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Research Article
Antioxidant Effects of Sesame Seed Oil (*Sesamum indicum*) on Semen Quality, Seminal Biochemistry and Hormonal Profiles in Male African Catfish (*Clarias gariepinus*)

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Abstract

Background and Objective: Fish are inherently susceptible to oxidative stress due to their continuous exposure to fluctuating environmental stressors, notably temperature. This physiological strain significantly impairs reproductive performance. Given its rich composition of bioactive molecules, *Sesamum indicum* seed oil (*Sio*) may serve as a potent dietary additive to mitigate these oxidative effects and enhance the overall reproductive performance of the species. This study was conducted to evaluate the impact of *Sio* on the reproductive performances, plasma seminal status and sexual hormones in *Clarias gariepinus*. **Materials and Methods:** A total of 48 catfish broodstock (*Clarias gariepinus*) with an average weight of 2.25 kg±15.20 g were randomly assigned to 4 dietary treatment groups in a completely randomised design. In 90 days, fish in group 1 (control) received diet without *Sio*, while the other diets were included with 0.5, 1 and 1.5% g/kg as treatment in T2, T3 and T4, respectively. At 13 weeks old, blood samples were collected from all fish of each treatment for sexual hormones analysis. In addition, 1.5 mL was collected from each fish per treatment, centrifuged and supernatant samples were collected for oxidative stress markers and biochemical analysis. On the other hand, 0.5 mL was also collected per treatment for semen characteristics. Data were analyzed using one-way ANOVA and Duncan's test ($p<0.05$) in IBM SPSS 21.0. **Results:** The sperm characteristics rate and the serum level of sexual hormones significantly increased ($p<0.05$) in fish receiving 1.5%/kg diet. In addition, the seminal plasma level significantly increased ($p<0.05$) with a dose of *Sio*. However, the seminal level of MDA was significantly decreased ($p<0.05$) with a dose of *Sio*. **Conclusion:** It was concluded that administration of 1.5% of *Sio*/kg diet enhances semen characteristics, plasma seminal status and sexual hormones in male fish.

Key words: *Sesamum indicum* seed oil antioxidant, semen characteristics status, sexual hormones, male *Clarias gariepinus*

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

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

INTRODUCTION

Water temperature is a primary environmental factor that profoundly influences the growth, survival and welfare of aquatic species[1]. Excessively high water temperatures or significant diurnal fluctuations induce thermal stress in fish, which can be exacerbated by cumulative effects[2]. Consequently, fish are subjected to continuous cycles of stress and recovery resulting from thermal oscillations[3]. Thermal variations are well-established triggers for oxidative stress, a condition arising when the surge in reactive oxygen species (ROS) overwhelms the body's natural defences[4]. This biochemical imbalance has profound implications for fish reproduction, where thermal fluctuations are known to undermine overall performance[5]. A major challenge lies in the unique architecture of the sperm membrane: Its high concentration of polyunsaturated fatty acids (PUFAs) creates a high-risk environment for oxidative decay. Coupled with a limited enzymatic antioxidant capacity, this makes the protection of sperm integrity a critical focal point in mitigating the effects of environmental stress[6]. Extensive literature has demonstrated that oxidative stress compromises several sperm functions, altering motility, viability[7] and DNA integrity[8], ultimately resulting in reduced fertilization rates[9]. To counteract these deleterious effects, the use of plants and their derivatives represents one of the most accessible, cost-effective and promising alternatives. Plants contain a diverse array of bioactive compounds with pharmacological properties beneficial to animal health[10] while remaining environmentally benign[11]. These plant-based products include extracts, powders, essential oils and vegetable oils.

Vegetable oil is a type of oil that's extracted from plants, like seeds, nuts, or fruits concentrates of bio-actifs compounds such as phenolics, polyphenols, terpenoids, saponins, quinines, esters, flavones, flavonoids, tannins and alkaloids which exhibit antioxidant, anti-inflammatory and immunostimulatory properties[12]. These molecules enhance stress resistance, growth and immune function in aquatic organisms[13]. Among the oil-bearing aromatic plants, sesame (*Sesamum indicum* L.) is notable for its exceptional oxidative stability, playing a crucial role in the food, pharmaceutical and cosmetic industries[14]. While the potential of sesame oil as a dietary additive to improve the reproductive performance and biochemical status of the Clariidae family remains unexplored, it has been observed that its use as a total replacement for fish oil in rainbow trout diets does not negatively affect semen volume, motility, or kinetic parameters[15]. In the Littoral Region of Cameroon, daily water temperatures in aquaculture ponds fluctuate between 22.4 and 32.8°C[16], exceeding the reported thermal neutrality zone for *Clarias gariepinus* 20-27°C[17]. Given the antioxidant properties of *Sesamum indicum* seed oil, it may mitigate the oxidative stress generated by these daily thermal variations and enhance reproductive outcomes. Therefore, the present study aimed to evaluate the effects of *Sesamum indicum* seed oil as a dietary additive on semen characteristics, seminal plasma antioxidant and biochemical status and sex hormones in male African catfish (*Clarias gariepinus*) broodstock.

