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Chasmanthera dependens Root Extract as a Dietary Supplement: Impacts on Reproduction Biomarkers in *Clarias gariepinus* Broodstock

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Abstract

This study investigated the effects of dietary supplementation with *Chasmanthera dependens* on the reproductive performance and histopathological health of *Clarias gariepinus* (African Catfish). A total of 75 healthy adult fish were randomly assigned to five dietary treatments: a control group with no additives and four groups receiving *C. dependens* at 10g, 20g, 30g, and 40g per kg of feed. The experimental duration lasted 90 days, during which parameters such as sperm quality, egg quality, and histopathological observations of gonads were assessed. Results indicated that the control group exhibited superior sperm quality, including the highest milt volume, motility, and live/dead ratio, compared to all treatment groups. Fecundity, egg size, fertilization rates, and hatchability percentages were also highest in the control group. Conversely, groups receiving higher levels of *C. dependens* demonstrated significantly lower ($p<0.05$) reproductive performance metrics, suggesting adverse effects linked to excessive supplementation. Histopathological analysis revealed no observable lesions in the testes and ovaries of the control and lower treatment groups, with healthy seminiferous tubules and advanced vitellogenesis. However, the group receiving the highest level of *C. dependens* exhibited signs of congestion in the ovaries, indicating potential detrimental effects. Overall, while *C. dependens* may offer reproductive benefits at moderate levels, but excessive dietary inclusion negatively impacts sperm and egg quality as well as gonadal health. Further research is recommended to establish optimal inclusion rates for maximizing the beneficial effects of *C. dependens* while minimizing adverse impacts on reproductive health.

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INTRODUCTION

The African catfish, *Clarias gariepinus*, is a major cultivated fish with significant commercial value in Nigeria, making it ideal for captive breeding (Arong and Eyo, 2017; Ondhoro *et al.*, 2015). However, several limitations affect fry production, necessitating the development of better broodstock management techniques (Anetekhai *et al.*, 2013; Hecht, 2013). One significant limitation is the inadequate understanding of broodstock nutrition, which is crucial for optimizing reproductive performance (Mandal *et al.*, 2013). Poor dietary formulations can lead to suboptimal spawning rates and reduced fry viability of offspring (Kumar *et al.*, 2018; Durigon *et al.*, 2019). Broodstock nutrition remains one of the least understood areas of finfish nutrition, often hampered by the need for specialized facilities and the associated costs of conducting extended feeding trials (de Oliveira *et al.*, 2014). Addressing this limitation through enhanced nutritional strategies could significantly improve both the quality and quantity of fish seed production, ultimately contributing to the sustainability of the aquaculture industry (Mabroke *et al.*, 2013).

Medicinal plants have been employed to enhance fertility, with research confirming the pro-fertility effects of various herbs in animals and fish (Dada and Aguda, 2015; Dada, 2012). Hence, incorporating medicinal plants into aquaculture diets can enhance fish reproductive health as they contain antioxidants that can enhance fertility, either directly or indirectly through improved sperm count, motility, and morphology (Dada *et al.*, 2019; Agbabiaka and Ezeafulukwe, 2013). In addition, medicinal plants enhance immunity and stress tolerance, providing an alternative to synthetic growth promoters (Dawood *et al.*, 2018; Valenzuela-Grijalva *et al.*, 2017). Furthermore, the use of medicinal plants is sustainable and cost-effective, potentially addressing the challenges of aquaculture while improving fish quality and yield (Jitendrasinh *et al.*, 2024; Emeka *et al.*, 2014).

