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IMPACTS ON SALMONID REARING PERFORMANCE WITH USE OF DIETARY BIOPROCESSED PLANT-BASED PROTEIN AND WATER VELOCITY

BY

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IMPACTS ON SALMONID REARING PERFORMANCE OF DIETARY BIOPROCESSED PLANT-BASED PROTEIN AND WATER VELOCITY

JILL M. VOORHEES

This thesis is approved as a credible and independent investigation by a candidate for the Master of Sciences in Natural Resources degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

ANOVA	analysis of variance
SE	standard error
K	Fulton’s condition factor
FCR	feed conversion ratio
SGR	specific growth rate
HSI	hepatosomatic index
SSI	splenosomatic index
VSI	viscerosomatic index
° C	degree Celsius
kg	kilogram
g	gram
mg	milligram
mm	millimeter
cm	centimeter
s	second
L	liter
mmt	million metric tons
ppm	parts per million
$CaCO_3$	Calcium Carbonate
MS-222	Tricaine methanesulfonate
bl/s	body lengths/second
BSM	bioprocessed soybean meal

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ABSTRACT

IMPACTS ON SALMONID REARING PERFORMANCE OF DIETARY BIOPROCESSED PLANTBASED PROTEIN AND WATER VELOCITY

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The inclusion of bioprocessed soybean (*Glycine max*) meal was evaluated in five experiments using either Rainbow Trout (*Oncorhynchus mykiss*) or Brown Trout (*Salmo trutta*). In the first experiment, adult Erwin x Arlee strain Rainbow Trout were fed diets formulated so that bioprocessed soybean meal (BSM) replaced 0, 60, or 80% of the dietary fishmeal. There were no significant differences in gain, percent gain, feed conversion ratio, specific growth rate, intestinal morphology, relative fin lengths, or organosomatic indices. In the second experiment, juvenile Plymouth strain Brown Trout were fed diets formulated so that BSM replaced 0, 60, 80, or 100% of the dietary fishmeal. Similar to the first experiment, there were no significant differences in gain, percent gain, specific growth rate, percent mortality, intestinal morphology, relative fin lengths, or organosomatic indices among the diets. Differing water velocities were an additional factor included in the final three experiments. The third experiment fed adult Erwin x Arlee strain Rainbow Trout diets where BSM replaced either 0 or 60% of the dietary fishmeal. Two velocity treatments of 3.6 or 33.2 cm/s were also included in the 2x2 study design. Neither diet nor velocity had any significant impact on gain, percent gain, specific growth rate, intestinal morphology, relative fin lengths, or organosomatic indices. However, feed conversion ratio was significantly lower in the lower velocity treatment compared to the higher velocity treatment. The 60% BSM diet also had a significantly lower feed conversion ratio than the fishmeal reference. There were also no interactions between diet and velocity. The fourth experiment examined juvenile Shasta strain Rainbow Trout fed three different where BSM replaced 0, 60, or 80% of the dietary fishmeal in conjunction with two different velocity treatments of 2.3 or 18.7 cm/s. The fish being fed the fishmeal diet ate significantly greater amounts of food than the 80% bioprocessed soybean meal diet. However, there were no significant differences among the diets in gain, percent gain, specific growth rate, percent mortality, relative fin length, intestinal histology, viscerosomatic index, or splenosomatic index among the diets. The fish at the lower velocity had significantly decreased growth compared to the fish at higher velocity. There was also a significant interaction between diet and exercise for the amount of food consumed. The fifth, and final, experiment examined juvenile Plymouth strain Brown Trout being fed diets where BSM replaced either 0 or 60% of the fishmeal and subjected to velocities of 2.8 or 16.1 cm/s. There were no significant differences in gain, percent gain, feed conversion ratio, specific growth rate, intestinal morphology, splenosomatic index, hepatosomatic index, or viscerosomatic index for the fish receiving either diet. However, gain, food fed, and specific growth rate were significantly higher for fish at the higher velocities. There were no significant interactions between diets and velocity in this experiment. In experiment three and experiment five towards the end of the experiments there was a significant decline in gain, percent gain, and specific growth rate for the fish reared in higher velocities, perhaps indicating exercise fatigue. Based on the results of these experiments, BSM can replace 100% of the fishmeal meal in diets of Brown Trout during normal rearing, and at least 60% of the fishmeal during continual

exercise. Bioprocessed soybean meal can replace at least 80% of Rainbow Trout diets, regardless of the exercise regimen.

CHAPTER 1: WHY SOYBEANS (*Glycine max*) IN AQUAFEEDS?

Introduction

Global population is continually increasing, increasing demand for sustainable protein sources for human consumption. In 2013, fish consumption accounted for about 17% of the global population intake of animal protein and 6.7% of all the protein consumed (FAO 2016). In 1960 the average per capita fish consumption was 9.9 kg, which rose to 14.4 kg in 1990, and reached a new record of 20.0 kg in 2014 (FAO 2016). With fish consumption increasing and capture fisheries stagnant, if not overfished, aquaculture will likely continue to grow (FAO 2016, 2017).

The fishery trade is a significant source of foreign currency earnings for many developing countries, with world exports of fish and fish products worth approximately $133 billion worldwide in 2014 (FAO 2016, 2017). In the United States, total aquaculture production was 608 million pounds in 2014, with a value of $1.33 billion (FUS 2016). The trout and salmon industries in alone the United States are worth about $152 million (FUS 2016).

One of the major impediments to aquaculture growth is the cost and unpredictability of the fish meal market (FAO 2016). Of the total world fisheries capture, 21 million tons of fish were destined for non-food products, and of that 21 million 76% were reduced to fishmeal and fish oil (FAO 2016). Capture fisheries fluctuations and increased aquafeed demand produces a need for cheaper, more sustainable, and more widely available feed-protein sources (Hardy 2010).

Fishmeal has historically been the primary protein source for aquafeeds for trout and other carnivorous fish (Satia 1974; Kim et al. 1991; Cheng and Hardy 2004), due to its high protein content, favorable amino acid profile, high palatability, high nutrient digestibility, few antinutritional factors, and wide availability (De Silva and Anderson 1995; Guillaume et al. 2001; Gatlin et al. 2007; NRC 2011). Small marine fishes such as Peruvian anchovy (*Engraulis ringens*), herring (*Clupea harengus*), or menhaden (*Brevoortia* sp.) are the primary species processed into fish meal, although a number of other species and fish offal byproducts are also used (Hertrampf and Piedad-Pascual 2000; Guillaume et al. 2001; FAO 2016). Fish meal is typically produced from whole fish, which after oil extraction, are chopped, cooked, decanted, pressed, shredded, and dried (De Silva and Anderson 1995, Guillaume et al. 2001).

Nutrient requirements for fish are primarily dependent upon species, age, and water quality (De Silva and Anderson 1995; Guillaume et al. 2001; NRC 2011). Fish feeding habits (herbivore, omnivore, or carnivore) typically dictate the proportion of dietary protein in fish diets (NRC 2011). For example, herbivorous fish like Tilapia (*Oreochromis*) or omnivorous fish like Channel Catfish (*Ictalurus punctatus*) have digestible protein requirements of 29% (dry-matter basis), but carnivorous fish like Rainbow Trout (*Oncorhynchus mykiss*) require at least 38% of their diets to be digestible protein (Guillaume et al. 2001; NRC 2011). When fish are intensively cultured, as in trout production, aquafeeds must provide fish with all the required nutrients (De Silva and Anderson 1995). Feed costs are often a major expense in intensive culture operations, and can account for 40-60% operating expenses (Fornshell et al. 2016). Protein is the single most important and expensive dietary component in aquafeeds, especially for carnivorous fish (NRC 2011).

With aquaculture demand growing, and fishmeal stocks stagnant and unreliable, there is a need to find alternative protein sources (Rana et al. 2009; FAO 2016). Many animal byproducts are used in feed manufacturing (NRC 2011), but are not likely to become a main protein source for aquaculture due to cost and unsuitable amino acid profiles. Some of the plant-based proteins that have been examined are soybeans (*Glycine max*), rapeseed (*Brassica* sp.), cotton (*Gossypium* sp.), common sunflower (*Helianthus annuus*), peanut (*Arachis hypogea*), oil palm (*Elaeis guineensis*), wheat (*Triticum aestivum*), corn (*Zea mays*), rice (*Oriza sativa*), beans (*Vicia faba*), lupins (*Lupinus* sp.), and peas (*Pisum sativum*) (Guillaume et al. 2001). Of these plant protein sources, soybeans have been one of the most researched for fishmeal replacement (Nordrum et al. 2000).

Soybeans

Soybeans are the leading oilseed crop produced globally (USDA 2017) and are relatively inexpensive, readily available, highly palatable, and contain a very suitable amino acid profile and high protein content (Sugiura et al. 1998; Refstie et al. 2000; Watanabe 2002; Gatlin et al. 2007; NRC 2011). However, soybeans have antinutritional factors that hinder fish digestion and may impact fish health (Salunkhe et al. 1992; Krogdahl et al. 1994, 2010, 2015; Kaushik et al. 1995; Bureau et al. 1998; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Gatlin et al. 2007; Iwashita et al. 2008; NRC 2011; Teng et al. 2012). Antinutritional factors are substances in feedstuffs that produce negative effects after ingestion, such as reduced feed intake, decreased growth, impaired nutrient digestibility and utilization, diminished internal organ function, or decreased disease resistance (Krogdahl et al. 2010; NRC 2011). Antinutritional factors in soybeans consist of at least five trypsin inhibitors (Salunkhe et al. 1992; Krogdahl et al. 1994; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Gatlin et al.2007; NRC 2011), lectins (Salunkhe et al. 1992; Francis et al. 2001; Gatlin et al. 2007; NRC 2011), saponins (Bureau et al. 1998; Salunkhe et al. 1992; Francis et al. 2001; Gatlin et al. 2007; NRC 2011; Krogdahl et al. 2015), phytic acid (Salunkhe et al. 1992; Francis et al. 2001; Gatlin et al. 2007; NRC 2011), antigenic proteins (Salunkhe et al. 1992; Kaushik et al. 1995; Gatlin et al. 2007; Teng et al. 2012), pytoestrogens (NRC 2011), antivitamins (NRC 2011), phytosterols (NRC 2011), and allergens (NRC 2011).

Trypsin inhibitors hinder proteases in the gastro-intestinal tract of monogastric animals (Francis et al. 2001; Gatlin et al. 2007; NRC 2011). Lectins bind specifically to carbohydrates, are not digestible, and are likely to cause immune responses in fish (Gatlin et al. 2007; NRC 2011). Saponins have an amphiphilic property to bind and form non-absorbable complexes with cholesterol (Krogdahl et al. 2015). Fish need phosphorous to survive, but two-thirds of the total phosphorous in soybeans is present in the form of phytic acid, which is not available to fish (Gatlin et al. 2007). Soybeans also have a large concentration of non-digestible carbohydrates (Salunkhe et al. 1992), specifically oligosaccharides such as stachyose and raffinose (van den Ingh et al. 1991, 1996; Bureau et al. 1998; Russert 2002; Gatlin et al. 2007). The antinutritional factors and carbohydrates limit the inclusion levels of soybean products in the diets of many carnivorous fish species (Fowler 1980; Reinitz 1980; Vielma et al. 2000; NRC 2011).

The use of high concentrations of dietary soybean products can induce enteritis and intestinal morphological changes in many salmonid species (van den Ingh et al. 1991; Rumsey et al. 1995; Burrells et al. 1999; Refstie et al. 2000). Shortened mucosal folds, increased epithelial vacuolization, increased numbers of inflammatory cells, macrophages, and neutrophilic granulocytes have all been observed in salmonids fed soybean meal (Baeverfjord and Krogdahl 1996; Bakke-McKellep et al. 2000; Heikkinen et al. 2006). Intestinal enteritis from soybean meal may also increase disease susceptibility (Krogdahl et al. 2000) and reduce nutrient absorption (Storebakken et al. 2000). However, Bureau et al. (1998) observed that salmonids might tolerate considerable intestinal inflammation and still maintain growth. The severity of the inflammation, the species of salmonid, and the processing techniques used for soybeans make inclusion rates inconsistent between species (Storebakken, et al. 2000).

Mechanisms exist to decrease or eliminate the antinutritional factors in soybeans. Heat occurring during the feed-extrusion process decreases lectins and proteinase inhibitors (Cheeke and Shull 1985; Liener 1994; Gomes et al. 1995; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Gatlin et al. 2007; Krogdahl et al. 2010; Bakke 2011). Saponins, sterols, and oligosaccharides can be reduced by alcohol extraction (Krogdahl et al. 2010). Bioprocessing, or fermentation, has also been shown to further decrease trypsin inhibitors, antigenic proteins, and non-digestible carbohydrates, while also increasing the protein concentration of the fermented product (Francis et al. 2001; Hong et al. 2004; Refstie et al. 2005; Gatlin et al. 2007; Yamamoto et al. 2010, 2012; Teng et al. 2012).

Aerobic Microbial Conversion

Fermentation is a metabolic process that consumes sugar in the absence of oxygen to produce organic acids, gases, or alcohol. Fermentation has been used to produce human food and drinks since the Neolithic times. Barley fermentation in beer processing is probably one of the most well-known fermentation processes (Loret et al. 2005).

Refstie et al. (2005) found that lactic acid fermentation of soybean meal eliminated sucrose, reduced levels of raffinose, and lowered trypsin inhibitor activities. Yamamoto et al. (2010) reported that fermented soybean meal in Rainbow Trout diets could replace 100% of dietary fishmeal in diets without any negative impacts on growth. The experiments described in this thesis evaluated a modified soybean meal produced using a proprietary microbial conversion process (SDSU, Brookings, SD, USA).

Exercise

In addition to specific dietary requirements, salmonids may react positively to exercise (resulting from increased water velocities) during hatchery rearing. During rearing in circular tanks, water velocity is typically adjusted to make tanks self-cleaning (Timmons et al. 1998; Davidson and Summerfelt 2004). However, higher rearing velocities may have a dramatic effect on feed consumption, feed conversion ratios (Davison and Goldspink 1977, Christiansen and Jobling 1990), growth (Leon 1986, Houlihan and Laurent 1987, Young and Cech 1993, Parker and Barnes 2014, 2015), fish physiology (Gallaugher, et al. 2001, Thorarensen and Farrell 2006), and immune competence (Takle and Castro 2013, Good, et al. 2016). Exercise may even increase post-stocking survival (Cresswell and Williams 1983, Parker and Barnes 2014, 2015), and potentially make them more aesthetically appealing to anglers due to reduced fin erosion (Christiansen and Jobling 1990).

Exercising salmonids by increasing water velocity has also led to improved swimming performance (Leon 1986; Gallaugher et al. 2001; Thorarensen and Farrell

2006) and reduced stress (Woodward and Smith 1985), although this may be dependent on the age or stage of the life cycle of a fish (Davison and Herbert 2013). Exercise has also been shown to decrease aggression, which may be partly responsible for increased growth (Davison and Herbert 2013). Salmonids are the main species examined in exercise studies, likely because exercise is a core component of their behavioral routine (Davison and Herbert 2013). Davison and Herbert (2013) stated the optimal swimming speeds for Rainbow Trout at 0.9-1.0 body length/s (bl/s) and 0.8 bl/s for Brown Trout (*Salmo trutta*). Rainbow Trout and Brown Trout have both shown improved growth during exercise (Davison 1997).

Diet and Exercise

While fishmeal alternatives have received considerable research focus, no previous studies have examined the use of plant-based diets in conjunction with exercise; the possible interaction of velocity and diet is unknown. Thus, the first objective of the experiment described in this thesis was to examine the possible inclusion levels of bioprocessed soybean meal in the diets of Rainbow Trout and Brown Trout. The second objective was to evaluate different rearing velocities in conjunction with dietary bioprocessed soybean meal.

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CHAPTER 2: DIRECT SUBSTITUTION OF FISHMEAL WITH BIOPROCESSED SOYBEAN MEAL IN RAINBOW TROUT (*Oncorhynchus mykiss*) DIETS

Abstract

This 125-day experiment evaluated the rearing performance of adult Rainbow Trout (*Oncorhynchus mykiss*) fed one of three isonitrogenous and isocaloric diets (46% protein, 16% lipid). Fishmeal was the primary protein source for the reference diet, which was compared to two other diets where bioprocessed soybean meal replaced 60 or 80% of the dietary fishmeal. At the end of the experiment, there were no significant differences in gain, percent gain, feed conversion ratio, nor specific growth rate among the dietary treatments. There were also no significant differences in intestinal morphology, splenosomatic index, hepatosomatic index, and viscerosomatic index among the diets. Based on these results, bioprocessed soybean meal can replace at least 80% of the fishmeal in adult Rainbow Trout diets.

Introduction

Plant-based proteins, like soybeans (*Glycine max*), have been extensively researched as alternatives to dietary fishmeal (Nordrum et al. 2000; Gatlin et al. 2007; Li and Robinson 2015). Alternative protein sources are needed due to exponential growth of aquaculture without a corresponding increase in fishmeal, which is primarily made from small pelagic fish (FAO 2016). Thus, there needs to be other suitable and cost effective protein sources to replace dietary fishmeal.

Soybean products are one of the leading alternatives to fishmeal in aquaculture diets (Nordrum et al. 2000; Li and Robinson 2015). Soybeans are highly palatable (Sugiura et al. 1998; Refstie et al. 2000; Watanabe 2002), high in protein, and have a balanced amino acid profile (Gatlin et al. 2007; NRC 2011). However, soybeans also have antinutritional factors that hinder fish digestion (Salunkhe et al. 1992; Krogdahl et al. 1994; Kaushik et al. 1995; Bureau et al. 1998; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Iwashita et al. 2008; NRC 2011; Teng et al. 2012), and can also cause gastro-intestinal issues, such as enteritis (van deh Ingh et al. 1991; Rumsey et al. 1995; Baeverfjord and Krogdahl 1996; Burrells et al. 1999; Bakke-McKellep et al. 2000; Krogdahl et al. 2000, 2015; Refstie et al. 2000; Heikkinen et al. 2006). Soybeans also have high levels of carbohydrates (Salunkhe et al. 1992; Gatlin et al. 2007), which can be deleterious to many fish species, but especially to carnivorous fish (NRC 2011). These antinutritional factors and carbohydrates limit the inclusion levels of soybean products in diets of many carnivorous species (Fowler 1980; Reinitz 1980; Vielma et al. 2000; NRC 2011).

Nevertheless, there are ways to decrease or eliminate the antinutritional factors in soybeans. Heat occurring during the feed-extrusion process decreases lectins and proteinase inhibitors (Cheeke and Shull 1985; Liener 1994; Gomes et al. 1995; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Gatlin et al. 2007; Krogdahl et al. 2010; Bakke 2011). Saponins, sterols, and oligosaccharides can be decreased by alcohol extraction (Krogdahl et al. 2010). Bioprocessing, or fermentation, has also been shown to eliminate or reduce many antinutritional factors (Hong et al. 2004; Refstie et al. 2005; Yamamoto et al. 2010, 2012).

Only a limited number of studies have examined bioprocessed soybean meal (BSM) in Rainbow Trout (*Oncorhynchus mykiss*) diets (Yamamoto et al. 2010, 2012; Barnes et al. 2012, 2013, 2014, 2015a, 2015b; Bruce et al. 2017a, 2017b). The objective of this study was to examine the effects of a novel BSM on the rearing performance of Rainbow Trout.

Methods

This feed trial was conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, using degassed and aerated well water at a constant temperature of 11° C (total hardness as $CaCO_3$, 360 mg/L; alkalinity as $CaCO_3$, 210 mg/L; pH, 7.6; total dissolved solids, 390 mg/L).

Seventy-two Erwin x Arlee strain Rainbow Trout (initial weight 130.7 ± 4.2 g, length 213.2 ± 2.0 mm, mean ± SE) were randomly selected and stocked into one of 12 semi-circular fiberglass tanks (190-L) on June 15, 2016, at six fish per tank. Flow rates were kept constant throughout the 125-day study.

Three different diets were used (Table 1), with modified soybean meal replacing 0, 60, or 80% of the fish meal as the primary protein source. The modified soybean meal was produced using a proprietary microbial conversion process (SDSU, Brookings, SD, USA). The isocaloric and isonitrogenous diets were manufactured by cooking extrusion (ExtruTech model 325, Sabetha, KS). Feed was analyzed according to AOAC (2009) method 2001.11 for protein, 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and AACC (2000) method 08-03 for ash content.

The individual fish weights were combined to obtain total tank weight. Fish were subsequently individually weighed and measured approximately every four weeks. Weight gain, percent gain, feed conversion ratio (FCR), and specific growth rate (SGR) were calculated by tank. Individual fish weights and lengths were used to calculate Fulton’s condition factor (K).

Fish were fed by hand daily, except on the days they were weighed and measured (days 30, 61, 92, and 125). Feeding amounts were initially determined by the hatchery constant method (Butterbaugh and Willoughby 1967), with planned feed conversion rates of 1.1 and maximum growth rate of 0.08 cm/day, which was based on historical maximum growth rate of Erwin x Arlee strain Rainbow Trout at McNenny State Fish Hatchery (Barnes et al. 2011), and then adjusted daily to be at or near satiation. Feed fed and mortalities were recorded daily.

To collect weight and length data on 30-day intervals, the fish were anesthetized using 60 mg/L MS-222 (Tricaine-S, tricaine methanesulfonate, Syndel USA, Ferndale, Washington). On day 125, fish were euthanized using a lethal dose of 250 mg/L MS-222 (AVMA 2013). In addition to weight and length measurements, fin lengths to the nearest 1.0 mm, and organ (spleen, liver, and visceral) weights to the nearest 1.0 mg, were recorded from three randomly selected trout per tank. Fin indices, hepatosomatic index (HSI) (Strange 1996), splenosomatic index (SSI) (Goede and Barton 1990), and viscerosomatic index (VSI) (Goede and Barton 1990) were calculated for individual fish.

The following equations were used:

$$\text{Gain} = \text{end weight} - \text{start weight}$$

$$\text{Percent gain }(\%) = \frac{\text{gain}}{\text{start weight}}$$

$$\text{FCR} = \frac{\text{food fed}}{\text{gain}}$$

$$\text{SGR} = 100 * \frac{\ln(\text{end weight}) - \ln(\text{start weight})}{\text{number of days}}$$

$$\text{K} = 10^5 * \frac{\text{fish weight}}{\text{fish length}^{3}}$$

$$\text{Fin indices} = \frac{\text{fin length}}{\text{fish length}}$$

$$\text{HSI (\%)} = 100 * \frac{\text{liver weight}}{\text{whole fish weight}}$$

$$\text{SSI (\%)} = 100 * \frac{\text{spleen weight}}{\text{whole fish weight}}$$

$$\text{VSI (\%)} = 100 * \frac{\text{visceral weight}}{\text{whole fish weight}}$$

A 2-mm wide section of the distal intestine was removed from three randomly-selected fish per tank to assess any soy-induced enteritis (Gu et al. 2017; Novriadi et al. 2017; Wang et al. 2017; Booman et al. 2018). After dissection, the intestinal tissue was immediately place in 10% buffered formalin, and stained with haematoxylin and eosin using standard histological techniques (Bureau et al. 1998; Burrels et al. 1999). Intestinal inflammation was assessed using an ordinal scoring system (Table 2) based on lamina propria thickness and cellularity, submucosal connective tissue width, and leukocyte distribution (Knudsen et al. 2007; Colburn et al. 2012; Barnes et al. 2014).

Data was analyzed using the SPSS (9.0) statistical analysis program (SPSS, Chicago Illinois), with significance predetermined at $P < 0.05$. One-way analysis of variance (ANOVA) was conducted, and if treatments were significantly different, post hoc mean separation tests were performed using Tukey's HSD test.

Results

At the end of this experiment there were no significant differences in gain, percent gain, feed fed, feed conversion ratios, specific growth rates, or percent mortality among the tanks of fish being fed the three different diets (Table 3). Overall mean (± SE) feed conversion ratio was not significantly different at 1.30 (± 0.04), 1.14 (± 0.03), and 1.25 (±

0.07) for the 0, 60, and 80% BSM diets, respectively. There was no mortality observed in any treatments.

There were no significant differences among the diets in gain, percent gain, or SGR overall and after the first rearing period (days 31-125). However, during the first rearing period, the fish in the tanks that were fed the reference (fishmeal) diet had significantly higher gain, percent gain, and SGR than the fish in the tanks receiving the 80% BSM diet, but were not significantly different than the fish receiving the 60% BSM diet. Mean (± SE) percent gain at the end of the first rearing period was 26.3 (± 1.7) %, 18.8 (± 3.3) %, and 9.9 (± 2.6) % for the fish being fed the 0, 60, and 80% diets, respectively.

Similarly, there were no significant differences overall in individual fish weight, length, or condition factor (Table 4). However, during rearing period 3, the mean (± SE) condition factor of the fish in tanks fed the fishmeal reference diet was 1.38 (± 0.01), which was significantly different from the fish in tanks being fed the 80% bioprocessed soybean meal diet at 1.28 (± 0.02). The condition factor of the fish in the tanks that were fed the 60% BSM was 1.31 (± 0.02), which was not significantly different from the other two diets.