MATERIALS AND METHODS

Study area: This work was carried out from February to May 2025 at the Cameroon Cooperative for Fisheries Development Unit in Ngodi Bakoko, Douala, located in the Littoral Region (Wouri Division, Douala III District). The Douala III municipal district is situated between Latitude 3°9'N and Longitude 9°08'E. Ngodi Bakoko lies at an elevation of approximately 1,420 m above sea level. The region is characterized by a high-altitude Guinean climate, with annual rainfall of approximately 4,200 mm distributed across two distinct seasons: A rainy season from mid-March to mid-November and a dry season from mid-November to mid-February. During the study period, ambient temperatures ranged from 22.4 to 32.8°C, with a mean of 27.6°C[18].

Plant material and *Sesamum indicum* seed oil extraction: The plant material, consisting of *Sesamum indicum* seeds, was procured from a local market in Obala, Central Region, Cameroon. The seeds were sun-dried and pulverized into a fine powder, which was stored in airtight nylon packaging until further use. To obtain the oil for the experimental diets, the seeds were processed using cold-pressing techniques. The extracted oil was then subjected to phytochemical screening following the protocols described by Ngbede *et al.*[19]. The qualitative phytochemical profile of the oil is summarized in Table 1.

Animal material and experimental design: A total of 24 male African catfish (*Clarias gariepinus*) broodstock, with an initial mean weight of 2.25 kg±15.20 g, were procured from reputable commercial hatcheries in Minkama, Obala (Central Region, Cameroon). The selection of broodstock was based on phenotypic maturity, specifically the presence of a reddish and elongated genital papilla. The fish were stocked in concrete tanks at a density of two individuals per tank, with each treatment performed in triplicate. Prior to the commencement of the trial, the fish underwent a two-week acclimatization period in 100 m^2 concrete tanks, during which they were fed a commercial diet containing 40% crude protein twice daily.

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

Constituents	Quantities (mg/100 g)
Alkaloids	4.95
Steroid	482
Flavonoid	18.50
Phenol	1.09
Lignans	1775

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

Ingredients	gr/kg diet
Fish meal	250
Corn meal	170
Soyabean meal	300
Blood meal	100
Vegetable oil	150
Vitamin-mineral premix	30
Proximate composition	
Crude protein	40.4
Crude lipid	13.5
Ash	12.1
Gross energy (mL/kg)	16.9

Vitamin-premix-A Pfizer livestock product containing the following per kg of feed: A: 4500 I.U, D: 11252 I.U, E: 71 I.U, K3: 2 m, B12: 0.015 mg, Pantothenic acid: 5 mg, Nicotinic acid: 14 mg, Folic acid: 0.4 mg, Biotin: 0.04 mg, Choline: 150 mg, Cobalt: 0.2 mg, Copper: 4.5 mg, Iron: 21 mg, Manganese: 20 mg, Iodine: 0.6 mg, Selenium: 2.2 mg, Zinc: 20 mg and Antioxidant: 2 mg

Experimental diet formulation and preparation: The basal feed ingredients, including fish meal, soybean meal, yellow maize, vitamins, premixes and binders, were sourced from the Obala market. An experimental diet containing 40% crude protein was formulated using the Pearson Square method. The chemical compositions of the diets were detailed in Table 2. Regarding processing, the soybeans were toasted to neutralize anti-nutritional factors, while the fish meal and maize were pulverized to a fine consistency. The dry ingredients were homogenized manually before the addition of warm water and a binder to achieve a dough-like consistency. The mixture was processed into pellets using a manual pelletizer and subsequently sun-dried. To incorporate the experimental treatments, specific concentrations of *Sesamum indicum* oil were applied to the respective rations via a top-dressing (spraying) technique. The final pellets were shade-dried to preserve the bioactive stability of the oil before being packaged for storage.

