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EFFECTS OF FEEDING LEVELS ON GROWTH PERFORMANCE, AND BODY COMPOSITION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM 1792)

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Abstract

In this study we investigate the effect of different feeding levels (FL1-2.5%BW day^{-1}, FL2- 3%BW day^{-1}, FL3-3.5% BW day^{-1}, FL4-4 %BW day^{-1}, FL5-4.5 %BW day^{-1} and, ad libitum-FL6) on the growth performance, morphologic indexes and body composition of rainbow trout (Oncorhynchus mykiss) reared in a recirculating aquaculture system. 360 juvenile rainbow trout (34.17±0.11 g, mean±SD) were randomly distributed to 12 rearing units for 44 days. At the end of the trial period, the ANOVA test showed that growth performance was significantly affected by feeding levels ($p<0.05$). Final mean body weight, weight gain, and specific growth rate(SGR) increased with increasing feeding level. Feed conversio rate (FCR) was below 0.7 for all groups, except FL6 were FCR registered a value of 0.83 (g/g). The protein efficiency ratio was significantly affected by the feeding level ($p<0.05$) and the best value was obtained for FL1 group (2.59±0.05 g/g). Significant changes ($p<0.05$) in ash, protein, lipids, and water content were observed at the end of the experimental period. Protein and lipids contents of rainbow trout meat increased with increasing feeding level, while moisture content and ash significantly decreased. Also, this increasing trend was observed in terms of the hepatosomatic index, but without significant differences between groups ($p>0.05$). The viscerosomatic index of fish the FL1, FL2, and FL3 was significantly lower than those from FL4, FL5, and FL6. Based on the obtained results, it is recommended, from an economic point of view, a feeding level of 2.5 % BW day^{-1} as optimum for rainbow trout, reared in a recirculating aquaculture system, from 30g to 130 g.

***Key words**: Feeding rate, growth performance, rainbow trout, body composition.*

INTRODUCTION

Feeding management practices (feeding level, feeding frequency or feeding delivery system) affect fish conversion rates and fish growth, size heterogeneity and has a significant contribution on feasibility and economics of commercial success of aquaculture (Du et al., 2006).
The main goal of trout aquaculture is to maximize growth at minimal cost because feed represents at least 40-60% of the total production costs (Hurye et al., 2010; Wendy et al., 2013). Of the feeding practices, feeding level is the most important variable and influences growth and feeds conversion rates. When fish are overfed, the digestive efficiency and the fish growth is reduced (Sanver, 2005) and poor water quality can occur (Mihelakakis et al., 2002; Serap and Fikret, 2009). On the other hand, underfed fish do not reach maximal growth, and may exhibit aggressive behavior due to limited feed availability, and the variability of fish sizes increases (Dwyer et al., 2002).
Feeding level ranges from the maintenance feeding level (at which fish neither gain nor lose weight), to the maximum feeding (maximum amount of fed the fish can consume). Over this range, feeding level reaches a maximum point beyond fish cannot consume the fed and the efficiency of feed conversion is reduced and weight gain no longer increases in direct proportion with the feeding level (Lovell, 1998).
The effects of feeding levels on fish growth and feed conversion efficiency have been studied for rainbow trout (Sanver, 2005; Kok and Siau, 2006; Bureau et al., 2006), but the results of these studies are quite variable because there are several factors which influence the feeding level, such as fish size (Mihelakakis et al., 2002), temperature (Azevado et al., 1998; Bailey and Alanärä, 2006), rearing systems (Cho et al., 2003).
In this context, the purpose of the present study

was to determinate the optimum feeding level on growth performance, feed efficiency and body composition of rainbow trout reared in a recirculating aquaculture system, from 30 g to 130 g.

MATERIALS AND METHODS

Experimental design and feeding trial. The experiment was carried out in a Recirculating Aquaculture System at „Dunărea de Jos" University, Galați, România. The experimental system consisted of 12 glass tanks, with a capacity of 132 L, each. 360 Trout fingerlings brought from fish farm Prejmer, Brașov, România, were stocked into a rearing tank for two weeks as an acclimatized period and then randomly distributed in the rearing units in such a manner to create homogenous groups with similar class frequencies and number. At the beginning of the experiment, the initial average weight±SD of fish was 34.17±0.11 g. All the fish were fed with extruded pellets with 54% protein content and 18% lipids (Table 1).

