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Effects of Dietary Protein and Energy Levels on Rainbow Trout *(Oncorhynchus mykiss)* Reared in Brackish Water

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Abstracts: A completely randomised design, with 3×3 factorial arrangement, was conducted to test various ratios of dietary protein to energy (P/E) for rainbow trout, reared in brackish water. Three crude protein levels (35, 40 and 45%) and three energy levels (370, 400 and 430 Kcal/100g) at each protein level were utilized. Semi-purified diet containing fish meal, casein and gelatin as the sources of protein and dextrin, starch and oil as the sources of energy were used. Each experimental diets was fed to triplicate groups of 20 fish with average weight of 81.5g, in nine 2000 lit flow trough fiberglass tanks, each partitioned into three sectors. Average water temperature, dissolved oxygen, pH and EC were between 15±2°C, 6.5-8.1 mg/L, 7.7-8.6 and 25400 µm/s, respectively. Fishes were fed for 84 days at a rate between 1.6-2% of body weight per day, in three equal amount, adjusted bi- weekly. At each protein level, weight gain (WG%), average daily growth (ADG%), protein efficiency ratio (PER), apparent net protein utilization (ANPU%), specific growth rate (SGR) and condition factor (CF) increased, but feed conversion ratio (FCR) decreased with increasing energy level from 370 to 430 Kcal/100g. The highest growth performance was obtained by fishes fed by 35% protein, 430 Kcal/100g energy diet with P/E ratio of 81.4 mg protein/Kcal energy. Carcass fat and moisture were affected by dietary protein and energy levels, whereas, carcass protein and ash levels were similar between dietary treatments.

Key Words : *Oncorhynchus mykiss;* brackish water; protein to energy ratio

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is mainly cultured in freshwater, but this species is euryhaline fish and can be reared in brackish water as in freshwater

Studies in Yazd Fisheries Research Station, central Iran, in recent years have demonstrated that rainbow trout can be cultured in desert areas using underground brackish water (salinity 10-20 gr/lit) in earthern ponds, during autumn and winter. In this system yield of about 3-4 ton/ha, without aeration, with 5-10% daily water exchange have been obtained in 4-5 months.

A wide range of opinions exists on protein, lipid and carbohydrate requirements for rainbow trout. The natural diets of salmonids containing a high proportion of protein, and part of the dietary protein is utilized as an energy source.

The non-protein energy level may also influence protein requirements of fish. Prather and Lovell (1973), postulated that high levels of protein without sufficient energy in the diet may be harmful to fish. Page and Andrews (1973), observed that larger fishes require more energy and less protein compared to smaller fishes

The protein sparing effect on fat and carbohydrate has been studied in rainbow trout (Cho & Kaushik, 1990). The protein to energy (P/E) relationships for a number of fishes, maintained under various culture condition, have been reported (Mukhopadhyay *et al*., 1986; Das *et al*., 1991; Seenappa, 1992; Samantaray & Mohanty, 1997; Hardy, 2002).

The present study was conducted to evaluate the response of rainbow trout reared in brackish water to diets containing various protein levels and P/E ratios and also to determine the maximum possible inclusion level of dietary lipid to spare protein for growth.

Material and Methods

Diets

Nine semi-purified experimental diets were formulated using fish meal, casein and gelatin as the sources of protein and dextrin, starch and oil as the sources of energy to produce three energy levels (370, 400 and 430 Kcal/100g) at three protein levels (35, 40 and 45%), (table 1). The energy level at each protein level was similar to determine the optimun P/E ratio for each energy level. The energy values of each diet was calculated based on standard physiological fuel value of

4,4 and 9 Kcal/g for protein, carbohydrate and lipid, respectively (Pike & Brown, 1967; Garling & Wilson, 1976; Nematipour *et al.*, 1992). Inevitably, there were slight differences in percentage of ingredients between the diets, primarily that of *a*-cellulose. Dietary energy levels were adjusted by changing the amount of fish oil, to produce low, medium, and high-energy diets.

Diets were prepared by mixing the dry ingredients in an electric grinder and then blending in the oils. Water was added and the moist mixture was pelleted using hand pelletizer. Size of pellets was 4.5 mm. The pellets were air- dried at 40°C. The dry pellets were packed and stored in a cool, dry place before being used.

