

Rearing Performance of Juvenile Brown Trout (*Salmo trutta*) Subjected to Exercise and Dietary Bioprocessed Soybean Meal

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Abstract

This 121-day experiment evaluated the rearing performance of brown trout *Salmo trutta* fed one of two isonitrogenous and isocaloric diets and reared at velocities of either 2.8 or 16.1 cm/s. Fishmeal was the primary protein source for the reference diet, and bioprocessed soybean meal 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, or 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, bioprocessed soybean meal can replace at least 67% of the fishmeal in brown trout diets, regardless of the rearing velocities used in this study. However, higher rearing velocities are recommended to maximize juvenile brown trout growth rates.

Keywords

Brown Trout, *Salmo trutta*, Bioprocessed Soybean Meal, Exercise, Diet

1. Introduction

With global human population expected to grow to 9 billion by 2050 [1], 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 [1]. However, the continuing growth of aquaculture is constrained by the cost and unpredictability of aquatic animal feedstuffs [1].

Fishmeal, primarily produced from marine pelagic fish [1] [2], has historically been the primary protein ingredient in carnivorous fish [3] [4] [5]. However, nearly 90% of the world marine fisheries are fully-fished or overfished [1], 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*) [6] [7], due to its relative low cost and worldwide availability [8]. Soybean products are highly palatable [9] [10] [11], have a high protein content (~48% crude protein), and also have a balanced amino acid profile [12] [13]. However, there are antinutritional factors associated with soybean which hinder fish digestion [13] [14] [15] [16], and can also cause gastro-intestinal issues, such as enteritis [10] [17] [18] [19] [20]. Soybean products also have large concentrations of non-digestible carbohydrates [12] [21]. These factors limit the inclusion levels of soybean products in diets for carnivorous fish species [13] [22] [23] [24]. However, some of these antinutritional factors are decreased or inactivated by heat, which occurs during the feed extrusion process [14] [25] [26]. Bioprocessing, such as fermentation, has also been shown to eliminate or reduce antinutritional factors [27] [28].

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 that evaluated fermented soybean products in brown trout diets. Sotoudeh *et al.* [29] replaced fishmeal 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 impact rearing performance [30] [31] [32] [33]. Exercise (increased velocities and forced swimming) has been shown to improve growth of rainbow trout and Atlantic salmon fed to satiation [31] [34]. If feed is limited however, growth can be impaired at higher velocities [30]. Davison and Goldspink [35] examined the effect of prolonged swimming on brown trout, and found that intermediate speeds (1.5 and 3.0 body lengths/second; bl/s) resulted in 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 diet. 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.

2. Methods

This experiment was conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, USA, using degassed and aerated well water at a constant temperature of 11°C (total hardness as CaCO₃, 360 mg/L; alkalinity as CaCO₃, 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 × 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 1**.

Water velocities were recorded using a flowmeter (Flowwatch, 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 2**), 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 [36] method 2001.11 for protein, 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and AACC [37] 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 a total tank weight. Weight gain, percent

Table 1. Study design for dietary and velocity treatments, and mean velocities (±SE).

Treatment	N	Diet (% BSM)		Velocity		Velocity (cm/s)
		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 2. Diet formulation and composition analyses of the diets used in the 121-day trial. Analysis conducted on post-extruded feed pellets.

Ingredients	Diet (%)	
	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, Alpharetta, GA; j. DSM Nutritional Products, Ames, IA; k. Virginia Prime Gold, Omega Protein, Houston, TX.

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 [38], 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 [39]. 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 [40]. 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) [41], splenosomatic index (SSI) [42], and viscerosomatic index (VSI) [42] were calculated.

The following equations were used:

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

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

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

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

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

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

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

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

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

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 [43] [44] [45] [46]. After dissection, the intestinal tissue was immediately put into 10% buffered formalin, and stained with haematoxylin and eosin using standard histological techniques [47] [48]. Intestinal inflammation was assessed using an ordinal scoring system (Table 3) based on lamina propria thickness and cellularity, submucosal connective tissue width, and leukocyte distribution [49] [50] [51].