Table 1. The composition of the experimental diet

Composition	U.M	Quantity
Crude protein	%	54
Crude lipids	%	18
Cellulose	%	1
Ash	%	10
Phosphorus	%	1.4
Digestible energy	Mj/kg	19.4
Vitamin A	UI	14000
Vitamin D3	UI	2300
Vitamin E	mg	250
Vitamin C	mg	500
Lysine	%	3.5
Methionine	%	1.5
Cystine	%	0.7
Ingredients: Fish meal, fish oil, hemoglobin, full-fat soy, soybean oil, wheat gluten, sunflower meal, wheat,and wheat products.		

Fish were fed twice daily at 9 a.m. and 6 p.m. at different feeding levels (FL1-2.5%BW day^{-1}, FL2- 3%BW day^{-1}, FL3-3.5%BW day^{-1}, FL4-4 %BW day^{-1}, FL5-4.5%BW day^{-1} and, ad libitum-FL6). In the *ad libitum* feeding, fish were fed until the first two or three pellet remains to the bottom of the rearing units, and usually, this action lasted for one hour. Fecal matter was removed by siphoning every morning, before feeding and if in the *ad libitum* feeding remains any uneaten feed, this was filtered, dried and weighed in order to quantify the exact amount of consumed feed.

All experimental variants were performed in duplicate.

The main physicochemical water parameters, temperature, pH and dissolved oxygen (DO), were measured daily with a Hach-Lange equipment Sc 1000. Nitrogen compounds (N-NO_2^-, N-NO_3^-, N-NH_4^-) were determined periodically with the Spectroquant Nova 400 type spectrophotometer, using kits from Merk. Water temperature was 17.37±1.08˚C, the oxygen content was 7.46±0.256 mg L^{-1} and pH was 6.93±0.206 pH units. Regarding the dynamics of the nitrogen compounds, the average values of nitrite, nitrate, and ammonium were, 0.09±0.05 mg L^{-1}, 142.251±40,935 mg L^{-1}, and 0.115±0.075 mg L^{-1}. Although nitrate concentrations were higher, many authors reported that concentrations around 200 mg L^{-1} may be acceptable for fish growth, with no serious consequences on the short term (Colt and Armstrong, 1981).

At the end of the feeding trial all the fish were individual weight and the growth and feed utilization parameters were calculated using the following standard formulas: weight gain (WG) = final body weight (W_{t1}) – initial body weight (W_{t0}) (g), feed conversion ratio (FCR) = total feed (F)/total weight gain (W), specific growth rate (SGR (%body weight day^{-1})) = [(Ln W_{t1} – Ln W_{t0})/t] × 100, coefficient of variation (%), protein efficiency ratio (PER) = total weight gain (WG)/amount of protein fed (g). For all equations, W_{t0} and W_{t1} are the initial and final body weight of fish and t is the experimental period of the trial.

Sample preparation and analysis of biochemical composition. At the end of the study, a sample of eight fish was taken from each experimental group randomly for the determination of body composition and for body condition indices such as Hepato-somatic index ((HSI)=100 (liver weight (g)/ body weight (g)) and Viscero-somatic index ((VSI)=100 (visceral weight (g)/body weight (g)). The determinations of biochemical composition of fish were performed on muscle tissue samples according to AOAC (2000): Proteins were determined with Gerhardt type equipment by using the Kjeldahl method (N × 6.25), fats were determined by Soxhlet solvent extraction method (petroleum ether) with Raypa extraction equipment, dry matter was determined by heating at a temperature of 105±2ºC using Sterilizer Esac and ash was evaluated by calcification at temperatures of 550±20°C in a Nabertherm furnace.

Statistical analysis. One-way ANOVA was used to compare the effects of feeding level on the growth performance and feed utilization. To determine the significant differences among the experimental variants, Duncan's test for multiple comparisons of means was performed. All statistical analyses were conducted using SPSS version 21 and were assessed at a significance level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Survival percent and growth performance of juvenile rainbow trout fed six different levels of feeding are presented in Table 2. No mortality was observed in any of the groups during the entire experimental period. At the beginning of the experiment, the normal distribution of the fish population (in terms of body weight and individual length) was statistically analysed and confirmed by statistical tests. The initial mean weight was between 34 ÷ 34.28 g and the total length was between 14.58 ÷ 14.72 cm, with no statistical differences between the experimental variants (ANOVA, $p>0.05$).