Experimental design and feeding trial: The male broodfish were randomly assigned to four dietary treatments in a completely randomized design, with each treatment performed in triplicate. The fish were housed in concrete experimental tanks (0.5×0.5×1.0 m depth). The experimental diets consisted of a control group (T1, 0% *S. indicum* oil) and three treatment groups supplemented with sesame seed oil at concentrations of 0.5% (T2), 1.0% (T3) and 1.5% (T4) per kg of feed. The fish were fed twice daily (08:00 and 18:00) at a rate of 5% of their total body weight for a duration of 90 days.

Sample collection and processing: At the end of the 90 day trial, three fish per treatment were randomly selected and anesthetized using clove oil (50 mg/L). Blood was drawn from the caudal vein using sterile 1 mL heparinized syringes (50 µL heparin). The blood samples were centrifuged (1075×g for 10 min at 4°C) and the resulting plasma was aliquoted and stored at -80°C for subsequent hormonal assays. Following blood collection, milt was harvested via abdominal massage (stripping), with careful measures taken to prevent contamination by water, feces, or urine, as described by Abascal *et al.*[20]. The milt was collected in 10 mL graduated centrifuge tubes and divided into two portions:

- A 0.5 mL aliquot was stored on ice (4°C) for immediate semen quality analysis
- A 2.0 mL aliquot was centrifuged (7063×g for 20 min at 4°C) and the supernatant (seminal plasma) was stored at -80°C for the assessment of redox status and biochemical composition

Evaluation of semen characteristics

Semen volume and pH: Milt volume was recorded directly from the graduated centrifuge tubes. The pH of the semen was measured in triplicate using a calibrated digital pH meter (Model pH ep®, Hanna Instruments, Italy).

Sperm motility and duration: Sperm motility and duration were assessed at room temperature (25.4°C) within one hour of collection. Activation was initiated by mixing 10 µL of milt

with 100 µL of an activation solution (0.3% NaCl) on a glass slide. Motility was observed under a light microscope (Leica DM 500) at 400 times magnification. Only spermatozoa exhibiting forward progressive movement were considered motile. The duration of motility was recorded using a stopwatch, beginning 3-5 sec post-activation and ending when all forward movement ceased.

Sperm density: Sperm density was determined using a hemocytometer counting chamber (ROHEM, India) after a 1000-fold dilution in 0.3% NaCl. A droplet of the diluted milt was placed on the slide, covered and allowed to settle for 3-5 min for sedimentation. Spermatozoa were counted in five large squares (area 0.04 mm^2 each) under 400 times magnification. The total concentration was calculated using the following formula:

$$\text{No. of cells/mL} = \text{Average No. of cells counted in the large squares} \times 10^4 \times \text{Dilution factor}$$

Biochemical analysis of seminal plasma

Composition of seminal plasma: To obtain seminal plasma, milt was centrifuged at 4000 rpm for 10 min at -20°C. The supernatant was collected and subjected to a second centrifugation to ensure the complete removal of spermatozoa. The resulting plasma was stored in Eppendorf vials at -20°C until analysis. Metabolite levels, including glucose, total protein, cholesterol, triglycerides and urea, were quantified using a UV-VIS spectrophotometer (Systronic 117). Specific diagnostic methods were employed for high sensitivity: Glucose was measured via the glucose oxidase/peroxidase (GOD/POD) method; total protein by the Biuret method; cholesterol via the cholesterol oxidase/phenol+aminophenazone (CHOD/PAP) method; triglycerides through the glycerophosphate oxidase-peroxidase (GPO-PAP) method; and urea using the modified Berthelot[21] method.

Oxidative stress and antioxidant assays: Total antioxidant capacity (TAC) was determined[22]. The reaction mixture comprised Na-benzoate (10 mmol/L), H_2O_2 (10 mmol/L), phosphate buffer (100 mmol/L, pH = 7.4) and a freshly prepared Fe-EDTA complex (2 mmol/L). After incubation with 10 µL of seminal plasma at 37° for 60 min, glacial acetic acid (20 mmol/L) and thiobarbituric acid (0.8% w/v) were added. Absorbance was measured at 532 nm following 10 min incubation at 100°C. TAC was calculated using the formula:

$$\text{Total antioxidant activity (mmol/L)} = \frac{(\text{CUA})\,(\text{K} - \text{A})}{(\text{K} - \text{UA})}$$

where, CUA represents uric acid concentration and K, A and UA represent the absorbance of the control, sample and uric acid solution, respectively. Lipid peroxidation (LPO) was assessed by measuring malondialdehyde (MDA) formation. Seminal plasma was mixed with trichloroacetic acid (TCA, 0.67 %) and heated in a boiling water bath for 45 min. After cooling in an ice bath and centrifuging at 2500 rpm for 10 min, the optical density of the supernatant was recorded at 532 nm.

