THESIS

GROWTH, PROXIMATE COMPOSITION, AND METABOLISM OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS)* CONSUMING DIETS CONTAINING ALTERNATIVE PROTEINS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2014

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ABSTRACT

GROWTH, PROXIMATE COMPOSITION, AND METABOLISM OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS)* CONSUMING DIETS CONTAINING ALTERNATIVE PROTEINS

Significant increases in the market price of fish meal during the mid to late-2000's spurred simultaneous increases in the cost of aquafeeds to fish production facilities. This intensified a search for less expensive but nutritionally sound alternatives to fish meal. In the first chapter of this study, eight experimental aquafeeds were formulated from 4 different ingredient groups, none of which included fish meal. These ingredient groups were classified as an animal plant diet, a novel plants diet, a plants with future potential diet, and a plant products diet. Each ingredient group was formulated into high protein (45% dry weight) and low protein (40% dry weight) diets. These diets were compared to two standard fish meal based diets formulated to the same protein:lipid ratios in a 5 × 2 factorial feeding trial at 15°C. Each diet was randomly assigned to a 65-L tank (for a total of 3 replicates per diet) containing 15 juvenile rainbow trout *Oncorhynchus mykiss* (mean ± S.E.M. initial wet weight: 91.3 ± 0.7 g). The 40% protein fish meal diet produced both the highest mean final wet weight (441 ± 5.7 g) and lowest feed conversion ratio (0.93 ± 0.02 g feed consumed g weight gain^{-1}). However, the best performing experimental diets had comparable values, with the 40% protein animal and plant diet (APD+) having an FCR of 0.95 and the 45% protein animal and plant diet (APD) having a SGR of 1.74. More statistical differences in growth metrics were noted when comparing different feed ingredient groups than when comparing between protein levels. Proximate analyses were used to determine proportions of moisture, protein, lipid, and energy within a subsample of fish from

each treatment at the conclusion of the trial. The most striking trend noted in these analyses was the apparent protein sparing and lipid sparing exhibited by fish consuming the 40% protein diets and 45% protein diets, respectively. The results indicate that although fish meal based diets generally promote the highest growth rates in rainbow trout, diets composed solely of plant or plant and animal based ingredients, when carefully formulated, promote similar rates of growth and are likely to be less costly to fish producers.

In the second chapter of this study, we examined one likely cause of the reduction in growth observed in fish consuming alternative proteins. It is generally believed that a deficiency in essential amino acids, particularly a lack of lysine, is a major factor limiting the effectiveness of plant and animal-based proteins for finfish. In this experiment, investigators examined the growth rates of juvenile rainbow trout (mean initial weight= 95.5 g) consuming plant-based feeds formulated to 7 graded lysine levels during a 90-day feeding trial at 15°C. Feeds used in this trial were formulated to include soybean meal, soy protein concentrate, corn protein concentrate, wheat flour, and menhaden fish oil and graded lysine levels of 1.35, 1.85, 2.34, 2.83, 3.32, 3.82, and 4.31 g lysine/100 g dry diet. Mean final wet weights ranged from 380 g to 436 g per fish, and mean feed conversion ratios ranged from 1.00 to 1.20 g weight gained g feed consumed^{-1}. Fish consuming the 2.34% lysine diet produced the best results in all examined growth metrics, suggesting that it approaches the optimum level for fish consuming plant-based feeds. Proximate analyses revealed a direct relationship between lysine content and biomass protein content, and an inverse relationship between lysine content and fat content. Results presented here suggest that feeds containing 2.34% lysine produce favorable growth rates in rainbow trout, and that supplementation of this amino acid at even higher levels is both unnecessary and has negative effects upon rainbow trout growth.

In the final portion of this study, two sets of experimental trials were conducted to determine the effects of different diets on the post-prandial metabolism of rainbow trout. Both sets of trials utilized 65-L tanks (respirometry chambers), fitted with specialized lids and sampling ports, to conduct intermittent static respirometry on a whole-tank basis on pre- and post-feeding rainbow trout. Metabolic responses examined included routine metabolism, specific dynamic action (SDA) maximum, SDA duration, time-to-peak SDA, total oxygen consumed during the SDA response, and SDA coefficient (the proportion of calories consumed during the SDA response). The first set of experiments included diets formulated from 5 ingredient groups (see Chapter 1), each of which was formulated to a 45:20 or 40:20 protein:lipid (P:L) ratio (5 × 2 factorial design). Results showed that metabolic responses were highly variable post-feeding, and few statistically significant results were observed. However, a significant relationship between time-to-peak SDA and dietary protein level was observed, with fish consuming the 45:20 P:L diets exhibiting their peak metabolic response 27 hours post-feeding, and those consuming the 40:20 P:L diets peaking after 19 hours.

The second set of trials examined three experimental diets. These feeds were plant-based, using soy, corn, and wheat-based ingredients, and formulated to 35:20 and 40:25 P:L ratios. The third diet was a standard fish-meal based feed, formulated to 45:20 P:L. Metabolic responses were, again, highly variable, due to varying activity levels of fish within the respirometry chambers. No statistically significant results were observed. There was a weak trend of greater SDA maximum as well as greater total oxygen consumed for fish consuming higher protein diets. These results highlight the substantial variability in metabolic rates of fish under conditions in which they are allowed volitional movement, as opposed to being confined within a more restrictive chamber. Due to the possibility of volitional movement, the results likely

represent more closely the metabolic response of fish within an aquaculture setting, in which they are allowed free movement within a raceway or pond, and suggest that oxygen requirements within these settings are not strongly correlated to the diet being offered.

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Chapter 1 - An evaluation of the growth performance and proximate composition of rainbow trout fed aquafeeds containing plant and animal-based proteins

1. Introduction

Finfish are consumed worldwide in human diets, and both demand and production continue to increase. Aquaculture of global fish supplies has increased from 3.9% of total production by weight in 1970-73 to 40.3% in 2010 (FAO, 2012). As demand for fish products has increased, so has demand for commercial feeds to sustain production. Commercial fish feeds, especially those for marine species and salmonids (Francis et al., 2001), commonly use fish meal and fish oil as the primary protein and lipid sources (Gatlin et al., 2007). Currently, roughly 25% of the dry weight of these feeds consists of fish meal (FAO, 2012), although levels may be as high as 45-50%. Fish meal and fish oil are often derived from marine species, such as anchovies (Family: Engraulidae), menhaden (*Brevoortia* spp.; Clupeidae), or eels (Order: Anguilliformes). Demand for fish meal and fish oil will likely continue to increase, paralleling aquaculture production (FAO, 2012; Tacon and Metian, 2008) and current harvest rates of these species may not be sustainable in the long-term.

Fish meal, historically, was the most economical and effective protein source used in fish feeds, due to its favorable amino acid profile and high digestibility (Lovell, 2002). Previous to 2006, the price of fish meal remained between US $400 and US $900 per metric ton, spiking to US $1500 in 2006, and remaining above US $1100 since (Hardy, 2010). As of March 2014, the price of fish meal is currently US $1622 per metric ton, down from US $2088 per metric ton in January 2013 (Indexmundi, 2013). The aquaculture industry presently consumes 65% and 89% of global annual fish meal and fish oil produced, respectively (Tacon and Metian, 2008; Hardy,

2010). Thus, most of the economic impact of increased prices will affect fish farming operations and hatcheries. Hardy (2010) suggests that increasing recovery and utilization of seafood processing wastes may increase total production of fish meal and oil by 15-20%. However, a point will likely be reached at which increasing feed costs will raise the price of farmed fish products to levels unacceptable to many consumers. This economic pressure has increased efforts to evaluate protein replacements for fish meal in commercial finfish feeds that are both effective and economically feasible.

Rainbow trout (*Oncorhynchus mykiss*) are a commonly raised fish species in the United States (Stickney, 1996). Currently, Idaho, North Carolina, California, and Pennsylvania account for much of the U.S. production of this fish for human consumption (Hardy, 2002). Farmed rainbow trout are preferred by many consumers, due to its familiarity as a sportfishing species, its organoleptic properties, and its perceived health benefits. Both the cardiovascular (Wang et al., 2006) and central nervous systems (Luchtman and Song, 2013) are generally believed to be positively affected by consumption of foods high in omega-3 fatty acids, such as trout and salmon. In addition to the fish raised for consumption, millions of rainbow trout are raised at private, state, and federal hatcheries, and are later stocked into lakes and streams to provide additional sportfishing opportunities where few may otherwise exist. Together, rainbow trout raised directly for human consumption, and those raised to enhance or provide sportfishing opportunities, account for a large proportion of aquaculture production in the United States, with total sales of eggs, fingerlings, stockers, and food size fish totaling over $86 million dollars in 2008 in the U.S. (USDA ERS, 2013). Production of these rainbow trout is directly dependent on wild fish stocks as a component of their feeds.

Efforts to find suitable protein ingredients to replace fishmeal in rainbow trout feeds has been pursued for at least the past 15 years (see Fontainhas-Fernandez et al., 1999, Gomes et al., 1995, Olivateles et al., 1994, Watanabe et al., 1996). Studies have focused on evaluating a range of different plant and animal protein ingredients and combinations. Plant-based protein ingredients include corn gluten meal, soy meal, soy protein concentrate, wheat gluten meal, barley protein concentrates, cottonseed meal, and canola (rapeseed) meal. Animal based ingredients include poultry by-product meal, feather meal, and blood meal. A common challenge of using alternative protein sources in rainbow trout diets is the greater likelihood of decreased growth rates (Bureau et al., 2000, Escaffre et al., 2007, Santigosa et al., 2008) and health problems (Burel et al., 2001, Rumsey et al., 1994), especially at high levels of fishmeal replacement. Interestingly, recent research by Overturf et al. (2013) demonstrated that certain strains of rainbow trout are better suited for production using fishmeal-free diets, suggesting that both diets and fish strains should be developed in concert.

Common obstacles associated with consumption of plant-based protein diets by rainbow trout include amino acid imbalances and deficiencies, high levels of indigestible carbohydrates present in certain grain products, and varying antinutritional factors (ANF) that negatively affect fish growth and health (Hardy, 2010). Certain grains may also require higher levels of processing to create a suitable protein ingredient, or to reduce or eliminate the negative effects of ANF (Hardy, 2010). In contrast, animal-based proteins are typically highly digestible, and generally contain favorable amino-acid profiles for fish growth (Bureau et al., 1999, El-Haroun et al., 2009). As early as 1957, Halver noted that Chinook salmon (*Oncorhynchus tshawytscha*) fed a diet based upon whole-egg protein exhibited favorable growth rates (Halver, 1957). Currently, many alternative feed ingredients are by-products from the processing of plant and animal

products for human consumption, helping to decrease the cost of these ingredients (Hardy, 2010, FAO, 2012).

The goal of this study was to evaluate the growth of standard (i.e., not selected for performance on fishmeal-free diets) rainbow trout fed diets composed of differing combinations of plant-based or plant and animal-based proteins, and to compare the results to those of rainbow trout fed a standard fish meal-based diet, comparable to many commercial diets currently available. For all experimental diet ingredient combinations in this study (5), we also compared the effects of feeding fish at two protein:lipid (P:L) ratios: 45:20 and 40:20 percent diet dry weight (Table 1.1). The 45:20 and 40:20 P:L ratios are recommended by Hardy (2002), and by the National Research Council (NRC, 1993), respectively.

2. Materials and Methods

2.1. Ingredients and Experimental Design

Ten experimental feeds were formulated from 5 different ingredient mixtures to either a high (45:20) or low (40:20) P:L ratio (Table 1.1) for a 5 × 2 factorial design. Diets listed with a three-letter code (e.g. PPD) were formulated to a 45% protein level, and those with a three-letter code followed by a plus symbol (e.g. PPD+) were formulated to a 40% protein level. Diets were not formulated to be isocaloric. However, most carbohydrates are not highly digestible by carnivorous fish, like rainbow trout (Sargent et al., 2002). Thus, the caloric portion of the diet composed of carbohydrates likely has a negligible impact on rainbow trout growth, and comparisons among treatments are still reasonable.

Table 1.1. Summary of diet types used.

Name of diet	Primary Ingredients (>10% of diet by dry weight)	Protein: Lipid ratio	Caloric Content (cal/g)
Animal Plant Diet (APD+)	Feather meal, chicken meal, blood meal	40:20	4605
APD		45:20	4931
Fish Meal Diet (FMD+)	Fish meal, soybean meal, corn protein concentrate, chicken meal, blood meal, wheat protein concentrate	40:20	4620
FMD		45:20	4938
Novel Plants Diet (NPD+)	Corn protein concentrate soybean meal, distillers dried grains and solutes, and Hamlet protein	40:20	4191
NPD		45:20	4513
Plants with Future Potential Diet (PFPD+)	Barley protein concentrate, Spirulina, full-fat soybean meal, wheat flour	40:20	3689
PFPD		45:20	3780
Plant Products Diet (PPD+)	Corn protein concentrate, soybean meal, soy protein concentrate, wheat protein flour	40:20	4598
PPD		45:20	4983

Eyed rainbow trout eggs (Fall steelhead strain) were received from Troutlodge, Inc. (Sumner, WA, USA) on 9 December 2010 and incubated at 10°C at the Foothills Fish Laboratory (Colorado State University, Fort Collins, CO, USA) until swim-up. Fish were initially fed a commercial starter diet (Trout and Salmon Starter, Silver Cup, Murray, UT, USA), and switched over to 1-3 mm pelleted feed supplied by the Bozeman Fish Technology Center (BFTC; U.S. Fish and Wildlife Service, Bozeman, MT) until the start of the trial. The grow-out period lasted 26 weeks, allowing the fish to reach a mean size of 91.3 ± 0.7 g (mean ± S. E. M.). Water temperatures were gradually increased to 15 ± 1° C and maintained at this temperature for the duration of the experiment. At the initiation of the feeding trial, thirty 65-L tanks were stocked with 15 rainbow trout, each of which had been marked with a unique visual implant alphanumeric (VI-alpha) tag (Northwest Marine Technologies, Shaw Island, WA) in the eyelid adipose tissue (Kincaid and Calkins, 1992). Tanks received 4 L/min of air-saturated water, and each tank was fitted with a fine pore diffuser connected to the laboratory compressed air supply to ensure high levels of dissolved oxygen. Fish were fed twice daily (0900 h and 1700 h) by hand to apparent satiation, which we defined as the point at which fish no longer actively responded to continued offerings of small portions of food. Tanks were fed chronologically, starting at a randomly selected tank at each feeding event.

At the start of the experiment and at three-week intervals thereafter, fish were fasted for 36 hours, rapidly anesthetized using MS-222 (100 mg/L, buffered to neutral pH with $NaHCO_3$), weighed (g) and measured (SL, FL, TL; mm). The overall feeding trial lasted 88 d. Laboratory lighting mimicked a natural photoperiod at 40.5853°N. All animal care and handling protocols were approved by the Colorado State University Institutional Animal Care and Use Committee (Protocol number 10-2318A) and personnel underwent the training required by the committee.

2.2. Growth Metrics

Growth metrics of interest were final wet weight, feed conversion ratio (FCR), and specific growth rate (SGR); the latter two were calculated using the following equations (Bureau et al., 2002, Stickney, 2005):

Feed conversion ratio (FCR) = $\frac{g\ dry\ feed\ fed}{g\ wet\ weight\ gain}$

Specific growth rate (SGR) = $\frac{(lnY_2 - lnY_1)}{(t_2 - t_1)} \times 100$,

where Y_1 and Y_2 represent weights at time 1 and 2, and t_1 and t_2 represent time 1 and time 2, respectively (Bureau et al., 2002). While final wet weight and specific growth rate were tracked on an individual fish level, mean per-tank values were used for all statistical analyses, as each tank represented one replicate per treatment.

2.3. Proximate Analyses

Proximate analyses were completed to determine overall body composition of fish consuming the experimental feeds. At the end of the trial, subsamples of 3 fish per treatment were euthanized, flash frozen, and held at -20°C until shipment to the BFTC for analysis using standard protocols (AOAC, 1995). The analyses provided data on wet biomass moisture content (% wet weight), wet biomass energy content (cal/g), wet biomass fat content (% wet weight), and wet biomass protein content (% wet weight). The BFTC also analyzed dry biomass energy content (cal/g), dry biomass fat content (% dry weight), and dry biomass protein content (% dry weight). Data for these analyses were grouped by tank.

2.4 Statistical Analyses

Two-way ANOVA was used to determine the effect of feed group, protein:lipid ratio, and their interaction for all growth metrics and body composition metrics. Tukey's HSD was used for all pairwise comparisons of means (Tukey, 1953, Kramer, 1956). Mean per-tank values of all

metrics were used for statistical analyses. Statistical analyses were run in SAS 9.2 or JMP 9.02 (SAS Institute Inc., Cary, North Carolina, USA).

3. Results

3.1 Feed Consumption

Estimated feed consumption rates varied between 4.47 (PFP) and 3.80 $g \cdot fish^{-1} \cdot d^{-1}$ (PPD) (Table 1.2). Because feed consumption rates of individual fish were not directly measured, these values are only approximations, and no statistical analyses were performed.

