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THE PROTEIN SPARING EFFECTS OF HIGH LIPID LEVELS IN DIETS FOR RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, W. 1792) WITH SPECIAL REFERENCE TO REDUCTION OF TOTAL NITROGEN EXCRETION

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Abstract

This research sought to develop low-protein, high-energy (less-polluting) diets for rainbow trout to properly utilize protein and reduce total nitrogen excretion. Duplicate groups of rainbow trout were fed one of four experimental diets with a protein to energy (P:E) ratio of 25, 24, 22 or 19 mg/kJ and a lipid content of 12, 13, 17 or 26%, respectively. The diets were given to fish with a mean initial weight of 181 g for 70 days. At the conclusion of the trial, mean weights ranged from 353.17 g (94% weight gain) to 394.75 g (118% weight gain) with 100% survival in all treatments. Weight gain, feed efficiency and protein retention increased as the dietary lipid content increased and as the P:E ratio decreased. Fish fed the 22 mg protein/kJ energy diet (17% lipid, 44% protein, 20.34 kJ/g gross energy) performed best. This indicates that the protein content in practical trout feeds can be reduced from the currently used 47% to around 44% without reducing the growth rate and feed efficiency, if high quality protein is used and the gross energy is increased by lipid to about 20.34 kJ/g diet. The low-protein, high-energy diets reduced the total nitrogen excretion from the rainbow trout by 27%.

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Introduction

Development of cost-effective nutritionally balanced diets for fish is the main factor affecting intensive aquaculture due to its influence on growth, health and production costs. Nutrients and energy are needed in the diet for growth and maintenance. Since protein sources are among the most expensive feed ingredients, it is economically desirable to minimize the protein level in the diet whilst keeping it high enough to sustain good growth. Excess protein can be replaced by an energy-rich compound such as lipid.

Fish, like other animals, eat to satisfy their energy requirements (Lee and Putnam, 1973). If the protein content of the diet is too high, the excess is catabolized to provide energy for growth, lowering the protein conversion efficiency (Lee and Putnam, 1973; Adron et al., 1976). However, when fish are fed an excess of energy, feed consumption decreases and growth is reduced (Ringrose, 1971; Lovell, 1998). Besides the high production costs associated with inadequate feed rations, deterioration of environmental waters due to wasted feed and excretion through fish gills and kidneys may also be a result of inadequate feeds.

It has been suggested that nitrogen excretion from fish can be reduced by increasing dietary protein retention. This can be accomplished by adjusting the total energy (protein:energy ratio) in the diet to an optimal balance (Watanabe et al., 1987a). Consequently, the objective of the current study was to evaluate the effects of high lipid contents in the diet on growth performance, feed utilization, protein utilization and sparing, lipid accumulation around the viscera, weight of the gastrointestinal tract and body composition in rainbow trout.

Materials and Methods

The study was conducted at the commercial trout farm of Inalti-Dorthavuzlar in Ayancik-Sinop, Turkey, from April through July 1996. Four hundred fish from the same broodstock and the same site were stocked into eight concrete ponds of 10,500 l (1.5 x 7 x 1 m) with fresh water of about 9ºC supplied at a flow rate of 120 l/min per pond.

Growth, feed utilization, protein utilization and body composition of rainbow trout were compared for 70 days. Three treatments received pelleted diets and the fourth received an extruded diet. Experimental diets were obtained from a commercial feed company (Pinar Yem Sanayii A.S., Turkey) and differed in lipid (12, 13, 17 and 26%) and protein (48, 47, 44 and 43%) levels with protein to energy ratios of 25, 24, 22 and 19 mg/kJ (Table 1). Proximate analyses of feed samples were performed following the Weende Methods of Analyses described by Akyildiz (1984). Analyses of fish muscle tissue were carried out according to Gökalp et al. (1993). Gross energy of the diets was estimated assuming 23.6 kJ/g protein, 39.5 kJ/g lipid and 17 kJ/g nitrogen free extracts

