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RESEARCH PAPER

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Extract of *Moringa oleifera* Leaves: Possible Useful Additives to Enhance Reproductive and Growth Performance of Male *Oreochromis niloticus*

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Abstract

The current study aimed to test *Moringa oleifera* leaves extract on the growth and reproductive performance of male Nile tilapia (*Oreochromis niloticus*). We purchased 420 healthy Nile tilapia (60.32±3.13 g) from the local fish farm for the feeding experiment. The fish were divided in triplicates into seven groups, including the control group, with each group consisting of 60 fish species (20 per glass aquarium). They were maintained in glass aquariums measuring 60×40×35 cm throughout the 60-day feeding trial. Experimental diets, incorporating various concentrations of *M. oleifera* extracts (0% 2%, 4%, 6%, 8%, 10%, and 12%), were administered to the fish at 3% of their body weight. The results revealed that experimental diets significantly ($P<0.05$) increased the growth and reproductive performance of the Nile tilapia compared to the control group. *M. oleifera* significantly ($P<0.05$) enhanced the levels of testosterone and luteinizing hormones. Furthermore, there was a progressive increase in the overall fertility rate with increasing concentrations of the plant extract. However, the overall performance of the fish decreased with increasing concentrations beyond 12%. The current study concluded that *M. oleifera* extract can be used to increase the fecundity and growth rate of the fish.

Introduction

The worldwide aquaculture sector has experienced rapid growth and currently provides approximately half of the world's food fish production (FAO, 2018). It is estimated that by 2030, the world will require an additional 27 million tons of fishery products to meet the increasing demand for fish as a food source (FAO, 2016). Among the most widely cultivated freshwater fish species is the Nile tilapia (*Oreochromis niloticus*), known for its adaptability to various environmental conditions and diverse diet options (Rind et al., 2023; Habib et al., 2021). Nile tilapia popularity in aquaculture stems from its numerous advantages, including rapid growth, disease resistance, and adaptability (Habib et al., 2023; Habib et al., 2022; Fazio et al., 2022a). However, limited research has been conducted on the reproductive biology and growth of this species using plant extracts. Diet content can significantly impact animal reproductive physiology and growth, with fish

embryos requiring adequate nutrients through their gametes for normal development (Singh et al., 2021).

The reproductive process and growth of fish significantly contribute to production yield, thus impacting the financial outcomes of aquaculture projects (De Silva et al., 2008). Various factors, including nutrition and environmental conditions, influence fish growth and reproductive processes (Ochokwu et al., 2015). Researchers have explored the effects of different plant extracts on fish reproductive processes and growth, such as ginseng root extract on the semen quality of *Oncorhynchus mykiss* (Sonmez et al., 2019), *Tribulus terrestris* extract on the reproductive performance of Nile tilapia (Hassona et al., 2020), and *Withania coagulans* on the growth of Labeo rohita (Fazio et al., 2022b).

As the demand for high-quality fish sperm increases, researchers are investigating natural alternatives to replace harmful chemicals in aquaculture. Medicinal plants, such as *Moringa oleifera*, are being explored for their potential as fertility enhancers in aquaculture (Abaho et al., 2022; Gabriel, 2019). *M. oleifera* is widely distributed in tropical and subtropical regions (Thapa et al., 2019) and is known for its nutrient-rich leaves containing significant protein, minerals, and vitamins (Arora and Arora, 2021). In addition to its leaves, Moringa seeds are also rich in proteins, fibers, and ash, with lipophilic compounds providing antimicrobial properties (Okiki et al., 2015; Arora and Arora, 2021). Moringa seed oil, valued for its medicinal properties, shares chemical similarities with olive oil and contains high levels of tocopherol (Leone et al., 2016; Sukarno et al., 2022). Previous studies have identified various secondary metabolites in Moringa extracts, including flavonoids, steroids, alkaloids, tannins, glycosides, saponins, and reducing sugars (Esther and Oladipo, 2012).

Despite numerous studies focusing on the use of herbal extracts to increase fish production, limited research exists on their effects on fish semen and sex hormones. Investigating the quality and quantity of fish semen and their sex hormones is crucial for fortifying diets with different plant extracts to enhance aquaculture productivity. Therefore, the current study aimed to assess the effects of *M. oleifera* leaf extracts on Nile tilapia (*Oreochromis niloticus*) sperm quality, sex hormones, and growth.

