Maintenance Energy Requirements in Rainbow Trout (*Oncorhynchus mykiss* W., 1792) Fed at Largely Varying Feeding Intensities

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Özet: *Geniş yoğunluk aralıklarında beslenmiş gökkuşağı alabalıklarında (*Onchorhynchus mykiss ***Walbaum, 1792) enerji gereksiniminin sağlanması.*** Bu çalışmada alabalıklarda yaşam ve verim payı için enerji ihtiyaçları araştırıldı. Yapılan 4 deneyde; geniş besleme aralığında (doygunluktan açlık sınırındaki kısıtlı yemlemeye kadar), farklı enerji seviyelerindeki (düşük ve yüksek) yemlerle beslenen, farklı başlangıç ağırlıklarına sahip (25 gr, 31gr, 65 g, 101 g, 176 g) ve yaşlarda (1, 2 yaş) gökkuşağı alabalıkları kullanıldı. Su sıcaklığı 15ºC olarak sabit tutuldu. En kısa süreli deney 50, en uzun süreli olanı 229 gün sürdü. Yem enerji ve besin maddeleri sindirimleri belirlendi. Deneme öncesi ve sonrasında homojenize edilmiş balıkların enerji, protein ve yağ oranları belirlendi. Sonuç olarak BW^x sabiti 0.21 olarak bulundu. Vücut ağırlık kazanımıyla depolanan enerji için kullanılan kısmi sindirilebilir enerji (BW sabiti 0.21 olarak hesaplandığında) tüketimi verimi 0.76 bulundu.

Anahtar Kelimeler: Besleme yoğunluğu, sindirilebilir enerji, kısıtlı yemleme, metabolik vücut ağırlığı.

Abstract: This study examined the maintenance and growth energy requirements of rainbow trout. In four experiments, trout of different ages (1 and 2) with different initial body weights (25g, 31g, 65g, 101g, 176g) were fed with different energy diets (high or low), on a wide range of feeding intensities (from satiation to restrictive feeding near hunger levels). Experiments lasted 50 days for the shortest and 229 days for the longest. Water temperature maintained at 15ºC. Energy and nutrient digestibilities were measured. Energy as well as protein and fat content were determined of the fish before and after the experiments from body homogenates. It is concluded that exponent of BW^x is 0.21. Retained energy as function of digestible energy (DE) intake (BW was raised to the power of 0.21), showed that partial efficiency of DE intake for retention was 0.76.

Key Words: Feeding intensity, digestible energy, restricted feeding, metabolic body size.

Introduction

Successful fish culture depends on the supply of diets containing optimal level of energy and nutrients for growth. As the feed represents the major cost for the fish farmer formulation must be based on sound knowledge of nutritional requirements for it to be economical. The feed that is not consumed will be lost to water surrounding and cause pollution. On the other hand, feeding should not be limited that the fish will suffer from underfeeding which could cause the decrease of fish quality. Many studies examined the effects of feeding regimes in terms of restrictive feeding having the above mentioned concerns to improve sound feeding systems with variable fat and protein levels and to define zero growth, which is needed to be applied to find the exact maintenance energy requirements. But such attempts of restrictive feeding have never been used beyond a few feeding levels (Storebakken *et al.* 1987, Austreng *et al.* 1987, Lupatsch *et al.* 1998), and is always done in terms of percent body weight per day and scarcely define the meaning and impact of varying a wide range of restrictive feeding intensities. Thus, more elaborated experimental feeding methods are essential than just finding the feeding level for below, at or above maintenance levels. The partitioning of digestible energy (DE) had been quantified by applying regression procedures to growth data, already done in many works for terrestrial livestock (ARC, 1981) in order to balance energy needs of animals and energy supply by feed. In fish species, factorial approach based on retention measurements has rarely been applied (Kirchgessner *et al.* 1984, Rodehutscord *et al.* 1999, Lupatsch *et al.* 1998, 2003). These evaluations to apply factorial approach for developing equations which allow the estimation for DE requirement for maintenance were limited with restricted data, for instance with regards to the different growth rates, or with regards to dietary fat, protein concentrations and fish weight. Therefore, this study is designed with wide range of feeding intensities using various energy level diets and trout of various ages and sizes to evaluate and determine the energy requirements for maintenance.