Fish receiving the 80% bioprocessed diets had significantly longer dorsal fins than those receiving the 60% diet, but were not significantly different from those fed the reference diet. No significant differences were observed among the dietary treatments for the pectoral and pelvic fin indices. There were also no significant differences in any of the organosomatic indices (HSI, SSI, and VSI), nor any of the histological scores (lamina propria, connective tissue, and vacuoles). Figure 1 show a representative image of the distal intestines that were scored for the histology sampling, this sample was from a fish fed the reference diet.

Discussion

The lack of significant differences in gain, percent gain, food fed, and feed conversion ratios indicates that at least 80% of the fishmeal can be replaced by BSM in adult Rainbow Trout diets. NRC (2011) states that most fish species exhibit reduced feed intake for a short period when their diets are changed. The initial rearing performance differences between the fish receiving the fishmeal diet compared to the fish receiving the 80% BSM diet could be due to the relative novelty of the 80% diet. Because the pre-trial feed was a commercial diet that did not contain any BSM, the relatively large amount of soy in the 80% fishmeal replacement diet likely required an acclimation period.

The overall results of this study are similar to other experiments feeding BSM to Rainbow Trout (Yamamoto et al. 2010, 2012; Barnes et al. 2012, 2013, 2014, 2015a; Bruce et al. 2017a, 2017b). Yamamoto et al. (2010, 2012) noted similar results replacing 100% of the fishmeal with fermented soybean meal, while Barnes et al. (2012, 2013, 2014, 2015a) found that a maximum of approximately 70% fishmeal substitution was possible without any deleterious effects. Bruce et al. (2017a, 2017b) reported 65% replacement of fishmeal by BSM could be attained with no effect on growth. Other species where BSM has been evaluated include: Atlantic Cod (*Gadus morhua*) (Refstie et al. 2006; Ringø et al. 2006), Atlantic Salmon (*Salmo salar*) (Refstie et al. 2005), Black Sea Bream (*Acanthopagrus schlegeli*) (Zhou et al. 2001; Azarm and Lee 2014), Brown Trout (*Salmo salar*) (Sotoudeh et al. 2016), Chinese Sucker (*Myxocyprinus asiaticus*)

(Yuan et al. 2012), Florida Pompano (*Trachniotus carolinus*) (Novriadi et al. 2017), Gilthead Sea Bream (*Sparus aurata* L.) (Kokou et al. 2012), Japanese Flounder (*Paralichthys olivaceus*) (Kader et al. 2012), Orange-spotted Grouper (*Epinephelus coioides*) (Shiu et al. 2015), Whiteleg Shrimp (*Litopenaeus vannamei*) (Chiu et al. 2015; Van Nguyen et al. 2018), Rockfish (*Sebastes schlegeli*) (Lee et al. 2016), White Seabass (*Atractosion nobilis*) (Trushenski et al. 2014), and Yellowtail Jack (*Seriola lalandi*) (Trushenski et al. 2014).

At 125 days, this experiment should have lasted long enough to determine any differences in fish rearing performance among the diets (Weatherup and McCraken 1999). NRC (2011) recommends feed trial durations of 56-84 days, with larger fish attaining at least a 200-300% gain. This experiment provided fish weight gains of approximately 250%, thereby meeting both requirements.

The FCR observed in this experiment was slightly higher than that reported in some other experiments involving Rainbow Trout (Barnes et al. 2012, 2013), but were also similar to other studies (Yamamoto et al. 2010, 2012; Barnes et al. 2014, 2015a; Bruce et al. 2017b). The SGR was slightly lower in this experiment (0.9-1.0) compared to the 1.0 to 1.3 reported by Bruce et al. (2017b) in a similar study, but were extremely low compared to 1.8 to 3.0 reported by Yamamoto et al. (2010, 2012) and Bruce et al. (2017a). The slower growth rate could possibly be due to the size of the fish or water temperatures differences. Yamamoto et al. (2010, 2012) and Bruce et al. (2017a, 2017b) used juvenile fish, while this study used adult Rainbow Trout, which have slower growth (Stickney 1994).

The condition factors observed in this experiment was higher than most of the other Rainbow Trout experiments (Barnes et al. 2012, 2013, 2014, 2015a, 2015b; Bruce 2017b). This could possibly be because the fish in this experiment were older and larger, and closer to sexual maturity (Barton et al. 2002).

Relative fin length can be influenced by several factors, including tank-induced abrasions (Bosakowski and Wagner 1995), rearing unit size and type (Bosakowski and Wagner 1994), aggressive behavior (Latremouille 2003), feeding rates (Wagner et al. 1996), rearing densities (Miller et al. 1995; Wagner et al. 1997; North et al. 2006), dietary nutritional differences (Lemm et al. 1988; Kindischi et al. 1991), environmental stress (Latremouille 2003), and fish health (Devesa et al. 1989). The lack of difference between the pectoral and pelvic fin indices in this experiment could be attributed to similar environmental stressors and adequate feeding rates. However, the significant differences seen in the dorsal fins between the 60 and 80% BSM could be due to nutritional differences in feed. Kindschi et al. (1991) found a significant difference in the dorsal fin measurement of Steelhead Trout fed diets containing either menhaden or herring oil. The overall pectoral fin values observed in this experiment are similar to those reported by Parker and Barnes (2015).

The lack of any differences in HSI between the dietary treatments indicates similar energy partitioning. HSI is an indirect measure of glycogen and carbohydrate levels, and can be used to indicate nutritional state of the fish (Daniels and Robinson 1986; Kim and Kaushik 1992; Barton et al. 2002). The HSI levels observed in this study were similar to those reported by Barnes et al. (2013, 2014, 2015b), and slightly higher than those reported by Yamamoto et al. (2010, 2012), Barnes et al. (2012), and Bruce et al. (2017a). Differences in HSI among the studies could be related to fish age. Barton et al. (2002) noted that the organosomatic indices can vary depending on a fishes life stage, and the Rainbow Trout use in this study were much larger and older than those used in other experiments.

The VSI indicates how lipids are being used or partitioned with VSI and lipids positively related (Jobling et al. 1998; Company et al. 1999; Yildiz et al. 2006). Thus, similar VSI values among the dietary treatments are likely due to similar dietary lipid levels. At 13.0 to 13.7 the VSI values are similar to the 12.0 to 13.8 values reported by Barnes et al. (2014, 2015a), but higher than those reported by Barnes et al. (2013, 2015b), Parker and Barnes (2014, 2015), Kientz and Barnes (2016), and Bruce et al. (2017a).

Similar SSI indicates the hematopoietic capacity of fish (Barton et al. 2002), and antibody production mostly occurs in the spleen (Smith 1991). Similar SSI values indicate that fish health was likely unaffected by dietary treatment. The SSI values observed were within the range reported by other studies (Barnes et al. 2015b; Parker and Barnes 2015; Kientz and Barnes 2016; Bruce et al. 2017b).

Enteritis was not observed in this study, despite the well-documented and potentially negative effects of soybean products to the distal intestine of Rainbow Trout (Rumsey et al. 1995; Burrels et al. 1999; Heikkeinen et al. 2006; Barrows et al. 2008; Iwashita et al. 2008; Romarheim et al. 2008a; Merrifield et al. 2009; Sealey et al. 2009). The BSM used in this study obviously decreased or eliminated the saponins (Krogdahl et al. 2015) and other antinutritional factors responsible for such enteritis (Yamamoto 2010, 2012; Barnes et al. 2012, 2013). The absolute intestinal scores observed in this study tended to be lower than those reported by Barnes et al. (2014, 2015a, 2015b) for Rainbow

Trout fed different fermented soybean meal diets. This could be due to the dietary differences among the studies or scoring difference between readers.

In conclusion, this study indicates that at least 80% of the dietary fishmeal can be directly replaced by BSM in diets of adult Rainbow Trout. It is unknown if the suitability of dietary BSM extends further during the trout life cycle, prior to spawning. Additional research is needed to determine if this BSM can replace all of the fishmeal in adult Rainbow Trout diets.

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Table 1. Diet formulation and composition analyses of the diets used in the 125-day trial. Analysis conducted on post-extrusion feed pellets.

	Diet (%)		
Ingredients	1	2	3
Fishmeal[a]	35.0	14.0	4.7
Bioprocessed soybean meal[b]	0.0	12.0	30.3
Wheat midds[c]	12.0	10.0	10.0
Whole wheat[c]	17.7	15.2	15.1
Poultry byproduct meal[d]	10.0	15.0	15.0
Blood meal[e]	2.0	2.0	2.0
Feather meal[d]	7.0	2.5	2.5
Vitamin premix[f]	1.3	1.3	1.3
Mineral premix[f]	0.8	2.0	2.0
Micro-mineral premix[f]	0.8	0.8	0.8
Choline chloride[g]	0.6	0.6	0.7
L-Lysine[h]	1.5	2.0	2.0
L-Methionine[i]	0.3	0.5	0.5
Stay-C 35[j]	0.2	0.2	0.2
Fish oil[k]	11.0	13.0	13.0
Total	100	100	100
Chemical analysis (% dry basis)			
Protein	43.18	43.85	43.84
Lipid	15.91	14.28	16.44
Ash	2.42	3.60	3.92
Nitrogen-free extract	20.48	24.33	23.96
Dry matter	93.00	95.20	96.25
Gross Energy (kJ/g)	16.5	16.0	16.8
Protein : Energy (MJ/g)	26.2	27.4	26.0

[a] Special Select, Omega Protein, Houston, TX; [b] SDSU; [c] Consumer Supply, Sioux City, IA; [d] Tyson Foods, Springdale, AR; [e] Mason City Byproducts, Mason City, IA; [f] NutraBlend, Neosho, MO; [g] Balchem, New Hampton, NY; [h] CJ Bio America, Fort Dodge, IA; [i] Adisseo USA, Alpharreta, GA; [j] DSM Nutritional Products, Ames, IA; [k] Virginia Prime Gold, Omega Protein, Houston, TX.

Table 2. Histological scoring system used on Rainbow Trout fed fishmeal or incremental amounts of bioprocessed soybean meal in diets (Barnes et al. 2014, modified from Geode and Barton 1990, Adams et al. 1993, and Barton et al. 2002).

Score	Appearance
	Lamina propria of simple folds
1	Thin and delicate core of connective tissue in all simple folds.
2	Lamina propria slightly more distinct and robust in some of the folds.
3	Clear increase in lamina propria in most of simple folds.
4	Thick lamina propria in many folds.
5	Very thick lamina propria in many folds.
	Connective tissue between base of folds and stratum compactum
1	Very thin layer of connective tissue between base of folds and stratum compactum.
2	Slightly increased amount of connective tissue beneath some of mucosal folds.
3	Clear increase of connective tissue beneath most of the mucosal folds.
4	Thick layer of connective tissue beneath many folds.
5	Extremely thick layer of connective tissue beneath some of the folds.
	Vacuoles
1	Large vacuoles absent.
2	Very few large vacuoles present.
3	Increased number of large vacuoles.
4	Large vacuoles are numerous.
5	Large vacuoles are abundant in present in most epithelial cells.

Table 3. Mean (± SE) gain, percent gain, food fed, feed conversion ratio (FCR[a]), specific growth rate (SGR[b]), and mortality of Rainbow Trout receiving fishmeal or incremental levels of bioprocessed soybean meal (BSM) as the main protein ingredient. Overall means with different letters in the same row differ significantly (*P* < 0.05).

Diet	1	2	3
BSM (%)	0	60	80
Initial			
Start weight (g)	772.7 ± 44.5	811.1 ± 29.3	761.5 ± 71.2
Days 1-30			
End weight (g)	974.0 ± 44.4	965.4 ± 54.7	837.3 ± 80.1
Gain (g)	201.2 ± 4.2 z	154.3 ± 30.0zy	75.8 ± 21.2 y
Gain (%)	26.3 ± 1.7 zy	18.8 ± 3.3 zy	9.9 ± 2.6 y
Food fed (g)	325 ± 8	300 ± 22	277 ± 24
FCR	1.61 ± 0.04	2.18 ± 0.42	4.67 ± 1.29
SGR	0.78 ± 0.05 z	0.57 ± 0.10zy	0.31 ± 0.08y
Days 31-61			
End weight (g)	1,368.6 ± 77.6	1,377.8 ± 100.6	1,171.0 ± 119.1
Gain (g)	394.6 ± 53.8	412.5 ± 52.4	333.7 ± 49.8
Gain (%)	40.6 ± 5.2	42.4 ± 3.6	39.7 ± 4.4
Food fed (g)	483 ± 39	468 ± 45	422 ± 62
FCR	1.25 ± 0.09	1.15 ± 0.09	1.28 ± 0.08
SGR	1.13 ± 0.12	1.18 ± 0.08	1.11 ± 0.10
Days 62-92			

End weight (g)	1,805.6 ± 85.7	1,948.6 ± 215.3	1,609.9 ± 156.5
Gain (g)	437.0 ± 17.2	570.7 ± 120.4	438.8 ± 51.0
Gain (%)	32.1 ± 1.7	40.3 ± 6.7	37.8 ± 3.3
Food fed (g)	631 ± 45	656 ± 79	534 ± 48
FCR	1.45 ± 0.11	1.29 ± 0.26	1.25 ± 0.14
SGR	0.93 ± 0.04	1.12 ± 0.17	1.07 ± 0.08
Days 93-125			
End weight (g)	2,538.7 ± 95.7	2,914.7 ± 317.1	2,449.5 ± 236.6
Gain (g)	733.1 ± 14.9	966.2 ± 1185	839.6 ± 94.3
Gain (%)	40.8 ± 1.7	49.9 ± 4.0	52.3 ± 3.4
Food fed (g)	855 ± 13	968 ± 168	866 ± 95
FCR	1.17 ± 0.03	0.98 ± 0.06	1.04 ± 0.08
SGR	1.14 ± 0.04	1.35 ± 0.09	1.40 ± 0.08
Overall (Days 1-125)			
Gain (g)	1,766.0 ± 70.2	2,103.6 ± 289.0	1,688 ± 178.1
Gain (%)	230.3 ± 13.0	256.7 ± 27.0	222.1 ± 14.5
Food fed (g)	2,294 ± 118	2,391 ± 304	2,100 ± 200
FCR	1.30 ± 0.04	1.14 ± 0.03	1.25 ± 0.07
SGR	0.95 ± 0.03	1.01 ± 0.06	0.93 ± 0.04
Mortality (%)	0	0	0

[a] FCR = feed conversion ratio = total food fed / total weight gain.

[b] SGR = 100 x [(Ln(final weight) – Ln(initial weight)) / days]

Table 4. Mean (± SE) condition factor (K[a]), fin indices[b], hepatosomatic index values (HSI[c]), splenosomatic index (SSI[d]), viscerosomatic index (VSI[e]), and histology scores for lamina propria, connective tissue, and vacuoles of Rainbow Trout fed one of three diets containing either fishmeal or incremental amounts of bioprocessed soybean meal (BSM) as the primary protein source. Means with different letters in the same row differ significantly ($P < 0.05$).

Diet	1	2	3
BSM (%)	0	60	80
Initial			
Weight (g)	128.8 ± 7.4	135.2 ± 4.9	126.9 ± 11.9
Length (mm)	210.8 ± 3.2	217.0 ± 2.4	210.4 ± 5.8
K	1.34 ± 0.03	1.31 ± 0.02	1.32 ± 0.02
Days 1-30			
End weight (g)	162.3 ± 7.4	160.9 ± 9.1	139.6 ± 13.4
End length (mm)	228.5 ± 2.8	231.0 ± 2.6	221.8 ± 7.0
K	1.33 ± 0.04	1.28 ± 0.03	1.24 ± 0.01
Days 31-61			
End weight (g)	228.1 ± 12.9	229.7 ± 16.8	195.2 ± 19.8
End length (mm)	252.5 ± 4.7	256.6 ± 4.6	244.4 ± 7.7
K	1.38 ± 0.01z	1.31 ± 0.02zy	1.28 ± 0.02 y
Days 62-92			
End weight (g)	301.0 ± 14.3	324.8 ± 35.9	268.3 ± 26.1
End length (mm)	277.3 ± 4.4	284.3 ± 7.5	267.4 ± 8.4

K	1.38 ± 0.02	1.35 ± 0.04	1.35 ± 0.02
Days 93-125 (Final)			
End weight (g)	423.1 ± 16.0	485.8 ± 52.9	408.3 ± 39.4
End length (mm)	308.4 ± 3.9	319.8 ± 10.3	301.1 ± 9.3
K	1.41 ± 0.03	1.42 ± 0.05	1.44 ± 0.02
Pectoral index (%)	10.1 ± 0.4	10.6 ± 0.6	11.1 ± 0.6
Pelvic index (%)	9.2 ± 0.2	8.8 ± 0.3	9.4 ± 0.4
Dorsal index (%)	6.4 ± 0.1zy	5.5 ± 0.2 y	6.8 ± 0.4 z
HSI (%)	1.22 ± 0.05	1.39 ± 0.08	1.34 ± 0.06
SSI (%)	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.01
VSI (%)	13.0 ± 0.2	13.1 ± 0.5	13.7 ± 1.0
Lamina propria [f]	1.33 ± 0.24	1.25 ± 0.16	1.50 ± 0.10
Connective tissue [f]	1.50 ± 0.10	1.42 ± 0.21	1.50 ± 0.17
Vacuoles [f]	1.92 ± 0.21	2.00 ± 0.00	2.00 ± 0.14

[a] K = 10^5 x [weight / (length^3)]

[b] Fin indices = 100 x (fin length / fish length)

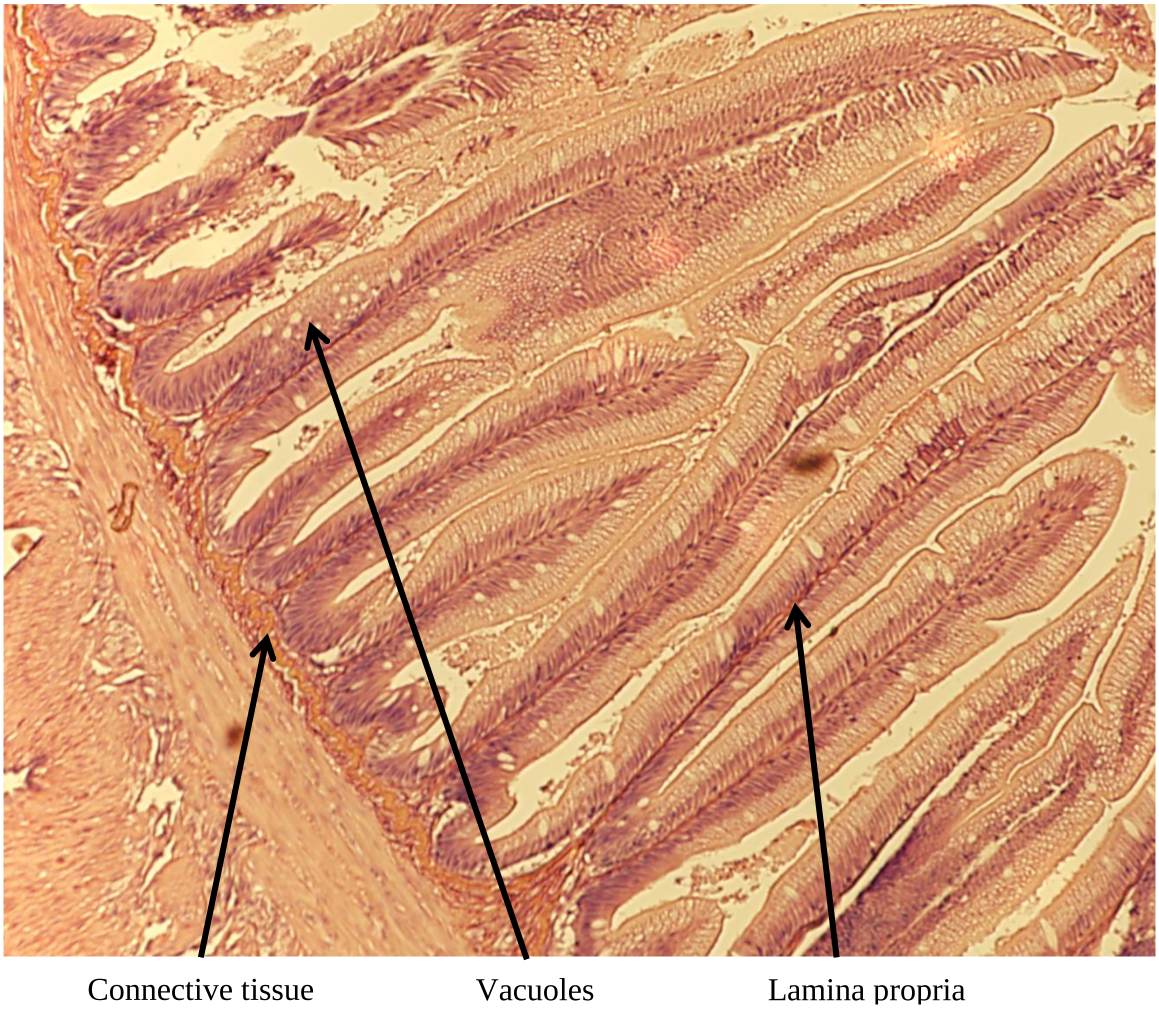
[c] HSI = 100 x (liver weight / body weight)

[d] SSI = 100 x (spleen weight / body weight)

[e] VSI = 100 x (visceral weight / body weight)

[f] Scoring Parameters in Table 2

Figure 1. Representative (fed reference diet) Rainbow Trout histology image used for scoring.



CHAPTER 3: DIRECT SUBSTITUTION OF FISHMEAL WITH BIOPROCESSED SOYBEAN MEAL IN BROWN TROUT (*Salmo trutta*) DIETS

Abstract

This 121-day experiment evaluated the rearing performance of juvenile Brown Trout (*Salmo trutta*) fed one of four isonitrogenous and isocaloric diets (46% protein, 16% lipid). Fishmeal was the primary protein source for the reference diet, which was compared to diets where bioprocessed soybean meal (BSM) that directly replaced 60, 80, or 100% of the dietary fishmeal. At the end of the experiment there were no significant differences in gain, percent gain, food fed, feed conversion ratio, nor specific growth rate among any of the dietary treatments. There were also no significant differences detected in intestinal morphology, relative fin lengths, or organosomatic indices (HSI, VSI, and SSI) related to dietary treatments. Based on these results, BSM can replace 100% of the dietary fishmeal in juvenile Brown Trout diets without any deleterious effects.

Introduction

Intensively cultured, carnivorous fishes (De Silva and Anderson 1995), such as many salmonids, require high levels of dietary protein (NRC 2011). Historically, the primary protein source in salmonid feeds has been fishmeal (Satia 1974; Kim et al. 1991; Cheng and Hardy 2004). However, the limited supply of fishmeal and rapid growth of aquaculture has led to an increase in price of aquafeeds (Tacon and Metian 2008; Hardy 2010; FAO 2016). Therefore, there is a need for lower-cost, sustainable protein sources to replace fishmeal in salmonid diets (Hardy 2010).

Soybean (*Glycine max*) products are some of the leading alternatives to dietary fishmeal (Nordrum et al. 2000; Li and Robinson 2015). Soybeans are highly palatable

(Sugiura et al. 1998; Refstie et al. 2000; Watanabe 2002), high in protein, and have a balanced amino acid profile (Gatlin et al. 2007; NRC 2011). However, there are antinutritional factors associated with soybeans which hinder fish digestion (Salunkhe et al. 1992; Krogdahl et al. 1994, 2015; Kaushik et al. 1995; Bureau et al. 1998; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Iwashita et al. 2008; NRC 2011; Teng et al. 2012), and can also cause gastro-intestinal issues, such as enteritis (van deh Ingh et al. 1991; Rumsey et al. 1995; Baeverfjord and Krogdahl 1996; Burrells et al. 1999; Bakke-McKellep et al. 2000; Krogdahl et al. 2000, 2015; Refstie et al. 2000; Heikkinen et al. 2006). Another facet limiting soybean use in fish diets is a large concentration of non-digestible carbohydrates (Salunkhe et al. 1992; Gatlin et al. 2007). These antinutritional factors and carbohydrates limit the inclusion levels of soybeans in diets for many carnivorous species (Fowler 1980; Reinitz 1980; Vielma et al. 2000; NRC 2011).

Nevertheless, there are ways to reduce or eliminate the undesirable characteristics of soybean products. Antinutritional factors such as proteinase inhibitors and lectins can be decreased by applying heat (Cheeke and Shull 1985; Liener 1994; Gomes et al. 1995; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Gatlin et al. 2007; Krogdahl et al. 2010; Bakke 2011), as typically happens during the feed extrusion process. Other antinutritional factors such as phytic acid or oligosaccharides are heat stable (Gatlin et al. 2007), but phosphorous from phytic acid can be made available to fish by hydrolysis (Gatlin et al. 2007). Another form of bioprocessing is fermentation, which has been shown to eliminate or reduce many antinutritional factors (Hong et al. 2004; Refstie et al. 2005; Yamamoto et al. 2010, 2012).

