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Effect of isoenergetic diets with different protein and lipid content on the growth performance and heat increment of rainbow trout

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Abstract

The heat increment of feeding (HiE) is the metabolic cost associated with feeding, typically measured in fish as the increase in oxygen consumption (MO_2) over some estimate of standard metabolic rate (SMR). The present study sought to accurately measure HiE using a variety of different methods in order to remove the influence of routine fish activity and excitement which could overestimate HiE. Protein handling makes up a large component of HiE. Thus, there is an expectation that variations in dietary protein content could influence HiE. Therefore, growth performance parameters were assessed in juvenile rainbow trout fed daily to satiation one of three isoenergetic diets with equivalent carbohydrate content (12%) but variable protein (P) and lipid (L) content [theoretical protein:lipid levels were: 55%:10% (HP:LL); 45%:15% (MP:ML) and 35%:20% (LP:HL)]. The estimated dietary digestible protein (DP) to digestible energy (DE) ratios of 19.8, 24.8 and 29.8 g/MJ bracketed the recommended levels of 22–25 g/MJ for juvenile rainbow trout. HiE values for the same groups of fish that were maintained on the test diets after the growth trial were subsequently assessed following a single meal (by gavage) of 2% of their body mass so that the growth performance parameters could be compared with the HiE estimates. Some growth performance parameters (*i.e.*, specific growth rate, feed efficiency and dry feed intake) did not vary significantly among fish fed the diets, whereas percent protein deposition was inversely related to dietary protein content and the dietary DP to DE ratio. The dissimilar diet treatments did not result in differences in values for SMR, RMR, peak MO_2 or time-to-peak MO_2 or in estimates for HiE. The mean SMR from all fish combined across treatments was 50.4±3.4 mg O_2/kg/h. MO_2 increased significantly above SMR by 4-h postprandial and peaked at 116.2±7.7 mg O_2/kg/h, representing an increase of 131%. The metabolic cost of the diets (as a % of DE) was low, and best estimates ranged between 4.0 and 4.8%.

Keywords: Heat increment of feeding; SDA; Rainbow trout; Isoenergetic diets

1. Introduction

Meal ingestion is followed by a concomitant increase in metabolic rate, variously referred to as the specific dynamic action (SDA), calorigenic effect, dietary thermogensis or the heat increment of feeding (HiE)

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(see review by McCue, 2006; Jobling 1981). Following the recommendations of the NRC (1993), HiE will be used here. HiE represents the cost associated with digesting a meal, which can be estimated as a percent of digestible energy intake (HiE coefficient; C_{HiE}). McCue (2006) recently summarized and categorized the various pre-absorptive, absorptive and post-absorptive processes that contribute to HiE, which are inevitably linked to one another and are thus difficult to isolate *in vivo*. The relative contribution of these processes to HiE varies greatly, with the mechanical component of HiE being a minor contributor (Tandler and Beamish, 1979) and handling of protein in growing fish being a major contributor to HiE. For example, trout hepatocytes used around 80% of their total oxygen consumption on protein synthesis (Pannevis and Houlihan, 1992). Moreover, HiE increases when amino acid deamination is excessive due to either suboptimal dietary amino acid balance (Brody, 1945; Beamish and Trippel, 1990), or inefficient use of amino acids to synthesize body protein when protein is excessive in relation to the dietary non-protein energy (Cho et al., 1976, 1982). Thus, protein content of a meal can influence HiE and since protein is the most expensive dietary component in aquaculture, there is great interest in the relationship between HiE and protein content of the diet.

McCue's (2006) review of HiE identified some significant problems when estimating HiE, two of which are addressed in the present work. HiE is usually calculated with respirometry by measuring the post-prandial oxygen consumption (MO_2) relative to some measure of SMR. McCue (2006) suggested that the methodology needs to be carefully scrutinized to ensure errors in estimating standard metabolic rate (SMR) are minimized. Therefore, the quality of the respirometry system used to estimate MO_2 and SMR affects the reliability of any HiE calculation. Furthermore, since spontaneous activity and diurnal rhythms elevate MO_2 both during pre-feeding and postprandial (Roe et al., 2004), accurate estimations of HiE must separate energy expended on activity and excitement from that associated with feeding (Brett and Groves, 1979). Here, we explore different approaches for estimating SMR and HiE in rainbow trout so that we can properly examine the effect of dietary protein on HiE.

Cost-effective salmonid production requires optimal dietary protein-to-lipid ratios to minimize amino acid catabolism and maximize anabolism (Hilton and Slinger, 1981; Cho, 1992). Nevertheless, McCue (2006) was concerned that not all studies utilize isoenergetic diets (although most control for relative meal size), bringing into question the validity of the conclusions and making comparisons among studies very difficult. Furthermore, protein requirements change with fish size and its stage of growth (Higgs et al., 1995), with young trout requiring more protein compared with larger trout on maintenance or production diets (Satia, 1974; Hilton and Slinger, 1981). Consequently, despite the extensive research on optimal dietary protein-to-lipid ratios in various cultured fish species (NRC, 1993), conflicting results still exist.

Studies using non-isoenergetic diets have estimated the optimal protein-to-lipid concentrations in grower diets for rainbow trout as 35–45% protein and 15–20% lipid, and an ideal digestible protein-to-digestible energy ratio (DP:DE) of between 22 and 25 g DP/MJ DE (Cho and Kaushik, 1990; Cho, 1992; Higgs et al., 1995). Some recent studies have observed improved growth of rainbow trout fed diets with reduced protein-to-lipid ratios (Yigit et al., 2002; Chaiyapechara et al., 2003; Morrow et al., 2004). In contrast, other studies using isoenergetic diets have not observed any significant effects of diets varying in protein-to-lipid ratio on growth of rainbow trout (Steffens et al., 1999; Azevedo et al., 2004a,b).

In view of the above differences in findings between studies, we reasoned that if protein turnover represented a large component of HiE, that trout fed isoenergetic diets containing considerable differences in levels of digestible protein and energy would exhibit dissimilar estimates for HiE. Therefore, our objective in this study was to assess the relationship between growth performance and HiE in rainbow trout fed three diets with varying protein and lipid concentrations but equal digestible carbohydrate and energy content. Our study differs from all previous studies on this theme in three important ways. First, we sought to assess HiE using a variety of methods and, by removing metabolic effects due to routine fish activity and excitement, we sought to provide accurate assessments of HiE in trout in relation to diet treatment. Second, we used isoenergetic diets in contrast to many previous studies. In addition, HiE was measured on the fish after several months following a comprehensive growth trial, *i.e.*, their diet treatments were not novel. Third, we used larger trout to permit comparisons of our results with those of previous studies which were conducted using smaller trout.

2. Materials and methods

2.1. Diet formulation and preparation

Three dry diets (refer to Table 1 for ingredient compositions) were formulated to be isoenergetic (16.7 MJ of estimated DE/kg) and equivalent in estimated digestible carbohydrate content

Table 1
Ingredient compositions of the three isoenergetic experimental diets

	HP:LL	MP:ML	LP:HL
Dietary protein (%)	55	45	35
Dietary lipid (%)	10	15	20
Ingredient (g/kg dry basis)			
Anchovy meal (low temperature-dried)	599.87	490.81	381.74
Blood flour (spray-dried)	49.36	40.38	31.41
Squid meal	49.97	40.88	31.8
Krill hydrolysate	18.95	15.5	12.05
Wheat gluten meal	51.5	42.14	32.78
Pregelatinized wheat starch	85.0	85.0	85.0
Raw wheat starch	73.86	73.86	73.86
Vitamin supplement[1]	20.0	20.0	20.0
Mineral supplement[2]	20.0	20.0	20.0
Anchovy oil (stabilized)	1.38	74.09	146.67
Soybean lecithin	10.0	10.0	10.0
Choline chloride (60%)	5.0	5.0	5.0
Vitamin C monophosphate (42%)	2.86	2.86	2.86
Permapell (lignin sulphonate binder)	10.0	10.0	10.0
DL-methionine	2.25	1.85	1.42
α-Cellulose	–	67.63	135.41
[2]Mineral supplement (mg/kg dry basis)			
Mn (as $MnSO_4$ H_2O)	75.0	75.0	75.0
Zn (as $ZnSO_4$ $7H_2O$)	54.6	71.5	89.0
Co (as $CoCl_2$ $6H_2O$)	3.0	3.0	3.0
Cu (as $CuSO_4$ $5H_2O$)	5.0	5.0	5.0
Fe (as $FeSO_4$ $7H_2O$)	50.0	50.0	50.0
I (as KIO_3)	5.0	5.0	5.0
(as KI)	5.0	5.0	5.0
Se (as Na_2SeO_3)	0.2	0.2	0.2
Mg (as $MgSO_4$ $7H_2O$)	400.0	400.0	400.0
K (as K_2SO_4)	–	654.0	1398.0
(as K_2CO_3)	–	654.0	1398.0
F (as NaF)	5.0	5.0	5.0

