Effects of dietary Capparis spinosa fruit powder on growth performance and reproductive traits of Sailfin molly (*Poecilia latipinna*).

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Abstract

This study was conducted to determine the effect of dietary supplementation with dried caper fruit (*Capparis spinosa*) powder at different percentages (0, 0.5, 1.0, 1.5%) on fertility indicators and reproductive performance of Sailfin molly (*Poecilia latipinna*). The experiment lasted 90 days and included 120 fish (60 males and 60 females), divided into four treatments with three replicates per treatment. The results indicated the superiority of the T2 treatment (1% caper fruit powder), recording the highest values for the number of offspring per birth (37.4 ± 2.6), offspring survival rate (88.3 ± 2.4%), and sperm viability (84.2 ± 2.7%), in addition to a significant increase in hormone levels (testosterone, estradiol, follicle-stimulating hormone/FSH). Luteinizing hormone (LH) was also affected. A significant improvement in feed conversion ratio (FCR) was observed in the same treatment ($P<0.05$). This positive effect is attributed to the rich content of phenolic, flavonoid, and alkaloid compounds in caper fruit, particularly rutin, quercetin, It can be concluded that adding 1% caper fruit powder represents the optimal level for improving fertility and reproductive performance in Sailfin molly

Keywords: *Capparis spinosa, Poecilia latipinna*, reproductive performance, sperm quality, dietary supplementation, aquaculture nutrition.

Introduction

The aquaculture sector has witnessed rapid development, making it a cornerstone of global food security. However, this growth has encountered numerous obstacles, such as the need to improve the reproductive performance of fish populations and to find safe alternatives to traditional chemical stimulants used in spawning (FAO, 2022). In the

context of scientific research, the Sailfin molly (*Poecilia latipinna*) stands out as an ideal biological model due to its unique reproductive characteristics including viviparity and a short life cycle.

This provides researchers a precise opportunity to investigate and understand the physiological mechanisms that regulate reproductive processes in cultured environments (Rosen and Bailey, 1963). Caper plant (*Capparis spinosa*), belonging to the Capparaceae family, is a perennial shrub with a wide geographical distribution extending from the Mediterranean basin to the Arabian Peninsula and is of great importance in traditional medical practices, especially in Iraq and the Levant, as it is attributed with multiple biological activities including antioxidant and anti-inflammatory Activities fertility-enhancing effects, and a role role in regulating blood glucose levels (Nabavi *et al*., 2016; Chedraoui *et al*., 2020).

Flavonoids derive their functional importance in reproductive physiology from the ability of some of their classes, such as isoflavones and flavonols, to mimic the molecular structure of estrogen. This structural similarity enables them to interact with hormone receptors, directly affecting the dynamics of the hypothalamic-pituitary-gonadal (HPG) axis and contributing to the regulation of sex hormone balance (Sepehrfar *et al*., 2023; Amulejoye *et al*., 2025). Regarding male fertility, quercetin stands out as a vital factor in improving the biochemical parameters of sperm, as it works to increase their motility and enhance their defensive capabilities against oxidative stress, thus protecting the structural and functional integrity of sperm from molecular damage (Qamar *et al*., 2023).

Recent studies have shown that plant extracts rich in antioxidants contribute to enhancing fertility indicators in fish through various mechanisms, most notably reducing the accumulation of reactive oxygen species (ROS) and activating oxidative defense systems such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes, which positively impact the quality of gametes in eggs and sperm (Martin-Hidalgo *et al*., 2019; Khademzade *et al*., 2022). Caper fruit powder also contains high levels of essential amino acids and unsaturated fatty acids, which play an important roles in improving the sperm membrane structure and increasing fertilization efficiency.

(Dada and Adeparusi, 2012). Despite the numerous studies that have addressed the medicinal and nutritional properties of the caper plant, the possibility of employing it as a food additive to enhance fertility in farmed fish, and especially in ornamental fish, remains a limited area of research.

