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Efficacy of medicinal herb *Asparagus racemosus* (shatavari) as an aphrodisiac feed additive for enhancing reproductive performance of female brood stock of freshwater carp fish, *Cyprinus carpio* Linn.

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ABSTRACT

Efficacy of shatavari (*Asparagus racemosus*) root powder (SRP) as an aphrodisiac fish feed additive was evaluated by feeding female brood stock of freshwater carp, *Cyprinus carpio* (Linn.) with experimental feeds (for 120 days), supplemented with SRP @ 1% (SRP_1), 2% (SRP_2), 3% (SRP_3) and 4% (SRP_4). Feed without SRP served as control (SRP_0) feed. SRP improved reproductive performance of fish significantly at 3 and 4% incorporation levels, but best results were obtained at 4% level (SRP_4), resulting in improved gonadosomatic index (41.23%↑) and relative fecundity (45.00%↑); supported with an upregulated hormone profile, viz. estradiol (53.36%↑) and progesterone (130%↑). Although, no significant changes were observed in the egg size, but egg quality was significantly improved in terms of protein and lipid content, indicating higher feed and energy reserve for larval development. The immuno-modulatory effect of SRP was indicated by 2.12 times higher haemoglobin and 1.61 times higher haematocrit values, with enhanced immunity [serum total protein (42.21%↑), albumin (53.91%↑), globulin (32.92%↑) and total immunoglobulin (68.48%↑)]; antioxidant markers [lipid peroxidation (44.56%↓) and superoxide dismutase (80.43%↑)]; and positive metabolic changes in respect to liver function [serum glucose (16.91%↓), alanine aminotransferase (27.88%↓) and aspartate aminotransferase (15.82%↓)] and lipid profile [serum cholesterol (17.33%↓) and triglycerides (13.10%↓)] of the shatavari fed fish (SRP_4). The results indicated that *A. racemosus* root powder can be incorporated in common carp brood stock feed @ 4% for boosting its reproductive potential, with a projected fry production enhancement of 45%.

Keywords: Antioxidant, *Asparagus racemosus*, *Cyprinus carpio*, Immuno-modulatory effects, Reproductive performance, Shatavari

With well-recognition of fisheries sector towards economic growth of the nation, India is the 2nd largest aquaculture producer in the world, with total fisheries production of 17.54 million metric tonnes (mmt) during 2022-23, including major share (74.74%) coming from the inland sector (DOF 2023). The 2024 assumed that production target can only be achieved through innovative technological interventions like use of feed additives in fish ration. However, these feed additives are documented to be associated with serious environmental and food safety concerns (Jangpangi *et al.* 2023). In this context, traditional knowledge of medicinal properties of various herbal plants (ashwagandha, garlic, amla, shatavari, etc.) have been used successfully in aquaculture feeds to stimulate reproductive performance in fish (Kaur *et al.* 2020, Kumar *et al.* 2021). These medicinal plants contain various bioactive substances offering multiple health benefits in human medication since ages, including fertility (Girish 2018, Kumar *et al*. 2022).

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Among the medicinal plants, shatavari (*Asparagus racemosus*), designated as "Queen of Herbs", is well-known ayurvedic rejuvenating tonic for multiple health benefits, like immuno-modulation and aphrodisiac properties (Kumar *et al*. 2019, Selvaraj *et al.* 2019). Shatavari contains many useful bioactive compounds (Dahiya *et al.* 2022, Singh and Sharma 2024), well-documented for above listed health advantages and action. Among all health functions, shatavari is more popular as an infertility tonic used to boost female health in traditional medicines (Thakur *et al.* 2021, Sharma *et al.* 2022).

Many reports on aphrodisiac use of shatavari in different animals are available (Kumar *et al*. 2019, Singh and Kapoor 2022). Although, some preliminary studies also reveal growth promoting efficacy of shatavari in fish (Parmar *et al.* 2020, Singh *et al.* 2024), but its application as a reproduction boosting supplement in fish still needs to be explored. Hence, the present study was conducted to estimate potency of shatavari root powder (SRP) as an aphrodisiac feed additive for female brood stock of widely cultured freshwater carp fish species, *Cyprinus carpio* Linn.