All diets were formulated to contain 16.7 MJ of digestible energy/kg dry diet and 12% digestible carbohydrate, but varying protein and lipid concentrations.
[1]The vitamin supplement was composed of the following per kg dry diet: D-calcium pantothenate, 168 mg; pyridoxine HCl, 49.3 mg; riboflavin, 54.2 mg; folic acid, 15.0 mg; thiamine mononitrate, 56 mg; biotin, 1.5 mg; vitamin B_{12}, 0.09 mg; vitamin K (as MSBC), 18.0 mg; vitamin E, 300 IU; vitamin D_3, 2400 IU; vitamin A, 5000 IU; inositol, 400 mg; niacin, 300.0 mg; BHT, 22 mg; Raw wheat starch was the carrier.

(12%). Moreover, they were formulated to contain one of three protein concentrations: 35% (LP), 45% (MP) or 55% (HP) with, respectively, either a high (20%, HL), medium (15%; ML) or low (10%; LL) lipid concentration. These formulations bracketed the established optimum DP to DE ratios for growth and protein utilization of rainbow trout, namely, 22–25 g DP/MJ DE (theoretical DP to DE ratios in the preceding diets were estimated to be 18.9, 24.3 and 29.7 g/MJ, respectively). To estimate dietary DP, DE, DP:DE and digestible carbohydrate content, we assumed that dietary protein and lipid were 90% digestible and that the digestibility of the carbohydrate provided by the animal protein sources, pregelatinized wheat starch and raw wheat starch, was 97%, 86%, and 49%, respectively (Hilton et al., 1982; Cho and Kaushik, 1990). However, actual estimates of DP, DE and DP:DE ratios in the diets (Table 2) considered the measured digestible protein concentrations in the diets (see below), together with the literature values for the digestibility of the lipid and carbohydrate sources, and the gross energy values of protein (23.64 kJ/g), lipid (39.54 kJ/g) and carbohydrate (17.15 kJ/g) (Maynard and Loosli, 1969). In addition, all diets had an identical balance of indispensable (essential) amino acids through proportional adjustment of the protein sources between the dietary protein concentrations. Supplemental vitamins and minerals exceeded the dietary needs of rainbow trout (NRC, 1993).

The diets were prepared at the Department of Fisheries and Oceans, University of British Columbia Centre for Aquaculture and Environmental Research (CAER) in West Vancouver, BC, Canada by first mixing all the finely ground dry ingredients together for at least 30 min in a Hobart Commercial Mixer (Hobart Manufacturing Company, Troy, OH, USA) with a portion of the supplemental lipid that was required in the case of diets MP:ML and LP:HL (lipid content in mash before pelleting was 8% on an air-dry basis). The dry mashes were then steam pelleted using a California model CL 2 Laboratory pellet mill equipped with a 4 mm ring die. Thereafter the pellets were dried immediately in a custom-made vertical cooler. The remaining supplemental anchovy oil was then added to the surface of the MP:ML and LP:HL diets using an electrically-operated sprayer and a cement mixer. Subsequently, the pelleted diets were stored overnight to allow the anchovy oil to absorb into the pellets and finally all diets were kept in air-tight containers at 4 °C until required.

2.2. Growth trial

In March of 2003, juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum) (120.7±1.6 g, mean±SEM) from Sun Valley Trout Farm (Mission, BC, Canada) were separated randomly into nine groups of 15 fish that were each held in 1100 l fiberglass tanks at CAER (West Vancouver, BC,

Table 2
Concentrations of proximate constituents and gross energy, estimated levels of digestible protein (DP) and digestible energy (DE), and estimated DP:DE ratios in the three experimental diets (dry weight basis)

Parameter	HP:LL	MP:ML	LP:HL
Dry matter (%)	91.2	92	92.5
Ash (%)	11.6	9.6	8
Lipid (%)	10.5	15.6	20
Protein (%)	55	46.8	37.6
Gross energy (MJ/kg)	18.7	19.8	20.9
DP (%)	49.5	42.1	34.2
DE (MJ/kg)	16.6	17	17.3
DP:DE	29.8	24.8	19.8

HP:LL refers to high protein, low lipid; MP:ML refers to medium protein, medium lipid; LP:HL refers to low protein, high lipid.
The apparent digestibility coefficients for protein in diets HP:LL, MP:ML and LP:HL were found to be 90%, 90% and 91%, respectively.

Canada). The tanks were supplied with aerated, flow-through (>10 l/min) well water. Temperature (11 °C±0.2 °C) and dissolved oxygen concentrations (>10.3 mg/l) were monitored daily. Photoperiod mimicked natural conditions. The fish were fed commercial trout chow (EWOS Canada Ltd., Surrey, BC, Canada) prior to the commencement of the study.

The three diet treatments were assigned randomly to the tanks. Fish were fed by hand to satiation twice daily (9:30 am and again at 1:00 pm) for 8 weeks. Two tanks of fish were fed at a time and the starting position was randomized each day. The fish in each tank were fed their prescribed diet until feeding activity stopped or fish regurgitated some of the pellets that they had eaten. After each feeding, the aquaria lids were closed and the fish were allowed to feed off the bottom of the tank for 10 min. Any remaining pellets on the bottom of the aquaria were then siphoned from the tanks and counted. Subsequently, the number of uneaten pellets was multiplied by their respective air-dry mean weight to obtain an estimate of waste feed, which was deducted from the weight of feed dispensed. Pellet recovery with the siphoning process was confirmed as 100% using a test with a fish-free tank.

Fish body mass and length were measured on days 0, 28 and 56 by draining half of the aquaria and adding clove oil (0.5 ppm; Hill Tech Canada Inc.) to sedate the fish for 15 min prior to their removal and complete anesthesia. Fish were anesthetized in aerated tricainemethanesulfonate (MS-222; 0.1 g/l that was buffered with sodium bicarbonate; Syndel Laboratories Ltd., Vancouver, BC, Canada) and then they were individually weighed and measured. General fish health was inspected at each sampling time. On day 56, three fish from each tank were taken at random for whole body proximate analysis. These fish were euthanized by cervical dislocation, vacuum sealed and stored at −40 °C until analysis.

Chromic oxide, an indigestible feed marker, was added to the diets (5 g per kg mash) during the final week of the growth trial to determine dietary protein and energy digestibility (Austreng, 1978; Hajen et al., 1993). Feces were stripped from the anesthetized fish on the final day of the growth trial (Hajen et al., 1993) and then the fecal samples were frozen until analysis. The chromic oxide-supplemented diets were continually fed to the fish as per the described growth trial for an additional 14 days and the feces were stripped on day 4 and 14 of this period and then frozen until analysis. Fecal samples were subsequently analyzed as described by Hajen et al. (1993), but the quantities of feces obtained from the replicate groups per diet treatment were insufficient to measure both dietary DP and DE. Hence, the feces from each treatment were pooled and analyzed together and only dietary DP was evaluated.

