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Effects of feeding levels on growth performance, feed utilization, body composition, energy and protein maintenance requirement of fingerling, rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792)

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Abstract

A 10-week feeding experiment was conducted to evaluate the effects of feeding level, energy and protein maintenance requirement of rainbow trout, *Oncorhynchus mykiss*, fingerling (5.65±0.45cm; 1.42±0.25g) by feeding casein-gelatin based purified diet (45% CP; 3.67 kcal g^{-1} GE) at six feeding levels from 2 to 7% of BW/day in two equal meals, at 0800 and 1700 h, in triplicate, with 20 fish per trough equipped with continuous water flow-through system water (60L volume). Maximum live weight gain, best feed conversion ratio (FCR), best specific growth rate (SGR) and highest protein efficiency ratio (PER) were reported in fish fed 4-5% BW day^{-1}. However, quadratic regression analysis for weight gain, FCR, PER, protein retention efficiency and energy retention efficiency data indicated the break-points occurred at 5.33, 4.50, 4.48, 4.63 and 4.74% BW day^{-1}, respectively. Body composition also produced significant (p<0.05) differences with respect to each feeding level, maximum protein, lowest moisture and intermediate fat contents were reported at 4-5% feeding levels. Protein and energy retention values also produced significant (p<0.05) differences among each feeding level with maximum values were reported at 5% feeding level. Based on the results, it is recommended that feeding in the range of 4.6 to 5.3% BW day^{-1}, corresponding to 2.07-2.39g protein and 16.88-19.45 kcal energy $g100g^{-1}$ of the diet day^{-1} is optimum for the growth and efficient feed utilization of rainbow trout, while 2-3% feeding levels (0.90-1.35g protein and 7.34-11.01 kcal energy) suggests that these amounts approximate the maintenance requirement of fish.

Keywords: *Oncorhynchus mykiss*, Feeding rate, Growth performance, Maintenance requirement

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Introduction

Supplementary feed being the most critical and major part of the production cost, and is thus considered as a determining factor in successful economy (Booth *et al.*, 2008; Zheng *et al.*, 2014). Therefore, judicious feed management may not only enhance the production performance, but also reduces the production cost and effluent production which help to mitigate any environmental impact on culture operation.

The growth of fish at all stages is largely affected by quality of food, feeding level, feeding frequency, feed intake and its ability to absorb the nutrients (Biswas *et al.*, 2006; Xie *et al.*, 2011). Since, feed is considered as one of the largest single item in aquaculture in terms of recurring expenditure of fish production. Therefore, quantification of feeding rate of cultured fish is necessary to achieve maximum production, and also useful for the determination of nutrient requirement in fishes (Garcia-Ortega *et al.*, 2009; Ahmed, 2010). Determination of the optimum feeding rate has a significant contribution in improving the feasibility and economic of commercial success of aquaculture through better feed utilization, reduce feed cost and also to minimize the feed loss, reduce water pollution (Mihelakakis *et al.*, 2001; Woods, 2005; Du *et al.*, 2006), and also help for reduction in size heterogeneity (Dwyer *et al.*, 2002). The relationship between growth rate and feeding level is very important because the expenditure of feed itself is at least 50% of the cost of intensive farming (Gunther *et al*., 1992).

The qualitative and quantitative aspects of feed consumed have a significant impact on growth rate, efficiency of feed utilization and chemical composition of fish (Lovell, 1989; Pickering, 1993; Biswas *et al.*, 2006), influence morphometry; such as lower condition factor and fat content which resulted changes in fatty acid profile of the fish (Kiessling *et al*., 2005; El-Saidy and Gaber, 2005). Optimum feeding rate and feeding frequency influence feed intake, feed utilization, and growth (Grayton and Beamish, 1977). At food intake levels higher than the optimum growth, increase is negligible (Tsevis *et al*., 1992), whereas sub-optimal ration may result in reduced growth and increased size variation (Johnston *et al*., 2003). Therefore, determination of optimum feeding rate is necessary to achieve the success of any aquaculture operation especially in early stage, where the fish is very susceptible to over and under feeding scenario, both resulting in increased incidences of disease and mortality (Yuan *et al*., 2010).

