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Effects of dietary protein levels on the growth of *Luciobarbus xanthopterus* juveniles (Heckel, 1843)

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Abstract - The aim of the present study is to investigate the effect of four fish dietary protein levels (30%, 35%, 40% and 45%) on the growth of *Luciobarbus xanthopterus* juveniles. About 240 fish of average weight of 18.4 g were kept in eight small letter tanks for 8 weeks at the Marine Science Center aquaculture room. Two replicates for each treatment with 30 fish for each replicate were used. A number of growth parameters were estimated during the experiment. The environmental factors measured were; water temperature, DO, salinity and pH. The highest average weight gained was 21.14 g ± 0.33 with the 40% dietary protein level, while the lowest average weight was 19.68 g ± 0.50. The highest weight gained was recorded at 40% dietary protein level. The highest weight gain and weight increase were significantly different ($p<0.05$) at 40% dietary protein level. Their values were 21.14 g ± 0.33 and 2.11 ± 0.06, respectively. The lowest weight gain and weight increase were at 45% protein level, with values of 19.68 g ± 0.50 and 1.09 ± 0.05, respectively. The weight increase was 2.11 ± 0.06. Significant differences ($p<0.05$) have been noticed in this protein level, however, the other dietary levels showed no significant increase. The highest rate of daily weight increase were recorded at the 30%, 35% and 40% levels and the significant differences ($p<0.05$) among these levels were in apparent. However, the weight increase rate of 2.12 was dropped at 45% level to 1.41 ± 0.033. The highest rate of specific growth was recorded at the 35% protein level with value of 0.31 g/day with no significant differences ($p<0.05$) between the various levels. Finally, it could be concluded from this study that the lowest specific growth 5.64% was recorded at the 45% protein level and the highest relative growth 11.06% was recorded at the dietary protein level of 35%.

Key words: Dietary, protein, level, growth, *Luciobarbus xanthopterus*.

Introduction

Protein is the most important source in fish nutrition since it affects the growth performance of fish and the production cost; in other words, increasing protein intake will improve fish production (Lovell, 1989). In order to know the nutritional requirements of a certain fish species, the necessary nutrients (needed for growth and performance of life activities) should be studied and estimated (Luo *et al.*, 2004; Lee *et al.*, 2006). Any increase in dietary protein intake will be metabolized [to generate energy] and produced as nitrogen excretion in water environment (Catacutan & Coloso, 1995; Tibbetis *et al.,* 2002), thus, it is important to know the optimum protein requirements so as to make balanced, cost-effective and environmental-friendly fish feed. Numerous studies have been carried out to study protein requirements for many fish species used in commercial farming, for instance: Carp, *Cyprinus carpio* (Ogino & Saito, 1970); grass carp, *Ctenophryngodon idela* (Dobrowski, 1977); *Tilapia aureus* (Davis and Stickney, 1978*); Luciobarbus* (Al-Shamma'a *et al.,* 1998) and the binni, *Mesopotmichthys sharpeyi* (Ysser *et al*., 2009). Furthermore, their broad-spectrum nutrition makes it easy for these fishes to feed on artificial food (Al-Rudainy *et al.,* 2004).

On the other hand, Al-Mahdawi *et al.* (1996) was able to get good growth rates when culturing these fishes in an earthen pond. It is, therefore, possible to have *L. barbus* cultured. Ideal nutrition for farm fishes is the economic and healthy way to have good breeding in terms of quantity and quality, and is one of the main and critical factors on which culture is dependent as it forms a big rate of cost (Craig & Helfrich, 2002). Moreover, Farnar (2010) was study the able to natural nutrition of larve cultured in two different environments.

Studying of the digestive system of any fish species gives initial impression of the food requirements of the species in question, and *L. barbus*, in particular (Al-Hamed, 1967). However, Al-Kanaani (1989) found that animal ingredients are high in the food of the fish and form 82.9%, while Polservice (1985) concluded that yellow fin barbells feed on broad-spectrum food. In general, *L. barbus* is regarded as mix-feed in the early stages and tend to animal feeding in advanced stages of their life.

However *L. Xanthopterus* has been targeted by a number of local studies which investigated some of their life aspects (Al-Rudainy *et al*., 1997; Al-Rudainy *et al*., 2001), their growth and feeding in various environments (Al-Rudainy *et al*., 2004; Ghazi, 2009) and fertility (Al-Mukhtar, 2008; Al-Mukhtar *et al*., 2009 and Farnar, 2014). In addition, yellow fin barbels' salinity adaptation has also been studied by Salman *et al.* (1993) and Al-Azawi *et al.* (1999). Growth rate is one of the most common standards in assessing validity of fish feed and its protein levels (Youssef and Abdulsame'a, 1996). A number of studies referred that yellow fin barbels are a local species that have the ability to grow, especially if they are farmed in earthen ponds. Al-Rudainy *et al.* (2001) mentioned that *L. barbus* farmed in earthen pond grew better. Farnar (2010) concluded that environmental factors and type of culture had a clear effect on the growth rates.