The concentrations of protein, lipid, moisture and ash in the whole fish and diets were assessed according to the procedures of Raven et al. (2006) and diet gross energy content was determined using bomb calorimetry (IKA-WERKE C5000, Staufen, Germany). The following growth and performance variables were calculated:

1. Diet protein digestibility = $[1-((F/D)\times(D_{cr}/F_{cr}))]\times 100$: where F = % protein in the feces, D = % protein in the diet, F_{cr} = % chromic oxide in the feces and D_{cr} = % chromic oxide in the diet
2. Weight gain (WG) = final mean wet mass (g) − initial mean wet mass (g)
3. Length gain (LG) = final mean fork length (cm) − initial mean fork length (cm)
4. Specific growth rate (SGR) = [(ln final mass (g) − ln initial mass (g))/# of expt days] × 100
5. Condition factor (CF) = [(body mass (g)/length $(cm)^3$) × 100]
6. Dry feed intake (DFI) = [total dry feed intake (g)/fish]
7. Feed efficiency ratio (FER) = [WG (g)/DFI (g)]
8. Protein efficiency ratio (PER) = [WG (g)/protein consumption (g)]
9. Percent protein deposited (PPD) = [(protein gained in fish (g)/total protein consumed (g)) × 100]: The initial protein concentration in the fish was estimated to be 17.7% (Weatherup and McCracken, 1999) in order to calculate percent protein deposited.
10. Hepatosomatic index (HSI) = [(liver mass (g)/body mass (g)) × 100]

2.3. Heat increment of feeding experiments

The HiE experiments were performed on a common stock of rainbow trout that had been maintained on their prescribed isoenergetic diets for many months and fed 4–5 days a week at a ration level of ~2% of body mass. Following the growth trial described above, the fish were transported from CAER, West Vancouver, BC and used for one of two respirometry studies, the first at Simon Fraser University, Burnaby, BC, Canada (SFU) and the second at the University of British Columbia, Vancouver, BC, Canada (UBC). At SFU, fish were maintained on the three diets in separate 2500 l outdoor tanks provided with aerated, dechlorinated fresh water (7.8–14.0 °C, dissolved O_2 >8.0 mg/l) and a natural photoperiod for 7 months (fish weight = 503.4 ± 10.7 g, mean ± SEM). At the conclusion of this experiment, the remaining fish were transported to UBC and maintained on the three diets in separate 1000 l indoor tanks supplied with aerated, dechlorinated fresh water (11.0–16.0 °C, dissolved O_2 >8.0 mg/l) and a 12 h light:12 h dark photoperiod for 8 months (fish weight = 646.7 ± 47.1 g, mean ± SEM).

2.4. Respirometry

Intermittent flow respirometry was used to measure the oxygen consumption (MO_2) of individual fish. The SFU experiment used an 8-chamber system to measure MO_2. The system has been fully described (Johansen and Geen, 1990; Janz et al., 1991). The 9.1–9.5 l glass vessels received aerated water (temperature ranged from 8.2 to 13.0 °C) at 0.95 l/min. The intermittent flow cycle was set such that each vessel was flushed for 25 min and closed for 5 min, during which the oxygen content of the water was recorded every minute using an Oxyguard O_2 probe (Point Four Systems, Richmond, B.C.).

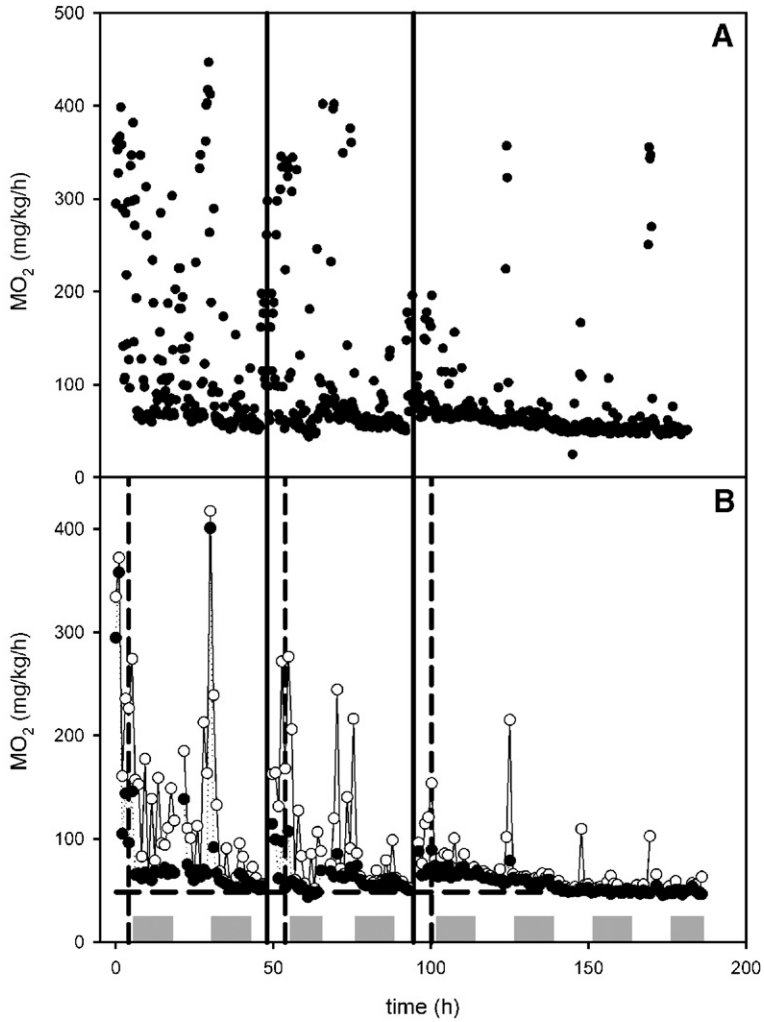

Fig. 1. A representative continuous recording of the MO_2 from a rainbow trout (575.4 g, MP:ML female) using the respirometry system at UBC. Shown in A are raw MO_2 data (each data point represents a 5 min average). The fish was placed in the respirometer at time 0 and allowed 48 h to adjust to the vessel. At the 1st vertical solid bar, the fish was removed, sham-fed and returned to the vessel. At the 2nd vertical solid bar, the fish was removed, force-fed 2% of its body weight and returned to the vessel. Shown in B are the calculated average (open circles) and minimum (closed circles) MO_2 values. The horizontal dashed line indicates the calculated SMR (average of the 6 lowest average values). The dashed vertical lines represent the 4 h recovery period after a fish was replaced into the respirometer following anesthesia which was not used for the analysis of SMR, RMR and HiE because MO_2 was subsiding. Prior to the experiment, the fish were held in a 12 h:12 h L:D photoperiod, which is indicated by the shaded boxes in panel B.

Propellers at the top of the chamber, adjacent to where the O_2 probe was located, ensured that the water in the chamber and around the probe was gently mixed. MO_2 was calculated from the slope of the declining O_2 content of the water during each 5 min closed period. The probes were calibrated with fully aerated water prior to each replicate.

The UBC experiment used a 4-chamber system (Loligo Systems, Hobro, Denmark). The 9.9 l plexi-glass vessels received aerated, 10.0–16.0 °C water at a flow rate of 5 l/min. These vessels had a recirculation pump to ensure proper mixing and minimize flow disturbances to the fish. The flush cycle was 10 min, the wait period was 30 s and the recirculation cycle was 5 min, during which the oxygen content of the water was measured every second using a MINI-DO probe (Loligo Systems, Hobro, Denmark). The oxygen probes were calibrated with oxygen-free distilled water and fully aerated water prior to each replicate. MO_2 was recorded from these measurements using LoliResp4 software (Loligo Systems, Hobro, Denmark).