Accordingly, this study aims to investigate the effect of introducing dried caper fruit powder in different proportions into the diet, compared to a control treatment, on fertility, reproduction, and growth indicators in Sailfin molly in addition to evaluating changes in hormonal levels, sperm quality, and the characteristics of the resulting individuals. The study hypothesizes that adding *Capparis spinosa* fruit powder to the diet of Sailfin molly improves growth performance and reproductive traits, and that the degree of improvement varies according to the level of inclusion in the diet.

Materials and Methods:

Experimental Fish

The experiment was conducted in the Aquaculture Unit Laboratory, College of Agriculture, University of Basrah The experiment lasted 90 days One hundred and twenty mature Sailfin molly (*P. latipinna*) of both sexes (60 males and 60 females) were used in the study, with an average weight of 2.12 ±0.08g. The fish were randomly distributed into 12 glass tanks, each with a capacity of 60 liters, with each experimental unit contained 10 fish (5 males and 5 females). Four treatments were applied with three replicates per treatment. The temperature was maintained at approximately 24°C using automatic electric heaters, and aeration was provided via electric pumps to keep dissolved oxygen levels above 5 mg/L. To maintain appropriate ammonia concentrations, pH levels, and water quality, approximately 75% of the aquariums water was replaced daily; a 12-hour lighting cycle was also implemented. The fish were acclimated for a period of two weeks.

Growth Performance Parameters

Daily Growth Rate (g/day) = (Final Weight gain- Initial weight)/Time =(W2-W1)/t
DGR(g/day) = FW – IW / days
Food Conversion Rate (FCR) = The amount of food / Weight gain Final
FCR= F/W.G
Survival Rate (SR%) :(Final number of fish / Initial number of fish) × 100
SR%= (No.2/No.1)×100

Preparation of Caper Fruit Powder

Mature caper fruits (*Capparis spinosa* L.) were collected from the Abi Al-Khasib district, Basrah Governorate, in July 2025. The fruits were washed with distilled water and dried in Ahot air oven at 50°C for 48 hours to preserve phenolic content and prevent thermal degradation of the active compounds (Said *et al*., 2010). The dried fruits were ground into a fine powder using an electric grinder and sieved through a 250µm sieve. The powder was then stored in dark airtight containers at 4°C until use.

The approximate chemical composition of the fruit powder was determined according to AOAC (2016). Protein 12.3% Fat 6.8%, Moisture 7.2%, Ash 8.1%, Crude Fiber 18.4%, and Nitrogen-free extract 47.2%. The total phenolic content was 34.2 mg GAE/g dry weight, and the total flavonoids were 18.6 mg QE/g dry weight.

Design of Experimental Diets

Four experimental diets were prepared, identical in their crude protein content (38%) and total energy (3820 kcal/kg), differing only in the concentration of added caper fruit powder, as follows:
Control (T0): 0% dried caper fruit powder.
T1: Diet supplemented with 0.5% dried caper fruit powder
T2: Diet supplemented with 1.0% dried caper fruit powder
T3: Diet supplemented with 1.5% dried caper fruit powder

Feeding and Management System

The experimental units were Fed three time daily diet, at a feeding rate of 5% of body weight.

Table (1): Composition of the experimental diets.

Ingredients	To	T1	T2	T3
Fish meal (g/kg)	450	430	410	390
(Soybean meal (g/kg)	250	250	250	250
Corn flour (g/kg)	200	185	170	155
Vegetable oil (g/kg)	50	50	50	50
Dried caper fruit powder (g/kg)	0	5	10	15
Vitamin and mineral premix (g/kg)	50	50	50	50
Crude protein(%)	38.2 ± 0.4	38.0 ± 0.3	37.9 ± 0.5	38.1 ± 0.4
Crude fat(%)	8.1 ± 0.2	8.0 ± 0.1	8.2 ± 0.3	8.0 ± 0.2
Total energy (kcal/kg)	3820	3810	3825	3815

Fertility and Reproductive Parameters Measured

Females were monitored daily to record parturition dates, number of offspring, and offspring vigor. Vigor was defined as the appearance of active swimming behavior within the first hour after birth. Gestation period (in days) was calculated from the date of insemination to the date of birth. Offspring were weighed using a high-precision electronic scale, and the total length of each offspring was measured using a digital caliper. The Reproductive success rate was recorded as the percentage of females that completed at least one pregnancy relative to the total number of females in each experimental treatment.