Several studies address the potential importance for determination of feeding level in fishes with manipulate feeding regimes on growth, survival and body composition in many cultured fish species in order to improve cultured efficiency and the details of these publications have been reviewed in previous study (Ahmed, 2010), excepting the work of Silva *et al*., 2007; Wang *et al*., 2007; Yuan *et al*., 2010; Guzel and Arvas, 2011; Xie *et al*.,

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2011; Zhang *et al*., 2011; De Riu *et al*., 2012; Abidi and Khan, 2014; Zheng *et al*., 2015 and Lee *et al*., 2016.

Despite having a potential to reduce both feed cost and environmental pressure in trout culture operation, however, no study so far been carried out to establish appropriate feed management practices on trout culture. Rainbow trout, *Oncorhynchus mykiss* is one of the most suitable fish for commercial farming in Jammu and Kashmir. However, the availability of good quality feed as well as appropriate feeding level to meet the nutritional requirements of trout for optimum production remains a major constraint under Kashmir climatic conditions. Jammu and Kashmir state basically a mountainous state covered by hills and mountains is virtually known to possess largest fresh coldwater resources of the country. However, due to the paucity of information about the nutritional requirements as well as the optimum feeding level of this species in the cold climatic condition of Kashmir, the success was marginal.

Although a comprehensive work related to feeding frequency and optimum feeding rate of trout have been carried out and reported from different parts of the world (Boujard *et al*., 2000; Bailey and Alanara, 2006; Blake and Chan, 2006; Blake *et al*., 2006; Bureau *et al*., 2006; Sevgili *et al*., 2006; Rasmussen *et al*., 2007; Guzel and Arvas, 2011; Sevgili *et al*., 2012 and Sevgili *et al*., 2013), however, no information is available on optimum feeding level, protein, and energy maintenance requirement of fingerling *O. mykiss* at fingerling stage from this region. The objective of study was therefore, undertaken to investigate the effects of optimum feeding rate on growth, feed utilization efficiency, and body proximate composition of fingerling *O. mykiss*, and to determine protein and energy maintenance requirement of this fish.

Materials and methods

Experimental diet

Casein-gelatin based purified (45% crude protein (CP); 367.5 kcal $g100g^{-1}$, gross energy (GE)) H-440 diet (Halver, 2002) was formulated (Table 1). The dietary protein level was fixed at 45% CP, reported optimum for growth of rainbow trout (NRC, 1993). The physiological energy values of 4.5, 3.5 and 8.5 kcal g^{-1} for protein, carbohydrate and fat, respectively (Jauncey, 1982) were utilized to calculate the energy content of the diet. The details for the preparation of diet up to the formation of dough used in the present study were as same as described earlier (Ahmed, 2007). The dough was passed through a pelletizer fitted with a 2 mm die to obtain pellets, which were dried in a hot air oven at 40 ºC to reduce the moisture content below 10%. The dry pellets were crumbled, sieved (0.20-0.25 mm) and stored at 4 ºC until used.

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Table 1: Ingredient and proximate composition of experimental diet

Ingredient*	g $100g^{-1}$, dry diet
Casein (vitamin-free)[1]	40.70
Gelatin[2]	13.40
Dextrin	25.12
α-Cellulose	2.78
Corn oil	6.00
Cod liver oil	3.00
Mineral mix[3]	4.00
Vitamin mix[3,4]	3.00
Carboxymethyl cellulose	2.00
Proximate composition	
Calculated crude protein	45.00
Analysed crude protein	44.91
Crude fat	8.93
Energy (kcal $g100g^{-1}$) [5]	367.02
E/P ratio (kcal $g100g^{-1}$)	8.17

*Loba Chemie, India; [1]Crude protein (80.0%); [2]Crude protein (93.0%); [3]Halver, 2002, mineral ($AlCl_3$. $6H_2O$, 15mg; $ZnSO_4$. $7H_2O$, 300mg; CuCl,10mg; $MnSO_4$.4 -$6H_2O$, 80mg; KI,15mg; $CoCl_2.6H_2O$,100mg; plus USP # 2 Ca $(H_2PO_4)_2$. H_2O, 13.58 g; $C_6H_{10}CaO_6$ 32.70 g; $C_6H_5O_7Fe.5H_2O$, 2.98 g; $MgSO_4.7H_2O$, 13.20 g; KH_2PO_4 (dibasic), 23.98 g; $NaH_2PO_4.2H_2O$, 8.72g; NaCl, 4.35g ($g100\ g^{-1}$), [3,4]1g vitamin mix (choline chloride, 500.00mg; thiamine HCl, 5.00mg; riboflavin, 20.00mg; pyridoxine HCl, 5.00mg; nicotinic acid, 75.00mg; calcium pantothenate, 50.00mg; inositol, 200.00mg; biotin, 0.50mg; folic acid, 1.50mg; ascorbic acid, 100.00mg; menadione (K), 4.00mg; α-tocopheryl acetate (E), 40.00mg; cyanocobalamine (B_{12}), 0.01mg ($g100g^{-1}$ +2g α-cellulose; [5]Calculated on the basis of physiological fuel values 4.5, 3.5 and 8.5 kcal g^{-1} for protein, carbohydrate and fat, respectively (Jauncey, 1982).