Materials and Methods

Preparation of Phytochemical Extract of *M. oleifera* Leaves and Experimental Diets

Fresh *M. oleifera* leaves were obtained from the local market for the current study. This plant was selected due to its known therapeutic properties and its successful application in previous studies as a feed ingredient in aquaculture. The phytochemical analysis of the moringa leaves followed the procedure outlined by Ojiako (2014) (Table 1). The fresh leaves were soaked overnight in a tank of water to remove saponins and other water-soluble anti-nutritional factors. After soaking, the leaves were drained on a wire mesh to remove excess water. They were then spread evenly on a plastic sheet and dried under shade to prevent the degradation of vitamins due to oxidation or photodynamic damage. To reduce crude fiber content, the stalks were manually separated from the leaves. The dried leaves were ground into a coarse powder and subjected to ethanol extraction. Initially, the powdered leaves were soaked in 75% ethanol using a Soxhlet extractor for 2 hours. This was followed by two rounds of heat reflux extraction with 75% ethanol, each lasting 3 hours. The resulting extract was concentrated at a controlled temperature range of 65–75°C to remove excess ethanol. The concentrated extract was then sterilized and stored in airtight containers in a cool, dry place. Experimental diets were formulated to maintain consistent nutrient composition while varying the levels of *M. oleifera* leaf powder (0%, 2%, 4%, 6%, 8%, 10%, and 12%) as shown in Table 2. The feed mixtures were pelleted using a fodder machine, dried in an oven, and allowed to cool. The cooled pellets were stored in sterile plastic bags at 4°C to preserve their nutritional quality. The nutritional analysis of the feed was performed according to the AOAC (2000) protocol.

Table 1. Phytochemical profile of *Moringa oleifera.*

Phytochemical	Test	*Moringa oleifera* leaves
Alkaloids	Mayer's test	++
Anthraquinones	Sulfuric acid test	Absent
Carbohydrates	Molisch's test	Absent
Fats and fixed oil	Filter paper press test	+
Flavonoids	Alkaline reagent test	++
Glycosides	Legal's test	+
Phytosterols	Liebermann–Burchard's test	+
Proteins and amino acids	Xanthoproteic test	+++
Reducing sugars	Fehling's test	+
Saponins	Foam test	+++
Steroids	Sulfuric acid test	++
Tannin	Gelatin test	Absent
Triterpenoids	Salkowski's test	Absent

Note: + available but low concentration, ++ medium concentration, +++ high concentration

Experimental Fish and Research Design

Experimental Fish Source

A total of 420 male Nile tilapia were sourced from a commercial fish farm in Faisalabad, Punjab, Pakistan. The fish were carefully transported in aerated containers to the Animal Experiment Laboratory to ensure minimal stress during transit.

Housing and Acclimation

The experiment utilized 21 identical glass aquariums, each with dimensions of 60×40×35 cm. Prior to the start of the experiment, the fish were randomly divided into seven groups, including a control group. Each group consisted of 60 individuals and was replicated in triplicate, ensuring statistical reliability. The fish were acclimated to the laboratory conditions for two weeks before the experimental feeding trial began. During this acclimation period, they were fed a standard basal diet containing 30% crude protein, provided twice daily at 8:00 a.m. and 3:00 p.m. to satiation. Uneaten feed and waste were removed daily using a siphon to maintain water quality.

Experimental Feeding Protocol

At the end of the acclimation period, physically healthy Nile tilapia with an average body weight of 60.32±3.13 g were selected for the feeding trial. The fish were fed experimental diets formulated to contain varying levels of the test ingredient (as described earlier). Feed was provided at 3% of the total body weight of the fish in each group, with quantities adjusted every two weeks based on observed growth. The feeding trial lasted for 60 days.

Tank Maintenance and Water Quality Management

Daily siphoning was performed to remove uneaten feed and fecal matter. Weekly, approximately 50% of the water in each tank was replaced with fresh, dechlorinated water to maintain optimal rearing conditions. A consistent 12-hour light/12-hour dark photoperiod was maintained throughout the experiment using controlled overhead lighting.

