Effects of Different Diets on the Growth of Zebra *Branchydanio rerio* Hamilton–Buchanan, 1822

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Abstract

Feeding experiments carried out to determine the effects of different diets on growth performance and survival rate on the fingerlings of Zebra *Branchydanio rerio* with different feed namely, diet I (dry food pellet) (Tokyu) as control, diet II (Tubefix), diet III (Beef heart) and diet IV (mixed feed), revealed that higher mean body weight (0.354+0.204 g) and mean specific growth rate (0.445) were achieved by diet II containing. No mortality occurred during the study. The mean value of condition factor (K) indicated good condition with all diets. The value of regression coefficient (b) was found to be lower than 3 indication negative allometric growth pattern with all diets. The highest correlation coefficient of length–weight relationship was found in specimens fed with diet III (r = 0908). Comparison on gain in weight among the fish fed with different diets, indicated that diet II (Tubefix) exert the best growth performance than the other diets.

Keywords: *Branchydanio rerio*, effect of diets, length–weight relationship (LWR), condition factor (k), weight gain, survival rate

Keywords: Growth performance, mortality, mean value

Introduction

Ornamental fish farming is an important primary industry. Ornamental fishes are often referred as living jewels due to their color, shape and behavior. They are peaceful, generally tiny, attractively colored and could be accommodated in confined spaces (Hatefi and Sudagar, 2013).

Aquarium keeping is among the most popular of hobbies with millions of enthusiasts worldwide. Together all countries of the European Union are the largest market for ornamental fish; how the United States (US) is the single largest importer of ornamental fish in the world (FAO 1996–2005; Chapman, 2000) (cited by Livengood, 2007).Zebrafish, *Danio rerio*, is a tropical freshwater teleost belonging to the Cyprinidae family. The species has become a major research model used in biomedical studies to investigate, for example, vertebrate development genetics, physiology, and behavior (Crunwald and Eisen, 2002) (cited by Nasiadk and Clark, 2012).

The zebrafish is omnivorous. Its natural diet consists primarily of zooplankton and insects, although phytoplankton, filamentous algae and vascular plant material, spores and invertebrate eggs, fish scales, arachnids, detritus, sand and mud have also been reported from gut content analysis (Spence *et al.,* 2008).

The feed of fish and their nutrient value is one of the most important factors in production cost and health of fish. Feeding habit of the fish is very different in the form of Carnivorous, Herbivorous, Omnivorous and also there is a large diversity in their feeding patterns (Yousefian, 2012).

In most case, fish only need to be fed once a day, and only need to feed a small amount. Feeding ornamental fish is more serious and difficult compared to pond fish culture due to limitation of space and lack of natural food (Yousefian, 2012).

The nutritional status of the broodstock can affect offspring quality. The accumulation of essential nutrients such as essential fatty acids and vitamins are dependent on the nutrient reserves in the mother animal, and consequently on the dietary input of broodstock in the period

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preceding gonadogenesis (Blom and Dabrowski, 1996; Bell *et al.,* 1997) (cited by Mehrad *et al.,* 2012).

Food or vitamin shortages may have caused suspension of vitellogenesis, resorption of oocytes, and decreased fecundity in the goldfish, *Carassius auratus* (L.) (Bekker, 1958) (cited by Mehrad *et al.,* 2012).

Understanding the importance of food in the growth and production of fish, lead to successful rearing of fish so that this research was conducted with the following objective;

- to determine the effect of different feeds on the growth of zebra fish in terms of body weight and length increment.
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Materials and Methods

Study Period

The study period lasted from July, 2016 to October, 2016.

Study Site

This study was carried out in the Laboratory of Zoology Department, Mandalay University.

Identification

Identification of fish was made following Tawlar and Jhingran (1991), Cliff Harrison (1998), Kanthaiyapparaju's (1999) and Jayaram (2013).