Materials and Methods

Experiments were carried out in a partial recycling Aquaculture System at the department of Animal Nutrition of Agricultural Faculty, Bonn University. The experimental system consisted of 24 circular shaped plastic culture tanks (in which the experimental fish were kept that were continuously supplied with water in parallel with about 70% of the out flowing water). Each culture tank had capacity of 250 l formed part of the culture system that had a water flow of 4-5 l per minute. Details of the system of circulation can be seen in

Sanver (2004). A sedimentation unit is attached to each tank which allows collection of faecal samples. The water temperature is adjusted and maintained at about 15ºC. All the rainbow trout (*Oncorhynchus mykiss*) were selected from a homogenous population at the Department of Animal Nutrition in Bonn.

For the first and second experiments 24 groups of 20 trout each, with an average initial weight of 101 g per trout; in the third experiment 6 groups had an average initial weight having 25 g per trout and trout of 12 groups weighed on average 31 g per trout; in the fourth experiment, all groups consisted of 20 fish with average initial weight 65 g per trout in 12 groups and 176 g per trout in the other 12 groups. Zero groups were killed by overdosed Benzocain (4-Ethyl-Aminobenzoat) and subsequently frozen for initial body composition analysis. Mean body weights per trout of zero groups were similar to initial body weights of experimental trout. At the end of the first experiment all groups were killed and then kept in deep freezer for the subsequent whole body analyses. In the second, third and fourth experiments after killing and weighing the group biomasses, fish were weighed individually and then half of each group were sorted out on weight basis to represent the whole group; these were combined and used for whole body analysis. Data on mortality (weight, date, group) was recorded individually.

In the first experiment four diets varying in fat and protein concentrations were used. Experimental diets were formulated by combining one of two fat concentrations (High Fat = about 300 g/kg vs. Low Fat = less than 200 g/kg) with one of two protein concentrations (High Protein = about 500 g/kg vs. Low Protein = about 400 g/kg). In experiments 2 and 4, High Fat and Low Protein diet (HFLP) was fed. In experiment 3, the diet with High Fat and High Protein concentration (HFHP) was fed. Diets fed in experiment 1, 2 and 3 were manufactured simultaneously on the other hand diet HFLP fed in experiment 4 was delivered later from a different charge. All the four diets consisted of fish meal, fish oil, soybean meal, wheat, vitamin and mineral premix of varying concentrations. The diets were formulated and produced by the company Nutreco. The feed ingredients were ground by using a hammer mill before mixing. Pellets were produced using a Wenger TX twin screw extruder. Oil was coated on the extruded pellets using a vacuum coater. Yttrium oxide was included as inert marker. The proximate composition of nutrients and energy (g or MJ per kg DM feed) of experimental diets are presented for each diet for each analysis respectively. HFHP (DM,%: 95.9; GE, MJ: 24.80; CP, g: 502; Lipid, g: 285; Ash, g: 106, Y_2O_3, mg: 96.5), HFLP (DM,%: 95.4; GE, MJ: 25.40; CP, g: 387; Lipid, g: 325; Ash, g: 83; Y_2O_3, mg: 83.6), LFHP (DM,%: 92.8; GE, MJ: 22.16; CP, g: 484; Lipid, g: 175; Ash, g: 100; Y_2O_3, mg: 81.2), LFLP (DM,%: 93.3; GE, MJ: 21.65; CP, g: 398; Lipid, g: 164; Ash, g: 81.6; Y_2O_3, mg: 86.6)

Fish were fed twice daily once in the morning and once in the evening but on weekend days feeding was done once a day resulting to 12 feedings per week. In the first experiment each diet was fed either to satiation or at a restricted rate. In feeding to satiation: feed was fed until the first pellet sank to the bottom of the tank. Consumption of three groups fed to satiation for each diet was calculated so as to determine the quantity to be fed to the fish that were fed at restricted rate. The dietary quantities to be fed at restricted rates were calculated as:

$$\frac{0.7 \times consumption\ when\ fed\ \ to\ satiation\ (g)}{(initial\ body\ weight\ (g) + accumulated\ consumption (g))}$$