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.

3. 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

Table 3. Histological scoring system used on brown trout fed fishmeal or bioprocessed soybean meal diets [51, modified from 42, 93].

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.

the fishmeal or BSM diet (**Table 4**). Overall mean (\pm 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 $928 (\pm 92)$ g of feed and the BSM diet tanks receiving $685 (\pm 88)$ 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 (\pm SE) percent gain in this rearing period (4) was $19.9 (\pm 1.2)\%$ and $15.1 (\pm 1.7)\%$ 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 5**). None of the fin indices (pectoral, pelvic, and dorsal), organismic indices (HSI, SSI, VSI), or gut histology scores were significantly different between diets. Representative

Table 4. Mean (\pm 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 same column or row differ significantly ($P < 0.05$). The absence of letters indicates no significant difference.

	Velocity	Diet (% BSM)		Overall
		1 (0)	2 (60)	
Initial				
Start weight (g)	Low	489.6 \pm 20.1	435.2 \pm 15.0	462.4 \pm 15.5
	High	444.4 \pm 36.3	409.1 \pm 28.1	426.7 \pm 22.3
	Overall	467.0 \pm 21.0	422.1 \pm 15.6	
Days 1 - 35				
End weight (g)	Low	511.6 \pm 16.4	458.6 \pm 21.4	485.1 \pm 16.0
	High	516.9 \pm 47.6	504.2 \pm 28.7	510.6 \pm 25.8
	Overall	514.2 \pm 23.3	481.4 \pm 18.7	
Gain (g)	Low	22.0 \pm 10.4	23.4 \pm 8.9	22.7 \pm 6.4 y
	High	72.6 \pm 12.1	95.1 \pm 9.1	83.8 \pm 8.2 z
	Overall	47.3 \pm 12.1	59.3 \pm 14.8	
Gain (%)	Low	4.7 \pm 2.2	5.3 \pm 2.0	5.0 \pm 1.4 y
	High	16.0 \pm 1.7	23.6 \pm 3.1	19.8 \pm 2.2 z
	Overall	10.3 \pm 2.5	14.5 \pm 3.9	
Food fed (g)	Low	192 \pm 5	144 \pm 8	168 \pm 10 y
	High	284 \pm 6	252 \pm 22	268 \pm 12 z
	Overall	238 \pm 18 z	198 \pm 23 y	
FCR	Low	-21.83 \pm 30.30	11.03 \pm 4.75	-5.40 \pm 15.50
	High	4.27 \pm 0.73	2.73 \pm 0.34	3.50 \pm 0.47
	Overall	-8.78 \pm 14.87	6.88 \pm 2.71	
SGR	Low	0.13 \pm 0.06	0.15 \pm 0.06	0.14 \pm 0.04 y
	High	0.44 \pm 0.04	0.62 \pm 0.07	0.53 \pm 0.05 z
	Overall	0.28 \pm 0.07	0.39 \pm 0.10	
Days 36 - 61				
End weight (g)	Low	533.2 \pm 16.1	499.4 \pm 34.3	526.3 \pm 20.3
	High	602.1 \pm 60.9	557.3 \pm 36.8	579.7 \pm 34.0
	Overall	577.6 \pm 30.6	528.4 \pm 25.7	
Gain (g)	Low	41.7 \pm 6.6	40.8 \pm 14.6	41.3 \pm 7.4 y
	High	85.1 \pm 13.7	53.1 \pm 11.3	69.1 \pm 10.2 z
	Overall	63.4 \pm 10.8	47.0 \pm 8.8	
Gain (%)	Low	8.2 \pm 1.4	8.6 \pm 2.7	8.4 \pm 1.4 y
	High	16.2 \pm 1.4	10.4 \pm 2.0	13.3 \pm 1.6 z
	Overall	12.2 \pm 1.8	9.5 \pm 1.6	
Food fed (g)	Low	112 \pm 6	81 \pm 17	97 \pm 10 y
	High	176 \pm 13	146 \pm 11	161 \pm 10 z
	Overall	144 \pm 14 y	114 \pm 15 z	

