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Production of an all-male population of guppy, *Poecilia reticulata* (Schneider)

Techniques of producing monosex population are imperative in guppy (*Poecilia reticulata*, Poecilidae) because the males possess a high market demand due to extreme variation in size and colour, particularly in their fins. It is well-known that phenotypic sex can be altered by the administration of oestrogens or androgens during sex differentiation, which transform genetically male or female individuals into phenotypic female or male. Since the use of steroid hormones for sex control in food fish evokes environmental and food safety concerns because of their residues, the use of non-steroidal aromatase inhibitors like letrozole (anti-estrogen used in the treatment of human breast cancer) for the production of all-male ornamental fish gains importance. Production of an all-male progeny would help aquarists derive higher profit.

A lot of success has been achieved in producing all-male or male-dominated progenies through the application of aromatase inhibitor in Chinook salmon, *Oncorhynchus tshawytscha*[1], Nile tilapia *Oreochromis niloticus*[2], zebrafish *Danio rerio*[3], golden rabbit fish *Siganus guttatus*[4] and Jawa tilapia[5]. In vertebrates, aromatase catalyses the conversion of C_{19} androgens into C_{18} estrogens. Two common non-steroidal aromatase inhibitors are fadrozole and letrozole. Letrozole inhibits the aromatase enzyme by competitively binding to the heme of the cytochrome P450 subunit of the enzyme, resulting in a reduction of estrogen biosynthesis in all tissues that can result in an elevated level of androgen (by preventing the conversion of androgens to estrogens), thus leading to sex reversal from genetic female to phenotypic male. Australian scientists have successfully produced 'daughterless carp' through the application of aromatase inhibitor to control common carp population, a pest in natural waterbodies. In India, the dietary administration of letrozole alone (100 or 200 ppm) or letrozole + 17α-MT (100 ppm + 25 ppm) induced 100% masculinization in *Oreochromis mossambicus*[5]. A previous study on the masculinization of *P. reticulata* employing androstenedione, 19-nor-ethynyltestosterone and 17α-ethynyltestosterone at doses of 200, 300 and 500 ppm reported the production of all-male populations respectively[6].

In the present study, three trials were conducted to evaluate the efficacy of letrozole on masculinization potential in *P. reticulata.* In Trial I, the letrozole-incorporated diet was fed at 0 (T_0), 25 (T_1), 50 (T_3), 75 (T_4) and 100 (T_5) mg/kg diet (ppm) to the two- to four-days-old fry for 30, 40 or 50 days. In Trial II, the letrozole-incorporated diet was fed at 0, 25, 50, 75 and 100 ppm to mature brooders for 8–12 days as well as the resultant fry for 30 days.

Letrozole incorporation into the diet was accomplished by dissolving the appropriate amount of letrozole in 50 ml of 95% ethanol and spraying it uniformly over the feed using a chromatogram column sprayer and allowing it to dry at room temperature.

In Trial III, an immersion treatment of letrozole in an aqueous solution was given at concentrations of 0, 2.5, 5.0, 7.5 and 10 mg/l (ppm) for 12 h to one-day-old fry. After the specified treatment period, the fry were transferred to outdoor cement tanks and fed on a letrozole-free diet till maturity. On termination of the experiments, all the surviving fish were harvested, counted and sexed based on the size and shape of the fish, the presence of gonopodium, body coloration, finnage, etc.

In general, letrozole treatment altered sex ratio, leading to the production of a dose-dependent increase in the percentage of males (Tables 1–3). The oral administration of letrozole at 100 ppm to 2–4-day-old fry, yielded 92%, 93% and 97% males when treated for 30, 40 and 50 days respectively. On the other hand, letrozole administration to brooders and the resultant fry at 75 and 100 ppm, produced an all-male population of guppy. When a dip treatment of letrozole in an aqueous solution was given, the highest percentage of males obtained was 93; the sex ratio of the untreated control was almost 1 : 1.

The results demonstrate that it is possible to produce an all-male population

Table 1. Number of fry stocked and recovered, average size, sex composition and survival of guppy fed varying doses of letrozole for different periods (Trial I)

	30 days					40 days					50 days				
	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
No. of fry (initial)	25	25	25	25	25	15	15	15	15	15	40	40	40	40	40
No. of fry (final)	20	22	22	23	24	14	15	15	15	15	37	36	36	36	37
Initial wt (mg)	6.0	6.1	6.0	6.1	6.0	7.0	7.1	6.9	7.0	7.2	4.0	3.9	4.2	4.1	4.0
Final wt (mg)	200	135	200	125	110	510	460	590	350	460	140	142	350	220	360
No. of males	9	19	20	21	22	6	13	12	13	14	17	33	34	34	36
No. of females	11	3	2	2	2	8	2	3	2	1	20	3	2	2	1
Males (%)	45	86.3	90.9	91.3	91.6	42.8	86.6	80.0	86.6	93.3	45.9	91.6	94.4	94.4	97.2
Survival (%)	80	88	88	92	96	93.3	100	100	100	100	92.5	90	90	90	92.5

Table 2. Number of fry stocked and recovered, sex composition and survival of guppy fed on different doses of letrozole to parents and the resultant fry (Trail II)

	Control	25 ppm	50 ppm	75 ppm	100 ppm
Let treatment to brooders (days)	5	10	8	12	12
No. of fry (initial)	15	10	15	12	12
No. of fry (final)	14	10	15	10	10
No. of males	8	9	14	11	11
No. of females	6	1	1	0	0
Males (%)	57.14	90.00	93.33	100.00	100.00
Survival (%)	93.33	100	100	91.67	91.67

Table 3. Number of fry stocked and recovered, sex composition and survival of guppy fry given an immersion treatment of letrozole (Trial III)

	Control	2.5 ppm	5 ppm	7.5 ppm	10 ppm
No. of fry (initial)	15	15	15	15	15
No. of fish (final)	13	12	12	14	14
No. of males	6	7	7	13	11
No. of females	7	5	5	1	3
Males (%)	46.15	58.33	58.33	92.86	78.57
Survival (%)	86.67	80.00	80.00	93.33	93.33

Figure 1. ***a***, An all-male population of guppy. ***b***, Control (left) and letrozole-treated (right) guppy brooders. ***c***, Control (left) and letrozole-treated (right) guppy brooders.

of guppy by oral administration of letrozole, a non-steroidal aromatase inhibitor. Under the given experimental conditions, 100% masculinization was successfully achieved at a dietary concentration of 75 and 100 ppm when fed to brooders for 12 days and the resultant fry for 30 days. However, the dietary administration of letrozole produced up to 97% males, while immersion treatment induced up to 93% male progeny. The letrozole-treated brooders displayed brighter colour (Figure 1). Earlier studies conducted in this laboratory revealed that it was possible to produce an all-male progeny of *O. mossambicus* by oral administration of letrozole (100 and 200 ppm), which produced 92–100% males when fed to 5–6-day-old fry for 30 days[5]. The present study reveals that the minimum dose of letrozole required to produce an all-male population of guppy is 75 mg/kg diet, to be fed to female brooders for 12 days and the resultant fry for 30 days.

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