At the end of the experiment, statistically significant differences were found between the fish weight and length from the six experimental variants (ANOVA, $p<0.05$). Generally, body weight and length increased in response to increasing of feeding levels.

In fact, Duncan's analysis divided the final weight of fish into five distinct groups (FL1; FL2; FL3; FL4, respectively FL5, and FL6) (Figure 1).

Significant differences ($p<0.05$) were registered in the final length of the fish.

Fish from FL1 have a significantly lower length than those from FL2 and FL3, while in the FL4, FL5 and FL6 groups, feeding level did not significantly influence the total length of the fish.

Table 2. Growth performance of juvenile rainbow trout fed with various feeding levels

Feeding levels	Initial body weight (IBW) (g/fish)	Initial body length (IBL) (cm/fish)	Final weight (FBW) (g/fish)	Final length (FBL) (cm/fish)	Individual weight gain (g/fish)	Survival (%)	SGR	FCR	PER
FL1	34.22 ± 5.62^{a}	14.59 ± 0.92^{a}	87.33 ± 14.18^{a}	19.83 ± 1.31^{a}	53.12 ± 1.88^{a}	100±0.0	2.13 ± 0.04^{a}	0.71 ± 0.02^{a}	2.62 ± 0.08^{a}
FL2	34.08 ± 6.41^{a}	14.66 ± 1.11^{a}	94.63 ± 15.62^{b}	20.26 ± 1.34^{b}	60.56 ± 0.54^{b}	100±0.0	2.32 ± 0.01^{b}	0.74 ± 0.01^{b}	2.50 ± 0.02^{b}
FL3	34.21 ± 5.32^{a}	14.60 ± 0.76^{a}	104.07 ± 15.83^{c}	20.73 ± 1.05^{b}	69.86 ± 0.84^{c}	100±0.0	2.53 ± 0.02^{c}	0.75 ± 0.01^{b}	2.46 ± 0.03^{b}
FL4	34.20 ± 4.60^{a}	14.88 ± 0.74^{a}	115.23 ± 17.35^{d}	21.40 ± 1.20^{c}	81.04 ± 0.68^{d}	100±0.0	2.76 ± 0.01^{d}	0.74 ± 0.01^{b}	2.50 ± 0.02^{b}
FL5	34.28 ± 6.01^{a}	14.59 ± 1.06^{a}	123.10 ± 23.01^{e}	21.75 ± 1.43^{c}	88.82 ± 1.38^{e}	100±0.0	2.91 ± 0.03^{e}	0.76 ± 0.01^{b}	2.43 ± 0.05^{b}
FL6	34.00 ± 6.41^{a}	14.58 ± 1.14^{a}	124.33 ± 23.11^{e}	21.67 ± 1.48^{c}	90.33 ± 0.39^{e}	100±0.0	2.95 ± 0.01^{e}	0.82 ± 0.02^{c}	2.25 ± 0.04^{c}

Mean value of 2 replicates ± SD; on the same row mean values with the same superscript are not significantly different ($p>0.05$)

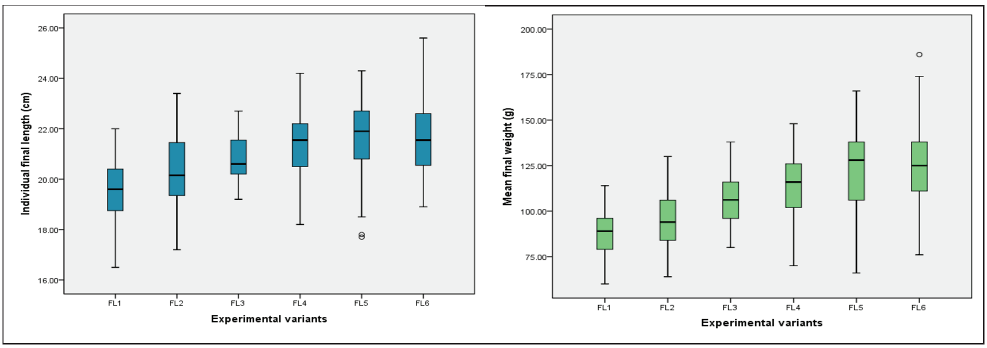

Figure 1. Individual final weight and the final length of juvenile's rainbow trout

Significant differences ($p<0.05$) were observed in weight gain, SGR, FCR, and PER of juvenile rainbow trout fed various feeding levels.