Experimental design and feeding trial

Rainbow trout fingerlings of the same batch and in good health condition brought to the laboratory in oxygen filled polythene bags from the nearest 'State Government Fishery Department Seed Hatchery, Dachigam, Laribal (Srinagar, Kashmir). These fingerlings were stocked in continuous flow-through system for a fortnight and were fed by a mixture of practical diet in the form of dry pellets. These fingerlings were then acclimatized for 2 weeks by artificial diet (Halver, 2002).

After acclimatization, the fingerling of rainbow trout (5.65±0.45cm;

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1.42±0.25g) were then randomly stocked in triplicate groups in 75 L circular polyvinyl troughs (water volume 60 L) fitted with a continuous water flow-through (2.5 L min^{-1}) system with 20 fish per trough for each dietary ration with three replicates. The fish were fed test diet in the form of dry pellets at a rate of 2, 3, 4, 5, 6 and 7% of their body weight day^{-1} on a dry to wet basis, for ten weeks. The daily ration was subdivided into two equal halves and fed at 0800 and 1700 h. No feed was offered to the fish on the day that the measurements were taken. Initial and weekly weights were recorded on a top loading balance (Sartorius CPA- 224S 0.1 mg sensitivity, Goettingen, Germany) and the weekly ration was adjusted accordingly. Fecal matter was removed by siphoning before feeding and uneaten feed, if any, was filtered over a screen soon after the active feeding, dried and weighed in order to measure the amount of feed consumed. Water temperature, dissolved oxygen, free carbon dioxide, total alkalinity and pH, were recorded following the standard methods (APHA, 1992), and based on daily measurements were 15.5-18.5 ºC, 7.6-8.8, 5-12, 60-80 mg L^{-1} and 7.1-7.5, respectively. At the end of feeding trial, fish were anaesthetized with tricaine methanesulphonate (MS 222) and final weight was measured. Weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using the following standard formulas:

$$\text{Weight gain (\%)} = \frac{\text{Final body weight - Initial body weight}}{\text{Initial body weight}} \times 100$$

$$\text{SGR} = \frac{\text{(In mean final weight) – (In mean initial weight)}}{\text{No. of Day}} \times 100$$

$$\text{FCR} = \frac{\text{Dry food fed (g)}}{\text{Wet weight gain (g)}}$$

$$\text{PER} = \frac{\text{Weight gain (g, wet weight basis)}}{\text{Protein intake (g, dry weight basis)}}$$

Chemical analysis

Proximate composition of casein, gelatin, and experimental diet, initial and final carcass in fish was estimated using standard AOAC (1995) methods for dry matter (oven drying at 105±1°C for 22 h), crude protein (N-Kjeldhal x 6.25), crude lipid (solvent extraction with petroleum ether B.P 40-60°C) by using soxhlet extraction technique (FOSS Avanti automatic 2050, Sweden), and ash (oven incineration at

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650 °C for 2-4 h). At the beginning of the feeding experiment, a pooled sample of 30 fish was collected for initial body composition analysis. At the end of the feeding trial, eight fish were randomly pooled from each replicate of dietary treatment and three sub-samples of each replicate from the pooled sample (n=3x3) were analyzed for final body composition.