Enzymatic antioxidants:

- **Superoxide dismutase (SOD):** The SOD activity was determined by the inhibition of pyrogallol auto-oxidation at 420 nm. One unit of SOD is defined as the amount of enzyme required to inhibit 50% of pyrogallol auto-oxidation in a 3 mL system. Results are expressed as U/min/mg protein
- **Glutathione peroxidase (GPx):** GPx activity was estimated by the rate of glutathione (GSH) consumption in the presence of H_2O_2. After 10 minutes of incubation, the reaction was stopped with 10 % TCA. Activity is expressed as micrograms of GSH consumed/min/mg protein
- **Catalase (CAT):** CAT activity was measured by monitoring the decomposition of H_2O_2 at 240 nm over 5 minutes. Activity is expressed as nmol H_2O_2 decomposed/min/mg protein

Endocrine assays: Plasma luteinizing hormone (LH, IU/L) was quantified using an automated Enzyme Immunoassay system (AIA-360; Tosoh India Pvt. Ltd.)[23]. Plasma testosterone concentrations (ng/mL) were measured using competitive Enzyme-Linked Immunosorbent Assay (ELISA) kits (DRG Instruments GmbH, Germany)[24]. The testosterone in the sample competed with a horseradish peroxidase-conjugated testosterone for binding sites on the microplate. Absorbance was read at 450 nm, with concentration being inversely proportional to optical density.

Statistical analysis: Data were analyzed using a one-way analysis of variance (ANOVA) to evaluate the effect of the different concentrations of *S. indicum* oil. Means were compared using appropriate *post-hoc* tests (Duncan) at a significance level of $p<0.05$. Linear correlation coefficient (R^2) was utilized to establish linear relationships between seminal redox status, reproductive parameters and biochemical markers. All analyses were performed using IBM SPSS Statistics (version 21.0).

RESULTS

Effects of *Sesamum indicum* seed oil on semen characteristics in male *C. gariepinus*: The physical and kinetic characteristics of sperm following treatment with varying concentrations of *S. indicum* seed oil are presented in Table 3. Overall, the supplementation of sesame seed oil led to a significant ($p<0.05$), dose-dependent improvement across all evaluated parameters.

There was a significant increase in sperm volume, rising from (2.46 ± 0.17^{a}) in the control group (T1) to (2.85 ± 0.25^{b}) treatment group (T4). Similarly, sperm density was significantly enhanced by the treatment; density values in T3 (57.35 ± 0.40^{b}) and T4 (57.70 ± 0.87^{b}) were significantly higher than those recorded in the control group (55.35 ± 1.57^{a}).

Sperm motility increased progressively with higher concentrations of oil, with T4 showing the highest percentage (78.32 ± 1.78^{c}) compared to the control (66.57 ± 4.17^{a}). Furthermore, the duration of motility was significantly extended ($p = 0.01$), reaching a maximum of 60.35 ± 2.01^{b} seconds in the T4 group.

The pH values of the semen rose from 7.24 ± 0.06^{a} in T1 to 7.57 ± 0.26^{b} in T4.

Effects of *Sesamum indicum* seed oil on semen biochemical in male *C. gariepinus*: The effects of *S. indicum* seed oil supplementation on seminal biochemical parameters are summarized in Table 4. A significant increase ($p<0.05$) dose-dependent was observed in the levels of energy substrates. Specifically, glucose and cholesterol levels rose significantly from the control T1 (0.52 ± 0.01^{a} and 0.19 ± 0.01^{a}, respectively) to the highest concentration group T4 (0.59 ± 0.01^{c} and 0.23 ± 0.01^{b}, respectively). Similarly, total protein and triglyceride concentrations were significantly augmented in the T3 (1.69 ± 0.05^{b}) and T4 (1.71 ± 0.01^{b} and 0.6 ± 0.01^{b}, respectively) groups compared to T1 (1.59 ± 0.01^{a} and 0.55 ± 0.10^{a}, respectively).

In contrast, urea levels exhibited a significant inverse relationship with the treatment concentration ($p = 0.03$). The highest concentration of urea was recorded in the control group (0.14 ± 0.02^{a}), while the lowest values were observed in the T3 (0.10 ± 0.01^{ab}) and T4 groups (0.10 ± 0.01^{b}).