In the first experiment, groups fed to satiation began to be fed one week earlier than groups fed at a restricted rate so as to enable calculations of the amount of feed to be fed to the fish fed at restricted rate in the proceeding week. Experimental feeding of the restricted rate fed fish started and ended one week later than the groups fed to satiation so as to enable same feeding durations. In the second experiment fish were offered feed at varying intensities. All the groups in the experiment consumed about 3 kg of feed which resulted in different experimental durations. The quantity of the feed to each group, corresponded to about 150% of its initial biomass, but the duration varied widely between treatments. The value of 3 kg feed fed to each group was drawn from the average consumption of groups fed to satiation in the first experiment. Feeding schemes were calculated according to the equation below. All variables in this equation are known except the duration in days (d) which could be calculated: Total consumption (g) = (IBW*DFI)*(1+DFI)^d IBW: Initial body weight (biomass) at the beginning DFI: Daily feed increase was from 2.00 until 0.5. At the highest feeding intensity experiment lasted 55 days while at the lowest intensity experiment lasted 229 days. For instance; The amount of feed consumed on the first day of the experiment by the groups fed at the highest intensity (2.00% of IBW) and the groups fed at the lowest intensity (0.5% of IBW) is calculated as follows:

Feed consumption on day no.1= (IBW*DFI)*(1+DFI)^d
                            = (2025 g * 0.02)*(1+0.02)^1
                            = 41.3 g (highest intensity)
in comparison to
                            = (2092 g *0.005)*(1+0.005)^1
                            = 10.5 g (lowest intensity)

In the third and fourth experiments the same equation was used to calculate feeding schemes. In the third experiment fish were fed with two different feeding intensities, with the aim of reaching two significantly different final body weights per trout at the end of 127 days of feeding, which will subsequently be used in experiment 4. In the fourth experiment the total amount of consumption for each group was almost equal to the initial biomass of the groups.

Identical analyses were applied for diets, faeces, and body homogenates. Dry matter was calculated weight loss after 24 h drying at 105ºC. Ash was analysed by overnight to constant weight at 550ºC. Crude protein was measured using the Dumas Method and multiplying N by 6.25. Total lipids

were determined by HCl digestion of samples followed by petroleum ether extraction. Energy was measured by Adiabatic calorimetry principle (Bomb calorimetry). Yttrium analyses were done in feed and faeces by ICP-AES-Inductively Coupled Plasma-Atomic Emission Spectroscopy.

In Fig.1, non-linear accretion of fat was defined by the following exponential model; in the GRAPHPAD PRISM 3.0 statistical package. $y = a (1-e^{-b (x-c)})$ (x = nutrient gain (g per trout); a = asymptote; b = parameter to write the curve bend; c = DFI of the nutrient accretion that is zero.) Determination of the exponential value (power) of BW which is referred to as metabolic body size was quantified by using the following equation: IDE $(kJ.d^{-1}$per trout) = a*RE $(kJ.d^{-1}$per trout) +b* BW^x (kg) (Equation) (IDE: Digestible Energy Intake, RE: Retained energy, BW: Body weight). This allometric equation was evaluated by using non-linear multiple regression with iterative by using Levenberg-Marquard-Method. Analyses were carried out with SAS statistical software package for Windows version 8. Graphs and diagrams used in Discussion section were done by Graphpad Prism 3.0.

Results

Growth, Lipid and protein accretion of trout in experiments:

Experiment 1: The first experiment was designed to test the hypothesis that diets containing high fat with simultaneously low protein concentration will have no negative influence on performance parameters in experimental as well as practical conditions. Primary performance results approved this hypothesis with a profound significant influence of dietary fat concentration and feeding in gain as well as in feed conversion efficiency.

