nearest 0.1 gr and the abdomen was cut with a pair of scissors and the ovaries were extracted. The ovaries were washed in normal saline solution to remove blood and ovarian fluid. Thereafter, the ovaries were weighed using an electronic scale (METLAR 5000D) to the nearest 0.1 gr. Gonadosomatic index (GSI) was calculated according to Mohammed *et al.* [41] as follows:

$$\%\ \text{Fertilization of eggs} = \frac{\text{Gonad weight}}{\text{Total weight of fish}} \times 100$$

Sub-samples of the ovaries weighing 1 gr were collected from each ovary from the anterior, posterior and mid part of the ovary. The sub-samples of the ovaries were fixed in Gibson fluid for 48 hours before counting the eggs. Fecundity was determined according to Mohammed *et al.* [41] as follows:

Fecundity = Total number of eggs in 1 gr of the ovary × Ovary weight (gr).

3.3.2. Eggs Characteristics

100 eggs were randomly selected weighed and preserved in 10% formalin for further egg biometric measurements. The diameter of ripped eggs was determined by using a simple ruler calibrated in millimetres scales.

3.3.3. Fertilization

Two grams of eggs, representing approximately 2000 oocytes, were collected from each fish and transferred into individual containers. The eggs were then fertilized with diluted milt and activated by the addition of 100 mL of 0.9% saline solution (9 g/L NaCl). After the first 5 minutes, the saline solution was decanted.

Incubation was conducted across four rearing tanks, each equipped with eight internal incubation basins. The fertilized eggs were uniformly spread in a mono-

layer on a kakaban (Shredded Nylon Sacks) and incubated in aerated indoor concrete tanks (during incubation, water levels were maintained at 30 cm^3 depth). Six hours after incubation, the color variations between the eggs were observed. Clear and transparent eggs were considered fertilized, while dead/white and opaque ones were regarded as unfertilized (dead eggs) and were siphoned out of the spawning tanks after 35 hours [42] [43].

The percentage fertilization was calculated by the average of five determinations of the fertilized eggs per 200 eggs siphoned based on color. Five different locations of the breeding tanks were marked, and 200 eggs were siphoned from each location, and the white eggs were recorded [42] [43]. The total larvae survival was determined by 10 days post-hatching. Percentage egg fertilization, hatchability and survival were determined as follows:

$$\%\ \text{Fertilization of eggs} = \frac{\text{Number of eggs fertilized}}{\text{Total number of eggs}} \times 100$$

$$\%\ \text{Hatchability} = \frac{\text{Number of hatchlings}}{\text{Total number of fertilized eggs}} \times 100$$

% Hatchability was obtained by direct counting of unhatched eggs as well as the number of eggs hatched in each incubating tank.

% Survival was calculated during initial feeding according to Otoh *et al.* [43] as the follows:

$$\%\ \text{Survival} = \frac{\text{Number of live larvae}}{\text{Total number of larvae hatched}} \times 100$$

3.4. Statistical Analysis

All data were subjected to a one-way analysis of variance (ANOVA) to determine the effects of sesame oil supplementation. The tank (n = 4 replicates per treatment) was defined as the experimental unit for all reproductive and biochemical parameters. Results are expressed as mean ± standard deviation (SD). Where significant differences were detected ($P < 0.05$), Duncan's Multiple Range Test was used to separate and compare the means. Additionally, Pearson's correlation coefficient (r) was employed to estimate linear relationships between the dietary sesame oil levels (0, 5, 10, and 15 g/kg) and the measured reproductive and biochemical parameters.

4. Results

4.1. Effects of *Sesamum indicum* Seed Oil on Growth Performance in Female *C. gariepinus*

The effects of *S. indicum* seed oil on Growth performances in Female *C. gariepinus* are shown in **Table 3**. *Sesamum indicum* seed oil had no significant effect ($P > 0.05$) on condition factor K and Gonad weight. Furthermore, there was a significant ($P < 0.05$) increase in both the final body weight and the gained body weight in female catfish in the group treated with 10 and 15 grammes of *Sesamum indicum* seed oil compared to the control group and which received 5 grammes.