Chasmanthera dependens (Hochst), a member of the Menispermaceae family, is typically found along forest margins and in secondary forests throughout West Africa. It is well known for its traditional applications in the treatment of various health conditions (Ogunlesi *et al.*, 2008). The availability of *C. dependens* throughout the year in tropical regions presents a practical solution for fish farmers looking to enhance fertility in their broodstock. Specifically, the aqueous extract of *C. dependens* has been shown to enhance fertility in male rats (Quadri and Yakubu, 2017), while the plant also exhibits analgesic and antiinflammatory properties (Kola-Mustapha *et al.*, 2020; Chidi and Assumpta, 2017). Furthermore, extracts from its roots demonstrate significant antifungal activity against various pathogens (Enenebeaku *et al.*, 2022; Aina *et al.*, 2019). Chemical screening of *C. dependens* has revealed the presence of various bioactive metabolites, including phenols, alkaloids, carbohydrates, flavonoids, terpenes, reducing sugars, tannins, and steroids (Ediah *et al.*, 2025; Tijani *et al.*, 2018). These compounds, particularly flavonoids and phenols, are recognized for their free radical-scavenging abilities and antioxidant properties. As such, these metabolites are thought to contribute significantly to the plant's therapeutic effects. Antioxidants, known for their ability to neutralize free radicals, are crucial in mitigating oxidative stress and related diseases. The leaf, stem, and root extracts of *C. dependens* have shown significant antioxidant activities (Asadu *et al.*, 2018). Additionally, *C. dependens* extracts exhibit ferric reducing antioxidant power, which reflects the bioactive components' ability to donate hydrogen (Shidduraju *et al.*, 2012), further supporting the plant's antioxidant properties.

The bioactive compounds in *C. dependens* offer a promising potential for enhancing fish reproduction. The plant's antioxidant properties, especially through its

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flavonoids and phenols, could play a role in improving reproductive health by reducing oxidative stress in fish. This reduction in oxidative stress can promote better cell function, egg quality, and overall reproductive performance. By harnessing these properties, extracts of *C. dependens* could be used to develop natural supplements or treatments aimed at improving reproductive outcomes in aquaculture, thus enhancing fish production efficiency and sustainability. Despite its potential, there is a lack of information on the efficacy of *C. dependens* roots as fertility enhancers in fish, highlighting the need for further research in this area.

The study investigated the dietary effects of *C. dependens* root on the reproductive biomarkers of *C. gariepinus* broodstock. It specifically examined its impact on sperm quality, fertilization success, and the histological characteristics of the tests and ovaries. Through this research, we aimed to deepen our understanding of how dietary supplementation with *C. dependens* influences reproductive factors in *C. gariepinus*, potentially providing a sustainable method for enhancing fish fertility and improving aquaculture practices in Nigeria.

METHODOLOGY

Ethical Approval

The study was conducted in line with the approved guidelines for animal handling in the Faculty of Agricultural Production and Renewable Resources of Olabisi Onabanjo University.

Place and Time

The study was conducted at the Fish Nutrition laboratory of the Faculty of Agricultural Production and Renewable Resources, Olabisi Onabanjo University, Nigeria, in 2023.

Research Materials

The devices employed for this study include a microscope (Olympus CX21), digital camera (AmScope, MU1803), and hemocytometer (Hawksley & Sons Ltd).

Research Design

The experiment employed a design consisting of five treatments, each replicated three times. Each replicate involved the random stocking of tanks with five brood stocks of *C. gariepinus* (three females and two males), each with an average weight of 700 g.

Work Procedure

Research Location

The experiment took place at the Teaching and Research Farm of the College of Agricultural Sciences, Olabisi Onabanjo University, located in Ayetoro, Ogun State, Nigeria. The university campus is situated in a deciduous/derived savannah zone at a latitude of 7°01’51” N and a longitude of 3°03’1” E. The climate in this region is classified as subhumid tropical, characterized by an annual rainfall of 1,909.3 mm. The rainy season typically spans from early April to late October, with a bimodal rainfall pattern that peaks in June and September. Maximum temperatures range from 29°C during the wet season to 34°C at the onset of the dry season, while the mean annual relative humidity is 81%.

Experimental Plant

Fresh roots of *C. dependens* were collected from the vicinity of the Teaching and Research Farm at the College of Agricultural Sciences, Ayetoro Campus, Ogun State. Identification and authentication of the plant material were conducted at the Department of Crop Production, College of Agricultural Sciences, Olabisi Onabanjo University. The collected roots were air-dried at room temperature, then mechanically ground into a powdered form using a household grinder (Silver Crest SC-9880) and sieved through a household sieve. The resulting powdered root was stored in a clean, dry container.

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Experimental Fish

A total of seventy-five healthy adult *C. gariepinus* were obtained from a local fish farm in Ayetoro, Ogun State. The fish were acclimatized in a prepared tank (1 x 2 x 1.5 m) for two weeks, during which they were fed a commercial diet containing 40% crude protein.