Experiment 2: The sole purpose of this experiment was to study the performance of the fish in a broad of feeding intensities. Therefore only one feed was chosen. Since the HFLP produced highest efficiencies of utilization of DE as well as digestible crude protein, this diet was chosen. After experimental data were used in regression analyses, accretion showed a non-linear course which follow the course of biological function. Curves of protein and lipid gain in relation to the eight DFI with their replications are shown in Fig.1 They follow the pattern of a biological curve in which the amount of accretion increases as the amount of lipid consumption increases and tends towards a plateau after a particular amount of lipid in the diet is consumed. And this explains why feeding at a restricted rate increased the overall protein deposition per unit gain. This plateau is an upper limit of lipid accretion. Relationship between DFI and protein gain as shown in Figure 1 levels out, making the relationship not of great interest to the study.

It is difficult and probably biologically erroneous to extrapolate the curves to the area of negative accretion of the negative weight gain, since experimentation was not carried in this area. The fourth experiment was therefore designed to cover this area so as to try and locate at what DFI accretion was zero. In order to effect variation and further validate the results, it was necessary to use fish of two significantly different initial body weights and added 4 DFIs lower than the ones used in the previous experiment.
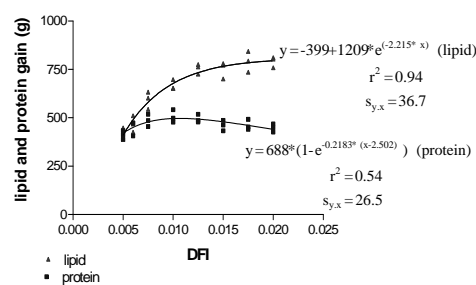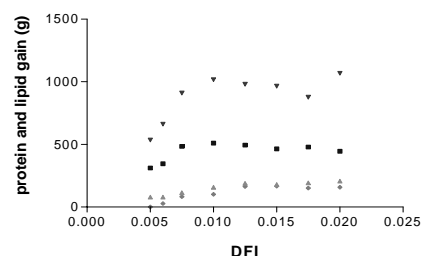

Figure 1. Relation of lipid and protein gain to DFI in experiment 2.

Experiment 3: This experiment was conducted with this sole purpose of attaining two different final weights for the experiment. To achieve this, only two DFIs were used in sufficiently large number of trout. Data on growth was measured and nutrient balances were also studied. At the end of the experiment two significantly different body weights of 65 and 176 g per trout as planned were achieved. The results of nutrient balances confirmed results discussed in the previous experiment wherein, the more the restriction the less fat was deposited.

Experiment 4: Within each of the two populations of initial BW, groups fed at the upper eight DFI consumed almost identical quantities of feed, though in widely varying durations. Accretion of lipids and protein of these groups during the experiment is shown in Figure 2.

▼ lipid gain heavy grp.
■ protein gain heavy grp.
▲ protein gain light grp.
◆ lipid gain light grp.

Figure 2. Relation of lipid and protein gain to DFI of the highest eight feeding intensities each which were fed comparable quantities in experiment 4.

In all the experiments in general, energy digestibility exceeded 80% and it was at its lowest at the highest feeding intensities and increased with decreasing feeding intensity and maintained a constant level. Digestibility ranges of energy and nutrients in this study are reflected in Table 1.

When all the data from all the experiments on the lipid and protein gain in g per kg BW per day and the digestible energy intake in kJ per kg BW per day (Figure 3 and Figure 4) are pooled into a linear regression the groups with light initial body weight in experiment 4 are seen to be remarkably far from the

general regression line. This could mean that body weight of the fish has a significant influence on the lipid accretion.

Table 1. Energy and nutrient digestibility ranges in this study.

Digestibility ranges in the study		
	Lowest (%)	Highest (%)
Energy	82	94
Protein	93	94
Lipids	78	98
Total carbohydrates	60	80

$RL = 0.014 * DEI - 1.05$

$r^2 = 0.83$

$s_{y.x} = 0.585$

- ▲ Exp.1
- ▼ Exp.2
- ✕ Exp.3
- ■ Exp.4 heavy grp.
- ● Exp.4 light grp.
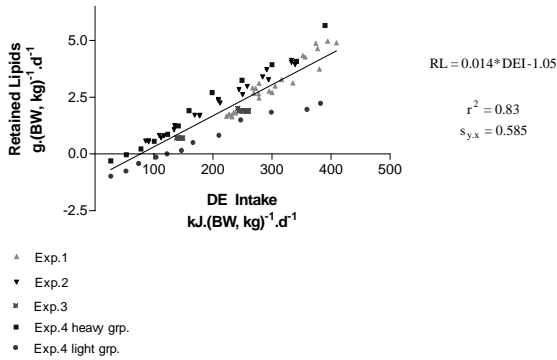
Figure 3.   Relationship between lipid gain in g.(BW, kg)$^{-1}$.d^{-1} and intake of digestible energy in kJ. (BW, kg)$^{-1}$.d^{-1} in the entire study.

