Effects of different protein sources on growth performance and food consumption of goldfish, *Carassius auratus*

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Received: February 2012 Accepted: August 2013

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Keywords: Aquarium, Goldfish, *Carassius auratus*, Food consumption, Growth performance

Protein is one of the most important components in a fish diet (De Silva and Andersoni, 1995; Kaushik, 1995). In aquaculture, the primary dietary animal protein source is fish meal, but its availability is limited and supply varies because of reductions in fish stocks related to factors such as climatic phenomena, and overexploitation and decline of ocean fisheries stocks. This variability can seriously affect aquaculture sustainability and provability, and therefore research in identifying alternative dietary protein sources has increased (Kissil et al., 2000; Maylor et al., 2000). Fish nutritionists have tried to use less expensive plant protein sources to partially or totally replace fish meal (Xie et al., 2000). In the context of research on substitutes for fish meal in diets of fish, numerous studies have been made in the past decades (Kaushik et al., 1995; Mambrini et al., 1999).

The market for ornamental fish in Turkey is increasing. To cater to the demand of quality feed for these species, a number of commercial feeds are available in the market. Feed formulation for goldfish is yet to be standardized. As a common practice in Turkey, commercial production of aquarium fish depends essentially on pelleted/granular form of fish feed.

Goldfish, *Carassius auratus* are the most popular choice of fish for a water garden. It can be found in a number of varieties and colors. This species reaches sexual maturity at a small size and the artificial insemination of goldfish eggs and rearing of larvae is comparatively easy (Wiegand et al., 1989). Therefore the goldfish provides a useful model for the study of nutrition in larval and juvenile Cyprinids. Some initial studies have been made on the nutritional requirements of warm water omnivore (Sales and Janssens, 2003).

The purpose of this study was to investigate food consumption and growth performance of goldfish fed diets containing only plant, only animal and plant-animal (50%-50%) derived protein source.

In the study, goldfish fingerlings were obtained from Kepez Research Institute of Aquatic Resource (Antalya/Turkey). The fish were acclimatized to experimental conditions for a period of two weeks before the start of the experiment. During this period, they were fed on a practical diet (Pinar Feed, 45% crude protein, 18% lipid) to satiation twice a day. In the trial, 81 fishes were divided into 9 aquariums (60 L for each aquarium) for the application of experimental diets. The aquariums were

aerated and filtrated with GAP II filter. Water temperature was 25 °C and pH 7.3 was around. The fish were exposed to normal photoperiod – 9 Light: 15 Dark for 8 weeks. Each was weighted (average weight of 4.8 ±0.05 g) at the beginning of the experiment. During feeding trial, fish were fed to satiation twice a day (at 08:30 and 15:30 h). The daily feed supplied was recorded and unconsumed feed were collected 20 min after feeding, and dried. The trial lasted for 8 weeks. At the end of the trial, all fish were weighted individually. Five fish from each tank were taken for proximate analysis. Chemical composition and formulation of three experimental diets are given in Table 1.

Table 1: Diet formulation and chemical composition of experimental diet (% of dry wieght)

Protein source	Diet		
	Plant	Animal	Plant-Animal
Wheat Starch	0	35	17
Corn Gluten Meal	29.45	0	15.00
Fish Meal	0	52	26
Wheat Meal	14.97	0	7.50
Soybean Meal	29.45	0	15.00
Sunflower Meal	14.97	0	7.50
Soybean Oil	4.99	0	6.00
[1]Vit-Min Premix	2.99	3	3.00
Binder	2.99	3	3.00
Fish Oil	0	0	7
Lysin	0.17	0	0.00
Crude Moisture	4.04±0.50	4.04±0.07	3.66±0.18
Crude Protein	36.32±0.24	37.08±0.34	36.05±0.13
Crude Lipid	10.67±0.40	11.63±0.50	10.69±0.60
Crude Ash	6.50±0.28	8.41±0.39	8.41±0.39
Gross Energy (MJ/kg)	20.82	20.75	20.48

[1] Vit-Min Premix (mg kg ^{1} , NRC 1977): vitamin A, 5500IU; vitamin D_3, 1000 IU; vitamin E, 50 IU; vitamin K, 10 mg; choline, 550 mg; niacin, 100mg riboflavin 20mg; pyridoxine, 20 mg; thiamine 20mg; biotin, 0,1mg; folacin, 5mg; B_{12}, 20μg; inositol, 100 mg; choline chloride, 5000 mg. Mineral premix (mg kg $^{-1}$ diets, H440): NaCl, 1,0; $MgSO_4$, 7; NaH_2PO_4 25; KIO_3 0,0003; $ZnSO_4$ 0,353; $MnSO_4$0,162.

Practical diets with three dietary animal-plant protein source ratios 1:0, 0:1 and 1:1 (animal, A; plant, P and animal-plant, A-P) were formulated. Dry ingredients were blended to make a homogenous mixture and were mixed with boiled water. After moderate cooling, pellets (1 mm size) were prepared by over-dried at 40 °C and stored at 4 °C.

Moisture, crude protein, lipid and ash of the experimental feed and fish were determined by methods as described in A.O.A.C. (1995) methods. The moisture content was analyzed by drying in a hot air oven at 105 °C. Crude protein was estimated by the Kjeldahl method and crude lipid was estimated by soxhlet extraction with petroleum ether. Ash contents were determined from the residue remaining after incineration of the samples at 550 °C in a muffle furnace. Dietary gross energy was calculated using the conversion factors of 23.7 kJ g for protein,

39.5 kJ g for lipid and 17.2 kJ g 1 for carbohydrate (Brett and Groves, 1979).