The feeding protocol was identical for each diet treatment and was as follows. Fish were starved for 48 h before being placed randomly in a vessel and then routine MO_2 was followed (for 24 h at SFU and 48 h at UBC). This period was used to habituate the fish to the respirometer, assess the variability in MO_2 and estimate SMR. Many and varied attempts to get the fish to feed voluntarily in the respirometer were unsuccessful. Therefore, a sham feeding was used to

habituate the fish to force feeding and to assess the handling effect on MO_2. For sham feeding, the fish were lightly anesthetized (a loss of their righting ability using buffered 0.1 g/l MS-222) and sham-fed by inserting plastic forceps and polyethylene tubing while the fish remained submerged in an aerated, buffered, anesthetic bath (0.08 g/l MS-222) for 5 min. MO_2 was followed for a further 24 h after the fish were replaced in the vessel. Fish were then re-anesthetized and force-fed their experimental diet (2% of their body mass) in pellet form using forceps in the same manner as the sham feeding to measure HiE. The fish were replaced and any lost pellets (<10%) were counted. Postprandial MO_2 was followed for 60–96 h. At the end of the experiment, the fish were removed, tagged and returned to their stock tanks. Background MO_2 in each vessel was monitored for at least one hour before and after each trial, and determined to be negligible. Water temperature and O_2 levels in the header tank were continually monitored throughout the experiment. Temperature never varied by more than 0.5 °C in a given experiment. The fish were kept in 24 h darkness throughout the experiment to minimize the effect of circadian rhythms.

2.5. Data analysis❖

MO_2 was recorded for a 5-min period every 30 min at SFU and for a 5-min period every 15.5 min at UBC. Four consecutive 5-min values were pooled to generate a data block that spanned either a 62-min (UBC) or 2-h (SFU) period. These blocks formed the basis for subsequent analyses (Fig. 1). Within each data block, the average MO_2 was determined as the mean of all four 5-min values, while the minimum MO_2 was the lowest 5-min value in a data block. The first 4 h after a fish was replaced in the vessel following anesthesia was deemed a recovery period, *i.e.*, MO_2 was subsiding to a routine level and therefore was not used for the analysis of SMR, routine metabolic rate (RMR) and HiE.

SMR was estimated for each fish as the average of the six lowest block average MO_2 values over the entire trial. RMR was estimated for each fish using equal "dark" and "light" periods prior to feeding. Although the fish were kept in the dark and were left undisturbed, this was a precaution for diurnal rhythms not subsiding and the "light" and "dark" periods corresponded to the times when the fish would have previously experienced light and dark conditions in their holding tanks. The peak postprandial MO_2 was defined for each fish as the highest block value after feeding and the time-to-peak postprandial MO_2 was the number of hours taken to reach the peak value.

The effect of sham feeding on MO_2 was assessed by inspecting individual data for each fish. Sham feeding typically, but not always, elevated MO_2 and was determined to have subsided after 4 h, similar to the initial introduction of fish into the vessel. In view of these results and the recommendation of Brett (1964) that recovery from exhaustive exercise in salmon takes 3–6 h, the first 4 h of postprandial data were not included in the analysis of HiE.

HiE is defined as the postprandial increase in MO_2 above SMR (Jobling, 1981; Beamish and Trippel, 1990). We estimated HiE in two ways: by integrating either the postprandial average MO_2 curve or postprandial minimum MO_2 curve and then subtracting SMR. We reasoned that because routine spontaneous activity elevates MO_2, the

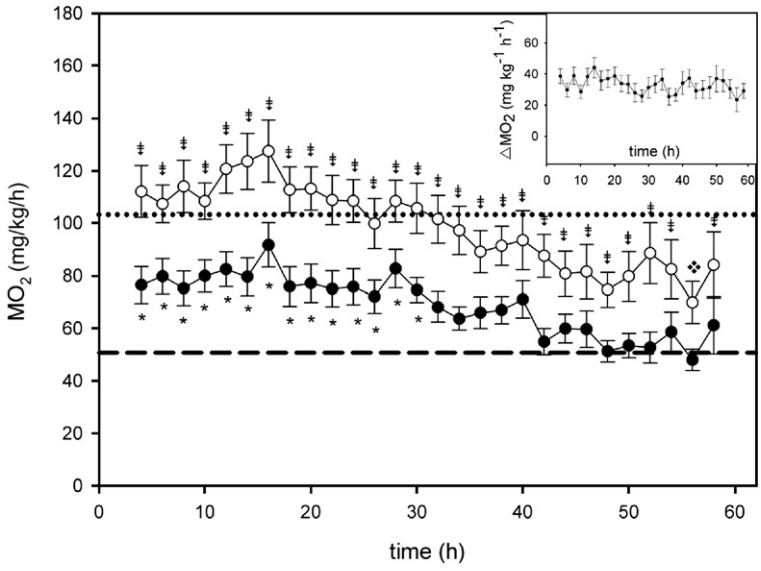

Fig. 2. Postprandial MO_2 average (open circles) and minimum (closed circles) for Simon Fraser University experiments ($n=24$). All diet treatments are combined as the mean±SE. Routine metabolic rate (dotted line) and standard metabolic rate (dashed line) are indicated. A significant difference in minimum MO_2 from SMR is indicated by an asterisk. A significant difference in average MO_2 from SMR is indicated by the symbol "‡" and a significant difference in average MO_2 from both SMR and RMR is indicated by the symbol "❖" ($P<0.05$). Inset: The difference between average MO_2 and minimum MO_2 is presented over time.

Table 3
Temporal changes in fish mass, fork length, weight gain (WG), fork length gain (LG), condition factor (CF), specific growth rates (SGR), and feed efficiency ratios (FER), and terminal hepatosomatic indices (HSI) in relation to diet treatment; $n=3$ for each diet

	HP:LL	MP:ML	LP:HL
Dietary protein (%)	55	45	35
Dietary lipid (%)	10	15	20
Day 0			
Body mass (g)	118.4±2.0[a]	124.5±2.0[a]	119.1±2.0[a]
Length (cm)	22.2±0.1[a]	22.5±0.1[a]	22.2±0.1[a]
CF[1]	1.1±0.0[a]	1.1±0.0[a]	1.1±0.0[a]
Interval 1: day 0 to 28			
WG (g)	54.4±8.4[a]	56.6±2.4[a]	52.5±4.5[a]
LG (cm)	2.2±0.2[a]	2.3±0.0[a]	2.1±0.2[a]
SGR (%/day)[2]	1.34±0.16[a]	1.34±0.05[a]	1.30±0.09[a]
FER (g/g)[3]	0.98±0.05[a]	0.96±0.03[a]	0.90±0.03[a]
Interval 2: day 28 to 56			
WG (g)	75.1±1.5[a]	84.5±2.9[a]	71.8±10.4[a]
LG (cm)	2.5±0.1[a]	2.4±0.1[a]	2.2±0.1[a]
SGR (%/day)	1.29±0.07[a]	1.37±0.03[a]	1.24±0.15[a]
FER (g/g)	0.82±0.04[a]	0.83±0.02[a]	0.81±0.03[a]
Entire trial: day 0 to 56			
WG (g)	129.4±8.1[a]	141.2±4.7[a]	124.2±13.3[a]
LG (cm)	4.7±0.2[a]	4.7±0.1[a]	4.3±0.3[a]
CF	1.3±0.0[a]	1.3±0.0[a]	1.3±0.0[a]
SGR (%/day)	1.32±0.05[a]	1.35±0.03[a]	1.27±0.11[a]
FER (g/g)	0.88±0.00[a]	0.88±0.02[a]	0.85±0.03[a]
HSI[4] (%)	1.5±0.1[a]	1.7±0.1[a]	1.4±0.1[a]

Means (±SE) with different superscript letters were significantly different ($P<0.05$).
HP:LL refers to high protein, low lipid; MP:ML refers to medium protein, medium lipid; LP:HL refers to low protein, high lipid.
[1]CF=[(body mass/length^3)×100].
[2]SGR=[(ln final mass−ln initial mass)/# expt days)]×100.
[3]FER=[wet mass gain (g)/dry feed consumption (g)].
[4]HSI=[(liver mass (g)/body mass (g))×100].

average postprandial MO_2 could overestimate HiE. Thus, the minimum MO_2 estimate was intended to remove this possibility. An alternative approach to account for activity when estimating HiE was to subtract RMR (rather than SMR) from postprandial average MO_2 values, *i.e.*, the assumption would be that the range of activity was similar both pre- and postprandial. However, estimating HiE using average postprandial MO_2 above RMR was rejected because of an unacceptable error was generated (postprandial MO_2 decreased below RMR after 56 h; Fig. 2) possibly because the fish became less active over time in the respirometry system. In calculating HiE and to account for the initial 4 h postprandial, we assumed a linear relationship between SMR and the measured 4-h postprandial value, an assumption that could slightly overestimate HiE if postprandial MO_2 was delayed appreciably. The cost of HiE as a % of digestible energy intake, termed the HiE coefficient (C_{HiE}), was estimated by assuming that 1 g of oxygen is associated with the release of 13.6 kJ of energy ($C_{HiE}=(E_{HiE}/E_{meal})*100$) (Cho et al., 1982).