Water Temperature and pH

Water parameter such as water temperature and pH were measured weekly using Thermometer and pH meter (MT–8062 EXACT INSTRUMENT pH meter) respectively.

Experimental Diets Preparation

Four kind of diet which included (minced beef heart muscles without fet and tendons), the usual dry food pellets (Tokyu), live food (Tubefix) and mixed feed were used in this experiment. Fish were tested by two feeding methods. (i) mono feed method (ii) mixed feed method.

(i) Mono Feed

Fish were fed specifically with only feed. Fish in Aquarium (I) the control were fed on; the usual dry food pellet (Tokyu). The experiment fish in Aquarium (II) were fed on live Tubefix (33% protein), in Aquarium (III) with minced beef heart muscle (30% protein).

(ii) Mixed Feed

Fish in aquarium (IV) were fed separately and all the three feed involved in the study, minced beef heart in the morning, live food in the afternoon and dry food pellets in the evening.

Feeding Reguime

For fish fry, 5% of egg yolk calculated based on the total body weight of the specimens was fed. Similarly, 5% of the body weight of total specimens of fingerlings was fed and only 3% was fed to the juveniles. Feeding was done at four hour intervals, three times per days.

Experimental Design

A total of 160 young zebra (one month old and approximately of the same length) were used in this experiment. These young fishes were selected from the fry hatched and maintained in the Laboratory of Zoology Department, Mandalay University. Four groups of glass aquarium tank (60 × 30 × 30 cm in dimension) were prepared stocking each tank with 20 fish fingerlings (1.4 to 2.8 cm in total length and 0.032 to 0.201 g in body weight). The fish in each tank were fed with each kind of prepared feed. Two replicas were made for each diet. To avoid water pollution, food remained at the bottom of each aquarium was siphoned off after feeding. Total length and body weight of sample fish were taken every two weeks. Body weight was taken by Digital Balance Setra, EI 200S and the length by a scaled ruler. The condition of fish was checked every day up to four months old.

Parameters Calculated

The following calculations were made

Survival rate (%) = 100x $\frac{\text{Final number of fish}}{\text{initial number of fish}}$ (Ricker, 1975)

Weight Gain (WG) = [(FW–IW)/ IW × 100] (cited by Debrnth *et al.,* 2006)

where, FW = final weight

IW = initial weight

Specific Growth Rate (SGR)% = $\frac{100\ [\text{In final weight} - \text{In initial weight}]}{\text{time (days)}}$

(Ricker, 1975) (Cited by Gokeek *et al.,* 2008)

Condition factor (K) = $\frac{W}{L^3}$ × 100 (Salam *et al.*, 1994)

Where; W = Weight (g)

L = Total body length (cm)

Relation between length and weight was calculated using regression equation given by Bailey (1968).

Statistical Analysis

In order to find out the significant differences between the treatments of different diets, the data were subjected to ANOVA test (SPSS version 21.0).

Results

Distinct Characters

Body is cylindrical form. Head is small and laterally compressed. Eye is large and rounded. Male zebra is web–shaped, with white stripes between the red colors. Male are always smaller than female. The female is larger. There are four kinds of colors varieties red, yellow, green and blue. Red color variety was used for breeding in the present study.

Feeding Experiment

During the experimental period, the water temperature recorded ranging from 22° to 28°C and pH value was 8(Table 1).

Body Length and Body Weight

One month old fingerlings, 40 in number with the mean initial length of 2.130 ± 0.352 cm and mean weight of 0.109 ± 0.049 g were fed with the routine feed of dry food pellet (diet I) represented the control group. Similar number of fingerlings with the mean length and weight of 2.130 ± 0.382 cm and 0.110 ± 0.053 g were fed with tubefix (diet II), the experimental group. Those with the mean length and weight of 2.060 ± 0.362 cm and 0.095 ± 0.050 g were fed with minced beef heart muscle (diet III) and lastly, all the above diet I, II and III were separately fed as (Mixed diet IV) on those where the mean length and weight of 02.070 ± 0.394 cm and 0.102 ± 0.055 g experiment fish group(Table 2, Plate 2).