Table 1: Ingredient and proximate composition of the test diets, expressed as dry Wt%

Ingredient	**Diet number**								
	1	2	3	4	5	6	7	8	9
Fish meal	40	40	40	40	40	40	40	40	40
Casein	6.3	6.3	6.3	10.2	10.2	10.2	10	10	10
Gelatin	1.1	1.1	1.1	3	3	3	8.3	8.3	8.3
Dextrin	14	14	14	9.5	9.5	9.5	5.2	5.2	5.2
Fish oil	6	8	9.9	5.5	7.6	9.5	5.3	7.3	9.2
Soybean oil	4	5.3	6.6	3.8	5	6.4	3.5	4.8	6.2
Starch	10	10	10	10	10	10	10	10	10
Yeast	5	5	5	5	5	5	5	5	5
Vitamin permix	2	2	2	2	2	2	2	2	2
Mineral premix	1	1	1	1	1	1	1	1	1
Binder	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Anti oxidan	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Vitamin C	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Anti fungus	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
A.Cellulose	9.1	5.7	2.4	8.4	5	1.8	8	4.6	1.8

Table 1 Continues:

Nutrient content									
Crude protein%	35	35	35	40	40	40	45	45	45
Crude lipid%	13.9	17.3	20.6	13.5	16.8	20.2	13.1	16.4	19.7
Carbohydrate%	26	26	26	22	22	22	18	18	18
Ash%	7.82	7.85	7.93	7.94	7.84	7.82	8.02	7.81	7.94
Moisture%	7.14	7.33	7.29	7.68	7.23	7.4	6.94	7.31	7.16
Energy [1]	370	400	430	370	400	430	370	400	430
P/E [2]	94.6	87.5	81.4	108.1	100	93	121.6	112.5	104.6

1- Available energy was calculated using physiological fuel values of 4.0, 4.0 and 9.0 Kcal/g for carbohydrate, protein and lipid, respectively (Pike & Brown, 1967; Halver, 1976; Nematipour *et al.*, 1992; Catacutan & Coloso, 1996)
2 – Protein to energy ratio in mg protein/Kcal

Experimental animal and design

Rainbow trout, average weight 81.5± 10.5 g., obtained from a local earth pond fish farm, were used for this experiment. The fish underwent a five days conditioning prior to the beginning of experiment. During this period the fish were fed using a commercial diet in 50×50% weight.

The experiment was conducted in nine 2000 lit flow through-fiberglass tanks each divided into three equal compartments by polyethylene mesh to obtain three replicates for each treatment contain 20 fish for each group. The experiment was conducted for 84 days. Water temperature, dissolved oxygen, pH were recorded daily. Those factors were 15 ±2°C, 6.5-8.1-mg/lit and 7.7-8.6, respectively. Water electric conductivity (EC) was nearly constant at 25400 µm/s. Light/dark cycle of 12:12 h was maintained using supplemental incandescent lighting.

The experimental fish were weighed at the beginning and at the end of the experiment and batch weighed at two week intervals for adjustment of feed level. The fish were fed at the rate between 1.6-2% of wet body weight per day depending to the water temperature and growth rate. Fishes were feed three times daily at 8.00, 12.00 and 16.00 o'clock. At the beginning of the experiment, five fishes were sacrificed and stored for carcass analysis. At the end of the experiment, six fishes (two from each replicate) from each feeding trials, were killed and frozen immediately for subsequent carcass analysis.

Analytical methods

Diet ingredients, experimental diets and carcasses were analysed using Association of Official and Analysis Chemists, AOAC (1990), methods. Mosisture was determined by oven drying at 100°C to constant weight. Crude protein was determined based on Kjeldahl nitrogen (crude protein = N×6.25). To determine crude fat samples together with petroleum ether were put for 8 h in a soxhlet extraction apparatus. Ash content was determined by leaving samples in a muffle fur nace for 6 h at 550°C.

The rate of weight gain (WG%), rate of average daily growth (ADG%), feed conversion ratio (FCR), protein efficiency ratio (PER), rate of apparent net protein utilization (ANPU%) or protein deposition, specific growth rate (SGR%) and condition factor (CF)were calculated.

Statistical procedure

All data analysed using statistic software, SAS (ANOVA model) and MSTATC. Multiple comparisons between means were made with Duncan's multiple range test (Duncan, 1955).

Results

The growth of rainbow trout fed with nine experimental diets, with three different protein and energy levels are shown in table 2. The growth rate increased with increase of dietary energy from 370 Kcal/100g (about 13.5% lipid) to 430 Kcal/100g (about 20% lipid) at each protein level (35%, 40% and 45%). At the 35% dietary protein level, growth rate was found to be highest with an energy level of 430 Kcal/100g (20.6%lipid) and was significantly different from diet 1 ($P<0.05$), but no significant differed it was found from diet with 400Kcal/100g energy. Diets 3, 6 and 9 with energy level of 430 Kcal/100g, at protein levels of 35%, 40% and 45%, respectively, showed higher growth performance compared to that of fish fed with the other diets (table 2). However, diet 3 was more efficient in terms of protein deposition or apparent net protein utilization (ANPU%), protein efficiently ratio (PER), specific growth rate (SGR%) and condition factor (CF) when compared to those fishes fed with diets 6 and 9 having higher protein levels.