2.6. Statistical analysis

Differences in fish body mass, WG, LG, CF, SGR, FER, DFI, PER, PPD, HSI, percentages for whole body ash, moisture, protein and lipid, SMR, RMR, peak, time-to-peak, HiE and C_{HiE} were compared using one-way Analysis of Variance (ANOVA) followed by the Holm–Sidak multiple comparisons test (SigmaStat 3.0). The effect of diet on postprandial MO_2 over time was assessed using 2-way repeated measures ANOVA (SigmaStat 3.0). The pooled data from all diet treatments were assessed using a one-way repeated measures ANOVA comparing MO_2 to SMR and RMR values over time. P values of less than 0.05 were considered statistically significant and the Holm–Sidak or Bonferonni multiple comparisons method was used to infer differences. Data that were not normally distributed or had

Table 4
Temporal changes in dry feed intake (DFI), protein efficiency ratio (PER), percent protein deposited (PPD), and final whole body concentrations of proximate constituents for trout in relation to diet treatment; $n=3$ for each diet

	HP:LL	MP:ML	LP:HL
Dietary protein (%)	55	45	35
Dietary lipid (%)	10	15	20
Interval 1: day 0 to 28			
DFI (g/fish)	55.17±6.04[a]	59.33±2.99[a]	58.08±3.42[a]
PER (g/g)[1]	1.78±0.10[a]	2.05±0.06[a]	2.39±0.08[b]
Interval 2: day 28 to 56			
Dry feed intake (g/fish)	92.39±4.38[a]	102.30±4.66[a]	87.83±9.29[a]
PER (g/g)	1.48±0.07[a]	1.77±0.05[a]	2.16±0.09[b]
Entire trial: day 0 to 56			
Dry feed intake (g/fish)	147.56±9.86[a]	161.63±6.96[a]	145.91±10.67[a]
PER (g/g)	1.60±0.01[a]	1.87±0.04[b]	2.25±0.08[c]
PPD[2] (%)	29.40±0.97[a]	31.90±0.61[ab]	37.57±2.21[b]
Final whole fish proximate composition (as is basis)[3]			
Moisture (%)	70.40±0.55[a]	68.16±0.14[b]	67.62±0.60[b]
Ash (%)	2.00±0.08[a]	1.87±0.05[a]	1.95±0.05[a]
Protein (%)	18.22±0.28[a]	17.06±0.06[b]	17.22±0.17[b]
Lipid (%)	9.05±1.02[a]	12.44±0.09[b]	12.69±0.63[b]

Means (±SE) with different superscript letters were significantly different ($P<0.05$).
HP:LL refers to high protein, low lipid; MP:ML refers to medium protein, medium lipid; LP:HL refers to low protein, high lipid.
[1]PER=[wet mass gain (g)/protein consumption (g)].
[2]PPD=[(protein gained in fish (g)/total protein consumed (g))×100].
[3]The initial protein concentration in the fish was estimated to be 17.7% (Weatherup and McCracken, 1999) in order to calculate values for percent protein deposited.

Table 5
Standard metabolic rate (SMR), routine metabolic rate (RMR), minimum and average peak postprandial MO_2 and minimum and average time-to-peak postprandial MO_2 for each diet treatment are presented as the mean±SE

	HP:LL	MP:ML	LP:HL
Dietary protein (%)	55	45	35
Dietary lipid (%)	10	15	20
n	9	8	7
SMR (mg O_2/kg/h)	50±5.6[a]	47.5±10.1[a]	55.5±5.1[a]
RMR (mg O_2/kg/h)	115.3±10.8[a]	79.1±16.3[a]	115.4±15.9[a]
Peak min MO_2 (mg O_2/kg/h)	115.7±12.1[a]	101.9±14.6[a]	148.3±17.5[a]
Peak avg MO_2 (mg O_2/kg/h)	185.9±14.8[a]	158.2±24.2[a]	194.4±23.5[a]
Time-to-peak min MO_2 (h)	18.9±4.6[a]	19.3±3.7[a]	21.7±6.4[a]
Time-to-peak avg MO_2 (h)	24.2±5.9[a]	21.8±4.3[a]	26.9±6.3[a]

Means with a common superscript letter in the same row indicate no effect of diet treatment within an experiment ($P>0.05$).

unequal variances were also assessed using a nonparametric rank test followed by Dunn multiple comparisons (SigmaStat 3.0). Mean values ±1 standard error of the mean (SE) are presented.

3. Results

3.1. Test diets

The protein and lipid concentrations in the test diets on a dry weight basis were determined to be close to expected values (Table 2). Likewise, the digestible protein contents of the HP:LL, MP:ML, and LP:HL diets (*viz.*, 49.5%, 42.1%, and 34.2%) were near expected values considering their respective determined protein concentrations and digestibility coefficients for protein (latter varied between 90 and 91%). Further, the gross energy values of the diets were within 11% of each other, *i.e.*, 18.7, 19.8 and 21.0 MJ/kg for the HP:LL, MP:ML and LP:HL diets, respectively (Table 2), and the estimated DE contents of the diets were almost identical, *i.e.*, 16.6–17.3 MJ/kg. DP to DE ratios in the HP:LL, MP:ML, and LP:HL diets were estimated as 29.8, 24.8, and 19.8 g/MJ, respectively.

3.2. Influence of diet treatment on fish growth performance and body composition

On day 0 of the feeding trial, fish mass, fork length and CF were not significantly different among fish assigned to the three diet treatments (Table 3). During the 8-week growth trial, the fish in each group more than doubled their weight and their final body mass, length and values for SGR and CF were unaffected by diet treatment throughout the trial. Similarly, DFI and FER values were not significantly influenced by the diet treatments throughout the trial (Table 4) and this was also true for terminal HSI values (ranged from 1.4 to 1.7%).

PER values varied significantly among fish given the dietary treatments (Table 4). Between day 0 and 28 and also between day 28 and 56, fish fed the LP:HL diet exhibited significantly higher PER values than those fed the HP:LL and MP:ML diets. Between day 0 and 56, there were significant differences in PER values among all three diet groups. Fish fed the HP:LL diet had the lowest PER value whereas that for fish fed the MP:ML diet was significantly higher. Fish fed the LP:HL diet had the highest PER value. Values for PPD were similarly inversely related to dietary protein concentration and fish fed the HP:LL diet had a significantly lower PPD value than that noted for fish fed the LP:HL diet.

Diet composition significantly altered the terminal concentrations of whole body proximate constituents (Table 4). The HP:LL fish had significantly more protein and moisture, and significantly less lipid compared with fish fed the other two diets (Table 4). However, percentages for whole body moisture, protein, and lipid were the same for fish fed the MP:ML and LP:HL diets.

Consequently, the HP:LL fish grew at the same rate and with the same feed efficiency as those ingesting the MP:ML and LP:HL diets. However, trout fed the two latter diets deposited significantly more lipid in their bodies and utilized dietary protein more efficiently for growth and protein deposition (LP:HL fish only) than those fed the HP:LL diet.