The antioxidant status of the semen was significantly ($p<0.05$) improved by the inclusion of *S. indicum* seed oil. The TAC and CAT levels were significantly higher in T3 (6.09 ± 0.10^{b} and 9.57 ± 0.43^{b}, respectively) and T4 groups (6.13 ± 0.14^{b} and 9.75 ± 0.50^{b}, respectively) compared to the control (5.84 ± 0.16^{a} and 8.5 ± 0.40^{a}, respectively). As what concerns the SOD and GPx the higher values (2.23 ± 0.26^{b} and 51.02 ± 1.54^{b}, respectively) were observed in T4 treatment and the lowest values (2.08 ± 40.05^{a} and 49.12 ± 124^{a}, respectively) in the T1 treatment.

Concomitantly, a significant reduction in LP was observed ($p = 0.01$). LP levels decreased from 2.06 ± 0.14^{a} in the control group to 1.86 ± 0.09^{b} in the T4 group.

Effects of *Sesamum indicum* seed oil on sexual hormones in male *C. gariepinus*: The influence of *S. indicum* seed oil supplementation on the reproductive endocrine profile is summarized in Table 5. The data reveal a significant ($p<0.05$), dose-dependent enhancement of both LH and Testosterone levels across the treatment groups.

Serum LH levels showed a significant upward trend associated with increasing concentrations of *S. indicum* oil. While no significant difference was observed between the control (T1: 4.7 ± 0.35^{a}) and the 0.5% treatment group (T2) (5.07 ± 0.46^{a}), the highest concentration (T4) resulted in a significant elevation of LH levels, reaching (5.99 ± 0.63^{b}).

Testosterone concentrations were significantly augmented by the treatment. Testosterone levels raised progressively from 6.95 ± 0.19^{a} in the control group to a peak of 7.54 ± 0.21^{b} in the T4 (1.5%) group. Statistical analysis indicates that the T4 group was significantly different from both the control (T1) and the low-dose (T2) groups.

Table 3: Effects of *Sesamum indicum* seed oil on semen characteristics in male *C. gariepinus*

	S. indicum seed oil (%)				
Characteristics	T1 = 0	T2 = 0.5	T3 = 1	T4 = 1.5	p value
Sperm volume (mL)	2.46 ± 0.17^{a}	2.54 ± 0.08^{a}	2.73 ± 0.11^{ab}	2.85 ± 0.25^{b}	0.02
Motility (%)	66.57 ± 4.17^{a}	69.78 ± 6.01^{ab}	75.83 ± 2.74^{bc}	78.32 ± 1.78^{c}	0.05
Motility duration (S)	57.20 ± 1.18^{a}	57.9 ± 1.47^{ab}	58.9 ± 1.43^{ab}	60.35 ± 2.01^{b}	0.01
Sperm density (×10 mL)	55.35 ± 1.57^{a}	56.50 ± 1.15^{ab}	57.35 ± 0.40^{b}	57.70 ± 0.87^{b}	0.04
pH	7.24 ± 0.06^{a}	7.30 ± 0.05^{a}	7.42 ± 0.1^{ab}	7.57 ± 0.26^{b}	0.01

The a, b and c: The means of each row marked with different letters are significantly different ($p<0.05$): T1 (0% *S. indicum* seed oil), T2 (0.5% (*S. indicum* seed oil), T3 (1% *S. indicum* seed oil), T4 (1.5% *S. indicum* seed oil)