3.2 Wet Weight

Mean final wet weights ranged from 382 g (PPD) to 441 g (FMD+) (Table 1.2). Feed group was a statistically significant predictor variable (DF=4, F=4.70, P=0.0002) with fish fed the FMD+ producing the highest mean weight, followed by the PFP+ (429.9 g) and FMD (423.9 g). Many of the means were not significantly different from each other, indicating that the performance of fish consuming these feeds was similar among many of the diets. P:L ratio, conversely, did not have a significant effect on final wet weight (DF=1, F>0.0001, P=0.9973), and the interaction between protein:lipid ratio × feed group was also not statistically significant (DF=4, F=0.96, P=0.4533).

3.3 Feed Conversion Ratio

Feed conversion ratios among feeds were generally low, ranging from 0.93 (FMD+) to 1.24 (NPD+) (Table 1.2). Feed group significantly affected FCR (DF=4,F=7.02, P=0.0011), while protein:lipid ratio (DF=1, F=0.004, P=0.9489) and the feed group × protein:lipid ratio interaction (DF=4, F=0.27, P=0.8938) did not. Fish fed the FMD+ and APD+ (0.95) had the lowest FCR values, with most mean FCR values being statistically indistinguishable.

3.4 Specific Growth Rate

Specific growth rates were significantly affected by feed group (DF= 4, F=9.38, P=0.0002), with the highest SGR observed in the FMD+ (1.82) and FMD (1.80) treatments (Table 1.2), while the PPD (1.66) and NPD (1.66) feeds had the lowest SGR. The protein:lipid

ratio (DF=1, F>0.001, P=0.9973), and the interaction between feed group and protein:lipid ratio (DF=4, F=9.551, P=0.4533) were not statistically significant.

3.5 Wet biomass moisture content

Wet biomass moisture content of fish at the end of our study ranged from 59.5% (PFP) to 63.7% (NPD+) of total wet body weight (Table 1.3). No statistically significant relationships were found between feed group (DF=4, F=2.615, P=0.0660), the protein:lipid ratio (DF=1, F=2.655, P=0.1189), and the interaction between these two terms (DF=4, F=2.279, P= 0.0967).

3.6 Wet biomass energy content

Mean energy contents range from 2723 (PFP+) to 2999 (PFP) cal/g (Table 1.3). Feed group (DF=4, F=0.2960, P=0.8771), protein:lipid ratio (DF=1, F=2.930, P=0.1024), and their interaction (DF=4, F=0.9775, P=0.4419) were all not significant predictor variables. Mean energy content for all 45% protein feeds was 2883 cal·g, while mean content for all 40% protein feeds was 2765 $cal \cdot g^{-1}$. Mean energy content for fish consuming all feeds can be found in Table 3.

3.7 Wet biomass fat content

Wet fat content of fish at the conclusion of our study ranged from 17.21 (NPD+) to 22.47% (PFP) (Table 1.3). Feed group was not statistically significant (DF=4, F=2.556, P=0.0706). However, protein:lipid ratio (DF=1, F=11.130, P=0.0033) and the interaction between feed group and protein:lipid ratio (DF=4, F=3.482, P=0.0259) were significant. Fish consuming feeds formulated to the 45:20 protein:lipid ratio had a mean wet fat content of 20.4%, whereas fish consuming feeds formulated to the 40:20 protein lipid ratio had a mean of 18.4%.

3.8 Wet biomass protein content

Mean wet protein contents of fish from our study ranged from 14.15% (PPD) to 16.16% (PPD+) (Table 1.3). Feed group (DF= 4, F=0.486, P=0.7461) and the feed group and

protein:lipid ratio interaction (DF=4, F=0.864, P=0.5024) were not significant. However, protein:lipid ratio was significant (DF=1, F=9.680, P=0.0055). Mean protein content of fish consuming feeds formulated to a 45:20 protein lipid ratio was 14.8%, while those consuming the feeds formulated to a 40:20 protein lipid ratio had a wet protein level of 15.7%.

3.9 Dry biomass energy content

Mean dry biomass energy contents of fish at the conclusion of our study ranged from 7161 $cal \cdot g^{-1}$ (PFP+) to 7697 $cal \cdot g^{-1}$ (NPD) (Table 1.3). Feed group (DF=4, F=0.384, P=0.8177) protein:lipid ratio (DF=1, F=0.577, P=0.4565), and the interaction between these two variables (DF=4, F=0.347, P=0.843) all lacked statistical significance at the α=0.05 level.

3.10 Dry biomass fat content

Dry biomass fat content of fish consuming the feeds used in our trial ranged from 46.9% (NPD+) to 56.5% (APD) (Table 1.3). Feed group (DF=4, F=2.207, P=0.1049) was not a statistically significant variable. However, protein:lipid ratio (DF=1, F=20.215, P=0.0002) and the interaction between feed group and protein:lipid ratio (DF=4, F=3.736, P=0.0199) were both significant. Fish consuming feeds formulated to the 45:20 protein lipid ratio had a higher dry fat content (53.0%) than those consuming the 40:20 P:L ratio feeds (49.0% mean dry fat content).

3.11 Dry biomass protein content

Mean dry biomass protein contents of fish at the conclusion of our trail ranged from 36.6% (PFP) to 44.3% (PPD+). Feed group (DF=4, F=1.349, P=0.2866) and the feed group and protein:lipid ratio interaction (DF=4, F=2.139, P=0.1135) were not statistically significant. The protein:lipid ratio of a given feed was significant (DF=1, F=17.891, P=0.0004), with fish consuming feeds formulated to a 45:20 level having a lower mean dry protein content (38.8%) than those consuming the 40:20 protein:lipid ratio diets (mean 42.4%, Table 1.3).

Table 1.2. Summary of the effects of diet type and protein:lipid ratio on fish size, mean daily food consumption rate, feed conversion rate, and SGR for rainbow trout. Values represent means (± S. E. M.) of the experimental diets. Values with different superscripted letters within a column denote statistically significant differences ($P < 0.05$).

Diet Code	Protein:Lipid Ratio[v]	Feed Consumed[w]	Final Wet Weight[x]	Feed Conversion Ratio[y]	Specific Growth Rate[z]
APD+	40:20	3.83	400.7 ± 13.2^{ab}	0.95 ± 0.00^{b}	1.69 ± 0.03^{ab}
APD	45:20	4.27	423.4 ± 8.8^{ab}	1.01 ± 0.01^{ab}	1.74 ± 0.03^{ab}
FMD+	40:20	4.15	440.6 ± 5.7^{a}	0.93 ± 0.02^{b}	1.82 ± 0.05^{a}
FMD	45:20	4.05	423.9 ± 6.7^{ab}	0.96 ± 0.03^{ab}	1.80 ± 0.02^{a}
NPD+	40:20	4.22	398.7 ± 16.1^{ab}	1.24 ± 0.10^{a}	1.65 ± 0.02^{b}
NPD	45:20	4.34	390.9 ± 5.8^{ab}	1.19 ± 0.04^{ab}	1.66 ± 0.01^{b}
PFP+	40:20	3.94	429.9 ± 16.6^{ab}	1.09 ± 0.11^{ab}	1.72 ± 0.02^{ab}
PFP	45:20	4.47	422.3 ± 15.4^{ab}	1.09 ± 0.05^{ab}	1.73 ± 0.01^{ab}
PPD+	40:20	4.06	422.6 ± 14.6^{ab}	1.03 ± 0.07^{ab}	1.71 ± 0.02^{ab}
PPD	45:20	3.8	382.3 ± 2.7^{b}	1.00 ± 0.01^{ab}	1.66 ± 0.02^{b}

[v] Values listed represent % diet protein: % diet lipid, by weight
[w] Values listed represent estimated g consumed $fish^{-1} \cdot day^{-1}$.
[x] Values listed represent final biomass (g).
[y] Values listed represent g biomass accumulated per gram of feed consumed (by tank).
[z] Values listed represent % biomass accumulated $fish^{-1} \cdot day^{-1}$.

Table 1.3. Effects of diet type and protein:lipid ratio on the proximate compositions of rainbow trout fed specific diets over a 12-week period. Values are means ± S. E. M. Differing superscripted letters represent means found to be statistically different at the α=0.05 level using a Tukey-Kramer adjustment for multiple comparisons (ANOVA using Tukey HSD).

Feed Group	Wet Moisture[y]	Wet Energy[z]	Wet Fat[y]	Wet Protein[y]	Dry Energy[z]	Dry Fat[y]	Dry Protein[y]
APD+	63.72 ± 0.58^{a}	2781 ± 102^{a}	17.50 ± 0.76^{c}	15.27 ± 0.93^{a}	7667 ± 188^{a}	48.12 ± 1.33^{b}	42.20 ± 2.64^{abc}
APD	60.71 ± 0.96^{a}	2930 ± 52^{a}	22.23 ± 0.61^{ab}	14.71 ± 0.58^{a}	7454 ± 106^{a}	56.50 ± 0.54^{a}	37.53 ± 1.19^{bc}
FMD+	60.71 ± 0.81^{a}	2907 ± 49^{a}	20.54 ± 0.77^{abc}	15.81 ± 0.25^{a}	7413 ± 38^{a}	52.01 ± 0.95^{ab}	40.36 ± 0.41^{abc}
FMD	62.76 ± 0.88^{a}	2786 ± 94^{a}	18.57 ± 0.63^{abc}	15.47 ± 0.57^{a}	7487 ± 283^{a}	49.76 ± 0.63^{ab}	41.61 ± 0.51^{abc}
NPD+	63.63 ± 1.54^{a}	2689 ± 92^{a}	17.21 ± 1.77^{c}	15.75 ± 0.15^{a}	7413 ± 98^{a}	46.93 ± 2.83^{b}	43.59 ± 1.87^{ab}
NPD	62.36 ± 1.46^{a}	2898 ± 178^{a}	19.57 ± 1.49^{abc}	14.83 ± 0.26^{a}	7697 ± 173^{a}	51.63 ± 2.11^{ab}	39.61 ± 0.94^{abc}
PFP+	61.79 ± 0.54^{a}	2723 ± 160^{a}	19.34 ± 0.55^{abc}	15.71 ± 0.51^{a}	7161 ± 487^{a}	50.23 ± 0.73^{ab}	41.48 ± 1.10^{abc}
PFP	59.46 ± 0.52^{a}	2999 ± 164^{a}	22.47 ± 0.86^{a}	14.71 ± 0.48^{a}	7447 ± 392^{a}	55.23 ± 1.45^{a}	36.59 ± 1.22^{c}
PPD+	63.41 ± 0.66^{a}	2729 ± 35^{a}	17.55 ± 0.40^{bc}	16.16 ± 0.34^{a}	7454 ± 183^{a}	47.77 ± 0.90^{b}	44.30 ± 0.30^{a}
PPD	63.18 ± 0.64^{a}	2805± 31^{a}	19.25 ± 0.63^{abc}	14.15 ± 0.33^{a}	7617 ± 139^{a}	52.08± 0.86^{ab}	38.61 ± 1.44^{abc}

[y] Values listed are % biomass ± S. E. M.
[z] Values listed are calories gram^{-1} ± S. E. M.

4. Discussion

The value of my experiment is its simultaneous evaluation of numerous experimental diets for rainbow trout, formulated from a wide range of possible plant and animal-based protein components. Other studies have often focused on one promising protein source (e.g. soy protein concentrate) at increasing levels of replacement of fish meal (sometimes up to 100% replacement). Instead, we only evaluated feeds that were formulated to 100% fishmeal replacement, and compared them to our two fishmeal-based control diets, to ascertain the viability of alternative proteins in diets devoid of fish meal. We observed a great deal of statistical overlap among growth metrics of the rainbow trout receiving different treatments in our feeding trial. This suggests that diet ingredient selection criteria should perhaps be more heavily influenced by the current market prices of the ingredients, as long as these ingredients are used carefully to formulate a nutritionally-balanced feed.

4.1 Growth metrics

Feed group formulation significantly affected all of the growth metrics examined (final wet weight, SGR, FCR), with the fishmeal-based (control) diets producing the highest mean SGR (1.80 and 1.82 for FMD and FMD+ diets respectively) the lowest mean FCR (0.96 and 0.93 for FMD and FMD+ respectively), and two of the highest final mean wet weights (423.9 g, and 440.6 g for FMD and FMD+, respectively). However, there was notable statistical overlap among all feed groups in many of the examined growth metrics (Table 1.2), suggesting that carefully-formulated feeds using animal and plant-based proteins can promote growth rates similar to those observed in rainbow trout consuming traditional, fishmeal-based diets.

Results observed in my study support those of previous research where growth performance is comparable to or slightly reduced in using plant-based diets (Burr et al., 2012,

Wacyk et al., 2012). Burr et al. (2012) noted that rainbow trout fed diets in which soy protein concentrate replaced fish meal (at levels of replacement of 63 and 82%), grew at similar rates to those fed a fishmeal-based diet (at levels of replacement of 63 and 82%). Fish fed on the 100% replacement diet experienced reduced growth. Similarly, Wacyk et al. (2012) noted reduced growth rates in trout consuming diets where 100% of fish meal was replaced with soy protein isolate and corn gluten, but comparable growth rates among those consuming the fish meal-based control diet and those in which small portions (8.4%, 20.2%) of the fish meal component was replaced with corn gluten. FCR values for fish raised in the feeding trial conducted by Wacyk et al. (2012) ranged between 1.2 and 1.5, whereas those observed in my feeding trials were slightly better, ranging from 0.93 (FMD+ diet) to 1.24 (NPD+ diet). Fish in the study by Wacyk et al. (2012) were smaller at the start of the study than our fish were initially, and ranged from a final mean weight of 82 $g \cdot fish^{-1}$ to 158 $g \cdot fish^{-1}$, depending upon treatment. Fish in our experiment attained a final mean weight of 382 $g \cdot fish^{-1}$ to 441 $g \cdot fish^{-1}$, depending upon diet. Caution should be used when making direct comparisons of growth rates between the two studies because younger fish typically have higher growth rates than older, larger fish (Fiogbe and Kestemont, 2003). It is also possible that some other factor, such as a strain-level effect, may be operating in this case, as certain strains of rainbow trout have been found to respond more favorably to different diet ingredients (Overturf et al., 2013).

Soybean meal, a readily available and relatively inexpensive protein source, has been associated with reduced growth in fish due to numerous ANF, including protease inhibitors, phytic acid, and oligosaccharides (Gatlin et al., 2007), and can lead to altered protein synthesis rates and increased catabolism of proteins (Sealey et al., 2009). Other plant-based ingredients, including cottonseed meal, canola, and barley, are also known to contain ANF, or be otherwise

limited in their nutritional profile. These deficiencies likely contributed to the reduced SGR values in the fish consuming the PPD, NPD, and NPD+ diets in our study, and in other studies where these ingredients were examined at high levels of fishmeal replacement. Interestingly, a recent study by Collins et al. (2012) examined the effects of diets formulated from mixtures including pea meal, pea protein concentrate, soybean meal, soy protein concentrate, canola meal, and canola protein concentrate at increasing levels of replacement (0 to 300 $g \cdot kg^{-1}$, at 75 $g \cdot kg^{-1}$ increments) for fishmeal and meat and bone meal. Among the diets included in the study, only the inclusion of soybean meal and canola meal led to reduced growth rates (Collins et al., 2012). This suggests that "concentrate" type protein ingredients may perform better than "meal" type ingredients, possibly due to lower concentrations of antinutritional factors and higher protein contents (Gatlin et al., 2007).

The results observed in our feeding trial suggest that plant-based feeds for rainbow trout, when formulated from multiple plant protein sources, with overall rainbow trout nutrient requirements and amino acid ratios in mind, have the potential to elicit high growth rates, comparable to standard commercial diets. Barrows et al. (2007) compared the performance of rainbow trout consuming 6 experimental diets containing either fish meal and barley meal, plant concentrates, or plant meals, each at a high nutrient formulation (48:18 P:L) or low nutrient formulation (43:13 P:L), against a standard fishmeal-based commercial diet (46:16 P:L). At the conclusion of this 12-wk study, the results were similar to those observed in our study. The standard fishmeal control diet produced the highest SGR (2.67 $g \cdot fish^{-1} \cdot d^{-1}$), and lowest FCR (0.88 g feed fed$\cdot g^{-1}$ weight gain) (Barrows et al., 2007). Interestingly, several of the test diets produced mean growth metrics that were statistically indistinguishable from the commercial diets. SGR values for high nutrient plant meals and both high and low nutrient fish and barley

meals (2.35, 2.6, and 2.3 g·fish^{-1} d^{-1}, respectively) were statistically indistinguishable from the control diet (Barrows et al., 2007). Similarly, FCR values for all three high nutrient experimental diets (0.84, 0.95, and 0.98 g feed fed·g^{-1} weight gain for fish and barley meal, plant concentrates and plant meals, respectively), and the low nutrient fish and barley meal diet (0.93 g·fish^{-1} d^{-1}) were statistically indistinguishable from the control diet. With further study and fine-tuning of formulations, and the use of strains of rainbow trout selectively bred to perform well on such diets (Overturf et al., 2013), it is likely that alternative protein-based aquafeeds for rainbow trout will elicit growth responses identical to those elicited by fishmeal-based diets.