Statistical analysis

Response of rainbow trout fingerling fed different ration levels were measured by growth data and by analyzing the whole body composition. The response variables were subjected to one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1967; Sokal and Rohlf, 1981). To determine the significant differences among the treatments, Tukey's multiple range test was employed. To predict more accurate responses to the feed intake, the optimum feeding level was estimated using second-degree polynomial regression analysis ($Y=ax^2+bx+c$) as described by Zeitoun *et al*. (1976). The break-point obtained represented the optimum feeding level for growth. Statistical analysis was done using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results

The influence of feeding rate on growth performance of *O. mykiss* fingerlings fed six different levels of feeding (2, 3, 4, 5, 6 and 7% BW day^{-1}) over the 10 week feeding trial is presented in Table 2. No mortality was observed in any of the groups during the entire experimental period. Significant (p<0.05) differences were observed in weight gain, SGR, FCR and PER of *O. mykiss* fed various feeding levels. After 10-week of the feeding trial, percent live weight gain of *O. mykiss* fingerlings increased linearly with increasing feeding rate up to 5% BW day^{-1}, but no further increases in weight gain (p>0.05) were observed when feeding rate was increased beyond 5% BW day^{-1}. However, there was no significant (p>0.05) difference in weight gain between 4% and 5% feeding levels. The fish fed at 5% BW day^{-1} had the highest weight gain, while the fish fed 1% BW day^{-1} exhibited the lowest weight gain among all the treatment levels. Best FCR and PER where obtained in fish fed 4% and 5% feeding levels. While the highest SGR was recorded at 5% feeding level. Fish fed various feeding levels produced significant differences in FCR, which ranged from 1.63 and 2.65 with the best FCR value 1.63 recorded at 4% feeding level, which was not significantly (p>0.05) different to that fed at 5% feeding level. However, further increase in feeding level resulted into no improvement or even poor FCR values as evident at 6% and 7% feeding levels. The fish fed 4 and 5% BW day^{-1} had the highest PER than that of the fish fed the other feeding levels and was found to be significantly (p<0.05) higher among all the feeding levels.

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Table 2: Growth and Conversion efficiency of rainbow trout, *Oncorhynchus mykiss* fingerling fed different feeding levels (*).

Ration level	Protein g kg^{-1}	Energy kcal kg^{-1}	Initial body weight (g)	Final body weight (g)	Weight gain (%)	SGR (%)	FCR (%)	PER (%)
2	90.00	73.40	1.397± 0.02	2.775± 0.05^{e}	98.64± 5.84^{e}	1.22± 0.05^{e}	2.65± 0.07^{a}	0.84± 0.02^{c}
3	135.0	110.10	1.396± 0.03	3.807± 0.15^{d}	172.48± 5.60^{d}	1.79± 0.04^{d}	2.04± 0.03^{b}	1.09± 0.01^{b}
4	180.00	146.80	1.403± 0.03	5.435± 0.10^{ab}	287.40± 6.59^{ab}	2.42± 0.03^{ab}	1.63± 0.05^{c}	1.36± 0.04^{a}
5	225.00	183.50	1.414± 0.01	5.605± 0.06^{a}	296.32± 5.18^{a}	2.46± 0.02^{a}	1.72± 0.06^{c}	1.29± 0.05^{a}
6	270.00	220.20	1.444± 0.03	5.286±0.19^{b}	265.81± 4.97^{b}	2.32± 0.02^{bc}	2.16± 0.02^{b}	1.03± 0.02^{b}
7	315.00	256.90	1.426± 0.02	5.040± 0.16^{bc}	253.16± 5.84^{bc}	2.25± 0.03^{c}	2.53± 0.05^{a}	0.88± 0.02^{c}

*Mean value of 3 replicates±SEM; Mean values sharing the same superscript are insignificantly different (p>0.05)

In order to generate more precise information on optimum feeding rate, second degree polynomial regression analysis was employed. On subjecting the live weight gain data to second degree polynomial regression analysis, a maximum weight gain was evident at 5.33% feeding level (Fig. 1), the relationship being:

$$Y= -18.1100x^2 +193.3200x- 221.4198$$

(r = 0.967; p<0.05).

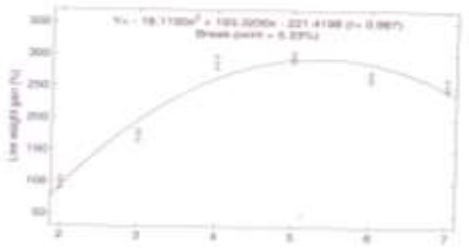


Figure 1: Second-order polynomial relationship between live weight gain (%) and feeding levels for rainbow trout, *Oncorhynchus mykiss* fingerlings fed with experimental diets for 10 weeks (n=mean of 3 replicates).

The FCR (Y) to feeding levels (X) relationship was best described by a second-degree polynomial regression analysis (Fig. 2). The equation being:

$$Y= 0.1483x^2- 1.3377x+ 4.7091$$

(r = 0.968; p<0.05).