Sperm Quality Assessment

At the end of the experiment, the males were anesthetized with a 0.1 ml/L osteogenic solution, and the seminal fluid was collected by gentle abdominal pressure. Total and progressive sperm motility were assessed using the Computer-Assisted Sperm Analysis (SCA Sperm Class Analyzer). Viability was determined using Eosin-Nigrosin staining, while sperm concentration was estimated using a Neubauer cell counter. Morphological evaluation was performed by preparing Papanicolaou-stainedsmears, which were then examined under a light microscope at 1000× magnification.

Hormonal Analysis

At the end of the experimental period (day 90) blood samples were collected from the caudal vein of the anesthetized fish, using the anesthetic substance clove oil. Blood samples were allowed to clot, and serum was separated by centrifugation at 3000 rpm for 15 minutes at 4°C Store the serum at -20°C until use. The concentrations of testosterone, 17β estradiol, FSH, LH, and cortisol were measured using Competitive ELISA with commercial assay kits from Abcam, UK, according to the manufacturer's instructions.

Statistical Analysis

Data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS, version 26). One-way ANOVA was used to determine the significance level between treatments. When significant differences were found at a probability level less than 0.05, Duncan's Multiple Range Test was performed for multiple comparisons. Results are presented as mean ± standard deviation (Mean ± SD).

Results

Fertility and Reproductive Parameters

Statistical analysis revealed significant differences between the various treatments for Sailfin molly ($P<0.05$), as shown in Table 3.

Table (3): Effect of adding caper fruit powder at different proportions on fertility and reproduction indicators in Sailfin molly.

Reproductive Parameters	To	T1	T2	T3
Number of offspring per birth	22.3 ± 1.8^{b}	29.5 ± 2.1^{ab}	37.4 ± 2.6^{a}	31.2 ± 2.3^{ab}
Offspring survival rate (%)	71.4 ± 3.2^{b}	78.9 ± 2.8^{ab}	88.3 ± 2.4^{a}	83.1 ± 2.6^{a}
Gestation period (days)	31.5 ± 1.2	30.8 ± 1.0	29.6 ± 0.9	30.1 ± 1.1
Offspring viability rate (%)	68.2 ± 4.1^{c}	76.5 ± 3.5^{bc}	89.1 ± 2.8^{a}	84.7 ± 3.2^{ab}
Birth weight (mg)	4.12 ± 0.22^{b}	4.58 ± 0.19^{ab}	5.21 ± 0.24^{a}	4.89 ± 0.21^{ab}
Birth length (mm)	9.4 ± 0.3^{b}	10.1 ± 0.4^{ab}	11.3 ± 0.3^{a}	10.8 ± 0.4^{a}
Number of parturitions per female (90 days)	1.4 ± 0.2^{b}	1.7 ± 0.2^{ab}	2.1 ± 0.3^{a}	1.9 ± 0.2^{ab}
Percentage of Reproductive success rate (%)	55.0 ± 5.0^{b}	65.0 ± 4.5^{ab}	80.0 ± 4.2^{a}	73.0 ± 4.8^{ab}

The different letters indicate significant differences ($P<0.05$).

Table (3) shows that treatment T2 (1%) recorded the highest number of offspring per birth (37.4 ± 2.6), significantly higher than the control treatment To (22.3 ± 1.8), while T1 and T3 did not differ significantly from either treatment. T2 also had significantly high setoff spring survival rate (88.3 ± 2.4%), offspring viability (89.1 ± 2.8%), and offspring birth weight. No significant differences were recorded in gestational period among the four treatments ($P>0.05$).

