Effect of dietary protein levels on reproductive performances of female viviparous ornamental fish, swordtail *Xiphophorus helleri* (Poeciliidae).

Alexander S.C. Chong*, Saraitulnis Ishak, Zulfaizuddin Osman, Roshada Hashim.

Laboratory of Fish Biology, Aquaculture Research Group, School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.

* corresponding author: Tel: 604-6533888 ext. 4009, Fax: 604-6565125;

e-mail: alex@usm.my

Abstract

A study to determine the effect of increasing levels of dietary protein on swordtail (*Xiphophorus helleri*), a popular freshwater live bearer was carried out. Five isocaloric semi pufired diets at 20, 30, 40, 50 and 60% dietary protein levels were used for this purpose. Broodstock performance was evaluated based on growth parameters, proximate content and fry production of female broodstock. Results showed that while the 20% and 30% growth rate gave lowest SGR (specific growth rate) values, there was no significant difference between 40-60% dietary treatments. The 20% dietary treatment also caused lowest protein content in both ovary and muscle of female. Fry production was highest from females fed with 50 and 60% levels, followed by 30 and 40% levels while diet 20% protein produced lowest number of fries. A significant correlation was also obtained between number of produced fries and weight of female fish, indicating that size is a major factor influencing production. In terms of relative fecundity, diet 20% protein showed lowest values, followed by diets 30-40% and 40-60%. There were no significant differences in both weight and length of produced fries among dietary treatment. Proximate analysis of fries also did not show any trend with different dietary protein levels of female broodstock. Dietary protein requirement of female swordtail during reproduction is therefore crucial for both somatic growth and reproduction process in order for fry production to be optimized. Depending on different farming conditions, we proposed dietary protein requirements of female swordtails to be at 30-40% levels

key words: broodstock nutrition, protein, swordtails, live bearers

1. Introduction

The culture of ornamental fish remains an important activity in several Asian countries. Ng and Tan (1997) estimated a total production value of US$ 80-150 million annually from Southeast Asian aquarium fish farms. Live bearing species from the family Poeciliidae such as guppy (*Poecilia reticulata*), molly (*P. latipinna, P. sphenops*) swordtail (*Xiphophorus helleri*), and platy (*X. maculatus*) are a popular group of species being produced in Singapore, Malaysia, Indonesia, Thailand, India and China. According to a survey on ornamental fish trade in the U.S., guppy and swordtail accounted for 25.8% and 5.4% of total number of ornamental fish imported in the United States in 1992 (Chapman et al., 1998). Farming of poeciliids is usually carried out in outdoor earthen ponds or net cages (Fernando and Phang, 1994). Feeding of broodstock in Asian farms still rely mainly on live feed such as bloodworms, *Tubifex* coupled with daily prepared paste consisting mixture of fish meal and skimmed milk powder (Fernando et al., 1991). Besides the risks of introducing harmful pathogens, these feeding practices may not provide adequate nutrient levels required by broodstock fish. These types of diets can also cause potential problems in terms of detrimental pond effluent. The use of fish meal in ornamental fish culture also means that potentially consumable protein is being converted to non-consumable luxury items (Tlusty, 2002).

Due to differences in biological processes, the nutrient requirement of broodstock will be different from growing juvenile animals. However, a full and comprehensive understanding on reproduction mechanisms such as gonadal maturation, fertilization success and larval quality is far from complete as these coordinated processes are very complex. Broodstock nutrition studies offer to provide applicable knowledge by determining if reproductive performance of a particular fish species can be improved by maternal dietary intake. However, the fish broodstock nutrition is still poorly understood due to difficulties in conducting studies involving proper feeding and reproduction of broodstock.

A review by Izquierdo et al. (2001) outlined lipid, protein, fatty acid profile, vitamin E, C and carotenoids as major nutrients influencing various reproduction processes such as fecundity, fertilization, hatching and larval development. Role of dietary protein on several reproduction parameters of tilapia has been well documented (De Silva and Radampola, 1990; Gunasekera et al., 1995; 1996a,b;1997a,b; Fontainhas-Fernandes et al., 2000; El-Sayed et al., 2003). The effect of dietary protein on reproduction of several marine species have also been studied (Watanabe et al., 1984; Cerda et al., 1994; Fernandez-Palacios et al., 1997).

