GROWTH, HEAT INCREMENT OF FEEDING AND GUT BLOOD FLOW IN RAINBOW TROUT

by

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Abstract

Juvenile rainbow trout were fed to satiation three different isocaloric diets of high (H), medium (M), or low (L) protein (P) and lipid (L) concentrations [protein: lipid ratios were: 55%:10% (HP:LL); 45%:15% (MP:ML) and 35%:20% (LP:HL)] during an 8-week growth trial. Fish fed the HP:LL diet were composed of significantly more protein (18.2%) and significantly less lipid (9.1%) compared to those fed the other two diets. However, growth performance parameters (specific growth rate, feed efficiency and dry feed intake) did not vary significantly among diets. Even so, the LP:HL diet resulted in a greater percent protein deposition (37.6 ± 2.2%) over the 8-week trial and a greater protein efficiency ratio (2.3 ± 0.1) compared to HP:LL diet (29.4 ± 1.0% and 1.6 ± 0.0, respectively), suggesting a protein sparing effect.

These same fish were maintained on the diet treatments and postprandial gut blood flow (GBF) and oxygen consumption (MO₂) were assessed following a single meal (by gavage) of 2% of their body mass. There were no significant differences among diet treatments for MO₂, GBF or heart rate. When the three diet treatments were pooled, standard metabolic rate (SMR), baseline GBF and baseline heart rate were 52.6 ± 2.5 mg O₂ kg⁻¹ h⁻¹, 4.0 ± 0.2 ml min⁻¹ kg⁻¹ and 34.2 ± 1.8 beat min⁻¹, respectively. Compared with these values and after accounting for the effect of handling for gavage, minimum MO₂, GBF and heart rate had increased significantly by 4-h postprandial, and peak increases were 119%, 153% and 75-115%, respectively. In fish equipped with a Transonic flow probe fed a second time, all three variables had returned to baseline levels between 24-h and 48-h postprandial, as was the case in un-probed fish. In contrast, fish with a GBF probe fed 48 h after surgery displayed elevated postprandial MO₂ and GBF for the entire
duration of the 80-h study. This extended elevation was likely due to prolonged digestion associated with surgery. The metabolic cost of the diets (as a % of digestible energy) was low (4.0 – 9.7%) and did not differ among these diet formulations.
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List of Abbreviations

AMR  active metabolic rate (mg O\textsubscript{2} kg\textsuperscript{-1} h\textsuperscript{-1})
DE   digestible energy
DFI  dry feed intake (g fish\textsuperscript{-1})
DP:DE digestible protein to digestible energy ratio (g MJ\textsuperscript{-1})
FER  feed efficiency ratio
GBF  gut blood flow (ml min\textsuperscript{-1} kg\textsuperscript{-1})
GSI  gonadosomatic index (%)
HL   high lipid
HP   high protein
HSI  hepatosomatic index (%)
HiE  heat increment of feeding (mg O\textsubscript{2} kg\textsuperscript{-1})
LL   low lipid
LP   low protein
ML   medium lipid
MO\textsubscript{2} oxygen uptake (mg O\textsubscript{2} kg\textsuperscript{-1} h\textsuperscript{-1})
MP   medium protein
PER  protein efficiency ratio (%)
PPD  percent protein deposition (%)
Q    cardiac output
Q\textsubscript{max} maximum cardiac output (ml min\textsuperscript{-1} h\textsuperscript{-1})
Q\textsubscript{rest} resting cardiac output (ml min\textsuperscript{-1} h\textsuperscript{-1})
RMR  resting metabolic rate (mg O\textsubscript{2} kg\textsuperscript{-1} h\textsuperscript{-1})
SGR  specific growth rate
SMR  standard metabolic rate (mg O\textsubscript{2} kg\textsuperscript{-1} h\textsuperscript{-1})
U\textit{crit} critical swimming velocity
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Dedication

I dedicate this thesis to ma, pa and Grandpa Eliason, whose love for fish and the great outdoors continues to inspire me daily.
Chapter 1: Introduction and literature review

Diet composition

Fish must ingest diets in order to obtain nutrients and energy that are essential for growth, health, reproduction and body maintenance (NRC, 1993). Researchers formulate diets with the goal of maximizing health and growth, and minimizing loss through excretion and heat production. Care must be taken to ensure that sufficient energy, protein (amino acids), lipid (fatty acids), carbohydrate, mineral and vitamin levels are obtained. In addition, these components all interact with each other; changing one nutrient inevitably varies the others in a complete diet.

Energy

Some of the energy ingested from a meal is lost while feed is digested and metabolized (Figure 1.1). Digestible energy (DE) represents the intake energy (IE) adjusted for the energy lost through the feces (FE). Metabolizable energy (ME) characterizes the DE adjusted for energy lost through gill excretion (ZE) and urine excretion (UE). The heat increment of feeding (HiE) is the difference between metabolizable energy and net energy (NE). This is attributed to the ingested energy lost through the digestion, absorption, waste and product formation processes directly associated with feeding. The remaining energy is first allocated to maintenance metabolism (HEm) to meet basal metabolism, then for voluntary activity and thermal regulation needs and, finally, the recovered energy is available for growth, fat deposition and reproduction.
Dietary nutrients

Protein is the most expensive and important nutrient for fish, meeting a vast array of biological functions. For instance, proteins provide energy and enzymatic function, and they are needed for tissue repair and maintenance, structural integrity of cells, elaboration of new tissue, glucose formation through gluconeogenesis, and for synthesis of antibodies and some hormones. Dispensable (non-essential) amino acids can be synthesized from other amino acids, while indispensable (essential) amino acids must be obtained from the feed. An optimal balance of both dispensable and indispensable amino acids, matching the needs of the fish, will result in the most efficient growth (NRC, 1993) provided that there is a sufficient and appropriate supply of dietary energy.

Dietary lipids are the highest energy-yielding nutrient and an important source of essential fatty acids. Essential fatty acids cannot be synthesized by fish de novo, and are vital for proper growth, membrane structure, reproduction and survival (Higgs et al., 1995). In particular, linolenic acid, 18:3(n-3), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential for rainbow trout growth (NRC, 1993). Moreover, lipids play an important role in the absorption of fat-soluble vitamins.

Glucose is a key fuel for normal nervous, gonad and erythrocyte function. It also acts as a precursor to synthesize various biologically relevant compounds (e.g. glycogen, chitin and nucleic acids) and can act to modulate endocrine function (Higgs et al., 1995). Glucose can be obtained either from dietary carbohydrate or it can be synthesized by the conversion of proteins and lipids through gluconeogenesis.

Minerals, or inorganic elements, play an important role in osmotic, ionic and pH equilibrium. Moreover, they are important structural components of various enzymes and form
skeletal structures. Some minerals are obtained directly from the surrounding water, while others must be obtained through the feed (NRC, 1993).

Vitamins are organic compounds that are essential for normal health, reproduction and growth. Classified as either fat- or water-soluble, vitamins act as co-enzymes in cellular metabolism, pre-cursors for other biologically important compounds (e.g. choline is a precursor for the neurotransmitter acetylcholine) and as structural elements in membranes (Halver, 2002). Fat-soluble vitamins can be stored in the fish, whereas water-soluble vitamins must be continually obtained through the diet.

Fish primarily use protein and lipid to meet their energy requirements as carbohydrates are not as effectively utilized (Higgs et al., 1995). The gross energy of protein, lipid and carbohydrates is 5.65, 9.45 and 4.11 kcal/g, respectively, although not all of that energy is bioavailable to the fish, as discussed above (NRC, 1993; Higgs et al., 1995). Nutrient digestibility is usually assessed through indirect means, using a non-digestible marker such as chromic oxide. The ratio of the non-digestible marker to nutrient in the feed and feces is calculated in order to estimate the apparent nutrient digestibility coefficient (Hajen et al., 1993).

**Determining optimal protein, lipid and DP:DE ratios**

It is imperative that fish feed has an appropriate protein, lipid and energy balance. Fish generally eat to meet their energy requirement; consequently, excessive dietary protein concentrations will result in amino acid deamination for energy use and inefficient growth (Higgs et al., 1995). Conversely, excess energy levels can lead to reduced feed consumption, decreased growth and excessive lipid deposition (NRC, 1993). Protein sparing is a commonly
demonstrated phenomenon where lipid or carbohydrate is utilized for energy, sparing protein for growth (e.g. Reinitz et al., 1978; Takeuchi et al., 1978; Medland and Beamish, 1985; Beamish and Medland, 1986; Yigit et al., 2002). Traditionally, optimal protein to lipid concentrations in grower diets for rainbow trout have been estimated to be 35% protein to 15-20% lipid (Cho and Kaushik, 1990; Cho, 1992; NRC, 1993; Higgs et al., 1995), with compromised growth and feed conversion efficiency occurring outside this range. The ideal digestible protein to digestible energy ratio (DP:DE) for rainbow trout has been estimated to be between 22-25 g DP MJ DE$^{-1}$ (Cho and Kaushik, 1990; Cho, 1992; NRC, 1993; Higgs et al., 1995). However, protein requirements change as a fish increases in size (Higgs et al., 1995). Specifically, young trout on starter diets require more protein (45-50%) compared to larger trout on maintenance or production diets (35-40%) (Satia, 1974; Hilton and Slinger, 1981). This is because the scope for growth (the difference between maximum ration and maintenance ration assuming a high quality diet) decreases as salmonids increase in size. Both maintenance and maximum ration decline as salmonids increase in size, however, maximum ration decreases at a faster rate (Higgs et al., 1995).

More recent studies on rainbow trout have shown that low protein and high lipid diets have resulted in improved growth (Yigit et al., 2002; Chaiyapechara et al., 2003; Morrow et al., 2004). Yigit et al. (2002) assessed growth in 181 g rainbow trout that were fed diets varying in protein (P) and lipid (L) content. They found that 44:17 and 43:26 P:L ratios resulted in superior growth rates compared to 47:13 and 48:12 P:L ratios. Similarly, Chaiyapechara et al. (2003) fed 100 g rainbow trout diets that varied in protein and lipid content and they discovered that fish fed diets containing a 40:30 P:L ratio had a significantly greater specific growth rate compared to fish fed diets containing a 40:15 P:L ratio. Finally,
Morrow et al. (2004) measured growth rates in 49 g rainbow trout that were fed three different isocaloric diets and they found that fish fed a 36:18 P:L diet experienced enhanced growth.

In contrast to the above studies, three other studies examining growth in rainbow trout fed isocaloric diets varying in protein and lipid content did not find a significant difference between dietary treatments (Steffens et al., 1999; Azevedo et al., 2004a; Azevedo et al., 2004b). Azevedo et al. (2004a; 2004b) fed 47 or 268 g rainbow trout four different isocaloric diets and found no significant difference in weight gain between diet treatments. Similarly, Steffens et al. (1999) found no difference in growth rates in 92 g rainbow trout fed isocaloric quantities of diets containing similar protein (47-48%) but different lipid (13% and 24%) levels. These findings contrast with the earlier notion that DP:DE ratios must be between 22-25 g DP MJ DE⁻¹ and protein to lipid ratios must be 35% protein to 15-20% lipid in order to maximize growth.

These studies elucidate the need to make careful comparisons between diet formulations. Non-isocaloric comparisons could have led to some of the discrepancies in the aforementioned studies; varying the DE of the diets confounds the interpretation of the effects of protein and lipid utilization. Additionally, varying one variable at time in an experimental diet (Cho et al., 1976) does not provide information on the interactions between different feed components. For example, it is known that the protein requirement is dependant upon the levels of other non-protein energy sources (Wilson, 2002; Ruohonen and Kettunen, 2004). It also unavoidably varies the energetic content of the diet. A recent approach to assessing fish nutrition recognizes the importance of a mixture design (Rouhonen and Kettunen, 2004). In light of this, the diets tested in the present study were formulated to have equivalent DE with
varying protein and lipid concentrations. The protein, lipid, carbohydrate, mineral and vitamin compositions were carefully formulated to meet all the dietary requirements of rainbow trout.

**Oxygen Consumption**

Indirect calorimetry is customarily used to estimate metabolic rate in fish by measuring oxygen uptake (MO₂). Standard metabolic rate (SMR) is defined as the minimum rate of oxygen consumption in a resting, thermally acclimated, non-digesting fish. This is typically estimated in fish using respirometry, by measuring the lowest level of oxygen consumption or by extrapolating to zero body lengths per second swimming speed using a swim tunnel respirometer. SMR has been estimated to be 48–80 mg O₂ kg⁻¹ h⁻¹ in rainbow trout (Webb, 1971; Kiceniuk and Jones, 1977; Pagnotta and Milligan, 1991; Alsop and Wood, 1997; Claireaux et al., 2005; Simonot, 2005). Routine metabolic rate (RMR) is SMR plus the energy spent on voluntary, spontaneous movement in an unfed fish. RMR varies considerably between fish, with activity, stress and the experimental set-up. Active metabolism (AMR) is the rate of oxygen consumption at maximal sustained swimming speed (Uₘₐₓ) measured using a swim tunnel (Jones and Randall, 1978) and can be 10-times SMR in salmonids. Thus, activity and stress can result in large differences between SMR and RMR.

After a fish ingests a diet, there is a concomitant increase in MO₂, variously termed as the specific dynamic action (SDA), calorigenic effect, dietary thermogensis or the heat increment of feeding (HiE). Following the recommendations of the NRC (1993), the heat increment of feeding will be used here. HiE is defined as the energy associated with 1) digestion and absorption of nutrients, 2) waste formation and excretion and 3) product formation. The term ‘apparent heat increment’ is sometimes used to incorporate the energy
associated with grasping, chewing and swallowing a meal which technically is not included under the HiE definition but is difficult to separate experimentally (Beamish and Trippel, 1990). HiE in fish is usually calculated using a respirometer as the integral under the postprandial MO$_2$ curve above some measure of SMR. As such, the reliability of any HiE estimate is strongly dependant upon the quality of the respirometry system used to estimate MO$_2$ and the method of SMR calculation. Spontaneous activity and excitement will elevate both pre- and postprandial MO$_2$, resulting in inaccurate estimates of HiE. In order to obtain useful, accurate estimations of HiE, it is imperative to separate energy expended on activity or due to excitation from the metabolism association with feeding (Brett and Groves, 1979). Beamish and Trippel (1990) describe one method of measuring a uniform and low activity MO$_2$ by forcing fish to swim against a minor workload in a respirometer and extrapolating back to SMR. However, caution must be employed when interpreting results obtained from these methods as exercise has been demonstrated to increase HiE and the duration of the postprandial MO$_2$ response (Blaike and Kerr, 1996).

There are the three key parameters of interest when discussing the postprandial MO$_2$: the duration of HiE, peak MO$_2$ level and magnitude of HiE.

*Duration of postprandial MO$_2$*

The duration of postprandial MO$_2$ above SMR has been demonstrated to be dependant upon fish species, temperature, fish size, quantity of feed and quality of feed (Jobling, 1981). Correspondingly, gastric evacuation time has been demonstrated to depend upon these same parameters (Fange and Grove, 1979). For example, the duration of the HiE response is clearly
correlated with gastric evacuation time in plaice (Jobling and Davie, 1980), although gastric evacuation time was slightly shorter than the duration of HiE.

Postprandial MO$_2$ varies considerably among fish species. In Antarctic plunderfish at -1.0 – +1.5°C (1.4 – 4.3 g), postprandial MO$_2$ remained elevated for a staggering 10 to 16 days after a meal of either 2.5% of body mass or to satiation (Boyce and Clarke, 1997). In contrast, juvenile haddock at 8 – 12°C (2-3 cm) demonstrated an elevated MO$_2$ for 5–12 h postprandial when fed between 1 and 20% dry body mass (Peck et al., 2005). Similarly, juvenile Atlantic cod at 10°C (1-8 g) fed 7.5% of their body mass showed an elevated MO$_2$ for 3–11 h postprandial (Hunt von Herbing and White, 2002). Many species, such as rainbow trout, largemouth sea bass, plaice and Nile tilapia, demonstrated an elevation in postprandial MO$_2$ for between 12 and 76 hours when fed 0.5 to 3.5 % of their body mass (Jobling and Davie, 1980; Tandler and Beamish, 1980; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Ross et al., 1992; Kaczanowski and Beamish, 1996).

The duration of HiE is inversely related to temperature (LeGrow and Beamish, 1986). Saunders (1963) found that postprandial MO$_2$ in Atlantic cod following a large meal of herring remained above baseline levels for 7 days at 10°C and for only 4-5 days at 15°C. Jobling and Davie (1980) found that postprandial MO$_2$ in plaice remained elevated above SMR for only 26 h at 20°C compared to 51 h at 10°C. Similarly, Brett and Higgs (1970) found that a decrease in water temperature from 23 to 10°C resulted in a doubling of gastric evacuation time in sockeye salmon.

The duration of the HiE has been shown to increase with increasing fish mass in sea bass, Antarctic plunderfish and Atlantic cod (Tandler and Beamish, 1981; Boyce and Clarke,
Likewise, gastric evacuation rate has been demonstrated to increase with increasing fish size (Fange and Grove, 1979).

Increasing meal size has been reported to increase the duration of HiE in aholehole, blenny, haddock and largemouth bass (Jobling, 1981; Tandler and Beamish, 1981; Peck et al., 2005), but not in the Antarctic plunderfish (Boyce and Clarke, 1997). Food particle size can also affect gastric emptying. Atlantic cod took longer to digest whole herring compared to minced herring (Dos Santos and Jobling, 1988).

Increasing the energy content of the diet has been shown to increase the gastric evacuation time in turbot, rainbow trout and Atlantic cod (Grove et al., 1978; Flowerdew and Grove, 1979; Jobling, 1981; Dos Santos and Jobling, 1988). Correspondingly, the duration of the HiE increased with increasing energy content in the meal in largemouth bass (Tandler and Beamish, 1981). However, LeGrow and Beamish (1986) demonstrated that regardless of protein level, lipid level or energy intake, the duration of the HiE in 10-15 g rainbow trout fed 2% of their body mass was $58 \pm 10$ h. Similarly, Kaczanowski and Beamish (1995) found that HiE lasted $21 \pm 2$ h in rainbow trout (250 – 450 g) fed diets with varying balanced and unbalanced essential amino acid profiles and was independent of energy intake and diet composition.