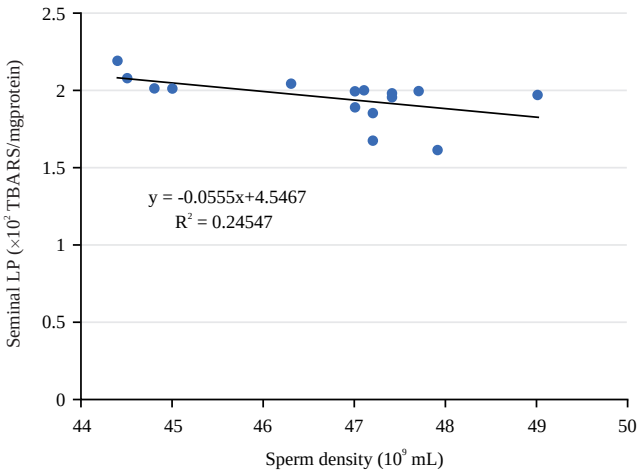

Fig. 1: Relationships between lipid peroxidation and spermatozoa density

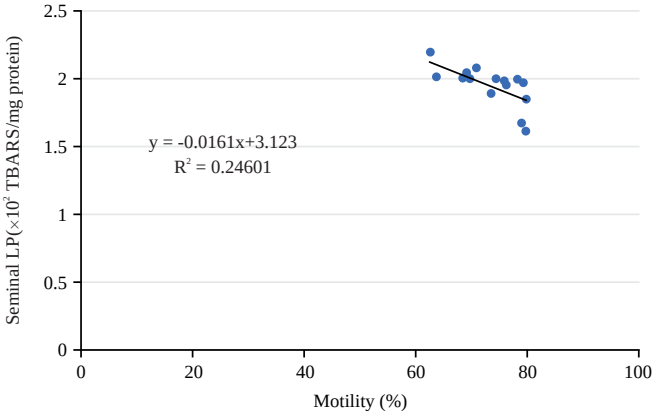

Fig. 2: Relationships between lipid peroxidation and spermatozoa mobility

Table 4: Effects of *Sesamum indicum* seed oil on seminal biochemicals in male *C. gariepinus*

	S. indicum seed oil (%)				
Seminal biochemical parameters (mg/mL)	T1 = 0	T2 = 0.5	T3 = 1	T4 = 1.5	p value
Glucose	0.52±0.01^{a}	0.55±0.03^{ab}	0.57±0.02^{bc}	0.59±0.01^{c}	0.01
Protein	1.59±0.01^{a}	1.63±0.04^{a}	1.69±0.05^{b}	1.71±0.01^{b}	0.00
Triglyceride	0.55±0.1^{a}	0.57±0.01^{a}	0.58±0.01^{ab}	0.6±0.01^{b}	0.01
Cholesterol	0.19±0.01^{a}	0.20±0.01^{ab}	0.21±0.01^{bc}	0.23±0.01^{c}	0.01
Urea	0.14±0.02^{a}	0.12±0.02^{a}	0.10±0.01^{ab}	0.1±0.01^{b}	0.03
Seminal oxidative					
Stress markers					
Total antioxidant (mmol/L)	5.84±0.16^{a}	6.04±0.14^{ab}	6.09±0.10^{b}	6.13±0.14^{b}	0.02
SOD (U/min/mg protein)	2.08±40.05^{a}	2.15±0.07^{ab}	2.22±0.07^{ab}	2.23±0.26^{b}	0.05
Gpx (ug/min/mg protein)	49.12±124^{a}	49.96±1.41^{ab}	50.32±0.46^{ab}	51.02±1.54^{b}	0.04
CAT (nm H_2O_2/mg protein)	8.5±0.40^{a}	9.1±0.26^{ab}	9.57±0.43^{b}	9.75±0.50^{b}	0.05
LP (×10² TBARS/mg protein)	2.06±0.14^{a}	2.01±0.17^{a}	1.86±0.02^{ab}	1.86±0.09^{b}	0.01

The a, b and c: The means of each row marked with different letters are significantly different (p<0.05): T1 (0% *S. indicum* seed oil), T2 (0.5% (*S. indicum* seed oil), T3 (1% *S. indicum* seed oil), T4 (1.5% *S. indicum* seed oil)

Table 5: Effects of *Sesamum indicum* seed oil on sexual hormones in male *C. gariepinus*

	S. indicum seed oil (%)				
Sexual hormones	T1 = 0	T2 = 0.5	T3 = 1	T4 = 1.5	p value
LH (mUl/mL)	4.7±0.35^{a}	5.07±0.46^{a}	5.4±0.02^{ab}	5.99±0.63^{b}	0.01
T (ng/mL)	6.95±0.19^{a}	7.19±0.24^{a}	7.26±0.15^{ab}	7.54±0.21^{b}	0.01

The a and b: The means of each row marked with different letters are significantly different (p<0.05): T1 (0% *S. indicum* seed oil), T2 (0.5% (*S. indicum* seed oil), T3 (1% *S. indicum* seed oil), T4 (1.5% *S. indicum* seed il) and T: Testosterone

