**Growth, proximate composition, and sensory characteristics of Rainbow Trout
Oncorhynchus mykiss consuming alternative proteins**

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Abstract

Increases in the price of fishmeal have spurred increases in the cost of aquafeeds to fish production facilities. This intensified a search for alternatives to fish meal. In this study, eight experimental aquafeeds were formulated from 4 ingredient groups that excluded fishmeal. These ingredient groups were designed as an animal product diet (APD), a novel plants diet (NPD), a plants with future potential diet (PFP), and a plant products diet (PPD). These diets were compared to two standard fishmeal based diets (FMD). Each ingredient group was formulated into high protein (45%) and low protein (40%) diets and used in a 5×2 factorial feeding trial. Diets were randomly assigned to three replicate tanks containing 15 juvenile Rainbow Trout *Oncorhynchus mykiss* (mean initial weight= 91.3 g). After the growth trial fish from each treatment were used in a sensory analysis, examining the aroma, flavor, and texture of the fillets harvested. The 40% protein FMD produced both the highest final wet weight: 441 ± 5.7 g (mean $\pm$ SEM) and lowest feed conversion ratio 0.93 ± 0.02 g feed consumed/g weight gain (mean $\pm$ SEM). However, the best performing experimental diets had comparable values. More differences in growth metrics were noted among different feed ingredient groups than between protein levels. Proximate analyses were also used to determine proportions of moisture, protein, lipid, and energy within a subsample of fish from each treatment. These analyses indicated protein sparing and lipid sparing by fish consuming the 40% protein diets and 45% protein diets, respectively. Sensory analyses identified few significant differences in fillet characteristics among the different treatments. The results indicate that diets composed solely of plant-based ingredients can promote similar rates of Rainbow Trout growth, produce high quality fillets, and be less costly to fish producers.

Keywords: Rainbow Trout; nutrition; growth; alternative proteins; sensory analysis

1. Introduction

Finfish are consumed worldwide, and both the demand for and the production of finfish 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). 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%. Demand for fish meal and fish oil will likely continue to increase, tracking aquaculture production (FAO, 2012; Tacon and Metian, 2008) and current harvest rates of the targeted fishes 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). The price of fish meal historically fluctuated between \$200 and \$400 per metric ton, but is now at approximately \$1500/ton (Hardy, 2010; Indexmundi 2016). The aquaculture industry recently consumed 65% and 89% of global annual fish meal and fish oil production, respectively (Tacon and Metian, 2008; Hardy, 2010). Thus, much of the economic volatility of fishmeal and fish oil prices will fall upon fish farming operations and hatcheries. This desire for sustainable protein sources and the economic pressure have increased efforts to evaluate protein replacements for fish meal in commercial finfish feeds that support efficient fish growth and that are economically feasible and ecologically sustainable.

The Rainbow Trout (*Oncorhynchus mykiss*) is a commonly raised fish species in aquaculture (Stickney, 1996). In addition to the fish raised for consumption, millions of Rainbow Trout are raised for stocking into ponds, lakes, streams, and rivers to provide additional

sportfishing opportunities where few may otherwise exist. Together, Rainbow Trout cultured directly for human consumption, and those cultured to enhance or provide sportfishing opportunities, account for a measurable proportion of global aquaculture production, with total production exceeding 810,000 tons in 2013 (FAO, 2015).

Efforts to find suitable protein sources to replace fishmeal in Rainbow Trout feeds have been pursued for well over 20 years (see Fontainhas-Fernandez et al., 1999; Gomes et al., 1995; Olivatel et al., 1994; Watanabe et al., 1996). Studies have focused on evaluating a variety of different plant and animal protein ingredients to meet the fish's nutritional needs. Plant-based protein sources that have been tested include corn gluten meal, soy meal, soy protein concentrate, wheat gluten meal, barley protein concentrates, cottonseed meal, and canola (rapeseed) meal. Animal-based protein sources tested to date include poultry by-product meal, feather meal, and blood meal. A common challenge to 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.