3.3. Heat increment of feeding experiments

The three dietary treatments did not result in significant differences for SMR, RMR, peak MO_2 or time-to-peak MO_2 (Table 5). Also, postprandial MO_2 was not significantly

Table 6
Minimum and average heat increment of feeding (HiE) values for fish from each diet treatment were calculated after 12, 18, 24, 36, 48 and 58 h postprandial

	HP:LL	MP:ML	LP:HL
Dietary protein (%)	55	45	35
Dietary lipid (%)	10	15	20
n	9	8	7
HiE[a] minimum (mg O_2/kg)			
12 h	368.9±50.2[a]	282.7±65.7[a]	305.8±64.7[a]
18 h	537.8±72.3[a]	457.1±94.5[a]	539.7±108.7[a]
24 h	668.3±94.9[a]	636.0±115.5[a]	674.5±127.4[a]
36 h	893.3±140.7[a]	929.1±113.7[a]	899.5±137.0[a]
48 h	946.1±180.4[a]	1254.8±87.7[a]	915.6±171.4[a]
58 h	898.3±201.6[a]	1274.9±101.1[a]	838.4±171.5[a]
HiE[a] average (mg O_2/kg)			
12 h	904.0±92.2[a]	653.8±76.2[a]	626.5±81.4[a]
18 h	1307.6±140.2[a]	1030.6±130.4[a]	1126.3±145.6[a]
24 h	1696.3±188.0[a]	1378.9±161.4[a]	1449.8±147.4[a]
36 h	2226.9±185.6[a]	2014.2±262.3[a]	2078.9±169.6[a]
48 h	2608.2±183.7[a]	2451.5±387.5[a]	2492.4±227.2[a]
58 h	2912.3±214.8[a]	2560.9±432.2[a]	2830.3±417.4[a]

Mean±SE with a common superscript letter in the same row indicate no effect of diet treatment within an experiment ($P>0.05$).

[a] Average or minimum HiE is calculated as the postprandial minimum or average MO_2 integral minus the SMR integral.

Table 7
Standard metabolic rate (SMR), routine metabolic rate (RMR), peak minimum and average postprandial MO_2, and time-to-peak minimum and average postprandial MO_2 for Simon Fraser University (SFU) and University of British Columbia (UBC) trout are presented as the mean±SE

	SFU	UBC
n	24	5
SMR (mg O_2/kg/h)	50.8±4.1[a]	48.8±2.1[a]
RMR (mg O_2/kg/h)	103.3±8.6[a]	92.6±7.9[a]
Peak min MO_2 (mg O_2/kg/h)	120.6±8.9[a]	94.8±8.4[a]
Peak avg MO_2 (mg O_2/kg/h)	179.2±11.8[a]	162.8±22.1[a]
Time-to-peak min MO_2 (h)	19.8±2.7[a]	13.4±2.4[a]
Time-to-peak avg MO_2 (h)	24.2±3.1[a]	12.4±2.2[a]

Means with a common superscript letter in the same row indicate no significant difference ($P>0.05$).

different among dietary treatments. Consequently, there were no significant differences for either minimum or average HiE at any time (12, 18, 24, 36, 48, 58 and 80 h) postprandial among fish fed the different diets (Table 6).

In the absence of differences among dietary treatments, data were pooled to increase the power of the analysis of HiE between the slightly different measurement protocols used at SFU and UBC.

For the pooled SFU experiments ($n=24$), SMR and RMR, respectively, were 50.8±4.1 mg O_2/kg/h and 103.3±8.6 mg O_2/kg/h (Table 7). For the pooled UBC experiments ($n=5$), SMR and RMR were 48.8±2.1 mg O_2/kg/h and 92.6±7.9 mg O_2/kg/h, respectively (Table 7). There were no significant differences in the estimates for SMR, RMR, and minimum or average peak MO_2 between the SFU and UBC experiments. Similarly, there were no significant differences in minimum or average time-to-peak MO_2 among experiments, although there was considerable variation (Table 7). RMR was about twice SMR at both locations.

The postprandial MO_2 data for both locations are illustrated in Figs. 2 and 3. By the 4th h postprandial (to account for the effect of handling) both minimum and average MO_2 were significantly elevated over SMR in both experiments. At SFU, average postprandial MO_2 remained significantly elevated above SMR for the entire duration of the trial, while minimum postprandial MO_2 returned to SMR after the first 30-h postprandial (Fig. 2). At UBC, average MO_2 remained consistently above SMR for the first 20-h postprandial, except for two significant spikes at 27 and 35 h. Minimum MO_2 remained elevated above SMR for 44-h postprandial (Fig. 3).

The insets for Figs. 2 and 3 show a more or less consistent difference between minimum and average MO_2 throughout the postprandial period, which represents a constant error term. An exception is between 11 and 16 h postprandial at UBC, when average postprandial MO_2 was 30–40 mg O_2/kg/h higher than minimum MO_2. Moreover, the difference between average and minimum postprandial MO_2 was statistically greater at SFU (around 30 mg O_2/kg/h, Fig. 2) than at UBC (around 10 mg O_2/kg/h, Fig. 3), which suggests that the fish at SFU were more active during the postprandial period.

Calculations of HiE and C_{HiE} are shown in Table 8. Using the minimum estimates, there was no significant difference in HiE among experiments. Notably, the minimum estimate of C_{HiE} was consistently between 4.0 and 4.8% of digestible energy consumed by fish in both experiments. Significant differences in average

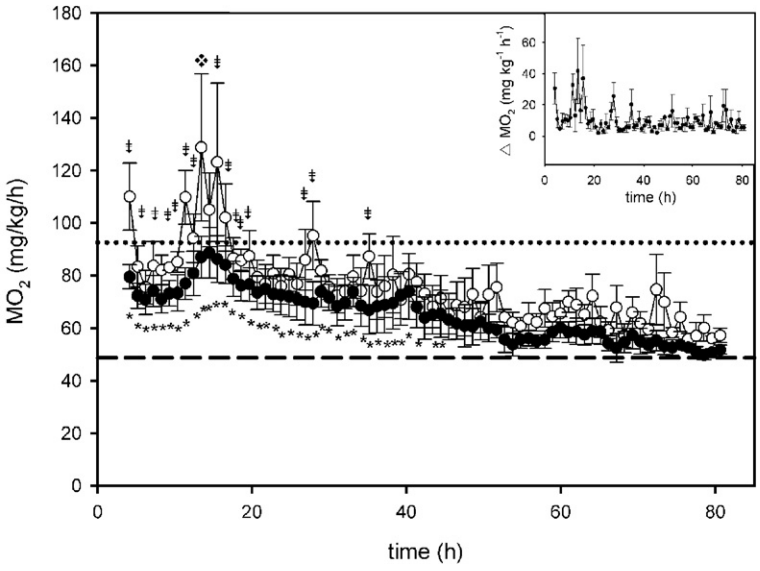

Fig. 3. Postprandial MO_2 average (open circles) and minimum (closed circles) for trout from the University of British Columbia experiments ($n=5$). All diet treatments are combined as the mean±SE. Routine metabolic rate (dotted line) and standard metabolic rate (dashed line) are indicated. A significant difference in minimum MO_2 from SMR is indicated by an asterisk. A significant difference in average MO_2 from SMR is indicated by the symbol "ǂ"and a significant difference in average MO_2 from both SMR and RMR is indicated by the symbol "❖" ($P<0.05$). Inset: The difference between average MO_2 and minimum MO_2 is presented over time.

