GROWTH AND FEED UTILIZATION OF LARGE SIZE RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*: DIET AND EFFECTS

— research paper —

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Abstract: Four diets differing in crude protein/crude lipid concentrations (CP/CL), 570/200, 510/220, 460/240, 430/260 (g kg^{-1} dry diet) were fed to near-satiety to rainbow trout (initial body weight, 1BW = 268 g,) for 308 days to determine the effect of diets, and fish size on efficiency of feed, nitrogen (N) and energy utilization. Weight gain, feed efficiency (FE), and energy retention efficiency (ERE, E gain/E intake) were not affected by diet ($P < 0.05$). N retention efficiency (NRE, N gain/N intake) increased linearly ($P < 0.05$) with decreasing CP/CL. There was a significant ($P < 0.05$) linear decrease in FE as fish grew, regardless of diet. NRE linearly decreased ($P < 0.0001$) and lipid to protein deposition ratio (LD/PD) increased ($P < 0.05$) as trout grew.

Keywords: Rainbow trout, diet, energy, growth

INTRODUCTION

Rainbow trout are one of the most important salmonid species of major economic interest for commercial culture worldwide and salmonid feed costs constitute more than 40% of the production costs. Over the last decade much effort has been and still is put into optimizing feed composition and feeding strategies for these species. Most of these studies have aimed at improving the dietary protein utilization for growth by replacing dietary protein by non-

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protein energy sources such as lipids and to a lesser extent digestible carbohydrates.
There is no doubt that non-protein energy sources can spare dietary amino acids from being utilized as energy sources, thereby improving efficiency of protein utilization for PD in rainbow trout (Cho et al., 1988), (Ruohonen et al., 1998), (Steffens et al., 1999). These findings have been of major importance for the economical and environmental sustainable development of commercial culture of that salmonid.
The objective of this study was to investigate the effects of different dietary protein/lipid ratios on growth and feed, N and energy utilization efficiency by rainbow trout. The second objective of this study was to investigate the effect of diet on the efficiency of utilization of feed, N and energy utilization for body gain, N and energy retention, respectively as fish grew.

MATERIALS AND METHODS

Diets

Four diets were formulated to be isoenergetic (gross energy, GE = 24 MJ kg^{*1}, approximately 20 MJ kg^{-1} digestible energy) but contain different protein to lipid ratios (Table 1). The dietary crude protein/crude lipid (CP/CL) ratios were 570/ 210, 510/220, 460/240 and 430/260 g kg^{-1} dry diet (Table 1). Ingredients were obtained from local suppliers. The diets were mixed using a mixer and pelleted to appropriate size using a laboratory steam pellet mill. The feed was subsequently dried in a forced air drier at room temperature for 24 h and then sieved. Part of the fish oil was incorporated in the mash during mixing and part was sprayed on the top of the dry pellets. The diets were kept at 4 °C until used and only the amount required for each week was kept at room temperature.

Fish and experimental conditions

Rainbow trout [1^{+}age (1-year-old fish or older), initial body weight (IBW) =268 g 3% CV (mean, coefficient of variation)] were obtained from the Doripesco Prod SRL, farm of Hărman.
Fifty-five fish were randomly allocated into each of 24 rectangular fiberglass tanks (1087 l) with three tanks per diet. Each tank was considered an experimental unit. The aquatic system was supplied with well water at 26 l min^{-1}. Water temperature averaged 8.5°C (±0.2) throughout the feeding trial. Oxygen and flow rates were measured weekly. Dissolved oxygen never fell below 7 mg l^{-1}.

Fish were acclimatized to the experimental conditions for a period of 2 weeks, during which time they were fed a commercial trout diet. Fish were then fed the experimental diets for 308 days. The fish were carefully hand fed to near-satiety the experimental diets twice a week as two meals a day. During the other days of the week, fish were fed the experimental diets in predetermined rations by belt-feeders, programmed to discharge feed twice a day at times similar to hand feeding. This ration was calculated to be slightly restricted (ca. 5-10% restriction) compared with the voluntary feed intake measured on the days the fish were handfed in order to avoid feed wastage. Mortality and temperature were checked daily, and feed intake weighed every day. Fish were weighed every 28 days.