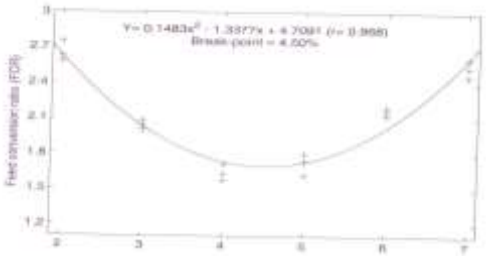


Figure 2: Second-order polynomial relationship between feed conversion ratio (FCR) and feeding levels for rainbow trout, *Oncorhynchus mykiss* fingerlings fed with experimental diets for 10 weeks (n=mean of 3 replicates).

The PER (Y) to feeding levels (X) relationship was best described by a second-degree polynomial regression analysis (Fig. 3). The equation being:

$$Y=- 0.0740x^2 + 0.6643x- 0.1941$$

(r = 0.938; p<0.05).

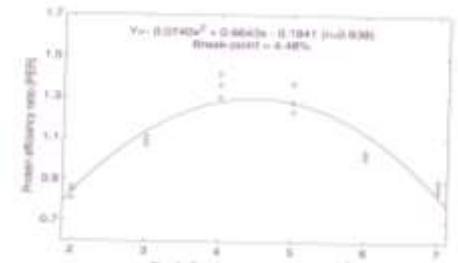


Figure 3: Second-order polynomial relationship between protein efficiency ratio (PER) and feeding levels for rainbow trout, *Oncorhynchus mykiss* fingerlings fed with experimental diets for 10 weeks (n=mean of 3 replicates).

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Based on the above equations, the best estimated FCR and PER occurred at 4.50 and 4.48 % feeding levels BW day^{-1}, respectively.

Body protein retention was found to be significantly (p<0.05) higher at 5% feeding level compared with the other feeding levels, excepting at 4% feeding level where no significant (p>0.05) differences in protein retention was observed. Second degree polynomial regression analysis was also employed to determine the break-point between protein retention (Y) and feeding levels (X) (Fig. 4). The relationship being:

$$Y = -1.7608x^2 + 16.3132x - 16.0171$$
$$(r = 0.943;\ p<0.05)$$

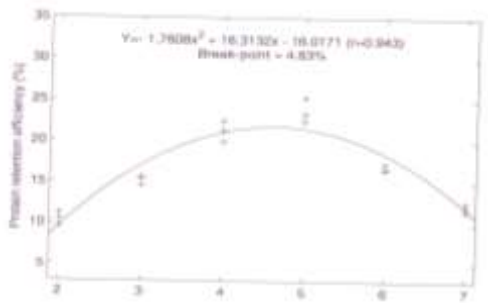


Figure 4: Second-order polynomial relationship between protein retention efficiency (PRE) and feeding levels for rainbow trout, *Oncorhynchus mykiss* fingerlings fed with experimental diets for 10 weeks (*n*=mean of 3 replicates).

Similarly energy retention was found to be significantly (p<0.05) higher at 5% feeding level BW day^{-1} when compared with the other feeding levels, except at 4% feeding level, where almost similar energy retention value was also achieved. Second degree polynomial regression analysis was also employed to determine the break-point between energy retention (Y) and dietary ration levels (X) (Fig. 5). The relationship being:

$$Y = -4.7873x^2 + 45.3171x - 23.4441$$
$$(r = 0.958;\ p<0.05)$$

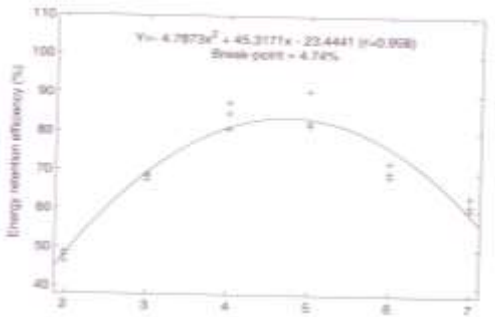


Figure 5: Second-order polynomial relationship between energy retention efficiency (ERE) and feeding levels for rainbow trout, *Oncorhynchus mykiss* fingerlings fed with experimental diets for 10 weeks (*n*=mean of 3 replicates).

Based on the above equations, the highest protein retention (21.77%) and energy retention (83.80%) occurred at 4.63% and 4.74% feeding levels, respectively.

