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Dietary Bioprocessed Soybean Meal Does Not Affect the Growth of Exercised Juvenile Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

Context: This 88-day experiment evaluated the rearing performance of juvenile rainbow trout (*Oncorhynchus mykiss*) fed one of three isonitrogenous and isocaloric diets and reared at velocities of either 2.3 or 18.7 cm s^{-1}.

Objective: Evaluate the effects of diet and exercise during rainbow trout rearing.

Design: Fishmeal was the primary protein source for one diet, with bioprocessed soybean meal (BSM) replacing either 60 or 85% of the fishmeal in the other two diets.

Setting: This study was performed at Cleghorn Springs State Fish Hatchery in Rapid City, South Dakota, USA.

Results: At the end of the experiment there were no significant differences among the dietary treatments in gain, percent gain, specific growth rate (SGR), 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 85% BSM diet. Intestinal histology was not affected by the inclusion of BSM. Fish reared at 2.3 cm/s^{-1} had significantly lower FCRs, gain, percent gain, and SGR than the fish reared at 18.7 cm/ s-1. There was a significant interaction in food consumed between diet and velocity, but no other significant interactions between the dietary and exercise treatments were observed.

Conclusion: Based on these results, BSM can replace at least 85% of the fishmeal in juvenile rainbow trout, even if the fish are exercised.

Keywords: Alternative protein; Plant-based diets; Salmonids; Feed formulation; Velocity

Abbreviations

BSM: Bioprocessed Soybean Meal; FCR: Feed Conversion Ratio; SGR: Specific Growth Rate; HSI: Hepatosomatic Index; SSI: Splenosomatic Index; VSI: Viscerosomatic Index; K: Condition Factor

Introduction

With the large increase in aquaculture production, there has been a corresponding increase in aquafeeds [1]. Fishmeal has historically been the primary protein source in feeds for carnivorous fish, like rainbow trout (*Oncorhynchus mykiss*) [2-4]. However, fishmeal is primarily made from small pelagic marine fish, and the supply of these capture fisheries has not increased correspondingly to the increase in aquaculture production, creating a need for alternative protein sources [1]. Plant-based proteins are a prime candidate due to wide availability and relatively favorable pricing in comparison to fishmeal [5].

Of the plant-based proteins, soybeans (Glycine max) are one of the leading alternatives to fishmeal [6,7], due to relatively high palatability [8-10], high protein levels, and balanced amino acid profiles [5,11]. However, soybean meal contains antinutritional factors that hinder fish digestion [11-13], and can cause gastro-intestinal issues, such as enteritis [8,14-16]. In addition, soybean meal has high carbohydrate levels [5,17], which can be especially deleterious to carnivorous fish [11]. For these reasons, soybean meal inclusion in carnivorous fish aquafeeds has been limited.

Soybean meal antinutritional factors can be decreased or eliminated. Heat applied during the feed extrusion process decreases lectins and proteinase inhibitors [5,18,19]. Saponins, sterols, and oligosaccharides can be decreased by alcohol extraction [19]. Bioprocessing, such as fermentation, has also been shown to eliminate or reduce antinutritional factors [20-23].

In addition to diet, exercise has been shown to affect fish rearing performance [24-27]. When fish are fed to satiation, exercise produces improved growth and feed conversion ratios (FCR), [27,28]. However, if feed is limited, growth can be impaired at higher velocities [26]. The interaction between dietary composition and exercise has not been investigated. Thus, there is no published information on how the replacement of fishmeal with plant-based proteins, such as soybean meal, may impact the response of fish forced to exercise by being subjected to higher water velocities. The objective of this experiment was to examine the effects of bioprocessed soybean meal (BSM) diets and velocity on juvenile rainbow trout rearing performance.

Materials and Methods

All experimentation occurred at Cleghorn Springs State Fish Hatchery, Rapid City, South Dakota, USA. Spring water (11°C; total hardness as $CaCO_3$, 360 mg L^{-1}; alkalinity as $CaCO_3$, 210 mg L^{-1}; pH, 7.6; total dissolved solids, 390 mg L^{-1}) was used throughout this 88-day study. On August 4, 2016, 20 randomly-selected Shasta strain rainbow trout (initial weight 48.8 ± 0.5 g, length 156.8 ± 0.5 mm, mean ± SE) were placed into each of 18 circular fiberglass tanks (1.8 m diameter, 0.6 m water depth). Three hundred and sixty total fish were used. The 3 × 2 experimental design used three diets and two water velocities, with three tanks per treatment **(Table 1).**

Table 1: Study design for dietary and velocity treatments (N=3), and mean (± SE) water velocities.