MATERIALS AND METHODS

The present study was conducted in outdoor cemented tanks (80 m^2) at relatively dry monsoon-influenced humid subtropical climate (Ludhiana, India) for a period of 120 days.

Preparation of shatavari root powder (Srp): The raw shatavari roots were procured from the Medicinal Plants Research Development Centre of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand and ground into powder form after drying, for incorporation in the fish feed. Four shatavari supplemented experimental fish feeds were prepared by incorporating SRP in the basal feed (de-oiled rice bran, 49%; mustard meal 49%; commercial multi-mineral mixture 1.5% and common salt 0.5%) @ 1% (SRP_1), 2% (SRP_2), 3% (SRP_3) and 4% (SRP_4), while feed without SRP served as control (SRP_0). Sinking pellets were made with the help of hand pelletizer and stored in air tight containers at a cool dry place after oven drying for 8 h at 40ºC.

Proximate composition of feeds: The proximate composition of feed ingredients (rice bran, mustard meal and SRP) and experimental feeds (SRP_0, SRP_1, SRP_2, SRP_3 and SRP_4), viz. crude protein, ether extract, ash, crude fibre, and nitrogen free extract, were estimated on dry matter (DM) basis (Table 1), following standard methods AOAC (2018).

Experimental design: Common carp brood stock was stocked in the outdoor tanks (80 m^2) @ 1500 kg/ha (12 kg/tank) in triplicates (male female ratio 1:1) for each feed treatment. The fish was fed with experimental feeds @ 3% body weight (BW) daily for 120 days and feeding ration was in accordance to weight gain after monthly intervals. Water quality parameters (*p*H, dissolved oxygen, alkalinity, hardness, ammonical-nitrogen) were monitored at monthly intervals to maintain optimum culture conditions (APHA 2018).

Reproductive parameters: The reproductive potential of the fish was estimated in terms of gonado-somatic index (GSI), absolute fecundity, relative fecundity and ova diameter by selecting three fish specimens randomly from each treatment. The GSI and fecundity were determined using a simple gravimetric method, while the ova diameter was measured using a stage and an ocular micrometre:

$$\text{GSI (\%)} = \frac{\text{Weight of Gonads (g)}}{\text{Weight of Fish (g))}} \times 100$$

$$\text{Absolute Fecundity (no.)} = \frac{\text{No. of eggs in sub-sample} \times \text{Total weight of ovary (g)}}{\text{Weight of sub-sample (g)}}$$

$$\text{Relative Fecundity (no.)} = \text{Absolute fecundity (no.)/ Weight of fish (g)}$$

Proximate composition of fish eggs: At termination of the experiment, eggs were collected from fish specimens (triplicate) selected randomly from each feed treatment for quality assessment in terms of protein, ash content (AOAC 2018) and lipid (Folch *et al*. 1957).

Reproductive hormone profile: After 120 days of rearing, the serum reproductive hormone profile of fish

Table 1. Proximate composition (%) of ingredients and experimental feeds (DM basis)

Ingredient/ experimental feed*	Crude protein	Ether extract	Crude fibre	Ash	Moisture	Nitrogen free extract
Rice bran	12.35	1.26	15.56	11.30	4.26	55.27
Mustard meal	38.64	1.86	11.43	8.13	5.10	34.84
SRP	8.08	0.73	2.36	6.23	4.26	78.34
SRP_0	$25.39^a±0.05$	$1.65^a±0.03$	$12.10^a±0.06$	$9.50^a±0.06$	$5.05^{ab}±0.03$	$46.31^c±0.01$
SRP_1	$25.39^a±0.03$	$1.60^a±0.06$	$12.00^{ab}±0.00$	$9.45^a±0.03$	$4.95^b±0.03$	$46.61^b±0.10$
SRP_2	$25.20^b±0.02$	$1.70^a±0.06$	$11.95^{bc}±0.03$	$9.40^a±0.06$	$5.10^{ab}±0.06$	$46.65^b±0.05$
SRP_3	$25.12^b±0.00$	$1.60^a±0.00$	$11.90^{bc}±0.00$	$9.45^a±0.03$	$5.20^a±0.06$	$46.79^{ab}±0.07$
SRP_4	$25.08^b±0.05$	$1.55^a±0.03$	$11.85^c±0.03$	$9.50^a±0.06$	$5.10^{ab}±0.06$	$46.92^a±0.01$