Table 1. Composition of experimental diets

Diet	1	2	3	4
Ingredients (g kg^{-1} as is basis)				
Fish meal, herring, 68% CP	340	290	250	220
Blood meal, spray dried, 84% CP	100	100	100	100
Corn gluten meal, 60% CP	340	290	250	220
Wheat middling, 17%CP	-	61	92	110
Whey, 10% CP	48	55	75	89
Celite AW521[1]	10	10	10	10
$CaHPO_4$	-	-	3	5
L-lysine	2	4	5	6
Vitamin premix	10	10	10	10
Mineral premix	10	10	10	10
Fish oil, herring	140	170	195	220
Determined diet composition, dry matter basis				
Dry matter (g kg^{-1}, as is basis)	941	936	949	948
Crude protein (g kg^{-1})	570	511	463	431
Crude lipid (g kg^{-1})	200	222	238	256
Ash (g kg^{-1})	86	79	77	75
Gross energy (MJ kg^{-1})	24.0	24.4	24.7	24.9
Calculated P/E ratio (g MJ^{-1}) [2]	23.7	20.9	18.7	17.3

[1] Celite AW521 (acid-washed diatomaceous silica) is a source of acid-insoluble ash.
[2] P/E ratio, protein/energy ratio, calculation based on crude protein and gross energy values determined for the experimental diets.

Fish sampling

On the first day of the experiment, 12 fish were randomly selected and frozen at -20°C, until analysis. This procedure was repeated at 84, 168 and 308 days in the feeding trial (5-10 fish sampled per tank). Whole fish, dressed carcass and viscera were weighed, and samples of dressed carcass

(including kidney) and of viscera (including liver and gonads) were pooled (per tank) and frozen for analysis of whole body chemical composition.
Whole fish bodies were cooked in an autoclave. After cooling, a few drops of liquid antioxidant were added to each pan. The autoclaved fish carcasses were then ground into homogeneous slurry in a blender. The ground samples were transferred into shallow dishes, frozen and subsequently lyophilized. These samples were then reground and stored at -20 °C prior to analysis.

Chemical analysis

Ingredients, diets and fish carcass were analysed for dry matter and ash according to Romanian standards, crude protein (CP, %N x 6.25) using a Kjeltec auto-analyzer, lipid using the method of Bligh & Dyer (1959) and gross energy (GE) using a automated bomb calorimeter.

Calculations and statistical analysis

Thermal-unit growth coefficient (TGC) were calculated as:

$(TGC) = [(FBW^{1/3}-IBW^{1/3}) / \Sigma (TxD)] \times 100$

where FBW, final body weight (g); IBW, initial body weight (g); T, water temperature (°C); and D, number of days.

Feed efficiency (FE) was calculated as

FE= [live body weight gain (g)/dry feed intake (g)].

Dressed carcass yield (DCY, %) was calculated as

DCY (%) = [(dressed carcass weight/total fish weight) x 100].

N-retention efficiency (NRE) was calculated on a tank basis according to the following formula:

$NRE\ (\%) = 100x\ [(FBWxN_{final}) - (IBWxN_{initial})]/$ Gross N intake

Energy retention efficiency (ERE) was calculated as follows:

$ERE\ (\%) = 100x\ [(FBW \times Energy_{final}) - (IBW \times Energy_{initial})]/$ Gross energy intake

where, FBW, final body weight (g); IBW, initial body weight (g)

Digestible N (DNI) and energy intakes (DEI) for each diet were calculated as:

DNI = gross N intake x ADCi (N) and

DEI = gross E intake x ADCi (energy)

where i = diet 1, 2, 3 and 4.

The DNI and DEI replaced gross N and energy intake, respectively to calculate the digestible N retention efficiency (DNRE) and digestible energy retention efficiency (DERE) on the previous NRE and ERE calculations.