Continued

	Low	2.85 ± 0.33	2.96 ± 1.16	2.90 ± 0.56
FCR	High	2.27 ± 0.49	3.16 ± 0.73	2.72 ± 0.44
	Overall	2.56 ± 0.30	3.06 ± 0.64	
	Low	0.29 ± 0.05	0.30 ± 0.09	0.30 ± 0.05 y
SGR	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				
	Low	669.2 ± 32.6	565.5 ± 67.8	617.3 ± 39.9
End weight (g)	High	738.3 ± 69.8	645.9 ± 51.3	692.1 ± 43.8
	Overall	703.7 ± 38.0	605.7 ± 42.2	
	Low	115.9 ± 24.3	66.0 ± 33.6	91.0 ± 21.4
Gain (g)	High	136.2 ± 17.6	88.6 ± 16.7	112.4 ± 14.4
	Overall	126.1 ± 14.4	77.3 ± 17.9	
	Low	20.9 ± 4.4	12.1 ± 5.4	16.5 ± 3.6
Gain (%)	High	23.1 ± 3.5	15.6 ± 2.2	19.4 ± 2.4
	Overall	22.0 ± 2.6	13.9 ± 2.8	
	Low	197 ± 26	133 ± 33	165 ± 23 y
Food fed (g)	High	311 ± 51	226 ± 29	269 ± 31 z
	Overall	254 ± 34	180 ± 27	
	Low	1.83 ± 0.22	3.18 ± 0.96	2.50 ± 0.52
FCR	High	2.28 ± 0.19	2.64 ± 0.16	2.46 ± 0.13
	Overall	2.05 ± 0.16	2.91 ± 0.46	
	Low	0.61 ± 0.12	0.36 ± 0.15	0.48 ± 0.10
SGR	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				
	Low	794.2 ± 47.0	650.6 ± 90.1	722.4 ± 56.4
End weight (g)	High	896.3 ± 49.4	752.1 ± 67.2	824.2 ± 57.5
	Overall	845.2 ± 49.4	701.3 ± 57.6	
	Low	125.0 ± 15.7	85.1 ± 28.7	105.0 ± 16.9
Gain (g)	High	158.0 ± 19.0	106.2 ± 17.1	132.2 ± 15.4
	Overall	141.5 ± 13.0 z	95.7 ± 16.0 y	
	Low	18.5 ± 1.7	14.0 ± 3.1	16.3 ± 1.9
Gain (%)	High	21.4 ± 1.7	16.2 ± 1.7	18.8 ± 1.5
	Overall	19.9 ± 1.2 z	15.1 ± 1.7 y	
	Low	229 ± 31	144 ± 32.4	187 ± 26 y
Food fed (g)	High	355 ± 36	245 ± 31	300 ± 30 z
	Overall	292 ± 32 z	195 ± 28 y	
	Low	1.83 ± 0.04	1.90 ± 0.21	1.86 ± 0.10 y
FCR	High	2.28 ± 0.15	2.36 ± 0.19	2.32 ± 0.11 z
	Overall	2.05 ± 0.11	2.13 ± 0.16	
	Low	0.58 ± 0.05	0.45 ± 0.10	0.52 ± 0.06
SGR	High	0.67 ± 0.05	0.52 ± 0.05	0.59 ± 0.04
	Overall	0.63 ± 0.04 z	0.48 ± 0.05 y	

Continued

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 = $100 \times [(\ln(\text{final weight}) - \ln(\text{initial weight}))/\text{days}]$.

Table 5. Mean (\pm SE) condition factor (K^a), fin indices^b, hepatosomatic index (HSI^c), splenosomatic index (SSI^d), viscerosomatic index (VSI^e), and histology scores for lamina propria, connective tissue, and vacuoles 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 same column or row differ significantly ($P < 0.05$). The absence of letters indicates no significant difference.