*Mineral mixture composition: SiO_2 (50-60%), Al_2O_3 (15-20%), CaO (15-20%) Fe_2O_3 (3-5%) and MgO (2-5%)

Table 2. Reproductive parameters of *C. carpio* female brood stock in different feed treatments

Parameter	Feed treatment				
	SRP_0	SRP_1	SRP_2	SRP_3	SRP_4
Average weight of fish (g)	$895.56^a±41.57$	$996.11^a±56.88$	$939.44^a±56.27$	$949.44^a±58.80$	$895.33^a±22.87$
Average weight of ovary (g)	$175.89^c±4.58$	$187.78^c±5.99$	$180.11^c±4.77$	$225.67^b±9.66$	$250.11^a±3.99$
Gonado-Somatic Index (GSI)	$19.84^c±0.65$	$19.07^c±0.55$ (-3.88%)	$19.50^c±0.78$ (-1.71%)	$24.03^b±0.68$ (+21.12%)	$28.02^a±0.58$ (+41.23%)
Absolute fecundity (eggs/ovary)	$2{,}04{,}530^d±2{,}931$	$2{,}28{,}270^c±4{,}457$	$2{,}22{,}940^{cd}±4933$	$2{,}67{,}270^b±10430$	$2{,}99{,}570^a±5530$
Relative fecundity (eggs/g BW)	$231.55^c±8.83$	$233.04^c±8.63$ (+0.64%)	$241.86^c±10.41$ (+4.45%)	$285.38^b±9.61$ (+23.25%)	$335.75^a±7.66$ (+45.00%)
Ova diameter (mm)	$0.71^a±0.01$	$0.72^a±0.01$	$0.71^a±0.01$	$0.72^a±0.01$	$0.72^a±0.01$
Estradiol (pg/ml)	$117.73^e±0.50$	$127.32^d±0.46$	$139.97^c±0.28$	$149.45^b±0.30$	$180.55^a±0.30$
Progesterone (pg/ml)	$297.78^e±1.27$	$384.22^d±1.24$	$447.00^c±1.77$	$535.78^b±0.98$	$684.67^a±1.54$

*, Values (mean±SE) with same superscripts in a row do not differ significantly (P ≤ 0.05), n=3.

(estradiol and progesterone levels) was estimated using competitive ELISA kit (Cayman chemical) with the help of ELISA plate reader.

Blood parameters: At termination of the experiment, the blood samples were collected from the caudal vein of the fish collected randomly (triplicate) from each feed treatment and centrifuged (3000 rpm for 15 min) to collect the serum for estimation of the haematological parameters, including haemoglobin (Hb), haematocrit value (Hct), total erythrocyte count (TEC) and total leucocyte count (TLC); immunological parameters (total proteins, albumin, globulin, A:G ratio and total immunoglobulins); lipid profile (cholesterol and triglycerides); liver function in terms of glucose level, alanine aminotransferase (ALT)/ aspartate aminotransferase (AST) activity; and the antioxidant markers including lipid peroxidation (LPO) and superoxide dismutase (SOD) activity. Estimation of serum total proteins, albumin, and globulin, glucose, cholesterol, triglycerides, ALT and AST levels was done with the help of Erba Diagnostic Mannheim GmbH Kit. The total immunoglobulins were estimated using 'BT LAB IgG ELISA' Kit, while LPO and SOD were estimated by the manual methods (Placer *et al*. 1966 and Nishikimi *et al*. 1972).