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). 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). 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 identify commercially available alternate ingredient combinations that can meet the production needs of Rainbow Trout. The study had the following objectives. First, to evaluate the growth of Rainbow Trout fed diets formulated with different combinations of plant-based or plant and animal-based proteins, and to compare the results to those of Rainbow Trout fed a fish meal-based diet. The second study objective was to determine if dietary protein levels could be reduced without incurring reductions in Rainbow Trout performance provided the reduced protein diets were supplemented with essential amino acids.

2. Methods

2.1. Feed Ingredients and experimental design

Diets were formulated on a digestible-energy and available-amino-acid basis based on data from Gaylord et al., 2008, 2010, and Barrows et al., 2014. Diets were not formulated to be isocaloric but digestible energy was allowed to vary between 4191 to 5194 cal/g. Five ingredients combinations were used: 1) Fishmeal Diet, (FMD) used as the control diet; 2) Animal Product Diet (APD); 3) Plant Product Diet, (PPD); 4) Novel Plant Protein Diet (NPD), and; 5) Plant Products with Future Potential (PFP). Detailed information on diet formulations is provided in Table 1.

Each ingredient combination was formulated to two nutrient concentrations (45% crude protein: 20% crude lipid; 40 – 42% digestible protein) to meet amino acid targets of Rainbow trout (Hardy, 2002), and 40% crude protein – 20% crude lipid (37 – 38% digestible protein) to meet the ideal amino acid balance of Rainbow Trout muscle for lysine, methionine and threonine (Table 1) (Gaylord and Barrows, 2009).

Eyed Rainbow Trout eggs (fall steelhead strain) were received from Troutlodge, Inc. (Sumner, WA, USA) on December 9, 2010, and incubated at 10°C at the Foothills Fisheries

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 $\pm$ SEM). Water temperatures were gradually increased to $15 \pm 1^\circ$ C and maintained at this temperature for the duration of the experiment. At the start of the feeding trial, thirty 65-L tanks (n = 3 tanks per diet $\times$ nutrient level) were stocked with 15 Rainbow Trout that were individually marked with a 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. Fish were fed twice daily (0900 hours and 1700 hours) 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 sequentially, 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 (tricaine methanesulfonate; 100 mg/L, buffered to neutral pH with NaHCO_3), individually weighed (g) and measured (SL, FL, TL; mm). The feeding trial lasted 88 days. Laboratory lighting mimicked a natural photoperiod at 40.585°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) calculated as per Bureau et al., (2002) and Stickney (2005). 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 for shipment to the BFTC. 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) were determined using standard methods (AOAC, 1995). Analyses were grouped by tank.

2.4. Sensory Analyses

Sensory analyses, examining the aroma, flavor, and texture of fillets were also conducted. At the conclusion of the trial, 3 fish/tank (6 fish/tank consuming the FMD and FMD+ diets) were harvested. Fillets were individually packaged, flash frozen, and shipped to Washington State University (WSU) at -20°C.

Fillets were skinned and individually packaged in 'Ziploc' freezer bags and stored at -23°C until analysis. Prior to analysis, fillets were partially thawed, just enough to cut with a knife; about 10 mm were removed from the head and ventral portions, and then 9.0 ± 1.0 g serving portions were sliced across the fillet from dorsal to ventral. Frozen fillet portions (six to nine pieces) were vacuum packed in 25 × 30 cm boil-in pouches. Vacuum-packed fillet samples were immediately returned to -23°C until sensory analysis. For sensory analyses, samples were

poached at 74°C for 6 min, then removed from the boiling pouch and held under the radiant heat unit at setting '6.5' for a maximum of 15 min before serving to consumers.

Sensory testing was conducted over six test dates in the WSU School of Food Science sensory laboratory. Two sensory evaluation studies were completed. Over the first four days of testing, all diets (APD, NPD, PFP and PPD, with the protein:lipid ratio held constant at either 40:20 or 45:20) were compared to the control diet (FMD) at the appropriate protein:lipid ratio. For these comparisons, 60 consumers participated. Consumers were recruited from the Washington State University community. Panelists were from a diverse ethnic background and ranged in age from 18 to 65, and 61% of consumers were female. All participants signed an Informed Consent Form and the project was approved by the Washington State University Institutional Review Board.