As first step to formulate effective low-cost feed for swordtail broodstock, we present here a study investigating the effects of dietary protein levels on various reproductive aspects of swordtail (*Xiphophorus helleri*) utilizing semi-purified diets. Since swordtail is a viviparous species where fertilization and hatching of embryos occurs internally, this study is also useful to understand the effect of dietary protein on broodstock possessing this type of reproductive strategy.

2. Materials and Methods

2.1 Diets

Five semi-purified isocaloric (395 kcal GE/100g) diets with increase levels of dietary protein (20%, 30%, 40%, 50% and 60%) were formulated utilizing casein, gelatin and fish meal as protein source (Table 1). Diets were also formulated to contain 9% lipid. Proximate analysis of diets was conducted according to AOAC (1990) for verification of nutrient levels.

2.2 Fish and tank system

Swordtail fries were obtained from populations bred and raised in our laboratory. Feeding of fries was carried out with *Artemia* nauplii for first 4 weeks, followed by frozen bloodworms. Throughout this period, males were separated from females at earliest sign of sexual differentiation to avoid reproduction activities. Virgin females aged 20 weeks old (1.1 -1.2 g) were used for experiment. Five replicate tanks were used for evaluation of each diet with a total of 5 females selected and stocked in each tank (3' x 2' x 1'). Prior to stocking, females were also weighed individually. Virgin males aged 20 weeks were kept separately in a large tank (4' x 3' x 3') and fed with frozen bloodworms twice daily. These males were randomly selected and 2 males were introduced to experimental tanks containing females every 30 days. Males were left in these tanks for 5 days before returning them to common holding tank. During feeding, males were separated from female using plastic sheets. Bundles of nylon strings tied together were also placed into each tank to offer shelter for new free-swimming fries from parental cannibalism.

2.3 Feeding and experimental design

Feeding was carried out till satiation twice daily at 0900 and 1700 hrs. Inspection for released fries from each tank was also carried out during feeding hours. Feeding trial lasted for 14 weeks. At end of both experiments, individual weight of females were measured before sacrificed for proximate analysis of muscle and ovary (AOAC, 1990). Parameters analyzed was

~~Specific growth rate (SGR %/day): [(ln W_t – ln W_i) / T)] x 100~~

where W_t = Mean final weight, W_i = Mean initial weight, T = total experimental days

Feed conversion ratio (FCR): total feed fed (g)/ total wet weight gain (g);

Total fry production: Total fry harvested throughout experimental period

Relative fecundity:

.Total fry production at throughout experimental period/mean weight of female (g)

Total fry produced/dietary protein intake:

Total fry production at end of experiment/ total protein given (g).

In order to investigate to effect of dietary protein content on fry quality, freshly sampled fries were immediately frozen at -20°C for measurement of total length, dry weight and proximate composition.

2.4 Data and Statistical analysis

~~Comparison of various growth and reproductive parameters from different dietary treatment~~ was carried out using analysis of variance (ANOVA) and where applicable, Tukey's HSD. Data in percentage was transformed to arc-sin before subjected to statistical analysis.

3. Results

Table 2 showed various growth parameters of female brood receiving different dietary protein treatment. Results showed that while there was no significant difference between final weight of female fed diet 20% and 30% protein, weight of females receiving 40-60% protein diets was significantly higher than the 2 lower levels ($P \leq 0.05$). Weight gain was also lowest in diet 20%, followed by 30% and 40%, while diet 50% and 60% provided highest weight increment. Specific growth rate (SGR) was also significantly lowest for diets 20% followed by 30%, and 40%-60% dietary levels. Results from the FCR values also showed that both the 40% and 50% dietary protein levels also provided the highest feed utilization efficiency in female brood.

Mean total fry production throughout experimental period is shown in Figure 1. Results showed an increase in overall fry production with increase in dietary protein levels. Highest fry production was obtained with dietary levels of 50% (167 $\pm$ 9) and 60% (182 $\pm$ 4), followed by 40% (129 $\pm$ 11) and 30% (100 $\pm$ 9). Diet 20% (41 $\pm$ 8) produced the significantly lowest number of fries ($P \leq 0.05$). Further analysis revealed that fry production is significantly correlated ($r = 0.80$) to final weight of female broodstock, indicating larger females produced higher number of fries (Figure 2).

