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Research Article

EXPLORING THE POTENTIAL OF VELVET BEAN, *MUCUNA PRURIENS* (L) SEED ON GROWTH AND GONADAL DEVELOPMENT OF MONO-SEX COMMON MOLY *POECILIA SPHENOPS* (VALENCIENNES, 1846)

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ABSTRACT: *Mucuna pruriens*, a rich source of L-dihydroxyphenylalanine, commonly known as L-DOPA and a precursor to dopamine, holds potential as a natural nutritional supplement. This study aimed to delve into the impact of incorporating *M. pruriens* seed powder (MpSP) into the feed on growth parameters and gonadal development of mono-sex common molly (*Poecilia sphenops*). The fish population was divided into three experimental groups, such as G1, G2, and G3, and a control group (C), each comprising 20 individuals. Over 45 days, the experimental groups were nourished with a commercial diet bolstered by MpSP in different concentrations (5, 7 and 10g/kg of feed, respectively). In contrast, the control group was provided with a regular diet devoid of the supplement. At the end of the experiment, MpSP demonstrated significant modulation ($p<0.05$) of growth performance metrics, including specific growth rate (SGR), length gain rate (LGR), body mass gain (BMG), and feed conversion ratio (FCR). Impressively, even the lower concentration of MpSP (5g/kg diet) yielded substantial increments in sperm count ($p <0.05$) and gonadosomatic index (GSI). These findings were corroborated by histological changes that reflected enhanced testicular development, consistently outperforming the control group. These outcomes collectively suggest the potential of velvet bean seed powder as a feasible, natural, and cost-effective dietary supplement for enhancing growth and testicular development in mono-sex *P. sphenops*.

Keywords: *Poecilia sphenops*, Velvet bean, Gonadal development, Growth performance, Diet supplementation.

INTRODUCTION

Successful reproduction plays a key role in ornamental fish culture, as the process increases population and thus profitability. However, uncontrolled reproduction typically causes overcrowding, a decline in growth, and a high percentage of fish become stunted reducing profitability [1]. Monosex culture is the most widely practised and cost-efficient means of limiting unregulated reproduction. It is labour-intensive and involves hand-to-hand separation of sexes, environmental changes, hybridization, sex reversal and genetic engineering techniques such as androgenesis, gynogenesis, polyploidy, and transgenesis [2]. Monosex culture has a significant role in the ornamental fish trade, as these fishes exhibit a marketable sexual dimorphism and in the majority of cases the male fishes are preferred over females due to their overtly pigmented bodies and well-developed fins [3]. A large economic benefit could result from the development of monosex (all-male) populations of ornamental fish due to this marketed preference.

Poeciliidae make up over half of the ornamental fish market in India [4, 5]. *Poecilia sphenops* (common molly) is one of the most popular feeder fishes due to

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its rapid growth, birth size, prolific reproduction, and high brood number [6]. There is a remarkable relevance for the development of quality males and females in molly culture. In recent times, plant-based products have been widely used to enhance reproductive indices in aquaculture [7]. The phytochemicals are proven to enhance sperm quality, egg size, induce sex inversion, and control unwanted breeding [8].

Mucuna pruriens, commonly known as the velvet bean, is a tropical legume belonging to the Fabaceae family. This herbaceous medicinal plant is globally recognized and boasts a diverse array of over 200 drug formulations. These formulations confer a range of health benefits including immunostimulatory, aphrodisiac, anthelmintic, anti-inflammatory, and antidepressant activities [9, 10]. These traits can be attributed to the presence of compounds like 3, 4-dihydroxy-L-phenylalanine (L-Dopa) and other phytochemicals such as alkaloids, glycosides, and saponins [11, 12]. Noteworthy constituents also include nicotine, physostigmine, serotonin, bufotenine, choline, N-N-dimethyl tryptamine, and certain indole compounds known for their neuroprotective effects [13]. L-DOPA has been investigated as a dietary supplement for addressing conditions like kidney failure, hypertension, and liver cirrhosis [14]. The impact of *M. pruriens* seeds has been explored in various animal species, revealing influences on reproductive indices such as sexual behaviour, gonad growth and gamete quality in rats [15] and rabbit bucks [3]. Similar studies in fish have investigated *M. Pruriens* efficacy in terms of growth performance, biochemical profiles, and immunity across a limited range of species [16, 17]. However, our understanding of how adding *M. pruriens* seed powder (MpSP) to the diet affects the growth and reproductive performance of fish is incomplete. The present study has examined the potential of MpSP as a dietary supplement for mono-sex common molly. The study has examined its effect on growth performance, sperm count, and gonadal histology within the framework of acceptable dietary inclusion levels.