Evaluations of animal-based aquafeeds for rainbow trout have also shown mixed results. Lee et al. (2001) evaluated trout consuming diets with high levels of a mix of leather meal, meat and bone meal, feather meal, squid liver powder, poultry by-product meal, and blood meal, in replacement of fish meal at graded levels (0, 20, 40, 60, and 100%). These investigators noted reduced weight gain (734% to 1275% mean weight gain over 16 weeks for feeds at 40%, 60%, and 100% levels of fish meal replacement, respectively) and SGR (2.34 to 1.89% mean biomass increase per day for the same feeds as above) of trout consuming these feeds (Lee et al., 2001). For comparison, fish consuming a standard fishmeal based reference diet increased in weight by 1370% and had a mean SGR value of 2.4 (Lee et al., 2001). Our study found similar results in that growth performance of rainbow trout consuming the APD ingredient based diets was slightly reduced but not statistically different than the trout consuming the control diets. Similarly, Yamamoto et al. (1995) noted slightly decreased growth and feed efficiency in fish consuming feeds containing (either singularly or in combination, at 60% or 80% levels of fish meal replacement) the following ingredients: soybean meal, malt protein flour, corn gluten meal, meat meal, and dried brewers' yeast. However, Yamamoto et al. (1995) recorded weight gains

that were statistically indistinguishable in fish consuming the fish meal control diet to those observed in fish consuming the soybean meal and malt protein flour diet at 60% replacement, the soybean meal and corn gluten meal diet at 60% replacement, the meat meal and malt protein flour diet at 60% replacement, and the soybean meal, meat meal, malt protein flour, and dried brewers' yeast feed at 80% replacement (Yamamoto et al., 1995). Weight gains for the 6-week trial were 265%, 268%, 269%, and 262%, respectively for the experimental feeds listed above, and 282% for the fishmeal control diet.

Interestingly, in our study growth metrics were not consistently influenced by the two feed formulation recommendations. Hardy (2002) recommends that practical diets for rainbow trout reared in freshwater be formulated to roughly 43% protein and 28% lipid, and further suggests that the protein components in the diets be reduced as the fish grow, from 45-50% for fry, then 42-48% in grower diets, to 35-40% in broodstock diets. Fat content recommendations were listed as 16-18% for fry, 20-24% for grower diets, and 14-16% for broodstock diets (Hardy, 2002). In our study, these recommendations were represented by diets formulated to a 45:20 P:L ratio. The National Research Council (1993) recommends slightly different proportions of fat and protein in rainbow trout feeds, endorsing dietary levels of 40% protein on an as-fed basis, with 20% lipid to ensure adequate energy availability for growth. Ideally, if one were using fishmeal as the protein component, it would be advantageous to reduce the overall amount of this expensive ingredient within the feed, while still maintaining high growth rates in the fish that consume it. Thus, financially it makes sense to use a more economical, lower protein diet. Furthermore, reduced protein contents in the feed may reduce nitrogenous and phosphorous wastes expelled by fish into ponds and raceways, especially when high-phosphorous ingredients (e.g. menhaden, herring, and anchovy fishmeal; Hardy, 2002) are used as a source of protein in

these feeds (Cho et al., 1994). High levels of these nutrients, especially when the phosphorous to nitrogen ratio is high, can have possible negative impacts (e.g. eutrophication) on waters downstream of culture facilities (Hardy, 2002). Many plant-based ingredients have lower overall phosphorous contents, allowing for the formulation of low-phosphorous feeds, which may be utilized to limit nutrient levels in wastewater discharges or settling ponds associated with culture facilities (Cho et al., 1994, Hardy, 2002).

4.2 Proximate analyses

Of the variables analyzed, P:L ratio of the feed consumed consistently affected the results of the proximate analyses, with the exceptions of wet biomass moisture content and dry biomass energy content. In general, rainbow trout fed diets formulated to the 45:20 protein:lipid ratio had higher wet energy contents (per gram) than did those fish consuming the diets formulated to the 40:20 ratio (2884 $cal \cdot g^{-1}$ mean wet biomass for the 45:20 diets; 2765 $cal \cdot g^{-1}$ mean wet biomass for the 40:20 diets), possibly due to the higher energy densities of the increased fat content generally found in fish consuming the 45:20 P:L diets (Table 1.3). The FMD feed group fed fish were a notable exception to this trend (2786 and 2907 $cal \cdot g^{-1}$ for fish consuming the FMD and FMD+ diets, respectively). Furthermore, fish fed the 45:20 diets generally had higher wet fat and lower wet protein contents when compared to the 40:20 diets. The same pattern was generally true for the metrics examining dry biomass, as fish consuming feeds formulated to the 45:20 protein lipid ratio generally had higher dry fat percentages and lower dry protein percentages than their 40:20 diet consuming counterparts. Interestingly, the proportions of fat and protein in the bodies of these fish were reversed in relation to the diets they consumed; i.e., fish on the higher protein diet stored less energy as protein and more as fat, and vice versa for the lower protein diet. These effects, termed “protein sparing,” and, “lipid sparing” i.e., an increase in the anabolic storage of protein when dietary lipid levels are increased, and a similar increase in the

storage of lipid when protein is increased, has been described by previous investigators (Beamish and Medland, 1986).

Final biomass protein and lipid levels observed in our study are somewhat unique, in that the final dry biomass fat contents (range: 46.9% to 56.5% biomass dry weight) were notably higher and final dry biomass protein contents (range: 36.6% to 44.3% biomass dry weight) were notably lower than those observed in other studies of plant-based proteins (Barrows et. al., 2008; Barrows et al., 2007; Yamamoto et al., 2012; Yamamoto et al., 1995). While this may partially represent a function of fish size at the onset of the trial, as those in our study were larger (91.3 g mean initial weight) than those in the studies listed above (mean initial weights of 4.8 g, 38 g, 15.7 g, 9.4 g, respectively), the size of the tank in which the fish were housed could have influenced this metric as well. While our 65-L aquaria were smaller than the 150-L aquaria used in the studies by Barrows et al. (2008, 2007), they were larger than the 60-L and 20-L tanks used by Yamamoto et al. (2012, 1995), suggesting that overall tank volume was not the primary cause of our unique results. Stocking density may be an additional influential factor, as our mean stocking density (21 g fish$\cdot L^{-1}$) was higher than initial stocking densities in the studies listed above (1, 7, 11, and 14 g fish$\cdot L^{-1}$, respectively). At our higher densities, our fish may have had reduced mobility within the tanks, decreasing the opportunities for voluntary movement, and thus leading to higher levels of fat storage and reduced muscle (protein) development.

It should be noted that a small numerical reduction in SGR and FCR of fish consuming plant and animal-based diets, instead of fish-based diets, when extended over a long grow-out period and extrapolated to a large number of trout within a production facility, could lead to notable differences in growth rates, total feed consumed during the production period, and, ultimately profit margins. Formal cost-benefit analyses are needed to determine break-even

points concerning the money saved by these less-expensive feeds versus the increased overall food consumption and fish care required during the extended grow out period. Our data show, however, that feeds formulated using plant and animal-based proteins using current knowledge of the nutritional requirements of rainbow trout produce favorable growth rates and should be considered for use by aquaculturists due to their economic benefits.

As a simplistic example, recent market prices (March 2014) of fish meal, soybean meal, unprocessed corn, and unprocessed wheat, were U.S. $1,622, $495, $220, and $319 per metric ton, respectively (Indexmundi, 2014). Costs of fish feeds are the largest variable expense to the producer, so if a feed were to have all fish meal replaced by a combination of the above ingredients, at a hypothetical cost of U.S. $345 per metric ton (the mean cost of the plant ingredients listed above), the feed manufacturer would be saving roughly 79% when compared to a fish meal-based diet, with comparable or slightly reduced growth rates. While slower growth rates would increase the amount of time fish would spend at the fish culture facility, the decreased feed cost would likely more than offset the increased costs of fish care during the extra weeks or months needed to grow the fish to market size. Admittedly, fish diet formulation is much more complex than this example. However, significant monetary savings can be accrued when large portions of fishmeal are replaced with less expensive plant and animal based ingredients. Diets containing alternative protein sources, like those examined in our trial, should be strongly considered for further utilization in rainbow trout production facilities due to these economic benefits.

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Chapter 2 - An evaluation of the growth of rainbow trout fed plant-based feeds at graded lysine levels

1. Introduction

Fish, like many animals, require an adequate intake of protein for optimum health and growth. When consumed, protein is broken down into amino acids, which are transported throughout a fish's body, and absorbed and used by various tissues and organs for the replacement of existing proteins as well as for growth (Wilson, 2002). An inadequate supply of protein can reduce growth rates, while an overabundance of protein in the diet will not further increase growth rates, and result in the excess protein being used as a source of energy (Wilson, 2002). Various investigators have suggested that protein contents (% of diet) should range from roughly 40% (Hardy, 2002) to 45% (NRC, 1993) in practical rainbow trout diets. Additionally, balanced ratios of the 10 essential amino acids in a given diet are required to ensure optimum growth rates are achieved, as a deficiency in any of these amino acids will negatively affect fish growth and health (Wilson, 2002).

As was succinctly summarized by Wilson (2002), pioneering work by Halver (1957) with juvenile Chinook salmon (*Oncorhynchus tshawytscha*) identified the following ten amino acids as essential for growth: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. These same 10 amino acids are generally considered "essential" for all species since studied (Kitagima and Fracalossi, 2011, Lee and Bai, 1997, Rawles et al., 2013, Wilson, 2002). The essential amino acids cannot be synthesized by fish in adequate quantities to support favorable growth rates, and must therefore be provided in their feeds (Wilson, 2002).

Lysine is generally the first limiting amino acid in formulated finfish diets (Robinson et al., 1980). For cultured rainbow trout (*O. mykiss*), lysine requirements have been reported to range from 1.3% (Kim et al., 1992) to 2.9% of the total diet formulation (Ketola, 1983) when examined in feeding trials using diets formulated from the same ingredients containing graded levels of lysine.

Fish meal, generally produced from processed marine fishes such as anchovies (family: Engraulidae) or menhaden (*Brevoortia* spp., family Clupeidae), is the most common source of protein in aquafeeds for finfish. Recently, in part catalyzed by an increase in the price of fish meal, researchers have sought to find less expensive plant or animal-based proteins for use in finfish diets that will still promote high growth rates and be nutritionally well-rounded. To be viable alternatives to fish meal alternative sources of protein must possess amino acid profiles approximating the amino acid requirements of the fish consuming them or must be combined with amino acid supplements.

Slight amino acid deficiencies can be compensated for by including supplements during the feed production process to ensure that minimum amino acid requirements are met. Prior studies have shown that this can be an effective means of producing a nutritionally-sound plant-based diet for finfish (Gaylord and Barrows, 2009, Yamamoto et al., 2012). In addition to meeting minimum requirements, it is important to establish the upper limits of amino acid supplementation, because over-supplementation would be an additional and unnecessary cost to the aquaculturist (Wilson, 2002). If possible, the ideal range of amino acid supplementation would be determined in feeding trials using a diet formulated from the same ingredients to be used in the actual diet. This helps ensure that possible amino acid interactions (Kaushik and Fauconneau, 1984) or the presence of antinutrients (Hardy, 2010) do not reduce the biological

availability of a given amino acid within the practical diet or negatively affect the growth of fish consuming them. For example, Kaushik and Fauconneau (1984) demonstrated a lysine-arginine interaction, but a subsequent study by different investigators failed to show a reduction in the growth of rainbow trout due to this interaction (Kim et al., 1992).

The purpose of our study was to evaluate the minimum lysine requirement of juvenile rainbow trout consuming plant-based diets formulated to contain seven graded lysine levels during a 12-week growth trial. Determination of these requirements is a necessary step in the formulation of cost-effective feeds that promote high growth rates and low feed conversion ratios in rainbow trout. Such feeds could also provide aquaculturists an alternative to fish meal-based diets. Furthermore, we sought to compare our observed optimum lysine values with those determined by other studies examining rainbow trout. Rainbow trout are highly adaptable to culture conditions and are, in fact, the most commonly raised salmonid in the world (Hardy, 2002). They are also considered a healthy addition to the human diet; the American Heart Association recommends each person consume two servings of fatty fish, such as trout, twice a week, as it is low in saturated fat, high in protein, and high in omega-3 fatty acids which have desirable impacts on the human cardiovascular system (AHA, 2010). In 2012, a total of 54 million trout, ranging in size from 1-12", were sold in the U.S., with a value of 79.7 million U.S. dollars (NASS, 2013).

2. Materials and Methods

2.1 Experimental Design and Diets

Eyed rainbow trout eggs (Fall steelhead strain) were received from Trout Lodge, Inc. (Sumner, Washington, USA) on 11 December 2011 and transported to the Foothills Fisheries Laboratory (Foothills Campus, Colorado State University, Fort Collins, Colorado). Eggs were incubated at 10° C, and were maintained at this temperature until hatching (28 December 2011). After swim-up, fry were fed appropriately sized commercial diets (Trout and Salmon feed, Skretting USA, Tooele, Utah) until the initiation of the feeding trial. The grow-out period lasted 37 weeks, allowing the trout to reach an initial weight of 95.5 ± 0.5 g (mean ± S. E. M.) During this grow-out period, temperatures were gradually increased to 15 ± 1° C and maintained here for the duration of the feeding trial. At the initiation of the trial, twenty-eight 65-L aquaria were stocked with 14-16 rainbow trout. Each of fish was marked with a unique visual implant alphanumeric (VI-alpha) tag (Northwest Marine Technologies, Shaw Island, WA) in the eyelid adipose tissue (Kincaid and Caulkins, 1992) to allow tracking of individual growth rates. Tanks were supplied with 4 $L \cdot min^{-1}$ of air-saturated water via a fine-pore diffuser connected to the laboratory air compressor to maintain near-saturation levels of dissolved oxygen. Fish were fed twice daily (0700 h and 1700 h) by hand to apparent satiation. Feeding started at a randomly selected tank at each feeding event and progressed chronologically until all tanks of fish had been fed.

At the start of the 90-d feeding trial (19 September 2012) and at three week intervals until the completion of the study (8 December 2012) fish were fasted for 36 h, anesthetized using MS-222 (100 $mg \cdot L^{-1}$, buffered to neutral pH using $NaHCO_3$), weighed (g) and measured (SL, FL, TL; mm). Lighting within the laboratory mimicked a natural photoperiod at 40.5853° N. All

animal care and handling protocols were approved by the Colorado State University Institutional Animal Care and Use Committee (Protocol number 10-2318A).

Seven experimental diets were formulated to graded lysine levels, ranging from 1.35 to 4.31 g lysine·g diet dry weight^{-1} (Table 2.1). These diets were randomly assigned to a total of 4 tanks per diet treatment. The diets were prepared using the same set of ingredients used in the Plant Products Diet (PPD, Chapter 1) by an interagency team (FWS, USDA) at the U.S. Fish and Wildlife Service Bozeman Fish Technology Center (BFTC, Bozeman, Montana, USA). All diets were formulated to a 40:20 P:L ratio, and were approximately isocalorific (range: 4604 to 4722 cal/g). In a previous study, diets formulated from these ingredients produced favorable growth rates in rainbow trout consuming them (G. Gaylord, U.S.F.W.S., Personal Communication). Researchers at Colorado State University conducting the lysine study were unaware of the lysine level of each diet being used until completion of the study (single blind study design).

Table 2.1. Lysine levels (g lysine·100 g of dry diet^{-1}) for diets used in this study.

Diet #	Lysine content (g·100 g dry diet^{-1})	Caloric Content (cal/g)
1	1.35	4722
2	1.85	4702
3	2.34	4682
4	2.83	4663
5	3.32	4644
6	3.82	4624
7	4.31	4604

2.2 Growth Metrics

Rainbow trout performance on the diets was quantified by comparing lysine effects on final wet weight, feed conversion ratio (FCR), and specific growth rate (SGR). FCR and SGR were calculated using the following equations: (Bureau et al., 2002, Stickney, 2005):

Feed conversion ratio (FCR) = $\frac{g\ dry\ feed\ fed}{g\ wet\ weight\ gain}$

Specific growth rate (SGR) = $\frac{(lnY_2 - lnY_1)}{(t_2 - t_1)} \times 100$,

where Y_1 and Y_2 represent weights at time 1 and 2, and t_1 and t_2 represent time 1 and time 2, respectively (Bureau et al., 2002). While fish growth was tracked at the individual fish level, metrics were averaged by tank, as this was the level at which each treatment was applied. .

2.3 Proximate Analyses

Proximate analyses were conducted to determine overall body composition of fish receiving each treatment at the conclusion of the study. A subsample of three fish per tank was euthanized, flash frozen, and held at -20° C before being shipped to the BFTC. There, fish were processed and analyzed using standard protocols (AOAC, 1995). Fish were grouped by tank before processing, giving an average value of the following metrics per tank, and giving 4 replicates per diet treatment. These analyses provided data on wet biomass moisture content (% wet weight), wet biomass energy content ($cal \cdot g^{-1}$), wet biomass fat content (% wet weight), and wet biomass protein content (% wet weight), dry biomass energy content ($cal \cdot g^{-1}$), dry biomass fat content (% dry weight), and dry biomass protein content (% dry weight).

2.4 Statistical Analyses

The effect of lysine level on the all growth metrics and body composition variables were compared using linear regression. Tank level means of all values were used for statistical analysis. Specific growth rate and feed conversion ratio were each fit with a 2nd order polynomial

equation, while all other variables were fit with a linear equation. Statistical analyses were run in JMP Pro 10.