While many studies have examined the inclusion of soybean products in Atlantic Salmon (*Salmo salar*) and Rainbow Trout (*Oncorhynchus mykiss*) diets (Collins et al. 2013), very little research has been done with Brown Trout (*Salmo trutta*). Worldwide Brown Trout food-fish production is a fraction of Rainbow Trout and Atlantic Salmon (FAO 2017) production, but there is still considerable production of Brown Trout for recreation and conservation (Belica 2007).

Only two studies have examined replacement of fishmeal with plant-based protein sources in Brown Trout diets. Michl et al. (2017) replaced fishmeal with a combination of numerous plant-based proteins, making comparison to other experiments difficult. Sotoudeh et al. (2016) replaced fish meal with different forms of processed soybean meal (untreated, gamma-ray, irradiated, and fermented) and found that Brown Trout fed fermented soybean meal grew larger than fish fed all of the other non-fermented soybean meal diet. However, this study did not have a fishmeal reference diet, again limiting comparisons to other experiments.

Because of the limited research on the use of plant-based meals in Brown Trout diets, the objective of this study was to examine the effects of bioprocessed soybean meals (BSM) on rearing performance.

Methods

This feeding trial was conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, using degassed and aerated well water at a constant temperature of 11° C (total hardness as $CaCO_3$, 360 mg/L; alkalinity as $CaCO_3$, 210 mg/L; pH, 7.6; total dissolved solids, 390 mg/L).

One-hundred twenty-eight Plymouth strain Brown Trout (initial weight 56.1 ± 1.6 g, length 167.2 ± 1.4 mm, mean ± SE) were randomly selected and placed into one of 16 circular fiberglass tanks (1.8 m diameter, 0.6 m depth) on September 15, 2016, at eight fish per tank. This study was conducted for a total of 121 days and flow rates were kept constant throughout the study, with average mean (± SE) velocity of 2.6 (± 0.3) cm/s. Velocities were measured using a Flowatch meter (JDC Electronic SA, Yverdon-les-Bains, Jura-Nord Vaudois, Vaud, Switzerland).

Four different diets were fed (Table 5), with modified soybean meal replacing 0, 60, 80, or 100% of the fishmeal as the primary protein source. The modified soybean meal was produced using a proprietary microbial conversion process (SDSU, Brookings, SD, USA). Diets were isocaloric and isonitrogenous and were manufactured by cooking extrusion (ExtruTech model 325, Sabetha, KS). Feed was analyzed according to AOAC (2009) method 2001.11 for protein, 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and AACC (2000) method 08-03 for ash content.

At the beginning of the experiment, fish were individually weighed to the nearest 0.1 g, measured to the nearest 1.0 mm, and then placed into the tanks. Fish were weighed and measured approximately every four weeks. The individual fish weights were combined to obtain total tank weight. Weight gain, percent gain, feed conversion ratio (FCR), and specific growth rate (SGR) were calculated. Individual fish weights and lengths were used to calculate Fulton’s condition factor (K).

Fish were fed daily for 121 days, except on days they were weighed and measured (days 35, 61, 92, and 121). Feeding amounts were initially determined by the hatchery constant method (Butterbaugh and Willoughby 1967), with planned feed conversion rates of 1.1 and maximum growth rate of 0.07 cm/day, which was based on historical maximum growth rate of Plymouth strain Brown Trout reared at McNenny State Fish Hatchery (Barnes et al. 2011). Fish were fed by hand daily and feed was adjusted daily to be at or near satiation. Feed and mortality were recorded daily.

To collect weight and length data on days 1, 35, 61, and 92, the fish were anesthetized using 60 mg/L MS-222 (Tricaine-S, tricaine methanesulfonate, Syndel USA, Ferndale, Washington). On day 121, fish were euthanized using a lethal dose of 250 mg/L MS-222 (AVMA 2013). In addition to weight and length measurements, fin lengths, to the nearest 1.0 mm; and spleen, liver, and visceral weights, to the nearest 1.0 mg, were recorded from three randomly selected Brown Trout per tank. Fin indices, hepatosomatic index (HSI) (Strange 1996), splenosomatic index (SSI) (Goede and Barton 1990), and viscerosomatic index (VSI) (Goede and Barton 1990) were calculated.

The following equations were used:

$$\text{Gain} = \text{end weight} - \text{start weight}$$

$$\text{Percent gain (\%)} = \frac{\text{gain}}{\text{start weight}}$$

$$\text{FCR} = \frac{\text{food fed}}{\text{gain}}$$

$$\text{SGR} = 100 * \frac{\ln(\text{end weight}) - \ln(\text{start weight})}{\text{number of days}}$$

$$\text{K} = 10^5 * \frac{\text{fish weight}}{\text{fish length}^{\,3}}$$

$$\text{Fin indices} = \frac{\text{fin length}}{\text{fish length}}$$

$$\text{HSI (\%)} = 100 * \frac{\text{liver weight}}{\text{whole fish weight}}$$

$$\text{SSI (\%)} = 100 * \frac{\text{spleen weight}}{\text{whole fish weight}}$$

$$\text{VSI (\%)} = 100 * \frac{\text{visceral weight}}{\text{whole fish weight}}$$

A 2-mm wide section of the distal intestine was removed from three randomly selected fish per tank to assess any possible soy-induced enteritis (Gu et al. 2017; Novriadi et al. 2017; Wang et al. 2017; Booman et al. 2018). After dissection, the intestinal tissue was immediately put into 10% buffered formalin, and stained with haematoxylin and eosin using standard histological techniques (Bureau et al. 1998; Burrels et al. 1999). Intestinal inflammation was assessed using an ordinal scoring system (Table 6) based on lamina propria thickness and cellularity, submucosal connective tissue width, and leukocyte distribution (Knudsen et al. 2007; Colburn et al. 2012; Barnes et al. 2014).

Data was analyzed using the SPSS (9.0) statistical analysis program (SPSS, Chicago Illinois), with significance predetermined at $P < 0.05$. One-way analysis of variance (ANOVA) was conducted, and if treatments were significantly different, post hoc mean separation tests were performed using Tukey’s HSD test.

Results

At the end of this experiment, there were no significant differences among the diets in gain, percent gain, food fed, or FCR (Table 7). However, significant differences were observed during specific rearing periods. During the first rearing period the tanks of fish receiving the reference, fishmeal based, diet received 192 (± 5) g (mean ± SE) of food, which was significantly great than the 144 (± 8) g, 147 (± 6) g, and 150 (± 9) g fed for the tanks receiving diets with 60, 80, and 100% BSM replacement, respectively. Fish receiving the fishmeal had a negative FCR, indicating weight loss during this first rearing period.

Individual fish weight, length, and condition factor were not significantly different between the dietary treatments at the end of the 121 day experiment or in any of the four rearing periods (Table 8). In addition, fin indices (pectoral, pelvic, and dorsal), organismic indices (HSI, SSI, and VSI), or gut histology scores were not significantly different among diets. Figure 2 is a representative image of the distal intestines that were scored for the histology sampling.

Discussion

The lack of significant differences in rearing performance among any of the four diets indicates BSM can replace 100% of the dietary fishmeal without any negative repercussions on Brown Trout. In the only other study examining BSM in Brown Trout diets, Sotoudeh et al. (2016) also indicated the suitability of fermented soybean meal. However, Sotoudeh et al. (2016) did not have a fishmeal-based reference. In addition, the fermented soybean meal only replaced 50% of the dietary fishmeal, making the results difficult to compare to this experiment. The results of this experiment are similar to those reported by Yamamoto et al. (2010, 2012) in Rainbow Trout, where fermented soybean meal replaced 100% of the fishmeal, without any negative effects. In other experiments with Rainbow Trout, BSM have successfully replaced the majority (~60-70%) of dietary fishmeal, but at higher concentrations fish rearing performance decreased (Barnes et al. 2012, 2014, 2015a; Bruce et al. 2017a, 2017b). BSM has been evaluated in Atlantic Salmon diets, but fishmeal replacement rates appear to be limited to 20% or less (Refstie et al. 2005). Other species where a fermented, or other BSM, have been evaluated include

Atlantic Cod (*Gadus morhua*) (Refstie et al. 2006; Ringø et al. 2006), Black Sea Bream (*Acanthopagrus schlegeli*) (Zhou et al. 2011; Azarm and Lee 2014), Chinese Sucker (*Myxocyprinus asiaticus*) (Yuan et al. 2012), Florida Pompano (*Trachniotus carolinus*) (Novriadi et al. 2017), Gilthead Sea Bream (*Sparus aurata* L.) (Kokou et al. 2012), Japanese Flounder (*Paralichthys olivaceus*) (Kader et al. 2012), Orange-spotted Grouper (*Epinephelus coioides*) (Shiu et al. 2015), Whiteleg Shrimp (*Litopenaeus vannamei*) (Chiu et al. 2015; Van Nguyen et al. 2018), Rockfish (*Sebastes schlegeli*) (Lee et al. 2016), White Seabass (*Atractosion nobilis*) (Trushenski et al. 2014), and Yellowtail Jack (*Seriola lalandi*) (Trushenski et al. 2014).

At 121 days, the duration of this study should have met the Weathercup and McCraken (1999) study length criteria to determine any differences in fish performance among the diets. It also met the NRC (2011) recommended duration of 56-84 days. However, even at 121 days, the Brown Trout in this experiment only gained approximately 150%, which did not attain the 200% gain recommend by NRC (2011). Despite not reaching a 200% gain, this study still lasted longer than most soybean meal feeding trials, with few lasting over 100 days (Vielma et al. 2000; Heikkinen et al. 2006; Barrows et al. 2008b; Merrifield et al. 2009; Johnsen et al. 2011; Barnes et al. 2012; 2014).

Even though there were no differences in fish growth among the diets, feed conversions were poor throughout the experiment. This was particularly evident in the fish receiving the fishmeal diet, which lost weight during the initial rearing period. Poor feed conversions could possibly be due to poor palatability of the diets. Poor palatability has been suggested to contribute to lower feed intake and reduced growth (Kissil et al.

2000; Bruce et al. 2017b). There are few published studies for Brown Trout that provide FCRs for comparison. Regost et al. (2001) reported an FCR of about 1.6 which was similar to the 1.5 to 2.1 values observed during rearing period four. Kizak et al. (2013) reported a feed conversion ratio of only 0.50. However, Kizak et al. (2013) fed a restricted ration, which has been shown to improve feed conversion ratio (De Silvia and Anderson 1995). Similar to the FCR results, SGR was poor at the start of the trial, but were similar to those reported for Brown Trout by Regost et al. (2001) and Kizak et al. (2013) in the final period.

Enteritis was not observed in any of the fish in this study. Even though soybean products in the diets of salmonids have caused well-documented and potentially deleterious effects of the distal intestine of Rainbow Trout and Atlantic Salmon (van den Ingh et al. 1991; Rumsey et al. 1995; Baeverfjord and Krogdahl 1996; Burrels et al. 1999; Bakke-McKellep et al. 2000; 2007; Heikkeinen et al. 2006; Barrows et al. 2008a; Iwashita et al. 2008; Romarheim et al. 2008a; Merrifield et al. 2009; Sealey et al. 2009). The BSM used in this study obviously decreased or eliminated the saponins (Krogdahl et al. 2015) and other antinutritional factors responsible for such enteritis (Yamamoto 2010, 2012; Barnes et al. 2012, 2013). There are no published studies where the intestinal scoring system was used with Brown Trout. However, intestinal scores observed in this study tended to be lower than those reported by Barnes et al. (2014, 2015a, 2015b) for Rainbow Trout fed different diets.

The lack of any differences in HSI between the dietary or velocity treatments indicates similar energy partitioning within the fish. HSI is an indirect measure of glycogen and carbohydrate levels, and can be used to indicate nutritional state of the fish

(Daniels and Robinson 1986; Kim and Kaushik 1992; Barton et al. 2002). The HSI in this study (0.9-1.1) is similar to that reported by in Sotoudeh et al (2011) (0.9-1.4), but are lower than that reported for Brown Trout in other studies (1.4-1.8) (Mambrini et al. 2006; Kizak et al. 2013; Sotoudeh et al. 2016). The relatively lower HSI values in this study may be due to different diets or may also be indicative of different stressors among the studies. Both HSI and VSI are used to indicate if energy is being diverted away from organ or tissue growth in order to combat stress, with stress is indicated by lower indices (Barton et al. 2002).

VSI indicates how lipids are being used or partitioned, and there is a positive relationship between lipid levels and VSI (Jobling et al. 1998; Company et al. 1999; Yildiz et al. 2006). Thus, similar VSI values among the dietary and velocity treatments is likely due to similar dietary lipid levels. VSI values in this experiment (5.0-5.7) are similar to Sotoudeh et al (2016) (4.9-6.0), but are extremely low compared to Mambrini et al. (2006) (8.9-10.6) or Kizzak et al. (2013) (12.9-14.2). Sotoudeh et al. (2016) is the only experiment examining processed soybean meal in Brown Trout diets.

SSI indicates the hematopoietic capacity of fish (Barton et al. 2002) and antibody production mostly occurs in the spleen (Smith 1991). Similar SSI values likely indicate that fish health was unaffected by diet. No literature values for Brown Trout SSI could be found, but dietary experiments with Rainbow Trout SSI had similar values to those observed in the Brown Trout in this study (Barnes et al. 2015b; Parker and Barnes 2015; Kientz and Barnes 2016; Bruce et al. 2017b).

The lack of difference in relative fin lengths among the dietary treatments indicates the suitability of the diets, as well as a lack of environmental stress

(Latremouille 2003), adequate feeding rates (Wagner et al. 1996), nutritional differences (Lemm et al. 1988; Kindischi et al. 1991), and good fish health (Devesa et al 1989). Fin erosion can be due to several factors, including tank-induced abrasions (Bosakowski and Wagner 1995), rearing unit size and type (Bosakowski and Wagner 1994), aggressive behavior (Latremouille 2003), feeding rates (Wagner et al. 1996), rearing densities (Miller et al. 1995; Wagner et al. 1997; North et al. 2006), and fish health (Devesa et al. 1989). Bosakowski and Wagner (1994) is the only other paper that has examined fin indices for Brown Trout, which had smaller pectoral and pelvic indices (9.8-9.8 and 9.5-9.9, respectively) compared to this study (13.2-13.5 and 10.7-11.1, respectively), but with much larger for dorsal indices (7.0 compared to 4.4).

In conclusion, this study is the first to verify the suitability of a BSM as a complete replacement of dietary fishmeal in Brown Trout diets. The BSM did not negatively affect growth, feeding efficiency, or fish health. Further research should be done to determine the reasons for the poor FCRs observed for all of the diets used in this study.

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Table 5. Diet formulation and composition analyses of the diets used in the 121-day trial. Analysis conducted on post-extrusion feed pellets.

	Diet (%)			
Ingredients	1	2	3	4
Fishmeal[a]	35.3	14.0	4.7	0.0
Bioprocessed soybean meal[b]	0.0	21.0	30.3	34.9
Wheat midds[c]	7.9	7.2	7.2	7.8
Whole wheat[c]	16.4	13.7	13.2	13.4
Poultry byproduct meal[d]	21.9	19.9	19.9	19.9
Blood meal[e]	2.6	2.5	2.5	2.5
Feather meal[d]	1.2	1.2	1.2	1.2
Vitamin premix[f]	0.8	2.0	2.0	1.1
Mineral premix[f]	0.8	2.0	2.0	1.1
Micro-mineral premix[f]	0.0	0.8	0.8	0.8
Choline chloride[g]	0.0	0.7	0.7	0.7
L-Lysine[h]	1.2	1.7	1.7	1.7
L-Methionine[i]	0.0	0.5	0.5	0.5
Stay-C 35[j]	0.0	0.3	0.3	0.3
Fish oil[k]	10.1	12.0	12.5	12.6
Total	100.0	100.0	100.0	100.0
Chemical analysis (% dry basis)				
Protein	46.98	45.76	45.55	45.3
Lipid	16.97	16.25	16.74	17.71
Ash	11.4	9.71	8.14	6.86
Nitrogen-free extract	18.79	20.63	23.84	22.89
Dry matter	96.48	95.85	94.26	92.76
Gross Energy (kJ/g)	17.8	17.2	16.0	16.3
Protein : Energy (MJ/g)	26.4	26.6	28.5	27.9

[a] Special Select, Omega Protein, Houston, TX; [b] SDSU; [c] Consumer Supply, Sioux City, IA; [d] Tyson Foods, Springdale, AR; [e] Mason City Byproducts, Mason City, IA; [f] NutraBlend, Neosho, MO; [g] Balchem, New Hampton, NY; [h] CJ Bio America, Fort Dodge, IA; [i] Adisseo USA, Alpharreta, GA; [j] DSM Nutritional Products, Ames, IA; [k] Virginia Prime Gold, Omega Protein, Houston, TX.

Table 6. Histological scoring system used on Brown Trout fed fishmeal or incremental amounts of bioprocessed soybean meal in diets (Barnes et al. 2014, modified from Geode and Barton 1990, Adams et al. 1993, and Barton et al. 2002).

Score	Appearance
	Lamina propria of simple folds
1	Thin and delicate core of connective tissue in all simple folds.
2	Lamina propria slightly more distinct and robust in some of the folds.
3	Clear increase in lamina propria in most of simple folds.
4	Thick lamina propria in many folds.
5	Very thick lamina propria in many folds.
	Connective tissue between base of folds and stratum compactum
1	Very thin layer of connective tissue between base of folds and stratum compactum.
2	Slightly increased amount of connective tissue beneath some of mucosal folds.
3	Clear increase of connective tissue beneath most of the mucosal folds.
4	Thick layer of connective tissue beneath many folds.
5	Extremely thick layer of connective tissue beneath some of the folds.
	Vacuoles
1	Large vacuoles absent.
2	Very few large vacuoles present.
3	Increased number of large vacuoles.
4	Large vacuoles are numerous.
5	Large vacuoles are abundant in present in most epithelial cells.

Table 7. Mean (± SE) gain, percent gain, food fed, feed conversion ratio (FCR[a]), specific growth rate (SGR[b]), and mortality of Brown Trout receiving one of four different diets containing fishmeal or incremental amounts of bioprocessed soybean meal (BSM) as the main protein ingredient. Overall means with different letters in the same row differ significantly ($P < 0.05$).

Diet		1	2	3	4
BSM (%)		0	60	80	100
Initial					
	Start weight (g)	489.6 ± 20.1	435.2 ± 15.0	464.0 ± 63.9	477.2 ± 9.6
Days 1-35					
	End weight (g)	511.6 ± 16.4	458.6 ± 21.4	477.1 ± 54.7	505.9 ± 14.5
	Gain (g)	22.0 ± 10.4	23.4 ± 8.9	13.1 ± 9.9	36.0 ± 10.2
	Gain (%)	4.7 ± 2.2	5.3 ± 2.0	3.6 ± 2.1	47.0 ± 40.9
	Food fed (g)	192 ± 5 z	144 ± 8 y	147 ± 6 y	150 ± 9 y
	FCR	-21.83 ± 30.30	11.03 ± 4.75	2.24 ± 4.20	5.39 ± 1.48
	SGR	0.13 ± 0.06	0.15 ± 0.06	0.10 ± 0.06	0.17 ± 0.04
Days 36-61					
	End weight (g)	553.2 ± 16.1	499.4 ± 34.3	519.4 ± 53.7	540.1 ± 16.1
	Gain (g)	41.7 ± 6.6	40.8 ± 14.6	42.2 ± 9.3	34.2 ± 12.7
	Gain (%)	8.2 ± 1.4	8.6 ± 2.7	9.3 ± 2.7	6.8 ± 2.6
	Food fed (g)	112 ± 6	81 ± 17	90 ± 13	82 ± 5
	FCR	2.85 ± 0.33	2.96 ± 1.16	2.34 ± 0.39	3.85 ± 1.31
	SGR	0.29 ± 0.05	0.30 ± 0.09	0.33 ± 0.09	0.24 ± 0.09

Days 62-92				
End weight (g)	669.2 ± 32.6	565.5 ± 67.8	596.5 ± 67.9	601.8 ± 21.7
Gain (g)	115.9 ± 24.3	66.0 ± 33.6	77.2 ± 19.0	61.7 ± 6.7
Gain (%)	20.9 ± 4.4	12.1 ± 5.4	14.6 ± 3.2	11.4 ± 1.1
Food fed (g)	197 ± 26	133 ± 33	120 ± 23	104 ± 7
FCR	1.83 ± 0.22	3.18 ± 0.96	1.72 ± 0.26	1.73 ± 0.16
SGR	0.61 ± 0.12	0.36 ± 0.15	0.44 ± 0.09	0.35 ± 0.03
Days 93-121				
End weight (g)	794.2 ± 47.0	650.6 ± 96.1	663.0 ± 81.2	695.3 ± 41.5
Gain (g)	125.0 ± 15.7	85.1 ± 28.7	66.4 ± 18.4	93.5 ± 20.2
Gain (%)	18.5 ± 1.7	14.0 ± 3.14	10.8 ± 2.9	15.2 ± 2.9
Food fed (g)	229 ± 31	144 ± 32	124 ± 34	127 ± 16
FCR	1.83 ± 0.04	1.90 ± 0.21	2.08 ± 0.33	1.52 ± 0.26
SGR	0.58 ± 0.05	0.45 ± 0.09	0.35 ± 0.09	0.49 ± 0.09
Overall (Days 1-121)				
Gain (g)	304.6 ± 40.3	215.4 ± 82.6	199.0 ± 39.4	218.1 ± 37.0
Gain (%)	62.4 ± 8.8	48.0 ± 16.7	44.6 ± 10.6	45.6 ± 7.4
Food fed (g)	730 ± 61	502 ± 87	480 ± 74	463 ± 28
FCR	2.45 ± 0.15	2.98 ± 0.59	2.66 ± 0.46	2.31 ± 0.38
SGR	0.40 ± 0.04	0.31 ± 0.09	0.30 ± 0.06	0.31 ± 0.04
Mortality (%)	1.0 ± 0.4	2.0 ± 0.7	1.5 ± 1.2	1.5 ± 0.6

[a] FCR = feed conversion ratio = total food fed / total weight gain.

[b] SGR = 100 x [(Ln(final weight) – Ln(initial weight)) / days]

Table 8. Mean (± SE) condition factor (K[a]), fin indices[b], hepatosomatic index values (HSI[c]), splenosomatic index (SSI[d]), viscerosomatic index (VSI[e]), and histology scores for lamina propria, connective tissue, and vacuoles of Brown Trout fed one of four diets containing either fishmeal or incremental amounts of bioprocessed soybean meal (BSM) as the primary protein source. Means with different letters in the same row differ significantly ($P < 0.05$).

Diet	1	2	3	4
BSM (%)	0	60	80	100
Initial				
Weight (g)	61.2 ± 2.5	54.4 ± 1.9	58.0 ± 8.0	59.6 ± 1.2
Length (mm)	171.9 ± 1.9	164.3 ± 1.9	169.1 ± 7.5	170.9 ± 1.9
K	1.19 ± 0.02	1.19 ± 0.01	1.16 ± 0.01	1.15 ± 0.01
Days 1-35				
End weight (g)	63.9 ± 2.1	57.3 ± 2.7	61.8 ± 6.8	63.2 ± 1.8
End length (mm)	178.2 ± 1.4	168.2 ± 3.1	175.5 ± 7.2	176.4 ± 2.2
K	1.12 ± 0.03	1.17 ± 0.02	1.10 ± 0.02	1.12 ± 0.01
Days 36-61				
End weight (g)	70.1 ± 3.0	66.5 ± 2.8	65.6 ± 6.5	68.2 ± 1.9
End length (mm)	185.3 ± 2.4	178.2 ± 2.6	180.4 ± 6.7	183.0 ± 1.4
K	1.11 ± 0.03	1.14 ± 0.01	1.07 ± 0.01	1.07 ± 0.01
Days 62-92				
End weight (g)	86.2 ± 5.5	77.1 ± 6.1	75.7 ± 8.0	77.1 ± 1.9
End length (mm)	195.8 ± 3.3	187.1 ± 4.2	188.2 ± 7.1	190.7 ± 2.2

K	1.13 ± 0.03	1.14 ± 0.01	1.09 ± 0.01	1.07 ± 0.01
Days 93-121 (Final)				
End weight (g)	103.7 ± 7.3	90.2 ± 8.9	85.8 ± 8.7	94.0 ± 4.2
End length (mm)	202.7 ± 6.6	192.7 ± 5.0	195.2 ± 6.7	201.7 ± 3.3
K	1.23 ± 0.06	1.21 ± 0.04	1.11 ± 0.02	1.10 ± 0.01
Pectoral index (%)	13.37 ± 0.35	13.54 ± 0.48	13.21 ± 0.49	13.51 ± 0.20
Pelvic index (%)	11.14 ± 0.15	11.15 ± 0.21	10.70 ± 0.36	10.70 ± 0.19
Dorsal index (%)	4.36 ± 0.58	4.66 ± 0.68	4.14 ± 0.52	4.17 ± 0.67
HSI (%)	1.14 ± 0.16	1.06 ± 0.12	1.06 ± 0.13	0.94 ± 0.11
SSI (%)	0.06 ± 0.01	0.07 ± 0.00	0.14 ± 0.06	0.14 ± 0.07
VSI (%)	5.29 ± 0.40	5.68 ± 0.27	5.00 ± 0.52	5.17 ± 0.32
Lamina propria	1.33 ± 0.14	1.50 ± 0.17	1.67 ± 0.14	1.58 ± 0.16
Connective Tissue	1.25 ± 0.08	1.33 ± 0.14	1.42 ± 0.08	1.58 ± 0.16
Vacuoles	2.50 ± 0.17	2.17 ± 0.22	2.08 ± 0.21	2.00 ± 0.19

[a] K = 10^5 x [weight / (length^3)]

[b] Fin indices = 100 x (fin length / fish length)

[c] HSI = 100 x (liver weight / body weight)

[d] SSI = 100 x (spleen weight / body weight)

[e] VSI = 100 x (visceral weight / body weight)

Figure 2. Representative (fed 60% BSM diet) Brown Trout histology image used for scoring.

