www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 12: 635-640 (2012) DOI: 10.4194/1303-2712-v12_3_11

Effects of Dietary Protein and Lipid Levels on Growth Performances of Two African Cichlids (*Pseudotropheus socolofi* and *Haplochromis ahli*)

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Received 21 October 2011
Accepted 24 June 2012

Abstract

Effects of experimental diets with varying protein and lipid levels on weight gain (WG), specific growth rate (SGR), survival rate (SR), feed conversion rate (FCR), hepatosomatic and viscerosomatic indices (HSI and VSI) of two popular ornamental cichlid species, omnivorous (*Pseudotropheus socolofi*) and carnivorous (*Haplochromis ahli*) were studied for 56 days. Two crude proteins (38%CP and 56%CP) and two crude lipids (9%CL and 16%CL) rates were applied to four formulated diets: R_1 (38%CP:16%CL), R_2 (38%CP:9%CL), R_3 (56%CP:16%CL) and R_4 (56%CP:9%CL). The SR was 100% in *H. ahli* while that of *P. socolofi* ranged from 66.6% to 93.3% in the two groups, respectively. The highest WG and SGR were seen in the *H. ahli* and *P. socolofi* groups fed with R_2 feed. The best FCR values were obtained in R_2 groups of *H. ahli* (1.64) and R_3 groups of *P. socolofi* (1.41). HSI values for *H. ahli* and *P. socolofi* were 2.73±0.29 to 3.25±0.34 and 3.30±0.19 to 4.02±0.09 respectively, while VSI values were 10.48±0.81 to 10.50±1.57 and 13.28±0.61 to 15.41±0.69, respectively. No significant differences were observed for HSI and VSI of *H. ahli* and *P. socolofi* (P>0.05). Our results suggest that R_2 (38%CP: 9% CL) is the most convenient feed for both species.

Keywords: Ornamental fish, feed utilization, nutrient requirement, growth.

Protein ve Yağ Seviyeleri Farklı Yemlerin İki Farklı Çiklit Türünün (*Pseudotropheus socolofi* ve *Haplochromis ahli*) Büyüme Performansları Üzerine Etkileri

Özet

Popüler süs balıklarından omnivor (*Pseudotropheus socolofi)* ve karnivor (*Haplochromis ahli*) beslenme özelliği gösteren iki farklı çiklit türünün 56 gün süreyle protein ve yağ oranları farklı deneme yemleri ile beslenmesinin ağırlık artışı, spesifik büyüme oranı (SBO), yaşama oranı, yem değerlendirme oranı (YDO), hepatosomatik indeks (HSI) ve viscerosomatic indeks (VSI) değerleri üzerine etkileri araştırılmıştır. İki farklı ham protein (%38 ve %56 HP) ve ham yağ oranı (%9 ve %16 HY) kullanılarak R_1 (%38 HP:%16 HY), R_2(%38HP:%9 HY), R_3 (%56HP:%16HY) ve R_4 (%56HP:%9HY) olmak üzere dört farklı yem hazırlanmıştır. Bu yemlerle deneme balıkları günde üç kez doyana kadar elle beslenmiştir. Deneme sonunda yaşama oranı tüm gruplarda *H. ahli* için %100 olurken, *P. socolofi* için %66,6-93,3 arasında değişiklik göstermiştir. *H. ahli* ve *P. socolofi* gruplarında en yüksek canlı ağırlık artışı ve en iyi SBO değerleri R_2 yemi ile beslenen gruplarda görülmüştür. En iyi YDO değeri ise *H. ahli* (1,64) için R_2 ve *P. socolofi* (1,41) için R_3 yemi ile beslenen gruplarda saptanmıştır. *H. ahli* ve *P. socolofi'*nin HSI değerleri sırasıyla 2,73±0,29-3,25±0,34 ve 3,30±0,19-4,02±0,09, VSI değerleri ise 10,48±0,81-10,50±1,57 ve 13,28±0,61-15,41±0,69 arasında belirlenmiş olup, gruplar arasındaki farklılık önemsiz bulunmuştur (P>0,05). Yapılan çalışmaya göre R_2 (%38 HP ve %9 HY) yeminin her iki tür için en uygun yem olduğu saptanmıştır.