Two month old fingerlings, 40 in number with the mean initial length of 2.873 ± 0.408 cm and mean weight of 0.228 ± 0.095 g were fed with the routine feed of dry food pellet (diet I) represented the control group. Similar number of fingerlings with the mean length and weight of 2.805 ± 0.386 cm and 0.229 ± 0.326 g were fed with tubefix (diet II), the experimental group. Those with the mean length and weight of 2.895 ± 0.399cm and 0.238 ± 0.083 g were fed with minced beef heart muscle (diet III) and lastly, all the above diet I, II and III were separately fed as (Mixed diet IV) on those where the mean length and weight of 02.895 ± 0.399 cm and 0.238±0.083 g experiment fish group(Table 3,Plate 2).

Three month old fingerlings, 40 in number with the mean initial length of 3.715 ± 0.444 cm and mean weight of 0.182 ± 0.655 g were fed with the routine feed of dry food pellet (diet I) represented the control group. Similar number of fingerlings with the mean length and weight of 3.785 ± 0.464 cm and 0.406 ± 0.163 g were fed with tubefix (diet II), the experimental group. Those with the mean length and weight of 3.753 ± 0.434 cm and 0.455 ± 0.171 g were fed with minced beef heart muscle (diet III) and lastly, all the above diet I, II and III were separately fed as (Mixed diet IV) on those where the mean length and weight of 3.533 ± 0.498 cm and 0.394±0.125 g experiment fish group(Table 3, Plate 2).

Four month old fingerlings, 40 in number with the mean initial length of 4.173 ± 0.519 cm and mean weight of 0.548 ± 0.210 g were fed with the routine feed of dry food pellet (diet I) represented the control group. Similar number of fingerlings with the mean length and weight of 4.238 ± 0.571 cm and 0.539 ± 0.173 g were fed with tubefix (diet II), the experimental group. Those with the mean length and weight of 4.100 ± 0.440 cm and 0.629 ± 0.181 g were fed with minced beef heart muscle (diet III) and lastly, all the above diet I, II and III were separately fed as (Mixed diet IV) on those where the mean length and weight of 3.375 ± 0.449 cm and 0.538 ± 0.167 g experiment fish group(Table 3, Plate 2).

Condition Factor (K), Correlation Coefficient (r) and Regression Coefficient (b)

At the age of one month old fish, the highest K value in Control (1.059 ± 0.176), r value in Treatment II (0.942) and b value in Treatment III (0.136) were recorded (Table 4). In the case of two–month old fish, the highest K value in Treatment III (1.071 ± 0.196), r value in Treatment I (0.898) and b value in Treatment II (0.248) were observed (Table 4).

Regarding with three–month old fish, the highest K value in Treatment III (0.885 ± 0.129),r value in Control (0.959) and b value in Treatment I(0.352)were recorded(Table 4).

Concerning with four–month old fish, the highest K value in Treatment I (0.893 ± 0.099), r value in Treatment III (0.932) and b value in Treatment I (0.389) were found respectively (Table 4).

Weight Gain (WG)

In this experiment, the mean weight gain (WG) were observed 0.040 g in Control (Tokyu), 0.056 g in Treatment I (Tubefix), 0.039 g in Treatment II (Beef heart) and 0.043 g in Treatment III (Mixed feed) (Table 2).

Survival Rate (SR)

The survival rate was 100 % since not a single mortality occurred throughout the experimental period in all groups (from July 2016 to October 2016) (Table 2).

Specific Growth Rate (SGR)

The highest specific growth rate (SGR) was found in those fed on Treatment I (0.445 %) followed by Control (0.366 %), Treatment III (0.363 %) and Treatment II(0.358 %) (Table 2).