The whole body composition of the fish fed different feeding levels at the end of trial is presented in Table 3. Fish fed lower feeding levels had significantly (p<0.05) higher moisture contents and was progressively declined with increasing feeding levels up to 5% BW day^{-1} and thereafter a significant increase in whole body moisture content was again noticed. Whole body protein content was found to be significantly (p<0.05) higher at 5% feeding levels compared to that fed the other dietary groups. A linear increase in whole body lipid content was evident with increasing feeding levels and a significantly (p<0.05) highest lipid content was noted at 7% feeding level BW day^{-1}. Contrary to protein and lipid

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no significant (p>0.05) differences were found in whole body ash content in fish fed by different feeding levels BW day^{-1}, except at 2% feeding level where significantly (p<0.05) highest body ash content was reported.

Based on the above second-degree polynomial regression analysis results and body composition, it is recommended that feeding rate in the range of 4.6% to 5.3% BW day^{-1}, corresponding to 2.07-2.39 g protein and 16.88-19.45 kcal energy 100 g^{-1} of the diet day^{-1} is optimum for the growth and efficient feed utilization of rainbow trout, while 2-3% feeding levels (0.90-1.35 g protein and 7.34-11.01 kcal energy g 100 g^{-1}) suggests that these levels approximate the maintenance requirement of this fish.

Table 3: Carcass composition and nutrient retention efficiency of rainbow trout, *Oncorhynchus mykiss* fingerling fed varying feeding levels (*)

Ration level	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Energy (kcal g^{-1}, dry weight)	Nutrient retention efficiencies (%)	
						Protein retention	Energy retention
2	76.72± 0.19^{a}	12.39± 0.11^{e}	5.10± 0.04^{f}	3.78± 0.07^{a}	4.56± 0.02^{e}	10.43± 0.36^{d}	47.90± 0.65^{d}
3	74.93± 0.12^{b}	13.42± 0.20^{d}	5.70± 0.06^{e}	2.22± 0.02^{b}	4.86± 0.01^{d}	15.49± 0.07^{c}	68.19± 0.37^{b}
4	72.67± 0.34^{c,d}	14.78± 0.08^{c}	6.32± 0.07^{d}	2.32±0.04^{b}	4.90± 0.01^{d}	21.17± 0.58^{b}	84.13± 1.62^{a}
5	72.09± 0.19^{d}	16.79± 0.10^{a}	7.30± 0.16^{c}	2.39± 0.02^{b}	5.11± 0.04^{c}	23.67± 0.68^{a}	84.59± 2.38^{a}
6	73.29± 0.18^{c}	15.32± 0.08^{b}	7.85± 0.04^{b}	2.13± 0.05^{b}	5.26± 0.02^{b}	16.89± 0.16^{c}	70.07± 0.91^{b}
7	74.15± 0.12^{b}	13.49± 0.06^{d}	8.59± 0.11^{a}	2.12± 0.03^{b}	5.39± 0.01^{a}	12.17± 0.24^{d}	61.89± 0.79^{c}
Initial	77.15± 0.05	12.38± 0.13	4.23± 0.08	3.31± 0.10	4.46± 0.01	-	-

*Mean value of 3 replicates ± SEM**;** Mean values with the same superscript are insignificantly different (p>0.05)

100 X [(Final body weight (g) X % nutrient of final body weight) – (Initial body weight (g) X % nutrient of initial body weight)]/[(amount of diet fed) X (% crude protein or total energy in the diet)

Discussion

The aim of this feeding experiment was to examine the exact utilization of feed which can promote best growth and also to know that how much quantity of protein and energy is required for rainbow trout in terms of both for optimum growth and for maintenance purposes. An optimal ration maximizes growth rate and minimizes size variability and reduces food waste (Booth *et al*., 2008; Oberg *et al*., 2014), and also reduces feed cost and improve water quality. Therefore, precise knowledge about the relationship between growth and ration is pre requisite not only for promoting good growth and feed efficiency ratio, but also useful for preventing incidence of water quality deterioration as a result of excess feeding. Thus, determination of appropriate feeding level is one of the key factors for better growth, feed conversion, nutrient retention efficiency and chemical composition of fish (Yuan *et al*., 2010; Zhang *et al*., 2015). Hence, a better understanding of the conversion of the dietary inputs in to fish biomass

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is a key factor to improving the economic and environmental sustainability of aquaculture operation including survival (Bureau *et al*., 2006; Zheng *et al.*, 2015).