**Peak postprandial MO$_2$**

The peak in postprandial MO$_2$ is usually observed within 12 h after a meal (Jobling, 1981). The height of the peak often depends upon the amount of feed consumed, usually increasing with larger rations (Beamish, 1974; Jobling, 1981). The peak MO$_2$ is typically reported to be between 1.5 and 2.5 times SMR in various fish species including blenny, plaice,
aholehole, largemouth bass, Atlantic cod, bluegill, Atlantic menhaden, rainbow trout, Antarctic plunderfish, haddock and Nile tilapia (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). This postprandial MO$_2$ effectively reduces the scope of activity available for the fish, by as much as 30-50% when fed at maximum ration (Jobling, 1981). As a result, in rainbow trout and chinook salmon, postprandial MO$_2$ decreased Ucrit without affecting maximum MO$_2$ (Alsop and Wood, 1997; Thorarensen and Farrell, 2006).

Studies on plasma amino acid levels after feeding rainbow trout 1% of their body mass have found that most plasma amino acids peaked between 4- and 12-h postprandial and had returned to baseline levels between 12- and 24-h postprandial (Murai et al., 1987; Ok et al., 2001). Similarly, most plasma amino acid levels in the dorsal aorta and hepatic portal vein peaked by 6 h and had returned to baseline by 48-h postprandial in rainbow trout fed 1% of their body mass (Karlsson et al., 2006 submitted). Thus, postprandial plasma amino acid patterns have been shown to closely parallel MO$_2$ and gastric evacuation.

**Magnitude of HiE**

The majority of HiE is due to the metabolic work for post-absorptive processes associated with dietary protein (Cho and Kaushik, 1990). Dietary amino acids are synthesized into proteins if adequate energy is available from dietary lipid or carbohydrate, termed protein sparing (e.g. Reinitz et al., 1978; Tandler and Beamish, 1980; Medland and Beamish, 1985; Beamish and Medland, 1986; Yigit et al., 2002). Amino acid deamination occurs when (1) excess amounts of protein have been ingested, (2) there is inadequate dietary non-protein energy, furnished mainly by lipid and to a lesser extent carbohydrate, and (3) there is poor
protein quality or balance between dietary essential and non-essential amino acids (Beamish and Trippel, 1990). Elevated carbohydrate levels have also been shown to increase HiE, particularly when digestible protein to digestible energy ratios (DP:DE) are lower than 20-21 g DP MJ DE\(^{-1}\) (Beamish et al., 1986; Bureau et al., 2002). In contrast, lipids do not have to be deaminated prior to being used as an energy source, thus, they are associated with a comparatively lower HiE. Minimizing HiE allows more energy to be available for growth and reproduction, as such, an appropriate balance of dietary amino acids, lipids and carbohydrates at a suitable energy level is desirable in order to obtain maximum efficiency.

HiE has been demonstrated to increase with increasing levels of dietary protein in a number of species, including rainbow trout (Cho et al., 1976; Jobling and Davies, 1980; Tandler and Beamish, 1980; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Cho and Woodward, 1989; Ross et al., 1992; Kaczanowski and Beamish, 1996). In contrast, Schalles and Wissing (1976) found no difference in HiE in bluegill sunfish when they increased dietary protein concentrations from 24 to 45%, although they noted significant individual variation which may have masked differences. The findings for rainbow trout are outlined below.

Cho et al. (1976) measured HiE in 96–145 g rainbow trout fed diets varying in protein (P) and lipid (L) content. They suggest that fish fed diets containing a 60:15 P:L ratio produced higher HiE compared to those fed the 40:15 or 40:25 P:L ratio diets.

Cho and Woodward (1989) examined the effect of varying DP:DE ratios on the HiE in rainbow trout (3.5–6.6 g). They found that DP:DE ratios of \(\geq26\) g DP MJ DE\(^{-1}\) produced higher HiE compared to lower ratios.
Rainbow trout (15 g) fed diets varying in protein (34, 48 and 58%) and lipid content (7 and 23%) demonstrated an increase in HiE with increasing dietary protein content at each lipid level (Medland and Beamish, 1985). However, these diets were not isocaloric and there was no significant difference in the cost of HiE as a percent of digestible energy with increasing dietary protein content.

LeGrow and Beamish (1986) fed rainbow trout (10-15 g, at 15°C) 2% of their body mass while they were continuously swimming at low speeds. Twelve experimental diets were assessed in the study and they were created by varying protein (34, 40, 48 and 60%) and lipid (7, 15 and 23%) levels with differing caloric values and DP:DE ratios. They found that the 34% protein and 23% lipid diet resulted in the lowest HiE, and suggested that this diet combination induced the most efficient protein utilization.

Kaczanowski and Beamish (1996) infused rainbow trout (250-450 g) with varying balanced and unbalanced essential amino acid profiles. The profiles closely mimicked trout whole body protein or those low in lysine produced the lowest HiE levels while diets deficient in branched amino acid chains or with excess lysine resulted in elevated HiE levels.

The magnitude of HiE can also vary with temperature, fish size and meal size (Bureau et al., 2002; Beamish and Trippel, 1990). The effect of temperature on HiE is unclear. Jobling and Davie (1980) found no difference in HiE in plaice fed equivalent amounts at varying temperatures. However, Cho and Slinger (1980) reported an increase in HiE expressed as a percent of digestible energy at 7.5 or 20°C compared to 15°C in rainbow trout. In contrast, Beamish et al. (1986) found that HiE was higher in fish at 15°C compared to fish at 8°C.
While HiE increases as fish size increases (Tandler and Beamish, 1981; Boyce and Clarke, 1997), HiE decreases or doesn’t vary with increasing fish mass when expressed as a percent of gross or digestible energy (Beamish and Trippel, 1990).

Finally, HiE has been shown to increase with larger meal sizes (Beamish, 1974; Vahl and Davenport, 1979; Jobling, 1981; Tandler and Beamish, 1981; Ross et al., 1992). While some researchers have demonstrated no difference in HiE with varying meal size when expressed as a proportion of gross or digestible energy (Beamish, 1974; Jobling and Davie, 1980; Tandler and Beamish, 1981; Ross et al., 1992), others have found an inverse or unclear relationship (Hamada and Ida, 1973; Beamish and MacMahon, 1988).

Cost of HiE as a percentage of energy consumed

HiE is often expressed as a percent of total energy consumed (either gross or digestible), facilitating comparison between species and diets. Values vary widely and are usually reported between 8 and 37% of DE (Cho et al., 1982; Beamish and Trippel, 1990; Cho and Kaushik, 1990). LeGrow and Beamish (1985) reported that the HiE in rainbow trout (10-15 g) fed 2% of their body mass cost 21-24% of the DE in fish fed 60% protein diets compared to a 15% cost in fish fed a diet with 34% protein and 24% lipid. Cho et al. (1976) reported a similar trend, with HiE cost increasing from 11 to 13% of the digestible energy intake when dietary protein increased from 40 to 60% in 96–145 g rainbow trout fed 1.5-2.2 % of their body mass. Cho and Woodward (1989) found that HiE in rainbow trout (3.5–6.6 g) cost between 33 and 36% of DE for DP:DE ratios of 20-24 g MJ$^{-1}$ and 37% for DP:DE ratios of 26-30 g MJ$^{-1}$. Kaczanowski and Beamish (1995) found that HiE represented 15% of gross energy for diets containing lysine in limiting proportions and up to 32% in diets deficient in
branched chain amino acids. Conversely, no relationship between dietary protein content and the cost of HiE relative to DE was demonstrated in 15 g rainbow trout fed 2% of their body mass diets varying in protein (34, 48 and 58%) and lipid content (7 and 23%) (Medland and Beamish, 1985). The average HiE was reported to be 18% of DE.

Increasing dietary lipid from 15 to 18% has been demonstrated to decrease HiE relative to DE (from 21 to 18%) in rainbow trout (LeGrow and Beamish, 1985). Cho et al. (1976) found a similar decrease in the cost of HiE in rainbow trout when dietary lipid increased from 15 to 25% (from 13 to 8% of the digestible energy). Correspondingly, increasing dietary lipid from 7 to 23% resulted in a decrease in the relative HiE to DE from 16 to 15% in rainbow trout (Medland and Beamish, 1985).

Gut blood flow

An important component of the physiological response to feeding is the cardiovascular adjustment that allows the transport of absorbed nutrients from the intestine to the liver via the hepatic portal vein for modification, storage and energy use. Not only is an increase in blood flow crucial for nutrient transport to the liver, blood flow must also deliver oxygen to the metabolically active tissues associated with feeding (e.g. for gut motility, membrane transport and intracellular processes) (Farrell et al., 2001). The splanchnic circulation is not a priority circulation, thus, it can be bypassed for more critical circulatory needs (e.g. the brain and skeletal muscle) when other physiological or environmental considerations are more pressing (Farrell et al., 2001). Consequently, much of the limited literature to date on the cardiovascular responses to feeding in fish has focused on the postprandial circulatory response to hypoxia, exercise and hypercapnia (for review, see Farrell et al., 2001). Regardless of the obvious role
of postprandial blood flow in digestion, no studies to date have examined the effect of diet composition on gut blood flow (GBF).

GBF can be measured by direct methods (e.g. electromagnetic, Doppler or Transonic flow probes), or via indirect methods (e.g. coloured or radiolabelled microspheres) (Bushnell et al., 1992). Despite requiring more invasive procedures, the direct methods have been shown to provide more reliable data on GBF (Bushnell et al., 1992; Kolok et al., 1993; Thorarensen et al., 1993; Crocker et al., 2000). Amongst the direct methods, Transonic flow probes have the advantage of providing absolute flow measurements, while Doppler and electromagnetic probes need to be calibrated after the experiment. However, Transonic flow probes have bulky heads, which limits the utilization of these probes for smaller fish or in tight quarters.

Resting gut blood flow

Resting GBF levels in unfed fish have been measured in a number of species including sea raven, Atlantic cod, chinook salmon, red Irish lord, white sturgeon, and sea bass. Routine GBF has been estimated to be between 15-40% of resting cardiac output ($Q_{rest}$). Specifically, resting GBF levels were found to be 2.9 ml min$^{-1}$ kg$^{-1}$ (celiac artery) or 15% of $Q_{rest}$ in the sea raven (Axelsson et al., 1989); 4.1 ml min$^{-1}$ kg$^{-1}$ (celiac artery) and 3.5 ml min$^{-1}$ kg$^{-1}$ (mesenteric artery) or 40% of $Q_{rest}$ in Atlantic cod (Axelsson and Fritsche, 1991); 12.0 -14.2 ml min$^{-1}$ kg$^{-1}$ (intestinal artery) or 36% of $Q_{rest}$ in chinook salmon (Thorarensen et al., 1993); 4.1 ml min$^{-1}$ kg$^{-1}$ (celiac artery) and 4.9 ml min$^{-1}$ kg$^{-1}$ (mesenteric artery) or 34% of $Q_{rest}$ in red Irish lord (Axelsson et al., 2000); 8.9 ml min$^{-1}$ kg$^{-1}$ (celiacomesenteric artery) or 20% of $Q_{rest}$ in white sturgeon (Crocker et al., 2000); and 9.6 ml min$^{-1}$ kg$^{-1}$ (ceolic and mesenteric arteries) or 24% of $Q_{rest}$ in sea bass (Axelsson et al., 2002).
Postprandial gut blood flow

Postprandial measurements in Atlantic cod, sea bass, chinook salmon, sea raven and red Irish lord demonstrated a 42-112% increase in blood flow to the splanchnic circulation. Atlantic cod fed 2.5-3.5% of their body mass experienced a 72 and 42% increase in postprandial flow in the coeliac and mesenteric arteries, respectively (Axelsson and Fritsche, 1991). Sea bass fed 2.9% of body mass demonstrated a 71% increase in postprandial flow in the coeliac and mesenteric arteries (Axelsson et al., 2002). Chinook salmon fed 2% of their body mass displayed a 90% increase in postprandial flow through the intestinal artery (Thorarensen and Farrell, 2006). Sea raven fed 10-20% of their body mass demonstrated a 100% increase in blood flow through the celiac artery (Axelsson et al., 1989). Red Irish lord fed 10-15% of body mass exhibited 112% and 94% increases in postprandial blood flow for coeliac and mesenteric arteries, respectively (Axelsson et al., 2000). The above variability has largely been credited to the size of the meal, lifestyle and species. Atlantic cod, sea bass and chinook salmon are pelagic species that more or less continuously feed in small amounts. In contrast, red Irish lord and sea raven are both benthic, sit-and-wait ambush predators.

The above-mentioned studies only examined the short-term effect of feeding on GBF (up to 24-h postprandial), with the exception of the studies on the red Irish lord (Axelsson et al., 2000) and chinook salmon (Thorarensen, 1994). Postprandial GBF was reported to remain elevated above control levels for 6 days in the red Irish lord following a meal equivalent to 10-15% of their body mass. The authors speculated that digestion lasted the entire 6 days because of the large meal size, low water temperature and the observation that small pieces of food and bones remained in the stomach at the conclusion of the study. The sequential pattern of an initial increase in the coeliac artery (supplying the stomach and liver) with a subsequent
decrease after 4 days and a delayed increase in the mesenteric artery (supplying the intestines), with its continued elevation above control levels for 6 days post-feeding, also supported their suggestion. In contrast, GBF in chinook salmon fed 2% of their body mass peaked at around 20 h and was back down to pre-feeding levels by 36 h postprandial. It was suggested that the discrepancy in the postprandial GBF time course between these two studies was due to differences in meal size, water temperature and the metabolic rates of the different species (Farrell et al., 2001).

Mechanisms elevating postprandial gut blood flow

All capillary beds cannot be simultaneously perfused. Therefore, an increase in GBF can occur via either a re-distribution of blood flow from other tissues to the gut, or an increase in $Q_{rest}$, or some combination of both (Farrell, 2001). The postprandial increase in GBF in Atlantic cod, red Irish lord and sea bass were all paralleled by an increase in $Q_{rest}$ (Axelsson and Fritsche, 1991; Axelsson et al., 2000; Axelsson et al., 2002). This differs from what is found in mammals where postprandial blood flow is largely due to a re-distribution of blood flow from other tissues (Vatner et al., 1974; Matheson et al., 2000).

The factors controlling GBF are not well understood. Gastrointestinal vasculature is under tonic $\alpha$-adrenergic control in the sea raven, dogfish, red Irish lord and white sturgeon (Axelsson et al., 1989; Holmgren et al., 1992; Axelsson et al., 2000; Crocker et al., 2000). Interestingly, while Atlantic cod demonstrated $\alpha$-adrenergic tonus in the mesenteric artery, none was found in the coeliac artery (Axelsson and Fritsche, 1991). Attempts to block $\alpha$-adrenergic control have had varying affects in postprandial fish. Postprandial GBF in Atlantic cod did not change following a phentolamine injection (Axelsson and Fritsche, 1991) while
postprandial blood flow in sea raven doubled subsequent to a phentolamine injection (Axelsson et al., 1989). This leads us to believe that the degree to which α-adrenergic vasoconstriction controls blood flow to the splanchnic system is variable between fish species. There are many other putative mechanisms controlling splanchnic resistance, such as tachykinins and substance P (Holmgren et al., 1992; Farrell et al., 2001), but the importance of these pathways is still unclear.

The postprandial pattern of GBF could have important implications for digestion. Theoretically, a sustained elevation in GBF would maximize oxygen delivery and nutrient uptake for transport to the liver. However, GBF has been shown to decrease in response to spontaneous struggling or agitation (Holmgren et al., 1992; Crocker et al., 2000). Any decrease in GBF could result in a decreased opportunity for the assimilation of nutrients, thus prolonging the duration of digestion, because decreases were not compensated for afterwards.

**Research Objectives and Hypotheses:**

1. To induce differential protein utilization by feeding fish isocaloric diets of varying protein and lipid levels. It is hypothesized that fish consuming low protein: high lipid diets will have greater percent protein deposition and superior protein efficiency ratio compared to fish fed high protein: low lipid diets.

2. To determine if fish with differential protein utilization accordingly alter their heat increment of feeding and postprandial blood flow to the gut. It is hypothesized that fish fed the high protein: low lipid diets will have greater HiE and increased GBF compared to those fed the low protein: high lipid diets.
3. To quantify postprandial MO$_2$ and GBF in rainbow trout fed a single meal, 2% of their body mass using improved methods. It is hypothesized that postprandial changes will last 24-36 h and be up to 100% higher than baseline levels.

4. To simultaneously measure MO$_2$ with GBF in order to assess the relationships between postprandial MO$_2$, GBF and heart rate. It is hypothesized that that GBF will be linearly related to postprandial MO$_2$. 
Excretory loss

Feces excretions (FE)

Gill excretions (ZE)
Urine excretions (UE)

Intake Energy (IE)

Digestible Energy (DE)

Metabolizable Energy (ME)

Net Energy (NE)

Recovered Energy (RE)

Growth
Fat
Reproduction

Heat loss

Heat Increment (HiE)
Waste formation and excretion (HwE)
Product Formation (HrE)
Digestion and absorption (HdE)

Maintenance (HEm)
Basal metabolism (HeE)
Voluntary activity (HjE)
Thermal regulation (HoE)

Figure 1.1: Schematic representation of the fate of dietary energy in fish. Source: National Research Council (1993).
References


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Chapter 2: Growth and digestibility in rainbow trout using isocaloric diets of varying protein and lipid concentrations

Introduction

Extensive research has examined the protein, lipid, carbohydrate and energy concentrations required to maximize growth and feed efficiency in various cultured fish species (NRC, 1993) Protein is the most expensive dietary component in aquaculture, supplying amino acids for growth and energy (Hilton and Slinger, 1981; Cho, 1992). Dry protein and lipid concentrations in feed for juvenile rainbow trout have been broadly recommended to be 35-36% and 15-20%, respectively (Cho and Kaushik, 1990; Cho, 1992; NRC, 1993; Higgs et al., 1995). Nevertheless, dietary protein and energy requirements of fish do vary with life history; very young trout on starter diets have a higher protein requirement (45-50%) compared to older trout on production or maintenance diets (35-40%) (Satia, 1974; Hilton and Slinger, 1981).

It is imperative for aquaculturists to optimize digestible dietary protein to digestible energy (DP:DE) ratios, not only because of the cost of protein but because excessive protein concentrations can result in inefficient metabolism and decrease growth rate. The optimal DP:DE ratio for rainbow trout has been suggested to be 22-25 g DP MJ DE^{-1} (Cho and Kaushik, 1990; Cho, 1992; NRC, 1993; Higgs et al., 1995). Conversely, elevated dietary lipid can improve energy and protein retention (e.g. Reinitz et al., 1978; Takeuchi et al., 1978; Beamish and Medland, 1986; Yigit et al., 2002), due to a protein-sparing effect whereby proteins (amino acids) are channeled into growth instead of being used for energy. However,
elevated dietary lipid typically increases lipid deposition and can decrease growth rate (Cho and Kaushik, 1990; Cho, 1992; Higgs et al., 1995).