Energy retained as lipid (LD, kJ day^{-1}) was calculated as lipid gain x 39.5 kJg^{-1} [3] where,

Lipid gain(g day^{-1})= [(FBWx$Lipid_{final}$)-(IBWx$Lipid_{initial}$)]/Day

Energy retained as protein (PD, kJ day^{-1}) = protein gain x 23.6-kJ g^{-1} (Brafield and Llewellyn, 1982)

where:

Protein gain (g day^{-1})= [(FBW x $Protein_{final}$) - (IBW x $Protein_{initial}$)]/ Day

All data were analysed using the GLM procedure from SAS (1990) and the Brown and Forsythe's test (SAS 1990) was used to test for homogeneity of variances for all the dependent variables prior to any other statistical analysis. Weight gain, TGC, feed intake (dry matter basis), FE, NRE and ERE, DCYs and whole body composition were calculated for the entire experiments and the responses were averaged on a tank basis. These dependent variables were analysed initially by analysis of covariance (ANCOVA) with initial fish body weight (or final body in the case of whole carcass composition) as the covariate factor and the main effects of species, diet and species x diet interaction, as well as interactions of covariate with each of the previous effects. If covariate effect was not significant or interaction of covariate factor with treatment effects were significant, the previous model was simplified by removing the covariate effect and any interaction effect of covariate with treatment factors.

When significant effects were found *(P* < 0.05) for treatment factors, the orthogonal polynomial (linear and quadratic) contrasts were provided for each dependent variable. A critical level of *P* < 0.05 was adopted for all the tests

The FE, NRE, ERE and LD/PD were analysed by a repeated measures ANOVA model to evaluate the responses over time as fish grew. The repeating variable was measured based on retentions between two consecutive sampling periods, with each tank being the experimental unit.

RESULTS

Growth and feed utilization: diet effects

Weight gain and growth rate (expressed as TGC) were not affected by diet *(P* > 0.05; Table 2). Despite of no effect of diet on weight gain and FE of trout, feed intake of these fish increased linearly *(P* < 0.05; Table 2) as dietary protein/lipid ratio decreased from 570/ 210 to 430/260.

Table 2. Growth and feed intake of rainbow trout (IBW=268g) fed diets with different protein/lipid (CP/CL,g kg^{-1} dry feed) ratios for 308 days at 8,5°C

	Gain (g $fish^{-1}$)	TGC	Feed intake (g $fish^{-1}$)	FE (gain $feed^{-1}$)
Rainbow trout				
Diet 1, CP/CL = 570/210	1249	0.198	1537	0.81
Diet 2, CP/CL = 510/220	1238	0.197	1560	0.79
Diet 3, CP/CL = 460/240	1313	0.204	1658	0.79
Diet 4, CP/CL = 430/260	1308	0.204	1647	0.79
Significance[1]				
Linear	NS	NS	$P < 0.05$	NS
Quadratic	NS	NS	NS	NS
SEM	52	0.00481	32	0.030
Effects				
Diet	NS	NS	$P < 0.05$	NS

[1] Significance of the orthogonal linear and quadratic contrasts of dependent variables across diets. TGC, thermal-unit growth coefficient [9; 5]; FE, feed efficiency; SEM, standard error mean (n=3); NS, not statistically significant (P > 0.05); IBW, initial body weight.

Dietary protein/lipid ratio had significant effect *(P* < 0.05) on NRE as indicated by the observed significant linear increase of NRE *(P* < 0.05; Table 3) as dietary protein/lipid ratio decreased. ERE was not affected by diet *(P* > 0.05; Table 3)

Table 3. **ANOVA** table for retention efficiency of nitrogen and gross energy for rainbow trout fed diets of varying protein/lipid (CP/CL, g kg^{-1} dry feed) ratios for 308 days at 8.5°C