Statistical Analysis

Differences of body length and weight of fish among different diets in each month of study period were statistically not significant in all diet ($p>0.05$) (Appendix I, II).

Table.1 Water quality parameters during the experimental period of Zebra fish, *Branchydanio rerio*

Parameters	July	August	September	October
Temperature range (°C)	26–28	25–28	24–27	23–26
pH	8	8	8	8

Table 2. Growth performance by different feed and survival rate of Zebra, *Branchydanio rerio* during the experimental period

Parameter	Diet I (Control, Tokyu)	Diet II (Tubefix)	Diet III (Beef heart)	Diet IV (Mixed feed)
Mean Initial Weight (IW)(g)	0.109 ± 0.049	0.095 ± 0.050	0.110 ± 0.053	0.102 ± 0.055

Mean Final Weight (FW) (g)	0.548 ± 0.211	0.629 ± 0.181	0.539 ± 0.173	0.538 ± 0.167
Mean Weight Gain (WG) (g) (%)	0.040	0.056	0.039	0.043
Mean Specific Growth Rate (SGR) (%)	0.366	0.445	0.358	0.363
Survival Rate (SR) (%)	100	100	100	100

Plate 1 Breeding pair, feeding tank and different diets

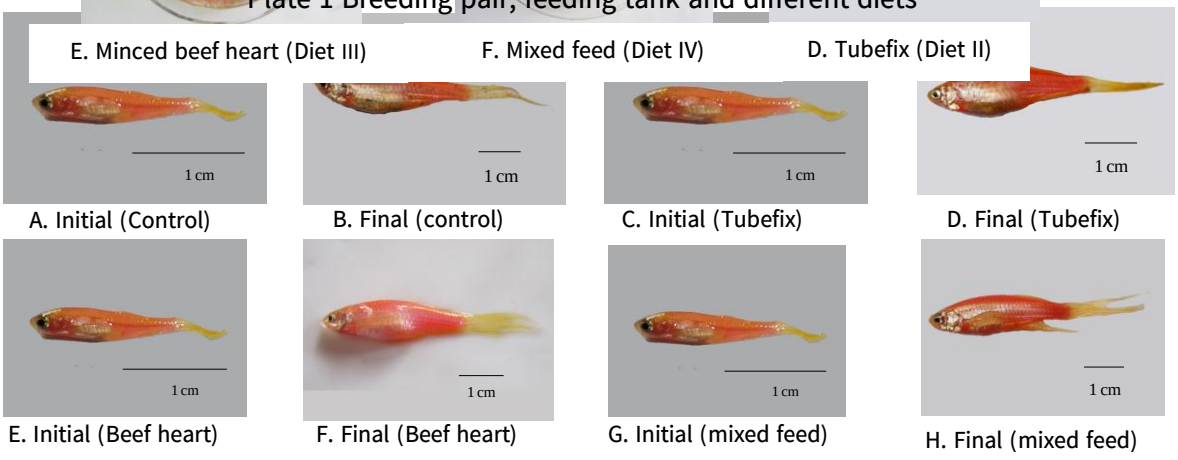

Plate 2 Relative gain in length of fish fed on different diets

Table.3 Monthly mean body weight and body length of Zebra, *Branchydanio rerio* fed with the different diets