MATERIALS AND METHODS

Collection and rearing of fishes

Adult mono-sex *Poecilia sphenops* (size: 4.877 ± 0.17cm, 2.311 ± 0.36 g) were sourced from a local commercial aquarium outlet situated in Bengaluru. The fish were transported to the laboratory in an oxygen pack. Subsequently, a five-day acclimatization period ensued, during which the fishes were housed in sanitized 500 L glass tanks with aeration and fed with a commercial diet at the rate of 3% of body weight. To ensure an optimal environment, the physicochemical parameters of the rearing water were diligently monitored. Daily assessments yielded temperature readings of 28 ± 2ºC, dissolved oxygen 5.7 ± 0.5 mg/L, ammonia 0.53 ± 0.07 mg/L, and pH7 ± 0.2 mg/L.

Preparation of *M. pruriens* seed powder (MpSP)

Seeds of *M. pruriens* were sourced from the Indian Institute of Horticulture Research (IIHR), Bengaluru. The seeds were washed thoroughly with distilled water, air-dried and ground to a fine powder which was blended at 3g, 5g and 10 g/kg with a commercial feed (obtained from TAIYO, Tokyu, Japan) containing 30% crude protein. The mixture was then moistened with deionized water, thoroughly mixed again and made into 2 mm diameter pellets using a pellet maker. The pellets were then air-dried at room temperature and constituted the experimental diet for the study.

Experimental design

The common molly fish, *P. sphenops*, was divided into four groups (with three replicates each), each containing 20 fingerlings. One group, labelled as the control group (C), received a regular commercial diet with no addition of MpSP. The experimental groups, namely, G1, G2, and G3, were fed with the prepared pellet feed containing 5g/kg, 7g/kg and 10g/kg of MpSP, respectively. The fish in all groups were fed an amount equal of feed at 3% of their body weight twice a day for 45 days (Table 1). Throughout the experimental period, a photoperiod of 10:14 (light: dark) was maintained. Adequate aeration was provided to all the fish tanks, and the water in each tank was manually replaced with fresh water once daily.

Growth performance and somatic indices

Growth parameters, such as specific growth rate (SGR %), feed conversion ratio (FCR), and length gain rate (LGR) were measured as per the formula given below:

$$\text{SGR} = \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{Experimental duration (days)}} \times 100$$

$$\text{FCR} = \frac{\text{Total feed intake}}{\text{Total weight gain}}$$

$$\text{LGR} = 100 \times \frac{\text{Final body length} - \text{initial body weight}}{\text{Initial bogy length}}$$

Sperm count in male gonad

To investigate the effects of MpSP on the male gonads of fish, the number of sperm present was counted at 15-day intervals. Upon reaching maturity, the male gonads were carefully removed using aseptic techniques, weighed and subjected to maceration in 0.1% saline buffer. For enhanced visibility and observation of sperm cells, a small quantity of acetocarmine (1-2 drops) was added. The macerated solution was then drawn into a WBC (white blood cell) pipette, introduced into a haemocytometer and the number of sperm cells present was counted under a microscope [18].

Gonado-somatic index

The gonado-somatic index was estimated by following Strum [19].

$$GSI = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

Gonadal histological study

Gonadal tissue sections from all the experimental groups were processed following the method of Bancroft and Cook with slight modifications [20]. Photomicrographs were captured using a digital imaging system (LYNX LM-52, Lawrence & Mayo, India) to assess the impact of MpSP on testes of the fish.