Anahtar Kelimeler: Süs balığı, yemden yararlanma, besin gereksinimi, büyüme.

Introduction

Ornamental fish have become increasingly important today. One of the most obvious indicators of this is that the ornamental fish trade in the world has a volume of US $1 billion annually (FAO, 2010). Cichlids, which are among the most popular ornamental fish, constitute approximately 95% of all ornamental fish in the world, and represent about 4000 species and varieties (Güroy *et al*., 2012). African cichlid species, *Pseudotropheus socolofi* and *Haplochromis ahli* are popular cichlid representatives commercially produced for the ornamental fish market (Loiselle, 1994; Smith, 2000).

Nutrients play an important role in maintaining good health and normal behavior in enhancing the external appearance and color of ornamental fish and in the development of ornamental fish. However, there is little published information on the nutrition of ornamental fish (Kruger *et al*., 2001; Miller and Mitchell, 2008; Güroy *et al*., 2012).

The commercial feeds formulated for salmon or trout containing often 45-55% crude protein and 12-17% crude lipid have been used in ornamental fish farms (Royes *et al*., 2006). The feeds are used without attention to the life cycle and natural feeding ecology of ornamental fish. This situation, thus, causes a decrease of growth performance and economic loss in fish.

Dietary protein plays a big role in the growth performance of fish. Therefore, it is very important to determine the protein requirement of fish. Another important issue is the high cost of the ornamental fish feeds. The biggest part of the cost of the unit of feed is composed of protein. The inclusion of protein in feed is of great importance for the growth and maintenance of fish while fat and carbohydrates are important to supply the energy needed by fish.

Many studies have shown that protein content in the diet can be reduced without decreasing growth if the caloric content of the diet is increased by manipulating the level of non-protein ingredients, such as carbohydrates and lipids (Winfree and Stickney, 1981; El-Sayed and Tashima, 1991; Royes *et al*., 2006).

On the other hand, the accumulation of lipid in the liver should be avoided. An excessive lipid and total energy level in the diet may result in liver damage (Ferguson, 1989). Therefore, the protein and non-protein energy in the feeds must be balanced (Winfree and Stickney, 1981).

In this study, the aim was to establish optimal protein and lipid levels without causing any negative effect on the best growth performance and health of omnivorous *P. socolofi* and carnivorous *H. ahli*. Effects of different protein and lipid levels in diets on growth performance, feed utilization, hepatosomatic and viscerosomatic indices of the two different cichlids were also investigated.

Materials and Methods

Experimental Diets

The combinations of two crude proteins (38%CP and 56%CP) and two crude lipids (9%CL and 16%CL) were used to formulate four different feeds i.e. R_1 (38%CP:16%CL), R_2 (38%CP:9%CL), R_3 (56%CP:16%CL) and R_4 (56%CP:9%CL). The formulation and proximate composition of experimental diets are given in Table 1. The feed ingredients were mixed in a food mixer for 30 minutes and 500 ml/kg of water maximum was added. The obtained mixture was pelleted to 1 mm diameter pellets, which were dried afterwards in an oven at 70°C, sealed in plastic bags and kept at -20°C until usage.

Experimental Conditions, Fish and Feeding

The experiment was carried out at the aquarium unit of Mugla University, Fisheries Programme of Ortaca Vocational School for eight continuous weeks. 240 *P. socolofi* individuals (with an average weight of 1.16 g) and 192 *H. ahli* (with an average weight of 1.68 g) were used. The experiment was planned in triplicate: 24 glass aquaria with 80x40x40 cm dimensions were stocked reserving 1.5 L water for each 1 cm of fish. Tap water was used in the aquaria. Fish were fed a control diet for one week for

Table 1. Feed formulation and proximate composition of experimental diets (n=3)