Experimental Diet

The powdered *C. dependens* was incorporated directly into the basal diet, which also contained 40% crude protein. The levels of the additive were set at 10 g/kg, 20 g/kg, 30 g/kg, and 40 g/kg, with a control diet that contained no additive. The basal diet was formulated using a mixture of fishmeal, soybean meal, groundnut cake, and yellow maize, along with fixed ingredients such as vitamin premix, lysine, methionine, salt, and vegetable oil. The required weights of each ingredient were determined using the Pearson square method. All five formulated diets underwent a careful preparation process that included measuring, grinding, mixing, and pelletizing with a hand-held pelletizing machine (using a 4 mm die), followed by sun drying, cooling in open air, and storage in opaque nylon bags according to treatment until the experiment commenced. The percentage composition of the feed ingredients is detailed in Table 1.

Table 1. Ingredients Composition (g/100g) of the Experimental Diets.

Ingredients (%)	T0	T1	T2	T3	T4
Fishmeal	31.2	31.2	31.2	31.2	31.2
Soybean meal	15.6	15.6	15.6	15.6	15.6
Groundnut cake	15.6	15.6	15.6	15.6	15.6
Yellow maize	30.5	30.5	30.5	30.5	30.5
Vitamin Premix	1.0	1.0	1.0	1.0	1.0
Lysine	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5
Vegetable oil	4.0	4.0	4.0	4.0	4.0
Methionine	0.5	0.5	0.5	0.5	0.5
DCP	0.5	0.5	0.5	0.5	0.5
C. dependens	0.0	1.0	2.0	3.0	4.0

Fish Feeding

Before the experiment commenced, all fish were starved for 24 hours to eliminate weight variation due to residual food in their guts and to enhance their appetite. The fish were fed according to the designated dietary treatments: T0 (Control diet without any additive), T1 (Addition of 10 g of *C. dependens* per 1 kg of basal diet), T2 (Addition of 20 g of *C. dependens* per 1 kg of basal diet), T3 (Addition of 30 g of *C. dependens* per 1 kg of basal diet), and T4 (Addition of 40 g of *C. dependens* per 1 kg of basal diet).

During the 90-day experiment, the fish were fed the dietary treatments to satiation twice daily, once in the morning (between 08:00 and 09:00 hours) and once in the evening (between 17:00 and 18:00 hours).

Reproductive Performance

After the 90-day feeding trial, several reproductive indices were assessed, including milt volume, motility duration, percentage motility, and spermatozoa concentration. Milt production and quality were evaluated at the end of the experiment by randomly selecting one male fish from each replicate of each treatment. These fish were sacrificed, and their testes were removed for analysis.

Determination of Sperm (Milt) Quality: Milt Volume

Milt volume was measured by making a small incision in the lobes of the testes and gently squeezing the milt into a Petri dish. The volume was then quantified using a plastic syringe in milliliters (ml).

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Motility Duration

To assess motility duration, 1 μl of milt from each male was placed on a cavity microscope slide, to which a drop of distilled water was added and covered with a cover slip. The sperm activity was observed under a microscope at 100x magnification, and the duration of motility was recorded until all sperm ceased movement, following the method outlined by Mims (1991).

Percentage Motility

Percentage motility was calculated immediately after adding 20 μl of distilled water as an activating solution, using a light microscope at 400x magnification.

Spermatozoa Concentration

Spermatozoa concentration was determined by counting the number of spermatozoa in a diluted sample (1:100) with distilled water, using a Burker hemocytometer under 400x magnification (Rainis *et al.*, 2003).

Fertilization Ability

To evaluate fertilization ability, a female *C. gariepinus* weighing 500 g was induced to spawn using 0.5 ml/kg of Ovaprim (containing 0.02 mg salmon gonadotropin-releasing hormone GnRHa and 10 mg domperidone). After 12 hours, the female was stripped, and 1 g of fresh eggs was placed in fifteen 2L circular bowls labelled according to treatment. A total of 2 ml of milt from each selected male was added to fertilize the eggs, with each treatment carried out in triplicate. The translucent eggs containing embryonic eyes at the time of polar cap formation (approximately 20 minutes postfertilization, before the 2-cell stage of first cleavage) were deemed fertilized and counted to calculate the percentage of fertilization. Opaque eggs were classified as unfertilized. The numbers of fertilized and unfertilized eggs were counted under a microscope at 40x magnification.