Table 3. Effects of *Sesamum indicum* seed oil on growth performances in female *C. gariepinus*.

Parameters	*S. indicum* seed oil (g/kg diet)				*P*
	T1 = 0	T2 = 5	T3 = 10	T4 = 15	
Initial body weight (gr)	154.39 ± 1.90^{a}	154.70 ± 2.62^{a}	155.76 ± 1.25^{a}	154.44 ± 4.19^{a}	0.91
Final body weight (gr)	632.00 ± 2.00^{c}	634.00 ± 2.00^{c}	643.33 ± 6.80^{b}	656.33 ± 4.16^{a}	0.00
Gained body weight (gr)	477.61 ± 1.78^{c}	479.30 ± 0.81^{c}	487.56 ± 5.63^{b}	501.88 ± 1.64^{a}	0.00
Condition factor K	1.00 ± 0.02^{a}	1.02 ± 0.04^{a}	1.05 ± 0.03^{a}	1.08 ± 0.05^{a}	0.15
Gonade weight (gr)	28.76 ± 1.72^{a}	29.00 ± 2.64^{a}	32.00 ± 3.00^{a}	33.41 ± 3.37^{a}	0.18

a, b and c: On the same line, means with the same letter are not significantly different ($P > 0.05$), P = probability; Values are presented as means ± standard deviation.

4.2. Effects of *Sesamum indicum* Seed Oil on Reproductive Performance in Female *C. gariepinus*

Data in Table 4 indicated that:

Table 4. Effects of *Sesamum indicum* seed oil on gonadosomatic index (GSI) and reproductive parameters in female *C. gariepinus*.

Reproductive parameters	*S. indicum* seed oil (g/kg diet)				*P*
	T1 = 0	T2 = 5	T3 = 10	T4 = 15	
GSI	4.33 ± 0.28^{b}	4.79 ± 0.08^{ab}	4.97 ± 0.42^{ab}	5.09 ± 0.52^{a}	0.04
Fecundity (No. of eggs)	6986.00 ± 109.98^{b}	7076.00 ± 188.41^{ab}	7097.33 ± 188.58^{ab}	7261.67 ± 159.85^{a}	0.04
Diamters oocyte (mm)	1.86 ± 0.03^{b}	1.91 ± 0.09^{ab}	2.10 ± 0.10^{ab}	2.20 ± 0.20^{a}	0.03
Fertilization (%)	53.82 ± 5.08^{b}	60.62 ± 5.59^{ab}	61.47 ± 5.73^{ab}	67.67 ± 5.13^{a}	0.04
Hatchability (%)	61.27 ± 5.03^{b}	75.94 ± 3.87^{ab}	78.92 ± 3.39^{ab}	81.42 ± 5.81^{a}	0.02
Survival (14 days)	77.59 ± 2.58^{b}	83.82 ± 3.15^{a}	85.30 ± 3.23^{a}	87.27 ± 2.03^{a}	0.01

a and b: On the same line, means with the same letter are not significantly different ($P > 0.05$), P = probability; Values are presented as means ± standard deviation.

- Gonado-Somatique Index, Fecundity, Diameters Ooocyte, fertilization and hatching increased significantly ($P < 0.05$) in fish that received *Seasamum indicum* seed oil at a dosage of 15 g grammes of *Sesamum indicum* seed oil compared to the control group and those that received 5 and 10 grammes of *Sesamum*

indicum seed oil.

- Survival rate significantly ($P < 0.05$) increased in fish that received *Sesamum indicum* seed oil compared to the control group.

4.3. Effects of *Sesamum indicum* Seed Oil on Reproductive Hormones in Female *C. gariepinus*

The effects of *S. indicum* seed oil on reproductive hormones, as shown in Table 5, reveal that the serum level of FSH, LH and Estradiol increased significantly ($P < 0.05$) in fish treated with 15 grammes of *Sesamum indicum* seed oil compared to the control group and those that received 5 and 10 grammes of *Sesamum indicum* seed oil.