Fry production was also calculated as relative fecundity (Figure 3). Although results still show a general trend of increased production with increasing levels of dietary protein, the differences between 40%, 50% and 60% are non significant. Figure 4 also showed that when production was calculated as number fry produced per dietary protein intake, there was actually no difference between diet 30%, 40%, 50% and 60% protein ($P \leq 0.05$). The 20% protein diet however still showed the lowest significant value.

Weekly fry production number showed that females fed higher dietary protein levels (40-60%) started producing high number of fries earlier (5^{th}-6^{th} week) than the 20% and 30% level (Figure 5). Results also showed that there was no significant difference in total length of produced fries among different dietary treatment (Table 3). There was also no clear pattern on the influence of dietary protein levels on larval weight, with the 40% level having the significantly highest weigh. Proximate analysis of female muscle, ovary and harvested fries are shown in Table 4. While there was no clear pattern in both lipid and ash content of all three samples, the 20% treatment caused significantly lower protein content in female muscle and ovary.

4. Discussions

This study showed that feeding female brood with 20% dietary protein level resulted in low production of fries. One of the most important contributions of dietary protein towards broodstock performance is in the effect on the brood size itself. It has been reported that in tilapia weight of female brood at first maturation increase linearly with higher dietary protein levels (De Silva and Radampola, 1990; El-Sayed et al., 2003). Gunasekera et al. (1995) revealed that female tilapia attained puberty and oocyte maturation earlier when fed higher levels of dietary protein and concluded that this due mostly to effect of diet on fish growth. Shim et al. (1989) also demonstrated that female dwarf gourami (*Colisa lalia*) broodstock showed highest weight increase and higher number of oocytes undergoing vitellogenesis when fed with higher dietary protein level. Larger broods have also been reported to display higher success in spawning rates (Gunasekera 1996a; El-Sayed et al., 2003). Milton and Arthington (1983) reported that in wild populations of swordtail, fecundity is linearly related to body size. Positive correlation between egg size and parental body size has also been reported in several freshwater species (Sehgal and Toor, 1991; Bromage et al., 1992). Our results demonstrated that feeding with higher protein levels resulted in significantly larger sized females, which correlates with higher fry production.

The lower feed utilization efficiency obtained with the 20% diet also indicates the poor feed utilization for growth by female swordtail. Washburn et al. (1990) reported that rainbow trout broodstock fed on a diet low in carbohydrate display reduced relative fecundity. Although the energy of requirement of broodstock fish has not been well studied, there is a possibility of high energy requirement to support important spawning related activities such as male attraction and overcoming competition for mate. Poeciliids have been known to display stringent male selection process for reproductive purposes.

Besides promoting growth, dietary protein is also essential for gonadal maturation and eventually, production of eggs. In teleosts, all the essential component of an egg must be incorporated during oocyte development as ovulated eggs take up very little or no nutrient. Besides intrinsic factors such as maternal gene quality, external factors such as nutrient intake, pollution have been identified as major factors influencing oocyte maturation in fish (Brooks et al., 1997). Gunasekera et al. (1997b) reported that a low 10% dietary protein resulted in lower protein content in latter oocyte stages of female tilapia, due to insufficient provision of protein for oocyte maturation by parental fish. Although we did not investigate the effect of dietary protein on the oocyte development in female swordtail, the lower protein content obtained in both muscle and ovary of fish with the 20% level probably indicates limited or insufficient availability of protein for both female body deposition and oocyte development. Continuous feeding with low dietary protein level also caused female tilapia to utilize body reserves during subsequent spawning seasons, compromising muscle deposition and growth (Gunasekera et al., 1996a). This is in agreement with Al Hafedh et al. (1999) who also reported lower protein content in tilapia broodstock fed with below optimal protein levels.

