Effect of ration levels on growth and gonad weight in red swordtail, *Xiphophorus helleri* (Poeciliidae)

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**Abstract**

Effect of ration levels (25, 50, 75 and 100%) on food intake, growth and gonad weight were studied in sexually immatures (SIM) and mature (SM) female red swordtail, *Xiphophorus helleri*. Maturity was determined based on the development of ova in the brood pouch. Rates of consumption and conversion and gonad weight of *X. helleri* were increased with an increase of ration in both SIM and SM groups. Consumption rate of SIM group was high as compared to SM group; however, the rate and efficiency of conversion and gonad weight showed the reversed trend. The optimum ration for SIM and SM female *X. helleri* was 122 and 80 mg g⁻¹ live fish day⁻¹ respectively. The energy allocation for growth and reproduction in relation to ration is discussed.

**Key words**: Ration levels, growth, sexual maturity, gonad weight, optimum ration, *Xiphophorus helleri*.

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**Introduction**

Feeding is one of the most important factors in ornamental fish farming because feeding regime would influence both growth and reproduction (James and Sampath, 2003; 2004). To achieve an efficient feeding regime in ornamental fish culture, continual adjustment of the ration level is necessary to compensate the changes in reproductive requirement. A precise knowledge of the relationship between food requirement and reproductive stages of a ornamental species and diet would be essential to avoid both over-feeding and restricted growth through sub-maximum rations.

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Red swordtail, *Xiphophorus helleri* is one of the important ornamental fish species. It has a short maturation period of 3-4 months and the female reproductive output is more sensitive to variations in food supply (James and Sampath, 2003). Female fish need adequate food energy for growth, egg/embryo development and reproduction. There is a need, therefore, to determine the optimum quantum of food to get maximum growth and gonad weight under culture conditions. Previous authors have studied the influence of ration (Vivekanandan, 1977; Sedgwick, 1979) and an interaction of ration and body weight (Sampath and Premila, 1995) on growth and its efficiency in edible fishes. There is no adequate information on the effects of ration
size on food intake, growth and gonad weight in sexually immature and mature female ornamental fish. Hence, the present paper reports on the effects of ration size on food intake, growth and gonad weight in ungravid and gravid female red swordtail, *Xiphophorus helleri*.

**Materials and Methods**

Two hundred and twenty five active sexually immature (0.71 \pm 0.03 g) and sexually mature (1.43 \pm 0.12 g) female *X. helleri* were separately collected from laboratory bred brooders. *X. helleri* was categorized into sexually immature and mature based on development of ova in the brood pouch (Meffe, 1985). Hereafter, they are represented as SIM and SM groups respectively. Both the groups were provided with five ration levels of 0, 25, 50, 75 and 100\% of feeding. Different ration levels were determined based on *ad libitum* food intake. Three replicates were maintained for each ration size. Each group consisted of 15 individuals and was reared in circular cement tanks (diameter : 60 cm \times height : 45 cm) containing 90 l of water. The water taken was unchlorinated well water. Water quality was monitored every 10 days. The tanks were completely drained twice a week and replenished with freshwater to remove accumulated feces from the bottom.

*X. helleri* is an omnivore and feeds on a variety of organisms. However, in the present experiment, an artificial food, beef liver was offered; it has been considered as whole food by previous authors (James and Sampath, 2004). Test fish maintained at 25 and 50\% ration levels were offered food once a day at 7.00 AM. To prevent food from remaining in the water too long, two groups with 75 and 100\% rations were fed twice a day at 7 AM and 5 PM. The water content of feed sample was estimated daily by drying a known weight of sample at 80\%C. Feed was given in a feeding tray for 1 hr after which unconsumed feed was removed and dried in a hot air oven at 80\%C. Feed consumption was estimated by subtracting the amount of unconsumed dry feed from the dry weight of feed offered. The consumption rate was computed as:

\[
\text{Consumption rate} = \frac{\text{Amount of feed consumed (mg)}}{[\text{mg g}^{-1} \text{ live fish day}^{-1} \times \text{Initial wet weight of fish (g)} \times \text{No. of days}]}.
\]

**Growth and gonad estimations**

Fish were weighed at the beginning of the experiment and on the termination of the experiment. The experiment lasted for 25 days. Growth or weight gain was calculated as the difference between the wet weight of the fish at the beginning and end of the experiment. Rate and efficiency of conversion (growth) were computed as:

\[
\text{Conversion rate} = \frac{\text{Weight gain (mg)}}{[\text{mg g}^{-1} \text{ five fish day}^{-1} \times \text{Initial wet weight of fish (g)} \times \text{No. of days}]}.
\]

\[
\text{Conversion efficiency(\%)} = \frac{\text{Weight gain (mg)}}{\text{Feed consumed (mg)}} \times 100.
\]

Five females from each treatment were sacrificed at the end of the experiment. Their ovaries were removed and weighed and the gonadosomatic index (GSI) were computed according to Dahlgren (1979).

\[
\text{Gonadosomatic index(\%)} = \frac{\text{Wet weight of gonad (mg)}}{\text{Wet weight of fish (mg)}} \times 100.
\]

Statistical analyses were done following Zar (1974). Student's 't' test was applied to determine the significance of difference between group means. Simple regression equation was computed for gonad weight against ration levels in SIM and SM groups following the least square method. ONE-way ANOVA was applied to find the significant effects of ration levels on consumption and conversion rates and gonad weight.
Results

An increase in ration levels significantly (ANOVA: p < 0.01) enhanced the feeding parameters (feed intake and consumption rate) and growth (rate and efficiency of conversion) parameters, gonad weight and gonadosomatic index in both SIM and SM groups. SIM groups showed significantly (p<0.05) higher feed intake and consumption rate as compared to SM groups in all ration levels (Table 1). However, growth parameters showed an opposite trend. It is interesting to note that, the conversion efficiency gradually increased with increase in ration levels in SIM groups; however, the trend was reversed in SM groups (Table 1). SM groups elicited the highest gonad weight; 2-5 times greater than those of SIM groups. The regression equation obtained for gonad weight vs ration levels in SIM and SM groups were:

\[ Y = -8.75 + 1.22x \text{ and } Y = 247.5 + 3.26x. \]

This shows that SM group had high b value (3.22) as compared to SIM group (1.22). Similar result was obtained in GSI also.