Table 5. Effects of *Sesamum indicum* seed oil on reproductive hormones in female *C. gariepinus.*

Reproductive Hormones	*S. indicum* seed oil (g/kg diet)				*P*
	T1 = 0	T2 = 5	T3 = 10	T4 = 15	
FSH (mUI/mL)	1.37 ± 0.15^{b}	1.37 ± 0.06^{b}	1.60 ± 0.16^{ab}	1.72 ± 0.20^{a}	0.05
LH (mUI/mL)	1.04 ± 0.06^{b}	1.08 ± 0.05^{b}	1.12 ± 0.02^{ab}	1.14 ± 0.01^{a}	0.02
Estradiol (pg/mL)	143.25 ± 3.85^{c}	145.13 ± 3.90^{bc}	149.96 ± 2.42^{ab}	151.81 ± 1.83^{a}	0.03

a, b and c: On the same line, means with the same letter are not significantly different ($P > 0.05$), P = probability; Values are presented as means ± standard deviation.

4.4. Effects of *Sesamum indicum* Seed Oil on Biochemical Parameters in Female Fish *C. gariepinus*

- **Serum Biochemical**

The effects of *S. indicum* seed oil on serum biochemical parameters are shown in Table 6. All biochemical serum characteristics increase significantly ($P < 0.05$) in catfish that received *Sesamum indicum* seed oil at dosages of 10 and 15 grammes of *Sesamum indicum* seed oil/kg diet compared to the control group that received 5 grammes of *Sesamum indicum* seed oil.

- **Egg Oxidative Stress Characteristics**

The effects of *S. indicum* seed oil on egg content in MDA and antioxidant enzymes, including GPx, CAT and SOD were shown in Table 6. The egg MDA concentration decreased significantly ($P < 0.05$) in catfish who received *Sesamum indicum* seed oil at dosages of 10 and 15 grammes of *Sesamum indicum* seed oil/kg diet compared to the control group and those received 5 grammes of *Sesamum indicum* seed oil.

The SOD activity increased significantly ($P < 0.05$) in female catfish at dosages of 10 and 15 grammes of *Sesamum indicum* seed oil/kg diet compared to the control group and which received 5 grammes of *Sesamum indicum* seed oil. The GPx and SOD activity increased significantly ($P < 0.05$) in female catfish at dosage of 15 grammes of *Sesamum indicum* seed oil/kg diet compared to the control group, and those received 5 and 10 grammes of *Sesamum indicum* seed oil/kg diet.

Table 6. Effects of *Sesamum indicum* seed oil on egg biochemical parameters and oxidative stress markers in female *C. gariepinus.*

Egg biochemical parameters (mg/dL)	*S. indicum* seed oil (g/kg diet)				*P*
	T1 = 0	T2 = 5	T3 = 10	T4 = 15	
Protein	15.98 ± 0.76^{b}	16.82 ± 0.26^{b}	17.81 ± 0.45^{a}	19.21 ± 0.56^{a}	0.00
Cholesterol	8.67 ± 0.21^{b}	9.14 ± 0.29^{b}	9.70 ± 0.35^{a}	9.71 ± 0.59^{a}	0.05
HDL	2.98 ± 0.14^{b}	3.26 ± 0.05^{b}	4.27 ± 0.11^{a}	4.55 ± 0.27^{a}	0.00
LDL	2.82 ± 0.72^{b}	2.99 ± 0.17^{b}	3.13 ± 0.02^{a}	3.61 ± 0.19^{a}	0.00
Triglycerides	22.29 ± 0.59^{b}	23.20 ± 1.06^{b}	25.01 ± 1.64^{a}	25.43 ± 2.16^{a}	0.03
Egg oxidative stress markers					
MDA (nmol/mL)	7.08 ± 0.33^{a}	6.97 ± 0.38^{a}	6.04 ± 0.54^{b}	5.80 ± 0.15^{b}	0.00
GPx (nmol/min/mL)	15.22 ± 0.59^{c}	16.84 ± 1.04^{bc}	17.60 ± 1.16^{b}	19.94 ± 0.92^{a}	0.00
CAT (U/mL)	20.92 ± 0.86^{b}	21.80 ± 0.74^{b}	22.42 ± 1.04^{b}	24.03 ± 0.31^{a}	0.00
SOD (U/mL)	16.63 ± 0.37^{b}	17.48 ± 0.71^{b}	19.21 ± 0.49^{a}	19,70 ± 0.24^{a}	0.00

a, b and c: On the same line, means with the same letter are not significantly different ($P > 0.05$), P = probability; Values are presented as means ± standard deviation.