In eggs, proteins are present as lipoproteins, hormones and enzymes. Poor egg quality is one of the major constraints in large scale fish farming. Parameters such as egg size and composition have been proposed to be useful indicators of seed production in terms of hatchability and larval quality (Brooks et al., 1997). Several studies have shown that, larger fish egg size will eventual result in larger fry at hatching (Sehgal and Toor, 1991; Ojanguren et al., 1996; Gisbert et al., 2000). Larger fries have been proposed to possess the advantage of better survival or growth through more efficient prey capture and tolerance to starvation (Miller et al., 1988). In swordtails, internal fertilization will be followed by hatching of eggs and a period of gestation period of approximately 27 days prior to release of free-swimming larva (Siciliano, 1972). Since it's not possible for us to measure egg size or content, we compared various parameters of newly released larva to determine if these parameters are influenced by maternal dietary protein intake. Our results showed that that length and weight of newly released swordtail larva were not influenced by dietary protein content. In tandem, maternal dietary protein intake also did not affect proximate composition of larval. Elsewhere, Gunasekera et al. (1996b) also reported that growth, moisture and protein content of tilapia larva were not correlated with dietary protein levels of broodstock. Our results also indicate that while lower dietary protein levels caused lower fecundity in swordtails, the quality of produced fries in terms of size, body content and growth were not affected. In tilapia, its is reported that although 20% dietary protein limits the size, oocyte maturation and fecundity of female tilapia, successful hatching of fertilized eggs from this protein level still produce larval of equal quality (Gunasekara et al., 1995b; 1996b). Therefore, tt's possible that in limiting nutritional conditions, swordtail will produce lesser quantity of offspring in order not to compromise quality, a strategy reportedly employed in other Poeciliids (Milton and Arthington, 1983).

Numerous studies have shown that once the dietary protein requirement of female broodstock is provided, further increase in dietary protein intake does not further enhance fish fecundity (Dahlgren 1980; Santiago et al. 1983; Gunasekera et al., 1996a; Al Hafedh et al., 1999; Emata and Borlongan 2003). Based on the similarity between requirement levels for young tilapia and brooders, De Silva and Radampola (1991) proposed that once the optimal requirement is met, brooding fish does not enhance their reproductive activities but utilized most of the energy for growth. In this study, we have shown that there is no significant difference in overall fecundity performances of swordtail between the 40-60% dietary levels. Since our results also indicated that feeding female swordtail with 20% dietary protein caused an overall lower growth rate, muscle body deposition, oocyte protein content and fry production, we proposed that the minimum dietary protein requirement for female swordtail to be at 30-40%, depending on farming conditions. In a series of studies with tilapia, Gunasekera et al. (1995; 1996a,b,; 1997a,b) concluded that female brood was able to maintain several reproductive parameters at 20%-30% dietary levels. Elsewhere, 40% dietary protein was recommended the requirement levels for tilapia (Hafedh et al., 1999; El-Sayed et al., 2003).

Swordtails have been reported to reach maturation stage as early as 2-3 months of age (Milton and Arthington, 1983). In cases where farmers utilize younger females as breeding stock, feeding with 40% dietary protein will probably ensure better growth rate of fish which will in turn result in higher fry production at shorter period. This is especially important in production of new strains, which generally have higher demand and higher price value when firstly introduced to market. In Singapore farms, 24-32 weeks old swordtails are used for breeding purposes for about 8 months to a year (Fernando and Phang, 1994). Therefore, provision of adequate dietary protein to ensure normal somatic and reproduction functions in female will be vital. Its also worth noting that commercial diets containing lower levels of protein (28-32%) than that proposed in this study has been used successfully in swordtail farms in Hawaii (Tamaru et al., 2001). However, these authors also stressed that these outdoor pond system also allows supplementation with natural feed sources such as zooplankton, which has been reported to stimulate breeding activities in swordtail (Kruger et al., 2001). In cases where older females are to be stocked for breeding purposes, a lower 30% dietary protein can be used to maintain good fry production without increasing feed cost unnecessary. Another useful alternative will be to utilize a mixed feeding schedule of high and low levels of dietary proteins, to ensure good growth of parent, fry production and cost effectiveness (Santiago and Laron, 2002). Since the actual dietary protein requirement fish are also affected by factors such as protein quality, levels of lipid and digestibility, further studies considering these factors will be needed for more precise determination.