The percentage of fertilization was calculated using the formula described by Britz and Hercht (1997):

Percentage egg fertilization

$$= \frac{\text{No. of eggs incubated} - \text{No. of opaque eggs}}{\text{Total number of eggs incubated}} x100$$

Percentage Hatchability

$$= \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs in the batches}} x100$$

Percentage survival rate

$$= \frac{\text{Total no. of hatchling}}{\text{Total no. of eggs counted}} x100$$

Histopathology Observation

At the end of the experiment, the fish were sacrificed, and the testes and ovaries were excised. These samples were fixed in Bouin's fluid and processed using standard histological procedures before being embedded in paraffin wax. The testes and ovaries were cleaned using xylene and then transferred to molten paraffin at temperatures between 58 - 60°C. Specimen blocks were prepared at a thickness of 3 microns using a rotary microtome, followed by staining. Haematoxylin and eosin staining was applied to colour the nuclei blue, while the other tissue components were stained pink.

Mounting

Each stained slide of the milt and ovary was mounted with paraffin. A drop of paraffin was placed on the cover slip, and the glass slide was inverted and left to dry for one day. These mounted slides were used for microscopic examination.

Microscopic Examination

High-resolution images of the tissue samples were captured using a Panasonic 7 megapixel digital camera. All slides were examined under low and high magnifications to assess their histological characteristics.

Fecundity Estimation

Fecundity was estimated using the gravimetric subsampling (wet method) as outlined by Bagenal (1978). The ovaries were weighed after excess water was

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removed with filter paper, and the number of eggs per gram was counted to calculate the total number of eggs. The total number of eggs per ovary was derived by multiplying the number of eggs counted by a factor based on the total weight divided by 10 g. Additionally, five fresh eggs were randomly selected from each dietary treatment for egg diameter measurement (in mm). For pear-shaped eggs, the mean of the long and short axes was used as the egg diameter (Ayinla, 1988). Data on egg diameter, along with the percentage of fertilization and percentage of hatching, were used to evaluate egg quality.

Determination of Fertilization Rate Percentage Fertilization

To calculate the percentage of fertilization, approximately 30 eggs from each replicate of each treatment were carefully placed on a Petri dish. A measured 0.1 ml of milt was added to fertilize the eggs in each replicate, and the number of fertilized and unfertilized eggs was counted under a microscope at 40x magnification.

Percentage Hatchability

The percentage of fertilized eggs, along with the percentage of eggs that hatched and the percentage of survival, was computed according to the method described by Ayinla (1988).

Percentage egg fertilised

$$= \frac{\text{No. of eggs incubated} - \text{No. of opaque eggs}}{\text{Total number of eggs incubated}} \text{x100}$$

Percentage of egg hatching

$$= \frac{\text{No. of whitish broken eggs}}{\text{No. of eggs fertilized}} \text{x100}$$

Data Analysis

The data collected from the parameters investigated were analysed using analysis of variance (ANOVA) according to the procedures outlined by SAS (2002). Duncan's Multiple Range Test was applied to compare differences among individual means.

RESULTS AND DISCUSSIONS

The influence of dietary components on fish growth has been well documented (Mahmoud *et al.*, 2017; Abdel-Tawwab *et al.*, 2010). In the present study, the control group (T0) exhibited the highest average weight (585 g), while the group with the lowest inclusion of *C. dependens* (T1) showed the lowest weight (510 g). However, the lower growth in T1 suggests that insufficient levels of this phytobiotic may not provide adequate nutritional benefits compared to a control diet.

Egg and sperm quality are significant for the production of high-quality fish larvae and for economical utilization of hatcheries (Coban *et al.*, 2011). The findings regarding the sperm quality parameters of *C. gariepinus* subjected to varying dietary treatments with *C. dependens* showed that the control group recorded superior values. The control group produced significantly ($p<0.05$) more milt (0.75 ml) than all other treatments, with T4 yielding the least (0.50 ml). Studies on *C. dependens* indicated that while it can enhance reproductive health due to its potential antioxidant properties (Madueke *et al.*, 2020; Quadri and Yakubu, 2017), high concentrations may have detrimental effects (George *et al.*, 2023; Sawe *et al.*, 2021). This finding aligns with earlier studies that have demonstrated adverse effects in fish resulting from high doses of phytobiotics, attributed to toxicity (Durojaiye *et al.*, 2023; Xavier and Kripasana, 2020).