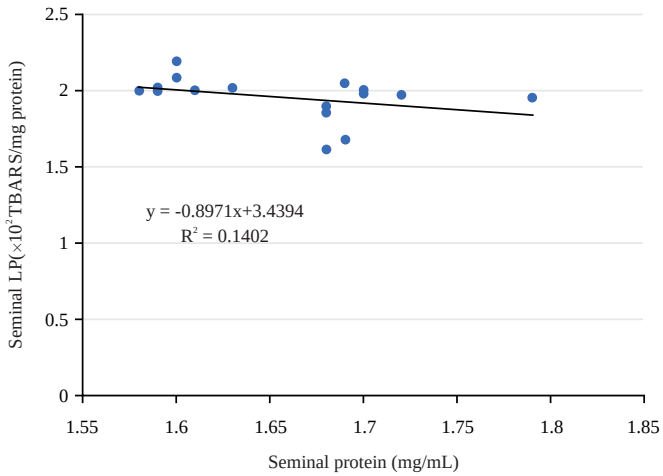

Fig. 3: Relationships between lipid peroxidation and seminal protein

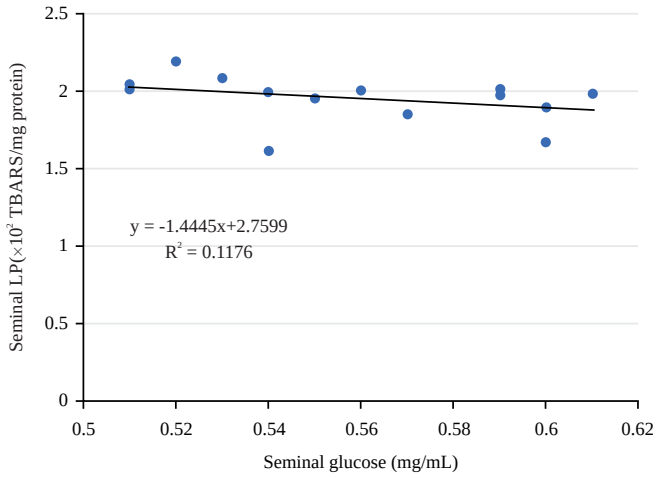

Fig. 4: Relationships between lipid peroxidation and plasma glucose

Relationships between seminal lipid peroxidation and semen characteristics, protein, glucose and total antioxidant activities

Relationships between lipid peroxidation and spermatozoa density: The relationships between seminal Lipid peroxidation and sperm density (Fig.1) reveal that sperm density showed a significant negative correlation with Lipid peroxidation levels ($R^2 = 0.24$; $p<0.05$).

Relationships between lipid peroxidation and motility: Figure 2 illustrated the relationships between lipid peroxidation and motility. It indicates that sperm motility was significantly and negative correlated with lipid peroxidation ($R^2 = 0.24$; $p<0.05$).

Relationships between lipid peroxidation and protein: The relationships between seminal lipid peroxidation and seminal protein (Fig. 3) reveal that seminal protein showed a significant negative correlation with Lipid peroxidation levels ($R^2 = 0.14$; $p<0.05$).

Relationships between lipid peroxidation and glucose: Figure 4 illustrated the relationships between lipid peroxidation and seminal glucose. It indicates that seminal glucose was significantly and negative correlated with lipid peroxidation ($R^2 = 0.11$; $p<0.05$).

Relationships between lipid peroxidation and total antioxidants activities: The relationships between seminal lipid peroxidation and Total antioxidants activities (Fig. 5) demonstrated that Total antioxidants activities showed a significant negative correlation with Lipid peroxidation levels ($R^2 = 0.09$; $p<0.05$).

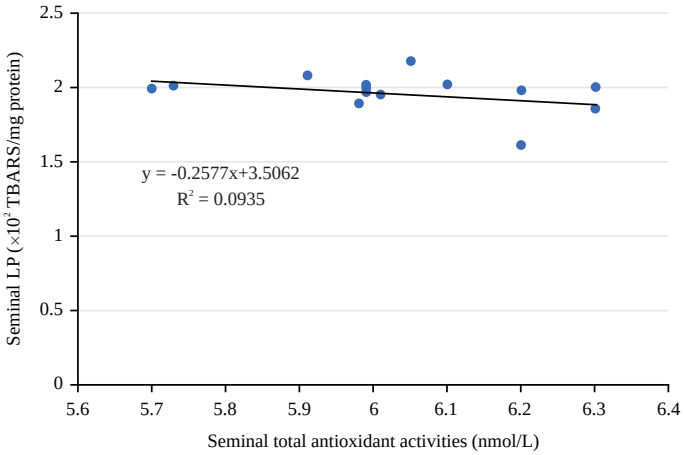

Fig. 5: Relationships between lipid peroxidation and Total antioxidants activities

DISCUSSION

Fish are inherently susceptible to oxidative stress due to their aquatic habitat and high metabolic rates[4]. Environmental stressors can detrimentally impact reproductive performance, gamete quality and larval viability. Because of its diverse properties, including antioxidant properties[25], *S. indicum* seed oil was given to female catfish *C. gariepinus* to alleviate the effect of oxidative stress to ameliorate its productive performances.