		Diet (% BSM[a])			Velocity		Velocity (cm s^{-1})
Treatment	N	1 (0)	2 (60)	3 (85)	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

[a]Bioprocessed soybean meal (BSM)

The three dietary treatments consisted of bioprocessed soybean meal replacing 0, 60, or 85% of the dietary fishmeal, with fishmeal being the primary protein source in the 0% treatment **(Table 2).**

Table 2: Diet formulation and composition analyses of the diets used in the 88-day trial. Analysis conducted on post-extrusion feed pellets.

Chemical analysis (% dry basis)	Diet (% BSM)		
Ingredients	1 (0)	2 (60)	3 (85)
Fishmeal[a]	35	14	4.7
Bioprocessed soybean meal[b]	0	21	30.3
Wheat midds[c]	12	10	10
Whole wheat[c]	17.7	15.2	15.1
Poultry byproduct meal[d]	10	15	15
Blood meal[e]	2	2	2
Feather meal[d]	7	2.5	2.5
Vitamin premix[f]	1.3	1.3	1.3
Mineral premix[f]	0.8	2	2
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	2
L-Methionine[i]	0.3	0.5	0.5
Stay-C 35[j]	0.2	0.2	0.2
Fish oil[k]	11	13	13
Total	100	100	100
Protein	43.18	43.85	43.84
Lipid	15.91	14.28	16.44
Ash	2.42	3.6	3.92
Nitrogen-free extract	20.48	24.33	23.96
Dry matter	93	95.2	96.25
Gross Energy (kJ/g)	16.5	16	16.8
Protein: Energy (MJ/g)	26.2	27.4	26

[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.

All three of the diets were isocaloric and isonitrogenous, and were prepared using an extruder (ExtruTech model 325, Sabetha, KS, USA). The bioprocessed soybean meal was produced using a proprietary microbial conversion process (SDSU, Brookings, SD, USA). Feed was analyzed according to Association of Official Analytical Chemists [29] method 2001. 11 for protein, 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and American Association of Cereal Chemists [30] method 08-03 for ash content.

The two water velocity treatments were either 2.3 or 18.7 cm s^{-1}, with in tank velocities recorded mid-depth behind the spraybar with a Flowatch flowmeter (JDC Electronic SA, Yverdon-les-Bains, Jura-Nord Vaudois, Vaud, Switzerland). Flow rates remained constant throughout the study.

At the start of the experiment and then approximately every four weeks thereafter, all of the fish in each tank were individually weighed to the nearest 0.1 g and measured (total length) to the nearest 1.0 mm. Total tank weights were obtained by summing the individual fish weights. Fish were fed daily for 88 days, except on days 29 and 60 when the fish were weighed and measured. Feeding amounts were initially determined by the hatchery constant method [31], using an anticipated feed conversion ratio of 1.1 and growth rate of 0.08 cm day^{-1}. These values were based on the previous rearing performance of Shasta strain rainbow trout at Cleghorn Springs State Fish Hatchery. Feed amounts were adjusted as needed to ensure that fish were fed at, or slightly above, satiation. Fish were fed by hand, with feed amounts and mortality recorded daily.

Prior to collecting weight and length data, the fish were anesthetized using 60 mg L^{-1} MS-222 (Tricaine-S, tricaine methanesulfonate, Syndel USA, Ferndale, Washington). At the end of the experiment, a lethal dose of 250 mg L^{-1} MS-222 was used to euthanize five fish per tank [32]. Data collected from these five fish included weight, length, fin lengths (to the nearest 1.0 mm), and spleen, liver, and visceral weights (to the nearest 1.0 mg). Relative fin lengths [33], hepatosomatic index (HSI) [34], splenosomatic index (SSI) [35], and viscerosomatic index (VSI) [35] were calculated using the following formulas:

Gain = end weight – start weight

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

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

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

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

$$\text{Relativefin length} = \frac{\text{fin length}}{\text{fish length}}$$

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

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

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

From the five fish sampled at the end of the study, a 2 mm wide section of the distal intestine was removed for histological examination [36-39]. This tissue sample was immediately preserved using 10% buffered formalin, prior to staining with haematoxylin and eosin using standard histological techniques [40,41]. Intestinal inflammation was assessed using an ordinal scoring system **(Table 3)** based on the thickness of cellularity of the lamina propria, the width of the submucosal connective tissue, and distribution of the leukocytes [42-44].