The geometric relationship between growth rate and ration levels is depicted in Fig. 1 and 2. The point at which the curve cuts the X-axis represents the maintenance level at which weight equilibrium is attained in the animal without weight change. A tangent to the curve from the origin, the ration provides the maximum growth with the least feed intake — optimum ration. The point at which the curve flattens gives the ration which stimulates the maximum growth — maximum ration. The maintenance, optimum and maximum rations of SIM groups were 47, 122 and 200 mg g\(^{-1}\) live fish day\(^{-1}\) and it significantly (p<0.01) declined to 27, 80 and 189 mg g\(^{-1}\) live fish day\(^{-1}\) respectively in SM groups (Fig. 1-2). The maintenance or optimum

Table 1: Effect of different levels of rations (%) on selected food utilization parameters, gonad weight and gonadosomatic index in sexually immatured and matured red swordtail, *Xiphophorus helleri*.

<table>
<thead>
<tr>
<th>Levels of rations(%)</th>
<th>Feed intake (g dry matter)</th>
<th>Consumption rate (mg g(^{-1}) live day(^{-1}))</th>
<th>Weight gain (g dry weight)</th>
<th>Conversion rate (mg g(^{-1}) live day(^{-1}))</th>
<th>Gross conversion efficiency %</th>
<th>Wet weight of gonad (mg)</th>
<th>Gonadosomatic index %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Starvation</td>
<td>0</td>
<td>-5.130</td>
<td>-14.880</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>18.80 ± 0.38</td>
<td>50.85 ± 4.35</td>
<td>1.21 ± 0.08</td>
<td>3.27 ± 0.25</td>
<td>6.44 ± 0.43</td>
<td>27.5 ± 2.04</td>
<td>3.65 ± 0.26</td>
</tr>
<tr>
<td>25</td>
<td>37.60 ± 3.25</td>
<td>115.70 ± 10.36</td>
<td>5.74 ± 0.48</td>
<td>17.66 ± 1.40</td>
<td>15.26 ± 1.21</td>
<td>52.5 ± 4.25</td>
<td>4.00 ± 0.13</td>
</tr>
<tr>
<td>50</td>
<td>55.99 ± 4.45</td>
<td>154.60 ± 10.34</td>
<td>8.42 ± 0.40</td>
<td>23.24 ± 1.61</td>
<td>15.05 ± 1.01</td>
<td>65.0 ± 4.08</td>
<td>6.27 ± 0.50</td>
</tr>
<tr>
<td>75</td>
<td>72.60 ± 6.24</td>
<td>199.58 ± 12.23</td>
<td>12.65 ± 0.72</td>
<td>34.78 ± 3.33</td>
<td>17.42 ± 1.21</td>
<td>125.0 ± 8.16</td>
<td>6.90 ± 0.73</td>
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<tr>
<td>100</td>
<td>169.2 ± 1.12</td>
<td>253.25 ± 4.12</td>
<td>14.33 ± 1.10</td>
<td>43.99 ± 3.81</td>
<td>23.30 ± 1.21</td>
<td>355 ± 14.48</td>
<td>14.54 ± 2.86</td>
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</tbody>
</table>

Sexually matured

<table>
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<tr>
<th>Levels of rations(%)</th>
<th>Feed intake (g dry matter)</th>
<th>Consumption rate (mg g(^{-1}) live day(^{-1}))</th>
<th>Weight gain (g dry weight)</th>
<th>Conversion rate (mg g(^{-1}) live day(^{-1}))</th>
<th>Gross conversion efficiency %</th>
<th>Wet weight of gonad (mg)</th>
<th>Gonadosomatic index %</th>
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<tbody>
<tr>
<td>0</td>
<td>Starvation</td>
<td>0</td>
<td>-4.570±0.32</td>
<td>-12.41 ± 1.12</td>
<td>30.61 ± 2.90</td>
<td>16.30 ± 1.41</td>
<td>14.93 ± 2.01</td>
</tr>
<tr>
<td>25</td>
<td>16.92 ± 1.12</td>
<td>53.25 ± 4.12</td>
<td>5.18 ± 0.14</td>
<td>16.30 ± 1.41</td>
<td>30.61 ± 2.90</td>
<td>265 ± 4.08</td>
<td>14.93 ± 2.01</td>
</tr>
<tr>
<td>50</td>
<td>33.84 ± 2.98</td>
<td>109.43 ± 9.21</td>
<td>9.80 ± 0.40</td>
<td>31.69 ± 2.24</td>
<td>28.96 ± 1.72</td>
<td>500 ± 4.92</td>
<td>24.87 ± 2.86</td>
</tr>
<tr>
<td>75</td>
<td>49.59 ± 3.59</td>
<td>172.94 ± 14.41</td>
<td>13.71 ± 1.30</td>
<td>47.81 ± 4.20</td>
<td>27.65 ± 1.31</td>
<td>505 ± 9.53</td>
<td>15.71 ± 2.04</td>
</tr>
<tr>
<td>100</td>
<td>61.49 ± 5.24</td>
<td>188.84 ± 13.21</td>
<td>14.33 ± 1.10</td>
<td>43.99 ± 3.81</td>
<td>23.30 ± 1.21</td>
<td>535 ± 14.48</td>
<td>14.54 ± 2.86</td>
</tr>
</tbody>
</table>

ND: Gonad not developed
Fig. 1: Geometric derivation of maintenance, optimum and maximum ration in sexually immated Xiphophorus helleri.

Fig. 2: Geometric derivation of maintenance, optimum and maximum ration in sexually matured Xiphophorus helleri.
ration of SIM group was 1.7 or 1.6 times more than that of SM group.

**Discussion**

The present study reveals that, ration was positively related to feed intake, weight gain and gonad weight in both SIM and SM groups. An increase in ration size from 25-100% increased the consumption rate by 2-4 times in SIM and SM groups. Working on *Mystus vittatus*, Arunachalam and Ravichandra Reddy (1981) observed enhanced consumption of *Tubifex tubifex* with increase in ration, supports the present study.