3. Results

3.1 Feed Consumption

Estimated overall mean feed consumption (g feed fish^{-1}) ranged from 3.7 (3.82 % lysine feed) to 4.3 (2.34% lysine feed) for the duration of the study (Table 2.2). These are only estimated consumption rates because we did not monitor these consumption levels directly at the individual fish level, and therefore no statistical analyses were conducted on this metric.

Table 2.2. Estimated mean feed consumption, mean final wet weight, mean feed conversion ratio, and mean specific growth rate of rainbow trout consuming plant-based diets formulated to graded lysine levels. Values represent means (± S. E. M.).

Diet Lysine Level[a]	Estimated Feed Consumption[b]	Final Wet Weight[c]	Feed Conversion Ratio[d]	Specific Growth Rate[e]
1.35	3.9	379.4± 6.2	1.2 ± 0.06	1.68 ± 0.02
1.85	3.9	391.0 ± 7.2	1.08 ± 0.02	1.71 ± 0.03
2.34	4.3	435.8 ± 3.7	1.00 ± 0.04	1.82 ± 0.01
2.83	3.8	405.5 ± 11.6	1.07 ± 0.05	1.76 ± 0.03
3.32	4.0	417.9 ± 8.4	1.03 ± 0.07	1.78 ± 0.02
3.82	3.7	399.7 ± 15.0	1.02 ± 0.07	1.72 ± 0.03
4.31	3.9	410.8 ± 6.0	1.03 ± 0.04	1.74 ± 0.03

[a] Values listed represent % of diet dry weight.
[b] Values listed represent g fish^{-1} day^{-1}.
[c] Values listed represent g fish^{-1} (± S. E. M.)
[d] Values listed represent g feed consumed·g biomass gained^{-1}.
[e] Values listed represent increase in body weight (%)· d^{-1}.

3.2 Final Wet Weight

Mean final wet weights ranged from 379 (1.35% lysine) to 436 g fish^{-1} (2.34% lysine) (Table 2.2). Lysine level did not significantly affect overall final tank biomass (DF=27, F=4.291, P=0.0250). The regression equation and R^2 value are shown in Table 2.4. Figure 2.1 represents the polynomial relationship between final wet weight and lysine percentage in the diet consumed.

3.3 Feed Conversion Ratio

The 2.34% lysine feed was the best performer, with a mean FCR of 1.00 (± 0.04) g feed g biomass gained^{-1}. Mean feed conversion ratios ranged from 1.00 (2.34% lysine diet) to 1.2 g feed consumed g biomass gained^{-1} (1.35% lysine diet) (Table 2.2). Lysine level did have a significant quadratic effect on FCR (DF=27, F=3.69, P=0.0396). The regression equation and R^2 value are shown in Table 2.4.

3.4 Specific Growth Rate

Mean SGR values ranged from 1.68 (± 0.02) for the 1.35% lysine diet to 1.82% (± 0.01) weight gain d^{-1} for the 2.34 lysine diet (Table 2.2). Fish consuming the 2.34% lysine diet exhibited the highest SGR. The relationship between lysine and SGR was statistically significant (DF=27, F=4.201, P=0.0267). The regression equation and R^2 value are listed in Table 2.4.

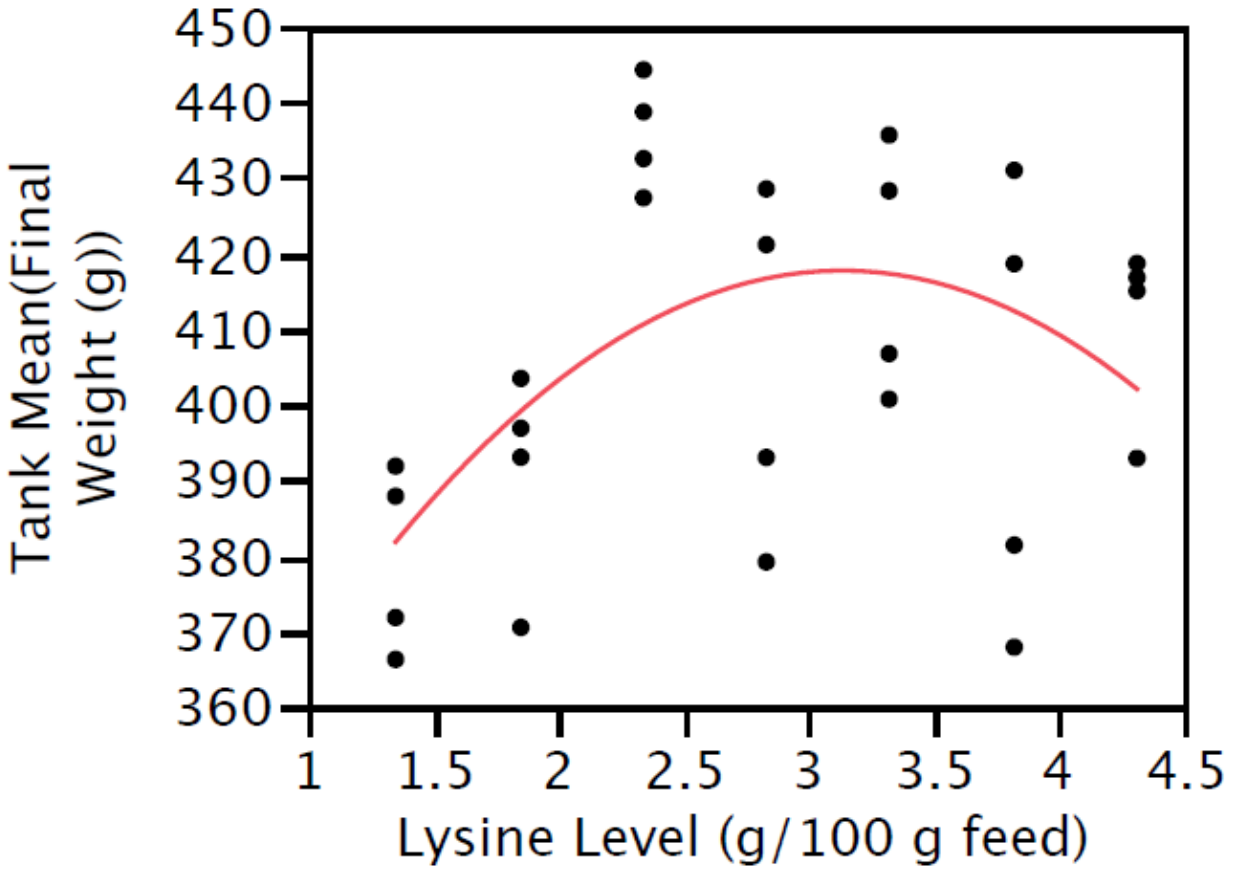

Figure 2.1. Relationship between lysine and mean final wet weight. The data were fit using a second-order polynomial ($y = -11.313 \times (\text{Lysine level} - 2.831)^2 + 6.808 \times \text{Lysine level} + 397.453$).

Table 2.3. Results of the proximate analyses of rainbow trout consuming diets formulated to graded lysine levels. Metrics examined include wet biomass energy content, fat content, protein content, and moisture content. Dry biomass energy content, fat content, and protein content were also examined. Values are means ± S. E. M.

Lysine Concentration (%)	% Moisture	% Fat-Wet	% Protein-Wet	Energy ($cal \cdot g^{-1}$)- Wet	% Fat-Dry	% Protein-Dry	Energy ($cal \cdot g^{-1}$)- Dry
1.35	63.0 ± 0.8	19.4 ± 0.9	14.7 ± 0.3	2792 ± 58	52.3 ± 1.3	39.9 ± 1.5	7558 ± 148
1.85	64.9 ± 0.6	17.1 ± 0.9	15.7 ± 0.3	2696 ± 47	48.6 ± 1.8	44.9 ± 1.4	7678 ± 47
2.34	63.9 ± 1.0	16.9 ± 1.0	16.2 ± 0.1	2653 ± 43	46.8 ± 1.6	45.0 ± 1.2	7353 ± 138
2.83	63.6 ± 0.5	16.6 ± 0.6	16.7 ± 0.1	2533 ± 48	45.5 ± 1.0	45.8 ± 0.5	6952 ± 146
3.32	64.9 ± 0.7	15.6 ± 0.6	16.7 ± 0.1	2540 ± 69	44.5 ± 0.9	47.5 ± 0.8	7241 ± 149
3.82	64.4 ± 0.7	16.3 ± 0.9	16.7 ± 0.5	2522 ± 31	45.6 ± 1.6	47.1 ± 2.1	7093 ± 88
4.31	63.5 ± 0.9	17.1 ± 0.9	17.1 ± 0.3	2559 ± 93	46.8 ± 1.5	47.1 ± 2.1	7014 ± 162

3.5 Wet biomass moisture content

Biomass moisture content was not related to dietary lysine level (DF= 27, F=0.36, P=0.56). Mean moisture contents ranged from 63.0 (± 0.8) for the 1.35% lysine diet, to 64.9% (1.85 and 3.32% lysine diets, with S. E. M. of 0.6 and 0.7, respectively, Table 2.3). Table 2.5 lists the regression equation and R^2 value.

Table 2.4. Regression equations for lines of best fit for FCR, SGR, and final wet weight. The regression expressions were in the form $y = ax^2 + bx + c$.

Growth Metric	ax^2	bx	c	P-Value	R-squared	DF
FCR	$0.038(L-2.83)^2$	-0.041(L)	1.141	0.0396*	0.23	27
SGR	$-0.033(L-2.83)^2$	0.012(L)	1.604	0.0267*	0.25	27
Final Wet Weight[b]	$-11.313(L-2.83)^2$	6.808(L)	397.453	0.0102*	0.26	27

Table 2.5. Linear regression equations calculated for each proximate analysis metric. The regression expressions are in the form $y = ax + b$.

Proximate Metric	a	b	P-Value	R-squared	DF
% Moisture	0.2	63.43	0.56	0.01	27
% Fat-Wet	-0.82	19.47	0.037*	0.16	27
% Protein- Wet	0.77	13.93	<0.0001*	0.58	27
% Fat- Dry	-1.99	53.16	0.0057*	0.26	27
% Protein- Dry	2.38	38.12	0.0009*	0.35	27

3.6 Wet biomass fat content

Wet biomass fat content was affected by dietary lysine level (DF=27, F=4.83, P=0.037), with increasing dietary lysine contents causing reduced lipid deposition (Table 2.3). Fish consuming the 1.35% lysine diet had the highest mean fat content, at 19.4% (± 0.9), while trout consuming the 3.32% lysine diet had the lowest (15.6 ± 0.6%). The regression equation and R^2 value are shown in Table 2.5.

3.7 Wet biomass protein content

Dietary lysine level had a highly significant effect on the wet biomass protein content of the rainbow trout (DF=27, F=35.30, P=<0.0001). Fish consuming diets higher in lysine generally had higher bodily protein contents (Table 2.3). We observed a range of mean protein contents from 14.7% (± 0.3, 1.35% lysine diet), to 17.1 (± 0.3, 4.31% lysine diet). Table 2.5 shows the regression equation and R^2 value for this metric.

3.8 Dry biomass fat content

Mean dry biomass fat contents of the trout observed in our study ranged from 44.5 % (± 0.9, 3.32% lysine diet), to 52.3% (± 1.3%, 1.35% lysine diet). Dry biomass fat content was strongly affected by diet lysine level (DF=27, F=9.10, P=0.006), with increasing lysine content generally causing decreased lipid storage within the fish (Table 2.3). The regression equation and R^2 value can be found in Table 2.5.

3.9 Dry biomass protein content

Increasing dietary lysine content was highly correlated with increased bodily protein percentages in the trout used in our study (DF=27, F=14.18, P=0.0009). The 3.32% lysine diet produced the highest mean dry biomass protein content (47.5 ± 0.8%), and the 1.35% diet caused the lowest (39.9 ± 1.5%, Table 2.3). Table 2.5 lists the regression equation and R^2 value for this metric.

4. Discussion

The results of my study clearly indicated that a dietary lysine level of 2.34% represents an optimum or near-optimum lysine level for rainbow trout consuming plant-based feeds. Fish consuming this diet exhibited both the highest SGR values and the lowest FCR values observed in our study, showing that our plant-based diet formulated to 2.34% lysine produced both the highest growth rates and was most efficiently utilized by the fish for tissue anabolism.

The 2.34% optimum observed in our study falls between the 1.3% optimum suggested by Kim et al. (1992) and the 2.9% optimum suggested by Ketola (1983). Mean final wet weights were generally higher among fish consuming diets with higher lysine levels (2.83 to 4.31%) than those of fish fed lower lysine diets (1.35 and 1.85%), although differences in means were not exceptionally large among mean specific growth rates (range: 1.68 to 1.78 for all diets except the 2.34% lysine diet) (Table 2.2). However, this small difference in FCR, when extended over a longer grow-out period with the large numbers of fish typical of a production facility, could have a large impact the overall productivity of the facility. Our results follow the pattern described by Wilson (2002) wherein fish exhibit increasing growth rates as lysine level increases to the perceived "optimum," then level off, without notable increases in growth as dietary lysine levels are further increased.

Admittedly, R^2 values for calculated linear regression equations were generally low (range: 0.58 to 0.01). The poor goodness-of-fit of the calculated regression lines highlights the high levels of variation often observed in many biological systems, and differing responses of the individual fish consuming these feeds. Fish in this study were not graded, a common practice in commercial culture operations, so it is likely that more intra-treatment variability was present than would be observed under commercial production, where the slowest-growing fish would

have been culled. Additionally, fish in this study were a standard production strain, and had not been selected for enhanced performance when fed on plant-based feeds (Overturf et al., 2013).

The results of the proximate analyses highlighted several trends. The percentage of biomass stored as fat generally decreased with increasing levels of dietary lysine (Table 2.3). Conversely, dry biomass energy content was highest in fish consuming the 1.85% lysine feed (7678 ± 47 cal·g dry biomass^{-1}, mean ± S. E. M.) and lowest in fish consuming the 4.31% lysine diet (7014 cal·g^{-1} ± 162), showing a generally inverse relationship between these two variables. This could be due to the higher energy content of fat when compared with protein (Blaxter, 1989); fish whose bodies contain more fat are likely to contain more energy than equally-sized fish having less fat. Wet biomass protein levels, conversely, generally increased as dietary lysine increased (range: 14.7 to 17.1%), suggesting that increasing levels of this amino acid facilitated increased protein anabolism. Kim et al. (1992) and Cheng et al. (2003) observed a similar pattern of increasing protein deposition and decreasing fat deposition as levels of dietary lysine increased. However, the fish used by Kim et al., (1992) were much smaller (mean initial weight: 13.7 g), than the fish used in our study, as were those used by Ketola (1983) (mean initial weight: 1.1 g), so direct comparisons of biomass protein and lipid levels among these three studies may not be advisable, as fish accrue biomass differently as they age.

Differences in observed optimum dietary lysine levels among studies could be influenced by factors such as different diet ingredients, laboratory rearing conditions, and disparate sizes or strains of fish used in the different feeding trials (Kim et al., 1992). Care should be taken when applying the results of my study or another in an attempt to optimize dietary lysine levels for a given situation. Thus, optimum dietary lysine levels for rainbow trout consuming plant-based diets, like those in our study, may not be the same as those for trout consuming a more

traditional, fish-based diet. Further research will help to illuminate differences in dietary ingredients and possible effects on the dietary needs of rainbow trout consuming them, particularly when strain-level differences, such as those noted by Overturf et al. (2013) are considered.

The performance of rainbow trout consuming the 2.34% lysine diet in my study is comparable to the performance of fish consuming several 40:20 P:L feeds in a previous study by the same investigators. Fish consuming an animal and plant-based diet exhibited an FCR of 0.95, and a SGR of 1.69 (Craft and Myrick, unpublished data). Those consuming a fish meal-based diet, comparable to most commercial feeds currently in use, exhibited a higher SGR of 1.82, and a lower FCR of 0.93 (Craft and Myrick, unpublished data). The FCR observed for the 2.34% diet in this study was close to 1.00, and the SGR was 1.82, highlighting the positive effects on growth of a diet with an appropriate amino acid profile.

The growth rates of rainbow trout observed in this study were slightly reduced when compared to fish consuming a standard fishmeal-based diet in another study with the same rearing conditions (Craft and Myrick, unpublished data). Similar results have been observed by other investigators. Cheng et al., (2003) noted that lysine supplementation in diets containing some plant-based proteins, replacing 50% of the fish meal in the diet, improved growth of rainbow trout consuming these diets. However, performance was still reduced when compared to trout consuming a fish meal-based control diet, suggesting that further research is needed to increase the growth performance of fish consuming fishmeal-free diets (Cheng, 2003). Possible causes of decreased growth rates may include antinutritional factors, which negatively impact the digestion, growth, or health of fish consuming these feeds. Gatlin et al. (2007) presented a thorough review of different plant-based ingredients for aquafeeds, highlighting the desirable and

undesirable qualities of each. Soybean meal, a readily-available ingredient, is known to contain multiple antinutritional factors, including high levels of indigestible oligosaccharides (carbohydrates), protease inhibitors, and phytic acid, which can limit the availability of zinc and may limit the digestibility of available protein (Gatlin et al., 2007). These antinutritional factors may explain the lag in growth rates observed by Cheng et al. (2003), and highlight another hurdle associated with the wholesale replacement of fish meal in aquafeeds. Improved methods of processing plant ingredients, such as the extraction of protein concentrates, may reduce levels of antinutritional factors and increase their utility (Hardy, 2010).

