AN INVESTIGATION ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF *POECILIA RETICULATA* (PETERS) (CYPRINODONTIFORMES: CYPRINIDAE) FED DIETS WITH DRIED INSECTS

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ABSTRACT: In the study, five feeds given to guppies (*Poecilia reticulata*) were evaluated for their effects on growth and reproductive performance of females. Feeds namely Diet I, II, III, IV and V formed flake food, blood worm, locust, rhino beetle and flour worm, respectively. Blood worm, locust, rhino beetle, flour worm were insects, and flake food was a commercial fish food. The growth and reproductive performance were investigated based on growth parameters, gonadal development and fry production. Fish fed Diet II, IV and V had a higher specific growth rate compared to the other groups. The ovary weight of guppies fed Diet II was significantly ($p<0.05$) higher than those with diets I, III, IV and V. The absolute fecundity values were 35.5±0.02, 58.8±0.04, 30.8±0.03, 50.2±0.05 and 48.1±0.02, respectively for the fish fed Diet I, II, III, IV and V. The number of fry produced was also significantly higher in fish fed Diet II. Between the groups, fish fed Diet II obtained the highest mean gonad weight and gonadal somatic index. The results of the present study demonstrated that the use of such dried insects is a reliable source for commercial guppy farming and the reproductive performance of the fish increased.

KEY WORDS: Insect, guppy, nutrition, fecundity, specific growth rate.

Aquarium fish are rapidly gaining importance due to their immense commercial value worldwide. Live bearing species of the family Poecilidae such as guppies and mollies are popular ornamental aquarium species. Guppies (*Poecilia reticulata*) are beautiful fish that are easy to keep and breed in aquaria. They readily eat dried food such as tropical flakes as well as *Tubifex* worms, small crustaceans such as brine shrimp, and plant matter. These feeds may not provide broodstock fish with adequate nutrients and promote optimal reproduction (Fernando et al., 1991). Broodstock nutrition is an important factor governing egg production and larval survival (Izquierdo et al., 2001). Also, gonad development and fecundity are affected by certain essential nutrients (Izquierdo et al., 2001). Dietary protein and lipid play major roles in growth and reproduction (Suting et al., 2013). Morimoto (1994) reported that for most aquatic organisms, the nutritional quality of diets given broodstock significantly affects the biochemical composition of eggs, total number of eggs spawned and the percentage of eggs hatched among other factors. Based on the afore-mentioned findings, the reproductive potentials, particularly the fecundity and quality of the guppy fry are presumably variable, depending on several factors such as the nutritional content of their diet.

Insecta is the biggest group of animals on earth. Insects are thought to be one of the biggest biological resources. Insects offer us many benefits, including their use in human and animal nutrition. The uses of many insect species as an important food source have become widespread in many parts of the world. More than 1,000 species of insects, mainly in developing countries, that are edible by humans at a certain stage of their life-cycle have been identified worldwide (Cerritos, 2009). Insects are essential agents feeding on organic matter in nature, and they efficiently exploit all organic sources. It is also important that insects are able to recycle organic wastes and provide nutrients for livestock. Therefore, they could be used as efficient biotransformers to conver abundant, low cost organic wastes into animal biomass rich in proteins and suitable for use in animal nutrition. Edible insects are one alternative resource to improve human and animal nutrition (Ramos-Elorduy, 2008). Conversely, edible insects may include contain vertebra toxins (Akinnawo et al., 2002). Therefore, the feeding of these insects may cause serious harmful effects to animals. In this context, the potential toxic effects of these edible insects need further investigation. According to our knowledge, no investigation has been carried out on the toxic or non-toxic effects of edible insects on fish species. Also, there is no study on the effects of dried insects and larvae on the reproductive performance of fish.

Therefore, the current study assess some growth and reproductive parameters of guppy fish *Poecilia reticulata* (Peters, 1859) fed four types of insect diets to study the influence of these diets on reproduction.

MATERIALS AND METHODS

Fish culture:

The guppies were obtained from commercial suppliers. Fish were acclimated in carbon- filtered city water, under standard laboratory conditions (25±1 ^{0}C, 14:10 light-to-dark photoperiod) and daily fed a commercial flake food during this period. The aquaria were cleaned and the water was changed every four days. All these procedures were made before the start of experiment. The average weight and length values of the female guppies were 0.0122-g and 1.02-cm, respectively.