Motility of the spermatozoan is the most used indicator of milt quality since high motility is a prerequisite for fertilization and correlates strongly with fertilization success (Li *et al.*, 2016). The control group's motility percentage (73.33%) and duration (4.55 seconds) were significantly higher ($p<0.05$) compared to the treatment groups. This contradicts the findings of Quadri and Yakubu (2017), which indicated that *C. dependens* can enhance sperm motility and vitality. The control group displayed the highest milt count (2.69 x 10^5 sperm/ml) and live/dead ratio (29.68), which were significantly lower ($p<0.05$) than those in the treatment groups that received a *C. dependens* based diet. This further supports the notion that *C. dependens* hurts the *C.*

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gariepinus.

Table 2. Sperm quality of *Clarias gariepinus* fed *C. dependens* based diet.

Parameters	T0	T1	T2	T3	T4
Fish weight (g)	585±7.45	510±7.91	550±8.34	530±7.55	550±6.65^{c}
Milt volume (ml)	0.75±0.05^{a}	0.58±0.05^{c}	0.54±0.10^{c}	0.66±0.07^{b}	0.50±0.08^{c}
Motility (%)	73.33±2.19^{a}	63.52±3.70^{b}	61.11±3.10^{b}	58.00±1.65^{d}	60.00±2.22^{c}
Motility duration (Sec)	4.55±0.40^{a}	3.06±0.10^{b}	2.88 ±0.69^{c}	3.00±0.58^{b}	3.00±0.55^{b}
Milt count (x 10^5 spzml)	2.69±0.28^{a}	1.780±0.42^{b}	1.76±055^{b}	1.73±0.76^{b}	1.69±0.60^{b}
Live/death ratio	29.68±4.44^{a}	19.09±3.20^{c}	21.11±4.89^{b}	20.81±4.31^{b}	17.17±4.02^{c}

Abcd means on the same row with different superscripts are significantly different (p<0.05).

The control group demonstrated significantly higher (p<0.05) fecundity (54.62 eggs) compared to the other treatments (T1: 34.63, T2: 39.29, T3: 37.30, and T4: 36.63). This does not align with the study of Dada (2012), who reported an increase in the fecundity of *C. gariepinus* fed kola seed-based diets. Differences in fecundity among a group of similarly sized fish species along a common trail may be influenced by factors such as hormone administration rate, breeding history, maturity stage, and various external environmental conditions (Ataguba *et al.*, 2009). The similar and lower fecundity across T1 to T4 suggests that higher levels of *C. dependens* might negatively affect reproductive capacity. In aquaculture, there has traditionally been a belief that larger eggs are of higher quality. The control group produced the largest eggs (1.76 mm), with T4 yielding the smallest eggs (1.19 mm). The significantly smaller egg sizes in the treatment groups further indicate that *C. dependens* may not provide the necessary nutrients for optimal egg development at higher inclusion levels.

The fertilization rate was highest in the control group (76.40%), with significantly lower (p<0.05) rates observed in all treatment groups (T1: 43.88%, T2: 39.10%, T3: 38.89%, T4: 33.76%). This finding reinforces previous research indicating that egg quality and fertilization success are closely linked to the composition of the diet (Valdebenito *et al.*, 2015). The sharp decline in fertilization rates among treatment groups suggests that the inclusion of higher levels of *C. dependens* may adversely impact egg viability.

The hatchability rate followed a similar pattern, with the control group achieving the highest rate of 54.00%, significantly higher (p<0.05) than the rates in the treatment groups (T1: 32.32%, T2: 37.76%, T3: 31.66%, T4: 30.00%). Research has consistently shown that hatchability can be influenced by the quality of eggs, which in turn is affected by maternal nutrition (Bobe and Labbé, 2010). The lower hatchability in the treatment groups suggests that the high levels of *C. dependens* may compromise the viability of eggs postfertilization.

Table 3. Egg quality of *Clarias gariepinus* fed *C. dependens* based diet.