Fifty randomly selected rainbow trout (average weight 181±0.53 g/fish) were stocked in each pond (stocking density 0.86 kg/m^3). Two ponds were randomly allotted to each diet. To prevent stress during handling, fish were anesthetized with a benzocaine solution of 5%; 100 ml ethyl alcohol (94%) was well mixed with 5 g crystalline benzocaine and 1 ml of the solution was made up to 1 l with tap water (Allen, 1988; Allen et al., 1994). The fish were individually weighed and measured at the beginning and at the end of the experiment. The fish were weighed in mass every two weeks during the trial. The fish were fed at a level of 2% of their body weight per day at 8:00 and 17:00 for 70 days under a natural light regime. The feeding amount was recalculated every two weeks after the mass weighing of the fish.

At the end of the experiment, ten fish were sampled from each treatment (five from each replicate) for subsequent analysis. Muscle tissues were sampled between the lateral line and the dorsal fin from both sides of the fish. All analyses were performed in duplicate.

Calculation of the total nitrogen retention and excretion rates followed the method of Hamada et al. (1979).

Table 1. Nutritional composition of the experimental diets (% air dried basis).

	Diet (% lipid level/P:E ratio in mg per kJ)			
	Diet 1 (12/25)	*Diet 2 (13/24)*	*Diet 3 (17/22)*	*Diet 4 (26/19)*
Moisture (%)	8.08	7.74	7.24	6.84
Crude protein (%)	47.71	46.54	44.14	43.07
Crude lipid (%)	11.48	13.39	16.71	25.82
Crude fiber (%)	2.18	2.57	1.87	0.95
Crude ash (%)	10.22	9.86	10.78	6.88
NFE (%)[1]	20.33	19.90	19.26	16.44
Gross energy (kJ/g)[2]	19.30	19.71	20.34	23.21
P:E (mg/kJ)[3]	24.71	23.61	21.70	18.56
PE/gross energy (%)[4]	0.58	0.56	0.51	0.44

1 Nitrogen free extracts = 100 - (crude protein - crude lipid - crude fiber - crude ash)
2 Gross energy is calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid and 17 kJ/g NFE.
3 P:E = crude protein/gross energy
4 Protein energy/gross energy = energy derived from protein/gross energy

The statistical significance of differences between measured parameters was computed using analysis of variance (ANOVA, SPSS 10.0 for Windows). Duncan's new multiple range test (SPSS 10.0 for Windows, General Linear Model - Univariate procedure, Post Hoc Tests) was applied to determine significant differences between individual treatments when ANOVA detected that factors were significant ($p<0.05$).

Results

Growth performance is shown in Table 2. Growth increased significantly as the dietary lipid and gross energy increased, with no evidence of a plateau. The final body weight, relative growth rate and specific growth rate of diets 3 and 4 were significantly ($p<0.05$) higher than those of diets 1 and 2. No mortality was observed in any treatment. The condition factor was significantly higher in diets with lower lipid levels and higher P:E ratios than in diets with higher lipid and lower P:E.

Feed utilization is shown in Table 3. Daily feed intake, daily energy intake and feed efficiency increased as the dietary lipid increased and the P:E ratio decreased. In contrast, daily protein intake decreased with the increasing dietary lipid level and decreasing P:E ratio. Apparent net protein retention (ANPR) increased as the lipid level increased from 12% to 17% but declined at the level of 26% dietary lipid. The ANPR for each lipid level was significantly different from the others at the 5% level. The protein efficiency ratio increased as the dietary lipid increased and the P:E ratio decreased.

The feed conversion ratio (Table 4) decreased with a higher lipid level and lower P:E ratio. Total nitrogen excretion per net wet

Table 2. Growth, survival and condition factor of rainbow trout fed test diets for 70 days.