Sperm Quality

Table (4): Effect of adding dried caper fruit powder in different proportions on sperm quality indicators in male Sailfin molly.

Sperm Quality Parameters	To	T1	T2	T3
Total Motility (%)	62.4 ± 3.1^{b}	71.8 ± 2.9^{ab}	84.2 ± 2.7^{a}	78.5 ± 3.0^{a}
Sperm Concentration ($\times 10^6$/mL)	18.3 ± 1.5^{b}	23.7 ± 1.8^{ab}	31.4 ± 2.1^{a}	27.9 ± 1.9^{ab}
Normal sperm morphology (%)	71.2 ± 2.8^{b}	78.6 ± 2.5^{ab}	88.9 ± 2.2^{a}	84.3 ± 2.4^{a}
Sperm viability (%)	69.5 ± 3.4^{b}	77.2 ± 2.8^{ab}	87.6 ± 2.6^{a}	82.4 ± 3.0^{ab}
Sperm velocity (µm/s)	48.3 ± 2.4^{b}	56.7 ± 2.2^{ab}	68.1 ± 2.6^{a}	62.4 ± 2.5^{ab}

The different letters indicate significant differences between treatments ($P<0.05$).

The results in Table (4) show a clear improvement in all sperm quality indicators in the T2 treatment compared to the control treatment. Total motility reached 84.2 ± 2.7% in T2 compared to 62.4 ± 3.1% in the control treatment To ($P<0.05$). T2 also outperformed T2 in concentration (31.4 × 10^6/ml), percentage of normal morphology (88.9%), viability (87.6%), and progressive motility (68.1 µm/s).

Hormonal Levels

Table (5): Effect of adding caper fruit powder in different proportions on hormonal levels in Sailfin molly (Mean ± SD).

Hormonal parameters	To	T1	T2	T3
Testosterone (ng/mL) - Males	3.21 ± 0.28^{b}	4.15 ± 0.32^{ab}	5.84 ± 0.41^{a}	5.12 ± 0.37^{a}
Estradiol E2 (pg/mL) Females	42.60 ± 3.8^{b}	56.30 ± 4.2^{ab}	74.80 ± 4.9^{a}	68.10 ± 4.5^{a}
FSH (mIU/mL)	2.14 ± 0.19^{b}	2.87 ± 0.23^{ab}	3.92 ± 0.31^{a}	3.45 ± 0.27^{a}
LH (mIU/mL)	1.84 ± 0.16^{b}	2.43 ± 0.21^{ab}	3.28 ± 0.26^{a}	2.97 ± 0.24^{a}
Cortisol (µg/dL)	12.40 ± 1.1^{b}	11.80 ± 0.9^{ab}	10.90 ± 0.8^{a}	11.3 ± 1.0^{a}

The different letters indicate significant differences between the different treatments ($P<0.05$).

The results in Table 5 showed a significant increase in testosterone levels in males, estradiol levels in females, and the both pituitary hormones FSH and LH in the T2 treatment compared to the control treatment ($P<0.05$). In contrast, no significant differences were recorded in cortisol levels between the treatments ($P>0.05$), indicating that the addition of dried caper fruit powder did not induce significant physiological stress.