CHAPTER 4: REARING PERFORMANCE OF ADULT RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FED A BIOPROCESSED SOYBEAN MEAL DIET WITH DIFFERING VELOCTY REGIMES

Abstract

This 90-day experiment evaluated the rearing performance of adult Rainbow Trout (*Oncorhynchus mykiss*) fed one of two isonitrogenous and isocaloric diets (46% protein, 16% lipid) and reared at velocities of either 3.6 or 33.2 cm/s. Fishmeal was the primary protein source for the reference diet, where-as bioprocessed soybean meal (BSM) directly replaced 60% of the dietary fishmeal in the experimental diet. At the end of the experiment, there were no significant differences in gain, percent gain, nor specific growth rate between the dietary treatments. However, the amount of food fed and feed conversion ratio (FCR) were significantly lower in the 60% bioprocessed soybean meal diet. There were also no significant differences in intestinal morphology, relative fin lengths, splenosomatic index, hepatosomatic index, or viscerosomatic index between the diets. Fish reared at 3.6 cm/s had a significantly lower FCR than fish reared at 33.2 cm/s. However, there was no significant differences in gain, percent gain, specific growth rate, or percent mortality between the two velocities. No significant interactions were observed between the diets and velocities. Based on these results, BSM can replace at least 60% of the fishmeal in adult Rainbow Trout diets, even if the fish are subjected to exercise at higher rearing velocities.

Introduction

Considerable research has evaluated soybean (*Glycine max*) use in Rainbow Trout diets (Storebakken et al. 2000; Collins et al. 2013). However, due to numerous antinutritional factors (Krogdahl et al. 1994, 2010, 2015; Arndt et al. 1999; Francis et al.

2001; Gatlin et al. 2007; Iwashita et al. 2008; Bakke 2011) and the possible harmful effects on overall health and growth (Rumsey et al. 1995; Burrels et al. 1999; Heikkinen et al. 2006; Barrows et al. 2008b; Iwashita et al. 2008; Romarheim et al. 2008; Merrifield et al. 2009; Sealey et al. 2009), soybean inclusion into carnivorous fish diets, such as Rainbow Trout, has been limited.

The use of heat, pressure, chemical treatments, and other processes has reduced or eliminated many of the antinutritional factors present in soybeans that induce undesirable effects in fishes (Cheeke and Shull 1985; Liener 1994; Gomes et al., 1995; Francis et al. 2001; Barrows et al. 2007). Bioprocessing, such as fermentation, has been shown to improve the suitability of soybean products as an alternative protein source in Rainbow Trout diets (Yamamoto et al. 2010, 2012; Bruce et al. 2017a).

In addition to dietary influences on fish rearing performance, exercise has also been shown to have impact (Davison and Goldspink 1977; Leon 1986; Houlihan and Laurent 1987; Christiansen and Jobling 1990; Young and Cech 1993; Gallaugher et al. 2001; Castro et al. 2011; Parker and Barnes 2014, 2015; Good et al. 2016; Liu et al. 2018). Parker and Barnes (2015) indicated that Rainbow Trout fed to satiation and exercised experienced improved growth. However, if feed is limited then growth was impaired (Parker and Barnes 2015).

Few studies evaluating bioprocessed soybean meal (BSM) in Rainbow Trout diets have been conducted, and novel BSM products continue to be developed. No research was found that has examined non-fishmeal based diets and exercise (rearing velocity) in any fish species. Therefore, the objective of this study was to examine the effects of a diet containing a proprietary BSM as the primary protein source, in conjunction with velocity (exercise), on the rearing performance and gastro-intestinal health of Rainbow Trout.

Methods

This experiment was conducted at Cleghorn Springs State Fish Hatchery, Rapid City, South Dakota, using 11° C spring water (total hardness as $CaCO_3$, 360 mg/L; alkalinity as $CaCO_3$, 210 mg/L; pH, 7.6; total dissolved solids, 390 mg/L).

Three-hundred twenty Erwin x Arlee strain Rainbow Trout (initial weight 139.0 ± 1.5 g, length 232.9 ± 0.8 mm, mean ± SE) were randomly selected and placed into one of 16, cement-bottom, aluminum-sided, circular tanks (6.1 m diameter, 73.7 cm water depth) on July 7, 2016 at a density of 20 fish per tank. This 90-day study used a 2 x 2 design (2 diets, 2 velocities), with four tanks per treatment. Study design and water velocities used are described in Table 9.

Water velocities were recorded using a flowmeter (Flowatch, JDC Electronic SA, Yverdon-les-Bains, Jura-Nord Vaudois, Vaud, Switzerland), with readings taken directly behind the spray bar, 60.0 cm from the side of the tank and about 36.1 m deep (mid-depth). Flow rates were set and kept constant throughout the study.

Two diets were used (Table 10), with BSM replacing 0 or 60% of the fish meal as the primary protein source. The BSM was produced using a proprietary microbial conversion process (SDSU, Brookings, SD, USA). Diets were isocaloric and isonitrogenous and were manufactured by cooking extrusion (ExtruTech model 325, Sabetha, KS). Feed was analyzed according to AOAC (2009) method 2001.11 for protein, 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and AACC (2000) method 08-03 for ash content.

At the start of the experiment all fish were individually weighed to the nearest 0.1 g, measured to the nearest 1.0 mm, and then placed into one of the sixteen tanks. Fish were subsequently weighed and measured approximately every four weeks. Individual fish weights were combined to obtain total tank weight. Fish were fed daily for 90 days, except on days when weight and length data were collected (days 31, 61, and 90). Feeding amounts were initially determined by the hatchery constant method (Butterbaugh and Willoughby 1967), with planned feed conversion rates of 1.1 and maximum growth rate of 0.08 cm/day, which was based on historical maximum growth rate of Erwin X Arlee strain Rainbow Trout at Cleghorn Springs State Fish Hatchery. Fish were fed by hand and feed was adjusted daily to be at or near satiation. The amount of food fed and mortality were recorded daily.

During data collection on days 1, 31, and 61 fish were anesthetized using 60 mg/L MS-222 (Tricaine-S, tricaine methanesulfonate, Syndel USA, Ferndale, Washington). On day 90, at the end of the experiment, fish were euthanized using a lethal dose of 250 mg/L MS-222 (AVMA 2013). In addition to weight and length measurements, fin lengths, to the nearest 1.0 cm, and spleen, liver, and visceral weights, to the nearest 1.0 mg, were recorded from five randomly selected Rainbow Trout per tank. Fin indices, hepatosomatic index (HSI) (Strange 1996), splenosomatic index (SSI) (Goede and Barton 1990), and viscerosomatic index (VSI) (Goede and Barton 1990) were calculated.

The following equations were used:

$$\text{Gain } = \text{end weight } - \text{start weight}$$

$$\text{Percent gain } (\%) = \frac{\text{gain}}{\text{start weight}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{food fed}}{\text{gain}}$$

$$\text{SGR} = 100 * \frac{\ln(\text{end weight}) - \ln(\text{start weight})}{\text{number of days}}$$

$$\text{K} = 10^5 * \frac{\text{fish weight}}{\text{fish length}^{\ 3}}$$

$$\text{Fin indices} = \frac{\text{fin length}}{\text{fish length}}$$

$$\text{HSI (\%)} = 100 * \frac{\text{liver weight}}{\text{whole fish weight}}$$

$$\text{SSI (\%)} = 100 * \frac{\text{spleen weight}}{\text{whole fish weight}}$$

$$\text{VSI (\%)} = 100 * \frac{\text{visceral weight}}{\text{whole fish weight}}$$

At the end of the experiment, a 2-mm wide section of the distal intestine was removed from five randomly-selected fish per tank to assess any possible soy-induced enteritis (Gu et al. 2017; Novriadi et al. 2017; Wang et al. 2017; Booman et al. 2018). After dissection, the intestinal tissue was immediately put into 10% buffered formalin, and stained with haematoxylin and eosin using standard histological techniques (Bureau et al. 1998; Burrels et al. 1999). Intestinal inflammation was assessed using an ordinal scoring system (Table 11) based on lamina propria thickness and cellularity, submucosal connective tissue width, and leukocyte distribution (Knudsen et al. 2007; Colburn et al. 2012; Barnes et al. 2014).

Data was analyzed using the SPSS (9.0) statistical analysis program (SPSS, Chicago Illinois), with significance predetermined at $P < 0.05$. Two-way analysis of variance (ANOVA) was conducted, and if treatments were significantly different, post hoc mean separation tests were performed using Tukey’s HSD test.

Results

At the end of the experiment there were no significant differences in gain, percent gain, SGR, and percent mortality between the tanks of fish receiving the fishmeal reference diet or the 60% BSM diet (Table 12). However, there was significant differences for food fed and FCR between the diets, with the fishmeal reference diet having higher values for both variables. The mean (± SE) FCR for Rainbow Trout fed the fishmeal diet was 1.10 (± 0.02), which was significantly higher than the 1.04 (± 0.03) value for fish fed 60% BSM.

There were no significant differences in gain, percent gain, or SGR in any of the rearing periods between the fish being fed the two different diets. Food fed and FCRs were significantly different between the diets in the first two rearing periods and overall, but were not significantly different for the last rearing period.

Individual fish weight, length, and condition factor were not significantly different between dietary treatments at the end of the experiment (Table 13). There were also no significant differences in fin indices (pectoral, pelvic, dorsal), organosomatic indices (HSI, SSI, VSI), or gut histology scores between the diets. Figure 3 shows a representative image sample of the distal intestines subjected to histology scoring.

Fish in the higher velocity tanks had a significantly higher FCR than the fish in the lower velocity tanks in each rearing period and overall. Gain, percent gain, food fed, SGR, and percent mortality at the end of the experiment were not significantly different between the two velocity treatments. However, during the third (final) rearing period gain, percent gain, and SGR were significantly different between the two velocity treatments, with mean (± SE) percent gain at 45.9 (± 0.7) for fish in lower velocity tanks, compared to 41.7 (± 1.6) for fish in higher velocity tanks.

Individual fish weight and length were significantly greater at the end of the experiment for fish reared at the lower velocity, with the mean (± SE) weights of 527.2 (± 15.2) g and 485.1 (± 9.6) g for the fish at low and high velocities, respectively. There were no significant differences in final fin indices (pectoral, pelvic, dorsal), organosomatic indices (HSI, SSI, VSI), or gut histology scores between the velocity treatments. There were also no significant interactions between diet and velocity in any of the variables measured at the end of the study or during any of the earlier rearing periods.

Discussion

The results of this experiment indicate that BSM can directly replace at least 60% of the dietary fishmeal in Rainbow Trout diets, even for fish subjected to higher velocities (exercise). Barnes et al. (2012, 2014, 2015b) also found fermented soybean meal could replace approximately 62% of the fish meal in Rainbow Trout diets without any deleterious effects on growth. Similarly, Bruce et al. (2017a, 2017b) replaced approximately 65% of fishmeal with a BSM without any significant difference in Rainbow Trout performance. Yamamoto et al. (2010, 2012) was able to replace 100% of the fishmeal with fermented soybean meal without any difference in growth, but Yamamoto studies were conducted in much warmer water (16.3 °C), and the diets were supplemented with many amino acids. Other species where BSM have been evaluated include Atlantic Cod (*Gadus morhua*) (Refstie et al. 2006; Ringø et al. 2006), Atlantic Salmon (*Salmo salar*) (Refstie et al. 2005), Black Sea Bream (*Acanthopagrus schlegeli*)

(Zhou et al. 2011; Azarm and Lee 2014), Brown Trout (*Salmo trutta*) (Sotoudeh et al. 2016), Chinese Sucker (*Myxocyprinus asiaticus*) (Yuan et al. 2012), Florida Pompano (*Trachniotus carolinus*) (Novriadi et al. 2017), Gilthead Sea Bream (*Sparus aurata* L.) (Kokou et al. 2012), Japanese Flounder (*Paralichthys olivaceus*) (Kader et al. 2012), Orange-spotted Grouper (*Epinephelus coioides*) (Shiu et al. 2015), Whiteleg Shrimp (*Litopenaeus vannamei*) (Chiu et al. 2016; Van Nguyen et al. 2018), Rockfish (*Sebastes schlegeli*) (Lee et al. 2016), White Seabass (*Atractosion nobilis*) (Trushenski et al. 2014), and Yellowtail Jack (*Seriola lalandi*) (Trushenski et al. 2014).

Weathercup and McCraken (1999) suggested that a feed trial should last long enough to determine any dietary-induced differences in fish performance. NRC (2011) suggests study duration of 56-84 days, or longer if needed for large fish to attain 200-300% gain. The 265% gain at the end of this 90-day study met or exceeded these requirements.

Negative effects on the distal intestine of Rainbow Trout from dietary soybean products are well-documented (Rumsey et al. 1995; Burrels et al. 1999; Heikkeinen et al. 2006; Barrows et al. 2008a; Iwashita et al. 2008; Romarheim et al. 2008; Merrifield et al. 2009; Sealey et al. 2009). However, the histological data in this study did not reveal any enteritis or histological changes in the fish receiving BSM. This is likely due to the additional processing of the defatted soybean meal (Yamamoto et al. 2010, 2012; Barnes et al. 2012, 2013), by depleting saponins (Krogdahl et al. 2015) and other compounds linked to enteritis. The histological scores observed in this study tended to be lower than those reported by Barnes et al. (2014, 2015a, 2015b). However, those studies used different dietary formulations and a different BSM.

HSI values are an indirect measurement of glycogen and carbohydrate levels, and can be used to indicate nutritional state of the fish (Daniels and Robinson 1986; Kim and Kaushik 1992; Barton et al. 2002). The similar HSI values in all of the treatments indicate similar energy partitioning in the fish. The HSI values observed in this study were 1.4 to 1.5, which is similar to those reported by Barnes et al. (2013, 2014, 2015b), Parker and Barnes (2014, 2015) and Kientz and Barnes (2016), but slightly higher than those reported by Yamamoto et al. (2010, 2012), Barnes et al. (2015b), and Bruce et al. (2017b). These inter-study differences in HSI could be related to a fish age and size. Barton et al. (2002) noted that the organosomatic indices can vary depending upon life stage, and the Rainbow Trout used in this study were much larger and older than sizes commonly used in the most fish nutrition experiments.

The VSI indicates how lipids are being used or stored with VSI and lipid levels positively related (Jobling et al. 1998; Company et al. 1999; Yildiz et al. 2006). Thus, the similar VSI values observed in this study are likely due to similar dietary lipid levels. The VSI values of about 13.3 in this study are similar to Barnes et al. (2014) who examined adult Rainbow Trout. However, the VSI values are higher compared to the values reported by Barnes et al. (2013, 2015a, 2015b), Bruce et al. (2017b), Parker and Barnes (2014, 2015), and Kientz and Barnes (2016) for smaller and younger Rainbow Trout.

SSI indicates the hematopoietic capacity of fish (Barton et al. 2002), with antibody production occurring mostly in the spleen (Smith 1991). The similar SSI values indicate that fish health was likely unaffected by dietary treatment. The SSI values observed in this study were within the range reported by others (Barnes et al. 2015b; Parker and Barnes 2015; Kientz and Barnes 2016; Bruce et al. 2017b).

In addition to diet, exercise has been shown to impact fish growth (Davison and Goldspink 1977; Leon 1986; Houlihan and Laurent 1987; Christiansen and Jobling 1990; Young and Cech 1993; Gallaugher et al. 2001; Castro et al. 2011; Parker and Barnes 2014, 2015; Good et al. 2016; Liu et al. 2018). Higher velocities seemed to improve fish rearing performance in this study, but these positive effects were limited to the first two months. In comparison to other studies on exercise with durations of 28 to 70 days, this study lasted approximately 30 to 60 days longer (Davison and Goldspink 1977; Leon 1986; Houlihan and Laurent 1987; Christiansen and Jobling 1990; Young and Cech 1993; Castro et al. 2011; Parker and Barnes 2014, 2015; Good et al. 2016; Liu et al. 2018). Only the Gallagher et al. (2001) experiment lasted 4 months, but did not report rearing performance. How can exercise influence growth rates for two months, and then disappear? Perhaps the fish could be exhibiting signs of exercise fatigue, which has been reported in humans after extended periods of intense exercise (Noakes 2000; Noakes et al. 2005; Crewe et al. 2008; Joyner and Coyle 2008).

The fish in the tanks at higher velocities had significantly higher (poorer) FCRs than the fish at the lower velocities. Parker and Barnes (2015) found that Rainbow Trout fed to satiation had similar FCRs, but FCR increased in exercised fish fed a restricted diet. It is possible that the fish in the higher velocity tanks, which were forced to exercise, did not receive enough food. Although food availability was increased daily in accordance with apparent satiation, it may not have been sufficient for the extra energy demands due to higher velocities.

The similar relative fin lengths observed in this study are another indirect indicator of the suitability of dietary bioprocessed soybean meal. In addition to nutritional influences (Lemm et al. 1988; Kindischi et al. 1991), fin length can also be due to tank-induced abrasions (Bosakowski and Wagner 1995), rearing unit size and type (Bosakowski and Wagner 1994), aggressive behavior (Latremouille 2003), feeding rates (Wagner et al. 1996), rearing densities (Miller et al. 1995; Wagner et al. 1997; North et al. 2006), dietary nutritional differences (Lemm et al. 1988; Kindischi et al. 1991), environmental stress (Latremouille 2003), and fish health (Devesa et al. 1989). The relative fin lengths in this experiment are similar to those reported by Bosakowski and Wagner (1994) and Arndt et al. (2002).

In conclusion, BSM can replace at least 60% of the dietary fishmeal in Rainbow Trout diets with no ill-effects, even if trout are subject to exercise. However, the time-related effects of exercise on rearing performance observed in this study indicate a need to examine potential exercise fatigue in fish subject to continuous high rearing velocities.

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Table 9. Study design for dietary and velocity treatments, and mean velocities (± SE).

		Diet (% BSM)		Velocity		
Treatment	N	1 (0)	2 (60)	Low	High	Velocity (cm/s)
1	4	X		X		3.6 ± 0.6
2	4	X			X	33.2 ± 1.8
3	4		X	X		3.6 ± 0.6
4	4		X		X	33.2 ± 1.8

Table 10. Diet formulation and composition analyses of the diets used in the 90-day trial. Analysis conducted on post-extrusion feed pellets.

	Diet (%)	
Ingredients	1	2
Fishmeal[a]	35.0	14.0
Bioprocessed soybean meal[b]	0.0	12.0
Wheat midds[c]	12.0	10.0
Whole wheat[c]	17.7	15.2
Poultry byproduct meal[d]	10.0	15.0
Blood meal[e]	2.0	2.0
Feather meal[d]	7.0	2.5
Vitamin premix[f]	1.3	1.3
Mineral premix[f]	0.8	2.0
Micro-mineral premix[f]	0.8	0.8
Choline chloride[g]	0.6	0.6
L-Lysine[h]	1.5	2.0
L-Methionine[i]	0.3	0.5
Stay-C 35[j]	0.2	0.2
Fish oil[k]	11.0	13.0
Total	100	100
Chemical analysis (% dry basis)		
Protein	43.18	43.85
Lipid	15.91	14.28
Ash	2.42	3.60
Nitrogen-free extract	20.48	24.33
Dry matter	93.00	95.20
Gross Energy (kJ/g)	16.5	16.0
Protein : Energy (MJ/g)	26.2	27.4

[a] Special Select, Omega Protein, Houston, TX; [b] SDSU; [c] Consumer Supply, Sioux City, IA; [d] Tyson Foods, Springdale, AR; [e] Mason City Byproducts, Mason City, IA; [f] NutraBlend, Neosho, MO; [g] Balchem, New Hampton, NY; [h] CJ Bio America, Fort Dodge, IA; [i] Adisseo USA, Alpharreta, GA; [j] DSM Nutritional Products, Ames, IA; [k] Virginia Prime Gold, Omega Protein, Houston, TX.

Table 11. Histological scoring system used on Rainbow Trout fed fishmeal or bioprocessed soybean meal diets (Barnes et al. 2014, modified from Geode and Barton 1990, Adams et al. 1993, and Barton et al. 2002).

Score	Appearance
	Lamina propria of simple folds
1	Thin and delicate core of connective tissue in all simple folds.
2	Lamina propria slightly more distinct and robust in some of the folds.
3	Clear increase in lamina propria in most of simple folds.
4	Thick lamina propria in many folds.
5	Very thick lamina propria in many folds.
	Connective tissue between base of folds and stratum compactum
1	Very thin layer of connective tissue between base of folds and stratum compactum.
2	Slightly increased amount of connective tissue beneath some of mucosal folds.
3	Clear increase of connective tissue beneath most of the mucosal folds.
4	Thick layer of connective tissue beneath many folds.
5	Extremely thick layer of connective tissue beneath some of the folds.
	Vacuoles
1	Large vacuoles absent.
2	Very few large vacuoles present.
3	Increased number of large vacuoles.
4	Large vacuoles are numerous.
5	Large vacuoles are abundant in present in most epithelial cells.

Table 12. Mean (± SE) gain, percent gain, food fed, feed conversion ratio (FCR[a]), specific growth rate (SGR[b]), and mortality of Rainbow Trout receiving one of two different diets containing fishmeal or bioprocessed soybean meal (BSM) as the main protein ingredient, and reared at two different velocities. Overall means with different letters in the same column or row differ significantly (P < 0.05).