	Diet (% lipid level/P:E ratio in mg per kJ)			
	Diet 1 (12/25)	*Diet 2 (13/24)*	*Diet 3 (17/22)*	*Diet 4 (26/19)*
Initial wet weight (g)	181.75±0.50	181.29±1.13	181.75±0.92	181.10±1.41
Final wet weight (g)	353.17±1.65[a]	355.75±0.12[a]	392.33±2.36[b]	394.75±0.35[b]
Weight gain (g)	171.42	174.46	210.58	213.65
Relative growth rate (%)[1]	94.31±0.38[a]	96.33±1.16[a]	115.87±2.39[b]	117.98±1.90[b]
Specific growth rate (%/day)[2]	0.95±0.00[a]	0.96±0.01[a]	1.10±0.02[b]	1.11±0.01[b]
Survival (%)	100	100	100	100
Condition factor[3]	1.44±0.06[a]	1.42±0.04[a]	1.31±0.08[b]	1.34±0.10[b]

Values (mean±SD) with different superscripts in the same row are significantly different at the 5% level.

1 Relative growth rate = % increase in weight = (final wet weight - initial wet weight/initial wet weight) x 100

2 SGR = % increase in body weight per day = [(Ln final wet weight - Ln initial wet weight)/days] x 100

3 Condition factor = (weight/length^3) x 100

gain and as a percent of the nitrogen intake was significantly ($p<0.05$) reduced by increasing the dietary lipid level and decreasing the P:E, up to the gross energy level of 20.34 kJ/g (diet 3). Total nitrogen retention per net wet gain and as a percent of the nitrogen intake rose with the increasing lipid and decreasing P:E, up to the levels in diet 3, then fell.

The hepatosomatic index (Fig. 1) was significantly higher ($p<0.05$) in fish fed diets 1, 3 and 4 than in fish fed diet 2. The visceral weight (as a percent of the body weight) and the weight of the lipids deposited around the viscera (as a percent of the body weight) increased in a linear fashion as the dietary lipid content rose and the P:E ratio decreased. The weight of the gastrointestinal tract (as a percent of the body weight) also increased in a linear fashion, but with a slight plateau at the dietary lipid level of 13%.

The effects of the diets on the proximate composition of the fish tissue are given in Table 5. In all groups, the body fat level increased during the trial. The amount of body fat positively correlated with the dietary lipid level, while an inverse relationship was noted for body protein. The moisture content of the fish body followed the same pattern as the body protein, with the exception of the group fed diet 4, which did not differ significantly ($p>0.05$) from that of the group fed diet 3. Increasing the lipid level from 17% to 26% did not significantly influence the crude protein or crude ash contents of the fish tissue.

Discussion

In the present study the growth and feed efficiency of rainbow trout tended to improve with an increase of dietary lipid and decrease of dietary P:E. When the lipid content increased from 12% to 17%, the relative

Table 3. Feed utilization in rainbow trout fed test diets for 70 days.

	Diet (% lipid level/P:E ratio in mg per kJ)			
	Diet 1 (12/25)	*Diet 2 (13/24)*	*Diet 3 (17/22)*	*Diet 4 (26/19)*
Daily dry feed intake (g/fish)	4.08±0.02[a]	4.10±0.03[a]	4.34±0.01[b]	4.38±0.02[b]
Daily dry protein intake (g/fish)	1.95±0.01[a]	1.91±0.01[b,c]	1.92±0.01[b]	1.88±0.01[c]
Daily dry energy intake (kJ/fish)	78.79±0.46[a]	80.87±0.53[b]	88.32±0.28[c]	101.56±0.40[d]
Feed efficiency (%)[1]	55.14±0.05[a]	56.07±0.69[a]	64.28±0.80[b]	64.94±0.79[b]
Protein efficiency ratio[2]	1.26±0.00[a]	1.31±0.02[b]	1.57±0.02[c]	1.62±0.02[d]
Protein retention (g)[3]	32.99±0.48[a]	34.86±0.68[b]	42.69±0.33[d]	38.21±0.06[c]
ANPR (%)[4]	24.20±0.50[a]	26.08±0.34[b]	31.83±0.14[d]	28.96±0.07[c]

Values (mean±SD) with different superscripts in the same row differ significantly at the 5% level.