In order to have a precise image of the relationship between the feeding level and weight gain and feeding level and SGR, second-degree polynomial regression analysis was performed. So, mean body weight gain = -0.6737 x^2 + 12.521x + 39.78; (r^2=0.984) and SGR = -0.0176 x^2 + 0.297x + 1.8265; (r^2=0.991), (where x= the feeding level) (Figure 2). Increasing of feeding level resulted in increased weight gain and SGR of juvenile rainbow trout, but at FL5 and FL6 (which was calculated at the end of the experiment at 5% BW day^{-1}), reached a plateau, fact which means that an increase of the feeding level does not necessarily lead to the somatic growth of the fish. The fish from the FL6 and FL5 had the highest weight gain, while the fish from the FL1 showed the lowest weight gain among all the experimental groups. However, there was significant ($p<0.05$) difference in weight gain between FL2, FL3, and FL4.

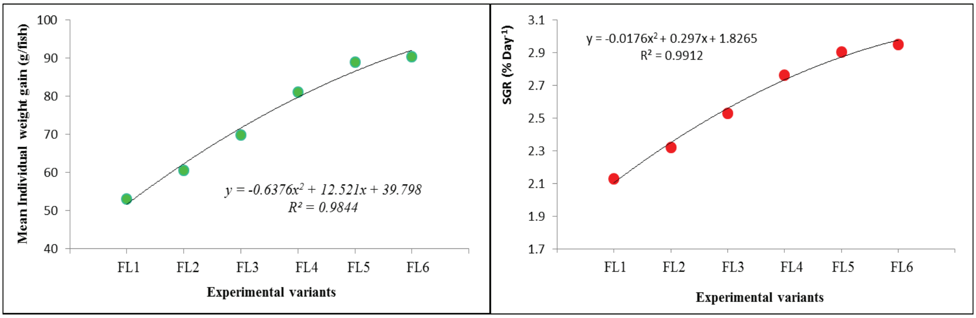

Figure 2. Changes in individual weight gain (g/fish) and specific growth rate (SGR) of juvenile rainbow trout fed with various feeding levels

Comparing the obtained values of SGR, the statistical analysis revealed no significant differences ($p>0.05$) between the experimental variants, FL5 and FL6, respectively significant differences ($p<0.05$) between FL1, FL2, FL3, and FL4.

Regarding the feed conversion ratio (FCR) and protein efficiency ratio (PER) ($p<0.05$), the best values was found in the case of FL1. No significant differences were reported between the FL2, FL3, FL4, and FL5, while in the case of FL6, it was registered the highest FCR and the lowest PER. However, a further increase in the feeding level resulted in no improvement of FCR and PER. According to Tvenning and Giskegjerde (1997), a poor FCR at higher ration levels appears because fish took a long time to consume food in order to reach satiation, thus causing the loss of nutrients and wastage of food.

Effects of feeding levels on body composition and morphological indices are shown in Table 3.

Table 3. Proximate composition (percentage of wet weight) of juvenile rainbow trout fed with various feeding levels

Feedinglevels	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	HSI (%)	VSI (%)
FL1	84.180±0.148^a	12.673±0.358^a	2.304±0.119^a	1.455±0.019^a	7.68±0.026^a	0.985±0.06^a
FL2	83.925±0.148^a	12.790±0.057^a	2.398±0.048^a	1.431±0.042^a	7.909±0.235^a	1.085±0.158^a
FL3	83.165±0.502^b	13.650±0.297^b	2.765±0.163^b	1.381±0.019^b	8.464±1.705^a	1.093±0.077^a
FL4	82.323±0.527^b	13.865±0.064^b	3.153±0.126^b	1.369±0.035^c	8.651±0.390^a	1.135±0.093^b
FL5	81.555±0.573^c	14.395±0.502^c	3.392±0.057^c	1.270±0.034^d	8.821±0.699^a	1.165±0.131^b
FL6	80.625±0.460^c	14.745±0.078^c	3.615 ±0.035^c	1.238±0.023^d	10.502±0.132^a	1.254 ±0.131^b

Mean value of 3 replicates ± SD; on the same row mean values with the same superscript are not significantly different ($p>0.05$)

Moisture, protein, lipid, and ash was significantly affected by the feeding level ($p<0.05$). Body moisture content decreased significantly ($p<0.05$) with the increasing of ration levels. The proteinand lipid content was found to be significantly high ($p<0.05$) in fish from FL5 and FL6 and a significant fall ($p<0.05$) was evident in fish from FL1 and FL2. Regarding the ash and moisture content, it was observed a significantly decreased ($p<0.05$) with the increase of feeding levels.