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Table 1. Composition and proximate analysis of test diets

	Diets				
	DP 20	DP 30	DP 40	DP 50	DP 60
Fish meal (70% CP)	20	20	20	20	20
Casein	3.3	14.3	25.2	36.1	47.0
Gelatin	3	3	3	3	3
Cod liver oil	2.9	2.9	2.9	2.9	2.9
Corn oil	5	5	5	5	5
Dextrin	51.3	40.5	29.8	19	8.3
Vitamin mix[a]	3	3	3	3	3
Mineral mix[a]	2	2	2	2	2
CMC	1.5	1.5	1.5	1.5	1.5
Cellulose	7.9	7.8	7.6	7.5	7.3
Proximate composition					
Crude protein (%)	20.6	30.4	39.3	51.3	59.3
Crude lipid (%)	9.5	9.1	9.4	9.1	9.5
Ash (%)	4.2	4.0	4.5	4.6	4.8
Fiber (%)	4.5	5.3	4.3	4.5	3.6
NFE[b]	61.2	51.2	42.5	30.5	22.8
GE (kcal/100g)[c]	394.4	394.5	395.0	396.4	396.5

[a] Content as according to Chong et al (2000)

[b] Nitrogen –free extract (calculated by difference)

[c] Gross energy, calculated based on 4.1, 9.5 and 5.65 kcal GE/g for carbohydrate, lipid and protein respectively.

Table 2. Mean values (± S.E.) of various growth parameters of female swordtail fed different levels of dietary protein. Mean values in similar row with different alphabet are significantly different (Tukey's HSD, $P < 0.05$).

	Diet				
	20P	30P	40P	50P	60P
Initial weight (g)	1.17 ± 0.04	1.13 ± 0.07	1.15 ± 0.08	1.20 ± 0.09	1.19 ± 0.08
Final weight (g)	2.95 ± 0.05a	3.52 ± 0.04ab	3.93 ± 0.19b	4.14 ± 0.10bc	4.35 ± 0.24b
Weight gain (g)	1.79 ± 0.04a	2.39 ± 0.05b	2.78 ± 0.15b	2.94 ± 0.09c	3.16 ± 0.17c
SGR (%)	0.94 ± 0.01a	1.16 ± 0.02b	1.25 ± 0.11bc	1.26 ± 0.09bc	1.32± 0.27c
FCR	2.45 ± 0.23a	2.28 ± 0.28a	2.07 ± 0.09b	2.02 ± 0.05b	2.22 ± 0.17ab

Table 3. Total length (mm), dry weight (mg) of larva produced by female swordtail fed different levels of dietary protein. Mean values in similar row with different alphabet are significantly different (Tukey's HSD, $P < 0.05$).

	Diets				
	DP 20	DP 30	DP 40	DP 50	DP 60
Total length (mm)	8.70 ± 0.08a	8.50 ± 0.12a	8.73 ± 0.09a	8.43 ± 0.12a	8.56 ± 0.05a
Dry weight (mg)	1.23 ± 0.08a	1.10 ± 0.05a	1.50 ± 0.14b	1.37 ± 0.09a	1.17 ± 0.07a

Table 4. Proximate composition of swordtail larva and female broodstock ovary and muscle fed different dietary levels of dietary protein. Mean values in similar row with ~~different alphabet are not significantly different (Tukey's HSD, P < 0.05).~~

	Diets				
	DP 20	DP 30	DP 40	DP 50	DP 60
Larva					
Crude protein (%)	55.8 ± 1.2a	56.5 ± 2.3a	56.7 ± 2.2a	55.0 ± 0.3a	57.2 ± 0.6b
Crude lipid (%)	31.2 ± 0.4a	30.3 ± 0.7a	32.2 ± 0.5a	33.5 ± 0.5b	31.7 ± 0.1a
Ash (%)	10.2± 0.2a	13.5 ± 0.1b	11.9 ± 1.2a	11.5 ± 1.7a	10.9 ± 0.9a
Female brood (muscle)					
Crude protein (%)	54.0 ± 0.3a	55.0 ± 0.3b	57.3 ± 1.2bc	58.2 ± 0.7c	57.4 ± 1.1bc
Crude lipid (%)	29.2 ± 0.2a	31.0 ± 0.2b	33.4 ± 1.6c	29.5 ± 1.7a	29.6 ± 2.1a
Ash (%)	12.03± 0.15a	13.02 ± 0.2a	13.2 ± 0.6a	12.5 ± 0.8a	11.9 ± 0.2a
Female brood (ovary)					
Crude protein (%)	55.0 ± 0.4ab	56.0 ± 1.1ab	57.2 ± 0.5b	57.5 ± 0.4b	57.4 ± 0.5b
Crude lipid (%)	31.2 ± 0.2a	33.2 ± 0.8b	32.8 ± 0.1ab	32.3 ± 1.7ab	31.5 ± 0.2b
Ash (%)	6.2 ± 0.2a	5.8 ± 0.1a	6.8 ± 1.0 ab	7.2 ± 0.3b	6.8 ± 0.5ab