The aim of the present study is to determine nutritional and protein requirements of the yellow fin barbels and the reason for adoption of four protein levels.

Materials and Methods

L. Xanthopterus were obtained from artificial breeding of fish at the MSC hatchery. The average weight of the juveniles fishes was 18.45 g ± 4.93, and they were distributed to 8 containers of 250L capacity. Thirty juveniles of *L. xanthopterus* were placed in each container and two replicates for each treatment were performed. The total weight of each fish was estimated at the beginning of the experiment, the containers were supplied with water of 16-20 °C using the hatchery boilers fitted with thermostat regulator to control the water temperature and the enclosure water was continuously replaced without affecting water temperature. Environmental factor such as dissolved oxygen, salinity, pH and temperature were measured weekly by YASI instrument.

The juvenile fishes were starved for five days then they were fed on experimental diet protein levels of 30, 35, 40 and 45 % (Table 1) at a feeding rate of 5% of their weight throughout the period of the experiment. The fishes were weighed every 10 days by Stanton electric scale to the nearest 0.1 g. The fish were fed twice a day. A number of growth parameters were measured depending on Jobling (1993).

Total weight gain WG/ g= Final weight (g)– initial weight (g)
Daily growth g/d= Final weight – initial weight / period of experiment (days)
Relative growth (%) (RGR)= [Final weight (g) / initial weight (g)] X 100
Specific growth rate (SGR) (%) (g/ d)= [Ln Final weight- Ln initial weight/ period of experiment (days)]X100.

Standard methods were followed in estimating the percentage of moisture, protein, fat and ash for the feed (A.O.A.C., 1990), and the carbohydrates were mathematically calculated from the following equation:
Carbohydrates = 100–(moisture % + protein % + fat % + fibers % + ash %) (Al-Aswad, 2000).

Completely Randomized Design (CRD) was applied and the significant differences were compared at a ($p<0.05$), (Al-Rawi and Khalaf-Allah, 2000), using SPSS computing software (SPSS, 2000). Table (1) shows the composition of the four different feed used in the experiment.

Table 1. Percentage of the ingredients of *L. xanthopterus* feed used in the experiment.

Ingredients	Percentage%			
Imported protein level	20	30	36	41
Soy bean	28	29	35	42
Yellow corn	25	19	13	8
Wheat bran	25	20	14	7
Vitamins and minerals	2	2	2	2
Total	100	100	100	100

Results

Table (2) shows the chemical composition for the diet introduced to *L. Xanthopterus*. Table (3) shows the data of some environmental factors during the experiment throughout four weeks. Temperatures ranged from 16°C to 20°C, the dissolved oxygen rate ranged from 7.1 to 7.6 mg/l., salinity ranged from 5.0 to 5.2‰ and pH ranged from 8.1 to 7.6. Actual moisture in feed 1 and 2 was less than the calculated; they were 1.60% and 3.49% instead of 6.88% and 6.44%, respectively, while the rate was increased in feed 4 (6.83%) instead of 5.43% in the calculated composition (Table 4). As for feed 3, the actual and calculated composition rates were close to each other, so as the actual and calculated rates of protein levels; however, fat content was clearly higher in the calculated, while the ash level was relatively high in the actual composition. Carbohydrates was remarkably higher than the calculated composition, and total energy was higher than the calculated in all feeds (Table 4).

Table (5) shows the final growth performance of *L. xanthopterus* fed on different protein levels. Fish fed on the 40% dietary protein level had the final weight of 21.1 g, while those fed on the 30% dietary protein level had the lowest average weight 19.68 g ± 0.50. The results of the statistical analysis indicate a significant difference ($p<0.05$) at protein level of 40%. However, the increase was 2.10 ± 0.06 g. Furthermore, the juveniles developed a daily growth on different protein levels. The highest daily rate of weight gain rate was achieved in the protein levels of 30%, 35% and 40%, but with no significant differences among the three levels as the increase

Table 2. Chemical composition of the feed used in the experiment of *L. xanthopterus*.