Just prior to serving, each fillet sample was transferred from the radiant warmer to a 4 oz soufflé cup identified with a 3-digit code, then sealed with a cap to trap aroma. Panelists were provided with reagent grade deionized water and unsalted-top saltine crackers for rinsing the palate. A cuspidor was provided for expectoration. Pair comparisons were presented in random order under white light. Using a paired difference test and paired preference test, panelists were asked to indicate which sample in a pair had more: 1) fishy aroma, 2) fishy flavor, 3) grassy flavor, 4) firm texture, and 5) which sample they preferred overall. Panelists were asked to comment as to why they preferred one sample over another.

In the second sensory evaluation study, over two days of sensory evaluation testing, the influence of protein: lipid ratio was determined. Specifically, within a given diet, the 45:20 protein:lipid ratio (denoted by a *) was compared to the 40:20 protein: lipid ratio. For these comparisons, 48 consumers participated. All other conditions are as described above.

Statistical Analyses.— Two-way ANOVA were 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).

Sensory evaluation results were analyzed using Compusense® Five software (Compusense Inc., Guelph, ON) and the binomial distribution tables for paired comparison (Roessler et al., 1978). Significance is reported as $p \leq 0.05$, representing minimum agreeing judgments of 39, if 60 consumers participated or 32 consumers, if 48 consumers participated in the study.

3. Results

3.1. Feed Consumption

Estimated feed consumption rates varied between 4.47 (PFP) and 3.80 g·fish/d (PPD). These correspond to rates of 2.12 to 2.47 % body weight per day (Table 2). Feed consumption rates of individual fish were not measured, but when analyzed in terms of whole tank consumption rates, there was a significant diet effect ($F = 11.38$; $df = 4$, $P < 0.0001$) wherein the fish fed NPD had higher consumption rates than fish fed any of the other diets. Similarly, there was a significant P:L ratio effect, where fish receiving the higher protein levels consumed slightly more food per day than those on the lower protein levels ($F = 39.49$; $df = 1$; $P < 0.0001$).

3.2. Fish Weight

Mean final fish weights ranged from 382 g (PPD) to 441 g (FMD+) (Table 2). Feed group was a statistically significant predictor variable ($F = 4.70$; $df = 4$; $P = 0.0002$) with fish fed FMD+ producing the highest mean weight, followed by the PFP+ (429.9 g) and FMD (423.9 g).

Most of the means were not significantly different, indicating that the performance of fish consuming these feeds was similar among most of the diets. The only difference in final fish weight was between the FMD+ and PPD treatments. P:L ratio did not have a significant effect on final wet weight ($F > 0.0001$; $df = 1$; $P = 0.9973$), and there was no significant interaction between protein:lipid ratio $\times$ feed group ($F=0.96$; $df=4$; $P=0.4533$).

3.3. Feed Conversion Ratio

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

3.4. Specific Growth Rate

Specific growth rates were significantly affected by feed group ($F = 9.38$; $df = 4$; $P = 0.0002$), with the highest SGR observed in the FMD+ (1.82) and FMD (1.80) treatments (Figure 1), while the PPD (1.66), NPD (1.66) and NPD+ (1.65) feeds delivered the lowest SGR. Neither protein:lipid ratio ($F > 0.001$; $df = 1$; $P = 0.9973$) or the interaction between feed group and protein:lipid ratio ($F = 9.551$; $df = 4$; $P = 0.4533$) significantly affected SGR.

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 3). No statistically significant relationships were found between feed group ($F = 2.615$; $df = 4$; $P = 0.0660$), the protein:lipid ratio ($F = 2.655$; $df = 1$; $P = 0.1189$), or the interaction between these two terms ($F = 2.279$; $df = 4$; $P = 0.0967$).