1 Feed efficiency = 100/feed conversion ratio (see Table 4)

2 Protein efficiency ratio = wet weight gain/dry protein intake

3 Protein retention = dry final body protein in g - dry initial body protein in g (See Table 5 for body composition)

4 Apparent net protein retention = [(final wet weight in g x final wet body protein in %) - (initial wet weight in g x initial wet body protein in %)/dry protein intake in g] x 100

growth rate increased significantly. The relative growth rate reached a slight plateau when the dietary lipid content increased from 17% to 26%, indicating that the required P:E ratio for maximum growth is at least 19 mg/kJ. Plateaus or decreases in weight gain of fish fed diets containing protein levels above the requirement have been observed in other species (Siddiqui et al., 1988; El-Sayed and Teshima, 1992).

Better growth or feed utilization of fish fed high energy diets, defined as the “protein sparing effect”, are similar to those reported for channel catfish (Page and Andrews, 1973), carp (Watanabe et al., 1987b), halibut (Helland and Grisdale-Helland, 1998) and Japanese flounder (Yigit, 2001). In contrast, negative effects of increased dietary lipid on growth and feed efficiency were observed for turbot (Caceres-Martinez et al., 1984) and flounder (Lee et al., 2000a; Lee et al., 2000b).

When protein is metabolized in high protein diets, energy might be lost in the form of excreted nitrogenous products, which can lead to low energy conversion efficiency. As the dietary protein content increases, protein efficiency is lowered because more protein is used as an energy source (Jobling and Wandsvik, 1983). Lee and Putnam (1973) reported that high energy diets containing 24% herring oil promoted excellent growth in rainbow trout. At this level of lipid, dietary protein was reduced one-third with no loss in weight gain, while feed conversion, energy conversion and protein utilization markedly improved (Lee and Putnam, 1973). In the present study, ANPR improved as the level of dietary energy increased. The high values of ANPR indicate that the digestible energy in

Table 4. Estimated total nitrogen excretion from rainbow trout fed experimental diets for 70 days.

	Diet (% lipid level/P:E ratio in mg per kJ)			
	Diet 1 (12/25)	*Diet 2 (13/24)*	*Diet 3 (17/22)*	*Diet 4 (26/19)*
Feed conversion ratio[1]	1.81±0.00[a]	1.78±0.02[a]	1.56±0.02[b]	1.54±0.02[b]
Total N intake (mg/g)[2]	127.23±0.11[a]	122.54±1.51[b]	101.92±1.27[c]	98.80±1.20[c]
N in 100 g diet (g)	7.63	7.45	7.06	6.89
N content in fish (%)	3.07±0.09[a]	3.14±0.07[a]	3.16±0.06[a]	2.96±0.31[a]
Total N retained (mg/g)[3]	30.79±0.66[a]	31.96±0.81[a,b]	32.44±0.26[b]	28.61±0.28[c]
Total N retained (% N intake)	24.20±0.50[a]	26.08±0.34[b]	31.83±0.14[c]	28.96±0.07[d]
Total N excreted (mg/g)[4]	96.44±0.55[a]	90.58±0.70[b]	69.49±1.01[c]	70.19±0.92[c]
Total N excreted (% N intake)	75.80±0.50[a]	73.92±0.34[b]	68.17±0.14[c]	71.04±0.07[d]

Values (mean±SD) with different superscripts in the same row differ significantly at the 5% level.