Ingredients	Protein Level			
	30%	35%	40%	45%
Moisture	6.88	6.40	5.92	5.43
Protein	30.06	35.27	40.19	44.96
Fat	7.14	7.87	8.90	9.97
Ash	9.45	12.32	14.40	16.26
Carbohydrates	47.48	41.38	34.33	28.26
*Energy (Kcal/g)	424.96	435.24	442.78	453.86

*Calculated energy: 5.5, 4.1and 9.1 cal/ g of proteins, carbohydrates and fats in a row as per New, 1987.

Table 3. Measurements of some environmental factors of the water of the enclosures during the period of the experiment.

Environmental Factors	Period (Week)			
	2	4	6	8
Temperature (°C)	20	20	18	16
Dissolved Oxygen (mg/l)	7.1	7.6	7.3	7.2
Salinity (‰)	5.1	5.2	5.0	5.2
pH	7.60	7.68	8.0	8.1

Table 4. Actual composition of the feed used in the experiment on *L. xanthopterus*.

Ingredients	Feeds			
	30%	35%	40%	45%
Moisture	6.10±0.02	3.49±0.32	5.86±0.13	6.83±0.20
Protein	30.59±0.13	35.99±0.32	42.33±0.53	46.38±0.13
Fat	14.49±0.16	15.53±0.35	15.40±0.06	16.27±0.10
Ash	7.45±0.03	8.28±0.02	8.82±0.01	9.47±0.04
Carbohydrates	45.87±0.02	36.73±0.32	27.60±0.74	21.06±0.39
*Energy (Kcal/g)	488.17±0.22	489.86±0.18	486.12±0.20	489.49±0.33

Table 5. Growth performance of *L. xanthopterus* during the experimental Period.

Growth Indicators	Feed of 30% Protein	Feed of 35% Protein	Feed of 40% Protein	Feed of 45% Protein
Initial weight (g)	18.49 ±0.58 a	19.06± 0.73 a	19.04 ± 0.39 a	19.26± 0.38 a
Final weight (g)	19.86± 0.50 a	20.71 ± 0.66a	21.14 ± 0.33 b	20.35 ±0.33 a
Weight gain (g)	1.37 ± 0.08 a	1.66 ±0.06a	2.11 ± 0.06 b	1.09 ± 0.05 a
Growth rate (g/ day)	2.12 ± 0.04 a	2.12 ± 0.051 a	2.10 ± 0.064 a	0.033± 1.41 a
Relative growth rate%	7.39 ± 0.65 a	8.69 ± 0.66 a	11.06 ± 0.57 a	5.64 ± 0.36 a
Specific growth rate (%/ day)	0.22 ± 0.02 a	0.25 ± 0.02 a	0.31 ±0.01 a	0.17 ± 0.03 a

*Means having different letters mean that they have significant differences under a probability level of≤ 0.05.

was from 2.10-2.12 g, while this rate reduced in the 45% protein level and reached 1.41 ± 0.33.

The highest value of specific growth rate was 0.31 g/day attained in the 40% protein level, but there was no significant differences ($p<0.05$) among the different levels. At the end of the experiment the lowest specific growth rate was 0.17 ± 0.03; recorded at the 45% protein level (Table 5). The highest relative growth rate was 11.06% ± 0.57 and recorded at the 40% protein level, while the lowest relative growth rate was 5.64% ± 0.03 and recorded in the 45% protein level (Table 5).

Discussion

Artificial food is used for juvenile fishes as alternative of natural food, and it should provide all nutritional ingredients that meet fishes' nutritional requirements of proteins, fats, vitamins, minerals and calories (Salama, 2000). The tables of the artificial feed composition, mentioned in the present study, showed that feed contains rates commensurate with the fish nutritional requirement suggested by a number of researchers, especially those related to fish juveniles protein levels which are between 30% and 45% (NRC, 1973). The highest weight gain, throughout the experiment, was found in the juveniles fishes fed on the 40% dietary protein; the weight increase was 2.11 ±0.06. Significant differences ($p<0.05$) have been noticed at this protein level, however, the other dietary levels showed no significant increase. At the end of the experiment, the highest average gained of weight was 21.1 g, using the 40% dietary protein level, while the lowest weight average was 19.68 g ± 0.50 in the 30% protein level. Statistical analysis of the present results showed significant differences ($p<0.05$) between weights gain at different protein levels. This is resulted from the fact that fishes are in little need for animal protein especially during the first weeks of their life, in order to use it in metabolism and growth process; this in an agreed with Farnar's (2010) result. Fish growth rate is variable and is dependent on fish species and their age. To reach the maximum growth, we must focus on nutrition and environmental conditions. Nutritional needs rely on temperature and feed quality, so they were considered in the experiment and they were found proper for fish growth.