Growth Indicators

Table (6): Effect of adding dried caper fruit powder on growth indicators in Sailfin molly during the experimental period (Mean ± SD)

Growth Performance Parameters	To	T1	T2	T3
Initial Weight (g)	2.12 ± 0.08^{a}	2.11 ± 0.07^{a}	2.13 ± 0.09^{a}	2.12 ± 0.08^{a}
Final Weight (g)	3.84 ± 0.15^{b}	4.21 ± 0.18^{ab}	4.87 ± 0.22^{a}	4.58 ± 0.20^{ab}
Daily Growth Rate (g/day)	0.019 ± 0.002^{b}	0.023 ± 0.002^{ab}	0.030 ± 0.003^{a}	0.027 ± 0.002^{ab}
Food Conversion Rate (FCR)	2.41 ± 0.12^{a}	2.18 ± 0.10^{ab}	1.89 ± 0.09^{b}	2.05 ± 0.10^{ab}
Survival Rate (%)	91.2 ± 2.1^{a}	93.5 ± 1.8^{ab}	95.8 ± 1.6^{b}	94.3 ± 1.9^{ab}

The different letters indicate significant differences between treatments (P<0.05).

Adding dried caper fruit powder significantly improved growth indicators in treatment T 2, with a final weight of 4.87 ± 0.22 g compared to 3.84 ± 0.15 g in the control treatment. The feed conversion ratio (FCR) also decreased from 2.41 in To to 2.18 in T1, 1.89 in T2 and2.05 in T3 indicating higher feed utilization efficiency. As for the survival rate, it was the highest in the treatmentT2 and the lowest in the treatment.

Discussion

The current results are consistent with recent research trends emphasizing the expanding role of antioxidant-rich The extract is concentrated and obtained using solvents, while the powder contains the complete components (fibers and crude compounds).in improving reproductive efficiency in fish. The findings suggest that the positive effect of dried caper fruit powder on reproductive indicators in Sailfin molly resulted from the interaction of multiple, integrated biochemical mechanisms.

First, and it applies to the rest hormonal regulation: caper fruits are characterized by their high content of quercetin and kaempferol, two flavonoids that are structurally similar to estrogenic compounds, allowing them to bind to estrogen receptors and activate the hypothalamic-pituitary-gonadal axis (Sepehrfar *et al.*, 2023). This property explains the significant increase in FSH and LH concentrations in the T2 treatment, consistent with as reported by Amulejoye *et al.* (2025) indicated regarding the the role of phenolic compounds and plant-derived saponins to enhance sex hormone secretion by stimulating the hypothalamic-gonadal axis.

Second Oxidative Protection of Sperm: Due to the nature of their membranes, which are rich in unsaturated fatty acids, sperm cells are more susceptible to lipid peroxidation and reduced motility. Phenolic compounds and flavonoids found in caper fruit, particularly quercetin, have have been reported to exhibit antioxidant scavenging reactive

oxygen species (ROS) and activating the oxidative defense system represented by SOD and CAT enzymes, thus preserving the integrity of the cell membrane and genetic material (Qamar *et al.*, 2023; Martin-Hidalgo *et al.*, 2019). This was clearly reflected in the improved sperm motility, count, and percentage of normal morphology in the T2 treatment.

Third Steroid Stimulation and Gonadal Development: Available evidence indicates that plant supplements containing phytosterols and saponins can activate steroid enzymes in Leydig cells, thereby promoting the endogenous synthesis of testosterone and estradiol (Amulejoye *et al.*, 2025). Elevated estradiol levels in females accelerated oocyte maturation.

Fourth Nutritional Value and Reproductive Effect: Caper fruits provide high-quality nutrients, including unsaturated fatty acids (omega-3 and omega-6), which enhance the flexibility and function of sperm cell membranes and contribute to increased metabolic efficiency. Treatment T2 also showed a significant decrease in the feed conversion ratio (FCR), indicating improved energy utilization for reproductive processes (Martin-Hidalgo *et al.*, 2019).

Fifth Dose Response and Paradoxical Effect: The T3 (1.5%) treatment showed lower performance than T2 in most reproductive parameters, despite outperforming the control treatment. This is consistent with the concept reported in phytochemical studies

of the paradoxical effect of high doses of phenolic and flavonoid compounds, which may induce anti-estrogenic properties or reduce the absorption of essential nutrients (Abaho *et al.*, 2022). This highlights the importance of determining the optimal dose for commercial use to avoid adverse physiological interactions.