3.6. *Wet biomass energy content*

Mean energy contents range from 2723 (PFP+) to 2999 (PFP) $\text{cal}\cdot\text{g}^{-1}$ (Table 3), and feed group ($F = 0.2960$; $\text{df} = 4$; $P = 0.8771$), protein:lipid ratio ($F = 2.930$; $\text{df} = 1$; $P = 0.1024$), and their interaction ($F = 0.9775$; $\text{df} = 4$; $P = 0.4419$) all lacked significant effects. Mean whole-body energy content for all 45% protein feed treatments was $2883 \text{ cal}\cdot\text{g}^{-1}$, while mean content for all 40% protein feed treatments was $2765 \text{ cal}\cdot\text{g}^{-1}$.

3.7. *Wet biomass fat content*

Whole-body fat content ranged from 17.2 (NPD+) to 22.5% (PFP) (Table 3). Feed group did not have a statistically significant effect ($F = 2.556$; $\text{df} = 4$; $P = 0.0706$), but protein:lipid ratio ($F = 11.130$; $\text{df} = 1$; $P = 0.0033$) and the feed group $\times$ protein:lipid ratio interaction ($F = 3.482$; $\text{df} = 4$; $P = 0.0259$) did. Fish consuming 45CP:20CL feeds had a mean wet fat content of 20.4%, while those fed the 40CP:20CL diets had a mean of 18.4%.

3.8. *Wet biomass protein content*

Mean whole-body protein content of fish from our study ranged from 14.2% (PPD) to 16.2% (PPD+) (Table 3). Feed group ($F = 0.486$; $\text{df} = 4$; $P = 0.7461$) and the feed group and protein:lipid ratio interaction ($F = 0.864$; $\text{df} = 4$; $P = 0.5024$) were not significant, but protein:lipid ratio was significant ($F = 9.680$; $\text{DF} = 1$, $P = 0.0055$). 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. *Sensory analyses*

The sensory panel found that across all diet comparisons, the FMD (control) diet had a firmer texture. When the FMD diet was compared to the APD or NPD diets, the FMD diet yielded a sample with a more intense fish aroma. The APD diet had a more grassy flavor

compared to the FMD diet, and when the FMD diet was compared to the PPD diet, the FMD was more preferred ($P < 0.05$). Figure 2A shows the results of the comparison between the control diet (FMD) and each treatment diet, all held constant at the 45:20 protein:lipid ratio.

Each treatment diet was also compared to the FMD control at the 40:20 protein:lipid ratio (Figure 2B). As seen with the 45:20 protein:lipid ratio, the FMD+ diet had a more intense fish aroma than the APD+ or NPD+ diets. The FMD+ diet was rated higher in fish flavor and lower in grassy flavor than the NPD+ diet. Unlike the 45CP:20CL diets, no significant differences in texture were observed among the comparisons.

The influence of the protein:lipid ratio on specific sensory properties was also determined (Figure 3). When the PPD diet at the two protein:lipid ratios was compared, the PPD+ diet was firmer and higher in grassy flavor, while the PPD was higher in fish flavor. Differences were also observed within the PFP diet, with the PFP diet being significantly higher in fish flavor and the PFP+ diet being significantly more firm.

4. Discussion

The value of this experiment is its simultaneous evaluation of numerous experimental diets for Rainbow Trout, formulated from a wide range of commercially available or potential plant and animal-derived protein sources. Other studies have often focused a single protein source at incremental levels of replacement of fish meal, but we evaluated Rainbow Trout feeds with 100% fishmeal replacement, and compared them to our two fishmeal-based control diets. We observed a great deal of statistical overlap among growth metrics of the Rainbow Trout fed different diets. This suggests that diet ingredient selection criteria could be more heavily influenced by ingredient prices and availability, provided these ingredients are used in a

nutritionally-balanced feed. From a consumer acceptance standpoint, fishmeal-free diets performed at similar levels.

4.1. Growth metrics

Feed group formulation significantly affected all of the Rainbow Trout growth metrics examined (final wet weight, SGR, FCR). Not surprisingly, the fishmeal-based (control) diets produced superior results in terms of SGR, FCR, and mean final wet weight. Nevertheless, the notable overlap among all feed groups in many of the examined growth metrics suggests that carefully-formulated feeds using animal and plant-based proteins can promote growth rates similar to those observed in fish consuming traditional, fishmeal-based diets.