Consumption rate of SIM group was significantly ($p<0.05$) higher than those of SM group; however, the rate and efficiency of conversion showed the opposite trend. Faster gastric evacuation would enable smaller SIM fish to digest more food per unit time and greater stomach volume to ingest a relatively greater quantity of food (Brett, 1971; Elliott, 1972). Comparatively, body weight of SIM group was lesser than SM group. An increase in metabolic rate was perhaps responsible for the higher rate of food intake in SIM group and left less feed energy resulting in low rate and efficiency of conversion. This was evidently confirmed from the data obtained for maintenance rations. The maintenance ration for SIM group was 48 mg g⁻¹ live fish day⁻¹ and it significantly declined to 24 mg g⁻¹ live fish day⁻¹ in SM group; it was reduced by 50% (Fig. 1-2).

On the other hand, SM group showed an efficient conversion of feed and it enabled the fish for effective allocation of more consumed feed energy for gonad development (See Table 1). The reproductive cycle of *X. belleri* is very short like other ornamental fishes (James and Sampath, 2003; 2004) and it requires more feed energy for gonad development and fry production. Townshend and Wooton (1984) found that females channeled a higher proportion of ingested food energy for gonad development than the males.

Consumption rate determines the conversion rate (Raghuraman, 1973). An increase in ration level from 25 to 100% enhanced the conversion rate of SIM group by 10 times and SM group by 3 times (Table 1). This observation contradict the observation made by Ponniah and Pandian (1977) in edible fishes. In the present study, though there was a linear increase in conversion rate with ration, maximum efficiency of conversion was exhibited at 50% ration level by SM groups and at 100% ration level by SIM group (Table 1; Fig. 1-2). For instance, SM female *X. belleri*, which received 50% ration level had significantly ($p < 0.01$) high gonad weight (500 mg wet weight) and GSI (25%) with a maximum conversion efficiency (29%) at low consumption rate of 80 mg g⁻¹ live fish day⁻¹ (Fig. 1-2) as compared to SIM group. The optimum ration for SIM and SM female *X. belleri* was 122 and 80 mg g⁻¹ live fish day⁻¹ respectively. The same trend was obtained for maintenance and maximum rations also. In ornamental fish culture, offering optimum ration to fishes would help to reduce the production cost sizeably by avoiding feed wastage and enhance their reproduction.

**References**


Dahlgren, B. T. : The effect of population density on fecundity and fertility in the guppy *Poecilia*


Effect of dietary *Spirulina* on reduction of copper toxicity and improvement of growth, blood parameters and phosphatases activities in carp, *Cirrhinus mrigala* (Hamilton, 1822)

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The effect of *Spirulina* supplementation on reduction of copper toxicity based on food utilization, phosphatases activities and selected haematological parameters was studied in a freshwater cultivable fish *C. mrigala*. Metal concentration in medium, body tissues and fecal matter was also analysed. Sublethal exposure of *C. mrigala* fed *Spirulina* – free diet significantly reduced the consumption and growth rates, phosphatases activities and haematological parameters. However, the above parameters enhanced in the copper exposed fish fed with *Spirulina* supplemented diets. A significant positive correlation was obtained for the relationship between supplementation of dietary *Spirulina* and copper defecation through feces. Among the doses of *Spirulina* supplementation, 6% addition has been considered optimum for *C. mrigala* since this dose produces maximum elimination of copper from the body and feces and better improvement on growth, phosphatases activities and haematological parameters.

Keywords: Blood parameters, *Cirrhinus mrigala*, Growth, Metal elimination, Phosphatases activity, *Spirulina*.

Indiscriminate discharge of industrial effluents and urban wastes into aquatic systems lead to contamination of the environment and affect the survival and physiological activities of organisms. The reduction of toxic elements in aquatic system/organisms by some acceptable methods is the need of the hour. The most widely used technique for removal of toxic elements involves the process of neutralization and metal hydroxide precipitation. The complete removal or reduction of toxic elements in polluted environments and fish body is of utmost importance. Certain chemical compounds [zeolite (sodium alumino-silicate), ethylene diamine tetra acetic acid (EDTA), nitrilo triacetic acid (NTA), sodium selenite etc.] can effectively remove toxic substances from industrial wastes or polluted medium, however the process is costly. The role of chemical substances in reducing toxic heavy metals and metabolites in aquatic environments, settlement of suspended solids, absorption of gases like CO$_2$, NH$_3$, SO$_2$ and H$_2$S in aquaculture ponds and improvement of mineral nutrition in fish and shrimp has been reported. However, information on the reduction of toxic elements by *Spirulina* is scanty.

James *et al.* have reported the therapeutic effects of *Spirulina* as a growth promoter, probiotic and booster of the immune system in animals including fishes. So far *Spirulina* is known for its nutritive value only; its role in alleviating metal toxicity in fishes and other cultivable organisms is not explored. Copper is a most common metal used in various day-to-day usages and it is the most common contaminant of freshwater systems. The present work has been designed to study the effect of the dietary *Spirulina* on the reduction of copper toxicity in fish and improvement of growth, phosphatases activities and selected haematological parameters in the freshwater fish, *Cirrhinus mrigala*.

**Materials and Methods**

*Cirrhinus mrigala* were collected from Manimuthar dam, Tirunelveli, Tamil Nadu and held for 30 days in laboratory conditions (DO : 4.27 ± 0.6 ml l$^{-1}$; 29.1 ± 0.6°C; pH : 7.7 ± 0.06; salinity : 0.13 ± 0.003 ppt and hardness (CaCO$_3$)90 ± 3.8 mg l$^{-1}$). During acclimatization, water was changed daily and fish were fed *ad libitum* with pelleted diet containing 35%...
protein. Acclimated fish (1.60 ± 0.10 g) were exposed to different concentrations (0, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 ppm) of copper (Analar grade copper sulphate-CuSO₄·7 H₂O (Merck)) and mortality was observed for 96 h. A static renewable bioassay method was adopted for the determination of 96 h median lethal concentration. Probit analysis was followed for the calculation of 96 h LC₅₀. Control group of fish was maintained in copper free-freshwater.