Growth trials have historically been the primary means of assessing diet quality for fish. There has recently been an interest in combining multiple means of assessing nutrient uptake and diet quality so as to better understand the digestive physiology in fish. In order to conduct these comparison experiments, it is essential to know the growth-related performance data associated with the experimental diets in question.

The objective of this study was to assess the growth and performance parameters of juvenile rainbow trout fed three diets with equal digestible energy and carbohydrate levels but varying protein and lipid concentrations. This information will not only be a valuable estimate of ideal protein and lipid levels in feed formulations for juvenile rainbow trout, but it will also be useful as a baseline comparison for studies assessing the digestive physiology in rainbow trout.

Materials and Methods

Three isocaloric (16.7 MJ of estimated DE kg\(^{-1}\)) diets with varying protein and lipid concentrations but equal digestible carbohydrate (12%) concentrations were prepared at the Department of Fisheries and Oceans, University of British Columbia Centre for Aquaculture and Environmental Research (CAER) in West Vancouver, B.C., Canada. The ingredient and proximate compositions of the diets are shown in Tables 2.1 and 2.2, respectively. Diet HP:LL was formulated to have a high protein (HP) and low lipid (LL) concentration (55% protein and 10% lipid). Diet MP:ML had a medium protein (MP) and medium lipid (ML) concentration (45% protein and 15% lipid). Diet LP:HL had a low protein (LP) and high lipid (HL)
concentration (35% protein and 20% lipid). Digestible protein to digestible energy ratios (DP:DE) were estimated to be 29.7, 24.3 and 18.9 g MJ$^{-1}$, respectively for the high, medium and low protein content diets, respectively.

The measured proximate compositions of the three experimental diets were within 9% of the targeted values (Table 2.2). Dry weight protein and lipid concentrations for the HP:LL diet were 55% and 11%, whereas for the MP:ML diet were 47% and 16% and for the LP:HL diet were 38% and 20%, respectively. The gross energy values were within 11% of each other (18.7, 19.8 and 21.0 MJ kg$^{-1}$ for the HP:LL, MP:ML and LP:HL diets, respectively). Dietary DE values were not determined but once again, were theoretically identical.

All dry ingredients were mixed together for at least 30 minutes in a Hobart Commercial Mixer (Hobart Manufacturing Company, Troy, OH). The dry mash was steam pelleted (Raven et al., 2006) under reduced moisture content at around 80-85°C using a 4 mm die and a California model CL 2 Laboratory pellet mill. The pellets were then immediately dried in a custom-made vertical cooler. Anchovy oil was added to the surface of the MP:ML and LP:HL diets using an electrically operated sprayer and a cement mixer. The diets were subsequently stored overnight to allow the anchovy oil to penetrate the pellets and finally they were kept in air tight containers at 4°C until required.

In March of 2003, juvenile rainbow trout (120.7 ± 1.6 g, mean ± SEM) from Sun Valley Trout Farm (Mission, British Columbia) were arbitrarily separated into nine groups of 15 fish that were held in 1,100 l fiberglass tanks. The tanks were supplied with aerated, flow-through (>10 l min$^{-1}$) well water. Temperature (11°C ± 0.2°C) and dissolved oxygen concentrations (>10.3 mg l$^{-1}$) were monitored daily. Photoperiod mimicked natural conditions.
The fish were fed commercial trout chow (EWOS Canada Ltd., Surrey, B.C.) prior to the commencement of the study.

The three diet treatments were randomly assigned to the tanks. Fish were fed by hand to satiation twice a day (9:30 am and again at 1 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 they no longer actively consumed the pellets as they sank or the fish regurgitated the pellets that they had eaten. After each feeding, the lids were closed and the fish were allowed to feed off the bottom of the tank for 10 minutes. The remaining pellets 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 was confirmed as 100% using a test with a separate, fish-free tank to assess whether some pellets were lost in the siphoning process.

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 anaesthesia. Fish were anesthetized in aerated MS-222 (0.1 g l\(^{-1}\) with buffered sodium bicarbonate; Syndel Laboratories Ltd., Vancouver) and then individually weighed and measured. General fish health was inspected at each sampling time. On day 56, three fish from each tank were randomly selected for whole body proximate analysis. These fish were euthanized by cervical dislocation, vacuum sealed and stored at -40°C until analysis.

Chromic oxide, a non-digestible feed marker, was added to the diets (5 g per kg mash) during the final week of the growth trial to assess protein digestibility (Austreng, 1978; Hajen
et al., 1993). Feces were stripped from the anaesthetised fish on the final day of the growth trial (Hajen et al., 1993) and 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 feces were stripped on day 4 and 14 of this period and then frozen until analysis. The frozen fecal samples were analyzed as described by Hajen et al. (1993). Insufficient quantities of feces were collected for the digestibility assessment in order to compare individual tanks. As such, feces from each treatment had to be pooled and analyzed together.

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 gross energy was determined using bomb calorimetry (IKA-WERKE C5000, Staufen, Germany). The following growth and performance variables were calculated:

1. Diet protein digestibility =

\[ 1 - \left( \frac{F \times D}{D_{cr} \times F_{cr}} \right) \times 100 \]

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. Specific Growth Rate (SGR) =

\[ \left( \ln \text{final mass (g)} - \ln \text{initial mass (g)} \right) \times \# \text{ of expt days}^{-1} \times 100 \]

3. Dry Feed Intake (DFI) =

\[ \text{total dry feed intake} \times \text{fish}^{-1} \]

4. Feed Efficiency Ratio (FER) =

\[ \text{wet mass gain (g)} \times \text{DFI (g)}^{-1} \]

5. Protein Efficiency Ratio (PER) =

\[ \text{wet mass gain (g)} \times \text{protein consumption}^{-1} \]
6. Percent Protein Deposition (PPD) =

\[
\left( \frac{\text{protein gained in fish (g) \times total protein consumed (g^{-1})}}{} \right) \times 100
\]

Initial protein concentrations in the fish were estimated to be 17.7% (Weatherup and McCracken, 1999) in order to calculate percent protein deposition.

Values are presented as the mean ± standard error of the mean (SEM). Statistical differences between means were assessed using analysis of variance (ANOVA) followed by the Tukey-Kramer Multiple Comparison test, when appropriate. All differences were considered to be statistically significant when \( p \leq 0.05 \).

**Results**

Fish body mass, length and condition factor did not differ significantly among the three diets at the commencement of the trial (Table 2.3). Fish more than doubled their weight during the 8-week growth trial without the final body mass, length and condition factor varying significantly among diet treatments at any time during the trial. Similarly, hepatosomatic indices (HSI; range 1.4 - 1.7%) did not vary significantly among diets. There was no significant difference in feed intake among fish given the different diet treatments (Table 2.4). Protein digestibility was 90% for fish ingesting diets HP:LL and MP:ML, and 91% for diet LP:HL. Consequently, neither specific growth rate nor feed efficiency differed significantly among diets.

Diet composition significantly altered body composition because whole fish proximate compositions varied significantly among diet treatments (Table 2.4). The HP:LL fish had significantly more protein \((18.2 \pm 0.3\% )\) and moisture \((70.4 \pm 0.6\% )\), and significantly less
lipid (9.1 ± 1.0%) compared to fish fed the other two diets (Table 2.4). However, percentages for whole body moisture (68.2 ± 0.1% and 67.6 ± 0.6%), protein (17.1 ± 0.1% and 17.2 ± 0.2%) and lipid (12.4 ± 0.1% and 12.7 ± 0.6%) were the same for fish fed the MP:ML and LP:HL diets, respectively.

The protein efficiency ratio (PER) varied significantly among diet treatments (Table 2.4). Between day 0 and 28 and also between day 28 and 56, the LP:HL diet resulted in a significantly higher PER compared to the HP:LL and MP:ML diets. Over the entire 56-day trial, there were significant differences in PER values among fish fed all three diets. Fish fed the HP:LL diet had the lowest PER (1.60 ± 0.01), whereas the PER for fish fed the MP:ML diet was significantly higher (1.87 ± 0.04). Fish fed the LP:HL diet had the highest PER value (2.25 ± 0.08). Values for percent protein deposition (PPD) followed a similar sequence and were respectively 29.4, 31.9 and 37.6% for fish fed the HP:LL, MP:ML, LP:HL diets. Thus, while fish on the HP:LL diet grew at the same rate and with the same feed efficiency as those ingesting the MP:ML and LP:HL diets, the latter diets of high lipid content resulted in a greater accumulation of body lipid and more efficient protein deposition.

Discussion

The specific growth rates (SGR, 1.27 – 1.35) and feed efficiency (FE, 0.85 – 0.88) values obtained in this study are indicative of good growth and feed conversion efficiency in rainbow trout. Indeed, the preceding values agree with those observed in other growth trials on rainbow trout (e.g. SGR: 1.18 – 2.06, Steffens et al., 1999; Lanari and D'Agaro, 2002; FE: 0.79 – 0.88, Brauge et al., 1994; Azevedo et al., 2004b).
Traditionally, the effect of diet quality on fish growth has been assessed using one of two ratios: the digestible protein to digestible energy (DP:DE) and the dietary protein to lipid ratio. The recommended DP:DE ratio has been suggested to be between 22-25 g DP MJ DE\(^{-1}\) for rainbow trout (Cho and Kaushik, 1990; Cho, 1992; NRC, 1993; Higgs et al., 1995). The DP:DE ratios of the diets tested in the present study (29.7, 24.3 and 18.9 g DP MJ DE\(^{-1}\)) bracketed this range. The present study clearly shows that our diets did not compromise any growth performance parameters (specific growth rate, feed efficiency or feed intake), suggesting that the earlier recommended range is too narrow for the rainbow trout used here.

Conventional reports recommend that dietary protein (P) and lipid (L) concentrations, expressed on a dry weight basis, should vary between 35-36% and 15-20%, respectively, for good growth of juvenile rainbow trout and caution that compromised growth and conversion efficiency occurs outside this range (Cho and Kaushik, 1990; Cho, 1992; NRC, 1993; Higgs et al., 1995). More recent studies have indicated that low protein: high lipid diets result in increased growth rates in rainbow trout (Yigit et al., 2002; Chaiyapechara et al., 2003; Morrow et al., 2004). Yigit et al. (2002) found that 44:17 and 43:26 P:L ratios resulted in increased growth rates in 181 g rainbow trout compared to 47:13 and 48:12 P:L ratios. Chaiyapechara et al. (2003) found that 100 g rainbow trout fed diets containing a 40:30 P:L ratio had a significantly greater SGR compared to fish fed diets containing a 40:15 P:L ratio. Morrow et al. (2004) used similar diets to the present study and found that 49 g rainbow trout grew best on the 36:18 P:L ratio diet during a 6-week growth trial. The discrepancies among the aforementioned studies could in part be explained by different caloric values among the various diets that were compared, a problem we controlled for by using isocaloric diets. Experiments that alter one variable at a time lack information on interactions between feed.
components. For example, it is known that the protein requirement is dependant upon the levels of other non-protein energy sources (Wilson, 2002; Ruohonnen and Kettunen, 2004). Additionally, varying multiple components is necessary in order to maintain caloric equivalence. A recent approach to assessing fish nutrition recognizes the importance of appropriate design of dietary mixtures (Rouhonen and Kettunen, 2004), varying one nutrient level inevitably alters the others in a complete diet.

My results are more in line with those of Azevedo et al. (2004a; 2004b) who recently showed that four isocaloric diets of varying protein and lipid ratios did not result in a significant difference in weight gain in either 47 g or 268 g rainbow trout. Steffens et al. (1999) also found no difference in weight gain in 92 g trout fed isocaloric quantities of diets of similar protein (47-48%) but different lipid levels (13% and 24%). Therefore, the commonly cited range for DP:DE ratios of 22-25 g DP MJ\(^{-1}\) DE, as well as the dietary dry matter protein and lipid concentrations of 34-35% and 15-20%, respectively, may be too conservative for 120-250 g juvenile rainbow trout. Consequently, there are now three independent studies that point to the possibility of aquaculturists being able to feed juvenile trout cheaper, higher lipid diets without sacrificing feed efficiency or growth.

While the present study supported the common finding that a high lipid diet increases the percent body lipid (e.g. Satia, 1974; Reinitz et al., 1978; Reinitz and Hitzel, 1980; Jobling, 1998; Azevedo et al., 2004a), it also provided evidence of an upper limit to lipid deposition. When LP:HL fish were fed 25% more lipid than the MP:ML fish, lipid content in the fish was unaffected. Instead, lipid content influenced protein utilization. The LP:HL diet resulted in a significantly higher protein efficiency ratio and percent protein deposition compared to the HP:LL diet fish. 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).

The results from this study provide a useful baseline comparison in order to use these diets to examine novel means of assessing nutrient uptake and digestion by combining MO$_2$ and gut blood flow studies. Given the previous recommendations for DP:DE ratios and the extreme protein and lipid concentrations used in the present study, we had anticipated significant differences in growth parameters among diet treatments. While this was not the case, the fish did demonstrate differential protein utilization and lipid deposition which may lead to distinct postprandial MO$_2$ and GBF patterns. In the event that this does not occur, the physiological responses of the fish will be pooled together to facilitate characterization of the overall relationship between MO$_2$, GBF and heart rate.
Table 2.1: Ingredient compositions for the three isocaloric experimental diets. All diets were formulated to contain 16.7 MJ kg\(^{-1}\) dry diet digestible energy and 12% digestible carbohydrate, but varying protein and lipid concentrations.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary protein (%)</td>
<td>55</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>Dietary lipid (%)</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ingredient (g kg(^{-1}) dry basis)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Anchovy meal (low temperature-dried)</td>
<td>599.87</td>
<td>490.81</td>
<td>381.74</td>
</tr>
<tr>
<td>Blood flour (spray-dried)</td>
<td>49.36</td>
<td>40.38</td>
<td>31.41</td>
</tr>
<tr>
<td>Squid meal</td>
<td>49.97</td>
<td>40.88</td>
<td>31.8</td>
</tr>
<tr>
<td>Krill hydrolysate</td>
<td>18.95</td>
<td>15.5</td>
<td>12.05</td>
</tr>
<tr>
<td>Wheat gluten meal</td>
<td>51.5</td>
<td>42.14</td>
<td>32.78</td>
</tr>
<tr>
<td>Pregelatinized wheat starch</td>
<td>85.0</td>
<td>85.0</td>
<td>85.0</td>
</tr>
<tr>
<td>Raw wheat starch</td>
<td>73.86</td>
<td>73.86</td>
<td>73.86</td>
</tr>
<tr>
<td>Vitamin supplement(^a)</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Mineral supplement(^b)</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Anchovy oil (stabilized)</td>
<td>1.38</td>
<td>74.09</td>
<td>146.67</td>
</tr>
<tr>
<td>Soybean lecithin</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline chloride (60%)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin C monophosphate (42%)</td>
<td>2.86</td>
<td>2.86</td>
<td>2.86</td>
</tr>
<tr>
<td>Permapell (lignin sulphonate binder)</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>2.25</td>
<td>1.85</td>
<td>1.42</td>
</tr>
<tr>
<td>(\alpha)-cellulose</td>
<td>-</td>
<td>67.63</td>
<td>135.41</td>
</tr>
</tbody>
</table>

\(^b\)Mineral supplement (mg kg\(^{-1}\) dry basis)

<table>
<thead>
<tr>
<th>Element (as (\text{mg kg}^{-1}))</th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn (as MnSO(_4) (\cdot) H(_2)O)</td>
<td>75.0</td>
<td>75.0</td>
<td>75.0</td>
</tr>
<tr>
<td>Zn (as ZnSO(_4) 7H(_2)O)</td>
<td>54.6</td>
<td>71.5</td>
<td>89.0</td>
</tr>
<tr>
<td>Co (as CoCl(_2) 6H(_2)O)</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Cu (as CuSO(_4) 5H(_2)O)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Fe (as FeSO(_4) 7H(_2)O)</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>I (as KIO(_3)) (as KI)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Se (as Na(_2)SeO(_3))</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Mg (as MgSO(_4) 7H(_2)O)</td>
<td>400.0</td>
<td>400.0</td>
<td>400.0</td>
</tr>
<tr>
<td>K (as K(_2)SO(_4)) (as K(_2)CO(_3))</td>
<td>-</td>
<td>654.0</td>
<td>1398.0</td>
</tr>
<tr>
<td>F (as NaF)</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

\(^a\)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.
Table 2.2: Proximate compositions of the three experimental diets (dry weight basis).