			Diet (% BSM)	
	Velocity	1 (0)	2 (60)	Overall
Initial				
Start weight (g)	Low	2,843.0 ± 91.0	2,769.0 ± 18.4	2,806.0 ± 45.2
	High	2,734.8 ± 48.9	2,771.2 ± 74.5	2,753.0 ± 41.8
	Overall	2,788.9 ± 52.0	2,770.1 ± 35.5	
Days 1-31				
End weight (g)	Low	4,637.4 ± 136.5	4,442.6 ± 88.3	4,540.0 ± 83.8
	High	4,294.6 ± 72.4	4,375.8 ± 164.7	4,335.2 ± 84.7
	Overall	4,466.0 ± 96.5	4,409.2 ± 87.4	
Gain (g)	Low	1,794.3 ± 55.7	1,673.6 ± 90.5	1,733.9 ± 54.2
	High	1,559.8 ± 39.4	1,604.6 ± 96.4	1,582.2 ± 48.9
	Overall	1,677.0 ± 54.4	1,639.1 ± 62.6	
Gain (%)	Low	63.2 ± 1.5	60.5 ± 3.4	61.8 ± 1.8
	High	57.1 ± 1.5	57.8 ± 2.2	57.4 ± 1.2
	Overall	60.1 ± 1.5	59.1 ± 1.9	
Food fed (g)	Low	1,796 ± 39	1,534 ± 46	1,665 ± 57
	High	1,792 ± 11	1,613.0 ± 53	1,702.8 ± 42

	Overall	1,794 ± 19 z	1,574 ± 36 y	
FCR	Low	1.00 ± 0.03	0.92 ± 0.03	0.96 ± 0.02 y
	High	1.15 ± 0.01	1.01 ± 0.05	1.08 ± 0.04 z
	Overall	1.08 ± 0.03 z	0.97 ± 0.03 y	
SGR	Low	1.63 ± 0.03	1.57 ± 0.07	1.60 ± 0.04
	High	1.51 ± 0.03	1.52 ± 0.05	1.51 ± 0.03
	Overall	1.57 ± 0.03	1.55 ± 0.04	
Days 32-61				
End weight (g)	Low	7,365.6 ± 337.6	6,978.8 ± 200.3	7,172.2 ± 195.9
	High	6,862.8 ± 60.3	6,917.4 ± 293.3	6,890.1 ± 139.0
	Overall	7,114.2 ± 185.0	6,948.1 ± 164.8	
Gain (g)	Low	2,728.2 ± 229.5	2,536.3 ± 115.5	2,632.2 ± 124.3
	High	2,568.2 ± 90.3	2,541.6 ± 144.1	2,554.9 ± 78.9
	Overall	2,648.2 ± 118.1	2,539.0 ± 85.5	
Gain (%)	Low	58.7 ± 3.9	57.0 ± 1.6	57.8 ± 2.0
	High	59.9 ± 2.9	58.0 ± 2.0	59.0 ± 1.7
	Overall	59.3 ± 2.3	57.5 ± 1.2	58.4 ± 1.3
Food fed (g)	Low	2,757 ± 204	2,318 ± 118	2,537 ± 137
	High	2,778 ± 110	2,438 ± 96	2,608 ± 93
	Overall	2,767 ± 107 z	2,378 ± 74 y	
FCR	Low	1.01 ± 0.03	0.91 ± 0.02	0.96 ± 0.02 y
	High	1.08 ± 0.01	0.96 ± 0.03	1.02 ± 0.03 z
	Overall	1.05 ± 0.02 z	0.94 ± 0.02 y	

SGR	Low	1.54 ± 0.08	1.50 ± 0.03	1.52 ± 0.04
	High	1.56 ± 0.06	1.52 ± 0.04	1.54 ± 0.04
	Overall	1.55 ± 0.05	1.5 ± 0.02	
Days 62-90				
End weight (g)	Low	10,791.1 ± 449.7	10,132.3 ± 338.2	10,461.7 ± 288.7
	High	9,842.9 ± 208.2	9,673.1 ± 281.8	9,758.0 ± 165.3
	Overall	10,317.0 ± 291.1	9,902.7 ± 221.5	
Gain (g)	Low	3,425.5 ± 121.3	3,153.5 ± 147.4	3,289.5 ± 102.2 z
	High	2,980.1 ± 184.4	2,755.6 ± 56.9	2,867.9 ± 98.9 y
	Overall	3,202.8 ± 132.4	2,954.6 ± 104.9	
Gain (%)	Low	46.6 ± 0.9	45.1 ± 1.1	45.9 ± 0.7 y
	High	43.4 ± 2.6	40.1 ± 2.0	41.7 ± 1.6 z
	Overall	45.0 ± 1.4	42.6 ± 1.4	
Food fed (g)	Low	3,780 ± 210	3,390 ± 126	3,585 ± 135
	High	3,653 ± 131	3,547 ± 70	3,600 ± 72
	Overall	3,716 ± 117	3,468 ± 73	
FCR	Low	1.10 ± 0.03	1.08 ± 0.03	1.09 ± 0.02 y
	High	1.23 ± 0.05	1.29 ± 0.05	1.26 ± 0.03 z
	Overall	1.17 ± 0.04	1.18 ± 0.05	
SGR	Low	1.28 ± 0.02	1.24 ± 0.02	1.26 ± 0.02 z
	High	1.20 ± 0.06	1.12 ± 0.05	1.16 ± 0.04 y
	Overall	1.24 ± 0.03	1.18 ± 0.03	
Overall (Days 1-90)				

Gain (g)	Low	7,948.0 ± 392.2	7,363.3 ± 341.3	7,655.7 ± 264.8
	High	7,108.1 ± 234.6	6,901.9 ± 215.7	7,005.0 ± 152.6
	Overall	7,528.1 ± 264.5	7,132.6 ± 206.2	
Gain (%)	Low	279.6 ± 11.2	266.0 ± 12.8	272.8 ± 8.3
	High	260.4 ± 11.7	249.1 ± 4.0	254.8 ± 6.1
	Overall	270.0 ± 8.3	257.5 ± 7.0	
Food fed (g)	Low	8,333 ± 443	7,241 ± 288	7,787 ± 320
	High	8,224 ± 239	7,599 ± 217	7,911 ± 190
	Overall	8,278 ± 234 z	7,420 ± 180 y	
FCR	Low	1.05 ± 0.01	0.98 ± 0.02	1.02 ± 0.02 y
	High	1.12 ± 0.01	1.10 ± 0.02	1.13 ± 0.02 z
	Overall	1.10 ± 0.02 z	1.04 ± 0.03 y	
SGR	Low	1.48 ± 0.03	1.44 ± 0.04	1.46 ± 0.02
	High	1.42 ± 0.04	1.39 ± 0.01	1.41 ± 0.02
	Overall	1.45 ± 0.02	1.41 ± 0.02	
Mortality (%)	Low	2.5 ± 2.5	0.0 ± 0.0	1.2 ± 1.2
	High	1.2 ± 1.2	0.0 ± 0.0	0.6 ± 0.6
	Overall	1.9 ± 1.3	0.0 ± 0.0	

[a] FCR = feed conversion ratio = total food fed / total weight gain.

[b] SGR = 100 x [(Ln(final weight) – Ln(initial weight)) / days]

Table 13. Mean (± SE) condition factor (K[a]), fin indices[b], hepatosomatic index values (HSI[c]), splenosomatic index (SSI[d]), viscerosomatic index (VSI[e]), and histology scores for lamina propria, connective tissue, and vacuoles of Rainbow Trout fed one of two diets containing either fishmeal or bioprocessed soybean meal (BSM) as the primary protein source, and reared at two different velocities. Means with different letters in the same column or row differ significantly (*P* < 0.05).

		Diet (% BSM)		
	Velocity	1 (0)	2 (60)	Overall
Initial				
Weight (g)	Low	142.2 ± 4.6	138.4 ± 0.9	140.3 ± 2.3
	High	136.8 ± 2.4	138.6 ± 3.7	137.6 ± 2.1
	Overall	139.4 ± 2.6	138.5 ± 1.8	
Length (mm)	Low	233.2 ± 2.9	232.9 ± 1.0	233.1 ± 1.4
	High	233.4 ± 1.8	231.8 ± 1.8	232.6 ± 1.2
	Overall	233.3 ± 1.6	232.4 ± 1.0	
K	Low	1.13 ± 0.03	1.08 ± 0.01	1.11 ± 0.02
	High	1.06 ± 0.01	1.10 ± 0.01	1.08 ± 0.01
	Overall	1.10 ± 0.02	1.09 ± 0.01	
Days 1-31				
End weight (g)	Low	231.8 ± 6.8	222.1 ± 4.4	227.0 ± 4.2
	High	212.0 ± 1.0	218.8 ± 8.2	215.4 ± 4.0
	Overall	221.9 ± 4.9	220.5 ± 4.4	
End length (mm)	Low	262.0 ± 3.2	260.6 ± 1.0	261.3 ± 1.6

	High	256.8 ± 0.9	256.8 ± 3.3	256.8 ± .16
	Overall	259.4 ± 1.8	258.7 ± 1.7	
K	Low	1.27 ± 0.01	1.24 ± 0.02	1.25 ± 0.01
	High	1.24 ± 0.01	1.27 ± 0.01	1.26 ± 0.01
	Overall	1.25 ± 0.01	1.26 ± 0.01	
Days 32-61				
End weight (g)	Low	371.9 ± 16.6	349.0 ± 10.0	360.4 ± 10.0
	High	339.0 ± 4.6	345.9 ± 14.7	342.4 ± 7.2
	Overall	355.4 ± 10.1	347.4 ± 8.2	
End length (mm)	Low	299.6 ± 4.2	295.4 ± 0.8	297.5 ± 2.1
	High	292.3 ± 1.1	291.0 ± 3.0	291.6 ± 1.5
	Overall	295.9 ± 2.4	293.2 ± 1.7	
K	Low	1.36 ± 0.01	1.34 ± 0.04	1.35 ± 0.02
	High	1.34 ± 0.02	1.39 ± 0.03	1.36 ± 0.02
	Overall	1.35 ± 0.01	1.36 ± 0.03	
Days 62-90 (Final)				
End weight (g)	Low	547.9 ± 22.7	506.6 ± 16.9	527.2 ± 15.2 z
	High	486.6 ± 15.1	483.6 ± 14.1	485.1 ± 9.6 y
	Overall	517.2 ± 17.1	495.1 ± 11.1	
End length (mm)	Low	336.2 ± 4.9	331.9 ± 2.7	334.1 ± 2.7 z
	High	326.8 ± 2.6	324.0 ± 3.8	325.4 ± 2.2 y
	Overall	331.5 ± 3.1	328.0 ± 2.6	
K	Low	1.41 ± 0.01	1.36 ± 0.02	1.39 ± 0.01

	High	1.38 ± 0.01	1.41 ± 0.02	1.39 ± 0.01
	Overall	1.40 ± 0.01	1.38 ± 0.01	
Pectoral index (%)	Low	11.74 ± 0.18	12.13 ± 0.19	11.94 ± 0.14
	High	11.92 ± 0.20	11.81 ± 0.09	11.87 ± 0.10
	Overall	11.83 ± 0.13	11.97 ± 0.12	
Pelvic index (%)	Low	10.13 ± 0.09	10.32 ± 0.25	10.22 ± 0.13
	High	10.42 ± 0.14	10.52 ± 0.04	10.49 ± 0.07
	Overall	10.27 ± 0.09	10.42 ± 0.12	
Dorsal index (%)	Low	7.43 ± 0.85	6.55 ± 0.25	6.99 ± 0.44
	High	5.74 ± 0.69	6.86 ± 0.65	6.30 ± 0.49
	Overall	6.59 ± 0.60	6.71 ± 0.33	
HSI (%)	Low	1.58 ± 0.05	1.50 ± 0.04	1.54 ± 0.03
	High	1.38 ± 0.12	1.45 ± 0.02	1.41 ± 0.06
	Overall	1.48 ± 0.07	1.48 ± 0.02	
SSI (%)	Low	0.078 ± 0.009	0.083 ± 0.006	0.080 ± 0.005
	High	0.083 ± 0.008	0.078 ± 0.005	0.080 ± 0.004
	Overall	0.080 ± 0.005	0.080 ± 0.004	
VSI (%)	Low	13.90 ± 0.56	12.89 ± 0.19	13.39 ± 0.33
	High	13.14 ± 0.62	13.2 ± 0.40	13.18 ± 0.34
	Overall	13.52 ± 0.41	13.05 ± 0.21	
Lamina propria[f]	Low	1.40 ± 0.28	1.43 ± 0.21	1.42 ± 0.16
	High	1.56 ± 0.26	1.60 ± 0.37	1.58 ± 0.21
	Overall	1.48 ± 0.18	1.52 ± 0.20	

Connective Tissue[f]	Low	2.10 ± 0.40	2.37 ± 0.28	2.23 ± 0.23
	High	1.93 ± 0.22	1.92 ± 0.34	1.93 ± 0.19
	Overall	2.02 ± 0.22	2.14 ± 0.22	
Vacuoles[f]	Low	1.95 ± 0.05	1.73 ± 0.09	1.84 ± 0.06
	High	1.76 ± 0.31	1.85 ± 0.34	1.81 ± 0.21
	Overall	1.86 ± 0.15	1.79 ± 0.17	

[a] K = 10^5 x [weight / (length^3)]

[b] Fin indices = 100 x (fin length / fish length)

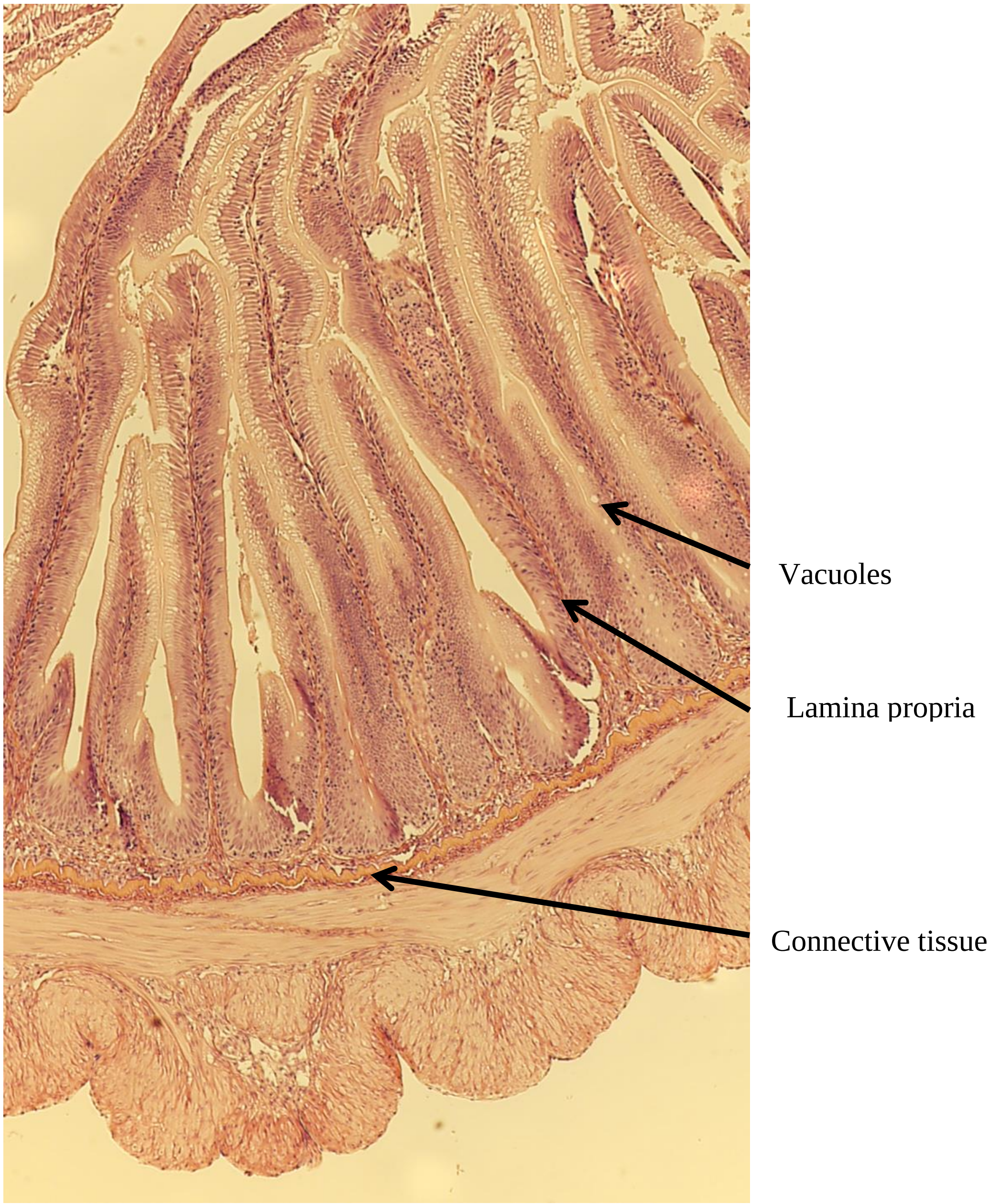
[c] HSI = 100 x (liver weight / body weight)

[d] SSI = 100 x (spleen weight / body weight)

[e] VSI = 100 x (visceral weight / body weight)

[f] Scoring parameters in Table 11

Figure 3. Representative (fed 60% BSM diet at slow velocity) Rainbow Trout histology image used for scoring.



CHAPTER 5: REARING PERFORMANCE OF JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FED A BIOPROCESSED SOYBEAN MEAL DIET WITH DIFFERING VELOCITY REGIMES

Abstract

This 88-day experiment evaluated the rearing performance of juvenile Shasta strain Rainbow Trout (*Oncorhynchus mykiss*) fed one of three isonitrogenous and isocaloric (46% protein, 16% lipid) diets and reared at velocities of either 2.3 or 18.7 cm/s. Fishmeal was the primary protein source for the reference diet, whereas bioprocessed soybean meal (BSM) directly replaced either 60 or 80% of the fishmeal in the experimental diets. At the end of the experiment there were no significant differences among the dietary treatments in gain, percent gain, specific growth rate, or percent mortality. However, fish fed the fishmeal based diet ate significantly more, experienced a significantly higher feed conversion ratio (FCR), and had a significantly higher hepatosomatic index than the fish fed the 80% bioprocessed soybean product diet. Relative fin length, splenosomatic index, and viscerosomatic index were not significantly different among the diets. Intestinal histology was not affected by the inclusion of BSM. Fish reared at 2.3 cm/s had significantly lower FCRs, gain, percent gain, and specific growth rates than the fish reared at 18.7 cm/s. There was a significant interaction for the amount of food consumed between diet and velocity. Based on these results, BSM can replace at least 80% of the fishmeal in juvenile Rainbow Trout, even if the fish are subjected to higher velocity flows.

Introduction

With the large increase in aquaculture production, there has been a corresponding increase in aquafeeds (FAO 2016). The primary protein source for carnivorous fish, like

Rainbow Trout (*Oncorhynchus mykiss*) has historically been fishmeal (Satia 1974; Kim et al. 1991; Cheng and Hardy 2004). Fish meal is primarily made from small, pelagic marine fish, and capture fisheries have not grown at an equal rate as aquaculture (FAO 2016). Increased demand coupled with unstable supply has created a need to find non-fishmeal protein sources for aquafeeds. Plant-based proteins are a prime candidate due to wide availability and favorable pricing (Gatlin et al. 2007).

Of the plant-based proteins, soybeans (*Glycine max*) are one of the leading alternatives to fishmeal (Nordrum et al. 2000; Li and Robinson 2015). Soybeans are highly palatable (Sugiura et al. 1998; Refstie et al. 2000; Watanabe 2002), high in protein, and have a balanced amino acid profile (Gatlin et al. 2007; NRC 2011). However, soybeans also have antinutritional factors that hinder fish digestion (Salunkhe et al. 1992; Krogdahl et al. 1994; Kaushik et al. 1995; Bureau et al. 1998; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Iwashita et al. 2008; NRC 2011; Teng et al. 2012), and can also cause gastro-intestinal issues, such as enteritis (van deh Ingh et al. 1991; Rumsey et al. 1995; Baeverfjord and Krogdahl 1996; Burrells et al. 1999; Bakke-McKellep et al. 2000; Krogdahl et al. 2000, 2015; Refstie et al. 2000; Heikkinen et al. 2006). Soybeans also have high levels of carbohydrates (Salunkhe et al. 1992; Gatlin et al. 2007), which can be especially deleterious to carnivorous fish (NRC 2011). Because of these antinutritional factors and carbohydrate levels, soybean inclusion in aquafeeds has been limited.

There are ways to decrease or eliminate many of the antinutritional factors. Heat, which is applied to feed during the extrusion process, decreases lectins and proteinase inhibitors (Cheeke and Shull 1985; Liener 1994; Gomes et al. 1995; Arndt et al. 1999;

Francis et al. 2001; Barrows et al. 2007; Gatlin et al. 2007; Krogdahl et al. 2010; Bakke 2011). Saponins, sterols, and oligosaccharides can be decreased by alcohol extraction (Krogdahl et al. 2010). Bioprocessing has also been shown to eliminate or reduce antinutritional factors (Hong et al. 2004; Refstie et al. 2005; Yamamoto et al. 2010, 2012).

Exercise, in addition to diet, has been shown to improve fish rearing performance (Davison and Goldspink 1977; Leon 1986; Houlihan and Laurent 1987; Christiansen and Jobling 1990; Young and Cech 1993; Gallaugher et al. 2001; Castro et al. 2011; Parker and Barnes 2014, 2015; Good et al. 2016; Liu et al. 2018). Exercise improves growth and feed conversion ratios (FCR), when fish are fed to satiation (Parker and Barnes 2015; Waldrop et al. 2018). However, if feed is limited, growth can be impaired at higher velocities (Parker and Barnes 2014).

This experiment is similar to the Chapter 4 experiment that examined bioprocessed soybean meal (BSM) and velocity in the diets of adult Rainbow Trout. However, this experiment used juvenile Rainbow Trout and also included an 80% BSM diet. Additionally, this experiment used a different strain of Rainbow Trout. Different Rainbow Trout strains have been shown to exhibit different responses to dietary ingredients (Barnes et al. 2006a, 2006b, 2007, 2015a; Overturf et al. 2013), and different sizes of fish can also impact nutritional acceptance (NRC 2011). The objective of this experiment was to examine the effects of BSM diets and velocity on juvenile Rainbow Trout rearing performance.

Methods

This experiment was conducted at Cleghorn Springs State Fish Hatchery, Rapid City, South Dakota, using 11° C spring water (total hardness as $CaCO_3$, 360 mg/L; alkalinity as $CaCO_3$, 210 mg/L; pH, 7.6; total dissolved solids, 390 mg/L).

Three-hundred sixty Shasta strain Rainbow Trout (initial weight 48.8 ± 0.5 g, length 156.8 ± 0.5 mm, mean ± SE) were randomly selected and placed into one of 18 circular fiberglass tanks (1.8 m diameter, 0.6 m water depth) on August 4, 2016, at twenty fish per tank. This 88-day study used a 3 x 2 design to evaluate the effects of water velocity and diet on Rainbow Trout rearing performance, with three tanks per treatment. Study design and water velocities used are described in Table 14.

Water velocities were recorded using a flowmeter (Flowatch, JDC Electronic SA, Yverdon-les-Bains, Jura-Nord Vaudois, Vaud, Switzerland), with readings taken directly behind the spray bar, 30.5 cm from the side of the tank and about 0.3 m deep (half way in water column). Flow rates were set and kept constant throughout the study.

Three different diets were used (Table 15), with modified soybean meal replacing 0, 60, or 80% of the fish meal as the primary protein source. The modified soybean meal was produced using a proprietary microbial conversion process (SDSU, Brookings, SD, USA). Diets were isocaloric and isonitrogenous and were manufactured by cooking extrusion (ExtruTech model 325, Sabetha, KS). Feed was analyzed according to AOAC (2009) method 2001.11 for protein, 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and AACC (2000) method 08-03 for ash content.

At the start of the experiment fish were individually weighed to the nearest 0.1 g, measured to the nearest 1.0 mm, and then placed into one of the eighteen tanks. Fish were weighed and measured approximately every four weeks. The individual fish weights were combined to obtain total tank weight.

Fish were fed daily for 88 days, except on days they were weighed and measured (days 29, 60, and 88). Feeding amounts were initially determined by the hatchery constant method (Butterbaugh and Willoughby 1967), with planned feed conversion rates of 1.1 and maximum growth rate of 0.08 cm/day, which was based on historical maximum growth rate of Shasta strain Rainbow Trout at Cleghorn Springs State Fish Hatchery. Fish were fed by hand daily and feed was adjusted daily to be at or near satiation. Feed and mortality were recorded daily.

Prior to data collection on days 1, 29, and 60, fish were anesthetized using 60 mg/L MS-222 (Tricaine-S, tricaine methanesulfonate, Syndel USA, Ferndale, Washington). On day 88, at the end of the experiment, fish were euthanized using a lethal dose of 250 mg/L MS-222 (AVMA 2013). In addition to weight and length measurements, fin lengths, to the nearest 1.0 cm, and spleen, liver, and visceral weights, to the nearest 1.0 mg were recorded from five randomly selected Rainbow Trout per tank. Fin indices, hepatosomatic index (HSI) (Strange 1996), splenosomatic index (SSI) (Goede and Barton 1990), and viscerosomatic index (VSI) (Goede and Barton 1990) were calculated.

The following equations were used:

$$\text{Gain} = \text{end weight} - \text{start weight}$$

$$\text{Percent gain (\%)} = \frac{\text{gain}}{\text{start weight}}$$

$$\text{FCR} = \frac{\text{food fed}}{\text{gain}}$$

$$\mathrm{SGR} = 100 * \frac{\ln(\text{end weight}) - \ln(\text{start weight})}{\text{number of days}}$$

$$\mathrm{K} = 10^5 * \frac{\text{fish weight}}{\text{fish length}^{3}}$$

$$\text{Fin indices} = \frac{\text{fin length}}{\text{fish length}}$$

$$\mathrm{HSI}\ (\%) = 100 * \frac{\text{liver weight}}{\text{whole fish weight}}$$

$$\mathrm{SSI}\ (\%) = 100 * \frac{\text{spleen weight}}{\text{whole fish weight}}$$

$$\mathrm{VSI}\ (\%) = 100 * \frac{\text{visceral weight}}{\text{whole fish weight}}$$

A 2-mm wide section of the distal intestine was removed from five randomly-selected fish per tank to assess any possible soy-induced enteritis (Gu et al. 2017; Novriadi et al. 2017; Wang et al. 2017; Booman et al. 2018). After dissection, the intestinal tissue was immediately put into 10% buffered formalin, and stained with haematoxylin and eosin using standard histological techniques (Bureau et al. 1998; Burrels et al. 1999). Intestinal inflammation was assessed using an ordinal scoring system (Table 16) based on lamina propria thickness and cellularity, submucosal connective tissue width, and leukocyte distribution (Knudsen et al. 2007; Colburn et al. 2012; Barnes et al. 2014).

Data was analyzed using the SPSS (9.0) statistical analysis program (SPSS, Chicago Illinois), with significance predetermined at $P < 0.05$. Two-way analysis of variance (ANOVA) was conducted, and if treatments were significantly different, post hoc mean separation tests were performed using Tukey's HSD test.

Results

At the end of this experiment there were no significant differences in gain, percent gain, SGR, or percent mortality among the three diets (Table 17). Food fed and FCR was significantly different between the fishmeal reference diet and 80% BSM, with overall mean (± SE) FCRs of 1.09 (± 0.04), 1.04 (± 0.01), and 0.97 (± 0.02) for the fishmeal, 60, and 80% diets, respectively. FCR was also significantly higher in the fishmeal treatment in rearing periods 2 (days 30-60) and 3 (days 61-88).