**Preparation of feed** — In the present experiment, 35% protein diet was used as basal diet for *Spirulina* supplementation. The ingredients of dried fish meal, ground nut oil cake, cod liver oil, egg yolk, tapioca flour, vitamins and mineral mixtures were used to prepare the 35% protein diet, with appropriate proportions by Square method. Five diets (0, 2, 4, 6 and 10%) were prepared with different *Spirulina* levels. The experimental diets were prepared by adding the appropriate levels of *Spirulina* with chosen ingredients to boiled water, mixed well and steam cooked for 15-20 min. After moderate cooling, pellets (2 mm) were prepared with a hand operated pelletizer and dried in sunlight. After drying, diets were separately stored in refrigerator.

Active and healthy fish (1.60 ± 0.10 g) were chosen from the acclimation tank and starved for 24 h prior to the commencement of the experiment. The fish were divided into 6 groups of 10 individuals each and were exposed to 0.63 ppm (50% 96 h LC₅₀ value) of copper for 21 days. Triplicates were maintained for each group. Group 1 served as control and reared in copper-free freshwater and fed with *Spirulina*-free diet. Test animals belonging to 2nd, 3rd, 4th, 5th and 6th groups were exposed to 0.63 ppm of copper. Group 2 individuals was fed with *Spirulina*-free diet; however, 3rd, 4th, 5th, and 6th groups were fed with 2, 4, 6 and 10% *Spirulina* diets respectively. The experimental groups 1, 2, 3, 4, 5 and 6 are designated as C, T1, T2, T3, T4 and T5 respectively. The experiment was conducted in epoxy coated cement tank (capacity:110 L) containing 100 L water. The water was not changed during the experiment but was aerated for 14 h to avoid depletion of oxygen. The hydrobiological parameters like dissolved oxygen, temperature, pH, salinity and hardness of water were estimated during non-aeration period. Two series of experiments were conducted in the present study.

**Series 1**: Feeding and growth — During the experimental period, the chosen test groups were fed with weighed quantities of experimental diets twice a day at 0700 and 1800 hrs. Unconsumed feed was removed after 1 h of feeding and dried in hot air oven at 80°C for two days. The sacrifice method was adopted to estimate the growth of the experimental fish. Calculation of selected food utilization parameters has been described in detail elsewhere.

**Series 2**: Like the first series of experiment, a parallel experiment was conducted simultaneously for 21 days to study the impact of dietary *Spirulina* on phosphatases (acid and alkaline) activities, selected haematological parameters and metal accumulation in *C. mirgala*. Test animals were fed *ad libitum* with chosen experimental diets to respective exposures twice a day at 0700 and 1800 hrs for 1 h each. Test animals were starved for 24 h prior to the conclusion of the experiment for the estimation of enzymes, haematological parameters and metal accumulation. Fecal matter was randomly collected by using feeding trays and dried in hot air oven at 60°C to estimate the copper content.

The acid and alkaline phosphatases were estimated according to the method of Bergmayer using p-nitrophenyl phosphate as a substrate. Three fish were removed from each experimental group at the end of the experiment; blood was collected and analysed for selected haematological parameters. Blood was collected in a watch glass containing the required amount of 6% EDTA as an anticoagulant from 3 experimental fish at a time by cutting the caudal peduncle using a sharp knife. Haematological parameters were estimated according to routine clinical method. RBC was counted by using an improved Neubauer counting chamber. Haemoglobinometer was used to determine the haemoglobin content of blood. Oxygen carrying capacity of blood was calculated by multiplying the haemoglobin content with 1.25, oxygen combining power of Hb g⁻¹(ref. 23). Oxygen consumption of test animal was estimated following Winkler’s method. Three samples were analysed for each parameter and the data were subjected to Student’s ‘t’ test and correlation and regression analysis.

Copper content in liver, muscle, gill, faeces and water were estimated at the end of the experiment on day 21. Three replicates of samples (except water) were digested with a mixture of concentrated nitric acid and perchloric acid in the ratio 1:2 until the formation of a white residue at 100°C in a water bath. The cooled residue was dissolved completely by
adding 1 N HCl and made up to 25 ml with distilled water\textsuperscript{25}. The copper concentration in water was estimated following the method of APHA\textsuperscript{26}. The solution was filtered through cotton wool and the filtrate was subjected to metal analysis in atomic absorption spectrophotometry (GBC Avantha model). The instrument was calibrated using standards prepared from copper sulphate.

Results and Discussion

The 96 h LC\textsubscript{50} value of copper for \textit{C. mrigala} was 0.126 ppm and its 95% confidence limits were 0.93 (lower limit) and 1.27 (upper limit). The results showed that sublethal exposure of \textit{C. mrigala} fed \textit{Spirulina}-free diet (T1 group) significantly reduced the food utilization parameters than those exposed to sublethal level of copper and fed \textit{Spirulina} supplemented diets (T2–T5 groups). The reduction of growth rate in \textit{C. mrigala} at sublethal level of copper was evidently due to the tissue burden of copper which in turn could cause reduction in the consumption rate, poor food conversion efficiency and increased oxygen consumption rate (Table 1–3). Lett et al.\textsuperscript{27} attributed the growth reduction in copper exposed \textit{Salmo gairdneri} partly due to increased metabolic costs and reduced food consumption. James et al.\textsuperscript{28,29} observed that reduction in RNA:DNA ratio in the copper exposed \textit{Oreochromis mossambicus} was due to the low production of RNA for protein synthesis by copper burden in tissues.

However, supplementation of \textit{Spirulina} in the diet, improved the food utilization parameters in copper exposed fish. The feed conversion ratio (FCR) value of fish belonging to T4 group was low (3.75) as compared to other groups and close to the FCR value of control fish (3.09) (Table 1). It was due to the \textit{Spirulina} which reduced the accumulation of copper in tissues and elimination of accumulated metal through feces, lessening the metal burden and its toxicity on fish. \textit{Spirulina} contains phycocyanin (14%) chlorophyll (1%) and carotenoids (0.37%) pigments\textsuperscript{30}. \textbeta-carotene of \textit{Spirulina} maintains the mucous membrane firmly\textsuperscript{30} and thereby entry of toxic element into the body is prevented. Chlorophyll of \textit{Spirulina} acts as a cleansing and detoxifying phytounrient against the toxic substances\textsuperscript{30}. It indicates that \textit{Spirulina} has the ability to eliminate and detoxify the accumulated copper and it was proved by improvement of feeding and growth parameters in sublethal exposure of \textit{C. mrigala} fed \textit{Spirulina} supplemented diets. Working on rainbow trout \textit{Salmo gairdneri}, Lanno et al.\textsuperscript{31} found that high level of dietary ascorbic acid (10 g kg\textsuperscript{-1} diet) improved the body weight gain in copper exposed fish as compared to fish fed on low levels of ascorbic acid (0.1–9 g kg\textsuperscript{-1} diet).