Experimental design and feeding:

There were five treatment groups, each with three replicates in five 30-L rectangular glass aquaria (30X20X50 cm). Each aquarium was stocked with 10 female fry of almost uniform size. Five types of feeds were used in this study. Tetramin fish flake (Figure 1A) that is a commercially available diet for aquaria fish served as Diet I. The dried insects diets are: Diet II is blood worm (larvae of *Phlebotomus*, Loew (Diptera: Psychodidae), Figure 1B and 1C). The commercial product was made by grinding of the dried larvae. Diet III is locust (adults of *Locusta migratoria*, Linnaeus (Orthoptera: Acrididae) Figure 1D). Diet IV is rhino beetle (adults of *Dynastes hercules*, Linnaeus (Coleoptera: Scarabaeidae) Figure 1E), and Diet V is flour worm (larvae of *Tenebrio molitor*, Linnaeus (Coleoptera: Tenebrionidae) Figure 1F). Diet I, II, IV and V were obtained from commercial suppliers. Diet III was obtained from an insect farming in Antalya, Turkey. All insect samples were dried and pulverized. The weight of all feed samples was measured before the experiment. Feeding was carried out until satiation, twice a day at 800 and 1700 h throughout the experiment. Dried insect powder and flake food were dropped into the tanks and repeated until satiation was observed. At the end of the week, the remaining amount of feed was weighed and the amount of food consumed was deducted. The water quality was monitored weekly throughout the experimental period.

Determination of the growth rate and reproductive performance:

The growth rate of female guppy fry was assessed after three months by initially recording their body length and weight. The fry weight was measured using an analytical balance device (OHAUS Adventurer™ Pro). Before the measurements, the fish were kept overnight without food. The specific growth rate and food conversion ratio were calculated with the below formulae:

Specific growth rate (SGR %/day) = 100 x (ln.final wt of fish – ln.initial wt of fish/trial day).

Food conversion ratio (FCR) = Total feed fed (g)/Total wet weight gain (g).

Reproductive performance was measured in terms of ovary weight, absolute fecundity, Gonadal Somatic Index (GSI) and the number of fry. Ten fish from each treatment were used to determine the reproductive indices. Dissections were carried out under a stereo microscope. The oviduct and mesovarium were separated and removed. The ovarian weight was measured using the same analytical balance device. All portions of ova were put into 4% formalin and the oocytes were counted under a stereo microscope. The absolute fecundity (F_a), which is the number of mature oocytes spawned by a female in a single spawning (Bagenal, 1973), was estimated as:

F_a = GW x D (GW: Weight of the ovary, D: Density of the mature oocytes = number of oocytes per g of ovarian tissue).

The GSI, which is the relation of gonad to somatic weight, was calculated by the formula (Arellano-Martínez and Ceballos-Vázquez, 2001):

GSI = (Individual gonad weight/Individual body weight) x 100.

Breeding and fry collection:

The 10 female fish were separated randomly from the experimental tanks for breeding. Each tank was stocked with 10 female and 5 male fish of the same size. Breeding tanks were provided with polythene strips arranged in bundles. After a gestation period, the newly born fry in each tank were collected daily with a hand net and kept in separate tanks. The number of fry was recorded daily during five months.

Statistical Analysis

Comparison of various growth and reproductive parameters from different dietary treatments was evaluated with analysis of variance (ANOVA) and Duncan's test by S.P.S.S. software program (version 15.0). All data was presented as mean ± S.E. of three replicates. The level accepted for statistical significance in all cases was $p<0.05$.

RESULTS

The mean values of total length (from nose to caudal fin) and final weight after 3 months rearing period are given in Table 1. The total length of the fish of the Diet II was higher than that of fish fed other diets. The total consumption of the dry diets (Diet I, II, III, IV and V) was 36.32-g. The initial and final weights were ranged from 0.0121-0.0124 and 0.144-0.215-g, respectively. The feed conversion values of the fish fed with Diet II had a high value. FCRs of Diet I, II and IV were significantly lower than with the other diets. The fish of Diet III had only a small increment in length and weight. SGR values for the fish fed all diets were 0.22 to 0.33. Differences between the SGR values were found insignificant, except for Diet III (Table 1).

The ovarian weight, absolute fecundity (F_a) and mean GSI values are given in Table 2. The fish fed Diet II had higher ovary weight, F_a and mean GS as compared to other diets. The differences between the diet groups were statistically significant.

The mean total fry production and live fry survival rate values are given in Table 3. Fry production was higher in fish fed with Diet II than with the other diets. Fry survival rates were not significantly different between the diets.