Ingredients (%)	*Experimental groups*			
	R_1	R_2	R_3	R_4
Fish meal (68.53% CP)	25.00	25.00	25.00	25.00
Sodium caseinate (89.60% CP)	20.00	20.00	40.00	40.00
Wheat starch	27.00	33.00	7.00	13.00
Maize gluten (61.28% CP)	3.00	3.00	3.00	3.00
Wheat flour	10.00	10.00	10.00	10.00
Sunflower oil	12.00	6.00	12.00	6.00
Vitamin[1]	1.0	1.0	1.0	1.0
Mineral[2]	1.0	1.0	1.0	1.0
Stay-C[3]	1.0	1.0	1.0	1.0
Proximate composition (%, on wet wt)				
Moisture	5.61	5.80	6.23	6.34
Crude protein	38.46	38.46	56.38	56.38
Crude lipid	15.44	9.53	15.84	9.53
Ash	5.74	5.92	5.74	5.92
Digestible energy (kJ/g feed) [4]	18.35	16.77	20.45	18.87

[1] Vitamin premix (Vit-A 4 000 000 IU/kg, Vit-D3 400 000 IU/kg, Vit-E 40 000 mg/kg, Vit-K 2 400 mg/kg, Vit-B1 4 000 mg/kg, Vit-B2 6 000 mg/kg, Niasin 40 000 mg/kg, Cal-D-Pantothenate 10.000 mg/kg, Vit-B6 4 000 mg/kg, Vit-B12 10 mg/kg, D-Biotin 100 mg/kg, Folic acid 1200 mg/kg, Vit C (Stay C) 40 000 mg/kg, Inositol 60 000 mg/kg)

[2] Mineral premix (Manganese 60 000 mg/kg, Iron 60 000 mg/kg, Zinc 80 000 mg/kg, Copper 5 000 mg/kg, Cobalt 200 mg/kg, Iodine 1 000 mg/kg, Selenium 150 mg/kg, Magnesium 80 000 mg/kg)

[3] Stay C : 40 000 mg/kg

[4] Estimated using values of protein: 20.9 kJ/g; lipid 37.7 kJ/g and carbohydrate:14.6 kJ/g (NRC, 1993).

acclimatization to the experimental conditions before initiation of the feeding trial. Tanks were randomly assigned to one of four diets. Fish in each tank were hand-fed so that they would consume in 20 min; such feeding was administered three times per day (08:30, 12:30 and 16:30). Records were kept of daily feed consumption. About 20% of the total water volume of the aquarium was exchanged and faeces and food waste extracted by siphoning every day.

Water temperature regulation in the experimental aquaria was maintained by means of an automatic heater (200-W). Aeration was provided by air stones (pad filtered). Measurement of water temperature was conducted with digital thermometers, pH with a WTW 315i pH meter, oxygen with a WTW 315i oxygen meter, while ammonia, nitrite and nitrate were measured with a Hanna C203 photometer. Live weight of experimental fish was obtained by means of a Scaltec digital balance with an accuracy of 0.01 g; total length was measured with fish measuring boards with 1 mm divisions. The photoperiod was maintained at 12 h light/12 h dark using a fluorescent lighting source (Gümüş *et al*., 2011).

Growth Parameters and Calculations

All fish from each replicate were individually measured and weighed at the beginning and every two weeks until the end of the experiment. Growth performance of African cichlids fed with the varying protein and lipid diets were evaluated through calculation of weight gain, feed conversion rate, specific growth rate and survival rate (Gümüş, 2011).

Weight gain (WG, g) = Final body weight (g) – Initial body weight (g)

Feed conversion rate (FCR) = Total amount of consumed feed (g) / weight gain (g)

Specific growth rate (SGR, %day^{-1}) = (Ln final body weight-Ln initial body weight)/days x 100

Survival rate (SR, %) = (Final fish number/Initial fish number) x 100.

A second order polynomial regression model between SGR and protein/lipid level was used for determination of protein/lipid level required for maximum growth (Chong *et al*., 2000).

At the end of the experiment, a random sample of the fish (n=3) from each aquarium was weighed, and then their internal organs and livers were removed and weighed to calculate hepatosomatic and viscerosomatic indices values (Gümüş, 2011).