Parameters	T0	T1	T2	T3	T4
Weight of female fish (g)	710±9.45^{a}	580±8.67^{b}	550±8.34^{c}	510±7.55^{d}	550±7.20^{c}
Fecundity estimation	54.62±1.59^{a}	34.63±2.98^{b}	39.29±7.22^{b}	37.30±7.59^{b}	36.63±2.58^{b}
Egg size (mm)	1.76±0.11^{a}	1.26±0.13^{c}	1.29±0.18^{b}	1.25±0.13^{c}	1.19±0.07^{d}
Fertilization (%)	76.40±1.25^{a}	43.88±2.91^{b}	39.10±1.70^{c}	38.89±2.22^{c}	33.76±3.21^{c}
Hatchability (%)	54.00±5.78^{a}	32.32±2.10^{c}	37.76±2.98^{b}	31.66±3.33^{c}	30.00±2.91^{c}

Abc means on the same row with different superscripts are significantly different (p<0.05).

According to Anayeokwu *et al.* (2023), histopathology deals with the study of pathological changes of the microscopic structure of the body tissue. However, the result in the present study revealed that inclusion of *C. dependens* at 23% in the diet of fish has no detrimental effect, such as sloughing, denudation, and necrosis, used in describing the damage done to the reproductive profile of Clarias gariepinus. This disagreed with the findings of some authors who worked on other phytobiotics like neem leaves (Jegede and Fagbenro, 2008), hibiscus leaves (Jegede, 2010), and Aloe vera latex (Jegede, 2011) at a rate of 23g on Tilapia fish (*O. niloticus*). The authors stated that observable indications, such as degenerative stroma, necrotic ovaries, and disruption of the endocrine system, lead to reduced fish reproduction by either affecting gonad differentiation or by delaying gonad maturation of the fish. However, the presence of spermatocytes in the seminiferous tubules, even in groups administered *C. dependens* is indicative of normal spermatogenesis, which is essential for male fertility. In addition, the increased density of spermatids and mature sperm in T2 suggests that moderate levels of *C. dependens* may positively influence spermatogenesis. Previous research by Jegede (2010) found that the inclusion of certain phytobiotics in fish diets can enhance reproductive performance by promoting gamete production. The findings in T4, where congestion in the testes was observed, echo concerns raised by other studies regarding the negative effects of excessive phytobiotic inclusion.

Inclusion of 4% of *C. dependens* in the diet of *C. gariepinus* in the present study revealed some moderate congestion in the ovaries, and a moderate area of the testis was congested. The findings in this present study have similarities to the work carried out by Omeje (2016) on pawpaw seed, which found degenerated stroma in ovaries and scanty spermatozoa in testes in the group fed 4 g/kg feed. Comparable results were obtained by Solomon *et al.* (2017), who reported continuous mild stroma degeneration in the ovaries of juvenile *O. niloticus* fed 2 g/kg feed; deformed seminiferous lobules and degenerated spermatozoa in the testes with increasing level of pawpaw seed meal from 2 g/kg to 8 g/kg feed.

In the treatment group receiving 1% *C. dependens,* moderate atrophy of the ovarian follicles was noted, yet there remained an advanced stage of vitellogenesis with many mature oocytes present. According to some studies, certain dietary supplements could lead to follicular atrophy while still promoting some level of oocyte maturation. In groups receiving 2% and 3% *C. dependens,* no observable lesions were reported, and the ovaries exhibited an advanced stage of vitellogenesis with numerous mature oocytes. However, in the group that received the highest dietary inclusion of 4% *C. dependens,* moderate congestion was observed in the ovaries, suggesting potential adverse effects from the higher dietary level.

Anayeokwu *et al.* (2023) stated that histopathology deals with the study of pathological changes in the microscopic structure of body tissue. In the present study, however, the inclusion of *C. dependens* at 23% in the diet of Clarias gariepinus did not result in detrimental effects such as sloughing, denudation, or necrosis, which are commonly used to describe reproductive damage. This contrasts with the findings of Jegede and Fagbenro (2008), Jegede (2010), and Jegede (2011), who worked with other phytobiotics like neem leaves, hibiscus leaves, and *Aloe vera* latex at a rate of 23g in *Oreochromis niloticus* (Tilapia). These studies reported observable damage, including degenerative stroma, necrotic ovaries, and disruption of the endocrine system, leading to reduced fish reproduction through altered gonad differentiation or delayed gonad maturation. These discrepancies in the findings suggest that while *C. dependens* may not have toxic effects at a low concentration, but treatments, especially at higher concentrations, may exhibit toxicity.