It is evident from the results of the present study that Diet II resulted in better growth and reproductive performance of female guppy compared to other diets. No diet caused any toxic effects (mortality, paralysis, abnormal swimming behavior, etc.). In general, the insect diets showed the same growth and reproductive effects of Diet I (control) and none of the insect products were toxic for the guppies.

DISCUSSION

The results of this study suggest that the effects of dried insect diets on the growth and reproductive performance of guppies were mediated. The average number of fry, total length and weight of fish were not negatively affected by the diets. The study showed that the insect products had enough essential nutrients for the guppy. Insects often contain more protein, fat and carbohydrates than equal amounts of beef or fish and a higher energy value than soybeans, maize, beef, fish, lentils, or other beans. The proximate compositions of the some insect groups are given Table 4.

As shown in Table 4, the edible insect products contain protein, lipid and carbohydrate levels. Crude protein levels are 22-66% in Ephemeroptera larvae, 40-65% in Odonata larvae, 42-73% in Hemiptera larvae, 40-57% in Homoptera larvae and eggs, 38-76% in Hymenoptera and 23-66% in Coleoptera larvae (DeFoliart, 1992; Zhang et al., 2008; Yang, 1998; Xiaoming and Ying, 1999; Ying et al., 2000; Ying et al., 2001). Blood worm used Diet II is a Diptera larva. Locust as Diet III and rhino beetle as Diet IV belong to Orthoptera and Coleoptera, respectively. Finally, flour worm as Diet IV is a Coleoptera larvae. Therefore, Diet I, II and III-IV may contain 59.39%, 44.10% and 50.41% protein, respectively. Likewise, lipid levels of the diets are 12.61%, 2.2% and 27.57%. Diet I, II and III-IV may contain 12.04%, 1.20% and 2.81% carbohydrates, respectively. The nutritional requirements for the ornamental fish have been reported by Swain (1999). The fish fry can be fed with 40-50% protein, 4-6% lipid and 40-50% carbohydrates. Accordingly, the insect diets, which used the study could be contained the recommended amount of dietary protein and lipid levels resulted in the growth and reproductive performance of female guppy.

It has been reported that the dietary protein and lipid levels play a major role in weight gain in fish and provision of adequate levels will lead to higher fry production (Milton & Arthington, 1983). Suting et al. (2013) reported that different dietary lipid sources had positive effects on growth and reproduction performance of guppy. Dahlgren (1980) conducted an experiment with three types of feed with different protein levels and recorded high growth and reproductive performance in female guppies fed 31% protein levels. In another study, it was found that 30-40% dietary protein was optimal level for breeding guppy and 9-10.5% lipid levels gained the high mean body weight, ovary weight, GSI and number of yolk oocytes (Shim & Ng, 1988). Shim & Chua (1986) also found that the diets with 30 to 40% protein appeared to be the best for gonadal development, since those diets resulted in the greatest mean ovary weight and in the largest mean number of yolk oocytes in the ovary of guppy. It has been reported that the diet contained highest levels of protein and lipid showed the maximum fry production in guppy females (Kithsiri et al., 2010). Compared to the recommended nutritional requirements, Diet II, III, IV and V contained higher protein percentages and required amount of lipid and carbohydrates. Also, the significantly low FCR recorded in the Diet II clearly indicated that those fish were fed a diet including the required amounts of protein and lipid. Therefore, by comparing the results of the present study with that of the required dietary protein and lipid levels reported, it is possible to establish the effect of the dried insect diets used on the growth and reproduction of guppies.

In conclusion, the commercial and edible insect products did not cause any health risk, conversely the products encouraged fish growth, development and reproduction. The results of the present study also indicated that Diet II (blood worms) contained highest protein level, showed the maximum fry production in guppies compared to the other diets. Diet IV and V probably had adequate levels of protein for proper maintenance of growth and reproduction. Finally, the use of feed with edible insect products is absolutely reliable for commercial guppy farming because the insects have high protein, lipid and carbohydrates levels.