		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	

Continued

Days 1 - 35				
	Low	63.9 ± 2.1	57.3 ± 2.7	60.6 ± 2.0
End weight (g)	High	64.6 ± 6.0	63.0 ± 3.6	63.8 ± 3.2
	Overall	64.3 ± 2.9	60.2 ± 2.3	
	Low	178.2 ± 1.4	168.2 ± 3.1	173.2 ± 2.5
End length (mm)	High	175.2 ± 4.6	172.6 ± 2.6	173.9 ± 2.5
	Overall	176.7 ± 2.3	170.4 ± 2.1	
	Low	1.12 ± 0.03	1.17 ± 0.03	1.15 ± 0.02
K	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			
	Low	70.1 ± 3.0	66.5 ± 2.8	68.3 ± 2.0
End weight (g)	High	75.3 ± 7.6	69.7 ± 4.6	72.5 ± 4.2
	Overall	72.7 ± 3.9	68.1 ± 2.6	
	Low	185.3 ± 2.4	178.2 ± 2.6	181.8 ± 2.1
End length (mm)	High	185.3 ± 5.1	180.5 ± 3.1	182.9 ± 2.9
	Overall	185.3 ± 2.6	179.4 ± 1.9	
	Low	1.10 ± 0.03	1.14 ± 0.01	1.12 ± 0.02
K	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			
	Low	86.2 ± 5.5	77.1 ± 6.1	81.6 ± 4.2
End weight (g)	High	92.3 ± 8.7	80.8 ± 6.4	86.5 ± 5.5
	Overall	89.2 ± 4.9	78.9 ± 4.2	
	Low	195.8 ± 3.3	187.1 ± 4.2	191.4 ± 3.0
End length (mm)	High	198.4 ± 5.2	190.4 ± 4.1	194.4 ± 3.4
	Overall	197.1 ± 2.9 ^z	188.7 ± 2.8 ^y	
	Low	1.13 ± 0.03	1.14 ± 0.01	1.14 ± 0.01
K	High	1.14 ± 0.02	1.12 ± 0.02	1.13 ± 0.01
	Overall	1.14 ± 0.02	1.13 ± 0.01	

a. $K = 105 \times [\text{weight}/(\text{length}^3)]$; b. $\text{Fin indices} = 100 \times (\text{fin length}/\text{fish length})$; c. $\text{HSI} = 100 \times (\text{liver weight}/\text{body weight})$; d. $\text{SSI} = 100 \times (\text{spleen weight}/\text{body weight})$; e. $\text{VSI} = 100 \times (\text{visceral weight}/\text{body weight})$.

images of the distal intestines from fish fed each diet, and velocities used for the scoring are shown in **Figures 1-4**. 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 (\pm SE) length of fish fed the fishmeal diet was 197.1 (\pm 2.9) mm compared to 188.7 (\pm 2.8) mm in the fish fed the bioprocessed soybean meal diet.