Aquaculturists would be well served to use aquafeeds with a lysine content that maximizes growth rate, to expedite growth of fish to market size and to reduce production costs associated with care and feeding before fish are stocked or sent to market. Our results identified 2.34% as near-optimal for juvenile rainbow trout consuming a plant-based feed formulated to 40% protein and 20% lipid. While our study utilized larger rainbow trout than those used by Kim (1992) and Ketola (1983), our predicted optimum lysine level fell between those calculated by these two studies. Future studies, examining feeds formulated from different sets of ingredients, will help to further fine-tune our understanding of the optimal amino acid requirements of rainbow trout, and other economically valuable finfish. Increased knowledge of these requirements will benefit the aquaculturist by keeping feeding costs low, which will in-turn benefit the consumer, reducing market prices while maintaining adequate profit margins for the producer. If aquaculture production is to continue to increase, despite the increasing price of fish meal, the nutritional requirements of fish consuming feeds containing alternative feedstuffs must be thoroughly researched to identify viable alternatives to fishmeal, and any special challenges

they may pose. This will facilitate further fine-tuning of the nutritional profiles of these feeds, and further increase performance of fish consuming them.

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Chapter 3 - Evaluation of the metabolic response of rainbow trout consuming feeds formulated with alternative protein ingredients

1. Introduction

The metabolism of fishes, as with many organisms, is influenced by a number of environmental, behavioral, and physiological factors (Cech, 1990, McCue, 2006). Water temperature (Brett, 1964), activity level (Alsop and Wood, 1997), rearing density (Lefrancois et al., 2001, Medland and Beamish, 1985, McKenzie et al., 2012), infection (Kumaraguru et al., 1996), and gonadal development can all influence the metabolic state of a fish. Feeding state can also have a significant impact on the metabolic status of a fish, as ingestion and digestion of food, as well as the egestion of wastes, elicit a suite of physiological responses that require energy (Cho et al., 1982). Of specific interest is the metabolic increase in fish due to the post-absorptive (post-feeding) processes that take place. This is known by various terminologies, including heat increment of feeding, calorigenic effect of food, and thermogenic action, but I will refer to it as "Specific Dynamic Action" (SDA). All organisms studied thus far are known to have an increase in metabolism post-feeding (McCue, 2006). McCue (2006) described how this increase is due to mechanical processes such as acid and enzyme secretion, peristalsis, increased blood flow, and the molecular-level processes such as deamination, ketogenesis, glycogenesis, and protein catabolism that follow food ingestion.

Metabolism is directly linked to oxygen consumption rates in fishes, with higher metabolism generally increasing respiratory rates (Cech, 1990). Furthermore, observation of oxygen consumption rates, either individually within a small chamber or on a larger, full-tank (multiple fish) scale, is generally presumed to be easier than measuring the relatively minor heat production of fish within an aquatic environment (Brett, 1962; Cech, 1990). Thus, studies

examining the metabolism of fishes typically use oxygen consumption rates as a proxy for metabolic rates. The effects of swimming (Dickson et al., 2012), temperature (Patterson et al., 2013), and toxicants (Alvarez and Fulman, 2005) on fish metabolism have all been examined using this methodology.

One practical concern for aquaculturists is how a given feed may affect their fishes' post-feeding oxygen consumption rate. If increases in oxygen consumption rates occur as a result of changes in feed composition, increased oxygenation may be required, especially in culture systems supporting high fish loadings. Otherwise, dissolved oxygen levels in these systems may become hypoxic, possibly reducing growth rates (Thorarensen et al., 2010) and overall health of the fish they contain. Therefore, investigations of the effects of SDA on the metabolism of fishes have an applied focus when thought of from the perspective of a fish culturist. This is especially relevant in instances where fish are repeatedly fed to satiation, where SDA responses can approach or exceed the oxygen consumption rates observed in fish swimming at their U_{Crit} velocities (Soofiani and Hawkins, 1982; Soofiani and Priede, 1985). This highlights the magnitude of the SDA response in relation to the overall metabolic scope of the fish.

The SDA responses of different fishes has, with varying success, been found to be dependent upon the formulation of a given diet, as well as the overall and relative quantities of certain ingredients (Beamish and Trippel, 1990). Alsop and Wood (1997) utilized a whole-tank intermittent static respirometry approach to determine that rainbow trout (*Oncorhynchus mykiss*) fed to satiation exhibit elevated routine metabolic rates more than 68% higher than fasted fish, illustrating the overall magnitude of the SDA response in rainbow trout. Early investigators examining the role of dietary composition on rainbow trout metabolism noted a generally increasing trend in the SDA response of fish as dietary protein content increased in graded

quantities (34%, 48%, or 58%); however, differences between diet treatments were not always statistically distinguishable (Medland and Beamish, 1985). Additionally, there were no differences in the SDA response between diets formulated to either 7% or 23% lipid (Medland and Beamish, 1985). One possible confounding factor in this early study may have been the differing digestible energy levels of diets used, because at higher protein and lipid ratios (P:L), diet digestible energy values increased from 56% to 92% (Medland and Beamish, 1985).

Eliason et al. (2007) compared the effects of three different isoenergetic diet compositions (55:10, 45:15, or 35:20 P:L, respectively) on the metabolic rates of adult rainbow trout (roughly 500- 650 g mean individual weights between two separate lots). They noted no discernable differences in the overall SDA response, the peak response (above both standard metabolic rate and routine metabolic rate), and the time to peak response among all diets examined. Additionally, supplementation of amino acids in unbalanced (nutritionally sub-optimal) proportions has been shown to affect the SDA response of rainbow trout (Kaczanowski and Beamish, 1995). Experiments examining the impacts of lipid content upon SDA have generally shown lipids to have a much smaller impact than that of proteins, as deamination of lipids before their catabolism is not necessary, as it is with proteins, making them a more efficient source of energy within aquafeeds (Cho et al., 1976). The impact of carbohydrates on SDA is generally thought to be low in rainbow trout, except at high levels of inclusion, at which the cost of breaking down and excreting these components may outweigh the nutritional value they provide, forcing fish to draw energy from other sources (i.e. tissues and dietary proteins) (Beamish et al., 1986, Beamish and Trippel, 1990).

We conducted a pair of respirometry studies investigating the effects of diet composition on whole-tank oxygen consumption rates. In the first study, we evaluated the oxygen

consumption rates of rainbow trout fed one of a set of 10 different plant and animal-based feeds. These feeds were formulated from 5 groups of experimental ingredients to either a 45:20 P:L ratio or a 40:20 P:L ratio, following the recommendations of Hardy (2002) and the National Research Council (NRC, 1993) respectively (Chapter 1, Table 2.1). We hypothesized that the higher protein (45:20 P:L) diets would elicit increased metabolic responses in trout as observed by previous investigators (Cho et al., 1976, LeGrow and Beamish, 1986).

In the second set of respirometry trials, three practical diets were evaluated, allowing us to increase our overall sample size from 3 tanks per treatment to 6 tanks per treatment, while still running trials for the same number of weeks. The ingredients used were influenced by the results of the feeding trials described in Chapters 1 and 2, and by a concurrent study conducted by co-investigators on our project. Similarly, the investigators attempted to address any possible sources of error that may have impacted the first round of trials.

2. Materials and Methods

2.1 Chamber design and sampling procedures

Whole-tank static respirometry trials were conducted at 15°C at the 6- and 12-week points during the multi-protein source feeding trial (see Chapter 1) to evaluate the specific dynamic action (SDA) of groups of fish fed the experimental feeds. Ten tanks of fish were tested per week over 3 consecutive weeks, to evaluate all 30 tanks of fish. Tanks of fish were randomly selected and assigned to one of ten 65-L whole-tank respirometry chambers. Each chamber consisted of an aluminum tank, (dimensions: 123 cm long, 14 cm wide, 15 cm deep), with a custom-built lid covering the whole tank. The drain end of the tank, which included a small settling basin and standpipe, was not covered. Ports allowed for the inflow of water at the head of the tank, and allowed introduction of a small (0.58 mm inner diameter) water-sampling cannula located within the center of the chamber. Additionally, a small feeding hatch was used to feed the fish within the chamber. This hatch was only opened during feedings, and fish were kept in darkness and as undisturbed as possible during the remainder of the trial.

For these trials, fish were fasted for 40 h, batch weighed without anesthesia, and loaded into the respirometry chamber. These fish were then allowed to recover for 4 h. During each subsequent hour, flows and supplemental aeration were shut off for a nominal period (week 6 trials: 10 minutes; week 12 trials: 5 minutes[1]). At the beginning and end of the static period, a water sample was taken and its dissolved oxygen concentration measured using a Strathkelvin 928 oxygen meter. These dissolved oxygen values were used to calculate the tank oxygen consumption rates using:

$$M0_2 = \frac{(Initial\ 0_2\ concentration - final\ 0_2\ concentration) x Volume}{Time}$$ (Cech 1990)

[1] The shorter duration of static interval in the week 12 trials was used because of the larger size (and thus oxygen demand) of the fish.

Oxygen consumption was then divided by the total biomass of fish (g) in the tank to determine the mass specific oxygen consumption rate of the fish being studied. Pre-feeding metabolic rates were monitored for approximately 16 h (40 h during week 1 due to mechanical difficulties). After 16 h had passed, fish were fed the respective feed assigned to their tank. After feeding, fish were allowed approximately 1 h to finish foraging and return to normal activity levels before sampling procedures resumed.

Since we could not observe feeding behavior, due to the design of our feeding hatch, we could not evaluate the amount of food needed for satiation. Additionally, we wanted to avoid excess feeding because this could increase bacterial oxygen consumption rates. Therefore, we used mean feed consumption levels calculated for all tanks during the two weeks previous to the respirometry trials. In a pilot study, we examined the utility of feeding fish at the maximum observed one-day consumption level. However, because large amounts of food accumulated in the settling basin at the drain-end of the tank, we used the lowest one-tank mean daily feed consumption rate observed (1.12% of the total fish biomass within the tank). During our trials, any wastes (feces, uneaten food pellets) observed within the settling basin were removed via siphoning. Care was taken to avoid disturbance of the fish during cleaning events, which were conducted at some point during the 55-minute flow-through period (i.e. tanks were not cleaned during the static, data collection interval).

After the completion of each trial, the respirometry lid was removed and fish were returned to their rearing tank of origin. Any remaining feces or food were left in the tank, and three blank readings were taken using the same procedures as when the fish were present in the tank. Mean blank MO_2 for all tanks was found to be 111.8 $mg \cdot h^{-1}$, suggesting that a small but measurable amount of oxygen consumption was occurring during each static period. This

accounted for approximately 15% of the average oxygen consumed on a by-tank basis each hour during this phase of trials. However, no correction factor was used during statistical analyses, as background oxygen consumption rates were variable, and the number of data points collected for this data set was relatively low.

2.2 Year 2 Respirometry Trials

Several procedural changes were made between the Year 1 and Year 2 respirometry work to try to reduce the amount of variation in whole-tank MO_2 readings, and thus increase the resolution of the whole-tank approach.

Fish were loaded and left undisturbed for roughly 20 h before sampling procedures were initiated (previously, recovery time after loading was 4 h). To further reduce the possibility of disturbing the fish during the collection of water samples, the arrangement of the tanks used for the trials was modified. Aisles were widened between the respirometry chambers, and tanks that were on the lower level (roughly knee high) were not used during this phase of the study. All tanks used in the study were roughly chest-high, allowing investigators to easily maneuver around the tanks and to draw samples without accidentally colliding with the tanks and disturbing the fish within. Additionally, trials were run at a lower temperature (mean: 13.7°C, range: 12.5- 15.3°C) due to water temperature fluctuations at the Foothills Fisheries Laboratory in January. During week one of trials, data collection ceased roughly 48 h post-feeding, due to an equipment malfunction. During weeks 2 and 3 of trials, fish were monitored for 72 h postprandial. Mean blank MO_2 was calculated to be 4.51 $mg \cdot h^{-1}$, suggesting that the lower water temperature reduced oxygen consumption within the tanks by microbes. However, fish oxygen consumption rates were also reduced, due to lower overall biomass of fish within the tank as well as the lower temperature, and background oxygen consumption rates accounted for roughly 15%

of the average oxygen consumed per tank per hour during the trials. No correction factor for background oxygen consumption was used during calculations.

The three feeds used during the study either utilized fish meal or plant-based ingredients as the main protein source, and were formulated to several different P:L ratios (Table 3.1).

Table 3.1. Primary ingredients and protein:lipid ratios of diets examined in the second round of respirometry trials.

Feed Number	Diet	Primary ingredients (>10% diet dry weight)	Protein:lipid ratio
1	low protein plant	corn protein concentrate, soybean meal, soy protein concentrate, wheat flour, menhaden fish oil	35:20
3	high lipid plant		40:25
2	standard fish meal	menhaden fish meal, soybean meal, corn protein concentrate, wheat flour, menhaden fish oil	45:20

These diets represent possible practical formulations that could be utilized by fish culturists. The ingredients used in the plant-based diets for this portion of the study are the same as those used to formulate the plant products diets (PPD, PPD+) used in the previous feeding trial. These are ingredients that are readily available to feed formulators, and trout consuming them performed favorably in a similar diet study conducted by colleagues at the Bozeman Fish Technology Center (U.S. Fish and Wildlife Service, Bozeman, Montana, U.S.A.). All other sample collection procedures and data analyses are identical to those used during the first year respirometry trials.

2.3 Statistical Analyses

Statistical analyses of respirometry data were conducted using a PROC mixed command in SAS 9.2. Metabolic oxygen consumption (MO_2) levels were averaged across 4-h blocks to

reduce variation due to fish activity levels. Predictor variables of interest during the first set of trials included the feed ingredient group and protein:lipid ratio, as well as the interaction between these terms. During the second set of trials, feed type was examined as the predictor variable. Tank and week of the trial were set as random effects. Statistical significance was set at an α-level of 0.05. Any tanks that experienced fish mortality during trials were removed from data sets before analyses were performed (year 1: n=1, year 2: n=0).

Dependent variables examined included several metrics. Routine (pre-feeding average; mg O_2 consumed$\cdot g^{-1} \cdot h^{-1}$) metabolic rates (RMR), were compared to examine possible differences in metabolic rates of fasted fish established during the concurrent feeding trial, possibly due to physiological differences influenced by the diet consumed. Next, we examined the difference between the routine metabolic rate and the mean peak MO_2 post-feeding, calculated by averaging the five highest post-feeding MO_2 values. This was termed "SDA Max" in our analyses. Third, we compared the time-to-peak SDA for all diets. Time-to-peak SDA was defined as the mean time of occurrence of each of the five MO_2 measurements used to calculate SDA Max. Fourth, we examined the total time elapsed from feeding to when MO_2 values returned to those equal to the RMR, termed "SDA Duration." This was done by fitting a quadratic equation of the form $y = ax^2 + bx + c$ to the data, and solving for the positive value of x when y is equal to 0. Fifth, we examined possible differences in area under the line plotted for SDA duration, to compare the total additional oxygen consumed above routine oxygen consumption levels due to SDA during the digestion process. This was termed "SDA oxygen consumed" in our analyses. Finally, we compared the energetic content of the feed consumed during the feed event (kcal) to the amount of calories burned during the SDA oxygen consumed, assuming that 1 mg of oxygen consumed is the equivalent of 0.0136 kJ or 0.00325 kcal of energy expended (Cho et al., 1982). We refer to

this metric as “SDA coefficient.” SDA max data were log-transformed to satisfy the assumption of normality in the ANOVA analysis.

3. Results – Year 1

3.1 Routine metabolic rate

Routine metabolic rates ranged from 0.11 to 0.13 mg $O_2 \cdot g^{-1} \cdot h^{-1}$ and were unaffected by feed group (F=0.33, P=0.8539), protein:lipid ratio (F=0.30, P=0.5981), and their interaction (F=0.51, P=0.7314). No pairwise differences were observed (Figure 3.1).