Water quality parameters including temperature: 27.35±0.42°C, pH: 7.48±0.33, dissolved oxygen (DO): 7.03±0.62 mg L^{-1}, ammonia: ≤0.001 mg L^{-1}, nitrite: ≤ 0.004 mg L^{-1} and nitrate: ≤1.02 mg L^{-1} were monitored daily to ensure optimal environmental conditions. Temperature, pH, and DO were measured using a YSI multiprobe water quality system (Yellow Springs Inc., USA). Ammonia, nitrite, and nitrate levels were analyzed weekly using HACH kits (Hach Company, USA) following standard spectrophotometric methods. Throughout the 60-day experimental period, no mortalities were recorded, indicating that the experimental conditions and diets were well-tolerated by the fish.

Growth Analysis

The growth of the fish was calculated according to the following formula:

Weight gain (WG)=final weight (Wf)–Initial weight (Wi)

Feed Conversion Ratio (FCR) and Specific growth rate (SGR) were calculated as follows:

FCR=total feed intake/weight gain

$SGR=(\text{Log } W_f-\text{Log } W_i)/$ study duration (in days)×100

Table 2. Formulation of the experimental diet

Basic Ingredients (%)	Control group	Concentration					
	Basal	2%	4%	6%	8%	10%	12%
Soybean meal	35.1	35.1	35.1	35.1	35.1	35.1	35.1
Wheat offal	6	6	6	6	6	6	6
Ca (PO4)2	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Palm oil	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Fish meal	22	22	22	22	22	22	22
Vitamin and mineral premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Common salt (NaCl)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Maize powder	34.5	32.5	30.5	28.5	26.5	24.5	22.5
Moringa leaves	-	2	4	6	8	10	12
Total	100	100	100	100	100	100	100
Crude protien %	30.61	31.02	31.25	31.58	31.89	31.00	31.58
Crude carbohydarte %	40.27	40.24	40.65	40.75	40.95	40.95	40.99
Moisture %	2.2	2.3	2.4	2.4	2.4	2.4	2.5
Crude fiber %	10	8.66	7.85	7.41	6.87	6.75	6.66
Crude lipid %	7.8	7.8	7.85	7.86	7.89	7.90	7.98
Ash %	10.02	10.11	10.08	10.04	10.08	10.12	10.09
Gross energy (kj.g^{-1} DM)	19.32	19.36	19.34	19.36	19.39	19.33	19.34

Semen and Blood Sample Collection

Sampling was done after the termination of the experiment and prior to semen collection, the fish were fasted for 12 hours to ensure empty digestive tracts and reduce contamination during handling. Using a hand net, the fish were individually captured and restrained securely with a cotton towel to minimize movement and stress.

Testicles were surgically extracted following a precise procedure. A small incision was made in the ventral region of the fish's abdomen using sterilized scissors. Once exposed, the testicles were carefully removed and processed for sperm extraction. Sperm was collected using the pressure method, where gentle pressure was applied to the abdominal area to release semen. The semen was collected in sterile polypropylene tubes with a volume of 2.0 ml. Each semen sample was properly labeled for seminal analysis, following the guidelines outlined by Sarmento et al. (2017). Blood samples were collected from the caudal vein of the fish in each group using a 3-ml sterile syringe. For accuracy and consistency, triplicate blood samples were taken from each individual. The blood was transferred to plain collection tubes without anticoagulants to allow clotting.

After clot formation, the samples were centrifuged at 3,000 rpm for 15 minutes to separate the serum. The supernatant serum was carefully pipetted into labeled plastic Eppendorf tubes and immediately stored at -20°C for subsequent hormonal analysis.

Hormonal Analysis

Testosterone

Serum hormone levels, including testosterone (ng/ml) and 11-ketotestosterone (pg/ml), were quantified using enzyme-linked immunosorbent assays (ELISA). Commercial ELISA kits (DRG Instruments GmbH) were employed for these assessments, following the manufacturer's protocol.

The quantification of testosterone levels was conducted based on the methodology described by Mansour et al. (2018). For the measurement of 11-ketotestosterone, the method described by Dzieweczynski et al. (2006) was followed. All assays were performed in duplicate to ensure accuracy and reliability of the results.

Luteinizing Hormone (LH) Determination

Luteinizing hormone (LH; IU/L) levels were measured in plasma samples using the Automated Enzyme Immunoassay system, specifically the Immulite/Immulite 1000 system (AIA-360; Tosoh India Pvt. Ltd., Goregaon, Mumbai, India). The analysis was performed according to the methods described by Beitins et al. (1976). Plasma samples were thawed and processed as per the manufacturer's guidelines to ensure accurate results.