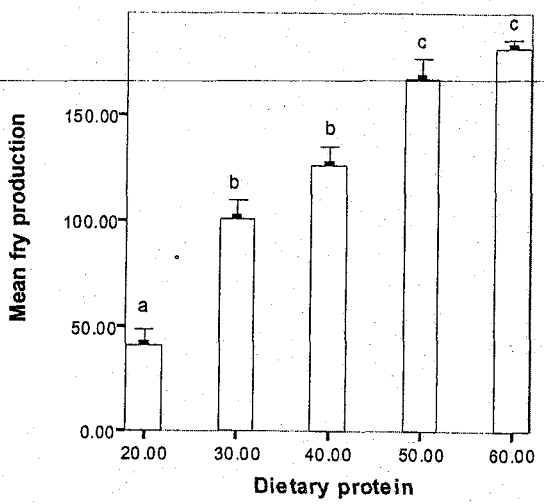


Figure. 1. Mean fry production (± S.E) of female swordtail fed different levels of dietary protein. Mean values with different alphabet are significantly different (Tukey's HSD, P < 0.05).

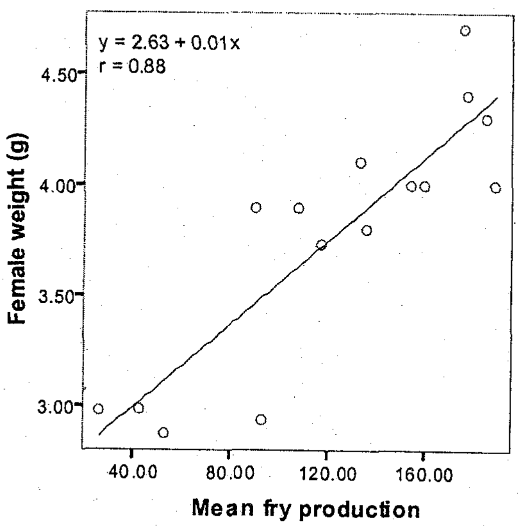


Figure. 2. Relationship between mean fry production and final weight of female swordtails.

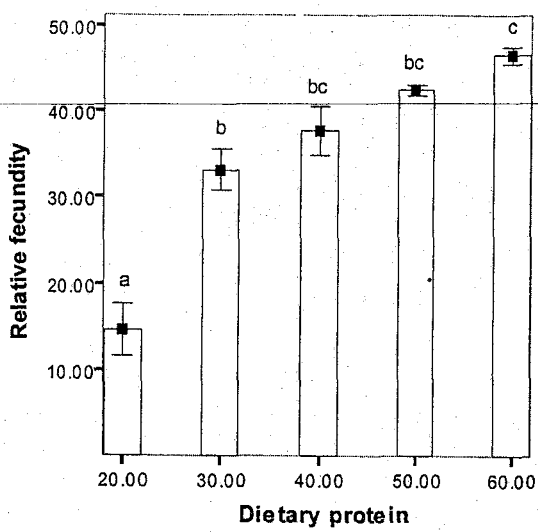


Figure 3. Relative fecundity (± S.E) of female swordtail fed different levels of dietary protein. Mean values with different alphabet are significantly different (Tukey's HSD, P < 0.05).