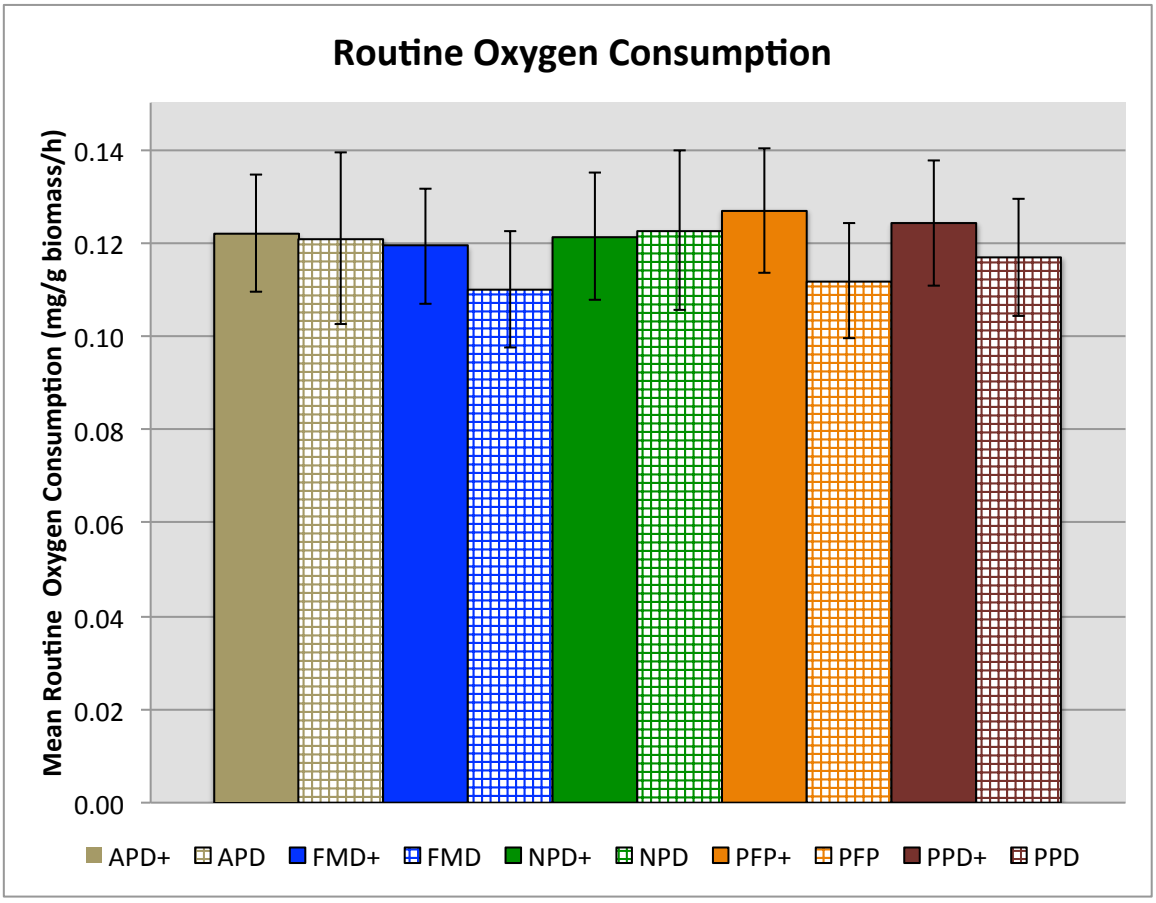

Figure 3.1. Mean (± S. E. M.) routine oxygen consumption (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) rates for tanks of rainbow trout assigned to each treatment during our trial.

3.2 SDA Max

SDA maximum values ranged from 0.015 (APD+) to 0.048 (NPD) mg $O_2 \cdot g\ biomass^{-1} \cdot h^{-1}$ (Figure 3.2). We observed no significant relationships between log SDA Max and feed group

(F=0.18, P=0.9453), protein:lipid ratio (F=1.09, P=0.3233), or the interaction of these two terms (F=0.39, P=0.8105).

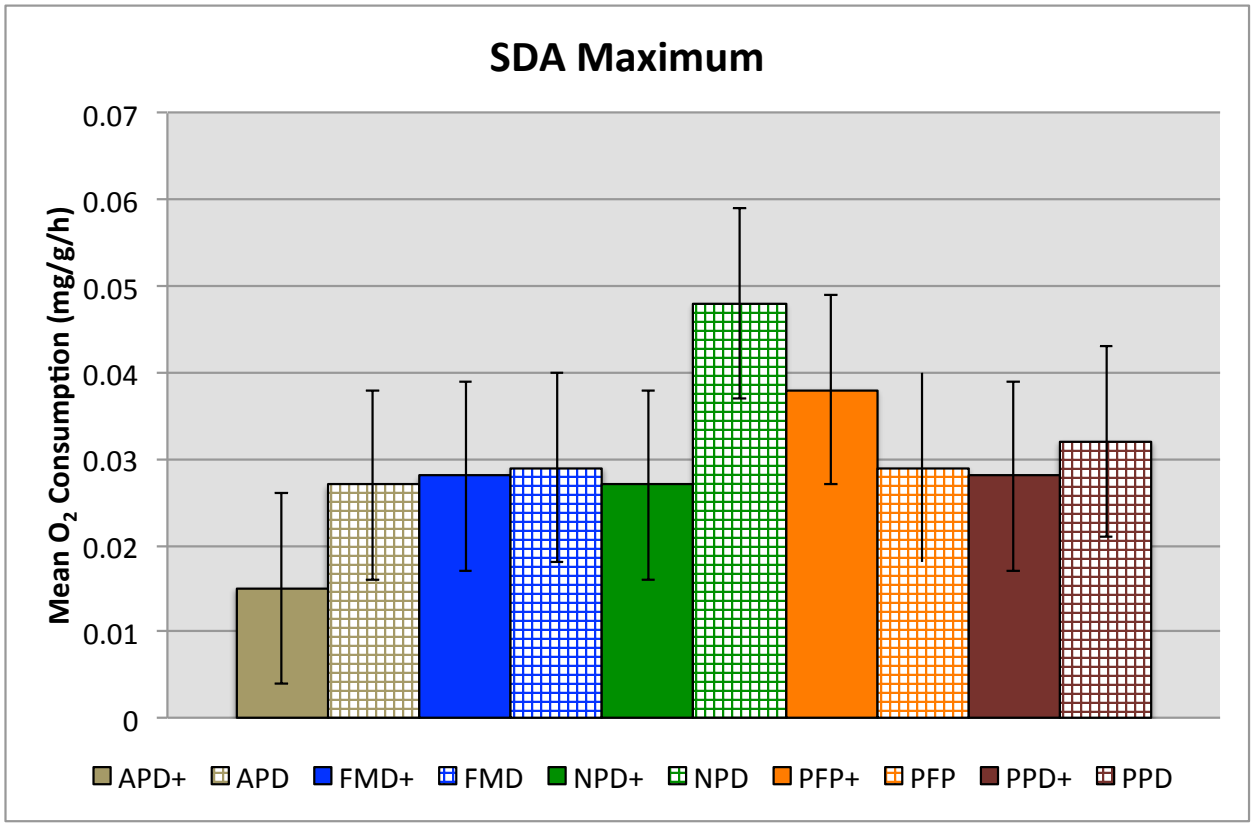

Figure 3.2. Mean (± S. E. M.) maximum increase in oxygen consumption rate (mg O_2 g^{-1} h^{-1}) above routine oxygen consumption rate observed in rainbow trout fed 1.12% of their biomass of the 10 experimental feeds.

3.3 Time-to-peak SDA

Mean time-to-peak SDA ranged from 0.73 to 1.31 d (17.5 to 31.44 h) during our trial (Figure 3.3), and were unaffected by feed group (F=0.76, P=0.5740) or the interaction between feed group and protein:lipid ratio (F=0.89, P=0.5039). However, the effect of the protein:lipid ratio (F=19.84, P=0.0012) was highly significant. Mean times-to-peak SDA were 1.12 d (26.88 h) and 0.81 d (19.44 h) for fish consuming the 45:20 and 40:20 feeds, respectively.

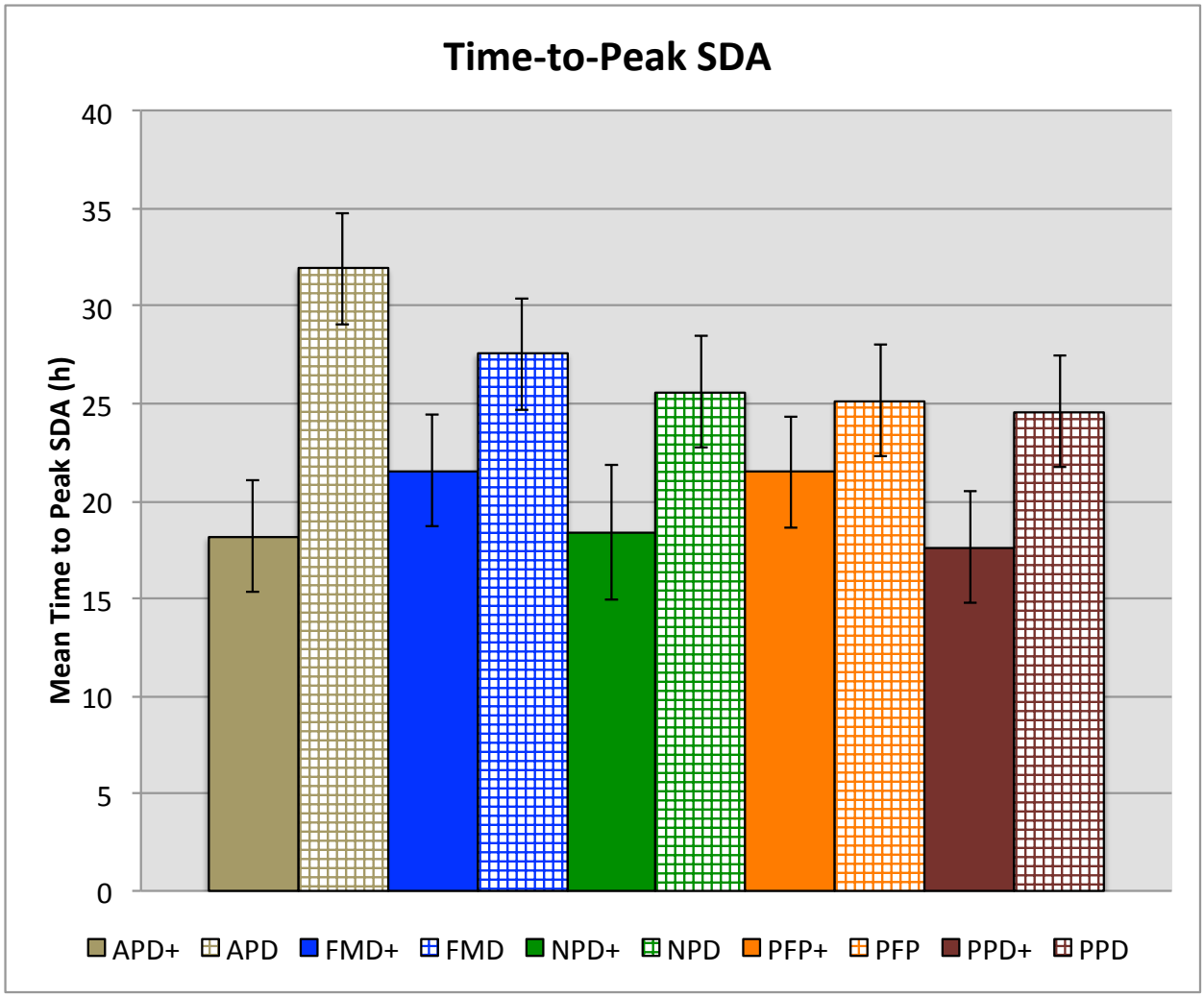

Figure 3.3. Mean time-to-peak SDA (± S. E. M.) for fish during respirometry trials examining the metabolic effects of 10 experimental feeds.

3.4 SDA Duration

Elevated oxygen consumption rates indicate that the SDA period lasted from 21.4 h (APD) to 61.0 h (PPD), depending on diet (Figure 3.4). However, there was a substantial amount of variability in SDA duration values, and thus no feed group ($F=2.64$, $P=0.1129$), protein:lipid ratio ($F=0.84$, $P=0.3872$), or interaction effects ($F=1.58$, $P=0.2693$) were detected.

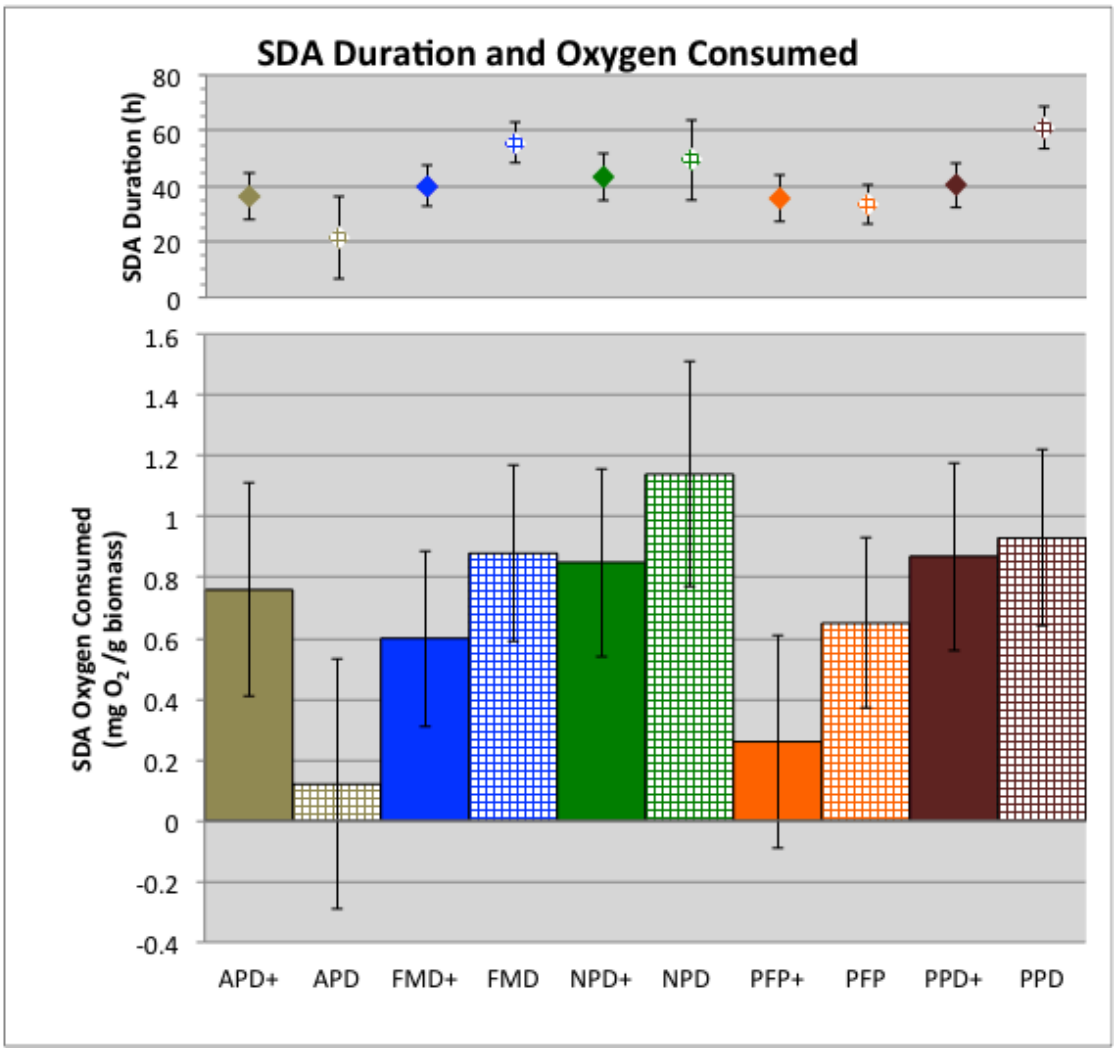

Figure 3.4. Mean SDA duration (h; ± S. E. M.) and SDA oxygen consumed (mg O_2·g biomass^{-1}) postprandial by rainbow trout consuming the ten experimental feeds examined in our study.

3.5 SDA Oxygen Consumed

The additional oxygen consumed post-feeding (above RMR; SDA oxygen consumed) ranged from 0.12 (APD) to 1.14 (NPD) mg O_2·g biomass^{-1} (Figure 3.4). These values were unaffected by feed group (F=1.11, P=0.4151), protein:lipid ratio (F=0.14 , P=0.7195), or their interaction (F=0.67, P=0.6298).

3.6 SDA Coefficient

Proportions of ingested calories consumed that were burned during the SDA response were calculated to range from 0.83% (APD+) to 8.78% (NPD+) (Figure 3.5). However, difficulties were encountered in accurately quantifying the amount of food consumed by the fish. In several instances, fish within the tanks did not consume a noticeable portion of the food they were offered. This food remained within the chamber until being flushed into the settling basin, where attempts were made to quantify the number of pellets removed. However, this proved difficult because the feed pellets had often partially or fully disintegrated due to prolonged saturation with water, making accurate counting of the number of pellets difficult. In light of this, statistical comparisons among the mean SDA coefficient values were not run, but the raw results are presented for informational purposes.