The essentiality of feeding rate for the normal growth of rainbow trout was clearly demonstrated in this study. In the present study, weight gain of fingerlings rainbow trout fed different feeding levels linearly increase with the increase of feeding levels and reached its plateau fed at 5% BW day^{-1}. The significant growth improvement was recorded with increasing feeding levels up to 5% BW day^{-1}, which was not significantly different from the values achieved in fish fed at 4% of BW day^{-1}. This showed that feeding rainbow trout in the range of 4-5% BW day^{-1} results in maximum feed utilization for growth and other activities. However, the fish fed at a feeding level of 5% BW day^{-1} produced maximum live weight gain and SGR, but produced insignificantly low FCR values compared to those fed 4% BW day^{-1} feeding level because at 4% feeding level fish probably also tended to optimize digestion to extract nutrient more efficiently. The best FCR obtained at 4 and 5% feeding level with maximum protein and energy retention values recorded at both levels clearly indicating the better utilization of feed at these levels, which were also supported by body composition of fish.

On the other hand, a gradual decline in conversion efficiency was noticed in fish fed at higher feeding levels i.e. 6 and 7% BW day^{-1} which can be the result of loss of nutrients and wastage of food, as fish took longer time to consume food to reach satiation (Tvenning and Giskegjerde, 1997). Increased feed wastage with increasing feeding rate has also been reported for tambaqui, *Colossoma macropomum* (Van der Meer *et al*., 1997), milk fish, *Chanos chanos* (Sumagaysay, 1998), bagrid catfish, *M. numurus* (Ng *et al*., 2000), Chinese long snout catfish, *Leiocassis longirostraris* (Han *et al*., 2004) and juvenile gross carp, *Ctenopharyngodon idella* (Du *et al*., 2006). Hassan and Jafri (1994) reported gradual decline in growth rate and feed conversion efficiency in fish fed at higher ration levels in *Clarias batrachus*. Ahmed (2007) also reported similar result in Indian major carp, *Labeo rohita*. In the present study, a similar trend in feed conversion efficiency was also noticed in rainbow trout fed at higher feeding levels than optimum. This may be due to the fact that the consumption of excess amount of feed put over burden on digestion process as nutrients are first involved in digestion instead of growth. The other reason for growth retardation in fingerling rainbow trout fed at 6% and 7% BW day^{-1} could be due to over burden of dietary nitrogen in the body requiring additional energy for its elimination which is done at the cost of deposited nutrients and the same has confirmed with low body protein content at these feeding rates. The nitrogen in the form of surplus amino acids resulting from the excess feeding levels at 6% and 7% are not stored in fish, they are to be deaminated requiring extra energy expenditure towards catabolism and hence adversely

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affected the growth of the fish at higher feeding levels.

The low growth rate and FCR reported at 2% and 3% BW day^{-1} suggests that these feeding levels could approximate only to the maintenance requirements of nutrients in rainbow trout, wherein the major portion of ingested nutrients is utilized to maintain life and a smaller portion is available for growth. Present findings on rainbow trout also seem in agreement with the observations of Hung and Lutes (1987) on *Acipenser transmontanus* and Ahmed (2010) on *Heteropneustes fossilis*. On the other hand, significant increase in growth of fish fed at 4% and 5% BW day^{-1} indicates that large portion of nutrients were utilized for growth and tissue building besides that utilized for maintenance.

The whole body composition of fish is often used as an indicator of fish health and flesh quality. Many studies have described the effect of diet and feeding rate on growth and body composition (Reinitz, 1983a; Han, 2004). Several other factors, including growth, ingredient used in the diet, and water temperature are also known to influence the body composition of fish. Body composition is also significantly influenced by feeding rate (Panda *et al*., 1999; Adebayo *et al.,* 2000; Khan *et al*., 2004; Du *et al*., 2006; Ahmed, 2007, 2010). In the present study, the whole body composition of rainbow trout fed varying feeding levels indicates that the composition parameters was significantly influenced by the feeding levels. Whole body moisture content gradually decreased with the increase of feeding levels up to 5% and thereafter, a significant increase in body moisture content was noted. Gatlin *et al.* (1986) also reported decrease in body moisture content of fingerling channel catfish with increasing feeding rate. Storebakken and Austreng (1987b) reported that variation in the content of moisture were mainly a direct results of ration level. Body protein content increased with the increasing of feeding levels up to 5% and thereafter a significant fall of body protein was also noticed. The lipid content of fish fed various feeding levels linearly increased with the increasing of feeding levels and maximum lipid content was reported at 7% BW day^{-1} feeding level. The similar results on lipid content of rainbow trout with different feeding levels has also been reported in the past study(Storebakken and Austreng, 1987a) and some other fish species such as *C. gariepinus* (Adebayo *et al*., 2000), and green sturgeon, *A. medirostris* (Zheng *et al*., 2015). Rasmussen and Ostenfeld (2000) reported that lipid accumulation is prevalent during fast growth at high rations, while lipid deposition is subtle during slow growth due to the presence of adequate energy in excess feed deposition as lipid (Reinitz, 1983a,b). At lower feeding level a slightly lower lipid content was observed, though at the same time the fish could manage to maintain relatively higher and constant amount of protein in their body tissue over the initial value, suggesting that in this fish, body lipid is mobilize as energy reserves to support fish as energy over protein. Similar results on body lipid