[1] Feed conversion ratio = g wet feed intake/g wet weight gain

[2] Total N intake = mg N/g net wet gain = total g protein intake/6.25/total g wet weight gain

[3] Total N retained = mg/g net wet gain = total g protein retained in fish/6.25/total g wet weight gain

[4] Total N excreted = mg/g net wet gain = total g nitrogen intake - total g nitrogen retained

the diets was sufficient. Similar conclusions were reported by Jobling and Wandsvik (1983) and Watanabe et al. (1987a).

The inverse relationship between the dietary protein level and feed intake agrees with Anguas-Velez et al. (2000). Our study shows that feed and protein utilization were affected by the dietary lipid level and the P:E ratio. The daily protein intake, estimated in grams of crude protein per fish per day, decreased while the ANPR improved as the lipid level increased from 12% to 17%, but declined at the lipid level of 26% and P:E ratio of 19 mg/kJ. This result can be explained by a possibly limited content of some essential amino acids in diet 4 which prevented further replacement of protein by lipids. The similar final weights and relative growth rates in fish fed diets 3 and 4 support this explanation. In the present study, the best FCR was obtained in fish fed diet 4, although it did not significantly differ with that of fish fed diet 3. It is possible that the maximum lipid toleration of this species is lower than 26%, when a more balanced diet is used.

The larger gastrointestinal tracts of fish fed higher lipid levels was mainly due to the increased size of the intestines and pyloric ceca. Similar results were reported by Lee and Putnam (1973). The increase in the size of the intestines and the pyloric ceca may have resulted from the slower passage of feed through the gut. Windell et al. (1972) reported that dietary lipid levels of more than 15% inhibited gastric motility.

The results of the present study demonstrate that the total nitrogen excretion (mg N

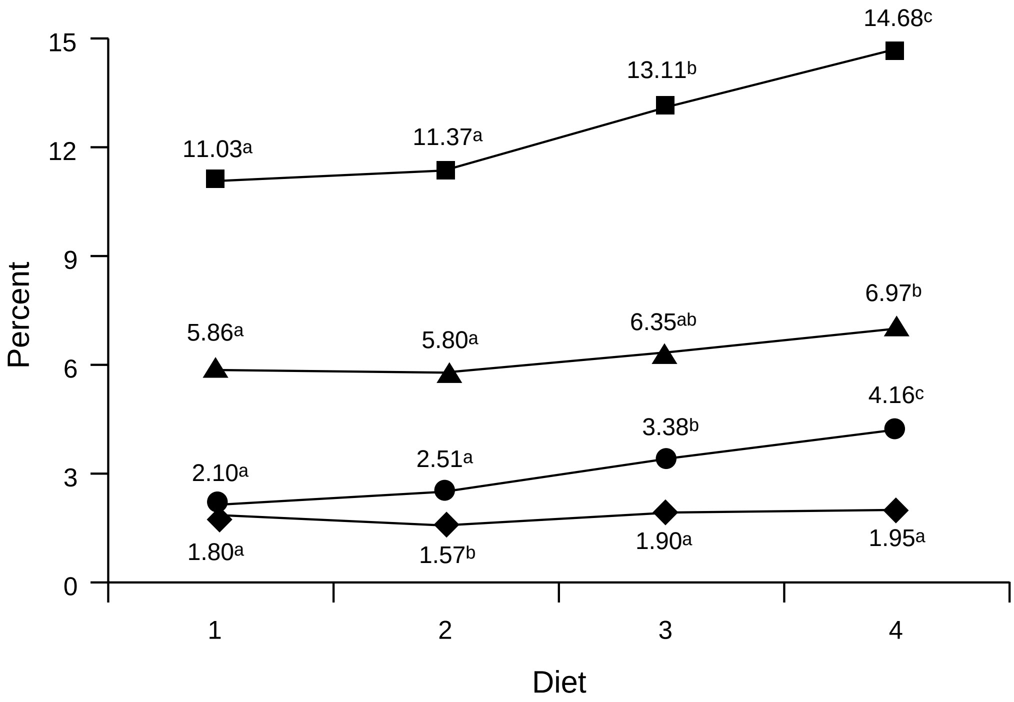

Fig. 1. Visceral weight (■), gastrointestinal tract weight (▲), lipid accumulation around the viscera (●) and hepatosomatic index (◆) of rainbow trout fed the experimental diets for 70 days. Values are the average of 10 fish/diet and are given as % of the body weight. Values with different superscripts on the same line differ significantly at the 5% level.

