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Effects of Different Protein and Energy Levels on Growth Performance of Caspian Brown Trout, *Salmo trutta caspius* (Kessler, 1877)

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Abstract: The objective of this study is to assess the effects of protein and energy levels of diet on fish performance and growth efficiency of Caspian brown trout in order to develop optimum protein and energy level during the preparation of diet for this species. Fish were fed with six experimental diets containing three protein levels (45, 50 and 55%) and two energy levels (3.5 and 4 kcal g^{-1}) according to a 3×2 factorial design. The diet was assigned to 18 tanks with 50 fish each, with three replicates for each diet. The experiment was conducted for 8 weeks with Caspian brown trout with an initial body weight of around 7 g. Protein content of diet influenced feed conversion ratio ($p<0.05$) and specific growth rate statistically ($p = 0.052$). Caspian brown trout demonstrated a better feed conversion ratio and a larger specific growth rate at lower protein levels (45 and 50%) in comparison to high protein level (55%). Protein efficiency ratio improved by a decrease in protein content of the diet ($p<0.05$), but energy content of diets does not affect any growth related parameter such as feed conversion ratio, specific growth rate and protein efficiency ratio. There was also no interaction between protein and energy levels in the growth related parameters, suggesting the effect of protein on the growth parameters in Caspian brown trout did not depend on energy levels of diet. In conclusion diet containing 50% protein can support the maximum growth and protein sparing by the use of high-energy diet did not occurred in this study.

Key words: Caspian brown trout, artificial production, growth, diet, protein, energy

INTRODUCTION

The Caspian brown trout is one of the nine subspecies of brown trout, *Salmo trutta caspius* (Kessler, 1877), around the world (Quillet *et al*., 1992). This species is distributed mainly at the western and southern coast of Caspian Sea (Kiabi *et al*., 1999) and showed a better growth and higher weight gain compared to other brown trout which are living in Caspian Sea (Sedgwick, 1995). According to Berg (1964) Caspian brown trout entered to Caspian Sea from the Arctic region in the past. Due to its fillet quality, it has been known a commercially valuable fish in the southern cost of Caspian Sea.

The Caspian brown trout natural population has showed a steady decline over the past two decades and it has been characterized as endangered species in the southern part of Caspian Sea (Kiabi *et al*., 1999). River pollution, over-fishing, poaching and destruction of natural spawning sites are known as main reasons for such a sharp stock decline. There is no evidence showing that this species is able to do a successful reproduction in the rivers. Therefore, artificial reproduction has been introduced as a solution for enhancement and protection of wild populations.

In the restocking centers, Caspian brown trout larvae were raised in the concrete ponds until releasing weight of 20 g. Due to lack of specific production system for this species, rainbow trout production model has been applied to grow Caspian brown trout in the region. This condition caused some problems in restocking program such as a lowered growth rate and a high mortality of fingerlings.

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Beside the stock enhancement program, there has been an attention toward artificial production of Caspian brown trout in Iran (Kalbassi *et al*., 2006). The aim is to introduce a new species into Iranian aquaculture industry. This species can be cultured in the cage or concrete pond along rainbow trout (Kalbassi *et al*., 2006). Understanding the requirements is a key step toward adjustment of this species to an artificial production system.

Preparation of a balanced diet and their adequate feeding is known as a key step for both restocking program and aquaculture. Nowadays, feed formulation for Caspian brown trout is based on information available for other salmonids. This solution however cannot be valid as different species show different requirement although they are classified within a family (Azevedo *et al*., 2004b, 2005).

Up to now, no nutritional studies have been conducted to produce artificial feeds for Caspian brown trout intensive culture. Growth, feed efficiency and body composition largely depends on protein and energy content in feed (Wang *et al*., 2006). Although dietary protein and energy requirements have been extensively studied in salmonids (Einen and Roem, 1997; Ruohonen *et al*., 1999; Lanari and Agaro, 2002; Azevedo *et al*., 2004a, b), those information for Caspian brown trout is still limited.

Therefore, the main goal of the study is to examine the effects of protein and energy levels of diet on fish performance and growth efficiency of Caspian brown trout. This data is needed to develop a proper diet to ensure the economical production of this species.

MATERIALS AND METHODS

Experimental Diets

In this study, three levels of dietary protein (45, 50 and 55%) and two levels of dietary energy (14.7 and 16.7 kJ g^{-1}) were examined in a 3×2 factorial design. This led to six experimental diets which were formulated by replacing casein as a pure protein source with fat as an energy source in the diets. Ingredients selection and feed formulation were to meet nutrients requirements for brown trout. Protein and energy levels were adopted according to salmonids nutrients requirement range (Einen and Roem, 1997; Azevedo *et al*., 2004a).