Table 8
Minimum and average heat increment of feeding (HiE) values for Simon Fraser University (SFU) and University of British Columbia (UBC) trout were calculated 12, 18, 24, 36, 48, 58 and 80 h postprandial

	Minimum		Average	
	SFU	UBC	SFU	UBC
n	24	5	24	5
HiE[1] (mg/kg)				
12 h	321.8± 33.9[a]	294.8± 63.2[a]	739.7± 53.9[a]	490.2± 61.1[a]
18 h	511.5± 50.5[a]	475.4± 98.8[a]	1162.4± 80.7[a]	791.8± 124.3[a]
24 h	659.3± 61.4[a]	629.2± 142.9[a]	1518.6± 98.8[a]	985.0± 160.5[b]
36 h	907.6± 72.1[a]	887.6± 237.3[a]	2112.8± 118.4[a]	1356.4± 253.2[b]
48 h	1021.7± 95.3[a]	1079.4±3 35.0[a]	2522.2± 154.2[a]	1619.9± 351.9[b]
58 h	987.9± 103.3[a]	1163.5± 82.1[a]	2771.2± 198.1[a]	1797.7± 396.4[b]
80 h	–	1010.4± 433.9	–	1827.0± 442.2
C_{HiE} (% of DE intake)				
12 h	1.4±0.1[a]	1.3±0.3[a]	3.3±0.2[a]	2.2±0.3[a]
18 h	2.3±0.2[a]	2.1±0.4[a]	5.1±0.4[a]	3.5±0.6[a]
24 h	2.9±0.3[a]	2.8±0.6[a]	6.7±0.4[a]	4.4±0.7[b]
36 h	**4.0±0.3**[a]	3.9±1.1[a]	9.4±0.5[a]	**6.0±1.1**[b]
48 h		**4.8±1.5**	11.2±0.7	
58 h			>**12.3±0.9**	
80 h				

The HiE coefficient (C_{HiE}) as a percent of dietary digestible energy intake (16.7 MJ/kg dry mass) was calculated assuming 1 g of O_2 is associated with the release of 13.6 KJ of energy (Cho et al., 1982). Mean±SE not sharing the same letter within a category (*i.e.* Minimum or Average) and row (timeframe) are significantly different ($P<0.05$). Bold font indicates the cost of HiE in each experiment estimated after postprandial MO_2 had returned to SMR.

HiE and average cost of HiE did exist, as might be expected from the greater difference between minimum and average postprandial MO_2 at SFU. In fact, the average HiE at SFU was significantly greater between 24 and 58 h compared with fish at UBC.

4. Discussion

4.1. Growth trial

The specific growth rate (SGR: 1.27–1.35%/day) and feed efficiency (FE: 0.85–0.88) values obtained in the present study agree well with previous growth trials involving rainbow trout (*e.g.* SGR: 1.18–2.06%/day, Steffens et al., 1999; Lanari and D'Agaro, 2002; FE: 0.79–0.88, Brauge et al., 1994; Azevedo et al., 2004b) and are indicative of good growth and feed conversion efficiency.

Traditionally, the effect of diet quality on fish growth is assessed using either DP:DE or the dietary protein-to-lipid ratio. The recommended DP:DE ratio is suggested as 22–25 g DP/MJ DE for rainbow trout (Cho and Kaushik, 1990; Cho, 1992; NRC, 1993; Higgs et al., 1995). The diets used in the present study (19.8, 24.8 and 29.8 g DP/MJ DE) bracketed this range and our results clearly show that while the specific growth rates, feed intakes and feed efficiencies of the groups were not compromised by any of the DP:DE ratios, those for dietary protein utilization were adversely affected when DP:DE was 29.8. Thus, while our findings agree with the maximum value for the recommended optimal range for dietary DP:DE for rainbow trout, they additionally suggest that the lower value of this range could be reduced to 20 when the body mass exceeds 100 g.

In this regard, previous studies while recommending dietary protein and lipid concentrations of 35–45% and 15–20% (expressed on a dry weight basis), respectively, for good growth of juvenile rainbow trout, have cautioned that compromised growth and/or protein utilization occur outside this range (Cho and Kaushik, 1990; Cho, 1992; NRC, 1993; Higgs et al., 1995). Yet, more recent studies show increased growth rates with rainbow trout fed diets of low protein and high lipid content (Yigit et al., 2002; Chaiyapechara et al., 2003; Morrow et al., 2004). The importance of isoenergetic diets, as used in the present study, has already been noted since varying one nutrient level inevitably alters the others in a complete diet. In addition, interactions between feed components are inevitable and important. For example, the protein requirement is dependent upon the levels of other non-protein energy sources (Wilson, 2002; Ruohonen and Kettunen, 2004) and when multiple components are varied it is essential to maintain energetic equivalence on a bioavailable basis.

Our growth results are more in line with those of Azevedo et al. (2004a,b) who observed a similar dietary protein utilization effect to the present study, where a protein-sparing effect was associated with increasing fish body fat content. Also, Steffens et al. (1999) found no difference in weight gain of 92 g trout fed isoenergetic diets of similar protein (47–48%) but different lipid levels (13% and 24%). Therefore, these studies together with the present one suggest that aquaculturists can feed juvenile rainbow trout less expensive diets of increased lipid content and a decreased ratio of digestible protein to lipid without sacrificing their growth and feed efficiency and, at the same time, improve their dietary protein utilization. Consequently, the commonly cited optimal dietary range for DP:DE ratios of rainbow trout *viz.*, 22–25 g DP/MJ DE is likely too conservative for 120–250 g juvenile rainbow trout and should be widened to 20–25 g DP/MJ DE.

In addition to supporting the common finding that trout fed a high lipid diet increase their whole body lipid content (*e.g.* Satia, 1974; Reinitz et al., 1978; Jobling, 1981; Azevedo et al., 2004a), the present study also provided evidence for an upper limit to lipid deposition. When LP:HL fish were fed 33% more lipid than the MP:ML fish, whole body lipid content was unaffected. Instead, the extra lipid content in the LP:HL diet favorably influenced the conversion of dietary protein into body protein. Indeed, trout consuming the LP:HL diet significantly increased the protein efficiency ratio and the percent protein deposited compared with the HP:LL diet. This phenomenon of protein sparing for growth, when lipid availability is at a high level, has been well documented in rainbow trout (*e.g.* Reinitz et al., 1978; Takeuchi et al., 1978; Medland and Beamish, 1985; Beamish and Medland, 1986; Yigit et al., 2002).

4.2. Heat increment of feeding

4.2.1. Calculating HiE

The method of estimating HiE varies among studies, and depends on the respirometry system and the SMR calculation method. Accurate estimates of HiE require a separation of metabolism associated with activity and stress from that associated with feed intake (Brett and Groves, 1979). In fact, the large range reported for HiE (8 to 29% of the digestible energy intake of fish fed formulated diets; Cho et al., 1982; Beamish and Trippel, 1990), could easily reflect an overestimate of HiE as a contributing factor. The present study addressed this concern by estimating HiE in several ways using intermittent flow-through respirometry and long measurement periods preceding the feeding trial. First, two different respirometry systems were used on the same group of fish. Second, we calculated HiE in three different ways. Of these, HiE calculated using the minimum postprandial MO_2 integral minus SMR most likely eliminated the influence of spontaneous activity. Even so, the average postprandial MO_2 integral minus SMR, which we also used, is a more common method for estimating HiE in the literature but it does not account for spontaneous activity. We rejected a third approach (subtracting RMR, rather than SMR, from postprandial average MO_2 values and assuming that the range of activity was similar both pre- and post-feeding) outright because postprandial MO_2 decreased below RMR. Therefore, only two estimates of HiE, both based on SMR, are presented here, although RMR is displayed for reference in the figures.

A priori there should not be a difference in comparable estimates of HiE. Our analysis clearly revealed that minimum rather than average postprandial MO_2 is a better estimate of HiE, as predicted. We base this conclusion on a comparison for the same fish but using different respirometry systems and a slightly different protocol, which revealed no significant difference in the estimates of minimum HiE. However, average postprandial MO_2 values at SFU were consistently higher than those at UBC, likely due to differences in levels of routine spontaneous activity in the respirometry systems. In fact, using average postprandial MO_2 overestimates C_{HiE} by 25–200%. While there is a risk that using minimum HiE may underestimate the true cost of HiE, the possibility of rainbow trout intermittently and substantially down-regulating their metabolism during the postprandial period is small, unlike the larger and more common error associated with spontaneous activity being included as part of HiE. Therefore, we recommend that future studies should use the minimum postprandial MO_2 to estimate HiE and thereby reduce the confounding effect of spontaneous activity of the fish in a respirometry system.