<table>
<thead>
<tr>
<th>Diet</th>
<th>% Dry matter</th>
<th>% Ash</th>
<th>% Lipid</th>
<th>% Protein</th>
<th>Gross Energy (MJ kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP:LL</td>
<td>91.2</td>
<td>11.6</td>
<td>10.5</td>
<td>55.0</td>
<td>18.7</td>
</tr>
<tr>
<td>MP:ML</td>
<td>92.0</td>
<td>9.6</td>
<td>15.6</td>
<td>46.8</td>
<td>19.8</td>
</tr>
<tr>
<td>LP:HL</td>
<td>92.5</td>
<td>8.0</td>
<td>20.0</td>
<td>37.6</td>
<td>20.9</td>
</tr>
</tbody>
</table>
Table 2.3: Growth performance data of fish from the three different experimental diets, n = 3 for each diet. Mean ± SEM with differing superscript letters were significantly different (p < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dietary protein (%)</strong></td>
<td>55</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td><strong>Dietary lipid (%)</strong></td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

**Day 0**

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body mass (g)</strong></td>
<td>118.4 ± 2.0a</td>
<td>124.5 ± 2.0a</td>
<td>119.1 ± 2.0a</td>
</tr>
<tr>
<td><strong>Length (cm)</strong></td>
<td>22.2 ± 0.1a</td>
<td>22.5 ± 0.1a</td>
<td>22.2 ± 0.1a</td>
</tr>
<tr>
<td><strong>Condition factor</strong></td>
<td>1.1 ± 0.0a</td>
<td>1.1 ± 0.0a</td>
<td>1.1 ± 0.0a</td>
</tr>
</tbody>
</table>

**Interval 1: Day 0 to 28**

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight gain (g)</strong></td>
<td>54.4 ± 8.4a</td>
<td>56.6 ± 2.4a</td>
<td>52.5 ± 4.5a</td>
</tr>
<tr>
<td><strong>Length gain (cm)</strong></td>
<td>2.2 ± 0.2a</td>
<td>2.3 ± 0.0a</td>
<td>2.1 ± 0.2a</td>
</tr>
<tr>
<td><strong>SGR</strong></td>
<td>1.34 ± 0.16a</td>
<td>1.34 ± 0.05a</td>
<td>1.30 ± 0.09a</td>
</tr>
<tr>
<td><strong>FER</strong></td>
<td>0.98 ± 0.05a</td>
<td>0.96 ± 0.03a</td>
<td>0.90 ± 0.03a</td>
</tr>
</tbody>
</table>

**Interval 2: Day 28 to 56**

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight gain (g)</strong></td>
<td>75.1 ± 1.5a</td>
<td>84.5 ± 2.9a</td>
<td>71.8 ± 10.4a</td>
</tr>
<tr>
<td><strong>Length gain (cm)</strong></td>
<td>2.5 ± 0.1a</td>
<td>2.4 ± 0.1a</td>
<td>2.2 ± 0.1a</td>
</tr>
<tr>
<td><strong>SGR</strong></td>
<td>1.29 ± 0.07a</td>
<td>1.37 ± 0.03a</td>
<td>1.24 ± 0.15a</td>
</tr>
<tr>
<td><strong>FER</strong></td>
<td>0.82 ± 0.04a</td>
<td>0.83 ± 0.02a</td>
<td>0.81 ± 0.03a</td>
</tr>
</tbody>
</table>

**Entire Trial: Day 0 to 56**

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight gain (g)</strong></td>
<td>129.4 ± 8.1a</td>
<td>141.2 ± 4.7a</td>
<td>124.2 ± 13.3a</td>
</tr>
<tr>
<td><strong>Length gain (cm)</strong></td>
<td>4.7 ± 0.2a</td>
<td>4.7 ± 0.1a</td>
<td>4.3 ± 0.3a</td>
</tr>
<tr>
<td><strong>Condition factor</strong></td>
<td>1.3 ± 0.0a</td>
<td>1.3 ± 0.0a</td>
<td>1.3 ± 0.0a</td>
</tr>
<tr>
<td><strong>HSI (%)</strong></td>
<td>1.5 ± 0.1a</td>
<td>1.7 ± 0.1a</td>
<td>1.4 ± 0.1a</td>
</tr>
<tr>
<td><strong>SGR</strong></td>
<td>1.32 ± 0.05a</td>
<td>1.35 ± 0.03a</td>
<td>1.27 ± 0.11a</td>
</tr>
<tr>
<td><strong>FER</strong></td>
<td>0.88 ± 0.00a</td>
<td>0.88 ± 0.02a</td>
<td>0.85 ± 0.03a</td>
</tr>
</tbody>
</table>

1 Condition factor = [(body mass / length) x 100] 
2 SGR denotes specific growth rate = [(ln final mass - ln initial mass) x # expt days] / 100 
3 FER denotes feed efficiency ratio = [wet mass gain (g) x dry feed consumption (g)] / [feed intake (g)] 
4 HSI denotes hematosomatic index = [(liver mass (g) / body mass (g)) x 100]
Table 2.4: Feed intake, protein efficiency*, protein digestibility and final whole fish proximate composition data from fish fed the three different experimental diets, n = 3 for each diet. Means ± SEM with differing superscript letters were significantly different (p < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary protein (%)</td>
<td>55</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>Dietary lipid (%)</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

**Interval 1: Day 0 to 28**
- Dry Feed Intake (g fish⁻¹)  
  - HP:LL 55.17 ± 6.04a
  - MP:ML 59.33 ± 2.99a
  - LP:HL 58.08 ± 3.42a

- PER 1.78 ± 0.10a  
  - HP:LL 2.05 ± 0.06a
  - MP:ML 2.39 ± 0.08b

**Interval 2: Day 28 to 56**
- Dry Feed Intake (g fish⁻¹)  
  - HP:LL 92.39 ± 4.38a
  - MP:ML 102.30 ± 4.66a
  - LP:HL 87.83 ± 9.29a

- PER 1.48 ± 0.07a  
  - HP:LL 1.77 ± 0.05a
  - MP:ML 2.16 ± 0.09b

**Entire Trial: Day 0 to 56**
- Dry Feed Intake (g fish⁻¹)  
  - HP:LL 147.56 ± 9.86a
  - MP:ML 161.63 ± 6.96a
  - LP:HL 145.91 ± 10.67a

- PER 1.60 ± 0.01a  
  - HP:LL 1.87 ± 0.04b
  - MP:ML 2.25 ± 0.08c

- PPD (%)  
  - HP:LL 29.40 ± 0.97a
  - MP:ML 31.90 ± 0.61ab
  - LP:HL 37.57 ± 2.21b

- Protein Digestibility (%)  
  - HP:LL 90
  - MP:ML 90
  - LP:HL 91

*Final whole fish proximate composition (as is basis)*
- Moisture (%)  
  - HP:LL 70.40 ± 0.55a
  - MP:ML 68.16 ± 0.14b
  - LP:HL 67.62 ± 0.60b

- Ash (%)  
  - HP:LL 2.00 ± 0.08a
  - MP:ML 1.87 ± 0.05a
  - LP:HL 1.95 ± 0.05a

- Protein (%)  
  - HP:LL 18.22 ± 0.28a
  - MP:ML 17.06 ± 0.06b
  - LP:HL 17.22 ± 0.17b

- Lipid (%)  
  - HP:LL 9.05 ± 1.02a
  - MP:ML 12.44 ± 0.09b
  - LP:HL 12.69 ± 0.63b

*Initial protein concentrations in the fish were estimated to be 17.7% (Weatherup and McCracken, 1999) in order to calculate percent protein deposition.

1PER denotes protein efficiency ratio = [wet mass gain (g) x protein consumption⁻¹]

2PPD denotes percent protein deposition = [(protein gained in fish (g) x total protein consumed (g⁻¹)) x 100]
References


Chaiyapechara, S., Casten, M. T., Hardy, R. W. and Dong, F. M. (2003). Fish performance, fillet characteristics, and health assessment index of rainbow trout (Oncorhynchus mykiss) fed diets containing adequate and high concentrations of lipid and vitamin E. Aquaculture 219, 715-738.


Chapter 3: Postprandial gut blood flow and the heat increment of feeding in rainbow trout with differential protein utilization

Introduction

The digestive physiology of fish is poorly understood. Much of the research to date has focused on optimizing diet formulations and defining nutrient and energy requirements in fish (NRC, 1993) and examining the consequences of diets on tissue composition (Halver and Hardy, 2002), issues directly relevant to aquaculture. Typically, these issues have been examined through the use of growth and digestibility trials. While these techniques have provided useful information, such as characterizing dietary nutritional requirements and investigating the effects of alternative dietary protein and lipid sources on growth, they rarely have provided quantitative or qualitative insights into the digestive physiology of single fish, after a single meal. However, metabolic studies have characterized the concomitant increase in metabolic rate when a fish feeds, termed heat increment of feeding (HiE). This increase in oxygen uptake (MO$_2$) is attributed to digestion and absorption processes, waste formation and excretion, and product formation (Jobling, 1981; Beamish and Trippel, 1990; NRC, 1993). A major portion of HiE stems from the deamination and excretion of amino acids (Cho and Kaushik, 1990; Pannevis and Houlihan, 1992). The HiE is typically estimated using respirometry by integrating under the average postprandial MO$_2$ curve and subtracting an estimate of standard metabolic rate (SMR). However, these methods vary widely.

The duration and magnitude of digestion, as measured by the gastric emptying rate and HiE, have been extensively studied (for review see Brett and Groves, 1979; Fange and Grove,
HiE and gastric emptying rate increase with meal size, fish size and increasing water temperature. In addition, the species, feeding history, period of food deprivation, food particle size and feed composition strongly affect the gastric emptying time. Force-feeding, surgical intervention and prolonged food-deprivation prior to a study have been shown to increase the gastric evacuation time (Fange and Grove, 1979; Olsson et al., 1999). HiE has been reported to range from 8 to 29% of the digestible energy (DE) consumed in fish fed formulated diets (Cho et al., 1982; Beamish and Trippel, 1990). Poorly formulated diets or diets with high digestible protein to digestible energy (DP:DE) ratios (> 25 g DP MJ DE\(^{-1}\)) have been shown to result in an increased HiE (Cho et al., 1976; Cho et al., 1982; LeGrow and Beamish, 1986; Cho and Woodward, 1989; Cho and Kaushik, 1990; Kaczanowski and Beamish, 1996). For example, LeGrow and Beamish, (1986) found that HiE in 10-15 g rainbow trout accounted for 24% of DE for high protein diets (59:14 P:L ratio) compared to just 15% of DE for low protein diets (34:24 P:L ratio). Cho et al. (1976) found HiE to vary between 8 and 13 % of DE for 96 – 145 g rainbow trout fed diets containing 40:25 and 60:15 P:L ratios, respectively.

In addition to HiE, an important response to feeding is the circulatory changes associated with the absorption and transport of nutrients from the stomach and intestine to the liver and rest of the body for modification, storage and energy use. However, only a handful of studies have looked at gut blood flow (GBF) in fish (for review, see Farrell et al., 2001). These studies show that ingestion of a meal typically results in a 42-100% increase in GBF, but none of them have examined the effects of diet composition on GBF. In fact, to the best of my knowledge no one has combined GBF measurements with metabolic studies. Therefore, the present study simultaneously measured postprandial metabolic rate (as oxygen consumption)
and GBF in rainbow trout reared on each of three isocaloric diets of varying protein and lipid, but equal carbohydrate levels. Because these diets resulted in differential protein utilization in the fish (Chapter 2), it was possible to examine for the first time: a) the effect of varying lipid and protein concentrations on both HiE and GBF; b) the general relationship between HiE and GBF.

**Materials and Methods**

*Diet Preparation*

Three isocaloric (16.7 MJ kg\(^{-1}\)) diets of varying protein and lipid concentrations but equal digestible carbohydrate (12%) concentration were prepared at the Department of Fisheries and Oceans, University of British Columbia Centre for Aquaculture and Environmental Research (CAER) in West Vancouver, B.C., Canada every 3 to 4 months as required. The ingredient and proximate compositions of the diets as well as the methods used for diet preparation have been outlined in chapter 2. Diet HP:LL was formulated to have a high protein (HP) and low lipid (LL) concentration (55% protein and 10% lipid). Diet MP:ML had a medium protein (MP) and medium lipid (ML) concentration (45% protein and 15% lipid). Diet LP:HL had a low protein (LP) and high lipid (HL) concentration (35% protein and 20% lipid). Consequently, the DP:DE ratios spanned almost a two-fold range and were 29.7, 24.3 and 18.9 g DP MJ DE\(^{-1}\), respectively for the high to low protein diets.
Fish Husbandry

Rainbow trout (120.7 ± 1.6 g, mean ± SEM) were initially used for an 8-week growth and digestibility trial at CAER in West Vancouver, B.C. beginning in March 2003. The fish were then used for one of two experiments, the first at Simon Fraser University, Burnaby, B.C. (SFU) and the second at the University of British Columbia, Vancouver, B.C. (UBC). The fish used in chapter 2 were transported at the conclusion of the growth trial to SFU and maintained on their same diets in aerated 2,500 l (7.8-14.0°C, dissolved O$_2$ > 8.0 mg L$^{-1}$) outdoor tanks under natural photoperiod for 7 months (503.4 ± 10.7 g, mean ± SEM, n = 24). At the conclusion of this experiment, the remaining fish were transported to UBC, and maintained on their same diets in aerated 1,000 l (11.0-16.0°C, dissolved O$_2$ > 8.0 mg L$^{-1}$) indoor tanks under 12:12 photoperiod for 8 months (807.6 ± 47.1 g, mean ± SEM; n = 24). Therefore, HiE and GBF experiments were performed on a common stock of rainbow trout that had been maintained on the isocaloric diets for many months. The fish were fed 4-5 days a week to near satiation (~2 % of body mass) during these experiments.

The parameters for fish used for the GBF measurements are given in Table 3.1. There was no difference in gonadosomatic index (3.0- 3.9) or hematocrit (37.0- 44.5%) among fish fed the diet treatments. Fish from the LP:HL diet weighed significantly more (29-38%) than those from the HP:LL and MP:ML diets, and MP:ML fish had a significantly greater condition factor and hepatosomatic index compared to HP:LL fish.

Respirometry

Intermittent flow respirometry was used to measure the oxygen consumption, MO$_2$, of individual fish. The SFU experiments 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 ranging between 8.2-13.0°C) at 0.95 l min⁻¹. 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 O₂ probe (Point Four Systems, Richmond, B.C.). Oxygen consumption (MO₂) was calculated from the slope of the declining O₂ content of the water during each 5 min closed period. The probes were calibrated to fully aerated water prior to each replicate.

The protocol for the feeding cycle at SFU was as follows (Figure 3.1). Fish from each diet treatment (n = 7 - 9 fish per diet) were starved for 48 h before being randomly placed in a vessel, typically between 3 and 6 pm. All bubbles were removed from the vessel and MO₂ was followed for 24 h. Between 3 and 6 pm, the fish were then anaesthetized (loss of their righting ability using buffered 0.1 g l⁻¹ MS-222) and sham-fed using plastic forceps and polyethylene tubing while being submerged in an aerated, buffered, anaesthetic bath (0.08 g l⁻¹ MS-222) for 5 min. MO₂ was followed for a further 24 h after the fish were replaced in the vessel. Between 3 and 6 pm, fish were then re-anaesthetized and force fed their experimental diet (2% of their body mass) in pellet form using forceps in the same manner as the sham feeding. The fish were replaced and any lost pellets were counted. The MO₂ was followed for 60-96 h postprandial. At the end of the experiment, the fish were removed, tagged and returned to their stock tanks. Background MO₂ in each vessel was monitored for at least one hour before and after each trial, and determined to be negligible. Water temperature and O₂ levels in the header tank were continually monitored throughout the experiment. The fish were kept in 24 hour darkness throughout the experiment to minimize the effect of circadian rhythms.
The UBC experiments used a 4-chamber system (custom-made by Loligo Systems, Hobro, Denmark). The 9.9 l plexi-glass vessels received aerated, 10.0-16.0°C water at 5 l min⁻¹. These vessels also had a recirculation pump so that even when the inflow water was off, the vessel received a water current. This design minimized intermittent flow disturbances to the fish. The flush cycle was 10 min, the wait period was 30 s and the recirculation cycle was 5 min. The oxygen content of the water was measured every second during the 5 min recirculation cycle 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. MO₂ was recorded using LoliResp4 software (Loligo Systems, Hobro, Denmark).

The UBC experiments measured a) GBF simultaneously with MO₂ for one feeding cycle in probed fish (n = 3 - 6 fish per diet), b) GBF simultaneously with MO₂ for two feeding cycles in probed fish (n = 5) c) GBF simultaneously with MO₂ in probed fish that were sham-fed twice instead of being fed and d) MO₂ in un-probed fish fed once (n = 5), (Figure 3.1). The respirometry software failed during the postprandial time period for the unfed fish, so no MO₂ data are available for those fish. The reason some fish with GBF probes were fed twice was because either the recording equipment malfunctioned during the first recording or it was observed that postprandial MO₂ and GBF were not returning to pre-feeding levels even after 80 h in probed fish. Thus, we sought to determine if a second feeding and therefore a longer recovery period from surgery and longer adjustment period in the vessel would allow postprandial MO₂ and GBF to return to pre-feeding levels.

The major difference in protocols between SFU and UBC experiments was that after the initial 24 h adjustment period in the vessel, the probed fish (n = 19) were removed and underwent GBF surgery (see below). MO₂ following recovery from surgery was monitored for
24 h and then the fish were removed first for sham feeding, and then 24 h later for regular feeding, as described above. At the end of the experiment, blood was sampled via caudal puncture to measure hematocrit in any fish that underwent surgery. All fish were euthanized by cervical dislocation. The tissues surrounding the probe were inspected for signs of inflammation but none were seen. Liver and gonads were removed and weighed and the stomach and intestines were checked for food and feces.

**Gut Blood Flow Surgery**

Fish were anaesthetized in buffered 0.1 g l\(^{-1}\) MS-222 and transferred onto a surgical table, ventral side up and right side exposed, where their gills were continually irrigated with a chilled, aerated, buffered anaesthetic solution (0.05 g l\(^{-1}\) MS-222). Surgical procedures to isolate the intestinal blood vessels followed those described by Thorarensen et al. (1993). An incision was made just posterior to the pectoral fin, extending from a few cm above the lateral line to a few cm below the midline. The gastric and intestinal arteries were carefully isolated so as not to disturb any nerves and either a 1.0RB or a 1.5RB Transonic flowprobe (Transonic Systems, Ithaca, NY, USA) was fitted around both arteries (Figure 3.2). The incision was closed using interrupted 2-0 silk sutures. The probe lead was secured at the incision site and sutured several times to the skin and at the dorsal fin using 2-0 silk. The incision was lightly dusted with powdered penicillin and then the fish was promptly returned to the experimental vessel where its recovery was visually monitored.
Data Analysis

MO₂ was assessed using both average and minimum values. Data blocks formed the basis of analysis. MO₂ was recorded for a 5 min period every 30 min at SFU and for a 5 min period every 15.5 min at UBC. Data blocks for both UBC and SFU consisted of four pooled 5 min values. Therefore, each SFU and UBC data block was 2 h and 62 min long, respectively. The average MO₂ was determined as the mean of all the 5 min values in a data block. The minimum MO₂ was determined as the lowest 5 min value in a data block. For GBF and heart rate, block averages over 1 h were determined using WINDAQ (Dataq Instruments, Akron, Ohio) or MP100 BioPac Acknowledge (BIOPAC Systems Inc., Santa Barbara, CA) software sampling at 20 Hz.

Standard metabolic rate (SMR), baseline GBF and baseline heart rate were estimated for each fish as the average of the six lowest average block values over the entire trial excluding periods I, III and V (see Figure 3.3), each of which correspond to a recovery period each time the fish was replaced in the vessel following anesthesia.

Routine metabolic rate (RMR), routine GBF and routine heart rate were estimated for each fish from periods II and IV using equal durations of “dark” and “light” periods (Figure 3.3). “Light” and “dark” periods correspond to the times when the fish would have experienced light and dark conditions in their holding tanks prior to the experiment.

