



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 plant protein diet (NPD), a plant products with future potential diet (PPF), and a plant product 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 being 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.

Statement of relevance

We show that alternative feeds efficiently grow trout.

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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

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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 a 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-Fernandes 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 (rape-seed) 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 (PPF). 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 ± 1 °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 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 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 h, 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 10-2318 A) 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

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