Hepatosomatic indices (HSI, %) = (wet liver weight/ final body weight) x 100

Viscerosomatic indices (VSI, %) = (visceral weight/ final body weight) x 100

Chemical and Statistical Analyses

Experimental feed and food ingredient analyses (dry matter, crude protein, crude lipid, and ash) were conducted via standard analysis methods (AOAC, 1990). The results are mean values (means±SD) of three replicates. Output data were evaluated using the SPSS 11.0 package program. The data were tested with variance analysis (ANOVA); Duncan's test was applied in comparison of group medians. P<0.05 was selected for determination of significance (Steel *et al*., 1996).

Results and Discussion

Water quality parameters in the aquaria were measured throughout the experiment. The water temperature ranged from 25 to 27°C, dissolved oxygen from 6.50 to 7.10 mg/L and the pH from 7.7 to 7.9. The ammonia, nitrite, and nitrate were determined as 0.17 mg/L, 0.03 mg/l, and 3.96 mg/L, respectively. Water quality parameters remained within acceptable ranges for the experimental fish (Loiselle, 1994).

At the end of 8 weeks of the feeding trial, WG, FC, FCR, SGR and SR of the *H. ahli* and *P. socolofi* juveniles are given in Table 2 and 3. FC values among the *P. socolofi* trial groups were insignificantly different (P>0.05). High-protein diets by *H. ahli* juveniles was consumed less. At the end of the experiment, SR for *P. socolofi* and *H. ahli* was determined to be between 66 to 93% and 100%, respectively (P>0.05).

This study indicates that 38% dietary protein and 9% dietary lipid levels were the optimal level for weight gain in juvenile African cichlids (*P. socolofi and H. ahli*) (Figure 1 and 2). The optimum dietary protein and lipid levels for *P. socolofi* and *H. ahli* juveniles determined by this study were lower than Royes *et al*. (2006), Kruger *et al*. (2001) and Chong *et al*. (2000). Royes *et al*. (2006) reported that 55% dietary protein and 19% dietary lipid levels for juveniles of omnivorous *P. socolofi* and *H. ahli* shown the best growth performance, whereas there were no significant differences between these levels for *H. ahli* and *P. socolofi*. A protein level of 45% at a low concentration of lipid 6% would provide the best growth rate in juvenile swordtails (*X. helleri*) (Kruger *et al*., 2001) and discus (*Symphsodon* spp.) (Chong *et al*., 2000). The present study indicated that the results ar

The highest SGR values for *P. socolofi* and *H. ahli* were obtained from the R_2 diet. In a previous study, in which the highest growth performance was obtained with 40% protein, SGR for electric yellow cichlid (*L. caeruleus*) was determined as 1.81 %day^{-1} (Ergün *et al*., 2010) being similar to those obtained in the present study.

Polynomial regression analyses relating to SGR are shown in Figure 3. Polynomial regression equations for *P. socolofi* (y = 0.0783 x^2

Table 2. Growth performance of juvenile *P. socolofi* for 56 days (means±SD; n=3)

Parameters	Experimental groups			
	R_1	R_2	R_3	R_4
Initial weight	1.18±0.02	1.15±0.01	1.17±0.02	1.16±0.04
Final weight (g)	2.57±0.28	3.13±0.16	2.96±0.21	3.10±0.19
Weight gain (g)	1.38±0.28	1.98±0.15	1.78±0.21	1.94±0.17
FC (g)[1]	2.45±0.28	2.89±0.11	2.48±0.19	2.95±0.14
FCR [2]	1.83±0.15^{b}	1.46±0.07^{a}	1.41±0.09^{a}	1.53±0.07^{ab}
SGR (%day^{-1}) [3]	1.36±0.19	1.79±0.08	1.64±0.13	1.75±0.09
Survival rate (%)	66.66±22.05	93.33±1.67	80.00±2.89	90.00±5.00

Different letters in the same column indicate significance ($P<0.05$)
[1] Feed consumption; [2] Feed conversion rate; [3] Specific growth rate