In comparison with previous studies, the current findings support the findings of Dada and Adeparusi (2012) regarding improved fertility in fish species fed plant-based ingredients rich in Antioxidant activity was not measured directly, as well as those of Khademzade *et al.* (2022) in their study on *Sparus aurata*, which demonstrated enhanced oxidative defenses associated with improved sperm quality and fertilization rates. These results contribute to the hypothesis that supporting the oxidative system through plant-based supplements is an effective pathway for improving reproductive efficiency in farmed fish.

Conclusions:

The results of this study have clearly demonstrated that including of 1% dried caper fruit powder in the daily diet of Sailfin molly yields the greatest improvements improvement in growth, fertility, and reproductive indicators. The main conclusions can be summarized as follows:

1-A concentration of 1% is considered optimal, providing a balance between positive effects and avoiding potential inhibitory effects at higher levels.

2-Caper fruit powder contributes to improved sperm quality in terms of motility, concentration, morphology, and viability, attributed to its antioxidant properties and its role in regulating hormonal activity.
3-The use of the powder leads to a significant increase in the secretion of reproductive hormones (FSH, LH, testosterone, and estradiol), which positively impacts reproductive efficiency and its various stages.
4-No negative effects of the supplementation on survival rates or cortisol levels (the hormone responsible for the stress response) were recorded.
5-The results highlight the promising potential of caper fruit as a natural feed additive in aquaculture, particularly for high-value ornamental fish.

The study recommends future research focusing on comparing the effects of purified extracts of the active compounds (such as quercetin and rutin) with the crude powder, as well as detailed histological studies of the reproductive glands and testing the plant's efficacy on other commercially important fish species, such as tilapia and carp.

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استخدام مسحوق ثمار نبات الشفلح *Capparis spinosa* المجففة بنسب مختلفة في تحسين خصوبة اسماك المولي شراعي الزعنفة *Poecilia latipinna*

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المستخلص

أجريت هذه الدراسة لتحديد تأثير إضافة مسحوق ثمار نبات الشفلح (*Capparis spinosa*) المجففة إلى العليقة بنسب متفاوتة (0، 0.5، 1.0، 1.5%) على مؤشرات الخصوبة والنمو والأداء التناسلي لأسماك المولي شراعي الزعنفة" (*Poecilia latipinna*). استمرت التجربة لمدة 90 يوماً وشملت 120 سمكة (60 ذكراً و60 أنثى)، قُسّمت إلى أربع معاملات بواقع ثلاث مكررات لكل معاملة. وأظهرت النتائج تفوق المعاملة T2 (التي تضمنت 1% من مسحوق ثمار الشفلح)، حيث سجلت أعلى القيم لكل من: عدد الصغار في الولادة الواحدة (37.4 ± 2.6)، ومعدل بقاء الصغار (88.3 ± 2.4%)، وحيوية الحيوانات المنوية (84.2 ± 2.7%)، بالإضافة إلى زيادة معنوية في مستويات الهرمونات (التستوستيرون، والإستراديول، والهرمون المنشط للحوصلة/FSH)، كما تأثر مستوى الهرمون الملوتن (LH) أيضاً. ولوحظ تحسن معنوي في معامل التحويل الغذائي (FCR) في المعاملة نفسها ($P<0.05$). ويُعزى هذا التأثير الإيجابي إلى المحتوى الغني لثمار الشفلح بمركبات الفينول والفلافونويد والقلويدات، ولا سيما مركبي "الروتين" و"الكيرسيتين". ويمكن استنتاج أن إضافة مسحوق ثمار الشفلح بنسبة 1% تمثل المستوى الأمثل لتحسين الخصوبة والأداء التناسلي لاسماك المولي شراعي الزعنفة.

الكلمات المفتاحية: *Capparis spinosa*، *Poecilia latipinna*، الأداء التناسلي، جودة الحيوانات المنوية، المكملات الغذائية، تغذية الاستزراع المائي.