	NRE (% nitrogen intake)	ERE (% energy intake)
Rainbow trout		
Diet 1, CP/CL = 570/210	26.5	42.7
Diet 2, CP/CL = 510/220	29.1	38.7
Diet 3, CP/CL = 460/240	31.6	41.2
Diet 4, CP/CL = 430/260	33.5	40.1
Significance[1]		
Linear	P < 0.05	NS
Quadratic	NS	NS
SEM	1.4	1.8
Effects		
Diet	P< 0.05	NS

[1] Significance of the orthogonal linear and quadratic contrasts of dependent variables across diets.NRE, nitrogen retention efficiency; ERE, energy retention efficiency; SEM, standard error mean *(n* = 3); NS, not statistically significant (P> 0.05).

Carcass traits and whole body composition: diet and effects

Whole body composition data was analysed initially with a covariate model where final fish weight was included in the model as the covariate factor. However, as this effect was not significant it was removed from the statistical model.

The DCY represented 88% of the whole body weight for trout and no effect of diet on DCY was observed ($P > 0.05$). The diet had no effect on CP of trout with decreasing dietary protein/lipid ratio. The whole body water and lipid contents of trout were not affected by diet ($P > 0.05$; Table 4).

Table 4. Whole body composition of rainbow trout (FBW = 1545 g) fed diets with different protein/lipid (CP/CL, g kg^{-1} dry feed) ratios for 308 days at 8.5°C

	Water (g kg^{-1})	CP (g kg^{-1})	Lipids (g kg^{-1})	Ash(g kg^{-1})
Rainbow trout				
Diet 1, CP/CL = 570/210	641	169	166	19
Diet 2, CP/CL = 510/220	660	170	150	21
Diet 3, CP/CL = 460/240	646	172	165	20
Diet 4, CP/CL = 430/260	634	170	166	20
Significance[1]				
Linear	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS
SEM	1,3	0.5	0.7	0.07
Effects				
Diet	NS	NS	NS	NS

[1] Significance of the orthogonal linear and quadratic contrasts of dependent variables across diets. CP, crude protein; FBW, final body weight; SEM, standard error mean ($n = 3$); NS, not statistically significant ($P > 0.05$).

Growth and feed utilization: responses as fish grew

A significant linear decrease of FE was observed fish as they grew and this decrease was not affected by diet. FE decreased for rainbow trout (from 0.93 to 0.72)

DISCUSSIONS

Diet effects

The diets used in this study did not affect growth and FE. This suggest that feeds with dietary protein/lipid ratios ranging from 570/210 to 430/260 can support good growth performance, at least for fish weighing more than 200 g. These results are in agreement with the results by other authors for large size rainbow trout (Steffens et al., 1999), (Lanari, 2002). The growth rate (TGC) of trout was similar to other studies with rainbow trout (Iwama,

1996). The NRE was significantly affected by diet indicating differences in dietary amino acid catabolism between fish fed the different diets. NRE significantly increased with decreasing dietary protein/lipid ratio, confirming a protein sparing effect from non-protein sources of energy as reported by numerous authors (Cho et al., 1988), (Johnsen et al., 1991), (Johnsen et al., 1993), (Ruohonen et al., 1998), (Steffens et al., 1999). ERE was not affected by diet indicating that the digestible energy was used as efficiently whether it was supplied as protein or non-protein energy sources (lipids and carbohydrates).

Dietary protein/lipid ratio had no effect on DCY. A number of studies have reported similar carcass yield in large salmonid fish fed diets with different lipid contents (Rasmussen et al., 2000). Conversely, the use of lipid-rich diets (low DP/DE ratios) has been found to decrease carcass yield of rainbow trout (Rasmussen, 2001) at least in fish of smaller sizes. Differences among these studies with fish of different sizes suggest that the effect of dietary protein/lipid ratio on carcass yield may be size specific. In addition, as different dietary protein/lipid ratios can be created by modifying diet composition in a variety of ways, different types of modifications of the diet to achieve different P/E may result in different responses for smaller fish but not for larger fish.