There were no significant differences among the diets in individual fish weight, length, condition factor, fin indices, splenosomatic index, viscerosomatic index, or any of the histology scores. However, the HSI was significantly different between the fishmeal and the 80% BSM diets, with mean (± SE) HSI values of 1.37 (± 0.05), 1.27 (± 0.02), and 1.16 (± 0.05) for the fishmeal, 60, and 80% BSM diets, respectively.

Gain, percent gain, food fed, FCR, and SGR, were all significantly greater in the higher velocity treatment overall, as well as in the last two rearing periods. At the end of the experiment, trout were significantly heavier in the high velocity tanks than the lower velocity tanks. The VSI was also significantly higher for the fish in the higher velocity tanks. There were no significant differences at the end of the experiment in fish length, condition factor, fin indices, hepatosomatic index, splenosomatic index, or gut histology scores. Figure 4 shows an image of the distal intestines that were scored for the histology sampling. Percent mortality was similar between velocity treatments.

Significant interactions were observed between diet and velocity in the amount of food fed. This interaction occurred in rearing periods 2, 3, and overall. In all cases, the fish at high velocities receiving either the 0 or 60% BSM diets were fed significantly more than the 80% high velocity fish and all of the low velocity dietary treatments.

Discussion

The results of this study clearly indicate the suitability of BSM as a fishmeal replacement in juvenile Rainbow Trout diets. This is evident by the similar growth observed among the diets and the improved FCR with 80% BSM diet.

Although there are antinutritional factors associated with soybeans, the lack of differences in growth, gut histology, fin indices, and organosomatic indices shows the bioprocessing technique used has decreased or eliminated many antinutritional factors. Yamamoto et al. (2010, 2012) examined fermented soybean meal and found that 100% of the dietary fishmeal could be replaced by fermented soybean meal without any impact on fish growth or health. Barnes et al. (2012, 2014, 2015a) replaced approximately 60% of the dietary fishmeal with fermented soybean meal, with no decrease in fish health or growth. Similarly, Bruce et al. (2017a, 2017b) examined a BSM and found that approximated 65% of the dietary fishmeal could be replaced without decreasing rearing performance. These studies are part of a growing body of literature indicating that BSM can effectively replace large percentages of dietary fishmeal, and thereby further reducing the stress on small pelagic marine fish. Other species where fermented or BSM have been evaluated include Atlantic Cod (*Gadus morhua*) (Refstie et al. 2006; Ringø et al. 2006), Atlantic Salmon (*Salmo salar*) (Refstie et al. 2005), Black Sea Bream (*Acanthopagrus schlegeli*) (Zhou et al. 2011; Azarm and Lee 2014), Brown Trout (*Salmo salar*) (Sotoudeh et al. 2016), Chinese Sucker (*Myxocyprinus asiaticus*) (Yuan et al. 2012), Florida Pompano (*Trachniotus carolinus*) (Novriadi et al. 2017), Gilthead Sea Bream (*Sparus aurata* L.) (Kokou et al. 2012), Japanese Flounder (*Paralichthys olivaceus*) (Kader et al. 2012), Orange-spotted Grouper (*Epinephelus coioides*) (Shiu et al. 2015), Whiteleg Shrimp (*Litopenaeus vannamei*) (Chiu et al. 2016; Van Nguyen et al. 2018), Rockfish (*Sebastes schlegeli*) (Lee et al. 2016), White Seabass (*Atractosion nobilis*) (Trushenski et al. 2014), and Yellowtail Jack (*Seriola lalandi*) (Trushenski et al. 2014).

Soybean products in the diets of salmonids have caused well-documented and potentially deleterious effects in the distal intestine (van den Ingh et al. 1991; Rumsey et al. 1995; Baeverfjord and Krogdahl 1996; Burrels et al. 1999; Bakke-McKellep et al. 2000, 2007; Heikkeinen et al. 2006; Barrows et al. 2008; Iwashita et al. 2008; Romarheim et al. 2008; Merrifield et al. 2009; Sealey et al. 2009). However, the lack of difference in gut histology among the diets is evidence that bioprocessing soybeans likely decreases antinutritional factors (Yamamoto et al. 2010, 2012; Barnes et al. 2012, 2013; Bruce et al. 2017a, 2017b). Saponins (Krogdahl et al. 2015), and possibly other gastro-inducing compounds, were evidently removed or decreased during bioprocessing. In comparison to other studies using a similar intestinal histology ranking system (Barnes et al. 2014, 2015a, 2015b) the histological scores in this study tended to be lower. However, dietary formulations and rearing conditions were different between the studies.

The results of this study support the observations that exercise has a positive impact on fish rearing performance (Davison and Goldspink 1977; Leon 1986; Houlihan and Laurent 1987; Christiansen and Jobling 1990; Young and Cech 1993; Gallaugher et al. 2001; Castro et al. 2011; Parker and Barnes 2014, 2015; Good et al. 2016; Liu et al. 2018). Although the higher velocity in this study produced a significantly poorer FCR, the relatively minor difference is not likely biologically significant. The increase in food fed to the higher velocity tanks in this study was due to more food being consumed to meet the increased energy demands from exercise (Kiessling et al. 1994; Azevedo et al. 1998; Rasmussen and Ostenfeld 2000; Parker and Barnes 2015). Parker and Barnes

(2015) also noted that as long as fish were fed adequate amounts of feed then the fish that were exercised had the greatest growth.

Fin erosion can be due to several factors, including tank-induced abrasions (Bosakowski and Wagner 1995), rearing unit size and type (Bosakowski and Wagner 1994), aggressive behavior (Latremouille 2003), feeding rates (Wagner et al. 1996), rearing densities (Miller et al. 1995; Wagner et al. 1997; North et al. 2006), dietary nutritional differences (Lemm et al. 1988; Kindischi et al. 1991), environmental stress (Latremouille 2003), and fish health (Devesa et al. 1989). The similar fin indices among the fish fed different diets and reared in different velocities indicate the suitable nutritional content of the diets and favorable rearing conditions. Although few studies have reported relative fin lengths, the overall pectoral fin values observed in this experiment are similar to those reported by Parker and Barnes (2015).

HSI is an indirect measure of glycogen and carbohydrate levels, and can be used to indicate the nutritional state of the fish (Daniels and Robinson 1986; Kim and Kaushik 1992; Barton et al. 2002). Although HSI values were significantly different among the diets, the ranges observed in this experiment are well within the range observed in other studies examining bioprocessed soybean products in Rainbow Trout diets (Barnes et al. 2013, 2014, 2015b). They are also similar to those values reported for Rainbow Trout in velocity studies (Parker and Barnes 2015; Kientz and Barnes 2016).

VSI indicates how lipids are being used or partitioned with VSI and lipid levels positively related (Jobling et al. 1998; Company et al. 1999; Yildiz et al. 2006). Thus, the similar VSI values observed in this experiment are likely due to similar dietary lipid levels. The levels observed in this study are similar to those reported in other studies evaluating bioprocessed soybean product diets for Rainbow Trout (Barnes et al. 2013, 2014, 2015a). Although there VSI was significantly affected by rearing velocity in this study, all of the values are in the range of those reported in other velocity studies with Rainbow Trout (Parker and Barnes 2015; Kientz and Barnes 2016).

SSI is an indicator of hematopoietic capacity (Barton et al. 2002) and antibody production (Smith 1991). The similar SSI values observed in this experiment indicate that fish health was likely unaffected by dietary or velocity treatments. The SSI values observed were within the range reported for Rainbow Trout by other studies (Barnes et al. 2015b; Parker and Barnes 2015; Kientz and Barnes 2016; Bruce et al. 2017b).

In conclusion, BSM can replace at least 80% of the dietary fishmeal in the diets of juvenile Rainbow Trout, even if the fish are subjected to exercise in high velocity water. In addition, exercise can be used, regardless of diet, to improve growth in fish as long as adequate rations are provided. Additional research examining the complete replacement of fishmeal with BSM in Rainbow Trout subjected to different exercise regimes is needed. In addition, research on the effects of dietary BSM and exercise with other species of fish is also needed.

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Table 14. Study design for dietary and velocity treatments, and mean velocities (± SE).

		Diet (% BSM)			Velocity		Velocity (cm/s)
Treatment	N	1 (0)	2 (60)	3 (80)	Low	High	
1	3	X			X		2.3 ± 0.3
2	3	X				X	18.7 ± 0.8
3	3		X		X		2.3 ± 0.3
4	3		X			X	18.7 ± 0.8
5	3			X	X		2.3 ± 0.3
6	3			X		X	18.7 ± 0.8

Table 15. Diet formulation and composition analyses of the diets used in the 88-day trial. Analysis conducted on post-extrusion feed pellets.

	Diet (%)		
Ingredients	1	2	3
Fishmeal[a]	35.0	14.0	4.7
Bioprocessed soybean meal[b]	0.0	12.0	30.3
Wheat midds[c]	12.0	10.0	10.0
Whole wheat[c]	17.7	15.2	15.1
Poultry byproduct meal[d]	10.0	15.0	15.0
Blood meal[e]	2.0	2.0	2.0
Feather meal[d]	7.0	2.5	2.5
Vitamin premix[f]	1.3	1.3	1.3
Mineral premix[f]	0.8	2.0	2.0
Micro-mineral premix[f]	0.8	0.8	0.8
Choline chloride[g]	0.6	0.6	0.7
L-Lysine[h]	1.5	2.0	2.0
L-Methionine[i]	0.3	0.5	0.5
Stay-C 35[j]	0.2	0.2	0.2
Fish oil[k]	11.0	13.0	13.0
Total	100	100	100
Chemical analysis (% dry basis)			
Protein	43.18	43.85	43.84
Lipid	15.91	14.28	16.44
Ash	2.42	3.60	3.92
Nitrogen-free extract	20.48	24.33	23.96
Dry matter	93.00	95.20	96.25
Gross Energy (kJ/g)	16.5	16.0	16.8
Protein : Energy (MJ/g)	26.2	27.4	26.0

[a] Special Select, Omega Protein, Houston, TX; [b] SDSU; [c] Consumer Supply, Sioux City, IA; [d] Tyson Foods, Springdale, AR; [e] Mason City Byproducts, Mason City, IA; [f] NutraBlend, Neosho, MO; [g] Balchem, New Hampton, NY; [h] CJ Bio America, Fort Dodge, IA; [i] Adisseo USA, Alpharreta, GA; [j] DSM Nutritional Products, Ames, IA; [k] Virginia Prime Gold, Omega Protein, Houston, TX.

Table 16. Histological scoring system used on Rainbow Trout fed fishmeal or bioprocessed soybean meal in diets (Barnes et al. 2014, modified from Geode and Barton 1990, Adams et al. 1993, and Barton et al. 2002).

Score	Appearance
	Lamina propria of simple folds
1	Thin and delicate core of connective tissue in all simple folds.
2	Lamina propria slightly more distinct and robust in some of the folds.
3	Clear increase in lamina propria in most of simple folds.
4	Thick lamina propria in many folds.
5	Very thick lamina propria in many folds.
	Connective tissue between base of folds and stratum compactum
1	Very thin layer of connective tissue between base of folds and stratum compactum.
2	Slightly increased amount of connective tissue beneath some of mucosal folds.
3	Clear increase of connective tissue beneath most of the mucosal folds.
4	Thick layer of connective tissue beneath many folds.
5	Extremely thick layer of connective tissue beneath some of the folds.
	Vacuoles
1	Large vacuoles absent.
2	Very few large vacuoles present.
3	Increased number of large vacuoles.
4	Large vacuoles are numerous.
5	Large vacuoles are abundant in present in most epithelial cells.

Table 17. Mean (± SE) gain, percent gain, food fed, feed conversion ratio (FCR[a]), specific growth rate (SGR[b]), and mortality of Rainbow Trout receiving one of three different diets containing fishmeal or bioprocessed soybean meal (BSM) as the main protein ingredient, and reared at two different velocities. Overall means with different letters in the same column or row differ significantly (*P* < 0.05).

		Diet (% BSM)			
	Velocity	1 (0)	2 (60)	3 (80)	Overall
Initial					
Start weight (g)	Low	975.3 ± 8.6	973.8 ± 16.4	999.7 ± 6.9	982.9 ± 7.1
	High	969.1 ± 29.8	980.5 ± 37.2	950.8 ± 26.4	966.8 ± 16.3
	Overall	972.2 ± 13.9	977.1 ± 18.2	975.2 ± 16.4	
Days 1-29					
End weight (g)	Low	1,375.0 ± 14.3	1,291.4 ± 38.0	1,343.4 ± 33.1	1,330.6 ± 18.1
	High	1,330.6 ± 36.9	1,333.1 ± 60.6	1,232.5 ± 42.1	1,298.7 ± 29.0
	Overall	1,343.8 ± 18.7	1,312.2 ± 33.3	1,288.0 ± 34.5	
Gain (g)	Low	381.7 ± 11.5	317.6 ± 25.3	343.7 ± 35.7	347.7 ± 16.1

	High	361.5 ± 19.0	352.6 ± 27.0	281.7 ± 25.2	331.9 ± 17.4
	Overall	371.6 ± 10.9	335.1 ± 18.3	312.7 ± 24.0	
Gain (%)	Low	39.1 ± 1.2	32.6 ± 2.3	34.4 ± 3.7	35.4 ± 1.6
	High	37.4 ± 2.1	35.9 ± 1.9	29.6 ± 2.5	34.3 ± 1.6
	Overall	38.3 ± 1.2	34.2 ± 1.5	32.0 ± 2.3	
Food fed (g)	Low	403 ± 8	338 ± 19	375 ± 20	372 ± 12
	High	455 ± 11	385 ± 28	354 ± 6	398 ± 17
	Overall	429 ± 13 z	362 ± 18 y	365 ± 10 y	
FCR	Low	1.06 ± 0.04	1.07 ± 0.03	1.10 ± 0.06	1.08 ± 0.02 y
	High	1.26 ± 0.04	1.09 ± 0.01	1.28 ± 0.11	1.21 ± 0.04 z
	Overall	1.16 ± 0.05	1.08 ± 0.02	1.19 ± 0.07	
SGR	Low	1.10 ± 0.03	0.94 ± 0.06	0.98 ± 0.09	1.01 ± 0.04
	High	1.06 ± 0.05	1.02 ± 0.05	0.86 ± 0.06	0.98 ± 0.04
	Overall	1.08 ± 0.03	0.98 ± 0.04	0.92 ± 0.06	
Days 30-60					

End weight (g)	Low	2,175.1 ± 50.4	2,110.4 ± 76.7	2,265.6 ± 44.8	2,183.7 ± 37.1
	High	2,295.4 ± 63.6	2,359.4 ± 116.7	2,113.4 ± 71.4	2,256.1 ± 57.0
	Overall	2,235.2 ± 45.2	2,234.9 ± 83.7	2,189.5 ± 50.8	
Gain (g)	Low	818.1 ± 46.7	819.0 ± 40.9	922.2 ± 14.8	853.1 ± 25.3 y
	High	964.8 ± 27.2	1,026.3 ± 57.9	877.5 ± 55.2	956.2 ± 32.6 z
	Overall	891.4 ± 40.8	922.6 ± 56.2	899.9 ± 27.4	
Gain (%)	Low	60.3 ± 3.5	63.4 ± 1.7	68.7 ± 1.2	64.1 ± 1.7 y
	High	72.5 ± 0.5	76.9 ± 1.6	71.3 ± 4.9	73.6 ± 1.7 z
	Overall	66.4 ± 3.1	70.1 ± 3.2	70.0 ± 2.3	
Food fed (g)	Low	783 ± 18	794 ± 43	801 ± 19	793 ± 15 y
	High	987 ± 13	1,018 ± 52	819 ± 37	941 ± 36 z
	Overall	885 ± 47 zy	906 ± 58 z	810 ± 19 y	
FCR	Low	0.96 ± 0.04	0.97 ± 0.00	0.87 ± 0.01	0.93 ± 0.02 y
	High	1.02 ± 0.04	0.99 ± 0.01	0.94 ± 0.02	0.98 ± 0.02 z
	Overall	0.99 ± 0.03 z	0.98 ± 0.01 z	0.90 ± 0.02 y	

SGR	Low	1.57 ± 0.07	1.64 ± 0.03	1.74 ± 0.02	1.65 ± 0.04 y
	High	1.82 ± 0.01	1.90 ± 0.03	1.79 ± 0.09	1.84 ± 0.03 z
	Overall	1.69 ± 0.06	1.77 ± 0.06	1.77 ± 0.04	
Days 61-88					
End weight (g)	Low	3,196.8 ± 157.0	3,076.4 ± 106.9	3,347.0 ± 71.6	3,206.7 ± 70.5
	High	3,492.5 ± 187.2	3,662.0 ± 161.4	3,238.9 ± 200.3	3,464.5 ± 107.1
	Overall	3,344.6 ± 127.7	3,369.2 ± 151.3	3,293.0 ± 98.2	
Gain (g)	Low	1,021.7 ± 107.8	966.0 ± 34.0	1,081.4 ± 46.8	1,023.0 ± 39.0 y
	High	1,197.1 ± 125.0	1,302.7 ± 25.6	1,128.8 ± 127.8	1,209.5 ± 57.9 z
	Overall	1,109.4 ± 83.6	1,134.4 ± 77.6	1,105.1 ± 61.8	
Gain (%)	Low	46.8 ± 4.0	45.8 ± 0.9	47.7 ± 2.0	46.8 ± 1.3 y
	High	51.9 ± 4.2	55.4 ± 2.5	53.2 ± 4.5	53.5 ± 2.0 z
	Overall	49.4 ± 2.8	50.6 ± 2.5	50.5 ± 2.5	
Food fed (g)	Low	1,099 ± 71	1,027 ± 36	1,037 ± 39	1,054 ± 28 y
	High	1,415 ± 41	1,421 ± 43	1,108 ± 96	1,315 ± 61 z

	Overall	1,257 ± 80 z	1,224 ± 92 zy	1,072 ± 49 y	
FCR	Low	1.09 ± 0.05	1.06 ± 0.02	0.96 ± 0.02	1.04 ± 0.02 y
	High	1.20 ± 0.10	1.09 ± 0.02	0.99 ± 0.3	1.09 ± 0.04 z
	Overall	1.14 ± 0.06 z	1.08 ± 0.01 zy	0.97 ± 0.02 y	
SGR	Low	1.28 ± 0.09	1.26 ± 0.02	1.30 ± 0.05	1.28 ± 0.03 y
	High	1.39 ± 0.09	1.47 ± 0.05	1.42 ± 0.10	1.43 ± 0.04 z
	Overall	1.33 ± 0.06	1.36 ± 0.05	1.36 ± 0.06	
Overall (Days 1-88)					
Gain (g)	Low	2,221.4 ± 148.4	2,102.6 ± 92.5	2,347.3 ± 78.2	2,223.8 ± 65.6 y
	High	2,523.4 ± 158.5	2,681.6 ± 94.2	2,288.1 ± 176.4	2,497.7 ± 93.2 z
	Overall	2,372.4 ± 118.3	2,392.1 ± 142.3	2,317.7 ± 87.3	
Gain (%)	Low	227.5 ± 13.3	215.8 ± 6.7	234.9 ± 9.4	226.1 ± 5.8 y
	High	259.9 ± 9.2	273.6 ± 1.1	240.1 ± 13.3	257.8 ± 6.8 z
	Overall	243.7 ± 10.2	244.7 ± 13.3	237.5 ± 7.4	
Food fed (g)	Low	2,285 ± 81	2,159 ± 94	2,213 ± 73	2,219 ± 46 y

	High	2,856 ± 26	2,823 ± 111	2,281 ± 131	2,654 ± 106 z
	Overall	2,570 ± 133 z	2,491 ± 162 zy	2,247 ± 69 y	
FCR	Low	1.03 ± 0.04	1.03 ± 0.01	0.94 ± 0.00	1.00 ± 0.02 y
	High	1.14 ± 0.06	1.05 ± 0.01	1.00 ± 0.02	1.06 ± 0.03 z
	Overall	1.09 ± 0.04 z	1.04 ± 0.01 zy	0.97 ± 0.02 y	
SGR	Low	1.35 ± 0.05	1.31 ± 0.02	1.37 ± 0.03	1.34 ± 0.02 y
	High	1.46 ± 0.03	1.50 ± 0.00	1.39 ± 0.03	1.45 ± 0.02 z
	Overall	1.40 ± 0.04	1.40 ± 0.04	1.38 ± 0.02	
Mortality (%)	Low	0.00 ± 0.00	1.67 ± 1.67	0.00 ± 0.00	0.56 ± 0.56
	High	1.67 ± 1.67	0.00 ± 0.00	3.33 ± 1.67	1.67 ± 0.83
	Overall	0.83 ± 0.83	0.83 ± 0.83	1.67 ± 1.05	

[a] FCR = feed conversion ratio = total food fed / total weight gain.

[b] SGR = 100 x [(Ln(final weight) – Ln(initial weight)) / days]

Table 18. Mean (± SE) condition factor (K[a]), fin indices[b], hepatosomatic index values (HSI[c]), splenosomatic index (SSI[d]), viscerosomatic index (VSI[e]), and histology scores for lamina propria, connective tissue, and vacuoles of Rainbow Trout fed one of three diets containing either fishmeal or bioprocessed soybean meal (BSM) as the primary protein source, and reared at two different velocities. Means with different letters in the same column or row differ significantly ($P < 0.05$).

		Diet (% BSM)			
	Velocity	1 (0)	2 (60)	3 (80)	Overall
Initial					
Weight (g)	Low	48.8 ± 0.4	48.7 ± 0.8	50.0 ± 0.3	49.1 ± 0.4
	High	48.4 ± 1.5	48.9 ± 1.8	47.5 ± 1.3	48.3 ± 0.8
	Overall	48.6 ± 0.7	48.8 ± 0.9	48.8 ± 0.8	
Length (mm)	Low	156.4 ± 0.8	157.0 ± 1.2	158.2 ± 0.6	157.2 ± 0.5
	High	156.4 ± 1.8	156.8 ± 1.6	156.1 ± 1.7	156.4 ± 0.9
	Overall	156.4 ± 0.9	156.9 ± 0.9	157.1 ± 1.0	
K	Low	1.26 ± 0.02	1.25 ± 0.01	1.25 ± 0.01	1.25 ± 0.01
	High	1.25 ± 0.01	1.25 ± 0.00	1.24 ± 0.01	1.25 ± 0.01

	Overall	1.25 ± 0.01	1.25 ± 0.00	1.25 ± 0.01	
Days 1-29					
End weight (g)	Low	67.9 ± 0.7	64.8 ± 1.7	57.2 ± 1.7	66.6 ± 0.9
	High	66.5 ± 1.8	66.7 ± 3.0	62.7 ± 1.8	65.3 ± 1.3
	Overall	67.2 ± 0.9	65.7 ± 1.6	64.9 ± 1.5	
End length (mm)	Low	177.4 ± 0.8	176.3 ± 1.3	178.5 ± 1.7	177.4 ± 0.7
	High	176.3 ± 2.1	176.8 ± 2.6	174.3 ± 2.1	175.8 ± 1.2
	Overall	176.8 ± 1.0	176.6 ± 1.3	176.4 ± 1.5	
K	Low	1.19 ± 0.00	1.17 ± 0.01	1.33 ± 0.16	1.23 ± 0.05
	High	1.19 ± 0.1	1.19 ± 0.00	1.17 ± 0.01	1.18 ± 0.01
	Overall	1.19 ± 0.01	1.18 ± 0.01	1.25 ± 0.08	
Days 30-60					
End weight (g)	Low	108.7 ± 2.5	106.4 ± 3.2	113.3 ± 2.25	109.5 ± 1.7
	High	114.8 ± 3.2	118.0 ± 5.8	107.5 ± 4.4	113.4 ± 2.8
	Overall	111.8 ± 2.3	110.4 ± 2.6	110.4 ± 2.6	

End length (mm)	Low	205.5 ± 2.6	204.6 ± 2.4	208.1 ± 2.0	206.1 ± 1.3
	High	206.5 ± 2.2	207.2 ± 2.0	201.9 ± 3.1	205.2 ± 1.5
	Overall	206.0 ± 1.5	205.9 ± 1.5	205.0 ± 2.2	
K	Low	1.22 ± 0.01	1.22 ± 0.01	1.24 ± 0.01	1.23 ± 0.01
	High	1.26 ± 0.01	1.38 ± 0.10	1.26 ± 0.01	1.30 ± 0.04
	Overall	1.24 ± 0.01	1.30 ± 0.06	1.25 ± 0.01	
Days 61-88 (Final)					
End weight (g)	Low	159.8 ± 7.8	155.4 ± 3.9	167.4 ± 3.6	160.9 ± 3.2 y
	High	177.4 ± 6.7	183.1 ± 6.6	167.5 ± 13.3	176.0 ± 5.2 z
	Overall	168.6 ± 6.1	169.2 ± 7.1	167.4 ± 6.18	
End length (mm)	Low	230.0 ± 5.7	232.0 ± 1.7	235.6 ± 2.3	232.5 ± 2.0
	High	233.5 ± 2.4	248.7 ± 12.7	231.8 ± 5.8	238.0 ± 4.9
	Overall	231.8 ± 2.9	240.4 ± 6.8	233.7 ± 2.9	
K	Low	1.36 ± 0.10	1.22 ± 0.01	1.26 ± 0.02	1.28 ± 0.03
	High	1.34 ± 0.02	1.31 ± 0.02	1.30 ± 0.02	1.32 ± 0.01

	Overall	1.35 ± 0.04	1.27 ± 0.02	1.28 ± 0.02	
Pectoral index (%)	Low	11.64 ± 0.70	10.76 ± 0.14	10.63 ± 0.17	11.01 ± 0.27
	High	10.65 ± 0.33	10.48 ± 0.18	10.82 ± 0.05	10.65 ± 0.12
	Overall	11.14 ± 0.41	10.62 ± 0.12	10.73 ± 0.09	
Pelvic index (%)	Low	9.14 ± 1.02	9.33 ± 0.06	9.10 ± 0.12	9.19 ± 0.30
	High	9.29 ± 0.14	8.66 ± 0.34	8.43 ± 0.40	8.79 ± 0.20
	Overall	9.22 ± 0.46	8.99 ± 0.22	8.76 ± 0.24	
Dorsal index (%)	Low	5.60 ± 0.64	4.74 ± 0.42	4.39 ± 0.04	4.91 ± 0.29
	High	4.82 ± 0.05	4.49 ± 0.14	4.68 ± 0.16	4.66 ± 0.08
	Overall	5.21 ± 0.34	4.62 ± 0.20	4.53 ± 0.10	
HSI (%)	Low	1.40 ± 0.06	1.28 ± 0.04	1.12 ± 0.08	1.27 ± 0.05
	High	1.33 ± 0.08	1.27 ± 0.02	1.21 ± 0.05	1.27 ± 0.03
	Overall	1.37 ± 0.05 z	1.27 ± 0.02 zy	1.16 ± 0.05 y	
SSI (%)	Low	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.06 ± 0.00
	High	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.05 ± 0.00

	Overall	0.06 ± 0.00	0.05 ± 0.01	0.06 ± 0.00	
VSI (%)	Low	11.38 ± 0.28	12.01 ± 0.41	11.29 ± 0.61	11.56 ± 0.25 y
	High	12.61 ± 0.34	13.30 ± 0.24	12.38 ± 0.84	12.77 ± 0.30 z
	Overall	12.00 ± 0.34	12.66 ± 0.36	11.84 ± 0.53	
Lamina propria[f]	Low	1.47 ± 0.29	1.67 ± 0.24	1.67 ± 0.19	1.60 ± 0.13
	High	1.67 ± 0.17	1.52 ± 0.26	1.72 ± 0.06	1.63 ± 0.01
	Overall	1.57 ± 0.16	1.59 ± 0.16	1.69 ± 0.09	
Connective tissue[f]	Low	1.25 ± 0.25	1.47 ± 0.29	1.42 ± 0.42	1.38 ± 0.17
	High	1.17 ± 0.17	1.45 ± 0.23	1.30 ± 0.10	1.31 ± 0.10
	Overall	1.21 ± 0.14	1.46 ± 0.17	1.36 ± 0.19	
Vacuoles[f]	Low	1.97 ± 0.17	2.13 ± 0.24	2.00 ± 0.00	2.04 ± 0.09
	High	2.17 ± 0.17	2.27 ± 0.27	1.80 ± 0.20	2.08 ± 0.13
	Overall	2.07 ± 0.11	2.20 ± 0.16	1.90 ± 0.10	

[a] $K = 10^5 \times [\text{weight} / (\text{length}^3)]$

[b] Fin indices = 100 x (fin length / fish length)

[c] HSI = 100 x (liver weight / body weight)

[d] SSI = 100 x (spleen weight / body weight)

[e] VSI = 100 x (visceral weight / body weight)

[f] Scoring Parameters in Table 16.