Data was analyzed using the SPSS (9.0) statistical analysis program (SPSS, Chicago Illinois). Two-way analysis of variance (ANOVA) was conducted with post hoc mean separation tests performed using Tukey's HSD comparison procedure. Significance was predetermined at P<0.05.

Table 3: Histological scoring system used on rainbow trout fed fishmeal or bioprocessed soybean meal in diets ([42]; modified from: [35]).

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 and most epithelial cells.

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 4).** However, food fed and FCR were significantly different between the fishmeal reference and 85% BSM diets. Overall mean (± SE) FCRs were 1.09 (± 0.04), 1.04 (± 0.01), and 0.97 (± 0.02) for the fishmeal, 60%, and 85% diets, respectively. FCR was also significantly poorer 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 **(Table 5).**

However, HSI was significantly different between the fishmeal and the 85% BSM diets. Mean (± SE) HSI was 1.37 (± 0.05), 1.27 (± 0.02), and 1.16 (± 0.05) for the fishmeal, 60, and 85% BSM diets, respectively.

Gain, percent gain, food fed, FCR, and SGR, were all significantly greater in the higher velocity treatment in each of the last two rearing periods, as well as for the entire study duration. At the end of the experiment, trout were significantly heavier in the higher velocity tanks compared to the lower velocity tanks. VSI was also significantly greater in the fish reared at the higher velocity. There were no significant differences at the end of the experiment in total fish length, condition factor, fin indices, hepatosomatic index, splenosomatic index, or gut histology scores. Representative images of the distal intestines from fish fed each diet used for the scoring are shown in **Figures 1-6**. Percent mortality was similar between velocity treatments.

There was a significant interaction between diet and velocity in food fed in rearing periods 2, 3, and for the entire study duration. The fish at high velocities receiving either the 0 or 60% BSM diets were fed significantly more than fish receiving any of the low velocity dietary treatments, as well as fish fed 85% BSM at high velocity.

Table 4: 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).

Parameters	Velocity	Diet (% BSM)			
		1 (0)	2 (60)	3 (85)	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 × [(Ln(final weight)–Ln(initial weight))/days]					

Table 5: 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).

Parameters	**Velocity**	**Diet (% BSM)**			
		1 (0)	**2 (60)**	**3 (85)**	**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	
Total 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	
Total 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	
Total 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	
Total 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=105 × [weight/(length3)]

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

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

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

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

[f] Scoring Parameters in **Table 3.**

Figure 1: Distal intestine of rainbow trout receiving 0% bioprocessed soybean meal, and in a low velocity tank.

Figure 2: Distal intestine of rainbow trout receiving 0% bioprocessed soybean meal, and in a high velocity tank.

Figure 3: Distal intestine of rainbow trout receiving 60% bioprocessed soybean meal, and in a low velocity tank.

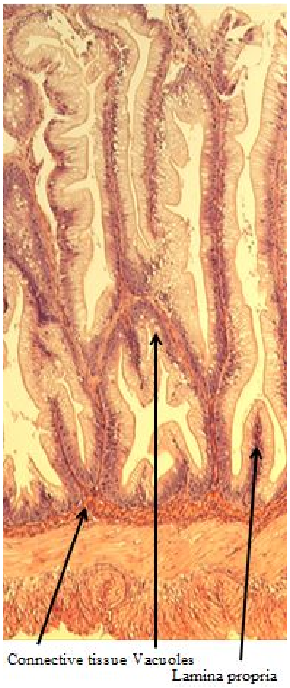

Figure 4: Distal intestine of rainbow trout receiving 60% bioprocessed soybean meal, and in a high velocity tank.

Figure 5: Distal intestine of rainbow trout receiving 85% bioprocessed soybean meal, and in a low velocity tank.