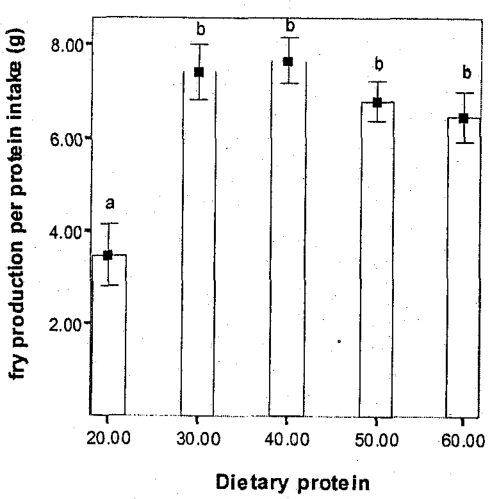
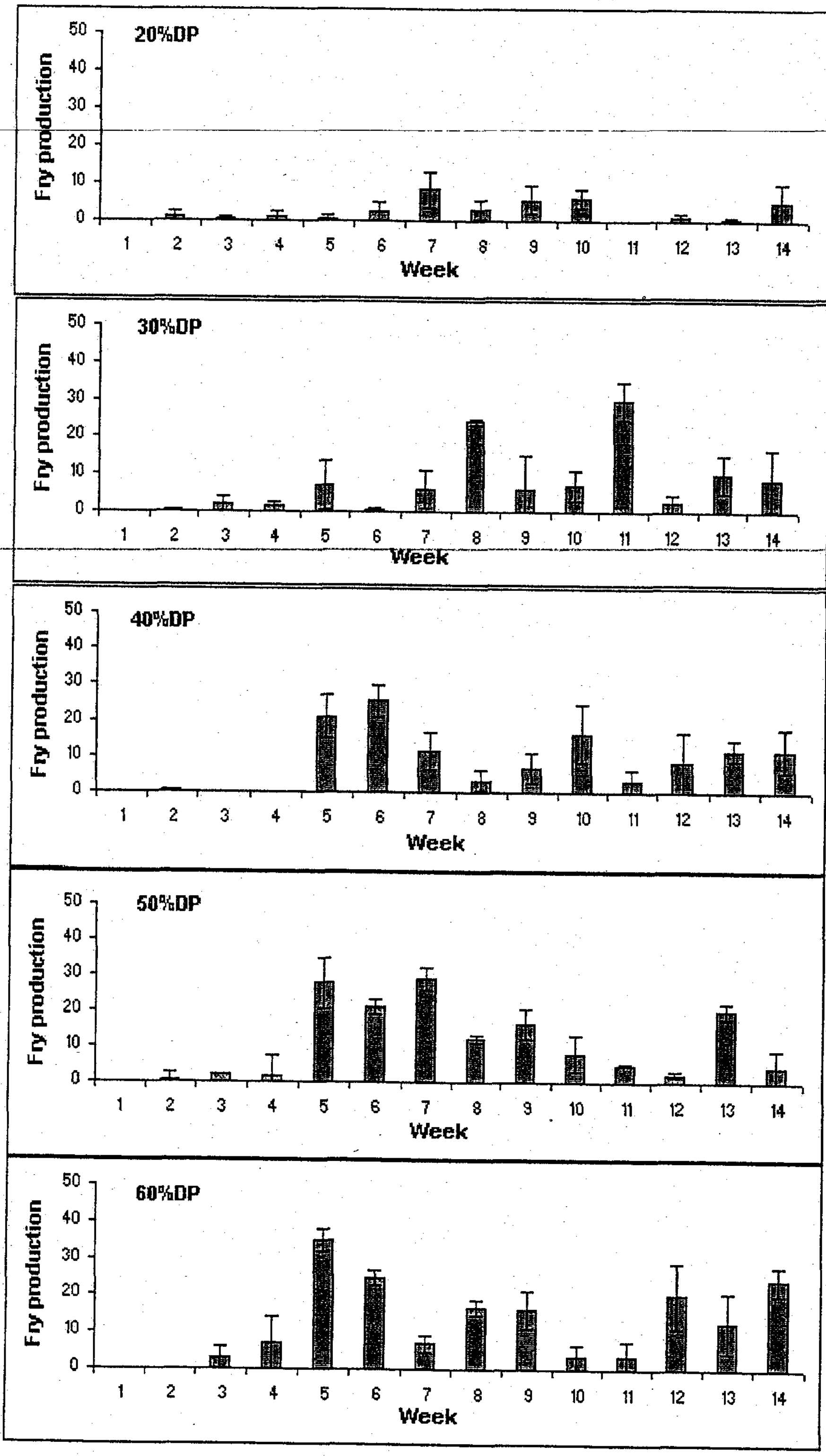


Figure 4. Mean fry production per dietary protein intake (± S.E) of female swordtail fed different levels of dietary protein. Mean values with different alphabet are significantly different (Tukey's HSD, P < 0.05).


20%DP
30%DP
40%DP
50%DP
60%DP
Fry production
Week
0 10 20 30 40 50
1 2 3 4 5 6 7 8 9 10 11 12 13 14