The results obtained by us are comparable to those reported by other authors. Storebakken and Austreng (1987) reported in the case of rainbow trout an increase in the lipid content, proportional to the level of feeding. Also, Palmegiano et al. (2008) obtained for rainbow trout with an individual weight of 180 g, a significant decrease in water content, while proteins and lipids significantly increased with feeding levels (1.2%, 1.4%, 1%, 8%, and *ad libitum*). In a recent study, Bureau et al. (2006) reported an increase of moisture content and a decreasing of the percentage of lipids in lower feeding levels (restricted by 25%, 50%, 75% or 100%).

Rasmussen and Ostenfeld (2000) reported that fat accumulation is prevalent during fast growth at high feeding levels, while fat deposition is subtle during slow growth due to the presence of adequate energy in excess feed deposition as fat. Also, according to Rasmussen and Ostenfeld (2000), the decline of the ash content at higher feeding levels is caused by relatively low skeletal growth compared with other tissue.

HSI and VSI of fish were gradually increased as the increasing feeding level. No significant ($p>0.05$) differences were found in HSI, while VSI from FL1, FL2, and FL3 was significantly lower than those from FL4, FL5,and FL6. Generally, HSI may provide information on growth, physical condition, energy reserves and the ability of fish to tolerate the environmental stresses. In a poor, stressful and unfavourable environment, fish usually have a smaller liver which means less energy reserved (Mihelakakis et al., 2002; MdMizanur et al., 2014). Increasing values of HSI and VSI at higher feeding levels were also confirmed by Storebakken and Austreng (1987), which after 6 weeks obtained a doubling of the HIS values from 0.71 ± 0.10% in the case of a feeding level of 0% to 1.40 ± 0.20% at a feeding level of 2%.

In aquaculture farms, feeding fish under the satiation level without affecting the growth is strongly recommended, mainly because of production costs. Thus, the determination of optimum feeding level is one of the key factors for better growth, feed conversion, nutrient retention efficiency and chemical composition of fish (Zhang et al., 2011). According to Jobling et al. (1994), the optimal feeding level corresponds to that feeding level at which the best FCR of the feed is obtained, while Cho et al. (2003) suggests that maximum growth occurs at the limit of voluntary food intake (satiation). In our study, increasing of the feeding level showed lower nutrient utilization and wastage of food, respectively a lower economic efficiency, since similar FCR is achieved between the FL2, FL3, FL4, and FL5 groups.

The results obtained by us are supported by the research of other authors (Van Ham et al., 2003), which, in the case of higher feeding levels, noticed a decrease of digestive efficiency, due to the increased metabolic consumption generated by the catching food processes, digesting and metabolizing the amount of feed that goes beyond the optimal nutritional level. Therefore, according to some authors (Van Ham et al., 2003; Ahmed, 2007) fish tend to optimize their digestion to extract nutrients more efficiently at lower feeding levels.

In the present study, it appears that the daily feed application feeding level of 2.5% BW day^{-1} was near to optimum when the fish grew from 30 to 130 g. Earlier studies have also reported different optimal feeding rate at rainbow trout. Storebakken and Austreng (1987) reported an optimum feeding level of 2% BW day^{-1} for rainbow trout weighing 0,5- 1,0 kg, while Imtiaz (2018) recommended for rainbow trout (5,65±0,45cm; 1,42±0,25g) a feeding level between 4,6 to 5,3% body weight. For rainbow trout with the weight of 300 g, Storebakken and Austreng (1987) reported that maximum feeding efficiency was obtained when the quantity of food was given 2% BW day^{-1}.

CONCLUSIONS

As a result of the present study it can be concluded that the increasing of the level of feeding, can lead to a qualitative production, from nutritional point of view, but the choice of the optimum feeding level should also take into account the efficiency of the feed utilization (FCR), and the fact that too much feed can lead to deterioration of water quality and the occurrence of mortality. Taking into account the higher cost of feeding for rainbow trout the results of this study suggest that the optimum feeding level for juvenile rainbow trout growing from 30 g to 130 g was 2.5% BW day-

[1] if we take in consideration the main growth parameters FCR and SGR.

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