The acid phosphatase activity of gill, liver and muscle of control fish was 0.240, 0.317 and 0.154 mg p-nitrophenol released mg\textsuperscript{-1} protein hr\textsuperscript{-1} and it significantly (P<0.01) declined to 0.123, 0.143 and

\begin{table}[h]
\centering
\begin{tabular}{lccccc}
\hline
\textbf{Parameters} & \textbf{C} & \textbf{T1} & \textbf{T2} & \textbf{T3} & \textbf{T4} & \textbf{T5} \\
\hline
Feed intake (g dry matter) & 25.26 ± 2.16 & 10.26 ± 0.83 & 12.95 ± 1.15 & 15.82 ± 1.36 & 18.96 ± 1.75 & 19.53 ± 1.53 \\
Consumption rate (mg live fish g\textsuperscript{-1} day\textsuperscript{-1}) & 73.26 ± 7.42 & 31.21 ± 3.43 & 39.48 ± 4.02 & 48.45 ± 4.92 & 56.38 ± 5.43 & 57.66 ± 5.83 \\
Weight gain (g wet weight) & 8.18 ± 0.73 & 1.71 ± 0.19 & 3.61 ± 0.36 & 5.20 ± 0.57 & 6.89 ± 0.65 & 6.76 ± 0.71 \\
Weight gain (%) & 49.82 ± 5.02 & 10.92 ± 1.20 & 23.11 ± 2.32 & 33.44 ± 3.52 & 43.03 ± 4.52 & 41.90 ± 4.21 \\
Growth rate (mg live fish g\textsuperscript{-1} day\textsuperscript{-1}) & 23.72 ± 2.38 & 5.20 ± 0.69 & 11.00 ± 1.29 & 15.92 ± 1.73 & 20.49 ± 2.03 & 19.95 ± 1.83 \\
Gross conversion efficiency (%) & 32.38 ± 3.47 & 16.66 ± 1.72 & 27.87 ± 2.69 & 32.86 ± 3.18 & 36.33 ± 3.72 & 34.61 ± 3.32 \\
FCR & 3.09 ± 0.28 & 6.00 ± 0.60 & 3.58 ± 0.31 & 3.04 ± 0.29 & 2.75 ± 0.26 & 2.88 ± 0.32 \\
Mortality (%) & Nil & 20 & 10 & 10 & 10 \\
Feeding rate (mg g\textsuperscript{-1} live fish day\textsuperscript{-1}) & = Feed consumption (mg) / initial wet weight of fish (g) x duration (days) \\
Conversion or growth rate (mg g\textsuperscript{-1} live fish day\textsuperscript{-1}) & = Weight gain (mg) / initial wet weight of fish (g) x duration (days) \\
Gross conversion efficiency (%) & = Weight gain (mg) / feed consumption (mg) x 100 \\
Feed conversion ratio (FCR) & = Feed consumption (mg) / weight gain (mg) \\
\hline
\end{tabular}
\caption{Effect of sublethal exposure of \textit{C. mrigala} levels on selected food utilization parameters in \textit{C. mrigala} (Values are mean ± S.D. of 3 observations) }
\end{table}
Table 2—Effect of supplementation of dietary Spirulina on chosen haematological parameters and oxygen consumption in C. mrigala exposed to sublethal concentration of copper
[Values are mean ± S.D. of 3 estimations]

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<thead>
<tr>
<th>Parameters</th>
<th>Exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Red blood corpuscles count (×10^6 mm⁻³)</td>
<td>2.57 ± 0.13</td>
</tr>
<tr>
<td>Haemoglobin (g%)</td>
<td>8.69 ± 0.42</td>
</tr>
<tr>
<td>Oxygen carrying capacity of blood (mg O₂ g⁻¹ Hb)</td>
<td>10.86 ± 0.94</td>
</tr>
<tr>
<td>Rate of oxygen consumption (mg O₂ g⁻¹ hr⁻¹)</td>
<td>0.507 ± 0.04</td>
</tr>
</tbody>
</table>

Table 3—Effect of dietary Spirulina on metal distribution in tissues and feces in C. mrigala exposed to sublethal level of copper for 21 days
[Values are mean ± S.D. of 3 estimations]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Gill</td>
<td>ND</td>
</tr>
<tr>
<td>Liver</td>
<td>ND</td>
</tr>
<tr>
<td>Muscle</td>
<td>ND</td>
</tr>
<tr>
<td>Feces</td>
<td>ND</td>
</tr>
<tr>
<td>Water</td>
<td>ND</td>
</tr>
<tr>
<td>Bioconcentration factor</td>
<td>ND</td>
</tr>
</tbody>
</table>

Biocenentrator factor = 21 days tissue concentration – 0 day tissue concentration/Copper concentration in water
ND: Not detected.

0.068 mg p-nitrophenol released mg⁻¹ protein hr⁻¹ respectively in T1 group (Fig. 1). Similarly, the alkaline phosphatase activity in gill, liver and muscle of control fish was 0.840, 0.987 and 0.710 mg p-nitrophenol released mg⁻¹ protein hr⁻¹ and it decreased to 0.365, 0.407 and 0.323 mg p-nitrophenol released mg⁻¹ protein hr⁻¹ in T1 group also. This showed that phosphatases activities were declined to 50-60% in T1 group as compared to control fish. However, the phosphatases activities were improved in copper exposed fish which fed Spirulina supplemented diets. An increase in the supplementation of Spirulina in the diets enhanced the both phosphatases activities upto a midpoint (6% Spirulina) and thereafter they did not show significant changes (Fig. 1). Student’s ‘t’ test revealed that excess level of Spirulina (10%) did not increase the phosphatase activities significantly (P>0.05) as against supplementation of 6% Spirulina in the diets. The decreased phosphatase activities in copper exposed C. mrigala may be due to the metal stress and agrees with previous studies. However, acid and alkaline phosphatases activities were improved in copper exposed fish which fed Spirulina supplemented diets. Copper exposed C. mrigala whenfed Spirulina supplemented diet may have eliminated the accumulated copper from body tissues through feces and thereby resulting in enhanced phosphatases activities (Fig. 1). Spirulina reduced the genotoxicity and oxidative stress of few antibiotics in mice and Pb toxicity in rats.