Figure 4. Representative (fed 80% BSM at slow velocity) Rainbow Trout histology image used for scoring.

CHAPTER 6: REARING PERFORMANCE OF JUVENILE BROWN TROUT (*Salmo trutta*) FED A BIOPROCESSED SOYBEAN MEAL DIET WITH DIFFERING VELOCTY REGIMES

Abstract

This 121-day experiment evaluated the rearing performance of Brown Trout (*Salmo trutta*) fed one of two isonitrogenous and isocaloric diets (46% protein, 16% lipid) and reared at velocities of either 2.8 or 16.1 cm/s. Fishmeal was the primary protein source for the reference diet, which was compared to a bioprocessed soybean meal (BSM) ingredient that replaced approximately 67% of the fishmeal in the experimental diet. At the end of the experiment, there were no significant differences in gain, percent gain, feed conversion rates, nor specific growth rates between the dietary treatments. There were also no significant differences in intestinal morphology, splenosomatic, hepatosomatic, and viscerosomatic indices related to diet composition. However, gain, percent gain, feed fed, and specific growth rate were all significantly greater in Brown Trout reared at the higher velocity. No significant differences in any of the other variables measured were observed between the velocity treatments. There were no significant interactions between diet and velocity in any of the variables. Based on the results of this study, BSM can replace at least 67% of the fish meal in Brown Trout diets, regardless of the rearing velocities used in this study. Higher rearing velocities are recommended to maximize juvenile Brown Trout growth rates.

Introduction

With global human population expected to grow to 9 billion by 2050 (FAO 2016), there is a need for increased and sustainable protein sources. Aquaculture production is rising to meet this demand, with the growth of aquaculture outpacing human population growth in the past five decades (FAO 2016). However, the continuing growth of aquaculture is constrained by the cost and unpredictability of aquatic animal feedstuffs (FAO 2016).

Fishmeal, primarily produced from marine pelagic fish (FAO 2016, 2017), has historically been the primary protein ingredient in carnivorous fish feeds (Satia 1974; Kim et al. 1991; Cheng and Hardy 2004). However, nearly 90% of the world marine fisheries are fully-fished or overfished (FAO 2016), and fishmeal risks becoming a limiting factor in aquaculture production. Thus, there is a need for sustainable proteins in aquafeed.

One of the leading plant-derived alternatives to dietary fishmeal is soybeans (*Glycine max*) (Nordrum et al. 2000; Li and Robinson 2015), due to its relative low cost and worldwide availability (USDA 2017). Soybean products are highly palatable (Sugiura et al. 1998; Refstie et al. 2000; Watanabe 2002), have a high protein content (~ 48% crude protein), and also have a balanced amino acid profile (Gatlin et al. 2007; NRC 2011). However, there are antinutritional factors associated with soybean which hinder fish digestion (Salunkhe et al. 1992; Krogdahl et al. 1994, 2015; Kaushik et al. 1995; Bureau et al. 1998; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Iwashita et al. 2008; NRC 2011; Teng et al. 2012), and can also cause gastro-intestinal issues, such as enteritis (van deh Ingh et al. 1991; Rumsey et al. 1995; Baeverfjord and Krogdahl 1996; Burrells et al. 1999; Bakke-McKellep et al. 2000; Krogdahl et al. 2000, 2015; Refstie et al. 2000; Heikkinen et al. 2006; Bakke 2011). Soybean products also have large concentrations of non-digestible carbohydrates (Salunkhe et al. 1992; Gatlin et al. 2007). These factors limit the inclusion levels of soybean products in diets for carnivorous fish species (Fowler 1980; Reinitz 1980; Vielma et al. 2000; NRC 2011). However, some of these antinutritional factors are decreased or inactivated by heat, which occurs during the feed extrusion process (Cheeke and Shull 1985; Liener 1994; Gomes et al. 1995; Arndt et al. 1999; Francis et al. 2001; Barrows et al. 2007; Krogdahl et al. 2010). Bioprocessing, such as fermentation, has also been shown to eliminate or reduce antinutritional factors (Hong et al. 2004; Refstie et al. 2005; Yamamoto et al. 2010, 2012; Barnes et al. 2012, 2013, 2014, 2015a, 2015b).

Studies have examined bioprocessed soybean meal (BSM) in Atlantic Salmon (*Salmo salar*) and Rainbow Trout (*Oncorhynchus mykiss*) diets. However, there is limited research examining BSM in the diets of Brown Trout (*Salmo trutta*). Only one study has been published on evaluating fermented soybean products in Brown Trout diets. Sotoudeh et al. (2016) replaced fish meal with different forms of processed soybean meal (untreated, gamma-ray, irradiated, and fermented) and found that Brown Trout fed fermented soybean meal grew larger than fish on the non-fermented soybean meal diet. However, this study did not have a fishmeal reference diet, making results difficult to compare.

In addition to dietary influences on fish rearing performance, exercise can also have impacts (Davison and Goldspink 1977; Leon 1986; Houlihan and Laurent 1987; Christiansen and Jobling 1990; Young and Cech 1993; Gallaugher et al. 2001; Castro et al. 2011; Parker and Barnes 2014, 2015; Good et al. 2016; Liu et al. 2018). Exercise (increased velocities and forced swimming) has been shown to improve growth of Rainbow Trout and Atlantic Salmon fed to satiation (Parker and Barnes 2015; Waldrop et al. 2018). If feed is limited however, growth can be impaired at higher velocities (Parker and Barnes 2014). Davison and Goldspink (1977) examined the effect of prolonged swimming on Brown Trout, and found that the intermediate speeds (1.5 and 3.0 body lengths/second; bl/s) shown greater growth than controls, but this study was very short (less than 30 days).

With only one uncontrolled study investigating BSM in Brown Trout diets, and only one study, of very limited duration, evaluating exercise during Brown Trout rearing, the need for further research is evident. More specifically, no research has been done to show the impacts velocity can possibly have on fish fed a BSM. Thus, the objective of this study was to examine the effects of both a diet with BSM, as the primary protein source, and velocity on the rearing performance and gastro-intestinal health of Brown Trout.

Methods

This experiment was conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, using degassed and aerated well water at a constant temperature of 11° C (total hardness as $CaCO_3$, 360 mg/L; alkalinity as $CaCO_3$, 210 mg/L; pH, 7.6; total dissolved solids, 390 mg/L).

One-hundred twenty-eight Plymouth strain Brown Trout (initial weight 55.6 ± 1.5 g, length 166.2 ± 1.3 mm, mean ± SE) were randomly selected and placed into one of 16 circular fiberglass tanks (1.8 m diameter, 0.6 m depth) on September 15, 2016, at eight fish per tank. This 121-day study used a 2 x 2 design to evaluate the effects of water velocity and diet on Brown Trout rearing performance, with four tanks per treatment. Study design and water velocities used are described in Table 19.

Water velocities were recorded using a flowmeter (Flowatch, JDC Electronic SA, Yverdon-les-Bains, Jura-Nord Vaudois, Vaud, Switzerland), with readings taken directly behind the spray bar, 30.5 cm from the side of the tank and about 0.3 m deep (half way in water column). Flow rates were set and kept constant throughout the study.

Two different diets were used (Table 20), with modified soybean meal replacing 0 or 60% of the fishmeal as the primary protein source. The modified soybean meal was produced using a proprietary microbial conversion process (SDSU, Brookings, SD, USA). Diets were isocaloric and isonitrogenous and were manufactured by cooking extrusion (ExtruTech model 325, Sabetha, KS). Feed was analyzed according to AOAC (2009) method 2001.11 for protein, 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and AACC (2000) method 08-03 for ash content.

At the start of the experiment fish were individually weighed to the nearest 0.1 g, measured to the nearest 1.0 mm, and then placed into one of the sixteen tanks. Fish were weighed and measured approximately every four weeks. The individual fish weights were combined to obtain total tank weight. Weight gain, percent gain, feed conversion ratio (FCR), and specific growth rate (SGR) were calculated. Individual fish weights and lengths were used to calculate Fulton’s condition factor (K).

Fish were fed daily for 121 days, except on the days they were weighed and measured (days 35, 61, 92, and 121). Feeding amounts were initially determined by the hatchery constant method (Butterbaugh and Willoughby 1967), with planned feed conversion rates of 1.1 and maximum growth rate of 0.07 cm/day, which was based on historical maximum growth rate of Plymouth strain Brown Trout at McNenny State Fish

Hatchery (Barnes et al. 2011). Fish were fed by hand daily and feed was adjusted daily to be at or near satiation. Feed and mortality were recorded daily.

To collect weight and length data on days 1, 35, 61, and 92, the fish were anesthetized using 60 mg/L MS-222 (Tricaine-S, tricaine methanesulfonate, Syndel USA, Ferndale, Washington). On day 121, at the end of the experiment, fish were euthanized using a lethal dose of 250 mg/L MS-222 (AVMA 2013). In addition to weight and length measurements, fin lengths, to the nearest 1.0 cm, and spleen, liver, and visceral weights, to the nearest 1.0 mg, were recorded from three randomly selected Brown Trout per tank. Fin indices, hepatosomatic index (HSI) (Strange 1996), splenosomatic index (SSI) (Goede and Barton 1990), and viscerosomatic index (VSI) (Goede and Barton 1990) were calculated.

The following equations were used:

$$\text{Gain} \ = \text{end weight} \ - \text{start weight}$$

$$\text{Percent gain (\%)} = \frac{\text{gain}}{\text{start weight}}$$

$$\text{FCR} = \frac{\text{food fed}}{\text{gain}}$$

$$\text{SGR} = 100 * \frac{\ln(\text{end weight}) - \ln(\text{start weight})}{\text{number of days}}$$

$$\text{K} = 10^5 * \frac{\text{fish weight}}{\text{fish length}^{\,3}}$$

$$\text{Fin indices} = \frac{\text{fin length}}{\text{fish length}}$$

$$\text{HSI (\%)} = 100 * \frac{\text{liver weight}}{\text{whole fish weight}}$$

$$\text{SSI (\%)} = 100 * \frac{\text{spleen weight}}{\text{whole fish weight}}$$

$$\text{VSI (\%)} = 100 * \frac{\text{visceral weight}}{\text{whole fish weight}}$$

A 2-mm wide section of the distal intestine was removed from three randomly-selected fish per tank to assess any possible soy-induced enteritis (Gu et al. 2017; Novriadi et al. 2017; Wang et al. 2017; Booman et al. 2018). After dissection, the intestinal tissue was immediately put into 10% buffered formalin, and stained with haematoxylin and eosin using standard histological techniques (Bureau et al. 1998; Burrels et al. 1999). Intestinal inflammation was assessed using an ordinal scoring system (Table 21) based on lamina propria thickness and cellularity, submucosal connective tissue width, and leukocyte distribution (Knudsen et al. 2007; Colburn et al. 2012; Barnes et al. 2014).

Data was analyzed using the SPSS (9.0) statistical analysis program (SPSS, Chicago Illinois), with significance predetermined at $P < 0.05$. Two-way analysis of variance (ANOVA) was conducted, and if treatments were significantly different, post hoc mean separation tests were performed using Tukey’s HSD test.

Results

At the end of the experiment there were no significant differences in gain, percent gain, FCR, SGR, and percent mortality between the tanks of fish receiving the fishmeal or BSM diet (Table 22). Overall mean (± SE) FCRs were relatively high for both the tanks receiving the fishmeal reference diet (2.50 ± 0.14) and in the BSM diet (2.78 ± 0.29). Food fed was significantly different between diets, with the fishmeal diet tanks receiving 928 (± 92) g of feed and the BSM diet tanks receiving 685 (± 88) g.

There were no significant differences between gain, percent gain, FCR, and SGR during any of the rearing periods. However, the amount of food fed was significantly different in all rearing periods, with the tanks of fish receiving the fishmeal diet consistently receiving more food. In rearing period 1 (first 35 days) the FCR was negative for the fish in tanks receiving the fishmeal diet, indicating that the trout actually lost weight. However, in rearing period 4 (days 93-121) gain, percent gain, and SGR were all significantly higher in the tanks of fish fed the fishmeal diet. Mean (± SE) percent gain in this rearing period (4) was 19.9 (± 1.2) % and 15.1 (± 1.7) % for the tanks of fish fed fishmeal and BSM diets, respectively. FCR for rearing period 4 was not significantly different.

Individual fish weight, length, and condition factor were not significantly different between dietary treatments at the end of the study (Table 23). None of the fin indices (pectoral, pelvic, and dorsal), organismic indices (HSI, SSI, VSI), or gut histology scores were significantly different between diets. Figure 5 shows a representative image of the distal intestines that were scored for the histology sampling. The only significant differences observed in any of the individual fish data in any of the rearing periods occurred in rearing period 3 (day 62-92), where the mean (± SE) length of fish fed the fishmeal diet was 197.1 (± 2.9) mm compared to 188.7 (± 2.8) mm in the fish fed the bioprocessed soybean meal diet.

Several significant differences in Brown Trout rearing performance were observed between the two velocity treatments. Gain, percent gain, food fed, and SGR were significantly higher in the tanks of fish reared at higher velocity overall and in the first two rearing periods. Mean (± SE) percent gain was only 55.2 (± 9.2) % in lower velocity tanks, but was 92.5 (± 6.0) % in the higher velocity tanks. However, in the last two rearing periods there is no a significant differences in gain, percent gain, nor SGR. In addition, mean (± SE) percent mortality was significantly higher in the lower velocity tanks at 18.8 (± 5.3) %, compared to 4.7 (± 3.3) % in the higher velocity tanks. Overall mean (± SE) FCRs were not significantly different, but were relatively poor at 2.72 (± 0.30) for the tanks of fish at the lower velocity and 2.56 (± 0.13) for the tanks of fish at higher velocity.

The amount of food fed was significantly higher in the higher velocity tanks for all of the rearing periods. FCR was only significantly different in rearing period 4, where tanks of Brown Trout at the faster velocity had a higher mean (± SE) of 2.32 (± 0.11) compared to 1.86 (± 0.10) for the slower velocity tanks. Similar to the dietary results, mean (± SE) FCRs in both velocities were extremely poor and inconsistent in rearing period 1, with the tanks of fish at the lower velocity having a FCR of -5.40 (± 15.50) compared to 3.50 (± 0.47) in the higher velocity tanks. Gain, percent gain, and SGR were only significantly greater in the higher velocity treatment during the first two rearing periods.

At the end of the experiment, and in every rearing period, individual fish weight, length, and condition factor were not significantly differences between the velocity treatments. In addition, no significant differences in fin index scores, hepatosomatic index, splenosomatic index, viscerosomatic index, nor any of the histological scores were observed between the low and high velocity treatments. There were no interactions between diet and velocity in any of the variables measured at either the end of the study or at the end of any of the rearing periods.

Discussion

The similarity in rearing performance response between the two diets indicates that BSM can replace at least 67% of the fishmeal in Brown Trout diets. Sotoudeh et al. (2016) also indicated the suitability of fermented soybean meal in Brown Trout diets. However, the Sotoudeh et al. (2016) study had no fishmeal-based reference diet, making it difficult to compare their results to this study. The results from this experiment with Brown Trout are consistent with those reported in Rainbow Trout by Bruce et al. (2017a, 2017b) who replaced 65% of the dietary fishmeal with BSM with no observed ill-effects. In addition, Barnes et al. (2012, 2014, 2015a) replaced approximately 62% of the fishmeal with a commercial fermented soybean product without any significant difference in Rainbow Trout performance. Yamamoto et al. (2010, 2012) also reported positive results with fermented soybean meal in Rainbow Trout diets. Different forms of BSM have been evaluated in Atlantic Salmon diets, but fish meal replacement rates appeared limited to 20% or less (Refstie et al. 2005). Other species where fermented, or other forms of bioprocessed, soybean have been evaluated include Atlantic Cod (*Gadus morhua*) (Refstie et al. 2006; Ringø et al. 2006), Black Sea Bream (*Acanthopagrus schlegeli*) (Zhou et al. 2011; Azarm and Lee 2014), Chinese Sucker (*Myxocyprinus asiaticus*) (Yuan et al. 2012), Florida Pompano (*Trachniotus carolinus*) (Novriadi et al. 2017), Gilthead Sea Bream (*Sparus aurata* L.) (Kokou et al. 2012), Japanese Flounder (*Paralichthys olivaceus*) (Kader et al. 2012), Orange-spotted Grouper (*Epinephelus coioides*) (Shiu et al. 2015), Whiteleg Shrimp (*Litopenaeus vannamei*) (Chiu et al. 2016; Van Nguyen et al. 2018), Rockfish (*Sebastes schlegeli*) (Lee et al. 2016), White Seabass (*Atractosion nobilis*) (Trushenski et al. 2014), and Yellowtail Jack (*Seriola lalandi*) (Trushenski et al. 2014).

There has been minimal research done on the long-term effects of soybean products in salmonid diets, with only a few experiments lasting over 100 days (Vielma et al. 2000; Heikkinen et al. 2006; Barrows et al. 2008b; Merrifield et al. 2009; Johnsen et al. 2011; Barnes et al. 2012; 2014). At 121 days, this study should have met the Weathercup and McCraken (1999) criteria for being long enough to determine any differences in fish performance among the diets. This study also met the NRC (2011) recommendation of lasting 56-84 days. However, even at 121 days, the Brown Trout only produced a 150% gain, short of the 200% gain recommended by NRC (2011) for feeding trial durations. Interestingly, gain, percent gain, and specific growth rate did not differ significantly between the diets for the first three months, but significantly improved in fish fed the fishmeal diet during the final rearing period. This is consistent with de Francesco et al. (2004), who did not see differences in rearing performance between fishmeal and plant-based diets until after 84 days. It is unknown if significant differences between the fishmeal and BSM would have occurred beyond the end of this experiment.

The poor initial growth rate and relatively poor FCRs throughout this experiment may be due to palatability problems. Poor palatability has been suggested to contribute to lower feed intake and reduced growth (Kissil et al. 2000; Bruce et al. 2017b). Overall, FCRs from the Brown Trout in this study are higher (worse) than that reported by Regost et al. (2001) or Kizak et al. (2013). However, Kizak et al. (2013) fed a restricted ration, which has been shown to improve FCR (De Silva and Anderson 1995). The SGR at the beginning being of the experiment was approximately 0.3, but improved to approximately 0.55 at the final rearing period. This is similar to the 0.6 SGR reported for Brown Trout by Regost et al. (2001).

It is unknown why the FCR was similar between the dietary treatments, despite the significant increase in feed consumption in fish fed the fishmeal diet. FCR is calculated by dividing the gain by the amount of food fed (Stickney1994), and any significant increase in food fed, with no change in gain, should produce a corresponding decrease in FCR. This enigma could be a statistical artifact, possibly due to small sample sizes (Pirhonen et al. 2000).

Soybean products in the diets of salmonids have caused well-documented and potentially-deleterious effects in the distal intestine of Rainbow Trout (Rumsey et al. 1995; Burrels et al. 1999; Heikkeinen et al. 2006; Barrows et al. 2008a; Iwashita et al. 2008; Romarheim et al. 2008; Merrifield et al. 2009; Sealey et al. 2009). Intestinal microbial communities may also be affected (Heikkinen et al. 2006; Barrows et al. 2008a; Merrifield et al. 2009; Bruce et al. 2017a). These issues have been reported in Atlantic Salmon (van den Ingh et al. 1991; Baeverfjord and Krogdahl 1996; Bakke-McKellep et al. 2000; 2007), a species closely-related to Brown Trout. However, the histological data in this study did not indicate any enteritis in any of the fish receiving the BSM diet or in the fish receiving the fishmeal diet. Fermentation decreases antinutritional factors (Yamamoto et al. 2010, 2012; Barnes et al. 2012, 2013), making it likely that the saponins (Krogdahl et al. 2015) and possibly other gastro-inducing compounds were removed during bioprocessing. The histological scores observed in this study tended to be lower than those reported by Barnes et al. (2014, 2015a, 2015b). However, the Barnes' studies examined Rainbow Trout which were fed different diets than those used in this study. In addition, Bruce et al. (2017a) also used the same scoring system with Rainbow Trout but compiled and averaged all numbers for an overall gut score.

The lack of any differences in HSI between the dietary or velocity treatments indicates similar energy partitioning. HSI is an indirect measure of glycogen and carbohydrate levels, and can be used to indicate nutritional state of the fish (Daniels and Robinson 1986; Kim and Kaushik 1992; Barton et al. 2002). The HSI of 1.1 to 1.2 found in this study is slightly higher than the Brown Trout HSI of 0.9 to 1.0 in Sotoudeh et al (2011), but lower for other studies (1.4 to 1.7) (Mambrini et al. 2006; Kizak et al. 2013; Sotoudeh 2016). The comparably lower HSI values in this study may be due to different diets or may also be indicative of different stressors among the studies. Both HSI and VSI are used to indicate if energy is being diverted away from organ or tissue growth in order to combat stress, and this is indicated by lower indices (Barton et al. 2002).

VSI values indicate how lipids are being used or and there is a positive relationship between lipid levels and VSI partitioned (Jobling et al. 1998; Company et al. 1999; Yildiz et al. 2006). Thus, similar VSI values among the dietary and velocity treatments are likely due to similar dietary lipid levels. While VSI values in this experiment were relatively low compared to Mambrini et al. (2006), Sotoudeh et al. (2011), and Kizak et al. (2013), they were similar to those reported by Sotoudeh et al. (2016), which is the only experiment examining processed soybean products in Brown Trout diets.