$RP = 0.009 * DEI - 0.47$

$r^2 = 0.89$

$s_{y.x} = 0.310$

- ▲ Exp.1
- ▼ Exp.2
- ✕ Exp.3
- ■ Exp. 4 heavy grp.
- ● Exp. 4 light grp.
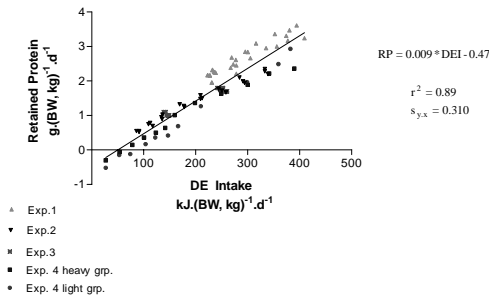
Figure 4.   Relationship between protein gain in g.(BW, kg)$^{-1}$.d^{-1} and intake of digestible energy in kJ.(BW, kg)$^{-1}$. d^{-1} in the entire study.

Relationship between retained energy and digestible energy intake in kJ.(BW, kg)$^{-1}$ in the entire study is shown in this Figure 5 A. The linearity of the relationship seems reliable since the r^2 is high. But the treatments of light groups in experiment 4

are relatively far from the regression line and tend to indicate another linear regression line from the general one which further confirms a significant effect caused by the significantly difference in initial body weights. The efficiency of utilization of digestible energy intake for energy retention is 0.75. But when the BW was raised to the power of 0.80 (the generally accepted BW power or the generally used metabolic body size for the fish) as shown in Fig. 5 B, treatments of these groups come a bit nearer to the regression line, r^2 gets higher. However, the values obtained here were still far from the general regression. Efficiency of utilization of digestible energy intake for energy retention is 0.74. If all the experimented data from all experiments were pooled and put into the following non-linear multiple regression analysis: IDE (kJ.d^{-1}per trout)= a*RE (kJ.d^{-1}per trout) + b*BW^x (kg) (Equation). The values of 1.31±0.053, 14.7±7.264, 0.21±0.219 were for a, b and x. Using these values one could generate the equation: IDE=1.31 ($\pm$0.053)*RE+14.7 ($\pm$7.264)*BW$^{0.21(\pm 0.219)}$ When BW was raised to the power of 0.21 (Figure 5 C), the regression line was in total agreement and the entire data set lies on the regression line. In Table 2, r^2 increases from 0.90 to 0.92 by changing the power of BW from 1 to 0.80 and even more so to 0.97 by changing BW power from 0.80 to 0.21 which is the exponent derived from these studies. However, r^2 is not the only criterion for evaluating. Standard error (S$_{y.x}$) is also an important criterion to evaluate whether the regression line improves from one transformation to another. When the body weight exponent is 1, DE intake ranges from 25 to 407 kJ. (BW, kg)$^{-1}$.d^{-1} and RE is from -33.5 to 253 kJ. (BW, kg)$^{-1}$.d^{-1}, RE has a mean value of 143 with the regression line's S$_{y.x}$ that is 24.3 and the standard deviation as percent of mean RE is 17. With raising the BW to the exponent 0.80, DE intake ranges from 27 to 542 kJ. (BW, kg)$^{-0.80}$.d^{-1} and RE varies from –41.6 to 339 kJ. (BW, kg)$^{-0.80}$.d^{-1}. RE has a mean value of 190.5, S$_{y.x}$ increases to 27.2. But the standard deviation as percent of mean RE decreases to 14. When the BW is raised to the power of 0.21, DE intake ranges from 28 to 1364 kJ. (BW, kg)$^{-0.21}$.d^{-1} and RE varies from -104 to 911 kJ. (BW, kg)$^{-0.21}$.d^{-1} with a mean value 508. S$_{y.x}$ is 40.5, and the standard deviation as percent of mean RE decreases to 8. This shows not only measure of determination but also mean standard deviation of the regression are optimised by choosing 0.21 as exponent for converting body weight into metabolic body size.
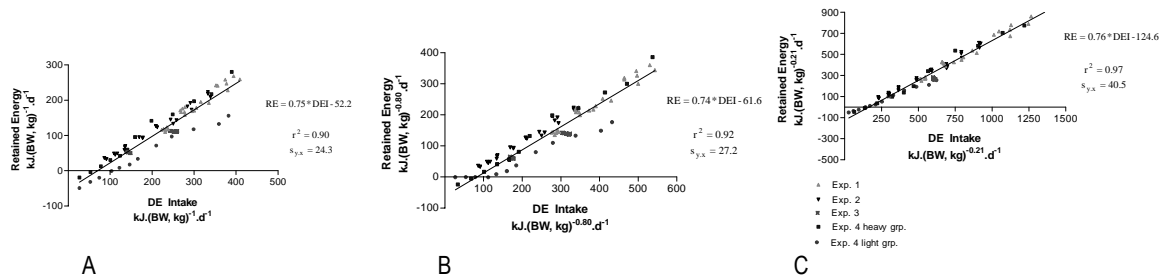