Statistical analysis

The experiments were carried out utilizing five separate replicates. The influence of MP seed powder on growth and reproductive parameters was assessed through a one-way analysis of variance (ANOVA). This analysis was performed using IBM SPSS Statistics, Version 11 (IBM Corp., Armonk, NY, USA). For making multiple comparisons, Duncan's test was employed. A significance threshold of $p<0.05$ was adopted, and the outcomes are presented as the mean ± SD.

RESULTS AND DISCUSSION

Traditional medicines are more cost-effective and useful in aquaculture than pharmaceutical medicines [21]. The current study evaluates the effects of an *M. pruriens* seed powder (MpSP)-supplemented diet on the growth and testicular development of mono-sex common molly (*P. sphenops*).

Growth performance

Results revealed that growth parameters such as SGR and LGR were noticeably enhanced in all the experimental groups (G1, G2 and G3) compared to the control ($p<0.05$). Similar result was reported with 20% of Mucuna seed meal by Elabd *et al.* [22]. However, no significant variations were observed among the experimental groups of fishes suggesting that the lowest dose used might improve the growth metrics of common mollyfish. Studies involving other fish species, such as *Oreochromis niloticus* [22], *Labeo rohita* [10], and *Clarias gariepinus* [16] indicated that even lower concentrations of MpSP (2g/kg) significantly improved their growth parameters. These beneficial effects are linked to MpSP's increased palatability and digestibility of minerals, resulting in an overall increase in growth rate [8].

Table 1. Composition of control and experimental diet.

Composition	Control group (C)	Experimental diets (g/kg)		
		G_1	G_2	G_3
Basal diet	√	√	√	√
MpSP	0	5	7	10

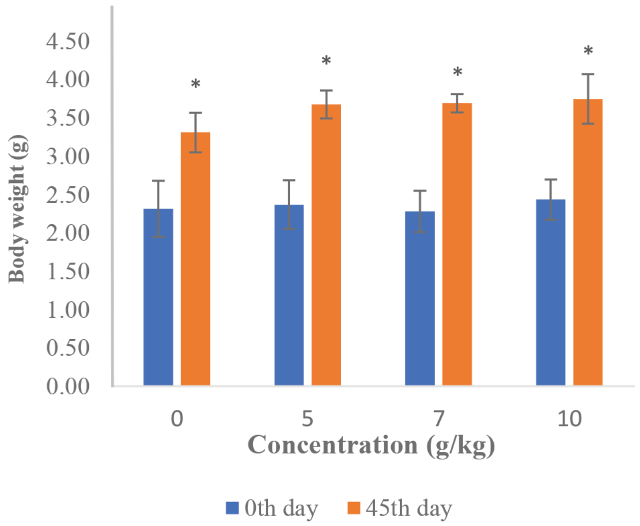

Fig. 1. Average weight gain in control and experimental groups after 45 days of feeding. (Data expressed as mean ± SD; * = $p<0.05$).

Testicular indexes

There were significant rises in testicular indexes, viz. GSI and spermatozoa count, in G2 (16.176%) and G3 (15.646%), compared to the control group (15.533%) (Table 1; Fig. 3). These beneficial effects might come from the action of L-DOPA present in MpSP on hypothalamus-pituitary-gonadal axis, and its effects on testosterone release [23]. Fish receiving the lowest dose of MpSp also displayed a pronounced development of testicular structure, as evidenced by

Table 2. Growth performance of *P. sphenops* fed a diet supplemented with different concentrations of MpSP.

Groups	Initial Wt. (g)	Final Wt. (g)	Initial length (cm)	Final length (cm)	SGR (%)	LGR (%)	GSI
C	2.31±0.36	3.31±0.26	4.88±0.17	5.50±0.36	3.323	12.77	15.533
G_1	2.31±0.36	3.67±0.18	4.88±0.17	5.88±0.24	4.543	20.56	16.176
G_2	2.31±0.36	3.69±0.12	4.88±0.17	5.85±0.22	4.596	19.95	15.641
G_3	2.31±0.36	3.74±0.30	4.88±0.17	5.73±0.42	4.776	17.49	15.646

Values are expressed as mean (n = 30) ± SE.