Despite these concerns, the present study found that the presence of spermatocytes in the seminiferous tubules in groups administered *C. dependens* indicated normal spermatogenesis, which is essential for male fertility. The increased density of

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spermatids and mature sperm observed in the group receiving moderate levels of *C. dependens* (T2) suggests that these levels may positively influence spermatogenesis. Additionally, Jegede (2010) found that certain phytobiotics in fish diets can enhance reproductive performance by promoting gamete production.

However, in the T4 group, where congestion in the testes was observed, the findings echo concerns raised by previous studies regarding the negative effects of excessive phytobiotic inclusion. Furthermore, the inclusion of 4% *C. dependens* in the diet of *C. gariepinus* revealed moderate congestion in both the ovaries and the testis. This is consistent with Omeje (2016), who found degenerative stroma in the ovaries and scanty spermatozoa in the testes in fish fed 4g/kg pawpaw seed meal, and with Solomon *et al.* (2017), who reported mild stroma degeneration in the ovaries and deformed seminiferous lobules with degenerated spermatozoa in the testes as pawpaw seed meal inclusion increased from 2g/kg to 8g/kg.

In groups receiving 1% *C. dependens,* moderate atrophy of the ovarian follicles was observed, though advanced vitellogenesis and many mature oocytes remained present. Some studies suggest that certain dietary supplements may cause follicular atrophy while still promoting oocyte maturation. No observable lesions were found in the groups receiving 2% and 3% *C. dependens,* and the ovaries exhibited an advanced stage of vitellogenesis with numerous mature oocytes. However, in the group with the highest dietary inclusion of 4% *C. dependens,* moderate congestion in the ovaries suggested potential adverse effects from the higher level of inclusion.

Table 4. Description of *Clarias gariepinus* testes fed *C. dependens* based diet.

Treatment	Observations
T0	1. No observable lesions 2. The seminiferous tubules are in the initial stages of development and contain spermatocytes (SC), indicating normal early development.
T1	1. No observable lesions 2. The seminiferous tubules remain healthy with no lesions present, showing an appropriate developmental stage.
T2	1. More densely packed with spermatids and sperm 2. The seminiferous tubules exhibit a higher density of spermatids and sperm, suggesting progression in spermatogenesis.
T3	No observable lesions Similar observations to T1 and T2, with healthy seminiferous tubules containing developing gametes and no signs of degeneration.
T4	Testes showed signs of congestion.

Table 5. Description of *Clarias gariepinus* ovaries fed *C. dependens* based diet.

Treatment	Observations
T0	1. No observable lesions. 2. Advanced stage of vitellogenesis with many mature oocytes and a few in the cortical alveolar state.
T1	Moderate atrophy of follicles; characterized by advanced stage of vitellogenesis and many mature oocytes (OC).
T2	1. No observable lesions 2. Advanced stage of vitellogenesis with many mature oocytes (OC).
T3	No observable lesions; advanced stage of vitellogenesis with many mature oocytes (OC).
T4	Moderate congestion observed in the ovary, indicating potential adverse effects.

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CONCLUSION

In conclusion, while *C. dependens* has the potential to enhance certain aspects of reproductive health in *Clarias gariepinus*, the results underscore the necessity for careful management of dietary inclusion rates. Optimal levels of phytobiotics can support gamete quality and reproductive success, while excessive amounts may lead to detrimental effects on both sperm and egg quality, as well as histological integrity. Future research should focus on fine-tuning the dietary formulations to maximize the benefits of *C. dependens* while minimizing potential negative impacts, ultimately contributing to more sustainable and productive aquaculture practices.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Ojetayo Teslim Asafe conceptualized the study, supervised the fieldwork, and conducted the laboratory analysis. Durojaiye Abiola Fadilat assisted with the laboratory analysis, compiled the field data, and helped develop the manuscript. Oshoke Omolegho Justina oversaw the fieldwork and supported the laboratory analysis.

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