A   B   C

Figure 5.   Relationship between retained energy and digestible energy intake in kJ.(BW, kg)$^{-1}$.d^{-1} (A), kJ. (BW, kg)$^{-0.80}$.d^{-1} (B), kJ. (BW, kg)$^{-0.21}$. d^{-1} (C) in the entire study, respectively.

Table 2. Comparison of fitness of curves using various exponents.

Exponent for Metabolic body size	r^2	$S_{y.x}$ as% of mean RE
1.0	0.90	17
0.8	0.92	14
0.21	0.97	8

Discussion

First detailed attempt to examine the partitioning of digestible energy for maintenance and growth in fast growing rainbow trout via multiple regression analysis (Rodehutscord and Pfeffer, 1999) evaluated data from 292 groups fed a large variety of different diets for which the DE intake was assumed to be the factor limiting growth and presented an equation. Multiple regression suggested that maintenance requirement for DE was dependent on the 0.43 power of body weight in grams. However, application of this equation was only suitable with data of feeding from high to moderate feeding intensities of this study and failed to validate lower feeding intensities. The light groups of experiment 4 show that the equation could not be relied on if the fish are lighter inweight. The equation is, therefore, not only restricted by the feeding regime but also by fish weight. Body weight exponent of 0.21 found in this study contradicts to almost the entire literature. This value is way below the generally used average. Considerable amount of research exists on the estimation of body weight power (exponent) which refers to metabolic body size. Huisman (1976) reported the value of 0.80 that both for rainbow trout and carp. Hepher (1988) reported the wide variation for this exponent in a review, 0.82 being the average value. Beck (1987) evaluated starvation experiments in trout and an exponent of 0.833 for fish weighing between 8 and 400 g. Hoogendoorn (1983) determined a value of 0.86 for African catfish *Clarias lazera*. Cui and Lui (1990) presented 0.855 in six different teleost species. Cho (1992) used 0.824 for trout. Lupatsch *et al.* (1998) found 0.83 for gilthead sea bream *Sparus aurata* L. However, the data set of these studies were not only restricted but also limited by the narrow variation in factors such as dietary protein, lipid concentrations and fish size. The only study with the exception of its result regarding to low body weight exponent was done by Rodehutscord and Pfeffer (1999). Value of 0.43 was derived from wide range of data set with variation in experimented factors on rainbow trout. This suggests that such a decrease in exponent presented in this study seems more credible for use since the variation in factors such dietary nutrients and fish size are studied largely. However, with future studies certain areas need to be covered to concretise the validity of this exponent. It is therefore recommended that further research should be carried out on fish with initial body weights lower or higher than those studied here, large variation ranges of protein and fat in the diet, using different breeds and possibly different species of freshwater fish.

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