Age	No. of fish	Diet I (Control, Tokyu)				Diet II (Tubefix)				Diet III (Beef heart)				Diet IV (Mixed feed)			
		Length (cm)		Weight (g)		Length (cm)		Weight (g)		Length (cm)		Weight (g)		Length (cm)		Weight (g)	
		range	Mean ± SD	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD	range	Mean ± SD
One month	40	1.5–2.7	2.130 ± 0.352	0.034–0.183	0.109 ± 0.049	1.5–2.7	2.060 ± 0.362	0.032–0.195	0.095 ± 0.050	1.5–2.8	2.130 ± 0.382	0.035–0.185	0.110 ± 0.053	1.4–2.7	2.070 ± 0.394	0.033–0.201	0.102 ± 0.055
Two month	40	2.2–3.6	2.873 ± 0.408	0.098–0.432	0.228 ± 0.095	2.2–3.6	2.895 ± 0.399	0.165–0.411	0.238 ± 0.083	2.1–3.4	2.805 ± 0.386	0.102–0.442	0.229 ± 1.326	2.1–3.4	2.895 ± 0.399	0.143–0.464	0.238 ± 0.083
Three month	40	3.1–4.5	3.715 ± 0.444	0.182–0.655	0.363 ± 0.145	3.0–4.4	3.753 ± 0.434	0.201–0.815	0.455 ± 0.171	3.0–4.6	3.785 ± 0.464	0.175–0.687	0.406 ± 0.163	2.7–4.3	3.533 ± 0.498	0.213–0.698	0.394 ± 0.125
Four month	40	3.4–5.1	4.173 ± 0.519	0.215–0.885	0.548 ± 0.210	3.2–4.8	4.100 ± 0.440	0.332–0.893	0.629 ± 0.181	3.3–5.2	4.238 ± 0.571	0.312–0.907	0.539 ± 0.173	3.3–4.8	3.975 ± 0.449	0.303–0.852	0.538 ± 0.167
Mean			3.223 ± 0.784		0.312 ± 0.163		3.202 ± 0.792		0.354 ± 0.204		3.239 ± 0.824		0.321 ± 0.164		3.118 ± 0.717		0.318 ± 0.164

Table.4 The results of condition factor K, correction coefficient r and regression coefficient b of Zebra fish during the experimental period

Age	Diet I (Control, Tokyu)			Diet II (Tubefix)			Diet III (Beef heart)			Diet IV (Mixed feed)		
	K	r	b	K	r	b	K	r	b	K	r	b
One month	1.059 ± 0.176	0.899	0.134	0.997 ± 0.142	0.878	0.130	1.058 ± 0.142	0.942	0.134	1.048 ± 0.120	0.930	0.136
Two month	0.933 ± 0.189	0.712	0.196	0.959 ± 0.141	0.898	0.196	0.979 ± 0.131	0.854	0.248	1.071 ± 0.196	0.816	0.195

Three month	0.670 ± 0.055	0.959	0.321	0.826 ± 0.171	0.799	0.352	0.707 ± 0.081	0.951	0.343	0.885 ± 0.129	0.895	0.238
Four month	0.722 ± 0.127	0.877	0.379	0.893 ± 0.099	0.883	0.389	0.703 ± 0.117	0.886	0.286	0.836 ± 0.078	0.932	0.359
Mean	0.846 ± 0.158	0.862	0.258	0.919 ± 0.065	0.865	0.267	0.862 ± 0.159	0.908	0.253	0.960 ± 0.101	0.893	0.232

Discussion

Zebra fish, *Danio rerio* is a tropical freshwater fish belonging to the minnow family (Cyprinidae) of order Cypriniformes. It is a popular aquarium fish, frequently sold under the trade name zebra danio. This species reaches sexual maturing at a small size and rearing of larvae is comparatively easy. Therefore, the zebra fish provides a useful model for the study of nutrition in larval and juvenile cyprinids.

Zebra fish are omnivorous and easy to feed and accept what is offered. They prefers most food sources including flake food, pellet, preserved food and are known to prefer natural food items such as living protozoa and tubefix worms.

This experiment was performed to find out the effects of different diets on the growth pattern and survival of zebra fish larvae. This experiment was conducted using four Diet I (Dry food pallet), Diet II (Tubefix), Diet III (Beefheart), Diet IV (Mixed feed). Raseduzzaman *et al.* (2014) suggested that feeding frequency has direct impact on the growth performance and survival of fry and larvae of ornamental fish. They found that a feeding frequency of three times in each day was best for rearing the fry and larvae of fish.