4.2.2. Effect of diet composition on HiE

Protein digestion and assimilation have a large contribution to HiE, and yet our large dietary range for DP:DE, which significantly altered protein utilization and deposition, had no effect on SMR, RMR, peak MO_2, time-to-peak MO_2 or HiE. While previous studies have reported that increases in dietary protein levels result in rises in HiE in rainbow trout (Cho et al., 1976, 1982; Jobling, 1981; LeGrow and Beamish, 1986; Cho and Woodward, 1989), these studies often have not provided detailed information on the methods that were used to calculate HiE. Moreover, the studies frequently did not use diets with equivalent DE or essential amino acid balance. In addition, few studies assessed the effect of diet on both HiE and growth using the same group of fish.

Despite the foregoing differences in conditions between the studies, several factors may account for most of the differences in their findings. For instance, smaller fish, because of their higher scope for growth, require more protein compared to larger fish (Satia, 1974; Hilton and Slinger, 1981) and maximum and maintenance rations both decrease as salmonids increase in size, with maximum ration decreasing at a faster rate (Higgs et al., 1995). Previous studies demonstrating higher HiE in trout fed diets of high protein content all used smaller (4–145 g) rainbow trout (Cho et al., 1976; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Cho and Woodward, 1989), than those used here (500–650 g). In addition, differences in the levels of dietary intake of the fish between and within studies could have influenced the findings. For instance, the

ration in the present study was ~2% of their body weight 4–5 days per week while HiE was assessed rather than the maximum ration used during the growth study. This change in protocol could have diminished the protein utilization differences that were observed between groups in the growth study where all fish were fed their respective diets daily to satiation. Further, as mentioned above, other studies did not pay close attention to formulating their test diets so that they contained equal concentrations of physiologically useful digestible carbohydrate (≤15%; Higgs et al., 1995), and DE as well as equivalent essential amino acid balance. Hence, it is conceivable that some of the elevation of HiE associated with the ingestion of high versus low protein diets in previous studies on trout can be attributed to increased catabolism of amino acids because of an inferior balance of essential amino acids in the high protein instead of low protein diets. Also, the diets in these former studies, unlike those in the present study, were likely not close in metabolizable energy content because of their dissimilar levels of digestible carbohydrate and this could have accentuated the differences in results for HiE between fish fed the diets of different protein content. Lastly, the low value of C_{HiE} found in this study may have made it difficult for us to detect subtle differences in HiE due to diet treatment.

The absence of a dietary effect on HiE is consistent with our findings of no effect of diet treatment on the specific growth rate, dry feed intake and feed efficiency of the fish during the growth trial. Moreover, these results collectively suggest that our test diets met the dietary needs of the fish and that their DP:DE ratios were generally within an acceptable range. Therefore, the energetic costs of the numerous processes that contribute to HiE were not found to be different over a wide range of dietary DP:DE ratios when the diets were isoenergetic and the fish were fed on a restricted ration basis.

4.2.3. Metabolic and postprandial states

Mean SMR from all fish combined (50.4±3.4 mg O_2/kg/h) was at the low end of the range reported for SMR (48–80 mg O_2/kg/h) in rainbow trout (Webb, 1971; Kiceniuk and Jones, 1977; Pagnotta and Milligan, 1991; Alsop and Wood, 1997; Claireaux et al., 2005; Simonot, 2005). Peak postprandial MO_2 for all fish combined was 116.2±7.7 mg O_2/kg/h (minimum) and 176.3±10.4 mg O_2/kg/h (average), which represent postprandial increases of 131% and 250% above SMR. These increases are within the range of peak postprandial MO_2 values obtained for many different fish of between 1.5 and 2.5 times SMR (Jobling, 1981; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Ross et al., 1992; Boyce and Clarke, 1997; Hunt von Herbing and White, 2002; Peck et al., 2005). Obviously, MO_2 has an upper limit and peak postprandial MO_2 can be expressed as a percentage of this value. Using an active metabolic rate for rainbow trout of this size range (371.9 mg O_2/kg/h; Kiceniuk and Jones, 1977), we estimated that peak postprandial MO_2 was 53% (minimum) and 69% (average) of maximum MO_2. Kaczanowski and Beamish (1996) estimated that peak postprandial MO_2 was 25–48% of the estimated active metabolic rate for 250–450 g rainbow trout that had been infused with various amino acid solutions. LeGrow and Beamish (1986) found that peak postprandial MO_2 was usually between 60 and 80% of active metabolic rate in 10–15 g rainbow trout fed 2% of their body mass diets of varying protein and lipid levels. Thus, large rainbow trout can often utilize around 50% of their aerobic scope (maximum MO_2 minus SMR) following a meal and this percentage may be higher in smaller fish.

Extensive research on gastric emptying time and duration of HiE (for review see Brett and Groves, 1979; Fange and Grove, 1979; Jobling, 1981) suggests that digestion lasts 24–36 h in rainbow trout held at intermediate temperatures (mean about 12 °C) and fed around 2% of their body mass almost daily. Our results suggest that HiE was completed for a meal of 2% of body mass between 30 and 44 h postprandial. Minimum MO_2 in fish at SFU returned to SMR 30-h postprandial. At UBC, average and minimum MO_2 returned to SMR after 35 and 44 h, respectively. These durations are consistent with earlier studies and therefore suggest that the rainbow trout handled gavage well. Indeed, MO_2 had largely subsided after 4 h in the present studies. Other studies show active digestion shortly after gavage. For example, following a force feeding of 1% of their body mass, the rise in amino acids in the circulatory system of rainbow trout peaked between 4 and 12 h postprandial and had returned to baseline by 24 h (Murai et al., 1987; Ok et al., 2001). Similarly, Karlsson et al. (2006) sampled plasma amino acids from both the dorsal aorta and hepatic portal vein in rainbow trout force fed 1% of their body mass and found a peak in amino acids between 6 and 24 h with a subsequent return to baseline by 48 h postprandial. Stress likely slows digestion for a variety of reasons, including a decrease in gut blood flow associated with handling and struggling (Farrell et al., 2001), and this was suggested as the reason for instrumented fish having a prolonged digestion unless they are allowed to recover for at least 7 days post-surgery (Eliason et al., submitted for publication).

The minimum C_{HiE} in fish at SFU and UBC was 4.0% and 4.8%, respectively. These values are appreciably

lower than those in the literature for several reasons. Foremost, minimum HiE essentially eliminated the overestimate associated with spontaneous activity in the respirometry vessels. Secondly, SMR was estimated over an extended period of time. Finally, well-formulated diets decrease C_{HiE} (Higgs et al., 1995). Not surprisingly, our estimates of average C_{HiE} were higher (from 6% to >12%) and also more in line with previous HiE estimates for rainbow trout fed 2% of their body mass (8–24%; Cho et al., 1982; Medland and Beamish, 1985; LeGrow and Beamish, 1986). These comparisons re-emphasize the importance of using minimum MO_2 for studies of HiE.

In conclusion, the present study devised a protocol that reliably and repeatedly estimated HiE and C_{HiE} in rainbow trout using the minimum postprandial MO_2. However, we were unable, under the conditions of this study, to detect any metabolic differences between trout fed diets that contained a wide range in DP:DE even though their protein utilization and protein deposition, but not growth performance, were inversely related to the dietary DP to DE ratio. The reason for this finding may be related to differences in the planes of nutrition and fish sizes that were employed between the growth and metabolic studies. While the HiE and C_{HiE} methods may prove useful for future laboratory studies examining the metabolic cost of digestion, large-scale field measurements of HiE, such as in aquaculture net pens, are difficult. Instead, it may be important to establish relationships between MO_2 and other digestion variables that can be measured more easily in the field in order to indirectly estimate HiE.

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