Other factors, such as pH, concentration of dissolved oxygen and salinity, were also within the rates suitable for survival of *L. xanthopterus*. Ysser *et al.* (2009) found that the weight gain of the yellow fin barbells larve (raised in aquarium and fed on artificial feed) ranged from 0.034 g to 0.06 g. As for specific growth rate of juveniles during the experimental period, the highest value of specific growth rate was 0.31 g/day in the 35% protein level, and showed no significant difference ($p<0.05$) between different protein levels, while Zarski *et al.* (2008) showed that the lowest recorded specific growth rate at the end of the experiment was 0.17 g/day using the feed of 45% protein level; which was lower than the rate obtained for the Shabout *Arasobarbus grypus*, which was 17.1 % g/day. Fishes well accepted the artificial feed after an adaptation period. Weight gain rates and relative growth rates of juveniles, *L. barbus*, fed on different-protein-level feeds, raised gradually up to the 40% protein level, then these rates reduced in feeds of higher protein levels. It is suggested here that 40% protein-level feed can be adopted to get better growth; in addition, growth results in this protein level are within the protein needs of the Carp. Therefore, these results are in accordance with the conclusion of Farnar and Al-Qatrani (2009) that feed ingredients affect fish growth rates.

The nutritional needs of yellow fin barbells reported here were in a close agreement with the results of Al-Shamma'a *et al.* (1998), despite the difference in size. Reduction of protein efficiency in the increasing protein levels, in the different-level feeds, might be attributed to age and its effect on protein needs (Wilson and Robinson, 1982). Reduction of protein efficiency in the increased-protein-level feeds is in agreement with the results of Al-Shamma'a *et al.* (1989). Juveniles, *L. xanthopterus,* protein needs should not be less than 40% when formulated optimum feed, and this is in accordance with the current study. Moreover, numerous studies have found significant differences in fishes cultured in the same conditions. There will also be differences in the growth of fishes if they consume the same food and are cultured in the same farming environment (Al-Hamdani, 2008). Farnar and Al-Qatrani (2009) showed that food ingredients have an effect on growth rates; this was clarified by the experiment when different-protein-level feeds have been used. This is resulted from the fact that fish in general are in bad need for animal protein since it is used in their metabolism and growth.

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تأثير مستوى بروتين العليقة على نمو صغار أسماك الكطان *Luciobarbus xanthopterus* (Heckel, 1843)

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المستخلص - تناولت الدراسة تأثير أربع مستويات بروتين في العليقة (30%، 35%، 40%، 45%) على نمو صغار اسماك الكطان *Luciobarbus*. استخدم 240 من صغار الاسماك بمعدل وزن 18.45 ± 4.93 غم في احواض ذات سعة 250 لتر وبمكررين لكل مستوى بروتيني وبواقع 30 سمكة لكل مكرر. كثرت الأسماك في مفقس مركز علوم البحار. حسب عدد من مؤشرات النمو، إذ أجريت القياسات البيئية وكانت ضمن المديات الملائمة لصغار الاسماك. سجلت الأسماك أعلى معدل وزن عند انتهاء التجربة، إذ بلغ 21.14 ± 0.33 غم عند العليقة ذات المستوى البروتيني 40% في حين كان اقل معدل وزن 19.68 ± 0.50. إن أعلى زيادة وزنيه سجلت خلال التجربة كانت للصغار التي تغذت على عليقة ذات نسبة بروتين 40% عن مثيلاتها إذ بلغت 2.11 ± 0.06. سجلت فروقا معنوية عند هذا المستوى من البروتين ولم تظهر بقية مستويات العليقة زيادة معنوية، وكانت أعلى زيادة وزنية يومية تحققت في المستويات 30% و 35% و 40% ولم تظهر فروق معنوية فيما بينها وبين بقية النسب حيث بلغت 2.12 في المستوى 30 % و 35% و 40% بينما انخفضت عند المستوى البروتيني 45% وبلغت 1.41 ± 0.033. سجل أعلى معدل نمو نوعي 0.31 غم/يوم في المستوى البروتيني 35% ولم تظهر أي فروق معنوية عن بقية المستويات، بينما سجل اقل نمو نوعي عند انتهاء التجربة وكان 0.17 عند مستوى العليقة 45%. بلغ أعلى معدل نمو نسبي لصغار أسماك الكطان عند التغذية على مستويات بروتين مختلفة في نهاية التجربة 11.06% في العليقة ذات المستوى البروتيني 35% بينما سجل اقل نمو نسبي عند انتهاء التجربة 5.64% في العليقة ذات المستوى البروتيني 45%.