- **Correlation between Egg Oxidative Stress Markers and Reproductive and Biochemical Parameters**

Correlation between egg oxidative stress markers and reproductive and biochemical parameters is shown in Table 7. This table reveals a negative and significant correlation was recorded between the MDA and: the total protein (r = −0.69; $P < 0.01$); lipid (r = −0.67; $P < 0.01$); Estradiol (r = −0.80; $P < 0.05$); the fertilization (r = −0.75; $P < 0.05$); the hatching (r = −0.69; $P < 0.01$) and the survival rate (r = −0.71; $P < 0.01$).

Table 7. Correlation between egg oxidative stress markers and reproductive and biochemical parameters.

Parameters	SOD	CAT	GPx	MDA
	***P*-value; r**	***P*-value; r**	***P*-value; r**	***P*-value; r**
Gonad weight (g)	0.53; 0.07	0.45; 0.14	0.65; 0.02	−0.42; 0.17
GSI	0.52; 0.08	0.41; 0.18	0.66*; 0.02	−0.55; 0.06
Fecundity rate	0.87; 0.05	0.74; 0.10	0.18; 0.56	−0.81; 0.07
Fertilization (%)	0.57; 0.05	0.71**; 0.01	0.50; 0.09	−0.75**; 0.00
Hatching (%)	0.83; 0.07	0.51; 0.08	0.43; 0.16	−0.69*; 0.01
Diamters oocyte (mm)	0.68*; 0.01	0.49; 0.10	0.58*; 0.04	−0.55; 0.06
Survival rate (21 days)	0.85*; 0.00	0.57; 0.05	0.69*; 0.01	−0.71*; 0.01
Protein	0.69*; 0.01	0.64*; 0.02	0.66*; 0.01	−0.74**; 0.00

Continued

Cholesterol	0.57*; 0.04	0.61*; 0.03	0.75** 0.00	−0.67*; 0.01
HDL	0.80**; 0.00	0.88 **; 0.00	0.95**; 0.00	−0.79**; 0.00
LDL	0.74**; 0.00	0.77**; 0.00	0.79**; 0.00	−0.69**; 0.01
TGS	0.33; 0.29	0.52; 0.07	0.69*; 0.01	−0.40; 0.19
FSH	0.58*; 0.04	0.73*; 0.00	0.70*; 0.01	−0.57; 0.05
LH	0.44; 0.14	0.41; 0.18	0.69*; 0.01	−0.39; 0.20
Estradiol	0.72**; 0.00	0.85**; 0.00	0.74**; 0.00	−0.80**; 0.00

*: Correlation is significant at the 0.05 level. **: Correlation is significant at the 0.01 level.

5. Discussion

Fish, constantly exposed to environmental stressors due to their aquatic habitat and high metabolic rates, are susceptible to oxidative stress [8]. In general, any kind of stressor can affect reproduction, gamete quality and progeny of a fish [8]. Because of its diverse properties, including antioxidant properties [31]-[33], *S. indicum* seed oil was given to female catfish *C. gariepinus* to alleviate the effect of oxidative stress to ameliorate its productive performances.

In this study, the incorporation of *S. indicum* seed oil in the basal feed at 10 - 15 grammes improved the growth performance, which corroborates the findings of Faheem *et al.* [44] and those of Al-Khalaifah *et al.* [45]. reported that, in African catfish (*Clarias gariepinus*), feeding Doum Palm fruit powder incorporated at 5 - 15 $g \cdot kg^{-1}$ of basal feed diets for 70 days improved growth parameters, including final body weight and body weight gain. Additionally, Eissa *et al.* [46] and Jastaniah *et al.* [47] discovered that curcumin and nano-curcumin respectively to red tilapia and European seabass (*Dicentrarchus labrax*) diets significantly enhanced the fish's growth performance. The observed growth enhancement can be attributed to the positive effects of *S. indicum* seed oil on various physiological processes. Ahmadi-far *et al.* [48] reported that phytochemicals enhance certain nutrient absorption, supporting overall health and reproductive success.