Semen Analysis

A hemocytometer was used for microscopic semen counting after dilution 1:4 with 0.9% NaCl. In addition to this, the following parameters were assessed; (The motility is divided into rapid and progressive phases, slowly progressive, non-progressive, and immotile), (The morphology of sperm that can be divided into normal sperm and abnormal sperm), Haematoxylin and eosin staining were performed to determine vitality (Navarro et al., 2014).

Reproductive Performance

After the experimental period, 5 male fish from each group were randomly selected to measure the gonadosomatic index (GSI). The testes of these fish were removed and weighed. Three males from each group were randomly selected for reproduction assessment, and eggs were placed in the recirculation system. We acquired tilapia fish eggs from commercial fish farm in Faisalabad, Punjab, Pakistan, to conduct fertilization rate experiments. The eggs were transported in oxygenated containers at controlled temperatures to maintain viability. Care was taken to minimize handling stress and prevent contamination. Upon arrival at the lab, the eggs were immediately acclimated to experimental conditions before initiating fertilization tests. Every day, the water of 10% was changed in the recirculation system, and the water temperature was fixed at 27°C. The following formulas were used to calculate reproductive parameters:

$$\text{GSI (\%)} = (\text{Gonad weight}/\text{Body weight}) \times 100$$

$$\text{Hatching (\%)} = (\text{Number of hatched larvae} / \text{Number of eggs laid}) \times 100$$

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

Statistical Analysis

In this study, One-Way ANOVA with Duncan's Multiple Range Test was used to compare the effects of different concentrations of *M. oleifera* leaf extract on fish growth, hormone levels, and reproductive performance. ANOVA helps determine if there are significant differences among the groups, while Duncan's test identifies which specific groups differ. To explore the dose-response relationship and determine the optimal concentration, polynomial-centered linear and quadratic regression analyses were employed. These regression models capture both linear and curvilinear relationships, allowing for the identification of the optimal extract dose where the effects on growth

and reproduction are maximized. PRISM GraphPad version 9.0 was used for statistical analysis, offering reliable tools for modeling and visualization. A significance level of 0.05 was set to ensure that results were statistically valid and unlikely to be due to chance. This combination of methods provided a robust framework for analyzing the effects of *M. oleifera* and determining the most effective doses for improving fish performance.

Results

The detailed results of fish growth performance are presented in Table 3. Analysis revealed that moringa leaf extract significantly ($P<0.05$) increased the weight gain of the fish, with the highest weight gain achieved at 10% extract concentration, reaching 83.97 g. The linear and quadratic relationships showed that increasing the extract dose led to an increase in weight gain. However, beyond a certain limit, weight gain decreased, resulting in another significant regression model. The optimum dose of the plant extract is depicted in Figures 1 and 2, where the optimal level was found to be 10.8 for improved weight gain, while for improved FCR, the optimum dose was 11.8. Furthermore, there was enhanced SGR with a plant extract concentration of 12%, followed by 10%, although the difference was not significant.

Sex Hormones

In the case of sex hormones, it was observed that after fish were fed on fortified feed with plant extracts, the concentrations of sex hormones (testosterone, 11k testosterone, and LH) increased in the experimental groups compared to the control groups. High concentrations of testosterone were observed in the 12% group (2.9), followed by the 10% and 8% groups (2.8). However, the optimum level of the extract for increasing testosterone levels to the optimum was found to be 11.2 This result is illustrated in Figure 3. Further details on the sex hormones are provided in Table 4.

Milt Quality and Fertilization Rate

The same results were found for milt quality analysis, wherein increasing the concentration significantly improved semen quality. However, concentrations above 8% slightly decreased the overall quality of milt and the overall fertilization rate of the species. Details are depicted in Tables 5 and 6. Figure 3 shows that the optimum dose to increase testosterone levels is 11.3. However, the fertilization rate (Figure 4) was found to be higher with the plant extract at 9.8%. This indicates a complex interplay between body physiological functions.