The growth parameters were calculated as follows: Specific Growth Rate (SGR) (% day^{-1}) = 100 x [(ln final fish weight) – (ln initial fish weight)] / days fed. Feed Intake (FI) (%) = daily feed intake (g) x 100 / biomass (g). Feed Conversion Ratio (FCR) = feed intake (g) / weight gain (g) x 100. Protein Efficiency Ratio (PER) = wet body mass gain/crude protein intake. Analyses were conducted using one-way ANOVA. For multiples comparisons of the means Duncan's multiple range was used (Zar, 1999). All the results were treated significant at the 5% level. The results of the study have been shown in Table 2.

Table 2: Growth performance, feed and nutrient utilization of goldfish fed the experimental diets

	A	P	P-A	±SEM*
Survival (%)	100	100	100	-
Initial Mean Weight (g)	4.8	4.8	4.8	0.05
Final Mean Weight (g)	10.1^{c}	6.4^{a}	8.6^{b}	0.54
Feed Intake (%)	2.57^{a}	2.34^{b}	2.21^{b}	0.05
Weight Gain (%)	110.4^{c}	33.3^{a}	79.2^{b}	1.05
Feed Conversion Ratio (%)	2.9^{a}	5.7^{b}	3.0^{a}	0.29
Specific Growth Ratio (%)	1.2^{c}	0.5^{b}	1.0^{a}	0.04
Protein Efficiency Ratio (%)	0.9^{a}	0.5^{b}	0.9^{a}	0.47

* Values in each row allocated common superscripts or without superscripts are not significantly different from each other (P > 0.05).

There were no statistically significant differences in survival rate (P>0.05) among the dietary treatments. Live weight gain and SGR significantly higher (P>0.05) for diet A than diets P and A-P. The mean feed intake of fish fed with diet A was significantly (P<0.05) higher than that of P and A-P fed fish. The FCR of P treatment was found highest (P>0.05), but there were not significant differences between A and A-P groups (P>0.05). The protein efficiency ratio of A and A-P groups approximately 80% higher than that of fish fed with the P diet. The proximate compositions of the fish carcasses at the end of the trial are given in Table 3. There were no significant differences (P>0.05) between groups in crude lipid. However, dry matter of A and A-P group were significantly lower than P group. In terms of crude protein, P and A-P were similar (P>0.05), but A was higher than the other groups (P<0.05). Ash content of P and A were established similar and lower than A-P (P<0.05).

Table 3: Proximate analysis of goldfish fed different protein sources

Grups	Dry Matter	Crude Protein	Crude Lipid	Ash
P	69.74 ± 3.77^{a}	40.92 ± 1.4^{a}	$37.15\pm-.68^{a}$	$2.72\pm-.52^{a}$
A	65.18 ± 1.31^{b}	$43.16\pm-.31^{b}$	34.79 ± 1.21^{a}	$2.65\pm-.22^{a}$
A -P	63.63 ± 1.34^{b}	39.56 ± 3.26^{a}	$35.77\pm-.95^{a}$	$3.53\pm-.07^{b}$

Values are in percentage wet weight. A: Animal source; P: Plant source; A-V: Animal and Plant source (% 50-50). Means with different superscripts (a, b, c) are significantly different from each other (P<0.05).

In most studies, decreased growth was observed in fish when a large proportion of the fish meal was replaced by plant proteins (Fournier et al., 2004; Espe et al., 2006, Palti et al., 2006). Factors suggested to be the causes for decreased growth in fish fed plant protein include low palatability (Mohsen and Lovell, 1990), deficiency amino acid such as methionin and lysine (Dabrowski et al., 1989; Tantikitti and Chimsung, 2001), poor utilization of protein (Boonyaratpalin et al., 1998), and low energy (Opstvedt et al., 2003). Generally, it has been examined that a partial replacement of fish meal by plant protein source is well supported by fish, so currently more than 30% is already replaced in most commercial fish feeds (Forster et al., 1999; Burel et al., 2000; Vielma et al., 2000; Opstvedt et al., 2003). The goldfish in the experimental are omnivore species but adult goldfish prefer to eat plant; the young ones may require a greater amount of protein in the diet for optimum growth. The inclusion of fish meal in the diet of young goldfish, with a maximum digestibility of 93%, could promote their growth of young goldfish (Degani et al., 1997; Bandyopadhyay et al., 2005). Growth in terms of weight gain (WG) and specific growth rate (SGR) were highest in fish fed with animal protein and lowest in fish fed with plant protein diet (Table 2) during the study period ($P<0.05$). The low-energy digestibility of plant feed ingredients can be attributed to their high-carbohydrate content and poor digestibility by carnivorous fish (Lupatsch et al., 1997). However, Abi-Ayad and Kestemont (1994) reported that the growth performance were highest on *Carassius auratus gibelio* when the fish fed feed animal protein, that results also similarly to our results. In the present study, total feed intake was lower in goldfish fed the plant and animal-plant diet than in the animal diet. Goldfish fed the animal diet and animal-plant diets had lower FCR than plant diet. The lower feed intake and highest FCR in plant diet fed fish were probably mainly caused by the existence of anti-nutritional factors and\or low palatability (Watanabe et al., 1992). Decreased growth of fish fed the diet with the inclusion of plant was reported to be due to low feed intake (Higgs et al., 1982), low digestibility of dry matter and/or protein (Hilton and Slinger, 1986), unbalanced amino acids and/or high fiber contents (Appler and Jauncey, 1983). In conclusion, the present study showed that inferior growth of fish fed animal protein was caused by different factors in goldfish fed diets with different protein contents.

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