per g of wet weight gain) decreased about 27 mg/g as the dietary lipid increased from 12% to 17% and the P:E ratio decreased from 25 to 22 mg/kJ diet, indicating that total nitrogen excretion of rainbow trout can be reduced by about 27% of the present value by feeding "low protein:high energy" diets. Furthermore, the dietary protein level can be reduced from the currently provided 47-48% to 44% by raising the energy level. Similar results were reported for other species. Watanabe et al. (1987a,b) reported a total nitrogen reduction of 30-40% in carp by feeding "low protein: high energy" diets containing 35-37% instead of 40% crude protein.

Oliva-Teles and Rodrigues (1991) reported that 60-69% of the nitrogen intake was excreted in 240 g rainbow trout fed a diet containing 44% protein. This result is close to the value obtained in the group fed diet 3 (68%) in the present study. Conversely, Smith and Thorpe (1976) reported values of 52.7-53.7% in 30-40 g rainbow trout. Lanari et al. (1993) and Dosdat et al. (1996) also found lower values (49% and 42%) in rainbow trout weighing 100 g fed dietary protein at levels of 39% and 55%, respectively. These discrepancies are probably due to different diet qualities and feeding conditions. Comparisons are difficult for salmonids, since the nitrogen balance is influenced by the proportion of the total energy that is supplied by lipid in the feed (Beamish and Thomas, 1984; Arzel et al.,1994).

Table 5. Proximate composition of fish tissues of rainbow trout fed the test diets for 70 days.

	Initial*	Diet (% lipid level/P:E ratio in mg per kJ)			
		Diet 1 *(12/25)*	*Diet 2* *(13/24)*	*Diet 3* *(17/22)*	*Diet 4* *(26/19)*
Moisture (%, wet weight)	74.45±1.13	74.45±1.13	73.52±0.52	72.82±0.45	73.18±2.63
Crude protein (%, dry basis)	75.16±1.10	75.16±1.10^{a}	73.98±0.18^{ab}	72.72±0.17^{b}	69.01±0.42^{b}
Crude lipid (%, dry basis)	13.04±0.62	13.04±0.62^{a}	16.09±0.05^{b}	20.12±0.98^{c}	23.34±0.47^{d}
Crude ash (%, dry basis)	5.35±0.14	5.35±0.14^{a}	5.20±0.08^{a}	4.53±0.13^{b}	4.13±0.28^{b}
Crude protein (%, wet basis)	19.2	19.2	19.59	19.77	18.51

* All fish in the experiment were fed diet 1 from the fingerling stage to the beginning of the trial. Group 1 continued to receive diet 1 during the trial. Hence, group 1 was considered the control group and the initial composition of all fish was assumed to be the same as the fish in group 1.

Ntrogen retention (as a % of the nitrogen intake) was highest (32%) with diet 3 (44% crude protein; 17% lipid; 20.34 kJ gross energy/g feed; P:E ratio 22 mg/kJ), indicating that digestible energy in this diet was sufficient. Our findings agree with results reported by Oliva-Teles and Rodrigues (1991), whose nitrogen retention was 30.6-40.1% in rainbow trout fed a diet containing 44.5% protein, 14.3% lipid and 19.6 kJ gross energy/g feed.

It can be concluded that for an optimum P:E ratio, the lipid level should be less than 26% and the protein content at least 44% to obtain maximum growth and best protein use in rainbow trout under the conditions applied in this study. It is suggested that future investigations study lipid levels lower than 26% and dietary protein of at least 44% to clearly establish the optimum protein to energy ratio for this species.

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