Discussion

Reproductive performance plays a crucial role in determining the profitability of fish. Several researchers focus on using phyto extracts to increase the final yield of aquaculture in different species (Reverter et al., 2014; Awad and Awaad, 2017; Bulfon et al., 2015). However, limited research is available on using moringa to

Table 3. Growth analysis of tilapia fed on fortified feed at different concentration

Parameters	Control	Concentration						ANOVA	Linear	Quardratic
	0%	2%	4%	6%	8%	10%	12%			
Initial weight	60.30	60.12	60.32	60.24	60.55	60.56	60.30	>0.05		
Final weight	120.32	125.34	129.14	133.54	139.54	144.53	140.05	<0.0001	NS	**
Weight gain	60.02	65.22	68.82	73.30	78.99	83.97	79.75	0.01	*	***
FCR	1.55	1.45	1.40	1.38	1.20	1.20	1.19	0.04	*	NS
SGR	2.79	3.12	3.24	3.66	3.99	3.99	4.00	0.02	**	NS

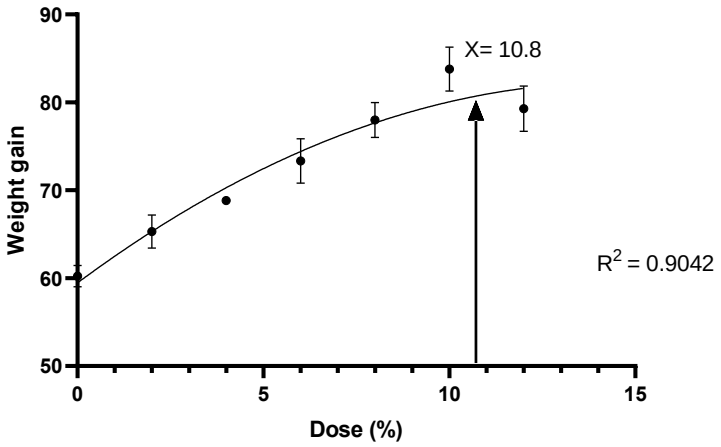

Figure 1. Dose-response of moringa leaves on the weight gain of tilapia.

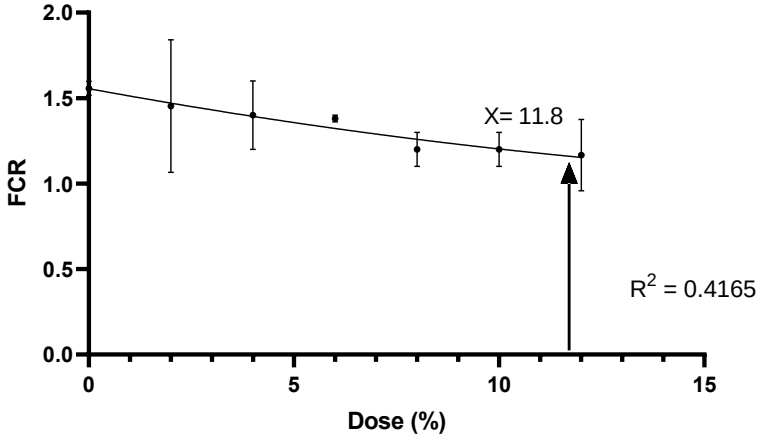

Figure 2. Dose-response of moringa leaves on the FCR of tilapia.

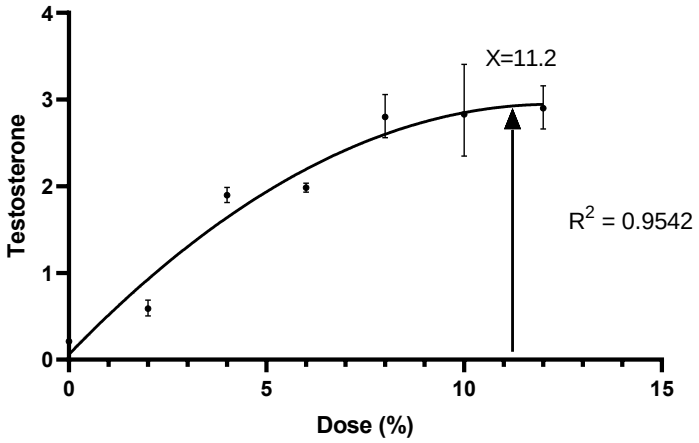

Figure 3. Dose-response of moringa leaves on the testosterone hormone of tilapia.

Table 4. Effect of fortified feed with moringa at different concentrations on tilapia sex hormones.