The peak MO₂, GBF and heart rate were defined for each fish as the highest postprandial block value for each variable. The time-to-peak is the number of hours postprandial to reach the peak value.

The effect of sham feeding on MO₂, GBF and heart rate was assessed by visually inspecting individual data for each fish. The sham effect was determined to have largely
subsided after 4 h. Therefore, the first 4 h of postprandial data were removed from analysis (period V from Figure 3.3).

The heat increment of feeding (HiE) is defined as the postprandial increase in \( \text{MO}_2 \) above SMR (Jobling, 1981; Beamish and Trippel, 1990). Here, HiE was assessed two ways: by subtracting SMR from the integration of either the postprandial average \( \text{MO}_2 \) curve or postprandial minimum \( \text{MO}_2 \) curve. Because active and excited fish exhibit elevated \( \text{MO}_2 \) levels, the average postprandial \( \text{MO}_2 \) could lead to an overestimation of HiE. Thus, the minimum \( \text{MO}_2 \) estimate could depict a more realistic HiE estimate. An alternative approach to account for this activity is to estimate HiE by subtracting RMR from postprandial average \( \text{MO}_2 \) values. However, inspection of the data revealed this to be an inappropriate method. Postprandial \( \text{MO}_2 \) decreased below RMR and was actually significantly lower than RMR at 56 h in un-probed fish at SFU (Figure 3.4). Postprandial average \( \text{MO}_2 \) in un-probed fish at UBC and probed fish fed the second time only significantly differed from RMR at 13 and 15 h, respectively (Figures 3.5 and 3.6). Therefore, the method of estimating HiE using average postprandial \( \text{MO}_2 \) above RMR was rejected. Since the first 4 h of postprandial data were not included, I assumed a linear relationship between SMR and the 4-h postprandial value. The cost of HiE as a % of digestible energy intake was estimated by the assumption that 1 g of oxygen is associated with the release of 13.6 kJ of energy (Cho et al., 1982). The HiE cost was estimated after postprandial minimum and average \( \text{MO}_2 \) had returned to SMR.

The total increase in postprandial blood flow to the gut was determined by integrating under the postprandial GBF curve and subtracting baseline GBF. Theoretically, routine GBF could have been subtracted from postprandial GBF, however, postprandial GBF actually decreased below routine GBF levels in some cases. Therefore, baseline GBF was determined
to be a superior comparison. Again, I assumed a linear relationship between baseline GBF and the 4-h postprandial value.

In order to increase statistical power and reduce the possibility of a Type II statistical error, the data were pooled for diet treatments after I first determined there were no statistical differences among diets for the pooled variables (see below). The relationship between MO$_2$ and GBF was assessed by comparing the difference between postprandial MO$_2$ from SMR ($\Delta$ MO$_2$) with the difference in postprandial GBF from baseline ($\Delta$ GBF). The change in minimum postprandial MO$_2$ from SMR was plotted against the change in postprandial GBF from baseline for each block interval. Delta GBF and delta heart rate were compared in a similar manner.

Statistical analysis

Mean values are presented ± standard error of the mean (SEM). The effect of diet on postprandial MO$_2$, GBF and heart rate over time was assessed using 2-way repeated measures ANOVA (SigmaStat 3.0). The pooled data from all diet treatments were assessed using a 1-way repeated measures ANOVA comparing MO$_2$, GBF and heart rate data to baseline and routine 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. Differences in fish body mass, condition factor, GSI, HSI, hematocrit, SMR, RMR, baseline GBF and heart rate, routine GBF and heart rate, peak, time-to-peak, HiE, cost of HiE and total GBF were compared using ANOVA followed by the Holm-Sidak multiple comparisons (SigmaStat 3.0). Data that were not normally distributed or had unequal variances were also assessed using a nonparametric rank test followed by Dunn multiple
There was no effect of the nonparametric rank test on the statistics apart from a few exceptions that will be noted.

Results

Fish Assessment

Less than 10% of the pellets were lost following force feeding in these experiments. All fish appeared to be in good health and had excellent hematocrit values (mean values 37 – 45% for each diet, range 28 – 50%) following each trial (Table 3.1). There were no signs of inflammation in the tissues surrounding the flow probe and the wound had already begun to heal in most fish. At the termination of the UBC experiment, only one fish had any food remaining in the stomach and eight fish had around 0.5-1.0 ml of feces remaining in the intestines.

Metabolic Rates

For experiments at SFU and UBC, there were no significant differences in SMR, RMR, peak MO$_2$ and time-to-peak MO$_2$ among fish given the different diets (Table 3.2). This allowed the pooling of these SFU and UBC data according to diet to increase the statistical power. Pooling the data had no consequence on any of these variables.

Postprandial MO$_2$ showed no significant difference among diet treatments (figures not shown). Consequently, there were no significant differences among fish given the different diets for either minimum or average HiE at any time (12, 18, 24, 36, 48, 58 and 80 h).
postprandial (Table 3.3). Notably, differences in average HiE did exist between SFU and UBC and in all cases, HiE at SFU was greater than at UBC. This difference will be revisited later.

The following analyses are based on pooled data for all three diet treatments to increase statistical power. There were four groups for comparison: un-probed fish from SFU, un-probed fish from UBC, probed fish fed once and probed fish fed twice.

For the SFU experiments (n = 24), SMR and RMR, respectively, were 50.8 ± 4.1 mg O₂ kg⁻¹ h⁻¹ and 103.3 ± 8.6 mg O₂ kg⁻¹ h⁻¹ when diets were pooled (Table 3.4). For the UBC experiments using un-probed fish (n = 5), SMR and RMR, respectively, were 48.8 ± 2.1 mg O₂ kg⁻¹ h⁻¹ and 92.6 ± 7.9 mg O₂ kg⁻¹ h⁻¹ (Table 3.4). Thus, RMR for un-probed fish was around 2-fold higher than SMR in both experiments in different locations with different apparatus. For fish with a flow probe and fed once in the respirometer (n = 11), SMR and RMR, respectively, were 60.5 ± 3.9 mg O₂ kg⁻¹ h⁻¹ and 85.0 ± 5.7 mg O₂ kg⁻¹ h⁻¹ (Table 3.4). In fish with a flow probe and fed twice in the respirometer (n = 6), SMR and RMR, respectively, were 48.4 ± 4.1 mg O₂ kg⁻¹ h⁻¹ and 63.9 ± 4.3 mg O₂ kg⁻¹ h⁻¹ (Table 3.4). In contrast to un-probed fish, RMR in probed fish was around 20 mg O₂ kg⁻¹ h⁻¹ higher than SMR. There was no significant difference among these four estimates of SMR. However, there was a significant difference in RMR between experiments. Un-probed fish at SFU had a significantly higher RMR (103.3 ± 8.6 mg O₂ kg⁻¹ h⁻¹) compared to probed fish fed for the second time at UBC (63.9 ± 4.3 mg O₂ kg⁻¹ h⁻¹). Furthermore, there were no significant differences in minimum or average peak MO₂ between experiments. Similarly, there were no significant differences in minimum or average time-to-peak MO₂ among experiments, although there was considerable variation (Table 3.4).
The postprandial MO$_2$ data for each experiment are illustrated in Figures 3.4 – 3.7. By the 4$^{th}$ h postprandial (to account for the effect of handling) both minimum and average MO$_2$ were significantly elevated over SMR in all experiments. In SFU experiments, average postprandial MO$_2$ remained significantly elevated above SMR for the entire duration of the trial while minimum postprandial MO$_2$ was significantly elevated above SMR for the first 30-h postprandial before returning to SMR levels (Figure 3.4). In fish without a probe from UBC experiments, average MO$_2$ remained consistently above SMR for the first 20-h postprandial, followed by 2 significant spikes at 27 and 35 h. Minimum MO$_2$ remained elevated above SMR for 44-h postprandial (Figure 3.5).

Fish that were probed showed very different MO$_2$ patterns depending whether they were fed the first or second time. Fish that were fed a second time, demonstrated a significant elevation in both average and minimum MO$_2$ above SMR for 41 h postprandial followed by a couple of subsequent significant peaks (Figure 3.6). Fish that were fed only once, 2 days after surgery, demonstrated a significant elevation in both average and minimum MO$_2$ above SMR for the entire duration of the 80-h trial (Figure 3.7).

The insets (Figures 3.4 – 3.7) show that there is a consistent difference between average and minimum MO$_2$ throughout the postprandial period, giving a constant error term. Moreover, the difference between average and minimum postprandial MO$_2$ was statistically greater at SFU (around 30 mg O$_2$ kg$^{-1}$ h$^{-1}$, Figure 3.4) compared to UBC (around 10 mg O$_2$ kg$^{-1}$ h$^{-1}$, Figures 3.5 – 3.7). This is consistent with an increased activity in SFU fish. An exception is in UBC fish without a probe, where average postprandial MO$_2$ peaked 30 – 40 mg kg$^{-1}$ h$^{-1}$ higher than minimum MO$_2$ between 11 and 16 h postprandial.
HiE and the cost of HiE expressed as a % of digestible energy consumed (after 12, 18, 24, 36, 48, 58 and 80 h postprandial) are shown in Table 3.5. There was no significant difference in HiE among experiments using minimum estimates. Notably, the minimum cost of HiE, once postprandial MO\textsubscript{2} values had returned to SMR, was consistently between 4.0 and 4.8% of digestible energy consumed in un-probed fish from SFU and UBC and in probed fish fed for the second time. Significant differences in average HiE and average cost of HiE did exist among experiments. Average HiE in un-probed fish from SFU was significantly greater between 12 and 58 h compared to probed fish from UBC. These same patterns are reflected in the cost of HiE.

*Gut Blood Flow*

There were no significant differences in baseline or routine GBF levels among fish given the diet treatments (Tables 3.6). Similarly, there were no significant differences in peak GBF or time-to-peak GBF among diet treatments (Table 3.6). Moreover, there was no significant difference in postprandial GBF among diet treatments (figures not shown). In light of the fact that there were no significant differences between diet treatments, values were pooled for comparison. This led to three comparison groups: fish that were fed once, fish that were fed twice and fish that were not fed.

There were no significant differences in baseline or routine GBF among groups of fish (Table 3.7), suggesting recovery after surgery. GBF in unfed fish did not differ from routine GBF levels. There was no significant difference in peak GBF between fish fed the first or second time (Table 3.7). In contrast, fish fed the first time experienced a significantly longer time-to-peak GBF (47.7 ± 8.2 h) compared to fish fed the second time (10.9 ± 4.7 h).
The time course of postprandial GBF is shown in Figures 3.8 and 3.9. GBF was significantly elevated above baseline for 35 h postprandial in fish fed the second time (Figure 3.8) with a few subsequent significant peaks. In contrast, fish fed the first time demonstrated a significantly elevated postprandial GBF above baseline for the entire duration of the 80 h trial (Figure 3.9). The pattern of postprandial GBF varied in fish that were fed the first time. Many fish \( (n = 8) \) demonstrated an elevated GBF early in the postprandial period (between 4 and 24 h), sometimes followed by a decrease in GBF (usually between 24 and 50 h), followed by a subsequent late increase in GBF (between 50 and 80 h). Other fish \( (n = 3) \) did not exhibit a noticeable peak early in the postprandial period. Instead, GBF tended to increase steadily over the postprandial period. These patterns were not associated with any discernable pattern in \( \text{MO}_2 \) or food remaining in the intestine.

There was no significant difference in the cumulative postprandial GBF above baseline between fish at any of the time periods (Table 3.7). However, the total cumulative postprandial GBF (after 36 h, once postprandial GBF had returned to baseline) in fish fed the second time was 6 L kg\(^{-1}\). In contrast, this amount was 3-times higher for fish fed the first time (after 80 h), at 17 L kg\(^{-1}\).

Throughout the trial, GBF was observed to be sensitive to disturbances and noises both before and after a meal. An example of a GBF trace following one of these disturbances is illustrated in Figure 3.10, showing ~ 10% decrease in total GBF during the 2 h time period. There was no compensatory increase in GBF above normal levels following one of these events. Therefore, these decreases could mean a lost opportunity for assimilation of nutrients from the gut.
Heart Rate

There were no significant differences in baseline, routine, peak heart rate or time-to-peak heart rate among fish given the diet treatments (Table 3.6). Therefore, the data for the three diets were pooled for comparisons among fish that were fed once, twice or not at all.

Baseline and routine heart rate did not significantly differ between these groups (Table 3.7). However, routine heart rate was significantly higher in fish fed once (50.9 ± 3.9 beat min⁻¹) compared to fish fed twice (34.5 ± 1.0 beat min⁻¹). Heart rate in unfed fish with two sham feedings did not differ from routine levels. Time-to-peak heart rate did not differ between fish that were fed once or twice (13.3 ± 2.0 h for all fish). However, peak heart rate was significantly higher in fish fed once (71.8 ± 2.1 beat min⁻¹) compared to fish fed twice (58.8 ± 2.5 beat min⁻¹) (Table 3.7). Therefore, the peak response of heart rate to feeding a second time was less than that of the first time, but the peak response was reached at the same time.

In addition, postprandial heart rate was significantly elevated above routine between 4 and 23 h for fish fed once and between 4 and 25 h for fish fed twice before returning to routine levels for the remainder of the trial (Figures 3.8 and 3.9). However, while postprandial heart rate in fish fed twice was elevated above baseline after 30 h postprandial with two small subsequent spikes, postprandial heart rate in fish fed once remained significantly elevated above baseline for the entire 80-h postprandial period.

Relationships between GBF, MO₂ and Heart Rate

The relationship between the postprandial increases in GBF and minimum MO₂ is shown in Figure 3.11. Fish fed twice demonstrated high delta GBF and high delta MO₂ for the first 24 h with a subsequent linear decrease for the remainder of the postprandial period. In
contrast, no obvious pattern existed for fish fed once. Both delta MO$_2$ and delta GBF remained high throughout the entire postprandial period. Delta GBF was highest at the end of the trial (36 – 80 h), and was independent of delta MO$_2$.

Figure 3.12 shows the relationship between delta GBF and delta heart rate. Fish fed twice demonstrate a similar pattern to what is seen with delta MO$_2$. An elevated delta GBF and delta heart rate is observed for the first 24 h with a subsequent linear decrease in both for the remainder of the postprandial period. Fish fed once also show an elevated delta GBF and delta heart rate for the first 24 h. However, as the postprandial period progressed, an inverse relationship between delta GBF and delta heart rate was observed. Thus, the late increase in GBF occurs without a concomitant increase in heart rate.

Discussion

As far as I am aware, the present study is the first to continuously examine the long-term effects of feeding on GBF simultaneously with MO$_2$ in fish. While a few studies have looked at postprandial cardiac changes after 24 h in the sea raven, Atlantic cod and sea bass (Axelsson et al., 1989; Axelsson and Fritsche, 1991; Axelsson et al., 2002), only one on red Irish lord has examined the chronic effects of feeding over 6 days (Axelsson et al., 2000), and recordings were not made continuously. This is also the first known study to examine the effect of diet on GBF in fish.

Effect of diet

Despite evidence of differential protein utilization among fish, there were no significant differences found in MO$_2$ (including SMR, RMR, peak MO$_2$, time-to-peak MO$_2$ or
HiE), GBF (including baseline, routine, peak GBF, time-to-peak GBF or total GBF) or heart rate (including baseline, routine, peak heart rate or time-to-peak heart rate) among fish given any of the three diet treatments.

HiE has been shown to increase with increasing protein levels (Cho et al., 1976; Jobling, 1981; Cho et al., 1982; LeGrow and Beamish, 1986; Cho and Woodward, 1989; Higgs et al., 1995). However, this occurs when the diets contain suboptimal protein quality or DP:DE ratios have been too high. The HP:LL diet in this study did have a DP:DE ratio (29.7 g DP MJ DE^{-1}) that was higher than the recommended level (22-25g DP MJ DE^{-1}) (Cho and Woodward, 1989; Cho and Kaushik, 1990). Nonetheless, there was no statistical difference in HiE among diet treatments.

There are several reasons why this may have occurred. First of all, the scope for growth decreases as fish grow. As salmonids increase in size, both maximum and maintenance ration decrease, however, maximum ration decreases at a faster rate (Higgs et al., 1995). As a result, smaller fish require more protein compared to larger fish (Satia, 1974; Hilton and Slinger, 1981). The studies that have demonstrated a higher HiE in rainbow trout fed diets with high protein levels have used 4 – 145 g rainbow trout (Cho et al., 1976; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Cho and Woodward, 1989). The trout used in the present study were much larger. Fish at SFU were 503.4 ± 10.7 g and fish at UBC were 807.6 ± 47.1 g. Furthermore, the fish were not fed daily to satiation but were fed ~ 2% of their body mass 4 – 5 days a week. As a result, the fish may not have been in an extreme state of protein utilization, unlike during the growth trial. In addition, HiE expressed as a percent of digestible energy was low, thus, the level of resolution required to detect differences between diets may not have been possible. Clearly, the lack of significance among diet treatments is consistent
with no significant differences in any of the growth parameters (specific growth rate, feed efficiency or dry feed intake) during the growth trial using these diets. Unlike the aforementioned studies that demonstrated significant differences in HiE with increasing protein content (Cho et al., 1976; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Cho and Woodward, 1989), the diets in the present study were formulated to be isocaloric. As was discussed in chapter 2, isocaloric comparisons are imperative if one wants to make informed interpretations of the effects of protein and lipid utilization. The results of the growth trial indicate that my diet formulations matched the needs of the fish and the DP:DE ratios of these diets were within an acceptable range. The absence of significant differences among diet treatments for MO$_2$, GBF and heart rate are also consistent with this consideration. Given that there were no significant differences among diet treatments, pooling of data from the diet treatments in order to increase the statistical power was considered a valid approach.