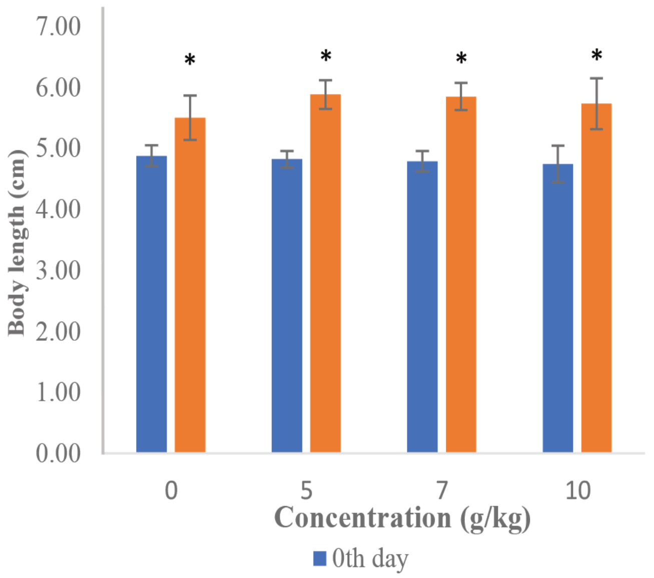

Fig. 2. Average length gain in control and experimental groups after 45 days of feeding. (Data expressed as mean ± SD; * = p<0.05).

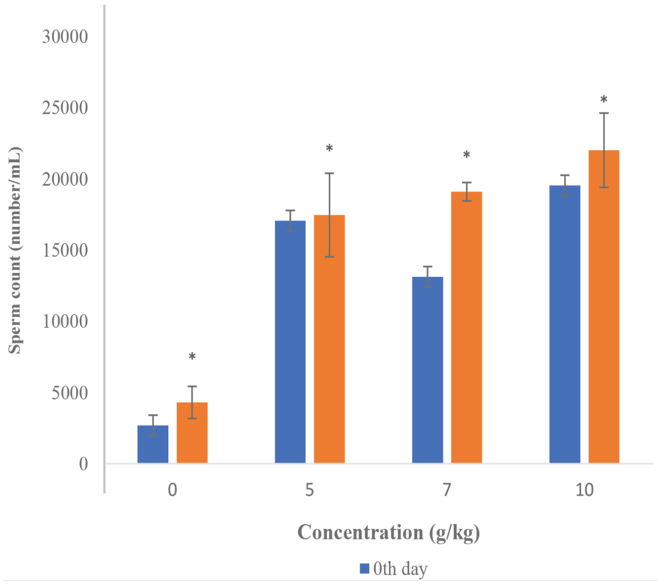

Fig. 3. Sperm count of control and experimental groups after 45 days of feeding trial. (Data expressed as mean ± SD; * = p<0.05).