The sublethal exposure of C. mrigala fed Spirulina-free diet resulted in significant (P < 0.05) decrease in RBC count and haemoglobin content. Oxygen carrying capacity of blood was also declined in copper exposed C. mrigala and it may be due to the reduction of RBC count and Hb content. James and Sampath found that the oxygen carrying capacity of blood of copper exposed Heteropeutes fossilis declined due to the reduction of RBC count and Hb content which reflected on tissue respiration. This may be evidently reflected on the overall oxygen consumption of animals exposed to copper (Table 2). The present results also revealed that, the haematological parameters were improved in copper exposed C. mrigala fed Spirulina supplemented diets as against copper exposed fish fed Spirulina-free diet. It suggests the protective role of Spirulina against copper toxicity in C. mrigala. Spirulina has 14% phycocyanin and it stimulates the erythropoietin (EPO) hormone production for hematopoiesis.
Phycocyanin also regulates the production of white blood cells even when bone marrow stem cells are damaged by toxic chemical or radiation. Sharma and Sharma reported that Spirulina added feed improved the tolerance of Poecilia reticulata when exposed to an azo dye methyl red by considerable reduction in the cytotoxic effects on RBC's count at higher concentrations of the dye.

The gill tissue elicits the highest copper accumulation followed by the liver and muscle in C. mrigala. Copper accumulation was significantly ($P < 0.05$) high in T1 group and it gradually decreased with an increasing the Spirulina levels in T2-T5 groups. The maximum reduction of copper accumulation in tissues occurred with the supplementation of 6% (T4) Spirulina diet followed by 10% (T5) and 2% (T2) Spirulina diet respectively; however, T4 and T5 groups did not show significant ($P > 0.05$) difference. The dietary ascorbic acid supplementation at a level of 2000 mg kg$^{-1}$ diet resulted in decreased copper accumulation in the gills and liver of rainbow trout and also decreased copper levels in the gills, hepatopancreas, kidney and intestine. These results demonstrated that dietary ascorbic acid decreased the toxicity of waterborne copper to rainbow trout and carp by preventing copper accumulation in the tissues. It is likely that, dietary Spirulina may also reduce the metal level in tissues and protect C. mrigala from copper toxicity.

The elimination of accumulated copper through feces increased with increasing the dietary levels of Spirulina in the diet (Table 3). Copper elimination through feces in T1 group was 0.053 µg g$^{-1}$ dry matter as against 1.62 and 1.69 µg g$^{-1}$ dry matter in T4 and T5 groups respectively. A positive correlation coefficient was obtained for the relationship between the supplementation of dietary Spirulina and elimination of copper through feces and it was statistically significant ($r = 0.831; P < 0.01; n = 18$). Elimination of copper through feces reduced the body burden of copper which directly improved the food utilization, phosphates activities and haematological parameters. James and Sampath reported that addition of ion-exchanging agent, zeolite to cadmium contaminated media significantly reduced the Cd level in water and fish body (metal elimination through feces) which reduced the Cd toxicity and improved the biochemical and growth parameters in O. mossambicus. The bioconcentration factor (BCF) was high in copper exposed fish fed Spirulina-free diet (T1) and it drastically declined in copper exposed fish fed Spirulina supplemented diets (Table 2).

The present study concludes that, the dietary supplementation of Spirulina reduced the metal toxicity in copper exposed C. mrigala and improved the food utilization, phosphates activities and haematological parameters significantly in a short period of time (21 days). However, feeding duration i.e. 21 days was not sufficient for complete removal of metal from the fish body and it requires still longer duration. Supplementation of 6% Spirulina diet (T4) produced the maximum reduction of copper uptake in tissues and elimination of more copper through feces which in turn improved the food utilization, phosphatase activity and haematological parameters than other Spirulina diets (2, 4 and 10%) and hence it is considered as the optimum dose.
References


Effect of Dietary Vitamin E on Growth, Gonad Weight and Embryo Development in Female Red Swordtail, *Xiphophorus helleri* (Poeciliidae)

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Key words: *Xiphophorus helleri*, vitamin E, growth, gonadosomatic index, embryo development

Abstract

The effects of different levels of dietary vitamin E (0, 250, 500, 750, and 1000 mg/kg diet) on growth, gonad weight, embryo development, and number of embryos produced were studied for 50 days in female red swordtail, *Xiphophorus helleri*. Food utilization, gonad weight, and gonadosomatic index increased with the increase in vitamin E levels up to 500 mg/kg and thereafter significantly \(p<0.05\) declined. The impact of vitamin E on gonad weight and gonadosomatic index directly reflected embryo development and production. Early embryo stages (immature ova, mature ova, primitive streak) dominated in fish fed diets containing 250 and 1000 mg vitamin E while late embryo stages (early and middle-eyed) were most frequent in fish fed the diet containing 500 mg vitamin E. Late-eyed embryos occurred only in fish fed the 500 mg diet. Thus, 500 mg vitamin E per kg diet is the optimum level for triggering gonad development and production of late embryo stages in female *X. helleri*.