**Baseline physiology**

SMR, baseline GBF and baseline heart rate values were within expected ranges. SMR from all fish (52.6 ± 2.5 mg O$_2$ kg$^{-1}$ h$^{-1}$) is at the low end of the reported range of 48 – 80 mg O$_2$ kg$^{-1}$ h$^{-1}$ for SMR in rainbow trout (Webb, 1971; Kiceniuk and Jones, 1977; Pagnotta and Milligan, 1991; Alsop and Wood, 1997; Claireaux et al., 2005; Simonot, 2005). Notably, RMR was significantly higher in SFU fish without a probe (103.3 ± 8.6 mg O$_2$ kg$^{-1}$ h$^{-1}$) compared to UBC fish with a probe and fed twice (63.9 ± 4.3 mg O$_2$ kg$^{-1}$ h$^{-1}$). This indicates that un-probed fish, particularly at SFU, were more active than probed fish. This trend has important implications for the interpretation of HiE data.
Baseline GBF from all fish was $4.0 \pm 0.2 \text{ ml min}^{-1} \text{ kg}^{-1}$, which translates to 23% of the expected resting cardiac output ($Q_{\text{rest}} = 17.6 \text{ ml min}^{-1} \text{ kg}^{-1}$, Kiceniuk and Jones, 1977; Figure 3.13). These values are within the range of reported values for sea raven, $2.9 \text{ ml min}^{-1} \text{ kg}^{-1}$ (celiac artery) or 15% of $Q_{\text{rest}}$ (Axelsson et al., 1989); Atlantic cod, $4.1 \text{ ml min}^{-1} \text{ kg}^{-1}$ (celiac artery) and $3.5 \text{ ml min}^{-1} \text{ kg}^{-1}$ (mesenteric artery) or 40% of $Q_{\text{rest}}$ (Axelsson and Fritsche, 1991); chinook salmon, $12.0 - 14.2 \text{ ml min}^{-1} \text{ kg}^{-1}$ (intestinal artery) or 36% of $Q_{\text{rest}}$ (Thorarensen et al., 1993); red Irish lord, $4.1 \text{ ml min}^{-1} \text{ kg}^{-1}$ (celiac artery) and $4.9 \text{ ml min}^{-1} \text{ kg}^{-1}$ (mesenteric artery) or 34% of $Q_{\text{rest}}$ (Axelsson et al., 2000); white sturgeon, $8.9 \text{ ml min}^{-1} \text{ kg}^{-1}$ (celiacomesenteric artery) or 20% of $Q_{\text{rest}}$ (Crocker et al., 2000); and sea bass, $9.6 \text{ ml min}^{-1} \text{ kg}^{-1}$ (coeliac and mesenteric arteries) or 24.0% of $Q_{\text{rest}}$ (Axelsson et al., 2002). Baseline heart rate from all fish ($34.2 \pm 1.8 \text{ beat min}^{-1}$) is slightly lower than most reported resting heart rate values in rainbow trout of $37.8 - 68.2 \text{ beat min}^{-1}$ (Kiceniuk and Jones, 1977; Gallaugher et al., 1995; Taylor et al., 1996; Simonot, 2005). Furthermore, routine heart rate was significantly lower in fish fed twice, indicating that prolonged habituation to the respiratory vessel may result in lower heart rates.

**Peak postprandial response**

Peak postprandial MO₂ for all fish was $115.1 \pm 5.4 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (minimum) and $164.6 \pm 7.4 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (average), which represent increases of 119% and 213% above SMR. These increases are within the range of peak postprandial MO₂ values obtained for many different fish of between 1.5 to 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). These peak postprandial MO₂ values would be
31% (minimum) and 44% (average) of the estimated active metabolic rate (defined as the highest \( \text{MO}_2 \) occurring at maximal sustained swimming speed; Figure 3.13). Kaczanowski and Beamish (1996) similarly estimated that peak postprandial \( \text{MO}_2 \) in 250-450 g rainbow trout, infused with various amino acid solutions, represented 25-48% of the calculated active metabolic rate, whereas, LeGrow and Beamish (1986) found that peak postprandial \( \text{MO}_2 \) 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.

Peak postprandial GBF for all fish was \( 10.1 \pm 0.9 \text{ ml min}^{-1} \text{ kg}^{-1} \), or a 153% increase from baseline GBF (Figure 3.13). This increase is higher than other reported ranges from various fish species: postprandial blood flow increased by 100% in the celiac artery of sea raven fed 10-20% of body mass (Axelsson et al., 1989); by 112 and 94% for coeliac and mesenteric arteries respectively in red Irish lord fed 10-15% of body mass (Axelsson et al., 2000); by 72 and 42% in the coeliac and mesenteric arteries, respectively, when Atlantic cod were fed 2.5-3.5% of body mass (Axelsson and Fritsche, 1991); and by 71% in the coeliac and mesenteric arteries when sea bass were fed 2.9% of body mass (Axelsson et al., 2002).

Postprandial heart rate peaked significantly higher in fish fed once (73.7 ± 2.0 beat \( \text{min}^{-1} \)) compared to fish fed twice (59.9 ± 1.8 beat \( \text{min}^{-1} \)), representing increases of 115% (first time) and 75% (second time) above baseline. These estimates represent 92% (first time) and 75% (second time) of maximal heart rate (estimated as 80 beat \( \text{min}^{-1} \) Simonot, 2005; Figure 3.13). Both increases represent greater responses than the ~ 10% postprandial increase in heart rate seen in sea bass (Axelsson et al., 2002) and are contrary to Atlantic cod, which exhibited no postprandial change in heart rate from pre-feeding levels (Axelsson and Fritsche, 1991). Previous studies also used gavage, therefore, stress is not a likely explanation for these
differences unless rainbow trout are more sensitive to gavage than Atlantic cod and sea bass. Consequently, there could be differences among species.

The time-to-peak postprandial response was assessed for MO$_2$, GBF and heart rate. Time-to-peak GBF was significantly longer in fish fed once (47.7 ± 8.2 h) compared to fish fed twice (10.9 ± 4.7 h). Therefore, a greater recovery period and habituation to apparatus and gavage appear to result in faster peak responses in GBF. In contrast, no differences in time-to-peak MO$_2$ or heart rate were found among experiments. Notably, there was a wide range in time-to-peak values within and among MO$_2$ experiments.

**Duration of the postprandial response**

The 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 would last around 24 – 36 h for rainbow trout at moderate temperatures and fed around 2% of their body mass almost daily prior to the experiment. Stress is likely to slow digestion for a variety of reasons, including a decrease in GBF associated with handling and struggling. Even so, trout seem to handle gavage well. As shown here, effects on MO$_2$, GBF and heart rate had largely subsided after 4 h. In addition, rainbow trout force fed 1% of their body weight display an amino acid profile in the circulatory system where most amino acids peak between 4 – 12 h postprandial and return to baseline levels by 24 h (Murai et al., 1987; Ok et al., 2001). Surgery is likely to add to these stresses. However, dual cannulation (including a similar incision to the present study) and a 24-h recovery in rainbow trout fed 1% of their body mass resulted in amino acid levels in the dorsal aorta and hepatic portal vein peaking at 6 or 24 h postprandial and taking 48 h to return to baseline levels (Karlsson et al., 2006 submitted). Nevertheless, sea
bass with GBF probes, as in the present experiments, showed a significantly longer gastric evacuation time compared to non-instrumented sea bass (Axelsson et al., 2002). Therefore, we would expect the duration of the postprandial response to be longer as a result of GBF surgery.

Our results suggest that digestion was completed in un-probed fish between 30 and 44 h postprandial. Minimum MO$_2$ in un-probed fish at SFU returned to SMR 30-h postprandial (Figure 3.4). At UBC, average and minimum MO$_2$ in un-probed fish returned to SMR after 35 and 44 h, respectively (Figure 3.5). These durations are consistent with earlier studies.

In probed fish fed once, both postprandial MO$_2$ and GBF remained elevated above SMR and baseline, respectively, for the duration of the 80 h study (Figures 3.7 and 3.9A). Considerable variation in GBF existed between fish fed the first time. Most fish (n = 8) exhibited an elevated GBF response immediately following feeding, usually lasting between 10 and 20 h, followed by a late peak in GBF that usually extended until the termination of the study at 80 h. Other fish (n = 3) did not show this initial peak in GBF, but instead demonstrated a steady increase in GBF from routine until the conclusion of the study. The individual MO$_2$ patterns were much more variable and less obvious.

In contrast, probed fish fed twice showed elevated average and minimum MO$_2$ above SMR for around 41 h, with a couple of subsequent significant peaks (Figure 3.6). GBF in fish fed a second time had returned to baseline levels after around 35 h, with a few additional significant peaks. Heart rate was consistently elevated for the first 30 h postprandial before returning to baseline levels (Figure 3.8).

There are two other chronic studies on the postprandial GBF response in fish (Thorarensen, 1994; Axelsson et al., 2000). GBF was reported to remain elevated above control levels for 6 days in red Irish lord (Axelsson et al., 2000). Similar to our study, these
fish were starved prior to surgery for one week, given a 24-h after recovery from surgery, and force-fed. However, they were given a large meal of pieces of raw fish muscle equivalent to 10-15% of their body mass, which would accordingly lead to longer digestion. The authors recorded cardiovascular variables once every 24 h and did not measure gastric evacuation rate or HiE, but speculated that digestion lasted the entire 6 days because of the large meal size, low water temperature and the observation that small pieces of food and bones remained in the stomach at the conclusion of the study. The sequential pattern of an initial increase in the coeliac artery (supplying the stomach and liver) and its subsequent decrease after 4 days and a delayed increase in the mesenteric artery (supplying the intestines) and its continued elevation above control levels 6 days post feeding also supported their speculation. Therefore, it is possible to create conditions that prolong digestion in fish for an excess of 130 h.

In contrast, Thorarensen (1994) starved chinook salmon for 24 h prior to surgery, and then after a 24 h recovery period from surgery, force-fed the fish 2% of their body mass at 8-11°C. GBF was recorded for 10 minutes, every hour, for 40 h postprandial and was determined to reach a peak at 23 h and remain elevated above pre-feeding levels for 36 h postprandial. The duration of the postprandial GBF response in the present study for fish fed twice is similar to these findings. Fish fed twice likely had a longer time to recover from the stress of the surgery and habituate to the apparatus and protocol. Therefore, they probably demonstrated a more typical postprandial response.

The elevation in GBF for 80-h postprandial in fish fed once is consistent with prolonged digestion due to stress associated with surgery. This prolonged elevation was associated with a 3-fold greater increase in total cumulative GBF compared to fish fed twice. It is unlikely that the late peak in GBF is due to an infection because the fish all displayed
healthy hematocrit levels and no signs of inflammation around the tissues adjacent to the probe. Furthermore, the different postprandial patterns were not associated with diet treatment or fish body mass, sex, condition factor, hepatosomatic index or gonadosomatic index.

An alternative explanation relates to how some of the fish fed once showed a “double” peak in postprandial GBF, both early and late in the trial (figures not shown). This leads me to speculate that it is also plausible that the second peak in GBF relates to a starvation response in the latter stages of the experiment with an increase in GBF related to mobilizing the extensive lipid stores located in the viscera. In addition, protein synthesis in the liver of fish has been shown to be stimulated by both feeding and fasting (McMillan and Houlihan, 1992). The hepatic artery branches off the intestinal artery, so it is equally possible that blood was being directed to the liver for protein metabolism due to the effects of starvation.

Magnitude of HiE

The method of calculating HiE varies from study to study, depending on the respirometry system used to estimate MO₂ and the method of calculating SMR. In order to obtain useful, accurate estimations of HiE, it is imperative to separate energy expended on activity and stress from the metabolism association with feeding (Brett and Groves, 1979). In fact, overestimation of HiE could be a contributing factor in the large variation of the reported metabolic cost of HiE, ranging from 8 to 29% of the digestible energy in fish fed formulated diets (Cho et al., 1982; Beamish and Trippel, 1990). The present study addressed this concern by estimating HiE in several ways. The minimum and average MO₂ were measured using an intermittent flow-through respirometer and SMR (the minimum MO₂ in a resting, unfed fish) and RMR (SMR plus voluntary activity) were estimated. HiE was calculated as 1) the average
MO₂ integral minus SMR, 2) the average postprandial MO₂ minus RMR and 3) the minimum MO₂ integral minus SMR. Average MO₂ assessments could result in an over-estimation of HiE because those average MO₂ values also include all the high MO₂ values associated with active or frightened fish.

I discovered that using RMR as the baseline condition to estimate HiE was unacceptable since both minimum and average postprandial MO₂ often decreased below RMR. Therefore, the only HiE analysis presented here is based on SMR, although RMR is shown in the figures for reference. My analysis also made it clear that using minimum postprandial MO₂ is more appropriate than average postprandial MO₂. The heat increment of feeding was estimated using two different respirometry systems, with and without GBF measurements. There was no significant difference in minimum HiE among experiments, providing support for minimum HiE as a superior estimate of HiE. As a result, future studies should estimate HiE using minimum estimates of MO₂ in order to reduce the likelihood of overestimating the cost of HiE.

This recommendation is supported by differences in average HiE that existed among experiments. Average HiE was significantly greater at SFU (no probe) compared to UBC (with a probe) after 12, 18, 24, 36, 48 and 58 h postprandial. There were four major discrepancies between the respirometry systems that may have led to these differences: (1) the temperature of the water at SFU was slightly colder than at UBC (8.2- 13.0°C compared to 10.0-16.0°C); (2) the fish were slightly smaller at SFU (503.4 ± 10.7 g compared to 807.6 ± 47.1 g); (3) the inflow water rate was 5-times faster at UBC; (4) the set-up at UBC had a recirculation pump, so the fish always experienced a continuous current, even when the inflow water was turned off. HiE has been shown to decrease with increasing water temperature.
(Jobling, 1981). In addition, HiE has been demonstrated to increase with decreasing fish mass (Hilton and Slinger, 1981; Higgs et al., 1995). Both of these factors may have played a role in the increased average HiE at SFU. Nonetheless, the faster inflow water rate and recirculation system at UBC most likely contributed to the lower average HiE. MO₂ values in the SFU fish were variable, occasionally reaching expected MO₂ maximum values (> 300 mg O₂ kg⁻¹ hr⁻¹), and the fish were visibly more active within their vessels. As a result, SFU experiments had a high RMR value and a large difference in average postprandial MO₂ compared to minimum postprandial MO₂ (Figure 3.4). These highly variable, elevated MO₂ values would lead to an overestimation of the average HiE associated with feeding. In contrast, the metabolic rates in fish at UBC were much less variable and well below expected MO₂ maximum values. The difference between average and minimum postprandial MO₂ values is much smaller in UBC fish (Figures 3.5 – 3.7).

In addition to differences in the respirometry systems used, the presence of the probe may have contributed to the discrepancies in average HiE. As discussed above, stressful conditions can prolong the time course of digestion. It is also possible that fish fitted with a probe were less active within their vessels, as demonstrated by lower RMR values and lower average HiE. However, there were no significant differences in RMR or average HiE among UBC fish with and without a probe, lending support to the proposal that the experimental set-up played a considerable role in the elevated average HiE in SFU fish. As such, future studies should utilize a respirometry system equipped with a recirculation pump to minimize activity.

As previously stated, the cost of HiE will clearly depend upon the method used to estimate HiE. There was very little difference in the minimum cost of HiE estimated after postprandial MO₂ had returned to SMR among unstressed fish. The minimum cost of HiE in
un-probed fish from SFU and UBC was 4.0% and 4.8%, respectively, and 4.6% for probed fish fed twice. In contrast, minimum HiE in probed fish fed once was around double the other estimates, at 9.7%, and HiE hadn't yet returned to SMR by 80 h. The percent of total digestible energy used for HiE was low in this study compared to other estimates of HiE in rainbow trout fed 2% of their body mass (8-24%, Cho et al., 1982; Medland and Beamish, 1985; LeGrow and Beamish, 1986), probably indicating the present experimental diets were well formulated for the fish.

*Relationships between postprandial GBF, MO$_2$ and heart rate*

Given the above discussion, it is clear that any examination of the relationships between GBF and postprandial MO$_2$ will be most revealing for probed fish fed twice. This proved to be the case with clearly positive relationships being evident (Figure 3.11A), unlike for fish fed once (Figure 3.11B). In general, postprandial GBF paralleled MO$_2$. In fish fed twice, both GBF and MO$_2$ were elevated immediately following feeding and declined back down to baseline before 48 h. However, the relationship is not a simple one. GBF peaked earlier (11 h) and returned to baseline sooner (35 h) than MO$_2$ (27 h and 41 h respectively). A similar phenomenon is seen when comparing gastric evacuation rates with HiE. They are closely related, however, HiE lasts a little longer (Jobling and Davie, 1980). This is likely because much of the increase in MO$_2$ associated with feeding is due to amino acid processing, a process that continues after nutrients have been absorbed from the intestine and wastes have been eliminated. Once all the nutrients have been transported to the liver, blood flow to the splanchnic circulation can be down-regulated, although some blood must still be directed to the liver via the hepatic artery to meet the oxygen requirements for nutrient processing.
Analysis of fish fed once showed that both MO$_2$ and GBF never returned to baseline for the entire postprandial period (Figure 3.11B). It is important to note that the peak GBF and MO$_2$ were similar to those in fish fed twice. A prolonged elevation in MO$_2$ is consistent with a stress response seen in other fish which resulted in a drawn out HiE and gastric evacuation rate (Axelsson et al., 2002; Claireaux, 2006 personal communication). A prolonged time course for digestion would necessitate a longer GBF response to facilitate transport of nutrients to the liver.

Most studies on postprandial GBF in fish indicate that the postprandial increase in GBF occurs primarily via an increase in Q rather than via a redistribution of blood from other tissues (Axelsson and Fritsche, 1991; Axelsson et al., 2000; Axelsson et al., 2002). Although the present study did not measure Q, the high heart rate for the first 24 h postprandial in all fish could indicate that the postprandial increase in GBF during this time period is due, at least in part, to an increase in Q. Though heart rate closely paralleled GBF in fish fed twice, this was not the case with fish fed once. The mean postprandial GBF remained high for the entire duration of the study in fish fed once and appeared to be increasing towards the end of the trial without a concomitant increase in heart rate. This late high GBF could either be due to an elevated Q due to an increased stroke volume or due to a redistribution of blood from other tissues. I am unable to distinguish between the two possibilities in the present study.
Table 3.1: Parameters for all probed fish are presented as the mean ± SEM; values within a row with differing superscript letters are significantly different (p < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
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<tbody>
<tr>
<td>Dietary protein (%)</td>
<td>55</td>
<td>45</td>
<td>35</td>
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<tr>
<td>Dietary lipid (%)</td>
<td>10</td>
<td>15</td>
<td>20</td>
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<td>5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>715.8 ± 57.1(^a)</td>
<td>766.3 ± 48.5(^a)</td>
<td>986.6 ± 93.0(^b)</td>
</tr>
<tr>
<td>Condition factor(^1)</td>
<td>1.1 ± 0.0(^a)</td>
<td>1.3 ± 0.0(^b)</td>
<td>1.2 ± 0.1(^{ab})</td>
</tr>
<tr>
<td>GSI(^2)</td>
<td>3.0 ± 0.4(^a)</td>
<td>3.5 ± 1.1(^a)</td>
<td>3.9 ± 2.1(^a)</td>
</tr>
<tr>
<td>HSI(^3)</td>
<td>1.1 ± 0.1(^a)</td>
<td>1.6 ± 0.1(^b)</td>
<td>1.3 ± 0.1(^{ab})</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.5 ± 2.0(^a)</td>
<td>37.0 ± 4.5(^a)</td>
<td>38.7 ± 1.8(^a)</td>
</tr>
</tbody>
</table>

\(^1\)condition factor = \([\text{body mass} / \text{length}^3] \times 100\)

\(^2\)GSI denotes gonadosomatic index = \([\text{gonad mass (g)} / \text{body mass (g)}] \times 100\)

\(^3\)HSI denotes hematosomatic index = \([\text{liver mass (g)} / \text{body mass (g)}] \times 100\)
Table 3.2: Standard metabolic rate (SMR), routine metabolic rate (RMR), minimum and average peak postprandial MO₂ and minimum and average time-to-peak postprandial MO₂ for each diet treatment from SFU, UBC and when the data were combined are presented as the mean ± SEM. Means with differing superscript letters in the same row indicate a statistically significant difference between diets within an experiment (p < 0.05). There were no significant differences for any of the parameters between SFU and UBC.