Statistical analysis: The analysis of data was done by one-way analysis of variance (ANOVA) at 5% level of significance (P≤0.05) between treatments and Tukey's b test was applied using SPSS (Version 16.0) and the data was expressed as mean±S.E.

RESULTS AND DISCUSSION

Water quality parameters, viz. temperature (27.56-33.33°C), *p*H (8.04-8.66), dissolved oxygen (9.77-13.11 mg/L), total alkalinity (127-192 mg/L), total hardness (154-213 mg/L) and ammonical nitrogen (0.017-0.024 mg/L), were well within the recommended range for carp culture (Boyd and Tucker 1998) in all the feed treatments (SRP_0-SRP_4) to support optimum fish growth and development throughout the experimental period.

Shatavari supplementation improved the reproductive potential of the fish significantly (P≤0.05) at 3% (SRP_3) and 4% (SRP_4) incorporation levels (Table 2), but maximum GSI (41.23%↑) and relative fecundity (45%↑) values were recorded at 4% inclusion level, supported with an upregulated hormone profile, viz. estradiol (53.36%↑) and progesterone (130%↑) levels. Although, no significant changes were observed in the egg size, but the egg quality improved significantly (Table 3) in respect to protein (6.65%↑) and lipid (25.56%↑) content, indicating extra food and energy reserve availability for larval development with higher survival rate expectancy.

Reproduction in fish is also coordinated by the hypothalamus-pituitary-gonadal axis (Ramezani-Fard *et al.* 2013). The present study indicates stimulation of the said axis, resulting in higher levels of reproductive hormones (estrogen and progesterone) in SRP fed fish. The phytoestrogens (steroidal saponins and isoflavones) present in shatavari are reported to resemble estrogen structurally or functionally and hence, expected to have modulated gonadal development in shatavari fed fish, boosting oogenesis (higher GSI and fecundity) and vitellogenesis (higher protein and lipid content). Although, supportive findings in respect to effect of Shatavari on reproductive performance of fish is not available, but it is well-documented in other animals and is reported to treat female infertility by enhancing folliculogenesis and ovulation (Kumar *et al*. 2019, Singh and Kapoor 2022).

Further, the antioxidant and immuno-modulatory properties of bio-active compounds, viz. shatavarins, flavonoids, immunoside, racemofuran, racemosol and asparagamine-A, present in the shatavari root (Singh and Sharma 2024) would also have indirectly contributed to the reproductive surge by improving the overall health status of the fish. The hypothesis is underpinned by positive changes in the haematological parameters (Fig. 1), viz. Hb (2.12 times↑) and HCT (1.61 times↑) and the immunological parameters (Fig. 2), viz. serum protein (42.21%↑), albumin (53.91%↑), globulin (32.92%↑) and total immunoglobulins (68.48%↑) in the SRP fed fish. With remarkable increase in Hb and Hct values, enhanced

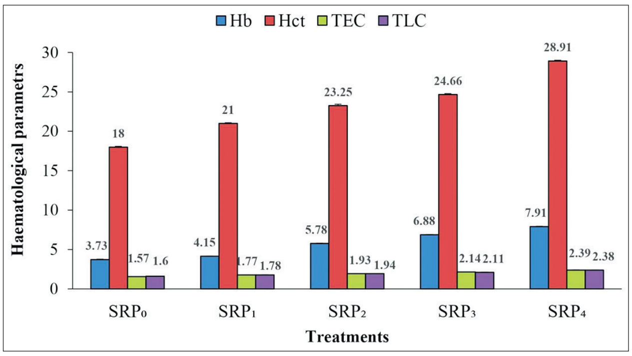

Fig. 1. Haematological parameters [Hb (g%), Hct (%), TEC (No. $\times10^6$/mm^3), and TLC (No. $\times10^3$/mm^3)] of *C. carpio* female brood stock under different feed treatments.