Sex hormones	Control	Concentration						ANOVA	Linear	Quardratic
	0%	2%	4%	6%	8%	10%	12%			
Testosterone (ng/ml)	0.21^{d}	0.59^{c}	1.89^{b}	1.98^{b}	2.8^{a}	2.8^{a}	2.9^{a}	0.03	**	*
11-keto testosterone (pg/ml)	1.32^{d}	1.61^{b}	1.71^{b}	1.89^{a}	1.54^{c}	1.55^{c}	1.56^{c}	0.02	*	NS
LH	0.43^{c}	0.48^{b}	0.50^{b}	0.55^{a}	0.57^{a}	0.57^{a}	0.58^{a}		**	*

increase the fertility and growth rate of Nile tilapia. Thus, the current study aimed to assess the effects of moringa leaves on the reproductive performance of male tilapia by evaluating semen quality, sex hormone levels, and fertilization rate. Moringa leaves contain flavonoid and phenolic compounds and show strong antioxidant activity (Ojiako, 2014). Previous studies on aquatic animals have indicated that incorporating a moringa leaf extract into the animals' diet could improve their growth and physiology and upregulate immune-related gene functions (Singh et al., 2018; Egwui et al., 2013). Morphological changes to sperm may directly influence fertility, affecting different sperm cell structures such as the head, mid-piece, and tail (Adamkovicova et al., 2016; Mishu et al., 2020). For fish, there is no standard classification of sperm abnormalities and their relationship to the fertility of brood fish. However, some researchers have attempted to classify sperm abnormalities in fish (Borowsky and Chen, 2022; Herman et al., 1994). The results of the present study revelaed that moringa ectract improve Nile tilapia weight gain, FCR and SGR. This might be because moringa is rich in essential nutrients such as proteins, vitamins, and minerals, providing a well-balanced diet for fish (Abdel-Latif et al., 2022). The high protein content supports muscle development, leading to faster growth rates and increased weight gain (Zhang et al., 2020). Additionally, their antioxidant properties help protect fish cells from damage, reducing stress and promoting healthier growth (Hamed and El-Sayed, 2019). The leaves are highly digestible, allowing fish to efficiently absorb and utilize the nutrients, further

Table 5. Effect of fortified feed with moringa on different parameters of tilapia milt quality

Milt analysis	Control	Concentration						ANOVA	Linear	Quardratic
	0%	2%	4%	6%	8%	10%	12%			
Sperm Count	163.0^{d}	169^{c}	175^{b}	179^{b}	185^{a}	185^{a}	184^{a}	0.034	NS	***
Rapid progressive motality (%)	5.668^{c}	5.9^{b}	6.2^{b}	6.9^{a}	7.2^{a}	7.1^{a}	7.2^{a}	0.04	*	**
Sluggish progressive motality (%)	42.00^{cd}	45^{bc}	47^{b}	49^{b}	55^{a}	56.2^{a}	56.9^{a}	0.03	**	NS
Non-progressive motality (%)	24.12^{cd}	27^{c}	30^{c}	36^{b}	39^{b}	40^{b}	45^{a}	0.04	**	NS
Immotile (%)	29.78^{b}	29.74^{b}	28.45^{b}	27.58^{b}	30.87^{ab}	31.76^{a}	33.4^{a}	0.04	**	NS
Normal sperm (%)	70.64^{ab}	74^{c}	77^{b}	79^{a}	80^{a}	80^{a}	78^{a}	0.02	*	*
Abnormal sperm (%)	29.35^{a}	25^{b}	22^{bc}	20^{c}	19^{c}	20^{c}	22^{bc}	0.03	NS	NS
Vitality (%)	72^{c}	75^{bc}	78^{b}	80^{a}	82^{a}	80^{a}	79^{ab}	0.02	**	***

Table 6. Effect of fortified feed with moringa on reproductive performance of tilapia

Reproductive performance	Control	Concentration						ANOVA	Linear	Quardratic
	0%	2%	4%	6%	8%	10%	12%			
	C									
Gonadosomatic index (%)	0.82^{b}	0.85^{ab}	0.85^{ab}	0.86^{a}	0.85^{ab}	0.85^{ab}	0.86^{a}	>0.05	NS	*
Fertilization ratio (%)	65^{d}	76^{c}	79^{c}	85^{b}	89^{a}	87^{a}	86^{ab}	0.04	NS	NS
Hatching ratio (%)	54^{c}	55^{c}	60^{b}	62^{b}	70^{a}	69^{a}	68^{a}	0.04	NS	*