In the present study, the feeding frequency of 3 times/day was adopted during the experimental period. Thus, it is assumed that the fish feeding is adequate for the growth of Zebra fish. Bilen (2013) indicated that protein is one of the most important components in a fish diet. The requirements for protein in larval fish are greater that in adult fish. The growth performances were the highest in ornamental fish when the fish fed with animal protein. Alam *et al.* (2010) stated that protein requirement varied from around 30–35% dietary protein for growing omnivorous fish.In this work, the different diets of Tokyu, Tubefix and Beef heart were used as the experimental diets containing 32%, 33% and 30% crude protein respectively covering the protein requirement of fish.

During this experiment, the highest specific growth rate (SGR) was found to be 0.445% shown in the zebra fish fry fed with Tubefix worms and thus assumed relevant feed for fish fry.

Khanna (1996) stated that growth of an organism can be defined as a change in its size (length and weight) over a period of time. The growth rate in fishes is highly variable and depends upon many environmental factors. Quality of food and its availability is one of the important factor influence growth rate of fish. In this experiment, the length gain and weight gain values showed slight variation among the different diets. The highest percent length gain (LG) was 0.0099% and highest weight gain (WG) was 0.056% respectively as observed in the fry fed with Tubefix diet. The result proved the above assumption that better growth was obtained by feeding with Tubefix which contain the highest 33% among the different diets fed.

Habib (2014) reported that nutrition's have significant effects on the survival rate and growth of fish species in aquarium. The results of the present experiments revealed the survival rates was 100% in all experimental diets. No mortality was found throughout the experimental periods.

Alex *et al.* (2012) stated that the condition factor (K) which showed the degree of well being of the fish in their habitat is expressed by coefficient of condition also known as length–weight factors. When condition factor value is higher, it means that the fish can be affected by a number of factors such as stress, season, feeds. The value of (K) (1) indicated that good condition of fish. The value of (K) is influenced by age of fish, season, type of food consumed and degree of growth pattern.

In this study, condition factors (K) approached the value of (1) in all experimental diets during the study period. Therefore, zebra fish (*Danio rerio*) fry in all aquaria of the present experiment, indicated that they were in good condition throughout the study period. Nehemia *et al.* (2012) demonstrated that fish can attain either isometric growth (b=3), negative allometric growth (b<3) or positive allometric growth (b>3). Isometric growth (b=3) is associated with no change of body shape as an organism grows. Negative allometric (b<3) growth implies the fish becomes more slender as it increases in weight while positive allometric growth implies the fish becomes relatively stouter or deeper–bodied as it increases in length.

In the present study, the regression coefficients (b) were lower than 3 in all diets. Thus (b<3) indicated negative allometric growth pattern and zebra fish became lighter for their length as described by Nehemia (2012). Romdhani *et al.* (2013) indicated that regression coefficient (r) of the total length weight relationship are closed to (1), reflecting a good correlation between the two parameters (length and weight).

In the present study, the regression coefficient (r) were closed to (1) in all diets. Therefore, it indicated good relationship between length and weight suggesting a good adjustment in growth of zebra fish in all experimental diets.

Dimitris *et al.* (2011) stated that temperature is known to be one of the most important environmental factors that strongly affect all developmental processes in fishes. It influences the growth performance and survivality.

Dimitris *et al.* (2011) stated that zebrafish can tolerate a wide temperature range. Zebrafish have a maximal thermal tolerance range 6.2 °C – 41.7°C. During this study water temperature ranged from 23°C–28°C as the study was conducted under laboratory condition and in which temperature appeared not to be a limited factor.

In conclusion, the survival rate was 100% for all experimental diets. The better growth rate was observed with Diet II (Tubefix worm), however, significant difference was not observed in the other three diets (p>0.05). It may thus be concluded that, as Tubefix diet fed subjects fared better growth, it is recommended as a relevant feed in the breeding of *Danio rerio*, the zebra fish.

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