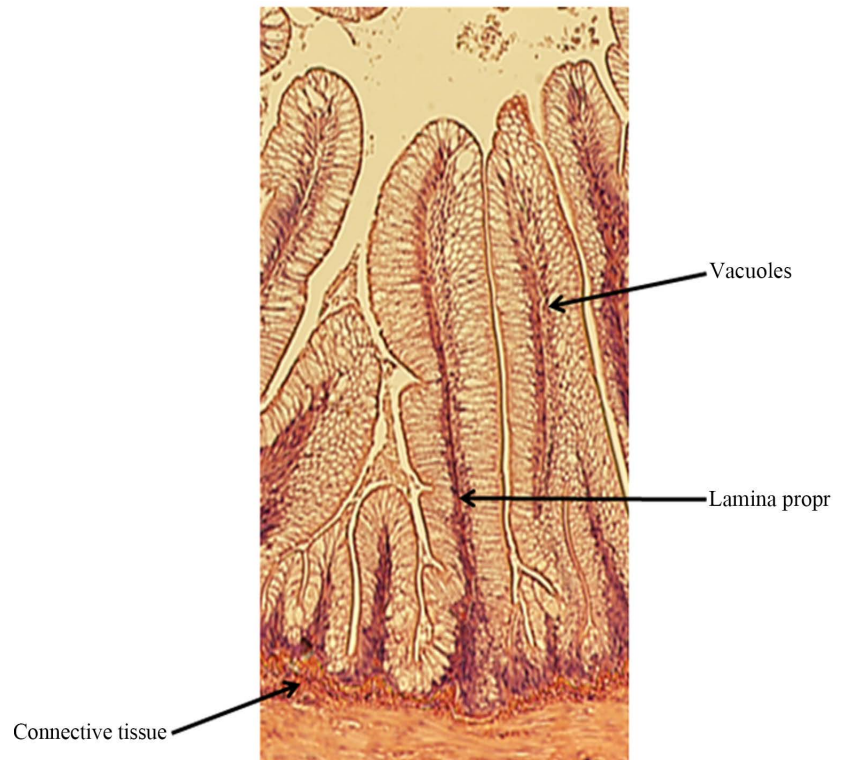


Figure 1. Distal intestine of brown trout receiving 0% bioprocessed soybean meal and reared at low velocity.

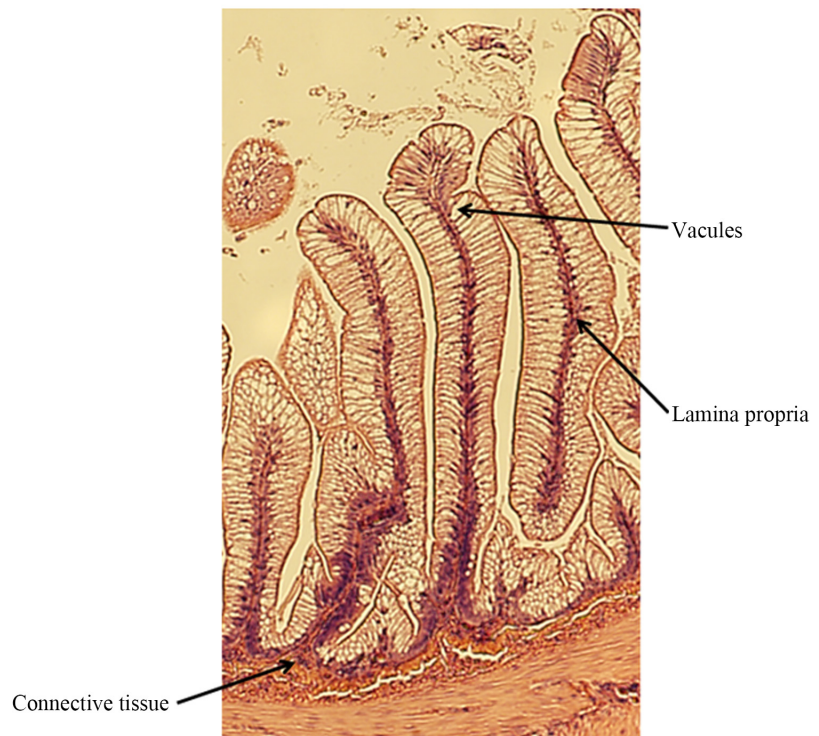


Figure 2. Distal intestine of brown trout receiving 0% bioprocessed soybean meal and reared at high velocity.