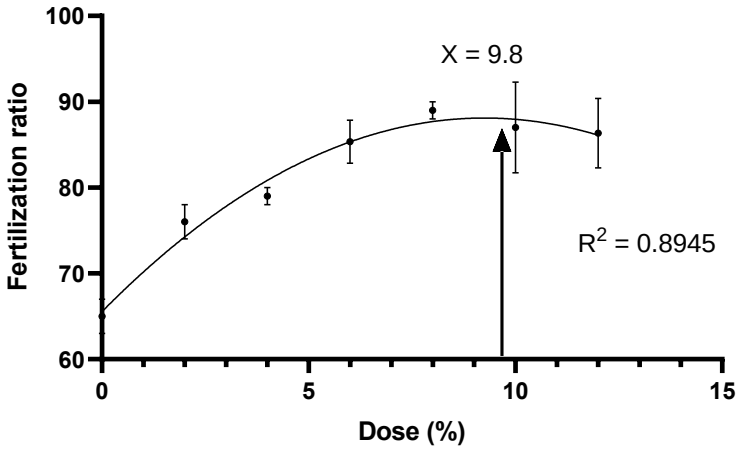

Figure 4. Dose-response of moringa leaves on the fertilization ratio of tilapia.

contributing to weight gain (Amer et al., 2024). Several studies on moringa revealed increased growth performance of fish species such as in *Pangasius bocourti* (Puycha et al., 2017), Nile tilapia (Ahmed et al., 2014; Emam et al., 2024), and *Poecilia reticulata* (Bisht et al., 2020). Improving FCR and SGR can contribute to sustainable aquaculture practices by reducing resource inputs, minimizing waste, and enhancing overall productivity without compromising fish welfare or environmental quality (Wilfart et al., 2023; Kumar et al., 2017).

The current study found that the fertility rate of the sperm increased with the increase in plant extract concentration. This suggests that the fecundity rate of tilapia can be improved with moringa leaf extract. Similar findings were reported by Abbas and El-Badawi (2014) and Gabriel (2019). Furthermore, it was also found that moringa leaf extract significantly improved sperm quality. This result may be attributed to the antioxidant agents in the leaves, which could combat oxidative stress within the sperm, thereby increasing sperm production and reducing DNA damage, resulting in fewer abnormal sperm (Sandoval-Vargas et al., 2021; Martin-Hidalgo et al., 2019). This was supported by the finding that sperm abnormality quantity decreased, with the optimum result achieved at 10% plant extract concentration. These findings are consistent with other studies where moringa extract improved the motility, concentration, morphology, and viability of sperm in rabbit bucks, Bali bulls, and Senduro goats (Wahjuningsih et al., 2019; El-Desoky et al., 2017; Syarifuddin et al., 2017). However, interestingly, sperm quality decreased above 10% extract concentration.

Moringa leaves contain rich vitamin C (Ahmed et al., 2016). This vitamin can stimulate the regeneration of other small antioxidant molecules from their respective

radical species, such as glutathione (GSH), α-tocopherol, and β-carotene, which protect sperm from oxidative stress (Chao et al., 2002), thus increasing sperm quality. Moreover, the growth rate of the fish was also improved with moringa. It might be possible that moringa is a rich source of protein and also acts as an appetizer.

Conclusions

The current study concludes that a 10% concentration of moringa leaf extract can improve Nile tilapia growth and sperm quality, ultimately increasing the final yield. Further research should be conducted with higher concentrations to determine the optimal level of these extracts and evaluate toxicity levels. To ensure the efficacy of these plants and their constituents, it is advisable to conduct experiments with a larger statistical population, prolong the administration period, compare with safe substances, and explore specific molecular mechanisms. In addition, since tilapia matures quickly and spawns 2–3 times per month, future studies should explore whether higher doses of moringa leaf extract accelerate spermatogenesis. Reproductive traits like sperm quality and egg development should be monitored at multiple points.

Ethical Statement

The research undertaken received ethical clearance from the Department of Zoology University of Lahore, with approval granted by the ethical committee. The trial registration number for this study is UOL/ZOO/08.

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Author Contribution

The conceptualization and design of the study were carried out by Saira Naz and Syed Sikandar Habib. Saira Naz prepared the original draft of the manuscript. Sampling was conducted by Mujeeb Ullah, Khayyam Khayyam, and Khalid Khan. Data analysis was performed by Saima Majeed. The manuscript was reviewed and edited by Ümit Acar, Francesco Fazio, Mohamed Mohany, and Osman Sabri Kesbiç. All authors have read and approved the final version of the manuscript for publication.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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