<table>
<thead>
<tr>
<th>Dietary protein (%)</th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
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</thead>
<tbody>
<tr>
<td>SFU</td>
<td>9</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>UBC</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Both</td>
<td>12</td>
<td>11</td>
<td>12</td>
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<table>
<thead>
<tr>
<th>Dietary lipid (%)</th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFU</td>
<td>55</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>UBC</td>
<td>10</td>
<td>15</td>
<td>20</td>
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<td>Both</td>
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<table>
<thead>
<tr>
<th>n</th>
<th>SFU</th>
<th>UBC</th>
<th>Both</th>
</tr>
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<tbody>
<tr>
<td>SFU</td>
<td>50 ± 5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.5 ± 10.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.5 ± 5.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>UBC</td>
<td>67.5 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.6 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.4 ± 7.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Both</td>
<td>54.4 ± 4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.1 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.5 ± 4.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<table>
<thead>
<tr>
<th>SMR (mg O₂ kg⁻¹ h⁻¹)</th>
<th>SFU</th>
<th>UBC</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFU</td>
<td>115.3 ± 10.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.1 ± 16.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.4 ± 15.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>UBC</td>
<td>97.1 ± 6.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.5 ± 12.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.1 ± 8.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Both</td>
<td>110.8 ± 8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.9 ± 12.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.3 ± 10.7&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
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<thead>
<tr>
<th>RMR (mg O₂ kg⁻¹ h⁻¹)</th>
<th>SFU</th>
<th>UBC</th>
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</tr>
</thead>
<tbody>
<tr>
<td>SFU</td>
<td>119.9 ± 10.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.9 ± 11.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.6 ± 11.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>UBC</td>
<td>110.8 ± 8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.9 ± 12.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>102.3 ± 10.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Both</td>
<td>119.9 ± 10.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.9 ± 11.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.6 ± 11.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<thead>
<tr>
<th>Peak min MO₂ (mg O₂ kg⁻¹ h⁻¹)</th>
<th>SFU</th>
<th>UBC</th>
<th>Both</th>
</tr>
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<tbody>
<tr>
<td>SFU</td>
<td>115.7 ± 12.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.9 ± 14.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148.3 ± 17.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>UBC</td>
<td>132.6 ± 21.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123.7 ± 15.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118.0 ± 6.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Both</td>
<td>119.9 ± 10.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.9 ± 11.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135.6 ± 11.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<thead>
<tr>
<th>Peak avg MO₂ (mg O₂ kg⁻¹ h⁻¹)</th>
<th>SFU</th>
<th>UBC</th>
<th>Both</th>
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</thead>
<tbody>
<tr>
<td>SFU</td>
<td>185.9 ± 14.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>158.2 ± 24.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>194.4 ± 23.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>UBC</td>
<td>164.2 ± 13.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>149.2 ± 8.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>153.9 ± 8.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Both</td>
<td>180.5 ± 11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.7 ± 17.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>177.5 ± 14.9&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<th>Time-to-peak min (h)</th>
<th>SFU</th>
<th>UBC</th>
<th>Both</th>
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<tbody>
<tr>
<td>SFU</td>
<td>18.9 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.3 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.7 ± 6.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>UBC</td>
<td>20.7 ± 12.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.3 ± 14.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.7 ± 12.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Both</td>
<td>19.3 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.7 ± 5.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.1 ± 6.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<th>Time-to-peak avg (h)</th>
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<th>UBC</th>
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<tbody>
<tr>
<td>SFU</td>
<td>24.2 ± 5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.8 ± 4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.9 ± 6.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>UBC</td>
<td>52.4 ± 20.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.2 ± 17.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.9 ± 9.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Both</td>
<td>31.3 ± 7.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.7 ± 5.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.7 ± 5.1&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>
Table 3.3: Minimum and average heat increment of feeding (HiE) values from each diet treatment for experiments at SFU and UBC were calculated after 12, 18, 24, 36, 48, 58 and 80 h postprandial. Means ± SEM with differing superscript letters in the same row indicate a statistically significant difference between diets within an experiment (p < 0.05); an asterisk indicates a statistically significant difference between experiments within a diet treatment (p < 0.05).
### Dietary protein (%)

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<tr>
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<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
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### Dietary lipid (%)

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<td>9</td>
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### HiE\(^1\) minimum (mg O\(_2\) kg\(^{-1}\))

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<tr>
<th></th>
<th>SFU</th>
<th>UBC</th>
<th>SFU</th>
<th>UBC</th>
<th>SFU</th>
<th>UBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>368.9 ± 50.2(^a)</td>
<td>282.7 ± 65.7(^a)</td>
<td>305.8 ± 64.7(^a)</td>
<td></td>
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<tr>
<td>UBC</td>
<td>234.1 ± 71.6(^a)</td>
<td>453.1 ± 176.1(^a)</td>
<td>251.8 ± 75.5(^a)</td>
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<tr>
<td>18 h</td>
<td>537.8 ± 72.3(^a)</td>
<td>457.1 ± 94.5(^a)</td>
<td>539.7 ± 108.7(^a)</td>
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<tr>
<td>UBC</td>
<td>360.2 ± 127.4(^a)</td>
<td>710.5 ± 229.2(^a)</td>
<td>363.0 ± 104.8(^a)</td>
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<tr>
<td>24 h</td>
<td>668.3 ± 94.9(^a)</td>
<td>636.0 ± 115.5(^a)</td>
<td>674.5 ± 127.4(^a)</td>
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<tr>
<td>UBC</td>
<td>471.4 ± 153.8(^a)</td>
<td>920.3 ± 263.6(^a)</td>
<td>572.6 ± 133.3(^a)</td>
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<tr>
<td>36 h</td>
<td>893.3 ± 140.7(^a)</td>
<td>929.1 ± 113.7(^a)</td>
<td>899.5 ± 137.0(^a)</td>
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<tr>
<td>UBC</td>
<td>661.0 ± 179.7(^a)</td>
<td>1351.0 ± 384.7(^a)</td>
<td>890.0 ± 177.1(^a)</td>
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<tr>
<td>48 h</td>
<td>946.1 ± 180.4(^a)</td>
<td>1254.8 ± 87.7(^a)</td>
<td>915.6 ± 171.4(^a)</td>
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<tr>
<td>UBC</td>
<td>889.5 ± 292.7(^a)</td>
<td>1808.8 ± 563.8(^a)</td>
<td>1465.6 ± 202.7(^a)</td>
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<tr>
<td>58 h</td>
<td>898.3 ± 201.6(^a)</td>
<td>1274.9 ± 101.1(^a)</td>
<td>838.4 ± 171.5(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBC</td>
<td>1079.2 ± 385.5(^a)</td>
<td>2128.1 ± 744.3(^a)</td>
<td>1361.2 ± 209.9(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### HiE\(^1\) average (mg O\(_2\) kg\(^{-1}\))

<table>
<thead>
<tr>
<th></th>
<th>SFU</th>
<th>UBC</th>
<th>SFU</th>
<th>UBC</th>
<th>SFU</th>
<th>UBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>904.0 ± 92.2(^a)</td>
<td>653.8 ± 76.2(^a)</td>
<td>626.5 ± 81.4(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBC</td>
<td>298.7 ± 30.6(^a)</td>
<td>547.7 ± 163.9(^a)</td>
<td>402.6 ± 72.1(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h</td>
<td>1307.6 ± 140.2(^a)</td>
<td>1036.8 ± 130.4(^a)</td>
<td>1126.3 ± 145.6(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBC</td>
<td>480.5 ± 98.0(^a)</td>
<td>878.8 ± 230.3(^a)</td>
<td>603.4 ± 110.6(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>1696.3 ± 188.0(^a)</td>
<td>1379.5 ± 161.4(^a)</td>
<td>1449.8 ± 147.4(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBC</td>
<td>665.9 ± 141.4(^a)</td>
<td>1137.2 ± 276.4(^a)</td>
<td>916.7 ± 137.4(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36 h</td>
<td>2226.9 ± 185.6(^a)</td>
<td>2014.2 ± 262.3(^a)</td>
<td>2078.9 ± 169.6(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBC</td>
<td>1013.5 ± 208.9(^a)</td>
<td>1637.1 ± 370.8(^a)</td>
<td>1371.9 ± 194.3(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 h</td>
<td>2608.2 ± 183.7(^a)</td>
<td>2451.5 ± 387.5(^a)</td>
<td>2492.4 ± 227.2(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBC</td>
<td>1419.5 ± 390.2(^a)</td>
<td>2200.9 ± 518.2(^a)</td>
<td>1755.2 ± 213.6(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>58 h</td>
<td>2912.3 ± 214.8(^a)</td>
<td>2560.9 ± 432.2(^a)</td>
<td>2830.3 ± 417.4(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBC</td>
<td>1740.1 ± 529.4(^a)</td>
<td>2566.3 ± 687.5(^a)</td>
<td>2019.8 ± 208.7(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Average or minimum HiE is calculated as the postprandial minimum or average MO\(_2\) integral minus the SMR integral.
Table 3.4: Standard metabolic rate (SMR), routine metabolic rate (RMR), peak minimum and average postprandial MO₂, and time-to-peak minimum and average postprandial MO₂ for fish with and without a flow probe are presented as the mean ± SEM. Means with differing superscript letters in the same row indicate a statistically significant difference (p < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>No probe</th>
<th>With probe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFU</td>
<td>UBC</td>
</tr>
<tr>
<td>n</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>SMR (mg O₂ kg⁻¹ h⁻¹)</td>
<td>50.8 ± 4.1ᵃ</td>
<td>48.8 ± 2.1ᵃ</td>
</tr>
<tr>
<td>RMR (mg O₂ kg⁻¹ h⁻¹)</td>
<td>103.3 ± 8.6ᵃ</td>
<td>92.6 ± 7.9ᵇ</td>
</tr>
<tr>
<td>Peak min MO₂ (mg O₂ kg⁻¹ h⁻¹)</td>
<td>120.6 ± 8.9ᵃ</td>
<td>94.8 ± 8.4ᵃ</td>
</tr>
<tr>
<td>Peak avg MO₂ (mg O₂ kg⁻¹ h⁻¹)</td>
<td>179.2 ± 11.8ᵃ</td>
<td>162.8 ± 22.1ᵃ</td>
</tr>
<tr>
<td>Time-to-peak min MO₂ (h)</td>
<td>19.8 ± 2.7ᵃ</td>
<td>13.4 ± 2.4ᵃ</td>
</tr>
<tr>
<td>Time-to-peak avg MO₂ (h)</td>
<td>24.2 ± 3.1ᵃ</td>
<td>12.4 ± 2.2ᵃ</td>
</tr>
</tbody>
</table>
Table 3.5: Minimum and average minimum heat increment of feeding (HiE) values for fish with and without a flow probe were calculated 12, 18, 24, 36, 48, 58 and 80 h postprandial. The cost of the meal as a percent of dietary digestible energy intake (16.7 MJ kg\(^{-1}\) 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 ± SEM not sharing the same letter within a timeframe (row) 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.
<table>
<thead>
<tr>
<th></th>
<th>SFU</th>
<th>UBC</th>
<th>first feeding</th>
<th>second feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No probe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HiE min (mg kg(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 h</td>
<td>321.8 ± 33.9(^a)</td>
<td>294.8 ± 63.2(^a)</td>
<td>301.8 ± 62.2(^a)</td>
<td>259.0 ± 62.2(^a)</td>
</tr>
<tr>
<td>18 h</td>
<td>511.5 ± 50.5(^a)</td>
<td>475.4 ± 98.8(^a)</td>
<td>457.0 ± 90.3(^a)</td>
<td>359.1 ± 96.4(^a)</td>
</tr>
<tr>
<td>24 h</td>
<td>659.3 ± 61.4(^a)</td>
<td>629.2 ± 142.9(^a)</td>
<td>639.8 ± 106.9(^a)</td>
<td>537.3 ± 141.5(^a)</td>
</tr>
<tr>
<td>36 h</td>
<td>907.6 ± 72.1(^a)</td>
<td>887.6 ± 237.3(^a)</td>
<td>953.3 ± 149.5(^a)</td>
<td>804.7 ± 207.9(^a)</td>
</tr>
<tr>
<td>48 h</td>
<td>1021.7 ± 95.3(^a)</td>
<td>1079.4 ± 335.0(^a)</td>
<td>1257.0 ± 205.0(^a)</td>
<td>1021.2 ± 260.6(^a)</td>
</tr>
<tr>
<td>58 h</td>
<td>987.9 ± 103.3(^a)</td>
<td>1163.5 ± 382.1(^a)</td>
<td>1493.4 ± 250.6(^a)</td>
<td>1162.3 ± 313.3(^a)</td>
</tr>
<tr>
<td>80 h</td>
<td>-</td>
<td>1010.4 ± 433.9(^a)</td>
<td>2201.5 ± 448.0(^a)</td>
<td>1429.4 ± 404.8(^a)</td>
</tr>
<tr>
<td><strong>HiE avg (mg kg(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 h</td>
<td>739.7 ± 53.9(^a)</td>
<td>490.2 ± 61.1(^ab)</td>
<td>413.8 ± 57.6(^b)</td>
<td>344.5 ± 75.6(^b)</td>
</tr>
<tr>
<td>18 h</td>
<td>1162.4 ± 80.7(^a)</td>
<td>791.8 ± 124.3(^ab)</td>
<td>645.0 ± 89.1(^b)</td>
<td>513.3 ± 110.4(^b)</td>
</tr>
<tr>
<td>24 h</td>
<td>1518.6 ± 98.8(^a)</td>
<td>985.0 ± 160.5(^ab)</td>
<td>908.4 ± 108.3(^b)</td>
<td>699.0 ± 158.5(^b)</td>
</tr>
<tr>
<td>36 h</td>
<td>2112.8 ± 118.4(^a)</td>
<td>1356.4 ± 253.2(^ab)</td>
<td>1346.5 ± 148.7(^b)</td>
<td>1038.9 ± 252.5(^b)</td>
</tr>
<tr>
<td>48 h</td>
<td>2522.2 ± 154.2(^a)</td>
<td>1619.9 ± 351.9(^ab)</td>
<td>1785.2 ± 199.1(^b)</td>
<td>1337.5 ± 331.4(^b)</td>
</tr>
<tr>
<td>58 h</td>
<td>2771.2 ± 198.1(^a)</td>
<td>1797.7 ± 396.4(^ab)</td>
<td>2092.6 ± 242.3(^ab)</td>
<td>1544.2 ± 394.2(^b)</td>
</tr>
<tr>
<td>80 h</td>
<td>-</td>
<td>1827.0 ± 442.2(^a)</td>
<td>2991.5 ± 421.0(^a)</td>
<td>1955.5 ± 507.2(^a)</td>
</tr>
</tbody>
</table>

**Cost of HiE (% of DE intake)**

**Min MO\(_2\)**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>1.4 ± 0.1(^a)</td>
<td>1.3 ± 0.3(^a)</td>
<td>1.3 ± 0.3(^a)</td>
<td>1.1 ± 0.3(^a)</td>
</tr>
<tr>
<td>18 h</td>
<td>2.3 ± 0.2(^a)</td>
<td>2.1 ± 0.4(^a)</td>
<td>2.0 ± 0.4(^a)</td>
<td>1.7 ± 0.4(^a)</td>
</tr>
<tr>
<td>24 h</td>
<td>2.9 ± 0.3(^a)</td>
<td>2.8 ± 0.6(^a)</td>
<td>2.8 ± 0.5(^a)</td>
<td>2.4 ± 0.6(^a)</td>
</tr>
<tr>
<td>36 h</td>
<td>4.0 ± 0.3(^a)</td>
<td>3.9 ± 1.1(^a)</td>
<td>4.2 ± 0.7(^a)</td>
<td>3.6 ± 0.9(^a)</td>
</tr>
<tr>
<td>48 h</td>
<td>-</td>
<td>4.8 ± 1.5(^a)</td>
<td>5.6 ± 0.9(^a)</td>
<td>4.5 ± 1.2(^a)</td>
</tr>
<tr>
<td>58 h</td>
<td>-</td>
<td>6.6 ± 1.1(^a)</td>
<td>&gt; 9.7 ± 2.0(^a)</td>
<td></td>
</tr>
<tr>
<td>80 h</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

**Avg MO\(_2\)**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>3.3 ± 0.2(^a)</td>
<td>2.2 ± 0.5(^ab)</td>
<td>1.8 ± 0.3(^b)</td>
<td>1.5 ± 0.3(^b)</td>
</tr>
<tr>
<td>18 h</td>
<td>5.1 ± 0.4(^a)</td>
<td>3.5 ± 0.6(^ab)</td>
<td>2.9 ± 0.4(^b)</td>
<td>2.3 ± 0.5(^b)</td>
</tr>
<tr>
<td>24 h</td>
<td>6.7 ± 0.4(^a)</td>
<td>4.4 ± 0.7(^ab)</td>
<td>4.0 ± 0.5(^b)</td>
<td>3.1 ± 0.7(^b)</td>
</tr>
<tr>
<td>36 h</td>
<td>9.4 ± 0.5(^a)</td>
<td>6.0 ± 1.1(^b)</td>
<td>6.0 ± 1.1(^b)</td>
<td>4.6 ± 1.1(^b)</td>
</tr>
<tr>
<td>48 h</td>
<td>11.2 ± 0.7(^a)</td>
<td>7.9 ± 0.9(^b)</td>
<td>7.9 ± 0.9(^b)</td>
<td>5.9 ± 1.5(^b)</td>
</tr>
<tr>
<td>58 h</td>
<td>&gt; 12.3 ± 0.9(^a)</td>
<td>9.3 ± 1.1(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 h</td>
<td>&gt; 13.2 ± 1.9(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Average or minimum HiE is calculated as the postprandial minimum or average MO\(_2\) integral minus the SMR integral.
Table 3.6: Baseline, routine, peak and time-to-peak postprandial gut blood flow (GBF) and heart rate for fish given each diet treatment (mean ± SEM). Values with differing superscript letters within a row indicate a statistically significant difference (p < 0.05) between diet treatments.