The trend of changes ADG% in relation to P/E ratio, shown in table 2, indicates that the diet with a P/E ratio of 81.4 mg protein $Kcal^{-1}$ (diet 3) was the most suitable diet for maximum growth of rainbow trout in the given condition. The growth remained almost constant and without any significant difference with increase in P/E ratio to 87.5, 93 and 104.6 mg protein $Kcal^{-1}$ (diets 2, 6 and 9). However, with further increases in P/E ratio to 94.6 mg protein $Kcal^{-1}$ in the 35% crude protein (diet1), 100 and 108.1 mg protein $Kcal^{-1}$ in the 40% crude protein (diet 4 and 5), 112.5 and 121.6 mg protein $Kcal^{-1}$ in the 45% crude protein (diets 7 and 8), growth rate was significantly reduced ($P<0.05$).

At each of the energy levels, the diet with lower protein level (35%) was more efficient in term of protein deposition than diets with higher protein levels (40% and 45%) (table 2). Protein deposition was highest with energy level of 430 Kcal/100g (about 19.3% lipid).

The mean PER and FCR in relation to dietary protein and energy levels are shown in table 2. At each of the dietary protein levels (35,40 and 45%), a significant increase in PER was observed with increase of dietary energy from 370 to 430 Kcal/100g . The highest PER and the lowest FCR values were obtained at a P/E ratio of 81.4 mg protein $Kcal^{-1}$ (diet 3).

Carcass moisture and fat content were affected by changes in dietary P/E ratio (table 3). At each of the dietary protein levels with an increase in energy from 370 to 430 Kcal/100g, carcass moisture content decreased and it's fat content increased significantly ($P<0.05$). Carcass protein and ash content were nearly constant with (no significant difference) in different treatments.

Table 2: Growth performance of rainbow trout fed the experimental diets (see table 1) with different P/E ratio after 84 days (x ± SE)

	Diet number								
	1	2	3	4	5	6	7	8	9
Initial Mean Weight (g)	81	82	81	80	81	82	83	81	82
Final Mean Weight (g)	133(9)	197(12.7)	206(12.1)	132(3)	183(5.5)	203(4.5)	161(7.4)	179(3.5)	202(2.5)
WG %	64.3^{d}(11.2)	140.7^{a}(13.6)	154.3^{a}(15.2)	65.3^{d}(4)	125.3^{b}(6.6)	146.7^{a}(5.5)	94^{c}(8.9)	121.3^{d}(4.5)	146.7^{a}(3)
ADG %	62.3^{d}(11.1)	136.3^{a}(15)	148.7^{a}(14.6)	62.3^{d}(3.6)	120.7^{b}(6.4)	143.3^{a}(5)	93^{c}(8.7)	116.7^{b}(4)	143^{a}(3)
FCR	2.6^{d}(0.5)	1.5^{d}(0.2)	1.3^{d}(0.11)	2.2^{ab}(0.2)	1.5^{d}(0.0)	1.3^{d}(0.0)	2^{bc}(0.15)	1.7^{cd}(0.1)	1.3^{d}(0.1)
PER	1.1^{a}(0.2)	1.8^{bc}(0.21)	2.1^{a}(0.17)	1.1^{d}(0.17)	1.7^{c}(0.0)	2^{ab}(0.0)	1.1^{d}(0.1)	1.3^{d}(0.1)	1.7^{c}(0.1)
SGR %	0.59^{d}(0.08)	1.04^{a}(0.07)	1.1^{a}(0.07)	0.6^{d}(0.02)	0.96^{b}(0.03)	1.07^{a}(0.03)	0.7^{c}(0.05)	0.94^{bc}(0.02)	1.07^{a}(0.01)
CF	1.05^{d}(0.03)	1.31^{a}(0.02)	1.32^{a}(0.05)	1.06^{d}(0.03)	1.22^{b}(0.02)	1.29^{a}(0.05)	1.16^{c}(0.05)	1.22^{bc}(0.05)	1.29^{a}(0.01)
ANPU %	18.9^{de}(4.2)	30.3^{ab}(5.2)	33.7^{a}(3.7)	16.9^{e}(2.8)	27^{bc}(0.8)	33.3^{a}(0.1)	18.7^{de}(1.3)	23.3^{cd}(1.8)	28.7^{abc}(1.8)

a,b,c,d,e,f Means with the same superscripts within the same row are not significantly different ($P<0.05$)