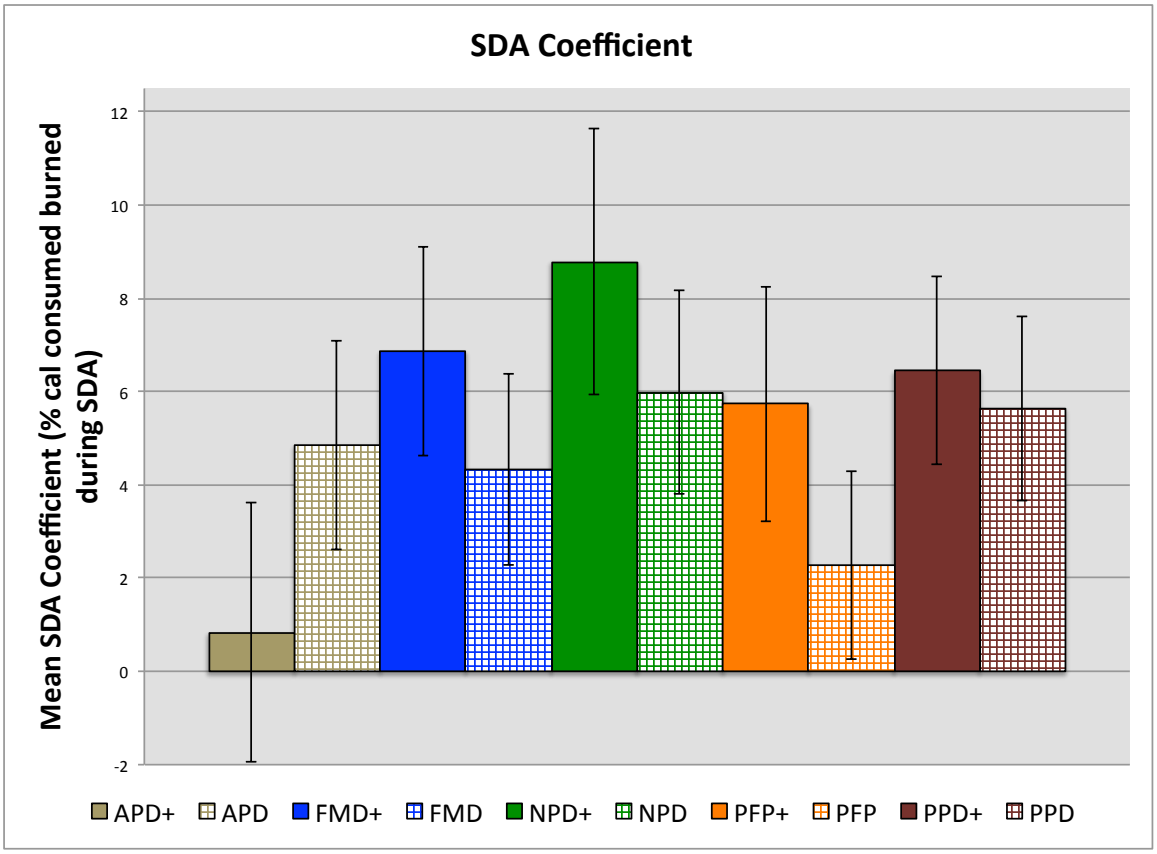

Figure 3.5. Mean SDA Coefficient values (± S. E. M.) in % of calories consumed burned during SDA. Statistical analyses were not run.

Results – Year 2

Mean routine oxygen consumption rates did not differ between treatments (F=0.72, P=0.5143). Mean routine metabolic rates were 0.1032, 0.1013, and 0.1142 mg $O_2 \cdot g^{-1} \cdot h^{-1}$ for feeds 1, 2, and 3 respectively (Figure 3.6). Similarly, peak oxygen consumption rates (above routine; SDA maximum) ranged from 0.45 to 0.63 mg $O_2 \cdot g^{-1} \cdot h^{-1}$ (Figure 3.7) but were not significantly related to feed type (F=1.38, P=0.2857). Mean time-to-peak ranged from 21.1 h, to 25.5 h (Figure 3.8), similar to results observed during year 1, but no statistically significant differences were found (F=1.20, P=0.3512). The duration of the SDA response for fish consuming feeds 1, 2, and 3 was 48.3 h, 56.6 h, and 60.7 h, respectively (Figure 3.9). Feed type did not have a significant effect (F=0.76, P=0.4898). Total oxygen consumption amounts ranged from 1.327 to 2.001 mg $O_2 \cdot g^{-1}$ (Figure 3.10). Analysis of the total oxygen consumed during the SDA response revealed that feed type did not have a statistically significant (F=0.59, P=0.5759) effect. Feed type was also not statistically significant (F=1.04, P=0.4324) when examined as a predictor variable for the SDA coefficient response. Mean SDA coefficient values for feeds 1, 2, and 3 were 1.75%, 2.88%, and 1.83%, respectively (Figure 3.11).

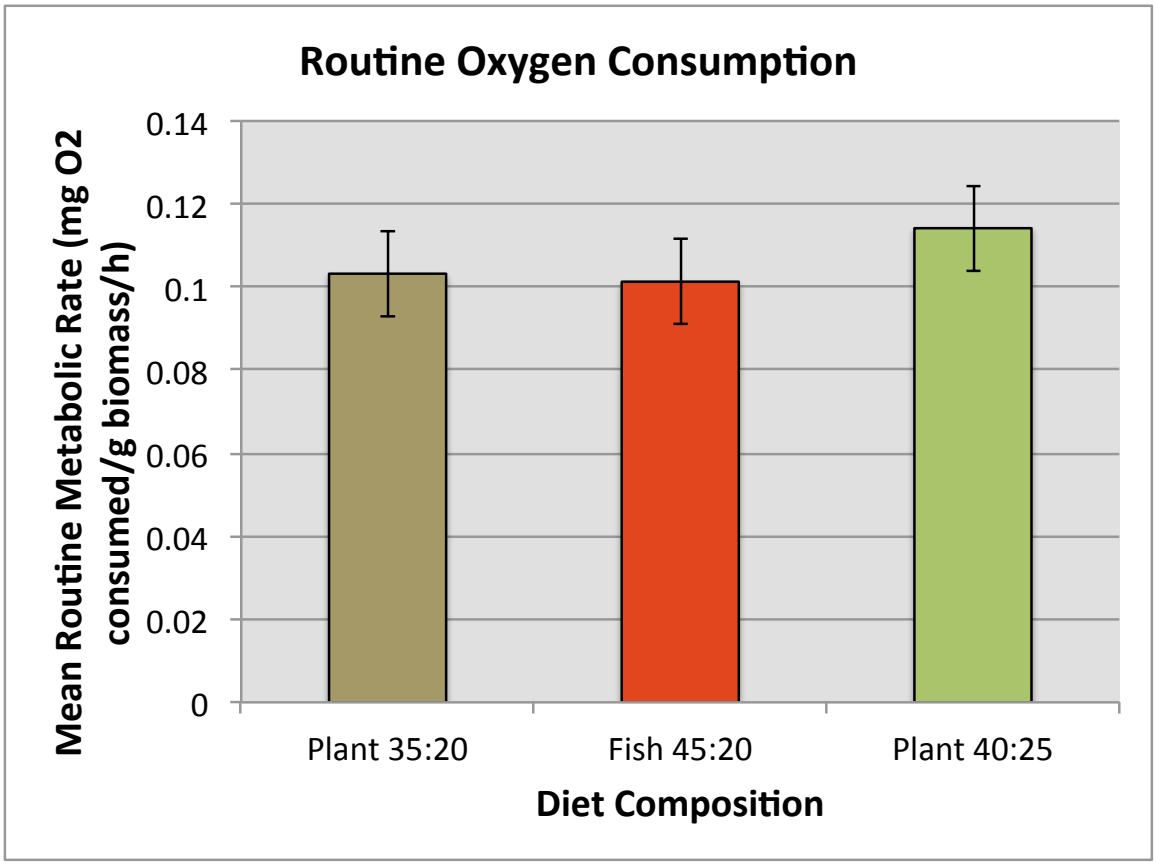

Figure 3.6. Mean (± S. E. M.) routine oxygen consumption (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) rates for tanks of rainbow trout assigned to each treatment (n=3) during our trial. There were no significant differences among treatments (ANOVA, $P > 0.05$).

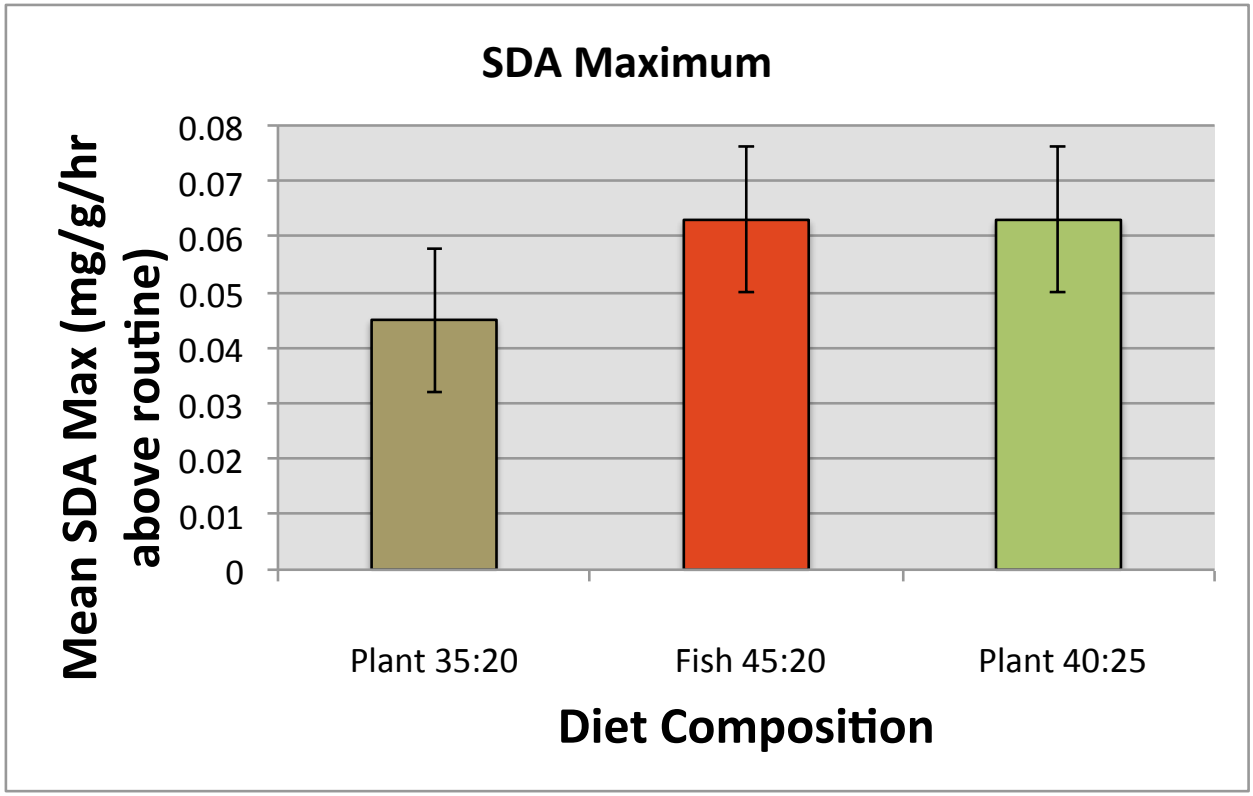

Figure 3.7. Mean (± S. E. M.) maximum SDA values (mg $O_2 \cdot g^{-1} \cdot h^{-1}$) for rainbow trout consuming each experimental feed. There were no significant feed-related differences in the degree of elevation above RMR.

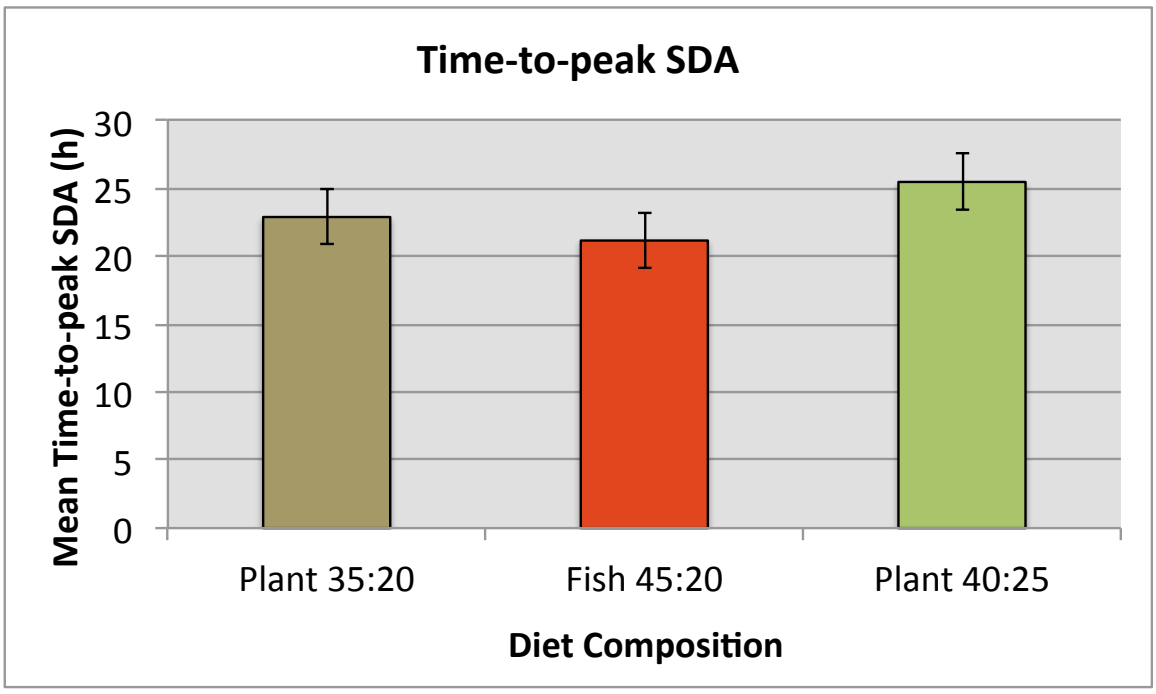

Figure 3.8. Mean time-to-peak SDA (± S. E. M.) (h) for rainbow trout consuming each of the three experimental feeds examined during our trial.

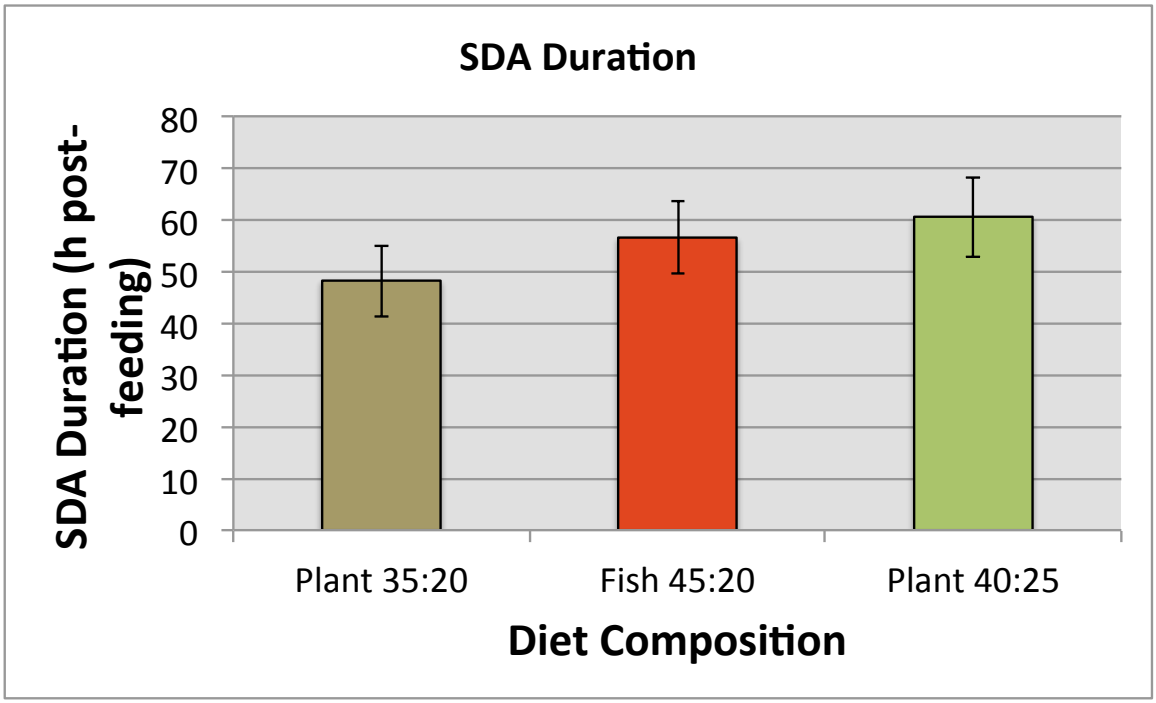

Figure 3.9. Mean SDA duration (± S. E. M.) (h) observed during our trials for each of the three experimental feeds.