<table>
<thead>
<tr>
<th></th>
<th>Dietary protein (%)</th>
<th>Dietary lipid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HP:LL</td>
<td>MP:ML</td>
</tr>
<tr>
<td>55</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GBF (ml min⁻¹ kg⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>4.1 ± 0.8ᵃ</td>
<td>4.8 ± 0.4ᵃ</td>
<td>3.2 ± 0.6ᵃ</td>
</tr>
<tr>
<td>routine</td>
<td>4.7 ± 0.9ᵃ</td>
<td>5.3 ± 0.4ᵃ</td>
<td>3.9 ± 0.7ᵃ</td>
</tr>
<tr>
<td>peak</td>
<td>11.3 ± 2.6ᵃ</td>
<td>12.1 ± 2.4ᵃ</td>
<td>8.0 ± 1.8ᵃ</td>
</tr>
<tr>
<td>time-to-peak (h)</td>
<td>30.3 ± 20.9ᵃ</td>
<td>67.3 ± 4.2ᵃ</td>
<td>42.4 ± 13.3ᵃ</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>HP:LL</th>
<th>MP:ML</th>
<th>LP:HL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate (beat min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>40.0 ± 1.1ᵃ</td>
<td>31.0 ± 4.9ᵃ</td>
<td>38.1 ± 5.7ᵃ</td>
</tr>
<tr>
<td>routine</td>
<td>49.5 ± 2.6ᵃ</td>
<td>42.3 ± 7.1ᵃ</td>
<td>58.5 ± 6.8ᵃ</td>
</tr>
<tr>
<td>peak</td>
<td>73.4 ± 1.6ᵃ</td>
<td>77.3 ± 0.5ᵃ</td>
<td>71.2 ± 5.0ᵃ</td>
</tr>
<tr>
<td>time-to-peak (h)</td>
<td>8.3 ± 1.9ᵃ</td>
<td>16.7 ± 1.2ᵃ</td>
<td>14.3 ± 3.3ᵃ</td>
</tr>
</tbody>
</table>
Table 3.7: Baseline, routine, peak and time-to-peak postprandial gut blood flow (GBF) and heart rate for unfed fish and fish fed once or twice (mean ± SEM). The total postprandial blood flow to the gut above baseline GBF levels after 12, 18, 24, 36, 48, 58 and 80 h are indicated. Values with differing letters within a row indicate a statistically significant difference (p < 0.05). Bold font indicates the total increase in GBF estimated after postprandial GBF had returned to baseline.

<table>
<thead>
<tr>
<th></th>
<th>First feeding</th>
<th>Second feeding</th>
<th>Unfed</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBF (ml min⁻¹ kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>4.0 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>routine</td>
<td>4.5 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>peak</td>
<td>10.2 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.9 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>time-to-peak (h)</td>
<td>47.7 ± 8.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.9 ± 4.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>Heart rate (beat min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>36.5 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.2 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.4 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>routine</td>
<td>50.9 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.5 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.7 ± 5.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>peak</td>
<td>73.7 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.9 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>time-to-peak (h)</td>
<td>13.2 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.5 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

Cumulative increase in GBF from baseline (l kg⁻¹)<sup>1</sup>

<table>
<thead>
<tr>
<th></th>
<th>12 h</th>
<th>18 h</th>
<th>24 h</th>
<th>36 h</th>
<th>48 h</th>
<th>58 h</th>
<th>80 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>1.9 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>routine</td>
<td>2.9 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>peak</td>
<td>3.9 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>36 h</td>
<td>6.3 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.9 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>48 h</td>
<td>8.7 ± 2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>58 h</td>
<td>11.0 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>80 h</td>
<td>&gt;17.1 ± 4.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>1</sup> Total increase in GBF is calculated as the integral under the postprandial GBF curve minus the baseline integral.
<table>
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<tr>
<th>Day 1</th>
<th>SFU</th>
<th>UBC</th>
<th>Unfed</th>
<th>First feeding</th>
<th>Second feeding</th>
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<td>sham feed</td>
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<td>GBF surgery</td>
<td>(GBF surgery)</td>
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Figure 3.1: Experimental protocol.
Figure 3.2: Schematic representation of the cardiovascular circulation in rainbow trout, highlighting the major vessels associated with digestion (adapted from Thorarensen et al., 1991).
Figure 3.3: Representative trace of a gut blood flow plot for a 699 g trout that was not fed (A) and a 965 g trout that was fed the MP:ML diet (B). The shaded boxes represent the light/dark cycles the fish had been exposed to prior to the experiment (although the fish were in 24 hour darkness during the experiment). The vertical solid lines indicate when the fish was removed from the vessels and the dashed lines indicate 4 h after the fish had been returned to the vessel. Periods I and II are from the first 24 hours after surgery while the fish was recovering. Recovery could involve an increase in GBF (B) or a decrease in GBF (A). The fish was sham fed between period II and III. Periods III and IV represent the sham response. Fish A was sham fed again between periods IV and V. The sham could result in an increase or no change in GBF, which had largely subsided by 4 h. Fish B was force fed 2% of its body weight between period IV and V and periods V and VI represent the postprandial GBF response. MO₂ and heart rate data were assessed in a similar manner.
Figure 3.4: Postprandial MO$_2$ average (open circles) and minimum (closed circles) for SFU experiments ($n = 24$). All diet treatments are combined as the mean $\pm$ SEM. 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 “$\bullet$” (p < 0.05). Inset: The difference between average MO$_2$ and minimum MO$_2$ is presented over time.
Figure 3.5: Postprandial MO₂ average (open circles) and minimum (closed circles) for non-probed fish from UBC experiments (n = 5). All diet treatments are combined as the mean ± SEM. Routine metabolic rate (dotted line) and standard metabolic rate (dashed line) are indicated. A significant difference in minimum MO₂ from SMR is indicated by an asterisk. A significant difference in average MO₂ from SMR is indicated by the symbol “†” and a significant difference in average MO₂ from both SMR and RMR is indicated by the symbol “◆” (p < 0.05). Inset: The difference between average MO₂ and minimum MO₂ is presented over time.
Figure 3.6: Postprandial MO₂ average (open circles) and minimum (closed circles) for probed fish fed for the second time from UBC experiments (n = 6). All diet treatments are combined as the mean ± SEM. Routine metabolic rate (dotted line) and standard metabolic rate (dashed line) are indicated. A significant difference in minimum MO₂ from SMR is indicated by an asterisk. A significant difference in average MO₂ from SMR is indicated by the symbol “i” and a significant difference in average MO₂ from both SMR and RMR is indicated by the symbol “★” (p < 0.05). Inset: The difference between average MO₂ and minimum MO₂ is presented over time.
Figure 3.7: Postprandial MO$_2$ average (open circles) and minimum (closed circles) for probed fish fed for the first time in UBC experiments (n = 11). All diet treatments are combined as the mean ± SEM. 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.
Figure 3.8: (A) Postprandial gut blood flow for all diet treatments pooled together (n = 7) for fish that were fed a second time (mean ± SEM). Routine gut blood flow (dotted line) and baseline gut blood flow (dashed line) are indicated. (B) Postprandial heart rate for all diet treatments pooled together for fish that were fed a second time (mean ± SEM). Routine heart rate (dotted line) and baseline heart rate (dashed line) are indicated. The symbol “#” indicates a statistically significant difference from both routine and baseline and an asterisk indicates a statistically significant difference from baseline (p < 0.05).
Figure 3.9: (A) Postprandial gut blood flow for all diet treatments pooled together (n = 12) for fish that were fed for the first time (mean ± SEM). Routine gut blood flow (dotted line) and baseline gut blood flow (dashed line) are indicated. (B) Postprandial heart rate for all diet treatments pooled together for fish that were fed for the first time (mean ± SEM). Routine heart rate (dotted line) and baseline heart rate (dashed line) are indicated. The symbol “‡” indicates a statistically significant difference from both routine and baseline and an asterisk indicates a statistically significant difference from baseline (p < 0.05).
**GBF (ml min⁻¹ kg⁻¹)**

![Graph showing GBF over time](image)

**Heart rate (beat min⁻¹)**

![Graph showing heart rate over time](image)

**Time (h)**

![Graph showing time](image)
Figure 3.10: Representative gut blood flow trace of an 811 g male rainbow trout, 2 days postprandial, responding to a disturbance.
Figure 3.11: The change in postprandial gut blood flow from baseline levels is plotted against the change in the postprandial minimum MO\textsubscript{2} from SMR in fish fed twice (A) and fish fed once (B). The filled circles, open circles and filled triangles represent values from 4 – 24 h, 24 – 36 h and 36 – 80 h postprandial, respectively.
Figure 3.12: The change in postprandial gut blood flow from baseline levels is plotted against the change in the postprandial heart rate from baseline levels in fish fed twice (A) and fish fed once (B). The filled circles, open circles and filled triangles represent values from 4 – 24 h, 24 – 36 h and 36 – 80 h postprandial, respectively.
Figure 3.13: The peak minimum MO₂, gut blood flow and heart rate are presented as a percent of maximum MO₂ (371.9 mg O₂ kg⁻¹ h⁻¹, Kiceniuk and Jones, 1977), maximum cardiac output (52.6 ml min⁻¹ kg⁻¹, Kiceniuk and Jones, 1977) and maximum heart rate (80 beat min⁻¹, Simonot, 2005), respectively, as estimated by swimming rainbow trout to Ucrit. Standard metabolic rate, baseline gut blood flow and baseline heart rate are indicated by a dotted line. Routine metabolic rate, routine gut blood flow and routine heart rate are indicated by a dashed line. The solid line in the gut blood flow graph indicates resting cardiac output (17.6 min⁻¹ kg⁻¹, Kiceniuk and Jones, 1977).
References


Kaczanowski, T. C. and Beamish, F. W. H. (1996). Dietary essential amino acids and heat increment in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry* 15, 105-120.


Chapter 4: Major findings and conclusions

This thesis examined the effects of varying dietary protein and lipid concentrations on growth, heat increment of feeding and gut blood flow in rainbow trout. There were four primary research objectives: (1) to induce differential protein utilization by feeding fish isocaloric diets of varying protein and lipid levels; (2) to determine if fish with differential protein utilization accordingly alter their heat increment of feeding and postprandial blood flow to the gut; (3) to quantify postprandial MO$_2$ and GBF in rainbow trout fed a single meal of 2% of their body mass using improved methods; and (4) to simultaneously measure MO$_2$ with GBF in order to assess the postprandial relationships.

Objective 1

Rainbow trout fed three isocaloric diets containing varying levels of protein and lipid did indeed have differential protein utilization. Fish fed the HP:LL diet had significantly more protein and significantly less lipid than those fed the medium or high lipid diets. However, fish fed the LP:HL diet had significantly improved protein utilization as indicated by higher values for protein efficiency ratio and percent protein deposition compared to values for HP:LL fish. This protein sparing effect due to high availability of lipid energy has been well established by other researchers (e.g. Reinitz et al., 1978; Takeuchi et al., 1978; Beamish and Medland, 1986; Yigit et al., 2002). Notably, I found no significant difference in any growth parameters (specific growth rate, feed efficiency or dry feed intake) among diet treatments. Similarly, Azevedo et al. (2004a; 2004b) and Steffens et al. (1999) found that isocaloric diets of varying protein and lipid ratios did not result in a significant difference in weight gain in rainbow trout.
trout. In contrast, other researchers have demonstrated differential growth using diets varying in protein and lipid content (e.g. Yigit et al., 2002; Chaiyapechara et al., 2003; Morrow et al., 2004). These discrepancies are likely due to differences in fish size, protein and lipid levels and, importantly, varying dietary energy content.

Objective 2

This study is the first to examine the effect of diet composition on GBF. Diets of suboptimal protein quality or diets with high digestible protein to digestible energy (DP:DE) ratios (> 25 g MJ⁻¹) have been shown to increase the HiE in fish (Cho et al., 1976; Jobling, 1981; Cho et al., 1982; LeGrow and Beamish, 1986; Cho and Woodward, 1989; Higgs et al., 1995). Thus, it was anticipated that feeding fish diets that bracketed the recommended DP:DE level would result in a significant difference in HiE and GBF. Despite evidence of differential protein utilization, there was no apparent difference in HiE, GBF or heart rate among fish given the diet treatments. There are several possible explanations. The fish used in this study were larger (503.4 ± 10.7 g for SFU fish and 807.6 ± 47.1 g for UBC fish) than those typically used in HiE studies (4 – 145 g; Cho et al., 1976; Medland and Beamish, 1985; LeGrow and Beamish, 1986; Cho and Woodward, 1989). The scope for growth decreases as fish grow (Higgs et al., 1995), which may have expanded the tolerable range of DP:DE. In addition, the fish were not fed to satiation prior to the MO₂ and GBF studies, so the decreased ration of ~2% of their body mass could have prevented the fish from being in extreme states of protein utilization. Finally, there was no significant difference in any of the growth parameters during the growth trial and the cost of HiE as a % of digestible energy was low (~ 4 – 10%), indicating that these diets were well formulated and matched the needs of the fish. Therefore, any differences in HiE, GBF or heart rate that may have existed may have been too small to
detect with the resolution of the equipment used. Consequently, any differences in these variables that do exist among diet treatments may have a negligible impact on the digestive physiology of the fish.

**Objective 3**

Standard metabolic rate, baseline GBF and baseline heart rate measured here (52.6 ± 2.5 mg O₂ kg⁻¹ h⁻¹, 4.0 ± 0.2 ml min⁻¹ kg⁻¹ and 34.2 ± 1.8 beat min⁻¹, respectively) were within established values. Four hours after a meal of 2% of their body mass, all three variables had increased significantly. Minimum MO₂, GBF and heart rate peaked by 119% above SMR, 153% above baseline GBF and 75-115% above baseline heart rate. Minimum postprandial MO₂ above SMR proved to be the superior method for estimating HiE. Digestion appeared to be complete before 48 h postprandial in un-probed fish, as predicted. Minimum MO₂ returned to SMR after 30 and 44 h postprandial in un-probed SFU and UBC fish, respectively. Probed fish fed a second time displayed similar postprandial MO₂ patterns to un-probed fish, returning to SMR by 41-h postprandial. Postprandial GBF and heart rate in fish fed a second time similarly returned to baseline levels within 35 and 30 h respectively. However, fish that were fed once, 48 h after surgery, demonstrated a different postprandial MO₂ and GBF pattern. MO₂ and GBF remained elevated above baseline levels for the entire duration of the 80 h study, likely due to prolonged digestion as a consequence of surgery. The minimum cost of HiE (as a % of digestible energy) once postprandial MO₂ had returned to SMR was similar for unstressed fish (4.0 – 4.8%), but around double in probed fish fed the first time (9.7%).
Objective 4

This is the first known study to simultaneously measure GBF with MO$_2$ in fish. Fish fed a second time showed a similar postprandial GBF response to chinook salmon (Thorarensen, 1994) and a similar MO$_2$ response to un-probed fish in the present study and were therefore considered to be a typical response. MO$_2$, GBF and heart rate were all more or less correlated. All three variables increased significantly from baseline within 4 h postprandial and returned to baseline before 48-h postprandial. There is a linear relationship between GBF and MO$_2$ and GBF and heart rate between 24 and 80 h postprandial, when all variables were decreasing back to baseline levels. However, postprandial MO$_2$ remained elevated above baseline longer (41 h) than GBF (35 h). This prolonged elevation in MO$_2$ could be attributed to nutrient processing, which continues after all the nutrients have been absorbed from the intestine and the waste has been eliminated (Jobling and Davie, 1980).

Recommendations

A variety of test systems, fish treatments and data analysis were performed in this thesis. These resulted in a number of recommendations.

1. The importance of estimating the HiE in calm, unexcited fish has been previously emphasized by other researchers (Brett and Groves, 1979; Cho et al., 1982). Agitated fish demonstrating excessive locomotion will have an elevated MO$_2$, leading to over-estimates of the HiE. To account for this, the present study examined two methods of estimating the metabolism associated with feeding, using minimum and average postprandial MO$_2$ estimates.
over one or two h blocks. The minimum MO₂ value obtained in a one or two h block proved to be a superior technique for estimating the true cost of HiE.

2. The present study used two different respirometry systems. The major difference was that the system used at SFU had a much lower flow and UBC’s system was equipped with a recirculation pump so that the fish were always exposed to a continuous current, even when the flush pump was turned off. Fish at SFU were observed to be much more active within their vessels than fish at UBC. Additionally, individual MO₂ points from fish at SFU occasionally reached expected maximum MO₂ levels (> 300 mg kg⁻¹ h⁻¹), which led to elevated RMR estimates. It is therefore recommended that respirometry vessels for salmonids be equipped with recirculation pumps with adequate flow rates to minimize aberrant activity.

3. Two drastically different postprandial patterns of MO₂ and GBF were observed depending upon whether the fish was fed immediately after surgery or whether it was re-fed a week later. Fish fed 48 h after surgery demonstrated a prolonged time course for digestion and elevated peak heart rate, suggesting a stress effect associated with surgery. The fish that were re-fed demonstrated a similar MO₂ pattern to un-probed fish, leading me to believe that this was the more accurate response. Therefore, it is recommended that rainbow trout should be given more than 48 h to recover from surgery prior to experimentation.

4. While diet did not have a significant effect on metabolism or gut blood flow in the present study, diets resulting in compromised growth could be associated with differential gut blood
flow and necessitate further investigation. It is recommended that isocaloric diets be used for such comparisons to prevent confounding results due to differences in energy content.
References


Chaiyapechara, S., Casten, M. T., Hardy, R. W. and Dong, F. M. (2003). Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentrations of lipid and vitamin E. *Aquaculture* 219, 715-738.