Table 3 : Composition of carcass of rainbow trout fed the experimental diets with different P/E ratio for 84 days (X ± SE) Values are expressed as wet weight % basis

	Diet number									
	Initial	1	2	3	4	5	6	7	8	9
Moisture %	74.9	75.5^{b} (0.36)	70.3^{e} (0.56)	68.9^{d} (0.33)	77.9^{a} (0.27)	71.2^{e} (0.22)	68.4^{e} (0.57)	71.9^{c} (0.72)	67.3^{e} (0.43)	67.2^{f} (0.59)
Lipid %	6.1	6.2^{f} (0.31)	9.6^{d} (0.6)	12.6^{c} (0.36)	3.3^{g} (0.06)	7.9^{e} (0.31)	12.6^{c} (0.31)	7.9^{f} (0.18)	14^{b} (0.43)	15.1^{a} (0.73)
Protein %	16.1	16.5^{ab} (0.16)	16.3^{bc} (0.55)	15.9^{cd} (0.33)	15.6^{g} (0.26)	16.1^{bcd} (0.38)	16.2^{bc} (0.02)	16.3^{bc} (0.07)	17^{b} (0.33)	16.5^{ab} (0.21)
Ash %	1.8	1.6^{a} (0.05)	1.5^{ab} (0.06)	1.5^{ab} (0.04)	1.6^{a} (0.03)	1.6^{a} (0.12)	1.4^{b} (0.06)	1.5^{ab} (0.05)	1.5^{ab} (0.03)	1.4^{b} (0.02)

A,b,c,d,e,f Means with the same superscripts within the same row are not significantly different ($P<0.05$).

Discussion

The importance of the level of dietary protein to energy for rainbow trout reared in brackish water was evident in the present study as was found earlier for several other species (Garling & Wilson, 1976; Murai *et al.*, 1985; Lovell 1989; Das *et al.*,1991; De Silva *et al.*, 1991; Garcia-Riera *et al.*, 1993; Webster *et al.*, 1994; Samantary & Mohanty, 1997). In the present study the rainbow trout grew best with a diet containing 35% protein, 430Kcal/100g energy and 20.6% lipid with a P/E ratio of 81.4 mg protein Kcal. However, lower, but not significantly different ($p>0.05$) growth was attained using diets containing 40 and 45 percent protein, the same energy level of 430 Kcal/100g, 20.2 and 19.7 present lipid and P/E ratios at 93 and 104.6mg protein Kcal, respectively. In the present study, at an optimum P/E ratio, maximum growth was obtained at the lower dietary protein level. This indicates that if the dietary energy level is not maintained using either lipid or carbohydrate, then protein will be utilized for energy rather than for growth.

The minimum level of lipid required for maximum growth, survival and feed conversion of rainbow trout has need reported to be 10% (Takeuchi *et al.*, 1978). In common carp growth was not depressed when 18% dietary lipid was fed (Jauncey, 1982). Channel catfish, however, showed growth depression when the dietary lipid level was increased form 12% to 16% of the dry diet (Dupree *et al.*, 1979). Red tilapia can effectively utilized a lipid level of 18% (De Silva *et al.*, 1991). No growth- depressing or pathological effects were observed where high levels of dietary lipid were fed to rainbow trout (Garcia-Riera *et al.*, 1993). From the present study, it can be concluded that a lipid level of 20.6% can be utilized by rainbow trout at growing stage.

The FCR and PER are known to decrease and increase respectively, with increasing dietary protein content up to a level that gives optimum growth (Wee & Tacon 1982; Wee, 1986). Results of the present study indicated that dietary energy with all protein levels influence PER and FCR.

The changes in carcass protein and ash levels in relation to dietary protein and energy levels were less consistent. The carcass lipid and moisture levels appeared to increase and decrease with higher dietary energy at all of protein levels (35, 40 and 45%), respectively. On the other hand, when carcass lipid level decreased, water replaced it and as a result of that carcass moisture level increased.

This study has demonstrated the capability of rainbow trout to spare protein and the degree to which the lipid content in the diet can be effectively used to obtain optimal growth.

Diets containing about 81.4 mg protein $Kcal^{-1}$ energy were adequate for rainbow trout in brackish water for maximum growth within certain dietary and energy concentrations.

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