The formulation and chemical composition of the six experimental diets are presented in Table 1 and 2, respectively. The chemical composition of the diets was different to examine the interactive effects of varying dietary protein and energy on growth performance of Caspian brown trout. Feed ingredients were provided from a local fish feed factory (Mazndaran Aqua-feed Company, Sari, Iran). The ingredients were separately ground and homogenously mixed. The pellet diets were made by the use of a cold feed pellet machine with a 2 mm diet. The resulting diets were then dried in an air dryer at 80°C and stored in a refrigerator until use.

Table 1: Percentage of ingredients used in the control diet and experimental diet

	Diets					
Variables	1	2	3	4	5	6
Protein (%)	45.00	50.00	55.00	45.00	50.00	55.00
Energy (kJ g^{-1})	14.60	14.60	14.60	16.70	16.70	16.70
Casein	33.00	38.00	43.00	33.00	38.00	43.00
Fishmeal	10.00	10.00	10.00	10.00	10.00	10.00
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00
Wheat flour	15.00	15.00	15.00	15.00	15.00	15.00
Fish oil	5.46	4.35	3.24	8.24	7.12	6.00
Soya oil	5.46	4.35	3.24	8.24	7.12	6.00
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix	3.00	3.00	3.00	3.00	3.00	3.00
Anti-oxidant	0.05	0.05	0.05	0.05	0.05	0.05
Cellulose	14.43	11.65	8.87	8.87	6.11	3.35
Binder	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin C	0.10	0.10	0.10	0.10	0.10	0.10

Table 2: Nutrient composition of the experimental diets in % or kJ g^{-1} based on dry matter basis

	Diets					
Variables	1	2	3	4	5	6
Dry matter (%)	94.1±0.2	94.2±0.3	94.8±0.1	94.2±0.3	94.8±0.2	94.6±0.1
Crude protein (%)	45.3±0.4	50.2±0.2	55.3±0.3	44.9±0.1	50.2±0.1	55.5±0.2
Crude fat (%)	13.9±0.3	12.2±0.2	14.2±0.1	13.9±0.1	14.9±0.2	13.2±0.2
Ash (%)	12.4±0.4	19.3±0.4	18.6±0.1	21.2±0.1	20.9±0.2	18.2±0.2
Energy (kJ g^{-1})	14.1±0.3	14.2±0.4	14.2±0.4	16.3±0.4	16.3±0.3	16.3±0.3

Experimental System and Animals

The experiment was conducted for 8 weeks with Caspian brown trout with an initial body weight of 6-8 g during fall 2007. The fish were bred at the reproduction facility of the Shahid Bahonar, Kelardasht, Iran.

The fish were grown in the 18 fibre-glass tank (2×2×0.3 m), with a volume of 1200 L. The water supply was maintained by a channel connected to a nearby river. The photoperiod regime during the experiment was 12 h light and 12 h dark. Water quality was checked every week after the first feeding in the outflow of the tanks. The measured parameters were: temperature, pH, oxygen content, NH_4^+. The water flow for all tanks was equal around 14 l min throughout the experiment. Ammonium was measured in the tank which was received diet containing maximum protein level.

The water temperature was kept at 13-16 (14.3±1.2)°C during the experiment. Oxygen concentration was measured in a randomly selected tank by a digital oxygen detector and always remained above 8.5 (9.4±0.9) mg L^{-1}. The water pH ranged between 7.45 to 7.62 (7.53±0.08) during the experiment. The ammonium concentration was always below 1 (0.33±0.11) mg L^{-1} during the experiment.

Experimental Procedure

On day one of the experiment, fish were randomly divided over 18 tanks (50 fish per tank). The fish were allowed to adapt to the experimental diets and to the feeding level for one week before the experimental period started.

The six diets were randomly assigned to 18 tanks with three tanks for each diet. The diets were given to the fish as soon as they were introduced into the tanks. The daily ration was calculated according to feeding chart adjusted for salmonids. The daily ration was divided into two feedings and fed by hand at 08:30 and 16:30. Before feeding, the pellets were sieved to remove dust and small particles. Fish were weighed at the beginning (day 0) and at the end of the experiment (day 56).