Table 3. Egg quality of *C. carpio* (on wet weight basis) in different feed treatments

Parameter (%)	Feed treatment				
	SRP_0	SRP_1	SRP_2	SRP_3	SRP_4
Total protein	$24.79^{c}\pm0.10$	$25.90^{b}\pm0.14$	$25.82^{b}\pm0.19$	$26.45^{a}\pm0.11$	$26.44^{a}\pm0.09$
Total lipid	$5.36^{c}\pm0.05$	$5.55^{bc}\pm0.06$	$5.58^{bc}\pm0.09$	$5.85^{b}\pm0.14$	$6.73^{a}\pm0.05$
Ash	$1.63^{b}\pm0.02$	$1.71^{a}\pm0.01$	$1.63^{b}\pm0.01$	$1.73^{a}\pm0.02$	$1.72^{a}\pm0.01$
Moisture	$68.18^{a}\pm0.15$	$66.80^{b}\pm0.13$	$66.93^{b}\pm0.28$	$65.93^{c}\pm0.09$	$65.08^{d}\pm0.07$

*, Values (mean±S.E.) with same superscripts in a row do not differ significantly (P ≤ 0.05).

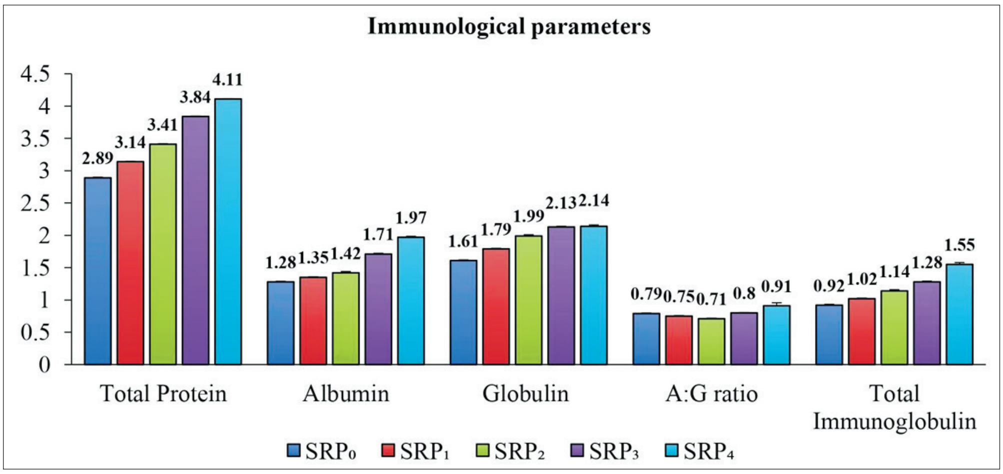

Fig. 2. Immunological parameters [(Total protein, Albumin, Globulin, A:G Ratio and Total Immunoglobulin (g/dL)] of *C. carpio* female brood stock under different feed treatmen.

oxygen carrying capacity of fish blood must have curtailed energy loss to environmental stressors to augment gonadal growth during the reproductive phase. Enhanced immunity is expected to channelise more energy towards gonadal development as compared to an immuno-compromised fish. Similar immunoglobulin boost was reported in fresh water carp, *Labeo rohita* (Sharma *et al.* 2018) and broiler chicks (Al-Subaihawi and Abbas 2021).

Further, beneficial changes in the lipid profile (cholesterol and triglycerides), liver function (glucose, ALT and AST) and oxidative stress markers (LPO and SOD) of SRP fed fish also substantiate health benefits of shatavari root (Table 4). Best results were recorded at 4% inclusion level (SRP_4). Significant decline in serum cholesterol (17.33%↓), triglycerides (13.10%↓) and glucose (16.91%↓) levels in SRP fed fish, indicates enhanced mobilization of energy reserve and cholesterol to support steroidogenesis for gonadal development, viz. estradiol lead vitellogenesis and progesterone conversion to MIH (Maturation-Inducing Hormone) to regulate oocyte maturation in fish (Yaron *et al.* 2003). Shatavari induced decline in blood glucose, triglyceride and cholesterol levels have also been reported in other animals (Al-Subaihawi and Abbas 2021).