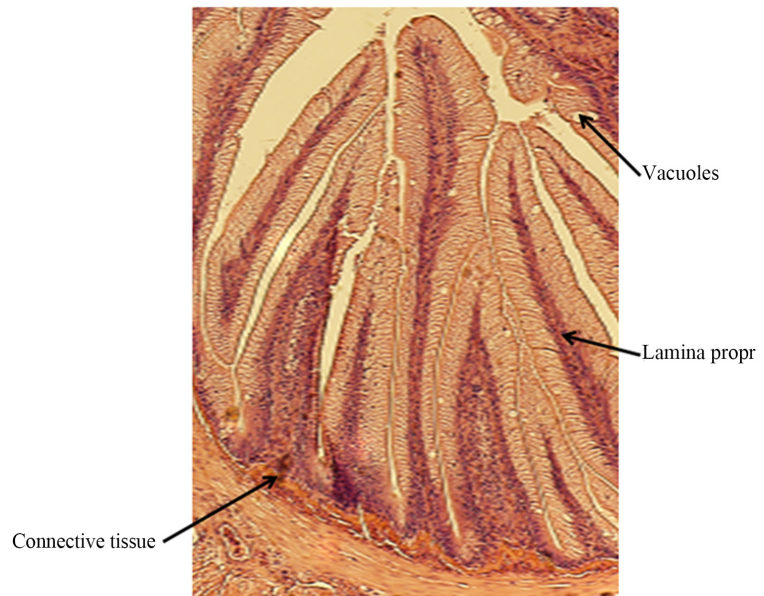


Figure 3. Distal intestine of brown trout receiving 60% bioprocessed soybean meal and reared at low velocity.

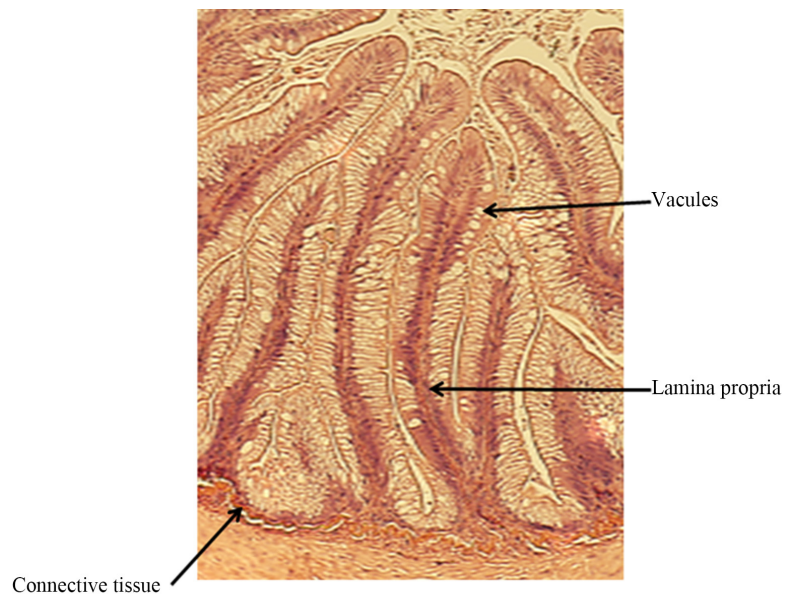


Figure 4. Distal intestine of brown trout receiving 60% bioprocessed soybean meal and reared at high velocity.

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 (\pm SE) percent gain was only 55.2 (\pm 9.2)% in lower velocity tanks, but was 92.5 (\pm 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 (\pm 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 (\pm 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 (\pm SE) of 2.32 (± 0.11) compared to 1.86 (± 0.10) for the slower velocity tanks. Similar to the dietary results, mean (\pm 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.