Chemical Analysis

Feed samples were collected at regular intervals (twice a week) during the experimental period and ground with a 1 mm screen before analysis. All chemical analysis were done in triplicate. Feed was analysed for dry matter by drying samples for 4 h at 103°C until constant weight (ISO, 1983). Ash content was determined by ashing the samples by incineration in a muffle furnace for 4 h at 550°C (ISO, 1978). Acid insoluble ash was measured by dissolving ash in hydrochloric acid (ISO, 1981). Crude protein (N×6.25) was measured by the Kjeldahl method after acid digestion, according to ISO (1979). Lipid was extracted by petroleum ether extraction in a Soxhlet apparatus (ISO/DIS, 1996).

Calculations and Statistical Analysis

Fish Performance

Weight gain was determined by the difference between initial and final body weight. Specific Growth Rate (SGR) was calculated from the natural logarithm of mean final weight minus the natural logarithm of the mean initial weight and divided by the total number of experimental days expressed as a percentage (Amirkolaie *et al*., 2005).

$$SGR = 100 \times (\text{Ln } W_{final} - \text{Ln } W_{initial})/\text{days}$$

The Feed Conversion Ratio (FCR) was calculated per tank from feed intake data and weight gain (Amirkolaie *et al*., 2005).

$$FCR = \text{Feed intake}/\text{Wet weight gain}$$

Protein Efficiency Ratio (PER) was calculated by dividing the fish weight gain to total protein ingested during the experiment. Total protein ingested was estimated from the daily feed ration multiplied by diets protein content.

$$PER = \text{Wet weight gain}/\text{Total protein ingested}$$

Statistical Analysis

Data are presented as means of each treatment with standard deviation. The data were verified for normality by the use of Kolmogorov-Smirnov test after transformation (ASIN). All data were analysed by a 2 way ANOVA for the effect of protein supplementation (45, 50 and 55%) and the effect of energy supplementation (3.5 and 4 kcal g^{-1}). Protein levels and energy levels are the factors in this study. For all statistical analysis, tank was considered as the experimental unit. The means were compared by a Tukey's post hoc test at five percent probability level.

RESULTS

Diet protein level influenced significantly FCR ($p<0.05$) in Caspian brown trout. Fish fed on diets containing 45 and 50% protein showed a better FCR than that of 55% protein. SGR is also affected by protein content of the diets ($p = 0.052$). Fish demonstrated a higher SGR by feeding on diet containing 45 and 50% protein.

Similar to FCR and SGR, Caspian brown trout showed a larger PER when fed on diets containing a lower level of protein ($p<0.05$; Table 3). Protein and energy levels did not influence final weight and weight gain statistically in Caspian brown trout, although feeding by diet containing 50% protein caused numerically a higher growth in fish.

In general, the energy levels did not affect the growth related parameters and fish showed similar performance at two experimental energy levels (Table 3). There was no interaction between protein and energy levels in the growth related parameters, suggesting that the effect of protein on growth parameters did not depend on energy levels of diet in Caspian brown trout.

Table 3: Growth performance±SD in brown trout fed with three levels of protein (45, 50 and 55%) and two levels of energy (3.5 and 4 kcal g^{-1})

	Diet						p-values of the factors		
Variables	1	2	3	4	5	6	Protein	Energy	Protein× energy
Protein (%)/Energy (kcal g^{-1})	45/3.5	50/3.5	55/3.5	45/4	50/4	55/4			
Initial weight (g)	6.57±0.32	8.04±0.5	8.4±0.61	7.33±1.1	6.40±1.2	8.20±0.44			
Weight gain (g)	5.37±0.04	5.24±3.8	4.23±1.5	4.58±0.71	6.30±0.91	4.44±0.04	0.363	0.826	0.791
Final weight (g)	11.9±0.42	13.2±5.6	14.6±2.0	12.1±0.95	12.9±1.18	14.7±0.38	0.346	0.767	0.853
FCR	1.90±0.63^{b}	2.74±0.6^{ab}	3.33±0.8^{a}	2.44±0.69^{b}	1.86±0.50^{b}	3.97±0.30^{a}	0.023	0.918	0.259
Specific growth rate (%)	1.95±0.01^{a}	1.42±0.6^{ab}	1.05±0.2^{ab}	1.50±0.43^{ab}	1.97±0.48^{a}	1.11±0.05^{ab}	0.052	0.707	0.172
Protein efficiency ratio	1.23±0.41^{a}	0.77±0.2^{bc}	0.57±0.1^{c}	0.96±0.25^{b}	1.11±0.27^{a}	0.48±0.37^{c}	0.018	0.939	0.216

DISCUSSION

In the current study, FCR and SGR of fish were improved as dietary protein level increased up to 50%. A better performance of FCR and SGR suggests that the optimum dietary protein level for Caspian brown trout to be between 45 and 50%. This finding is similar to the experiments in which different protein levels were evaluated in order to determine optimum protein requirement of salmonids. Based on those studies, protein requirement for salmonids vary between 40 and 50% (Webster and Lim, 2002; Lovell, 1989; Lee and Kim, 2001; Chan *et al*., 2002).