The results in this study support prior research where growth performance was comparable to or slightly reduced when Rainbow Trout were fed plant-based diets (Burr et al., 2012; Wacyk et al., 2012). Burr et al., (2012) noted that Rainbow Trout fed diets where soy protein concentrate replaced fish meal (63 and 82% replacement) grew at similar rates to those fed a fishmeal-based diet. Fish fed a 100% replacement diet experienced reduced growth in their study. Similarly, Wacyk et al. (2012) saw reduced growth rates in trout consuming diets with 100% replacement of fish meal by soy protein isolate and corn gluten, but comparable growth rates among those consuming the fish meal-based control diet and those in which smaller proportions (8.4%, 20.2%) of the fish meal were replaced with corn gluten. FCR values for the Wacyk et al. (2012) study ranged between 1.2 and 1.5, whereas those observed in the current study were notably better for most of the feed formulations tested, ranging from 0.93 (FMD+ diet) to 1.24 (NPD+ diet). Wacyk et al. (2012) used smaller fish at the start of their study than in the current study, and their fish did not reach the same range of final sizes. 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); nevertheless, given this trend, one would expect that if our study had used younger, smaller fish, the difference in FCR might have been even greater. It is also possible that some other factor, such as a strain 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 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 promote altered protein synthesis rates and increased catabolism of proteins (Sealey et al., 2009). Other plant-based ingredients, including cottonseed meal, canola, and barley, contain ANF, or are otherwise limited in their nutritional profiles. These factors likely contributed to the reduced SGR of fish fed the 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/kg, at 75 g/kg 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).

Our feeding trial results support the notion 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 six 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-week study, the results were similar to those observed in our study. The standard fishmeal control diet produced the highest SGR (2.67 g per fish per day), and lowest FCR (0.88 g feed fed per g 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 for both high and low nutrient fish and barley meals (2.35, 2.6, and 2.3 g per fish per d, respectively) were statistically similar to 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 per g 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 per fish per day) 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 can support growth rates identical to those generated 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% vs. 1275% mean weight gain over 16 weeks for feeds at 40%,

60%, and 100% levels of fish meal replacement, respectively) and decreased 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) recommended slightly different proportions of fat and protein in Rainbow Trout diets (40% protein with 20% lipid). 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 impact waters receiving culture facility effluent (Hardy, 2002). Many plant-based ingredients have lower overall phosphorous contents, allowing for the formulation of low-phosphorous feeds, can minimize 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 energy contents than did those fish consuming the diets formulated to the 40:20 ratio), possibly due to the higher energy densities of the increased fat content generally found in fish consuming the 45:20 P:L diets (Table 3). The FMD feed group fed fish were a notable exception to this trend. Furthermore, fish fed the 45:20 diets generally had higher wet fat and lower wet

protein contents when compared to the 40:20 diets. 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” have 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 were notably higher and final dry biomass protein contents 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 per L) was higher than initial stocking densities in the studies listed above (1, 7, 11, and 14 g fish per L, respectively). At our higher densities, our fish may have had reduced mobility within the tanks, decreasing activity levels, and thus leading to higher levels of fat storage and reduced muscle (protein) development; there may also have been a strain-level difference, though this was not evaluated.

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

430 a large number of trout within a production facility, could lead to notable differences in growth
431 rates, total feed consumed during the production period, and, ultimately, profit margins. Formal
432 cost-benefit analyses are needed to determine break-even points concerning the money saved by
433 these less-expensive feeds versus the increased overall food consumption and fish care required
434 during the extended grow out period. Our data show, however, that feeds formulated using plant
435 and animal-based proteins using current knowledge of the nutritional requirements of Rainbow
436 Trout produce favorable growth rates and should be considered for use by aquaculturists due to
437 their economic benefits.