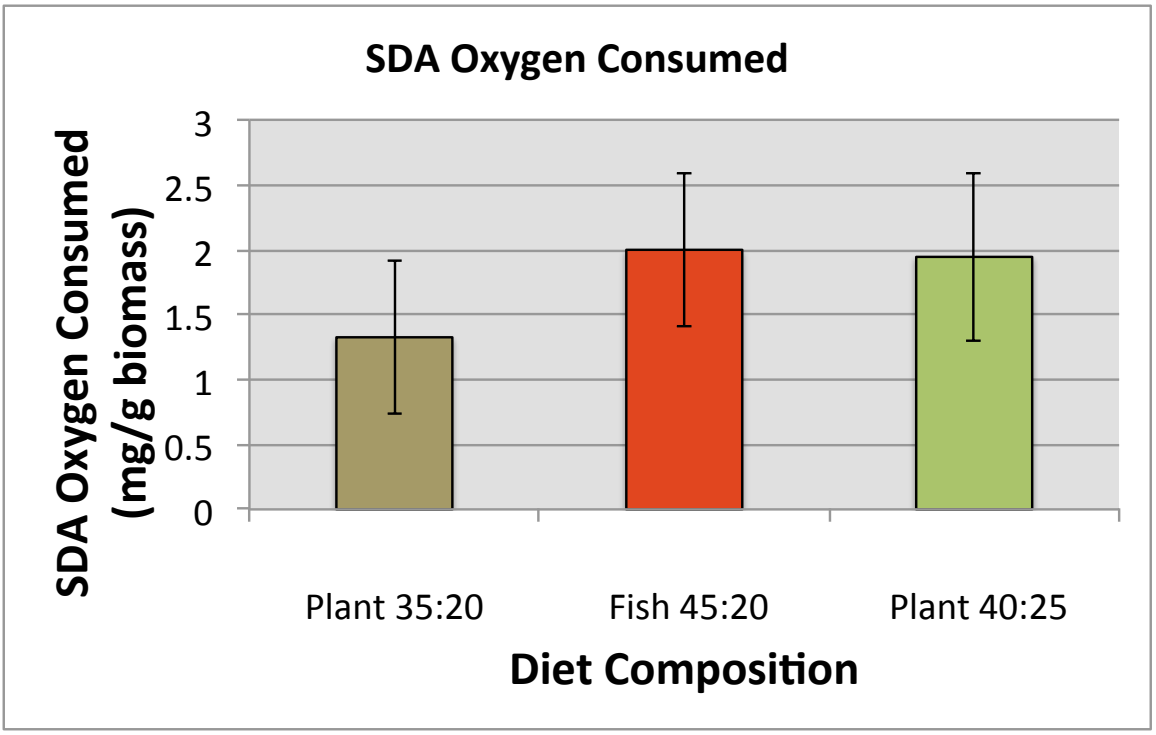

Figure 3.10. Mean total oxygen consumed (mg $O^2 \cdot g$ biomass^{-1}) (± S. E. M.) by rainbow trout consuming each experimental feed examined.

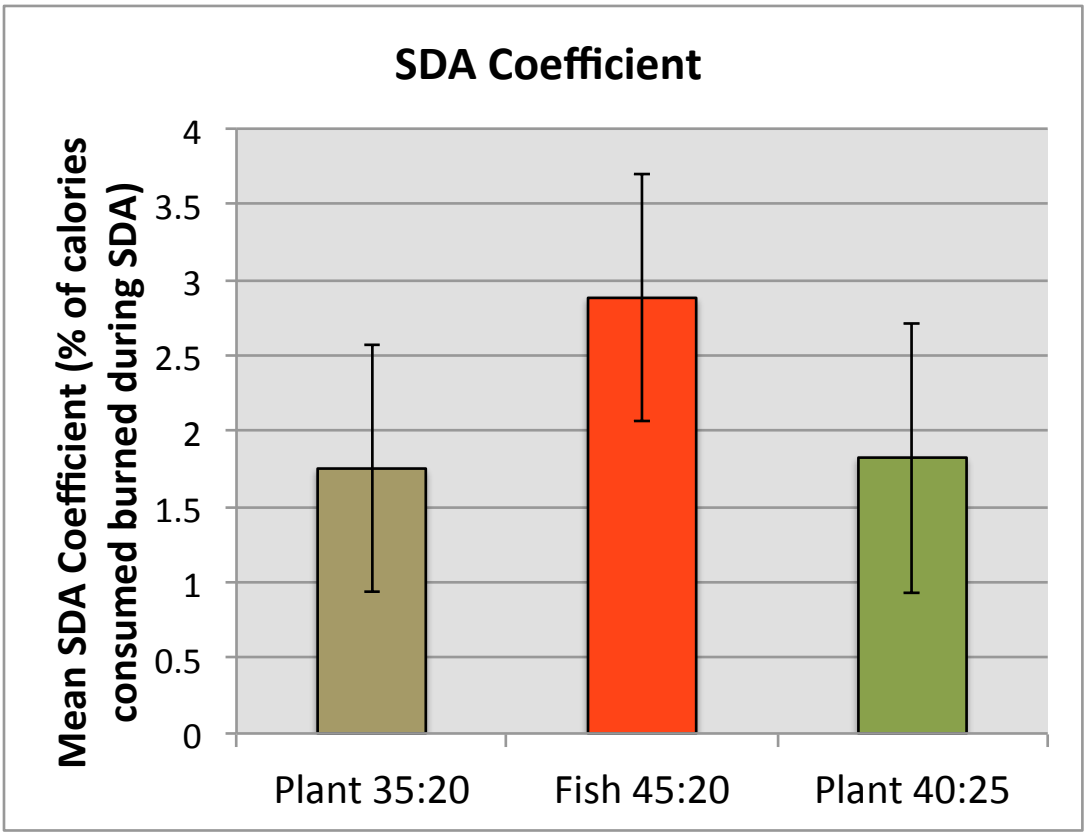

Figure 3.11. Mean SDA coefficients (± S. E. M.) calculated from respirometry data and feed consumption amounts of fish used during our trial.

4. Discussion

4.1 Effects of Alternative Protein Sources on Oxygen Consumption Rates

We used whole-tank respirometry to measure the change in oxygen consumption rates associated with feeding rainbow trout diets containing different protein sources and P:L levels. While we did not detect many diet or P:L level differences in oxygen consumption rates, fish did show the expected responses wherein MO_2 was elevated post-feeding and then declined to routine levels after an extended period.

A comparison of the routine metabolism of the groups of fish examined pre-feeding was found to be statistically indistinguishable among all treatments during our trials, suggesting that the groups of fish did not have differing baseline metabolic rates due to factors unrelated to SDA. Likewise, we were unable to identify statistically significant differences among SDA maximum, SDA duration, and SDA oxygen consumed of the fish consuming the experimental feeds during our study. Furthermore, no probable trends below the threshold of statistical significance were apparent within these three response variables being examined.

Mean routine SDA observed during our study was found to be 0.12 mg $O_2 \cdot g^{-1} \cdot hr^{-1}$. This is slightly higher than the back-calculated standard metabolic rate (0.071 mg $O_2 \cdot g^{-1} \cdot hr^{-1}$) for a 50 g sockeye salmon (*Oncorhynchus nerka*) held at 15° (Brett 1964). As these fish were smaller than ours, and a direct relationship between increasing fish size and increasing standard metabolic rate has been established (Smith et al., 1978), there appears to be no disagreement between these observations. Similarly, Eliason et al. (2007) conducted two sets of trials on individual rainbow trout (mean weights of 503.4 and 646.7 g, respectively) in small (9.1 to 9.9 L) vessels, at temperatures from 8.2 to 13.0°, and 10.0 to 16.0° C, respectively. These investigators found the routine metabolic rate of fasted rainbow trout to be 0.103 and 0.0926 mg. These RMR values are very close to those calculated within our study suggesting that overall

differences in RMR between individual fish within a relatively restrictive chamber and groups of fish within a less restrictive chamber may be minimal.

SDA maximum values within our study ranged from 0.015 (APD+) to 0.048 (NPD) mg $O_2 \cdot g^{-1} \cdot hr^{-1}$ above the routine metabolic rates observed (a 12.5% to 40% increase in oxygen consumption), with an overall mean increase of 0.03 mg $O_2 \cdot g^{-1} \cdot hr^{-1}$ above baseline (25.1%). This is similar to the 18% increase observed by Seth et al. (2008) in rainbow trout injected with a predigested 50:25 P:L diet. In contrast, Eliason et al. (2007) noted a larger increase (73.7%) in peak SDA above routine metabolic rates. As the oxygen sensing apparatuses used by Eliason et al. (2007) were fully automated and operated at a finer time scale, utilizing either 25-minute flow through and 5-minute static periods, or 10-minute flow through and 5-minute static periods in the two locations (Simon Fraser University, Burnaby, British Columbia, Canada and University of British Columbia, Vancouver, British Columbia, Canada) at which they collected data at during their study, comparisons between this study and others run with fewer intervals may not be advisable. Alsop and Wood (1997) reported a 68% increase in metabolism of small (4-8 g) rainbow trout fed to satiation, at a temperature of 15° C and a flow rate of $10\ L \cdot min^{-1}$. In this study, fish voluntarily consumed approximately 3% of their body mass, perhaps illustrating the increased metabolic impact associated with higher feed consumption rates (Alsop and Wood, 1997).

Interestingly, the effects of the different protein:lipid ratios used during the first year of trials were found to be statistically significant when examining the time-to-peak SDA response. Fish consuming the high protein (45%) feeds took longer to reach their peak response than those consuming the low protein (40%) feeds. These results follow the pattern reported by Cho et al. (1976) showing that protein is the main component contributing to the SDA response in rainbow

trout. The delay in peak SDA response due to high levels of protein in rainbow trout diets was elegantly shown by Seth et al. (2008), and both redistribution of blood flow and increased cardiac output were shown to be contributing factors. While differing lipid levels were not examined in this part of our study, they are believed to have a relatively minimal impact upon the SDA response (Cho et al., 1976, Seth et al., 2008). This is likely due to the relatively simple physiological responses associated with either catabolism or anabolism of lipid components, while more complex processes are required to lyse and assimilate protein to replace old tissues or form new ones.

Mean observed SDA duration for all treatments was 1.74 d (41.8 h). Similarly, Eliason et al. (2007) observed elevated metabolic rates (above standard metabolism) for either 30 h (Simon Frasier University) or the duration of their trial (60-96 h post-feeding, University of British Columbia) for fish force-fed 2% of their body weight of three different diets. An earlier study by Beamish et al. (1986) concluded that rainbow trout offered 1.5% body weight of feed on a daily basis, for either 3 or 8 consecutive days, returned to pre-feeding metabolic rates after 2-3 d of fasting. One should note that these fish were forced to swim at a slow speed (20 $cm \cdot s^{-1}$), so the speed at which consumed calories were burned are likely slightly faster than fish held in a static water bath and allowed to regulate their own activity levels (Beamish et al., 1986). Conversely, a study examining the SDA of rainbow trout injected directly with differing mixes of amino acids showed that metabolic rates returned to pre-feeding levels after an average duration of approximately 21 (± 2) h, suggesting that digestion of the whole protein chains as well as the handling of fiber and other low-digestibility components within practical diets prolongs the SDA response in rainbow trout (Kaczanowski and Beamish, 1996).

SDA coefficient was roughly between 0.8% and 8.8% of the calories consumed during the feeding event. Eliason et al. (2007) observed SDA coefficient values between 4% and 12% of the digestible energy consumed by rainbow trout at similar temperatures (8.2-16.0° C) during a study examining feeds formulated to either 35:20, 45:15, or 55:10 P:L ratios. The fully-automated systems used by this group of investigators allowed for a collection of a higher number of samples as well as decreased disturbance of the fish, which in turn generated much smaller S. E. M. (0.3 to 1.5%) than was observed during our study (1.9 to 3.0%), highlighting possible bias due to the activities of the investigators during data collection and manipulation of valves controlling the flow of water (Eliason et al., 2007).

Another possible reason for the variability in MO_2 measurements were the volitional movement of individual fish within our tanks; i.e., one or multiple trout within a given respirometry tank. Fish in the tanks could move on their own accord, or perhaps when disturbed by vibrations or movement of the investigators around the tank; the increased activity levels would raise their oxygen consumption rates. Interactions between the active trout and others within the tank would likely further increase activity levels and, thus, observed MO_2. Much of the "noise" observed in our data was likely due to these spontaneous variations in activity. Previous investigators (Beamish et al., 1986, Medland and Beamish, 1985) have suggested that flows within respirometry chambers be kept at low velocities, but high enough to reduce spontaneous activity of fish within the chambers. While the respirometry chambers used during our study did not allow this type of flow, the inhibition of spontaneous activity of the fish in our experiment was not our intent. The full-tank approach utilized in this study more closely mimics the rearing environment used in aquaculture facilities, where flow velocities in tanks or raceways are generally quite low, and voluntary movements are not inhibited due to the confining nature of

a small respirometry chamber. Additionally, Bureau et al. (2002) has expressed skepticism as to the applicability of the results of studies in which fish are forced to maintain a given activity level, as these likely affect observed SDA responses due to possible energy partitioning.

Furthermore, variation in meal portion size among individual fish within a tank could further confound accurate determination of SDA metrics, as larger quantities of feed are associated with both increased duration of SDA as well as an increase in the magnitude of the peak (Alsop and Wood, 1997, McCue, 2006). While not quantified, investigators on this study did note that some fish routinely consumed more than other fish in the same tank. This variation among individual feed consumption rates, varying activity levels within a tank during a given trial, and possible diel fluctuations in metabolism (McCue, 2006, Brett, 1962) could further mask the effects of feeding upon postprandial metabolism during our study.

This study illustrates the challenge of quantifying the SDA response in rainbow trout in a group setting, but may, in a way, represent good news to fish culturists. Inland trout farms typically use small ponds and raceways, which can be thought of as larger versions of the tanks utilized in this study. Often the fish are stocked at high densities to maximize total fish output from the rearing space available. Under these conditions, it's likely that the daily fish husbandry and facility maintenance activities momentarily increase activity levels of fish exposed to them. As observed in our study, these spontaneous activities of fish due to these external stimuli and volitional movement unrelated to external stimuli overshadow the SDA response discussed in the literature (Kaczanowski and Beamish, 1995). Whole-tank (group) respirometry may be less-than-ideal as a tool to examine and quantify the SDA response of salmonids, when compared to individual respirometry within a confined chamber, as described by Cech (1990). However, a whole-tank approach is useful. Results outlined here suggest that alternative protein sources are a

secondary concern when compared with metabolic oxygen demands due to the activity levels of fish within the rearing environment. These activity levels are influenced by both intra-fish encounters and interactions between the rearing environment and activities proximal to it. It may be advantageous for culturists to limit activities that cause stress to their fish whenever possible, while still maintaining appropriate feeding and cleaning schedules. During severely stressful situations (i.e. collection of a subsample of fish for weighing and measuring, collection for transport and stocking, significant cleaning and maintenance of facilities) increased aeration and mild sedation of the fish would be advisable to decrease possible problems associated with hypoxia, as well as physical damage to the slime coat that may be caused due to abrasions.

4.2 Comparison of Plant-based and Fish-based feeds Formulated to Different Protein and Lipid Ratios

The respirometry trials we conducted successfully measured the metabolic rates of rainbow trout, pre and post-feeding. As in Year 1, no statistically significant differences in post-feeding metabolic indices were noted among fish consuming the three feeds tested during out study. However, a post-feeding increase in metabolic rates, followed by a gradual decrease to routine metabolic rates over a period of several days (range: 48.3 to 60.6 h) was observed, illustrating the classic SDA response observed in fasted fish.

Seth et al. (2008) found that trout force-fed predigested diets containing differing levels of protein, lipid, and carbohydrate after a period of fasting exhibited differing SDA responses, with the high protein diet (P:L=70:5) eliciting a delayed and increased spike in oxygen consumption, when compared to the balanced diet (P:L=50:25). The high lipid diet used in this study (P:L=15:60) failed to elicit a significant SDA response (Seth et al., 2008). The fish-based 45:20 diet used in our study likewise caused the highest SDA maximum value (Figure 3.7), the largest SDA oxygen consumed value (Figure 3.10), and the largest SDA coefficient value

(Figure 3.11), however, no statistical differences were noted among all three feeds examined in our respirometry trials. A previous trial examining the SDA response in rainbow trout fed diets with differing P:L ratios were likewise unable to find any statistically significant differences when examining feeds formulated to 55:10, 45:15, and 35:20 P:L ratios (Eliason et al., 2007). Furthermore, similar studies examining the SDA of southern catfish (*Silurus meridionalis*) (Luo and Xie, 2008), as well as Atlantic cod (*Gadus morhua*) and haddock (*Melannogramus aeglefinus*) (Perez-Casanova et al., 2010) fed diets differing in P:L ratio were also unable to find any significant differences in similar SDA metrics. An early study by Medland and Beamish (1985) observed few statistically significant differences, but noted generally increasing SDA responses as dietary protein level increased (protein levels: 34, 48, and 58%) and no relationship between SDA and dietary lipid ratio except in the low protein feed, where fish consuming the 23% lipid diet had a generally greater SDA response than those consuming the 7% lipid diet.

To date, studies comparing the SDA response of rainbow trout consuming diets formulated from plant-based ingredients to those consuming fish-based diets are lacking, aside from these trials. No differences in SDA metrics were observed, suggesting that these ingredients will not have a differing affect on the SDA response of fish that consume them. Further studies may be necessary, with increased sample sizes, to elucidate any possible differences in SDA responses of rainbow trout consuming diets containing proteins from different sources. However, it appears that aquaculturists need not be overly concerned with the increase in post-feeding metabolic rates of rainbow trout consuming plant-based feeds. As described in this study, post-feeding metabolic rates are comparable between plant and fish-based diets, and problems associated with increased oxygen consumption rates are unlikely to arise, given that adequate aeration is already being supplied.

Works cited

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