The reduction of growth at higher protein levels (55%) may be caused by catabolism of excessive amino acid by the fish. It has long been known that feeding protein in excess beyond requirement lead to catabolism of amino acid which associated with excretion of ammonia and loss of energy (Lloyd *et al*., 1978). In other words, since protein is the preferential energy source in fish metabolic routes (Hepher, 1988), brown trout utilize the extra protein for other metabolic functions not related to weight gain. This action cost a high energy loss in fish. Similar observation was made by Meyer and Fracalossi (2004) in *Rhamdia quelen* at high protein utilization level.

The main cause of larger energy loss is an increase in heat production followed by consumption of diet containing higher levels of protein. An increase in total energy expended on the ingestion, digestion, absorption and biochemical processing of a meal is main responsible for such a higher energy loss (Houlihan *et al*., 1995; Fu *et al*., 2007). Smitth *et al*. (1978) and Fu *et al*. (2005, 2007) showed that heat production associated with feeding protein to fish was larger than that for fat or carbohydrate. Therefore, high level protein diet may have increased energy cost of production, leading to a lower growth.

The reduction of growth performance may be also caused by poor water quality induced by high protein content diet. Amino acid degradation for energy supply increased ammonium excretion by the fish, leading to higher ammonia concentration in the water. Chakraborty and Chakraborty (1998) observed that ammonia loss by *Labeo rohita* was directly dependent on the amount of dietary protein. Ammonia build up in water is toxic to fish (El-Shafai *et al*., 2004) and may reduce the growth rate in the tanks at which fish were fed by diet containing maximum protein level (55%). Relatively higher ammonia concentration in the tank with the largest protein level (55%) may support the idea.

In the present study, surprisingly the growth related parameters were not affected with increase of dietary energy. This is in contrast to a number of earlier studies on different species including Lee and Kim (2001) in masu salmon ; Chan *et al*. (2002) in coho salmon; Meyer and Fracalossi (2004) in *Rhamdia quelen*; Wang *et al*. (2006) in cuneate drum; Salhi *et al*. (2004) in black catfish. There was also no interaction between dietary protein and energy suggesting that protein requirement dose not depends on energy content of the diets. This situation is also in contrast to the hypothesis that dietary protein requirements of fish are closely related to dietary energy levels and by proper use of non-protein energy sources, such as lipid and carbohydrate, dietary protein in fish feed can be spared (Shiau and Lan, 1996; Santinha *et al*., 1999; McGoogan and Gatlin, 2000). It appears that Caspian brown trout energy requirement was met by diet containing 3.5 kcal g^{-1} energy and extra energy does not lead to further growth. However, the idea can be debated by the fact that salmon generally rely more heavily on lipids as an energy source and that divert/spare more amino acids for protein deposition (Azevedo *et al*., 2004a). The opposite results gained in this study might be an issue that needs further investigation.

Protein efficiency ratio decreased with increasing dietary protein level. This is in agreement with the earlier findings for salmonids Lee and Kim (2001) in masu salmon and Chan *et al*. (2002) in coho salmon. The range of PER in this study was 0.48 to 1.23. This results are lower than the reported range of PER for coho salmon (1.8 to 2.4; Chan *et al*., 2002) and for masu salmon (1.1 to 1.8 ; Lee and Kim, 2001). This may support the idea that Caspian brown trout utilizes protein as main energy source.

Like growth rate, energy content of the diet did not have an impact on PER. This may suggest that Caspian brown trout heavily depends on protein to supply energy for maintenance and growth. It appears that a high energy diet by the use of non-protein sources cannot spare a bigger protein portion for growth.

In conclusion, the addition of increasing protein level affected growth performance in Caspian brown trout and the best FCR and SGR were attained by diet containing 50% protein. An extra heat production related to the high protein diet may have reduced growth performance in this treatment. An increased dietary energy content of diet to spare the bulk of dietary protein for the growth appears not to work very well in the fish, as dietary energy dose not have any significant impact on growth, PER, FCR and SGR.

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