Table 3. Growth performance of juvenile *H. ahli* for 56 days (means±SD; n=3)

Parameters	Experimental groups			
	R_1	R_2	R_3	R_4
Initial weight	1.70±0.05	1.69±0.08	1.68±0.01	1.65±0.02
Final weight (g)	3.56±0.14^{bc}	4.23±0.23^{a}	3.75±0.13^{ab}	3.13±0.16^{c}
Weight gain (g)	1.86±0.10^{bc}	2.54±0.25^{a}	2.07±0.13^{ab}	1.48±0.15^{c}
FC (g)[1]	1.61±0.03^{ab}	1.86±0.09^{a}	1.58±0.08^{ab}	1.48±0.12^{b}
FCR [2]	1.94±0.10^{ab}	1.64±0.07^{a}	1.72±0.20^{a}	2.23±0.15^{b}
SGR (%day^{-1}) [3]	1.32±0.04^{bc}	1.63±0.12^{a}	1.43±0.06^{ab}	1.14±0.08^{c}
Survival rate (%)	100.0±0.00	100.0±0.00	100.0±0.00	100.0±.00

Different letters in the same column indicate significance ($P<0.05$)
[1] Feed consumption; [2] Feed conversion rate; [3] Specific growth rate

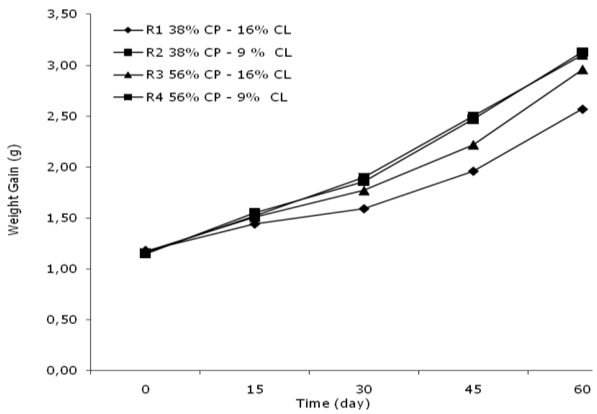

Figure 1. Periodic weight gain of juvenile *P. socolofi*.

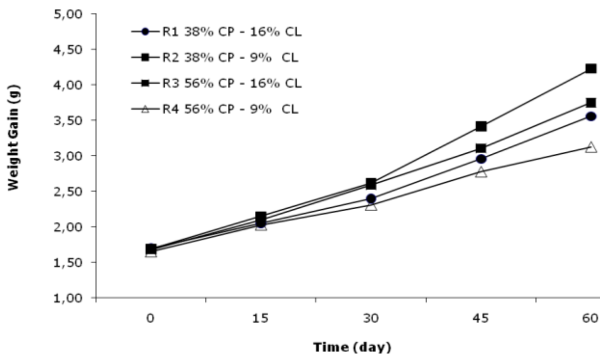

Figure 2. Periodic weight gain of juvenile *H. ahli*.

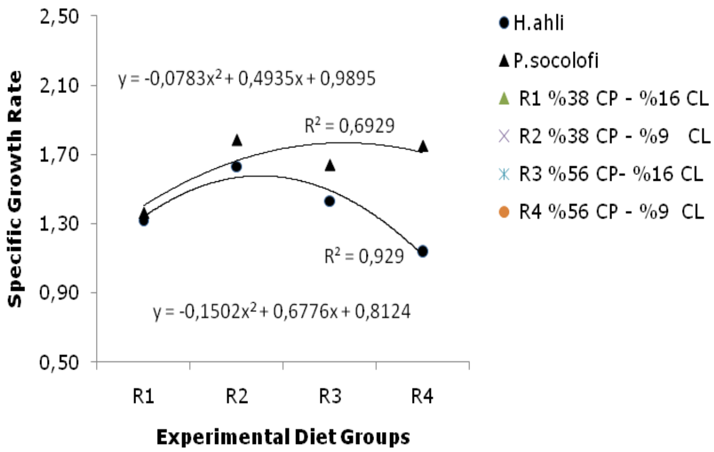

Figure 3. Effects of dietary protein-lipid level on the specific growth rate (SGR) of juveniles *P. socolofi* and *H. ahli*.