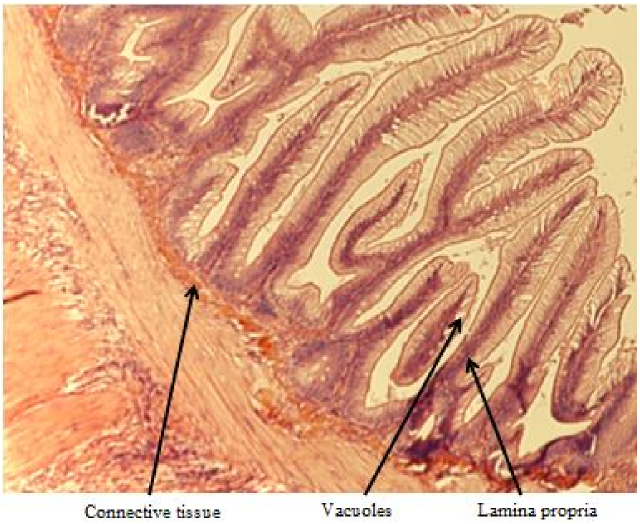

Figure 6: Distal intestine of rainbow trout receiving 85% bioprocessed soybean meal, and in a high velocity tank.

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 85% 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. [22,23] 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. [42,45,46] replaced approximately 60% of the dietary fishmeal with fermented soybean meal, with no decrease in fish health or growth. Similarly, Bruce et al. [47,48] examined BSM and found that approximated 65% of the dietary fishmeal could be replaced without decreasing rearing performance, while Voorhees et al. [49] observed that BSM could replace 100% of the fishmeal in juvenile brown trout (*Salmo salar*) diets. 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*) [50,51], Atlantic salmon (*Salmo salar*) [21], black sea bream (*Acanthopagrus schlegeli*) [52,53], Chinese sucker (*Myxocyprinus asiaticus*) [54], Florida pompano (*Trachniotus carolinus*) [38], gilthead sea bream (*Sparus aurata L.*) [55], Japanese flounder (*Paralichthys olivaceus*) [56], largemouth bass (Micropterus salmoides) [57], orange-spotted grouper (*Epinephelus coioides*) [58], whiteleg shrimp (*Litopenaeus vannamei*) [58-60], rockfish (*Sebastes schlegeli*) [61], white seabass (*Atractosion nobilis*) [62], and yellowtail jack (*Seriola lalandi*) [62].

Soybean products in the diets of salmonids have caused well-documented and potentially deleterious effects in the distal intestine [12,63-67]. However, the lack of difference in gut histology among the diets is evidence that bioprocessing soybeans likely decreases antinutritional factors [22,23,43,47,48,65]. Saponins [16], 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 [42,48,68] 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 [24-27]. Voorhees et al. [69] also observed increased growth in exercised brown trout, but noticed that this positive result disappeared after approximately 60 days, possibly due to exercise fatigue. These results were not observed in this study, indicating that there may be species-specific responses to exercise. 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 [27,70-72]. Parker and Barnes [27] 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 [73], rearing unit size and type [74], aggressive behavior [75], feeding rates [76], rearing densities [77-79], dietary nutritional differences [80,81], environmental stress [75], and fish health [82]. The similar fin indices among the fish fed different diets and reared at different water 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 [27].

HSI is an indirect measure of glycogen and carbohydrate levels and can be used to indicate the nutritional state of the fish [83-85]. 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 [42,46,67]. They are also similar to those values reported for rainbow trout in velocity studies [27,86].

VSI indicates how lipids are being used or partitioned with VSI and lipid levels positively related [86-88]. 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 [42,46,67]. Although the 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 [27,87-89].

SSI is an indicator of hematopoietic capacity [83] and antibody production [90]. 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 [27,47,68,86].

In conclusion, the results of this experiment indicate that juvenile rainbow trout respond similarly to exercise even when dietary fishmeal is replaced by at least 85% BSM. The suitability of BSM as a primary protein source provides additional ingredient options when formulating trout diets. Such flexibility in diet formulation is important given the large variation in market pricing for protein fish feed ingredients, as well as the geographic variation in ingredient availability. Additionally, regardless of diet, exercise can be used to improve growth in fish as long as adequate rations are provided. Future research should examine the complete replacement of fishmeal with BSM in rainbow trout subjected to different exercise regimes. Similar research should be conducted on other fish species. Lastly, the timing and duration of exercise to produce maximum rearing efficiencies should be evaluated for a wide variety of salmonid and other fish species as well.

Conflict of Interest

There are no conflicts of interest in this experiment.

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