4. 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.* [29] also indicated the suitability of fermented soybean meal in brown trout diets. However, the Sotoudeh *et al.* [29] 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.* [52] [53] who replaced 65% of the dietary fishmeal with BSM with no observed ill-effects. In addition, Barnes *et al.* [51] [54] [55] replaced approximately 62% of the fishmeal with a commercial fermented soybean product without any significant difference in rainbow trout performance. Yamamoto *et al.* [56] [57] also reported positive results with fermented soybean meal in rainbow trout diets. Different forms of BSM have been evaluated in Atlantic salmon diets, but fishmeal replacement rates appeared limited to 20% or less [28]. Other species where fermented, or other forms of bioprocessed, soybean have been evaluated include Atlantic cod (*Gadus morhua*) [58] [59], black sea bream (*Acanthopagrus schlegelii*) [60] [61], Chinese sucker (*Myxocyprinus asiaticus*) [62], Florida pompano (*Trachniotus carolinus*) [46], gilthead sea bream (*Sparus aurata* L.) [63], Japanese flounder (*Paralichthys olivaceus*) [64], largemouth bass (*Micropterus salmoides*) [65], orange-spotted grouper (*Epinephelus coioides*) [66], whiteleg shrimp (*Litopenaeus vannamei*) [66] [67] [68],

rockfish (*Sebastes schlegeli*) [69], white seabass (*Atractosion nobilis*) [70], and yellowtail jack (*Seriola lalandi*) [70].

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 [19] [24] [51] [54] [71] [72] [73]. At 121 days, this study should have met the Weathercup and McCracken [74] criteria for being long enough to determine any differences in fish performance among the diets. This study also met the NRC [13] 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 [13] 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.* [75], 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 [52] [76]. Overall, FCRs from the brown trout in this study are higher (worse) than that reported by Regost *et al.* [77] or Kizak *et al.* [78]. However, Kizak *et al.* [78] fed a restricted ration, which has been shown to improve FCR [79]. The SGR at the beginning 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.* [77].

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 amount of food fed by the gain [80], and any significant increase in food fed, with no change in gain, should produce a corresponding increase in FCR. This enigma could be a statistical artifact, possibly due to small sample sizes [81].

Soybean products in the diets of salmonids have caused well-documented and potentially-deleterious effects in the distal intestine of rainbow trout [15] [72] [82] [83]. Intestinal microbial communities may also be affected [19] [53] [72] [84]. These issues have been reported in Atlantic salmon [85] [86] [87] [88], 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 [54] [56] [57] [89], making it likely that the saponins [18] 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.* [51] [55] [90]. However, the Barnes' studies examined rainbow trout which were fed different diets than those used in this study. In addition,

Bruce *et al.* [53] also used the same scoring system with rainbow trout but compiled and averaged all numbers for an overall histology 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 [91] [92] [93]. 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.* [94], but lower for other studies (1.4 to 1.7) [29] [78] [95]. 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 [93].

VSI values indicate how lipids are being used and there is a positive relationship between lipid levels and VSI values [96] [97] [98]. 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.* [95], Sotoudeh *et al.* [94], and Kizak *et al.* [78], they were similar to those reported by Sotoudeh *et al.* [29], which is the only experiment examining processed soybean products in brown trout diets.

SSI indicates the hematopoietic capacity of fish [93] and antibody production mostly occurs in the spleen [99]. 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 [52] [55]. In two velocity studies that reported SSI in rainbow trout, SSI values were approximately 25% higher than those observed in this study [31] [100].

In addition to diet, exercise has been shown to impact fish growth [30] [31] [32] [33]. 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 [30] [31] [32] [33] [35] [101] [102] [103] [104] [105]. Only one other velocity experiment has lasted 4 months, but growth data was not reported [106]. 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 [107] [108] [109] [110].

As expected, fish at the higher velocity ate significantly more food than 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 [31] [111] [112] [113]. This was also observed in the present study where, similar to other studies [101] [105], 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 bet-

ter at a ratio of 1.86, while 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 [114], adequate feeding rates [115], no nutritional differences [116] [117], and good fish health [118]. Fin erosion has been found to be due to several factors, including tank-induced abrasions [119], rearing unit size and type [120], aggressive behavior [114], feeding rates [115], rearing densities [121] [122] [123], and fish health [118]. Bosakowski and Wagner [120] 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 [120] was over 35% greater than in this experiment.

5. Conclusion

In conclusion, BSM can replace fishmeal in brown trout diets with no ill-effects, even if the trout is 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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