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Introduction
The major function of vitamin E is to prevent peroxidation of polyunsaturated fatty acids of phospholipids and cholesterol in cellular and subcellular membranes. In fish, most deficiency signs (such as nutritional muscular dystrophy, fatty liver degeneration, anemia, erythrocyte hemolysis, hemorrhage, depigmentation, and reduction of fertility) are related to peroxidative damage to cellular membranes (NRC, 1983). As a membrane-bound antioxidant, vitamin E appears to scavenge free radicals at the site of their formation. Many authors have studied the impact of vitamin E on growth (Gupta et al., 1987) and immune response (Hardie et al., 1990) in various organisms but studies related to the effects of vitamin E on reproduction (James et al., 2008), particularly on gonad weight and embryo development in ornamental fishes, are scanty. The present study examines the effect of different levels of dietary vitamin E on growth, gonad weight, and embryo development in an ornamental fish, the red swordtail *Xiphophorus helleri*.

Materials and Methods
*Fish and maintenance.* Three hundred healthy 60-day-old juveniles of *X. helleri* (18±0.96 mm, 240±13 mg) were obtained from a laboratory where they were bred by a single pair of brooders. They were divided into five groups, corresponding to five levels of vitamin E: 0 (control), 250, 500, 750, and 1000 mg/kg diet. The levels of vitamin E were chosen, based on a pilot study. Each diet was tested in triplicate groups consisting of 20 individuals, reared in circular cement tanks (diameter 58.5 cm, height 40 cm) containing 100 l static water. Average hydrological parameters were temperature 28.3±1.1°C, hardness 325.13±13 mgCO\textsubscript{3}/l, pH 7.8±0.05, dissolved oxygen 4.10±0.13 ml/l, and salinity 0.58±0.02 ppt. The tanks were drained twice a week and replenished with fresh water to remove accumulated feces from the bottom.

*Feed and feeding.* Diets were prepared from fishmeal, ground nut oil cake, tapioca flour, wheat flour, mineral mix, and cod liver oil (lipid source). The source of vitamin E was α-tocopherol acetate, a precursor of vitamin E. The dried and powdered ingredients were blended to obtain a homogenous mixture, mixed with an aliquot of boiled water, and steam cooked for 15-20 min. The required level of vitamin E was prepared by dissolving an appropriate quantity of α-tocopherol acetate in 10 ml acetone and spraying it on the corresponding diets. Diets were prepared every fortnight and stored in a refrigerator to minimize nutrient loss.

Fish were fed *ad libitum* twice a day for 50 days. Feed was given in a feeding tray and left for one hour after which unconsumed feed was removed and dried in a hot air oven at 80°C. Feed consumption was estimated by subtracting the amount of unconsumed dry feed from the dry weight of the offered feed. The daily feeding rate (mg/g live fish/day) was computed as the amount of feed consumed/initial wet wt of fish/no. days.

*Growth and gonad estimation.* Fish were weighed at the beginning of the experiment and on days 25 and 50. Before beginning the experiment, the total wet weight of the test fish in each tank was measured with an electrical monopan balance. Five fish from the same stock were sacrificed to estimate the initial water content (Maynard and Loosli, 1962) and the initial dry weight of the experimental fish was determined using this estimation. All animals were collected on day 25 and at the termination of the experiment (day 50) and wet weight was measured. Wet weight was converted to dry weight using the percent water content of the fish sacrificed at the beginning of the experiment. Growth was calculated according to the difference in dry weight between the previous weighing and that on the day of calculation. The daily growth rate (mg/g live fish/day) was calculated as growth/initial wet wt of fish/no. days. Gross conversion efficiency (%) was calculated as growth/feed intake x 100.

Three females from each treatment were sacrificed on days 25 and 50. Their ovaries (brood pouch) were removed and weighed and the gonadosomatic index (GSI) was computed as wet wt of gonad/wet wt of fish x 100 (Dahlgren, 1979).

After that, the ovaries were dissected, and eggs and embryos were separated, counted, and staged according to Meffe (1985) using a dissection microscope. Eggs and
embryos were sorted into six categories, primarily to detect superfetation: immature ova (ova in the process of yoking but submature in size), mature ova/fertilized eggs (full-sized ova, unfertilized or recently fertilized), primitive streak (primitive streak present but eyes not yet distinguishable), early-eyed (eyes present but not full-sized, little dorsal pigmentation), middle-eyed (eyes full-sized, moderate to extensive dorsal pigmentation), and late-eyed (little yolk remaining, near parturition).

Fish, feed samples, unconsumed feed, and ovaries were weighed in an electric monopan balance to an accuracy of 1 mg. Student’s t test was applied to determine the significance of differences between group means (Zar, 1984).

**Results**

In general, food utilization and weight increased as the level of vitamin E increased up to 500 mg/kg and thereafter declined (Table 1). Likewise, gonad weight and GSI increased up to a level of 500 mg/kg, then dropped (Fig. 1). The feeding and growth parameters significantly decreased with time while gonad weight and GSI showed the opposite trend.

<table>
<thead>
<tr>
<th>Vitamin E level (mg/kg diet)</th>
<th>0</th>
<th>250</th>
<th>500</th>
<th>750</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feed consumption (g dry matter)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-25</td>
<td>38.12±0.09</td>
<td>38.02±0.40</td>
<td>35.59±0.36</td>
<td>36.60±0.36</td>
<td>38.51±0.40</td>
</tr>
<tr>
<td>26-50</td>
<td>35.44±0.46</td>
<td>35.06±0.60</td>
<td>35.25±0.70</td>
<td>34.31±0.78</td>
<td>33.35±0.05</td>
</tr>
<tr>
<td><strong>Feeding rate (mg/g live fish/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-25</td>
<td>250.32±18.41</td>
<td>247.19±12.85</td>
<td>276.37±9.54</td>
<td>277.83±3.58</td>
<td>274.49±0.87</td>
</tr>
<tr>
<td>26-50</td>
<td>96.04±6.59</td>
<td>92.19±3.50</td>
<td>92.61±6.81</td>
<td>90.39±3.80</td>
<td>82.11±0.32</td>
</tr>
<tr>
<td><strong>Weight gain (g dry weight)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1-25</td>
<td>2.30±0.39</td>
<td>2.38±0.08</td>
<td>2.65±0.01</td>
<td>2.56±0.06</td>
<td>2.51±0.08</td>
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<tr>
<td>26-50</td>
<td>1.41±0.02</td>
<td>1.29±0.06</td>
<td>1.35±0.08</td>
<td>1.22±0.17</td>
<td>0.99±0.13</td>
</tr>
<tr>
<td><strong>Growth rate (mg/g live fish wt/day)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-25</td>
<td>73.37±7.68</td>
<td>73.56±2.67</td>
<td>98.13±2.85</td>
<td>87.67±2.50</td>
<td>85.21±2.06</td>
</tr>
<tr>
<td>26-50</td>
<td>18.20±1.71</td>
<td>16.07±0.15</td>
<td>17.70±1.61</td>
<td>15.35±2.41</td>
<td>11.61±1.49</td>
</tr>
<tr>
<td><strong>Gross conversion efficiency (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-25</td>
<td>28.77±4.95</td>
<td>29.80±0.47</td>
<td>33.74±1.26</td>
<td>31.54±0.49</td>
<td>31.04±0.65</td>
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<tr>
<td>26-50</td>
<td>18.90±0.49</td>
<td>17.46±0.50</td>
<td>19.07±0.34</td>
<td>16.86±1.96</td>
<td>14.12±1.76</td>
</tr>
</tbody>
</table>