In the present study, dietary supplementation with *S. indicum* seed oil at 1.0-1.5% significantly enhanced sperm motility, density, volume and pH. These findings align with[26], who reported improved semen parameters in Nile tilapia (*Oreochromis niloticus*) supplemented with pumpkin seed oil and[26], observed similar improvements in *Mugil liza* following Vitamin C administration. The enhancement of semen characteristics is likely attributable to the high concentration of phytosterols and polyphenols in sesame oil. These bioactive compounds, including Vitamin C, flavonoids and lignans (e.g., sesamin and sesamolin), function as potent antioxidants[27]. By mitigating lipid peroxidation (LPO), which is known to cause structural damage to sperm membranes[28] these antioxidants preserve sperm functionality. This is further supported by the significant negative correlation observed between LPO levels and both sperm density (R^2 = -0.24; $p<0.05$) and motility (R^2 = -0.24; $p<0.05$), suggesting that as oxidative damage decreases, gamete quality concurrently improves.

Dietary inclusion of 1.5% *S. indicum* oil resulted in a significant increase in serum Luteinizing Hormone (LH) and testosterone levels. This mirrors the results[29], noted increased gonadotropin and androgen levels in male Japanese quail supplemented with essential oils. The phytosterols and polyphenolic fractions of sesame oil may modulate the hypothalamic-pituitary-gonadal (HPG) axis, stimulating the synthesis and release of these key reproductive hormones[30]. The upregulation of testosterone is particularly critical, as it directly governs spermatogenesis and the development of secondary sexual characteristics in male teleosts.

The administration of *S. indicum* oil increased the concentrations of glucose, total protein, triglycerides and cholesterol in the seminal plasma. These results are consistent with studies on *Labeo gonius* and *Labeo rohita* using herbal additives[31]. These metabolites serve as essential energy substrates and structural components for spermatozoa; for instance, seminal glucose and lipids provide the necessary fuel for motility duration[32]. A significant negative correlations found between LPO and total antioxidant capacity (R^2 = - 0.09; $p<0.05$); total protein (R^2 = 0.14; $p<0.05$) and glucose (R^2= 0.11; $p<0.05$).

The antioxidant defence system, comprising enzymes such as SOD, CAT and GPx, is vital for neutralizing reactive oxygen species (ROS) under physiological conditions. In this study, 1.5% sesame oil inclusion significantly increased SOD, CAT and GPx activities while reducing MDA levels. Similar antioxidant improvements have been documented with pumpkin seed oil and *Tribulus terrestris* in tilapia[32,33]. Our results suggest that lipid peroxidation (LPO) remains a critical factor in the loss of membrane integrity and the subsequent decline in sperm quality. As fish spermatozoa are particularly rich in Polyunsaturated Fatty Acids (PUFAs), they are highly susceptible to oxidative deterioration[34]. The observed reduction in MDA concentrations, a chemical signature of phospholipid peroxidation, indicates that the lignans and phenolic compounds derived from sesame oil successfully integrated into the seminal plasma. These bioactive compounds likely functioned as potent radical scavengers,

shielding the sperm membranes from LPO-induced damage and preserving vital cellular functions[30,35]. This protective mechanism is highlighted by the significant negative correlations found between LPO and total antioxidant capacity (R^2= - 0.09; $p<0.05$).

CONCLUSION

The findings of this study demonstrate that dietary supplementation with *Sesamum indicum* seed oil significantly improves reproductive performance, seminal plasma biochemistry and the antioxidant status of male *Clarias gariepinus*. Given its high concentration of bioactive lignans (sesamin, sesamolin...) and phytosterols, *S. indicum* oil at a dose of 1.5% per kg of diet is a promising natural feed additive for enhancing male broodstock productivity.

Further research is warranted to evaluate the effects of this oil on sperm DNA integrity, larval quality and sperm kinetics.

SIGNIFICANCE STATEMENT

Aquatic environmental stressors significantly impair fish reproduction through oxidative damage. Our research identifies *Sesamum indicum* seed oil as a key dietary additive that enhances male *Clarias gariepinus* fertility. Study show that sesame oil's bioactive compounds not only boost the systemic antioxidant defense system (SOD, CAT, GPx) but also improve sperm motility and hormonal profiles. This study provides a practical nutritional strategy to combat lipid peroxidation in fish spermatozoa, ensuring better seed production and sustainability in the catfish industry.

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