Dietary protein/lipid ratio had only small effects on whole body composition of trout. Despite some statistical significant effects detected, the differences were in general small. A dietary lipid content varying from 200 to 260 g kg^{-1} (DM basis) had no effect on lipid content of trout carcass.

Basis for difference in FE between fish of different sizes

This study provides solid evidence that FE is size dependent in salmonids.

Difference in FE between fish of different sizes could be mainly related to (i) differences in digestibility of nutrients and energy (ii) differences in body composition (amount of nutrient accreted/unit of weight gain) and (iii) differences in cost and efficiency of growth (energy requirement per unit of weight gain, efficiency of conversion of nutrients into body components, e.g. dietary amino acids into body protein).

Differences in digestibility

The nutrient and energy digestibility of the diets used in the present study were determined with rainbow trout of smaller sizes (< 200 g fish) than fish used in the present study (Azevedo et al., 2004). This study found

significant interactions between diet and species for digestibility of lipids, energy and dry matter. When N and energy retention results from the present study were analysed as a function of digestible protein and energy intakes, rather then the gross intakes, the effect of diet was the same for NRE. Digestible N retention efficiency linearly increased from 28, 32, 34 to 36% of rainbow trout as the dietary protein/lipid ratios decreased from 570/210, 510/220, 460/ 240 to 430/260, respectively. This decrease was in agreement with the NRE response to diet as ADC of N was also not affected by species (Azevedo et al., 2004). Higher calculated DNRE for salmon compared with trout suggests better efficiency of N utilization for growth by salmon compared with trout when fed the same dietary protein/ lipid ratios.

Differences in body composition

At similar ERE, more dietary energy is required to support the same weight gain for fish of higher carcass energy concentrations, resulting in lower FE (Bureau et al., 2002).

As trout grew, carcass lipid and energy concentration increased while protein concentration remained relatively constant and, consequently, LD/PD ratio increased as trout grew. These changes in body composition (i.e. increase in energy density *of* whole body), correlate well with the linear reduction of FE observed as trout grew.

Differences in energetic cost of growth and efficiency of conversion of dietary nutrients into body components

Difference in FE could also be linked to differences in terms of cost of growth or efficiency of conversion of dietary nutrients into body components. As such, examining body composition (protein and lipid gains), as well as NRE and ERE, is essential to understand conversion efficiency of dietary nutrients into body components and, consequently, FE. Changes in cost of growth may well explain part of the decrease in FE of salmon as they grew as ERE was observed to decrease with fish size. It has been suggested that maintenance energy requirement of larger fish may be greater than that of smaller fish per unit of energy deposited (Cho, 1992) Consequently, ERE would be expected to slightly decrease with increasing body weight.

The metabolic/biochemical cost for protein accretion is generally higher than the cost of lipid accretion (per unit of energy deposited) (Reeds, 1991). This should result in a lower efficiency of energy utilization for PD (k_p)

compared with that for LD (k_f). Values found in the literature appear to support this assumption, as reported k_p values for fish range from 0.4 to 0.6 while k_f- values range from 0.7 to 0.9 (Meyer-Burgdorff et al.,1995), (Schwarz et al., 1995), (Lupatsch et al., 1998), (Rodehutscord, 1999), (Lupatsch et al., 2001).
The efficiency of conversion of dietary protein into body protein is yet another factor that could explain the difference in terms of FE between fish of different sizes. PD is associated with substantial water deposition whereas lipid depots contain little water (Cho et al., 1990). Protein gain consequently appears to be what drives weight gain (Cho et al., 1990), (Bureau et al.2002) Similar protein intakes will result in a higher PD and weight gain in an animal with higher NRE. As NRE decreased in trout as fish grew, the size-specific decrease in FE in this species could also be explained in a similar fashion.

CONCLUSIONS

The different response of NRE and ERE between fish of different sizes is a clear demonstration that the efficiency of amino acids and other energy-yielding nutrients utilization for protein and other body component accretion is size dependent.
Energy source had no effect on energy utilization efficiency by rainbow trout.

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