+0.4935x+0.9895, r^2=0.6929) and for *H. ahli* (y= -0.1502x^2 +0.6776x+0.8124, r^2 =0.929) were fitted to describe the effects of dietary protein-lipid levels on the SGR of *P. socolofi* and *H. ahli* juveniles. The polynomial regression analyses of the SGR in our study show that diet R2 (38%CP and 9%CL) was the optimum diet level for the best growth in both species of fish, when the dietary energy value was 16.77 kJ/g diet. In earlier studies, polynomial regression analyses have been applied to determine the optimum protein level for discus (*Symphsodon* spp.) (Chong *et al*., 2000) and blue streak hap (*Labidochromis caeruleus)* (Ergün *et al*., 2010).

The best FCR for *P. socolofi* was obtained with diet R_3 (1.41) and for *H. ahli* with diet R_2 (1.64), but no significant differences were found between R_2 and R_3 diets (P>0.05). FCR value for *P. scolofi* was stated as 2.75-3.35 by Royes *et al*. (2005), while it was found to be 2.09-2.58 and 2.2-3.8 for freshwater cichlids angelfish (*Pterophyllum scalare*) by Zuanon *et al*. (2006) and discus (*Symphysodon* sp.) by Chong *et al*. (2000), respectively. In this study, FCR values were found to be better than those of other studied cichlid species. These findings also indicate that both species have converted feeds relatively well when fed with diet R_2.

Considered an important indicator for health, HSI and VSI values in both fish fed with diets including high lipid levels are relatively higher compared to other groups, but this difference is insignificant (P>0.05, Table 4). The highest HSI values were observed in groups fed with high lipid level feeds but differences among the other groups were not significant. R_1 feed provided growth without any negative effects on liver. The findings are similar to those of Chong *et al*. (2003) on discus (*Symphysodon aequifasciata*) and Zhou *et al*. (2005) on cobia (*Rachycentron canadum*). Royes *et al*. (2006) determined a higher HSI rate in groups fed with low lipid feeds, noting that differences between groups were insignificant. They also stated that carnivorous *H. ahli* was more tolerant than *P. socolofi* to a higher lipid rate in feed, but lipid content higher than 10% could lead to fattening of liver and internal organs. A similar result for striped bass (*Morone chrysops x Morone saxatilis*) fed with feed having a 16% lipid rate was found (Gallagher, 1996).

As perfect energy sources, lipids make better energy sources when compared to proteins and carbohydrates (Robinson and Wilson, 1985; Barrows and Hardy, 2001). In many species, depending on the feed protein level, lipids largely provide a sparing effect in protein (Wilson, 1989). But a high protein and lipid combination in feeds often leads to fattening in liver and visceral tissue (Stoskopf, 1993). In this study, optimal weight gain and feed conversion rates were obtained with R_2 feed and the health of fish in experimental groups was not affected negatively as evidenced by hepatosomatic and viscerosomatic indices. Studies on protein-lipid requirements of ornamental fish should be carried out on different species. Determination of optimal feed requirements of ornamental fish can economically improve farming of popular species and lead to termination of the collection of ornamental fish from the wild.

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Table 4. Hepatosomatic and viscerosomatic indices of juveniles *P.socolofi and H. ahli* for 56 days (means±SD; n=3).

	HSI (%)		VSI (%)	
Experimental groups	*P. socolofi*	*H. ahli*	*P. socolofi*	*H. ahli*
R_1	4.02±0.09	3.25±0.34	15.41±0.69	10.50±1.57
R_2	3.14±0.53	2.80±0.29	14.89±0.76	10.48±0.48
R_3	3.93±0.17	3.19±0.45	14.32±0.73	11.15±1.19
R_4	3.30±0.19	2.73±0.29	13.28±0.61	10.48±0.81

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