The ALT and AST enzyme activity in liver represents protein metabolism, while raised concentrations in the blood indicates liver cell damage (Yan *et al*. 2007). Significantly declined serum ALT (27.88%↓) and AST (15.82%↓) levels in SRP fed fish (SRP_4) indicate improved liver health and attributed to hepatoprotective properties of shatavari, as proved in case of rats challenged with diethylnitrosamine (DEN) induced hepatotoxicity and oxidative stress (Selvaraj *et al*. 2019). Further, LPO refers to oxidative lipid degradation, which reduces antioxidant capacity of the cells due to loss of membrane fluidity, integrity and function, while SOD is an enzyme, which helps in breakdown of potentially harmful oxygen molecules (free radicals) in the cells. Decreased LPO (44.56%↓) and increased SOD (80.43%↑) serum levels in SRP fed fish indicate enhanced protection against free radical damage, due to antioxidant properties of shatavari phyto-active compounds, viz. shatavarins, racemoside, racemofuran and asparagamine-A (Dahiya *et al.* 2022, Singh and Sharma 2024). Shatavari induced decline in cholesterol, AST and ALT levels has also been documented in chicken broilers (Kant *et al.* 2015); while significant improvement in LPO (↓) and SOD (↑) activity was observed in rats (Bhatnagar *et al.* 2005).

The anti-oxidant, anti-hepatotoxic, immuno-modulatory, anti-microbial activity of phytochemical constituents, viz. saponins (Shatavarin I-VI), alkaloids (Asparagamine-A), flavonoids (Quercetin), poly-phenols, Racemofuran, present in *A. racemosus* (Selvaraj *et al.* 2019, Dahiya *et al.* 2022, Singh and Sharma 2024), offer multiple health benefits boosting liver health, growth, immunity

Table 4. Blood metabolic profile of *C. carpio* female brood stock in different feed treatments

Parameter	Feed treatment				
	SRP_0	SRP_1	SRP_2	SRP_3	SRP_4
Glucose (mg/dL)	91.58^a±0.14	87.53^b±0.13	84.74^c±0.22	79.91^d±0.14	76.09^e±0.23
Cholesterol (mg/dL)	168.82^a±0.19	162.82^b±0.23	151.57^c±0.18	145.85^d±0.13	139.56^e±0.16
Triglycerides (mg/dL)	152.86^a±0.13	151.33^b±0.15	147.92^c±0.15	142.03^d±0.22	132.84^e±0.27
ALT (U/L)	35.22^a±0.10	33.12^b±0.06	30.82^c±0.05	28.48^d±0.13	25.40^e±0.09
AST (U/L)	148.76^a±0.24	146.08^b±0.14	139.23^c±0.13	134.35^d±0.11	125.23^e±0.11
LPO (nmol MDA g/Hb)	1.93^a±0.007	1.72^b±0.003	1.53^c±0.008	1.28^d±0.007	1.07^e±0.019
SOD(U mg/Hb)	0.46^e±0.003	0.51^d±0.004	0.68^c±0.002	0.76^b±0.003	0.83^a±0.005

*, Values (mean±SE) with same superscripts in a row do not differ significantly ($P \leq 0.05$).

and reproduction in human beings (Selvaraj *et al.* 2019) and animals like cow (Muwal *et al.* 2020), and chicken (Kumar *et al.* 2022). The shatavari root, commonly called "Satavar", is traditionally referred as female tonic, being a potential source of phytoestrogens (Shatavarins) and is used since ages to promote female health and reproduction (Thakur *et al.* 2021, Sharma *et al.* 2022). The present study reveals that 'Satavar' can also be used as a 'Fish Tonic' to boost health and reproductive performance of carp fish, in special reference to fecundity (more seed production per kg brood stock) and egg quality (higher fry survival).

Shatavari (*A. racemosus*) root powder can be used as a potential aphrodisiac fish feed additive at 4% inclusion level for rearing common carp brood stock, with an anticipated fry production enhancement by 45%. Robust seed production with better survival from shatavari fed fish is also foreseen, which needs to be investigated for optimised economic utilization of shatavari in carp fish brood stock management.

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