The results of this study suggest that dietary *S. indicum* seed oil at 15 g/kg diet improved fecundity, ovary weight, egg size, GSI, fertilization and hatchability. The current results are in agreement with the Enayat *et al.* [49], who observed similar results when the extract of *Vitex agnus-castus* was supplemented to Zebrafish and those of Abdel-Aziz *et al.* [18] reported the positive effect on the growth of gonads, ovarian maturation and fertility in female fish. The increase in the value of reproductive characteristics in the female catfish group that received *S. indicum* seed oil at 15 g/kg diet was related to the phytosterols and polyphenols compounds of *S. indicum* seed oil, which may lead to an increase in gonad weight, fecundity, egg size, GSI, fertilization and hatchability. In fact, the *S. indicum* seed oil contains several antioxidant compounds such as Vitamin C, Zinc, lignans, and flavonoids [19] [26]-[28]. The presence of these antioxidant compounds increased oxidative metabolism, especially in the final few days before hatching, as a normal result of

the high survival larvae rate. It is reported that over-increased lipid peroxidation may lead to tissue damage [50], whereas diets with antioxidant properties, such as *S. indicum* seed oil, may protect the embryo and therefore increase hatchability and larvae survival rate. A negative and significant correlation was recorded between the MDA and: survival rate ($\rho = -0.71$; $P < 0.05$); hatchability of fertile eggs ($r = -0.69$; $P < 0.05$) and fertility ($\rho = -0.75$; $P < 0.01$).

The present study indicated a significant ($P < 0.05$) increase in the serum levels of FSH, LH and Estradiol with the increase of *S. indicum* seed oil dose. The current results are in agreement with the findings of Qiang *et al.* [51] and, Shastak and Pelletier [52] who reported that adding Astaxanthin's at doses of 150 (mg/kg diet) during 60 consecutive weeks in female Nil tilapia significantly ($P < 0.05$) increased LH and FSH, and Estradiol serum level in this dosage as compared with the control group. The increase in the value of these reproductive hormones is due to the phytosteroid and phenols contained in *S. indicum* seed oil. These phytosteroids and polyphenols may stimulate the synthesis of estradiol by acting on the hypothalamic-pituitary-gonad axis [53], which in turn increases the activity of FSH and LH.

Concerning the effect of *S. indicum* seed oil on fish egg biochemical parameters, the results presented in this study revealed that administration of *S. indicum* seed oil increased the level of egg total proteins, cholesterol, LDL, HDL and triglycerides. These results are consistent with those of Yogeshwari *et al.* [54] and Debnath *et al.* [55] in female *Labeo gonius* and *Labeo rohita* treated respectively with turmeric extract and herbal diets. The increase of total proteins, cholesterol, LDL, HDL and triglycerides value observed in egg may be due to a number of antioxidant content in *S. indicum* seed oil, such as Vitamin C, flavonoid and lignans (Sesamin, Sesaminol…). These antioxidant compounds could act as a safeguard, shielding gametes from oxidative damage and upholding their functionality and viability [52]. Pintus and Ros-Santaella [56] reported that ova are exquisitely sensitive to oxidative damage due to their lipid-rich composition and limited antioxidant defences.