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content were also reported in the previous study on different fish species (Huges and Lutes, 1987; Brown *et al.*, 1990; Hassan and Jafri, 1994; Khan *et al*.. 2004; Ahmed, 2007, 2010). In contrast to protein and lipid contents, the whole body ash showed no significant differences in their ash values among various feeding levels and remained constant with each other, excepting at initial feeding level i.e. 2% BW day^{-1}, where significantly higher ash content was reported. The decline in ash content with higher rations could be due to relatively low skeletal growth compared with other tissue (Resmussen and Ostenfield, 2000). Similar results on ash content with varying feeding level has also been reported in cuneate drum, *Nibea miichthioides* (Wang *et al*., 2007). Contrary to this, the higher body ash content reported the groups fed at lower feeding levels were probably due to higher proportions of bone mass and less muscle mass thus resulting to potentially higher ash content in fish fed lower feeding levels.

The protein and energy retention of fish fed various feeding levels showed an increasing trend with increasing feeding level and reached its maximum values at 5% feeding level BW day^{-1} and thereafter a decrease in protein and energy retention values were noted. On the other hand, the low protein and energy retention values obtained at sub-optimal feeding levels indicates that a substantial amount of the reduced energy intake was used to compensate the maximum requirement at the expanse of somatic growth. Similar trends on protein and energy retention values has also been reported in juvenile turbot, *Scophthalmus maximus* (Van Ham *et al*., 2003), because energy and protein consumption is the product of feed intake and feed composition.

On subjecting the growth data such as weight gain, FCR, PER, protein and energy retention to second-degree polynomial regression analysis, break-points were occurred at 5.33, 4.50, 4.48, 4.63 and 4.74% feeding levels, respectively. The break-points indicate that the feeding rate in the range of 4.60% to 5.30% BW day^{-1} is optimum for growth of rainbow trout.

Based on the results of the present study on optimum feeding rate, body composition, and energy and protein maintenance requirement of rainbow trout, it is recommended that provision of feeding rate in the range of 4.60% to 5.30% BW day^{-1}, corresponding to 2.07-2.39g protein and 16.88-19.45 kcal energy g 100g^{-1} of the diet day^{-1} is optimum for the growth and efficient feed utilization. Whereas poor FCR and live weight gain obtained at 2-3% feeding levels, corresponding to 0.90-1.35g protein and 7.34-11.01 kcal energy g 100g^{-1} of the diet day^{-1} suggests that these levels approximate the maintenance requirement of the fish. The optimum feeding level recommended in the present study for rainbow trout (4.60%-5.30%) is slightly higher than 2% feeding level reported earlier for rainbow trout weighing 0.5-1.0 kg (Storebakken and Austrung, 1987a) and is lower than the feeding level reported for other fish species such as brown trout, *Salmo trutta* (Elliot., 1975). However, when

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compared the feeding level recommended in the present study with other fish species, a wide range of results have been achieved in similar groups of fish examined.

The variation in feed intake among fish species or within the species may be due to the difference in the methodologies used such as the nature of diet composition, water temperature, flow rate, stocking density, size, feeding frequency, habitat, photoperiod, etc (Brett, 1979; Fiogbe and Kestemont, 2003; Bailey and Alanara, 2006). Taking the above factors in to the consideration in the present study a standard protocol and diet has been chosen for determination of feeding level and energy and protein maintenance requirement of rainbow trout and the data found in the present study would be useful for its maximum production as well as nutrient requirement determination such as protein, amino acids, vitamins, minerals and fatty acids.

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