Table 1. Effect of different levels of vitamin E on selected food utilization parameters in red swordtail, *Xiphophorus helleri* (means±SD; n = 3).

![Fig. 1](image1.png)  
**Fig. 1.** Effect of different levels of vitamin E on (a) gonad weight and (b) gonadosomatic index in female red swordtail (*Xiphophorus helleri*). White bars show increase during days 1-25, black bars during days 26-50.
Fish fed the 500 mg/kg diet had more early, middle, and late-eyed embryos than fish fed any other diet (Fig. 2). The increase of vitamin E up to 500 mg/kg triggered more rapid embryo development and a greater number of embryos (Table 2).

![Fig. 2. Development stages of ova and embryos in female red swordfish, Xiphophorus helleri, fed diets containing different concentrations of vitamin E for 50 days.](image)

**Table 2. Effect of vitamin E level on embryo stage in brood pouch of female red swordfish, Xiphophorus helleri** (means±SD; n = 9) on day 50.

<table>
<thead>
<tr>
<th>Embryo stage</th>
<th>0</th>
<th>250</th>
<th>500</th>
<th>750</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature ova</td>
<td>26.00±2.88</td>
<td>21.67±1.80</td>
<td>20.25±1.29</td>
<td>19.00±1.06</td>
<td>25.50±2.19</td>
</tr>
<tr>
<td>Mature ova</td>
<td>29.75±1.98</td>
<td>40.25±2.70</td>
<td>30.00±2.05</td>
<td>27.10±1.53</td>
<td>30.00±2.24</td>
</tr>
<tr>
<td>Primitive streak</td>
<td>7.50±0.06</td>
<td>9.00±0.39</td>
<td>18.25±1.48</td>
<td>23.00±1.24</td>
<td>22.80±1.70</td>
</tr>
<tr>
<td>Early eyed</td>
<td>9.75±0.80</td>
<td>18.25±1.50</td>
<td>42.75±4.10</td>
<td>17.75±1.12</td>
<td>15.75±1.17</td>
</tr>
<tr>
<td>Middle eyed</td>
<td>8.50±0.65</td>
<td>21.75±2.11</td>
<td>29.00±1.24</td>
<td>11.00±1.20</td>
<td>9.75±1.10</td>
</tr>
<tr>
<td>Late eyed</td>
<td>0</td>
<td>0</td>
<td>5.50±0.50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total production</td>
<td>81.5±4.55</td>
<td>110.92±6.80</td>
<td>145.75±7.15</td>
<td>97.85±8.18</td>
<td>103.80±5.67</td>
</tr>
</tbody>
</table>

*Student’s t test*

<table>
<thead>
<tr>
<th></th>
<th>500 vs 250</th>
<th>500 vs 750</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total embryo production</td>
<td>$t = 9.77; p&lt;0.01$</td>
<td>$t = 12.47; p&lt;0.01$</td>
</tr>
</tbody>
</table>
**Discussion**

Fish fed the 500 mg vitamin E diet had the best growth rate and gonad weight. In addition, fish fed this diet produced more embryos and the embryos developed faster than in fish fed the other diets. Vitamin E requirements vary with species. The growth rate was significantly higher in rainbow trout (*Salmo gairdneri*) that consumed a diet containing 200 mg vitamin E per kg than in trout that consumed a diet lacking vitamin E (Frigg et al., 1990). Diets containing 60-120 and 300 mg α-tocopherol acetate per kg, respectively, were sufficient to enhance the survival and growth in *Salmo salar* (Hamre and Lie, 1995) and *Carassius auratus* (James et al., 2008).

The growth rate was higher on day 25 than on day 50 while an opposite trend was observed in gonad weight and GSI. The lower growth rate during days 26-50 might be due to diversion of assimilated energy to ovary development and embryo production, suggesting that vitamin E has a specific function in reproduction and that 500 mg vitamin E/kg diet stimulates the development of gonad weight and GSI in *X. helleri*. Similarly, female convict cichlid (*Cichlasoma nigrofasciatum*) channeled a higher proportion of ingested food energy to gonad development (Townshend and Wooton, 1984). Growth, gonad weight, GSI, and egg production were enhanced in female goldfish (*Carassius auratus*) fed a diet containing 300 mg vitamin E per kg (James et al., 2008). Being an ovo-viviparous fish, red swordtail may require a higher dose of vitamin E than the oviparous goldfish to obtain optimum growth, gonad weight, and reproduction.

The significance of vitamin E in fish reproduction has been confirmed. Incorporation of vitamin E and growth hormone in the diet of freshwater fish (*Cyprinus carpio*) produced a higher gonadosomatic index, larger ova, and more eggs (Gupta et al., 1987). Inclusion of vitamin E in the diet caused complete spawning whereas a vitamin E free diet showed partial spawning (Gupta et al., 1987). Better gonad development and complete spawning were observed in goldfish (*C. auratus*) fed diets containing vitamin E than in control fish (Sutjaritvongsanon, 1987).

In conclusion, the inclusion of 500 mg vitamin E per kg diet produced the highest gonad weight, GSI, and number of matured embryos. Therefore, this is considered the optimum level for maximum growth and reproductive performance in female *X. helleri*.

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**References**