Antioxidant defence systems are the main reason behind the quenching of ROS under a natural physiological state. *S. indicum* seed oil has outstanding antioxidant properties due to its inherently present bioactive compounds, mainly vitamins C, minerals (Zinc, Selenium…), flavonoids and lignans (Sesamin, Sesaminol…) [26]-[29] and other organic acids (linolenic, linoleic, and decanoic acids) [57], which help in the heavy metal chelating properties and amelioration of oxidative stress markers. In the present investigation, the dietary inclusion of *S. indicum* seed oil at a dose of 15 g/kg of feed has resulted in increased activity level of the SOD, CAT, GPx and decreased MDA in eggs in female catfish. A similar observation was reported by Xie *et al.* [58], Eissa *et al.* [46] and Ngoumtsop *et al.* [59] who indicated that Astaxanthin, Curcumin powder and *Curcuma longa* essential oil increased activity of SOD, GPx and decreased MDA level respectively for the golden pompano (*Trachinotus ovatus*), Red tilapia (*Oreochromis* sp.) and the yolk eggs in female Japanese quail. The increase of SOD, CAT and GPx activity level in

eggs in treated catfish is related to the phenolic compounds because of their antioxidant property, which would reduce the peroxidation of cells or nutrients involved in the production of eggs and subsequently promote their good formation [59]. The high-performance indices observed in the fish that receive *S. indicum* seed oil may be explained by the inhibition of the deleterious effect of lipid peroxidation brought about by the decreased reagent oxidative species (ROS) generation as reflected by low egg MDA and high SOD, GPx and CAT activities. To maintain homeostasis, fish eliminate ROS to counteract oxidative stress and prevent or repair oxidative damage by antioxidant defense system, which includes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) [60] [61].

Lipid peroxidation represents a deleterious biochemical process characterized by the oxidative degradation of polyunsaturated fatty acids (PUFAs) within cellular membranes, which compromise structural integrity and essential cellular functions [50]. Consequently, malondialdehyde (MDA), a primary byproduct of phospholipid peroxidation, is frequently employed as a biomarker for assessing oxidative damage [50] [62]. In the present study, egg MDA concentrations were significantly reduced ($P < 0.05$) in fish supplemented with *Sesamum indicum* oil compared to the control group. This attenuation is likely attributable to the systemic transfer and subsequent yolk deposition of bioactive antioxidant constituents, specifically flavonoids and lignans (e.g., Sesamin and Sesaminol), which are prevalent in *S. indicum* oil and known to scavenge superoxide anions and hydroxyl radicals. Furthermore, a strong inverse correlation was established between MDA levels and oocyte diameter ($\rho = -0.98$, $P < 0.05$), as well as between MDA and egg cholesterol content ($\rho = -0.67$, $P < 0.05$). Our findings indicate that a reduction in lipid peroxidation, as evidenced by lower MDA levels, is associated with a concomitant increase in gonad weight and elevated egg cholesterol levels.

6. Conclusion

The results of the present study suggested that the inclusion of *S. indicum* seed oil in the female catfish diet can improve the reproductive, biochemical parameters and oxidative stress markers in female catfish. Based on these findings, *S. indicum* seed oil due to its various antioxidant compounds as flavonoids, lignans (Sesamin, Sesaminol…) and pharmacologically active compounds (phytosterols and polyphenols) can be considered a promising feed ingredient for managing reproductive processes in female fish at a level of 15 g/kg of diet. We address the fact that while sesame oil substitution improved reproductive performance, further research is required to evaluate the long-term effect on embryo development and larval survival beyond the yolk-sac stage.

Authors' Contributions

Conceptualization, H. V. N.; methodology, H. V. N., H. T., and F. N.; software, H. V. N., H. T. and F. N.; validation, H. V. N., H. T., and F. N.; formal analysis, H. V.

N., H. T., and F. N.; investigation and resources, H. V. N., H. T., S. N. D., D. A. K., G. T. N., and F. N.; data curation, H. V. N., H. T., S. N. D., D. A. K., G. T. N., and F. N.; writing original draft preparation, G. T. N. and S. N. D.; writing—review and editing, H. V. N., H. T., S. N. D., D. A. K., G. T. N., and F. N. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Experimental protocols used in this study were approved by the ethical committee of the Department of Animal Science of the University of Dschang (ECDAS-UDs 23/02/2015/UDs/FASA/DSAES) and were in conformity with the internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24 November 1986.

Data Availability Statement

Qualified researchers can obtain the data from the corresponding author.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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