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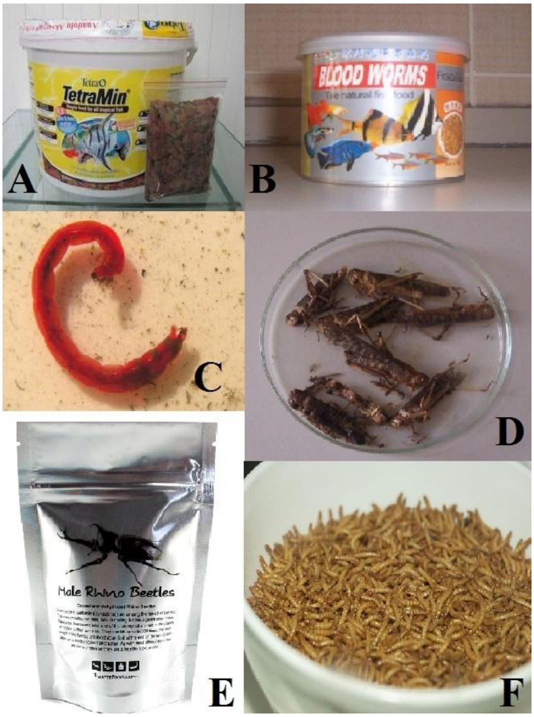

Figure 1. The feeds used in the experiment: A) Tetramin fish flake food as Diet I, B) Blood worm as Diet II (commercial) C) Blood worm larva (*Phlebotomus*), D) Locust as Diet III (*Locusta migratoria*), E) Rhino beetle as Diet IV (*Dynastes hercules*) (commercial), and F) Flour worm as Diet V (*Tenebrio molitor*).

Table 1. Growth parameters of guppy fed different diets.

Parameter	Diet I	Diet II	Diet III	Diet IV	Diet V
Initial length (cm)	1.01±0.01	1.03±0.02	1.02±0.02	1.01±0.02	1.00±0.01
Final length (cm)	1.56 ± 0.02^{a}	2.07 ± 0.07^{b}	1.40 ± 0.03^{a}	1.93 ± 0.04^{b}	1.91 ± 0.06^{b}
Length gain	0.55±0.01	1.04±0.05	0.38±0.01	0.92±0.02	0.91±0.05
Initial weight (g)	0.0121±0.001	0.0123±0.001	0.0124±0.002	0.0124±0.002	0.0121±0.002
Final weight (g)	0.188 ± 0.001^{b}	0.215 ± 0.003^{c}	0.144 ± 0.001^{a}	0.208 ± 0.005^{c}	0.204 ± 0.003^{c}
Weight gain	0.176±0.001	0.202±0.002	0.131±0.003	0.195±0.003	0.191±0.002
SGR (%)	0.29 ± 0.03^{b}	0.33 ± 0.01^{b}	0.22 ± 0.02^{a}	0.32 ± 0.02^{b}	0.31 ± 0.01^{b}
FCR	2.68 ± 0.10^{b}	1.15 ± 0.09^{a}	6.57 ± 0.13^{c}	1.34 ± 0.08^{a}	1.38 ± 0.09^{a}

SGR: Specific growth rate, FCR: Feed conversion ratio. Values within a row with different superscript letters are significantly different ($p<0.05$).

Table 2. Reproductive parameters of guppy fed different diets.

Parameter	Diet I	Diet II	Diet III	Diet IV	Diet V
Ovarian weight (g)	0.25 ± 0.02^{a}	0.49 ± 0.01^{c}	0.20 ± 0.02^{a}	0.40 ± 0.02^{b}	0.39 ± 0.01^{b}
F_a	35.5 ± 0.02^{a}	58.8 ± 0.04^{c}	30.8 ± 0.03^{a}	50.2 ± 0.05^{b}	48.1 ± 0.02^{b}
Mean GSI%	24.07 ± 0.021^{b}	30.17 ± 0.045^{d}	20.81 ± 0.032^{a}	28.82 ± 0.024^{c}	28.40 ± 0.013^{c}

Values within a row with different superscript letters are significantly different ($p<0.05$).

Table 3. Fry production and fry survival during the breeding period.

Parameter	Diet I	Diet II	Diet III	Diet IV	Diet V
Mean total fry production	351 ± 6.5^{c}	384 ± 5.4^{d}	312 ± 7.1^{a}	353 ± 2.3^{c}	336 ± 5.6^{b}
Fry survival rate	93.14±8.2	91.57±12.6	94±5.6	93.57±4.32	92±5.61

Values within a row with different superscript letters are significantly different ($p<0.05$).

Table 4. Proximate composition of some insect groups (100 g dry matter).

Insect ordo	Crude protein (%)	Crude lipid (%)	Carbohydrates (%)
Odonata	58.83	25.38	3.75
Orthoptera	44.10	2.2	1.20
Homoptera	51.13	27.73	2.17
Hemiptera	55.14	30.43*	3.23
Coleoptera	50.41	27.57	2.81
Lepidoptera	44.91	24.76	8.20
Diptera	59.39*	12.61	12.04*
Hymenoptera	47.81	21.42	3.65

* showed the highest values