SSI indicates the hematopoietic capacity of fish (Barton et al. 2002) and antibody production mostly occurs in the spleen (Smith 1991). Similar SSI values indicate that fish health was unaffected by diet or velocity. No literature values for Brown Trout SSI could be found, but dietary experiments with Rainbow Trout SSI had similar values to those observed in the Brown Trout in this study (Barnes et al. 2015b; Bruce et al. 2017b). In two velocity studies that reported SSI in Rainbow Trout, SSI values were approximately 25% higher than those observed in this study (Parker and Barnes 2015; Kientz and Barnes 2016).

In addition to diet, exercise has been shown to impact fish growth (Davison and Goldspink 1977; Leon 1986; Houlihan and Laurent 1987; Christiansen and Jobling 1990; Young and Cech 1993; Gallaugher et al. 2001; Castro et al. 2011; Parker and Barnes 2014, 2015; Good et al. 2016; Liu et al. 2018). Higher velocities improved fish rearing performance in this study, but the positive effects were primarily limited to the first 8 weeks. Nearly all of the other studies investigating exercise have lasted between 4 and 10 weeks (Davison and Goldspink 1977; Leon 1986; Houlihan and Laurent 1987; Christiansen and Jobling 1990; Young and Cech 1993; Castro et al. 2011; Parker and Barnes 2014, 2015; Good et al. 2016; Liu et al. 2018). Only one other velocity experiment has lasted 4 months, but growth data was not reported (Gallaugher et al. 2001). Why did the influence of exercise on growth rates and gain disappear after eight weeks? Perhaps the fish could be exhibiting exercise fatigue, which has been reported in humans after extended periods of intense exercise (Noakes 2000; Noakes et al. 2005; Crewe et al. 2008; Joyner and Coyle 2008).

As expected, fish at the higher velocity ate significantly more food then fish at the lower velocities. It is well documented that although more food must be consumed to meet the increased energy demands from exercise, feed efficiency will be the same or better at lower velocities (Kiessling et al. 1994; Azevedo et al. 1998; Rasmussen and Ostenfeld 2000; Parker and Barnes 2015). This was also observed in the present study where, similar to other studies (Leon 1986; Castro et al. 2011), the FCR was not significantly different in the overall data, or for the first three rearing periods. However, the final rearing period saw a significant difference in FCRs between the two velocities. The fish in lower velocity tanks converted better at a ratio of 1.86, but the higher velocity fish converted at 2.32. This could potentially be another indicator of exercise fatigue.

The lack of difference in the fin indices among the dietary or velocity treatments indicates dietary suitability, as well as a lack of environmental stress (Latremouille 2003), adequate feeding rates (Wagner et al. 1996), nutritional differences (Lemm et al. 1988; Kindischi et al. 1991), and good fish health (Devesa et al 1989). Fin erosion has been found to be due to several factors, including tank-induced abrasions (Bosakowski and Wagner 1995), rearing unit size and type (Bosakowski and Wagner 1994), aggressive behavior (Latremouille 2003), feeding rates (Wagner et al. 1996), rearing densities (Miller et al. 1995; Wagner et al. 1997; North et al. 2006), and fish health (Devesa et al. 1989). Bosakowski and Wagner (1994) is the only other paper that has examined fin indices for Brown Trout, which had relative pectoral and pelvic lengths approximately 30% less than observed in this study. However, the relative dorsal length reported by Bosakowski and Wagner (1994) was over 35% greater than in this experiment.

In conclusion, BSM can replace fishmeal in Brown Trout diets with no ill-effects, even if the trout are subjected to exercise. In addition, regardless of diet, exercise improves fish rearing performance, at least initially. Additional research on complete fishmeal replacement with BSM in Brown Trout diets is needed. There is also a need to examine potential exercise fatigue in fish forced to swim continuously for extended periods of time.

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Table 19. Study design for dietary and velocity treatments, and mean velocities (± SE).

		Diet (% BSM)		Velocity		Velocity (cm/s)
Treatment	N	1 (0)	2 (60)	Low	High	
1	4	X		X		2.8 ± 0.4
2	4	X			X	16.1 ± 1.0
3	4		X	X		2.8 ± 0.4
4	4		X		X	16.1 ± 1.0

Table 20. Diet formulation and composition analyses of the diets used in the 121-day trial. Analysis conducted on post-extrusion feed pellets.

	Diet (%)	
Ingredients	1	2
Fishmeal[a]	35.3	14.0
Bioprocessed soybean meal[b]	0.0	21.0
Wheat midds[c]	7.9	7.2
Whole wheat[c]	16.4	13.7
Poultry byproduct meal[d]	21.9	19.9
Blood meal[e]	2.6	2.5
Feather meal[d]	1.2	1.2
Vitamin premix[f]	0.8	2.0
Mineral premix[f]	0.8	2.0
Micro-mineral premix[f]	0.0	0.8
Choline chloride[g]	0.0	0.7
L-Lysine[h]	1.2	1.7
L-Methionine[i]	0.0	0.5
Stay-C 35[j]	0.0	0.3
Fish oil[k]	10.1	12.0
Total	100.0	100.0
Chemical analysis (% dry basis)		
Protein	46.98	45.76
Lipid	16.97	16.25
Ash	11.4	9.71
Nitrogen-free extract	18.79	20.63
Dry matter	96.48	95.85
Gross Energy (kJ/g)	17.8	17.2
Protein : Energy (MJ/g)	26.4	26.6

[a] Special Select, Omega Protein, Houston, TX; [b] SDSU; [c] Consumer Supply, Sioux City, IA; [d] Tyson Foods, Springdale, AR; [e] Mason City Byproducts, Mason City, IA; [f] NutraBlend, Neosho, MO; [g] Balchem, New Hampton, NY; [h] CJ Bio America, Fort Dodge, IA; [i] Adisseo USA, Alpharreta, GA; [j] DSM Nutritional Products, Ames, IA; [k] Virginia Prime Gold, Omega Protein, Houston, TX.

Table 21. Histological scoring system used on Brown Trout fed fishmeal or bioprocessed soybean meal diets (Barnes et al. 2014, modified from Geode and Barton 1990, Adams et al. 1993, and Barton et al. 2002).

Score	Appearance
	Lamina propria of simple folds
1	Thin and delicate core of connective tissue in all simple folds.
2	Lamina propria slightly more distinct and robust in some of the folds.
3	Clear increase in lamina propria in most of simple folds.
4	Thick lamina propria in many folds.
5	Very thick lamina propria in many folds.
	Connective tissue between base of folds and stratum compactum
1	Very thin layer of connective tissue between base of folds and stratum compactum.
2	Slightly increased amount of connective tissue beneath some of mucosal folds.
3	Clear increase of connective tissue beneath most of the mucosal folds.
4	Thick layer of connective tissue beneath many folds.
5	Extremely thick layer of connective tissue beneath some of the folds.
	Vacuoles
1	Large vacuoles absent.
2	Very few large vacuoles present.
3	Increased number of large vacuoles.
4	Large vacuoles are numerous.
5	Large vacuoles are abundant in present in most epithelial cells.

Table 22. Mean (± SE) gain, percent gain, food fed, feed conversion ratio (FCR[a]), specific growth rate (SGR[b]), and mortality of Brown Trout receiving one of two different diets containing fishmeal or bioprocessed soybean meal (BSM) as the main protein ingredient, and reared at two different velocities. Overall means with different letters in the same column or row differ significantly ($P < 0.05$).

		Diet (% BSM)		
	Velocity	1 (0)	2 (60)	Overall
Initial				
Start weight (g)	Low	489.6 ± 20.1	435.2 ± 15.0	462.4 ± 15.5
	High	444.4 ± 36.3	409.1 ± 28.1	426.7 ± 22.3
	Overall	467.0 ± 21.0	422.1 ± 15.6	
Days 1-35				
End weight (g)	Low	511.6 ± 16.4	458.6 ± 21.4	485.1 ± 16.0
	High	516.9 ± 47.6	504.2 ± 28.7	510.6 ± 25.8
	Overall	514.2 ± 23.3	481.4 ± 18.7	
Gain (g)	Low	22.0 ± 10.4	23.4 ± 8.9	22.7 ± 6.4 y
	High	72.6 ± 12.1	95.1 ± 9.1	83.8 ± 8.2 z
	Overall	47.3 ± 12.1	59.3 ± 14.8	
Gain (%)	Low	4.7 ± 2.2	5.3 ± 2.0	5.0 ± 1.4 y
	High	16.0 ± 1.7	23.6 ± 3.1	19.8 ± 2.2 z
	Overall	10.3 ± 2.5	14.5 ± 3.9	
Food fed (g)	Low	192 ± 5	144 ± 8	168 ± 10 y
	High	284 ± 6	252 ± 22	268 ± 12 z

	Overall	238 ± 18 z	198 ± 23 y	
FCR	Low	-21.83 ± 30.30	11.03 ± 4.75	-5.40 ± 15.50
	High	4.27 ± 0.73	2.73 ± 0.34	3.50 ± 0.47
	Overall	-8.78 ± 14.87	6.88 ± 2.71	
SGR	Low	0.13 ± 0.06	0.15 ± 0.06	0.14 ± 0.04 y
	High	0.44 ± 0.04	0.62 ± 0.07	0.53 ± 0.05 z
	Overall	0.28 ± 0.07	0.39 ± 0.10	
Days 36-61				
End weight (g)	Low	533.2 ± 16.1	499.4 ± 34.3	526.3 ± 20.3
	High	602.1 ± 60.9	557.3 ± 36.8	579.7 ± 34.0
	Overall	577.6 ± 30.6	528.4 ± 25.7	
Gain (g)	Low	41.7 ± 6.6	40.8 ± 14.6	41.3 ± 7.4 y
	High	85.1 ± 13.7	53.1 ± 11.3	69.1 ± 10.2 z
	Overall	63.4 ± 10.8	47.0 ± 8.8	
Gain (%)	Low	8.2 ± 1.4	8.6 ± 2.7	8.4 ± 1.4 y
	High	16.2 ± 1.4	10.4 ± 2.0	13.3 ± 1.6 z
	Overall	12.2 ± 1.8	9.5 ± 1.6	
Food fed (g)	Low	112 ± 6	81 ± 17	97 ± 10 y
	High	176 ± 13	146 ± 11	161 ± 10 z
	Overall	144 ± 14 y	114 ± 15 z	
FCR	Low	2.85 ± 0.33	2.96 ± 1.16	2.90 ± 0.56
	High	2.27 ± 0.49	3.16 ± 0.73	2.72 ± 0.44
	Overall	2.56 ± 0.30	3.06 ± 0.64	

SGR	Low	0.29 ± 0.05	0.30 ± 0.09	0.30 ± 0.05 y
	High	0.55 ± 0.04	0.36 ± 0.07	0.56 ± 0.05 z
	Overall	0.42 ± 0.06	0.33 ± 0.06	
Days 62-92				
End weight (g)	Low	669.2 ± 32.6	565.5 ± 67.8	617.3 ± 39.9
	High	738.3 ± 69.8	645.9 ± 51.3	692.1 ± 43.8
	Overall	703.7 ± 38.0	605.7 ± 42.2	
Gain (g)	Low	115.9 ± 24.3	66.0 ± 33.6	91.0 ± 21.4
	High	136.2 ± 17.6	88.6 ± 16.7	112.4 ± 14.4
	Overall	126.1 ± 14.4	77.3 ± 17.9	
Gain (%)	Low	20.9 ± 4.4	12.1 ± 5.4	16.5 ± 3.6
	High	23.1 ± 3.5	15.6 ± 2.2	19.4 ± 2.4
	Overall	22.0 ± 2.6	13.9 ± 2.8	
Food fed (g)	Low	197 ± 26	133 ± 33	165 ± 23 y
	High	311 ± 51	226 ± 29	269 ± 31 z
	Overall	254 ± 34	180 ± 27	
FCR	Low	1.83 ± 0.22	3.18 ± 0.96	2.50 ± 0.52
	High	2.28 ± 0.19	2.64 ± 0.16	2.46 ± 0.13
	Overall	2.05 ± 0.16	2.91 ± 0.46	
SGR	Low	0.61 ± 0.12	0.36 ± 0.15	0.48 ± 0.10
	High	0.67 ± 0.09	0.47 ± 0.06	0.57 ± 0.06
	Overall	0.64 ± 0.07	0.41 ± 0.08	
Days 93-121				

End weight (g)	Low	794.2 ± 47.0	650.6 ± 90.1	722.4 ± 56.4
	High	896.3 ± 49.4	752.1 ± 67.2	824.2 ± 57.5
	Overall	845.2 ± 49.4	701.3 ± 57.6	
Gain (g)	Low	125.0 ± 15.7	85.1 ± 28.7	105.0 ± 16.9
	High	158.0 ± 19.0	106.2 ± 17.1	132.2 ± 15.4
	Overall	141.5 ±13.0 z	95.7 ±16.0y	
Gain (%)	Low	18.5 ± 1.7	14.0 ± 3.1	16.3 ± 1.9
	High	21.4 ± 1.7	16.2 ± 1.7	18.8 ± 1.5
	Overall	19.9 ± 1.2 z	15.1 ± 1.7 y	
Food fed (g)	Low	229 ± 31	144 ± 32.4	187 ± 26 y
	High	355 ± 36	245 ± 31	300 ± 30 z
	Overall	292 ± 32 z	195 ± 28 y	
FCR	Low	1.83 ± 0.04	1.90 ± 0.21	1.86 ± 0.10 y
	High	2.28 ± 0.15	2.36 ± 0.19	2.32 ± 0.11 z
	Overall	2.05 ± 0.11	2.13 ± 0.16	
SGR	Low	0.58 ± 0.05	0.45 ± 0.10	0.52 ± 0.06
	High	0.67 ± 0.05	0.52 ± 0.05	0.59 ± 0.04
	Overall	0.63 ± 0.04z	0.48 ±0.05y	
Overall (Days 1-121)				
Gain (g)	Low	304.6 ± 40.3	215.4 ± 82.6	260.0 ± 45.8 y
	High	452.0 ± 53.0	343.0 ± 45.1	397.5 ± 38.2 z
	Overall	378.3 ± 41.5	279.2 ± 49.8	
Gain (%)	Low	62.4 ± 8.8	48.0 ± 16.7	55.2 ± 9.2 y

	High	101.4 ± 6.6	83.6 ± 8.6	92.5 ± 6.0 z
	Overall	81.9 ± 9.0	65.8 ± 11.0	
Food fed (g)	Low	730 ± 61	502 ± 87	616 ± 65 y
	High	1,127 ± 96	868 ± 81	998 ± 76 z
	Overall	928 ± 92 z	685 ± 88 y	
FCR	Low	2.45 ± 0.15	2.98 ± 0.59	2.72 ± 0.30
	High	2.55 ± 0.26	2.58 ± 0.13	2.56 ± 0.13
	Overall	2.50 ± 0.14	2.78 ± 0.29	
SGR	Low	0.40 ± 0.04	0.31 ± 0.09	0.35 ± 0.05 y
	High	0.58 ± 0.03	0.50 ± 0.04	0.54 ± 0.03 z
	Overall	0.49 ± 0.04	0.40 ± 0.06	
Mortality (%)	Low	12.5 ± 5.1	25.0 ± 8.8	18.8 ± 5.3 y
	High	0.0 ± 0.0	9.4 ± 6.0	4.7 ± 3.3 z
	Overall	6.2 ± 3.3	17.2 ± 5.8	

[a] FCR = feed conversion ratio = total food fed / total weight gain.

[b] SGR = 100 x [(ln(final weight) – ln(initial weight)) / days]

Table 23. Mean (± SE) condition factor (K[a]), fin indices[b], hepatosomatic index values (HSI[c]), splenosomatic index (SSI[d]), viscerosomatic index (VSI[e]), and histology scores for lamina propria, connective tissue, and vacuoles of Brown Trout fed one of two diets containing either fishmeal or bioprocessed soybean meal (BSM) as the primary protein source, and reared at two different velocities. Means with different letters in the same column or row differ significantly (*P* < 0.05).

			Diet (% BSM)	
	Velocity	1 (0)	2 (60)	Overall
Initial				
Weight (g)	Low	61.2 ± 2.5	54.4 ± 1.9	57.8 ± 1.9
	High	55.5 ± 4.5	51.1 ± 3.5	53.3 ± 2.8
	Overall	58.4 ± 2.6	52.8 ± 1.9	
Length (mm)	Low	171.9 ± 1.9	164.3 ± 1.9	168.1 ± 1.9
	High	166.3 ± 4.4	162.3 ± 2.8	164.3 ± 2.5
	Overall	169.1 ± 2.5	163.3 ± 1.6	
K	Low	1.19 ± 0.02	1.19 ± 0.01	1.19 ± 0.01
	High	1.18 ± 0.02	1.15 ± 0.02	1.16 ± 0.01
	Overall	1.18 ± 0.01	1.17 ± 0.01	
Days 1-35				
End weight (g)	Low	63.9 ± 2.1	57.3 ± 2.7	60.6 ± 2.0
	High	64.6 ± 6.0	63.0 ± 3.6	63.8 ± 3.2
	Overall	64.3 ± 2.9	60.2 ± 2.3	
End length (mm)	Low	178.2 ± 1.4	168.2 ± 3.1	173.2 ± 2.5

	High	175.2 ± 4.6	172.6 ± 2.6	173.9 ± 2.5
	Overall	176.7 ± 2.3	170.4 ± 2.1	
K	Low	1.12 ± 0.03	1.17 ± 0.03	1.15 ± 0.02
	High	1.16 ± 0.02	1.17 ± 0.03	1.17 ± 0.02
	Overall	1.14 ± 0.01	1.17 ± 0.02	
Days 36-61				
End weight (g)	Low	70.1 ± 3.0	66.5 ± 2.8	68.3 ± 2.0
	High	75.3 ± 7.6	69.7 ± 4.6	72.5 ± 4.2
	Overall	72.7 ± 3.9	68.1 ± 2.6	
End length (mm)	Low	185.3 ± 2.4	178.2 ± 2.6	181.8 ± 2.1
	High	185.3 ± 5.1	180.5 ± 3.1	182.9 ± 2.9
	Overall	185.3 ± 2.6	179.4 ± 1.9	
K	Low	1.10 ± 0.03	1.14 ± 0.01	1.12 ± 0.02
	High	1.14 ± 0.02	1.15 ± 0.02	1.15 ± 0.02
	Overall	1.12 ± 0.02	1.15 ± 0.01	
Days 62-92				
End weight (g)	Low	86.2 ± 5.5	77.1 ± 6.1	81.6 ± 4.2
	High	92.3 ± 8.7	80.8 ± 6.4	86.5 ± 5.5
	Overall	89.2 ± 4.9	78.9 ± 4.2	
End length (mm)	Low	195.8 ± 3.3	187.1 ± 4.2	191.4 ± 3.0
	High	198.4 ± 5.2	190.4 ± 4.1	194.4 ± 3.4
	Overall	197.1 ± 2.9 z	188.7 ± 2.8 y	
K	Low	1.13 ± 0.03	1.14 ± 0.01	1.14 ± 0.01

	High	1.14 ± 0.02	1.12 ± 0.02	1.13 ± 0.01
	Overall	1.14 ± 0.02	1.13 ± 0.01	
Overall (days 1-121)				
End weight (g)	Low	103.7 ± 7.3	90.2 ± 8.9	97.0 ± 5.9
	High	112.0 ± 10.8	94.9 ± 8.0	103.4 ± 7.0
	Overall	107.9 ± 6.2	92.6 ± 5.6	
End length (mm)	Low	202.7 ± 6.6	192.7 ± 5.0	197.7 ± 4.3
	High	209.4 ± 4.9	198.5 ± 5.6	203.9 ± 4.0
	Overall	206.0 ± 4.0	195.6 ± 3.6	
K	Low	1.23 ± 0.06	1.21 ± 0.04	1.22 ± 0.03
	High	1.18 ± 0.03	1.16 ± 0.01	1.17 ± 0.01
	Overall	1.20 ± 0.03	1.18 ± 0.02	
Pectoral index (%)	Low	13.37 ± 0.35	13.54 ± 0.48	13.46 ± 0.28
	High	12.94 ± 0.21	13.25 ±_ 0.35	13.10 ± 0.20
	Overall	13.16 ± 0.21	13.40 ± 0.28	
Pelvic index (%)	Low	11.15 ± 0.15	11.15 ± 0.21	11.15 ± 0.12
	High	10.84 ± 0.10	10.83 ± 0.26	10.84 ± 0.13
	Overall	11.00 ± 0.10	10.99 ± 0.17	
Dorsal index (%)	Low	4.36 ± 0.58	4.66 ± 0.68	4.51 ± 0.42
	High	4.39 ± 0.48	4.52 ± 0.64	4.45 ± 0.37
	Overall	4.37 ± 0.35	4.59 ± 0.43	
HSI (%)	Low	1.14 ± 0.16	1.06 ± 0.12	1.10 ± 0.09
	High	1.20 ± 0.12	1.17 ± 0.11	1.18 ± 0.07

	Overall	1.17 ± 0.09	1.12 ± 0.08	
SSI (%)	Low	0.06 ± 0.01	0.07 ± 0.00	0.07 ± 0.00
	High	0.10 ± 0.03	0.06 ± 0.00	0.08 ± 0.02
	Overall	0.08 ± 0.02	0.06 ± 0.00	
VSI (%)	Low	5.30 ± 0.40	5.68 ± 0.26	5.49 ± 0.24
	High	6.16 ± 0.20	6.04 ± 0.43	6.10 ± 0.22
	Overall	5.73 ± 0.26	5.86 ± 0.24	
Lamina propria[f]	Low	1.33 ± 0.14	1.50 ± 0.17	1.42 ± 0.10
	High	1.50 ± 0.17	1.17 ± 0.17	1.33 ± 0.13
	Overall	1.42 ± 0.10	1.33 ± 0.13	
Connective tissue[f]	Low	1.33 ± 0.14	1.25 ± 0.16	1.29 ± 0.10
	High	1.50 ± 0.17	1.42 ± 0.21	1.46 ± 0.13
	Overall	1.42 ± 0.10	1.33 ± 0.13	
Vacuoles[f]	Low	2.50 ± 0.17	2.17 ± 0.22	2.33 ± 0.14
	High	2.33 ± 0.14	2.25 ± 0.25	2.29 ± 0.13
	Overall	2.42 ± 0.10	2.21 ± 0.15	

[a] $K = 10^5 \times [\text{weight} / (\text{length}^3)]$

[b] Fin indices = 100 x (fin length / fish length)

[c] HSI = 100 x (liver weight / body weight)

[d] SSI = 100 x (spleen weight / body weight)

[e] VSI = 100 x (visceral weight / body weight)

[f] Table 21. Explains scoring curriculum

Figure 5. Representative (diet is 60% BSM at a slow velocity) Brown Trout histology image used for scoring.

CHAPTER 7: IMPLICATIONS AND SUGGESTIONS FOR FURTHER STUDY

Overall, the results from the five experiments described in this thesis consistently indicate that bioprocessed soybean meal (BSM) used can replace at least 80% of the fishmeal in the diets of juvenile or adult Rainbow Trout (*Oncorhynchus mykiss*) and 100% of the fish meal component of juvenile Brown Trout (*Salmo trutta*) diets without deleterious effects. In addition, in comparison to fish-meal based diets, the inclusion of BSM in trout diets does not appear to have any influence on the rearing performance of trout subjected to continual exercise.

BSM process improvements may produce new ingredients that provide additional performance benefits beyond results observed in this study. In particular, the Brown Trout growth and feed conversion ratios were extremely poor in this study, and could likely be improved by using higher quality dietary ingredients. The results of these experiments, particularly those involving Brown Trout, may have also been influenced by the texture and physical characteristics of the feed pellets. Qualitatively the pellets appeared hard and very dense, and quantitatively had floating ranks of less than 30%. Thus, further investigation of extruder conditions used to manufacture plant-based feeds should be done to develop softer pellets with a higher float rate. This research would likely result in improved feed palatability and consumption, which in turn should improve growth and feed conversion ratios.

The observations showing an elimination of exercise benefits after 60 days and the hypothesis of possible exercise fatigue are unique to this study. Nearly all of the prior published experiments involving exercise in fish had a much shorter duration, with no indications of possible fatigue. Additional experimentation is needed to determine if exercise fatigue after continual swimming longer than 60-days is actually occurring. In addition, cyclical exercise regimes incorporating rest periods could be evaluated. Intermittent exercise, typically performed on humans, responses appears to be a potential area for additional research. There are numerous possibilities, but options such as subjecting a fish to high velocities for only a set number of hours in a day, with rest hours included, or high velocities for a set number of days, again with rest days included, would be initial experiments. In addition to collecting data on growth and feed utilization, evaluating physiological, immunological, and stress responses should be done.

The experiments conducted in this thesis provide an important step in improving the growth, health, and post-stocking survival of cultured Brown Trout and Rainbow Trout. Additional experimentation with other ingredients and exercise regimes would likely lead to further improvements in rearing efficiencies and fish quality. Ideally, future research into both diet and exercise for fish reared at recreational hatcheries, such as those run by state conservation agencies, may translate into better fish fitness that could result in an improved stocking product (e.g., better survival, return to creel, and fighting ability), which in turn would increase angler satisfaction.