438

Acknowledgements

This project was supported by Western Regional Aquaculture Center Grant nos. 2006-38500-17048 and 2010-38500-21758 from the USDA National Institute of Food and Agriculture. Personnel from the Western Regional Aquaculture Center assisted with all phases of the study and report. The authors thank J. Callison, K. Oles, B. Robey, T. Swarr, B. Welk, N. Wilson, I. Wilson., and R. Winkelman for their considerable assistance with tank system construction, fish care, data collection, and troubleshooting of any laboratory problems that arose, even at inconvenient times. Additional thanks to O. Aon, J. Anderson, R. Dana, S. Dugan, A. Ficke, E. Gardunio, S. Hoyer, L. Hargis, A. Schwindt, A. Herdrich, A. McMahon, A. Milano, D. Pruitt, A. Riverman, S. Sedillo, N. Vieira, J. Wardell, Z. Underwood for their assistance. We also wish to thank A. Hess and J. zumBrunner of the Franklin A. Graybill Statistical Laboratory at Colorado State University for help with statistical consulting and data analysis. All animal care and handling protocols were approved by the Colorado State University Institutional Animal Care and Use Committee protocol 10-2318A, and personnel were trained as required by the committee. The authors have no conflicts of interest to disclose.

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Table 1. Formulations and nutrient targets of diets fed to rainbow trout to determine the ability to remove fishmeal from feeds and reduce dietary protein levels.

Ingredient index	1	2	3	4	5	6	7	8	9	10
	FMD	FMD+	APD	APD+	PPD	PPD+	NPD	NPD+	PFP	PFP+
Menhaden fishmeal	22.00	17.60								
Poultry by-product, pet food	6.00	5.30	27.00	25.00						
Blood meal	4.00	3.20	5.00	3.00						
Feather meal			5.00	3.00						
Soybean meal 48%CP	15.00	12.00	15.00	11.71	15.00	15.00	0.00	12.00		
Corn protein concentrate	15.00	12.00	9.00	7.03	23.00	17.00	22.00	9.60	12.00	9.60
Soy protein concentrate-Profine VF					23.00	17.00				
High protein distillers dried grains							15.00	12.00		
Soy protein concentrate-Hamlet Protein							35.00	28.00		
Soy full fat, Schillinger Gen.,									30.00	24.00
Spirulina-Earthrise									15.00	12.00
Barley protein concentrate									12.00	9.60
Wheat flour	16.36	22.81	17.65	23.90	11.99	19.18	1.71	7.26	5.42	14.40
Monocalcium Phosphate	0.50	1.40	1.10	1.50	3.40	3.80	3.10	3.55	2.30	2.80
DL-Methionine	0.22	0.58	0.44	0.74	0.37	0.70	0.39	0.81	0.31	0.66
Lysine HCl	0.00	2.79	0.00	2.95	0.19	3.16	0.54	3.20	0.00	2.94
Threonine	0.00	0.77	0.00	0.91	0.00	0.82	0.00	0.78	0.00	0.74
Tryptophan	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Menhaden fish oil	15.99	16.62	14.88	15.33	18.12	18.41	17.33	17.89	18.04	18.33
Choline Cl 50%	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin premix ARS 702	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Potassium chloride	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Taurine	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
NaCl	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Biofix Plus	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay-C 35	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
TM ARS 640	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Astaxanthin	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Magnesium Oxide	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06

Formulated nutrient content	FMD	FMD+	APD	APD+	PPD	PPD+	NPD	NPD+	PFP	PFP+
CP dry	45.4	41.3	45.1	40.2	45.2	40.4	45.7	40.5	45.3	41.0
Lipid	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
DP dry	40.2	36.9	39.9	37.3	42.5	38.1	40.8	36.8	41.8	38.0
Digestible Energy	4919	4613	4788	4605	4991	4618	4519	4191	5194	4825
Total Phosphorus	1.19	1.18	0.98	1.01	1.03	1.07	1.05	1.14	1.17	1.14
Available-Phosphorus	0.53	0.53	0.53	0.52	0.52	0.53	0.53	0.53	0.53	0.53
Arg	2.55	2.11	2.86	2.36	2.86	2.33	2.52	2.35	2.77	2.26
His	1.02	0.83	0.93	0.86	0.96	0.76	1.03	0.85	0.94	0.75
Ile	1.58	1.30	1.42	1.43	1.78	1.43	1.73	1.43	1.91	1.56
Leu	4.62	3.78	3.95	3.58	5.14	4.04	5.44	3.83	4.36	3.55
Lys	2.02	3.82	1.85	3.82	1.82	3.81	1.80	3.82	1.89	3.82
Met	1.10	1.30	1.10	1.30	1.14	1.30	1.10	1.30	1.10	1.30
Phe	2.21	1.82	2.08	1.84	2.52	2.01	2.52	1.94	2.41	1.97
Thr	1.67	2.14	1.53	2.14	1.64	2.14	1.64	2.14	1.72	2.14
Val	2.28	1.87	2.17	2.02	2.11	1.70	2.13	1.73	2.32	1.90