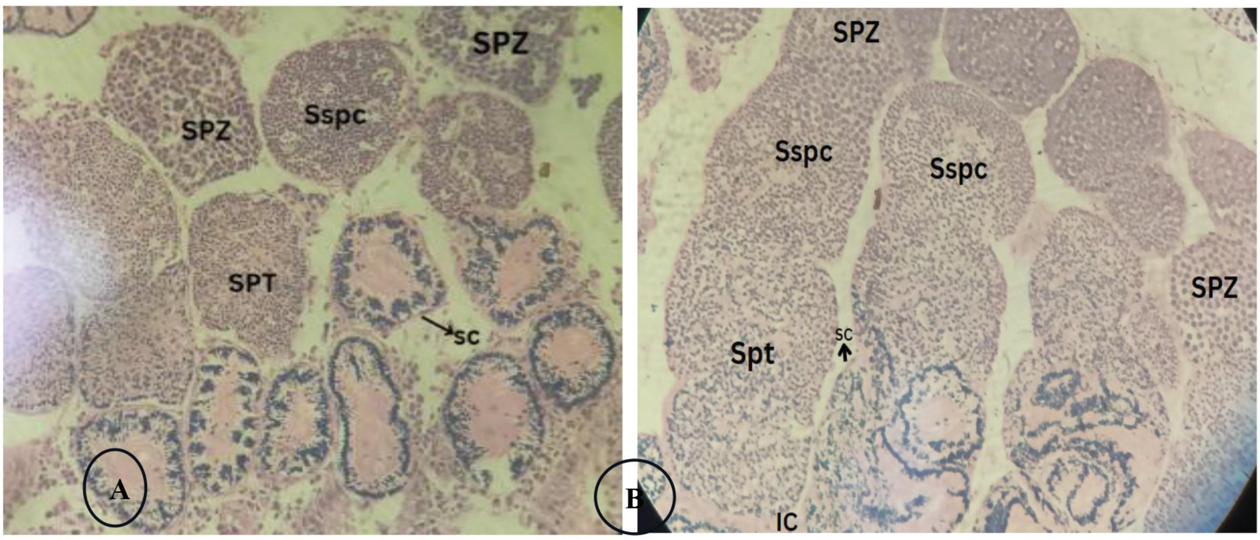

Fig. 4. Transverse section of testes of control (A) and G3 group of fish (B). (SPZ: Spermatozoa; Sspc: Secondary spermatocytes; SPT: Spermatids; SC: Sertoli cells; IC: Interstitial cells).

gonadal histology. This favourable outcome might be attributed to MpSP's capacity to stimulate the growth of spermatocytes and its modulation of hypothalamus-pituitary-gonadalaxis, resulting in dose-dependent improvements in testicular structure. However, contrary to these observations, studies by Farizah *et al.* [24] and Elabd *et al.* [22] found that higher doses led to degenerative changes, accompanied by counteractive effects of higher concentrations of L-DOPA in combination with melastoma leaf extract and *M. pruriens* powder.

The transition of spermatocytes to sperm may explain the enhanced histological structure in fish receiving only 5g of MpSP supplementation. Previous studies [25, 26] showed that MpSP's high antioxidant activity protects testicular architecture. Thus, feeding MpSP at a dose of 5g/kg to mono-sex common mollies resulted in increased growth and improved testicular histopathology. The results suggest that MpSP has the potential of use as a cost-effective way to improve fish growth and reproductive performance which would benefit the aquarium fish trade.

Testicular histology

Histological examination of the testis showed presence of the connective tissue and lobulation inside the testicular structure in the control group, after 45-day feeding. In control fish, most of the spermatogenic cells, along with certain spermatogenic cysts were observed on the periphery of the testicular lobules (Fig. 4a). Whereas in experimental groups, a significant increase in sperm count (20766.60 ± 1925.20) was noted in G3 fish and with a dose-dependent increase among the experimental groups.These findings underscore the ability of MpSP supplementation to modulate sperm production rate and masculinization in a dose-dependent manner.

Analysis of phytochemical composition of MpSp revealed presence of vitamin E (tocopherol), a recognized nutrient for male fertility as well as an antioxidant. It is capable of scavenging free radicals that could potentially impact sperm cells and consequently lead to an elevation in sperm count [20]. MpSP also contains prurienine and prurienidine, potent pro-fertility enhancers that stimulate testosterone secretion and augment spermatozoa concentration and testes size in human males [30]. Also, it has the potential to enhance daily sperm production, guard against reproductive damage, and counteract apoptosis induced by alterations in pivotal functional proteins within the testis and sperm [27]. These compounds might also exert similar effects on fish, as suggested by [28]. It was also observed to elevate testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin hormone levels, as well as sperm count and motility in infertile obese mutant rat models [29].

It is notable that testicular tissue development was more pronounced in G1 and G2 (5g and 7g MpSP/kg diet) groups. Additionally, spermatozoa were observed within the lobule lumens (Fig. 4a). In the G3, the presence of spermatozoa within the lobule lumens was accompanied by the appearance of primary and secondary spermatocytes, along with degeneration in spermatid cysts (Fig. 4b). Concurrently, a decrease in the amount of connective tissue was noted, accompanied by more distinct testicular lobulation (Fig. 4b).

CONCLUSION

This study highlights the noteworthy role of MpSP in enhancing both growth and reproductive processes. *M. Pruriens* enhances the masculinization of mollyfish, suggesting its potential use as a natural alternative to chemicals in inducing monosex populations in aquaculture settings.

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