2 Table 2. Summary of the effects of diet type and protein:lipid ratio on fish size, and mean daily food consumption rate for Rainbow
3 Trout used in a 12-week growth study. Values represent means ($\pm$ SEM) of the experimental diets. Values with different superscripted
4 letters within a column denote statistically significant differences ($P < 0.05$).

Diet Code	Protein:Lipid Ratio ^a	Feed Consumed ^b	Final Wet Weight ^c	Food Consumption Rate ^d
APD+	40:20	3.83	400.7 $\pm$ 13.2 ^{yz}	2.12 $\pm$ 0.04 ^w
APD	45:20	4.27	423.4 $\pm$ 8.8 ^{yz}	2.30 $\pm$ 0.03 ^{xy}
FMD+	40:20	4.15	440.6 $\pm$ 5.7 ^y	2.24 $\pm$ 0.05 ^{wxy}
FMD	45:20	4.05	423.9 $\pm$ 6.7 ^{yz}	2.25 $\pm$ 0.04 ^{wxy}
NPD+	40:20	4.22	398.7 $\pm$ 16.1 ^{yz}	2.30 $\pm$ 0.05 ^{xyz}
NPD	45:20	4.34	390.9 $\pm$ 5.8 ^{yz}	2.47 $\pm$ 0.01 ^z
PFP+	40:20	3.94	429.9 $\pm$ 16.6 ^{yz}	2.14 $\pm$ 0.03 ^{wx}
PFP	45:20	4.47	422.3 $\pm$ 15.4 ^{yz}	2.39 $\pm$ 0.02 ^{yz}
PPD+	40:20	4.06	422.6 $\pm$ 14.6 ^{yz}	2.14 $\pm$ 0.03 ^{wx}
PPD	45:20	3.8	382.3 $\pm$ 2.7 ^z	2.21 $\pm$ 0.02 ^{wx}

^a Values listed represent % diet protein: % diet lipid, by weight

^b Values listed represent estimated g consumed per fish per day.

^c Values listed represent final biomass (g).

^d Values listed represent mean daily food consumption rate in percent body weight per day.

13 Table 3. Effects of diet type and protein:lipid ratio on the proximate compositions of Rainbow Trout fed diets with varying protein
14 sources over a 12-week period. Values are means $\pm$ SEM. Differing superscripted letters represent means found to be statistically
15 different at the $\alpha = 0.05$ level using a Tukey-Kramer adjustment for multiple comparisons (ANOVA using Tukey HSD).

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

16

17 ^a Values listed are % biomass $\pm$ S. E. M.

18 ^b Values listed are calories per gram $\pm$ S. E. M.

1 Figures
2 Figure 1. Effects of diet type and protein:lipid ratio on feed conversion rate (columns) and
3 specific growth rate (open circles) for Rainbow Trout used in a 12-week growth study. Values
4 represent means ($\pm$ SEM) of the experimental diets. Columns or points with different
5 superscripted letters denote statistically significant differences ($P < 0.05$).

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Figure 3. Consumer (n = 48) scores indicating the more intense attribute and the preferred sample in a pair comparing a specific diet at two protein:lipid ratios (45:20 vs. 40:20). Darker shades of gray reflect greater consumer selection or agreement. The closer the level of gray between pairs, the more similar the consumer scores. Within a given comparison of the diet with the control diet (FMD), significant differences ($P < 0.05$) are indicated by different subscripted letters.