



## Research Article

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# Direct Substitution of Fishmeal with Bioprocessed Soybean Meal in Brown Trout Diets

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## Abstract

This 121-day experiment evaluated the rearing performance of juvenile brown trout (*Salmo trutta*; initial weight 56.1±1.6 g, length 167.2±1.4 mm, mean ±SE) fed one of four isonitrogenous and isocaloric diets (46% protein, 16% lipid). Fishmeal, the primary protein source for the reference diet, was compared to diets where bioprocessed soybean meal directly replaced approximately 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. Gains for the 0, 60, 80, and 100% treatments were 304.6 ± 40.3 g, 215.4±82.6 g, 199.0±39.4 g, and 218.1±37.0 g, respectively. There were also no significant differences observed in intestinal morphology, relative fin lengths, viscerosomatic index, hepatosomatic index, or splenosomatic index among the dietary treatments. Based on these results, bioprocessed soybean meal may be able to replace 100% of the dietary fishmeal in juvenile brown trout diets without any deleterious effects.

**Keywords:** Alternative Protein; Rearing Performance; Salmonids; *Salmo trutta*

## Introduction

Intensively cultured carnivorous fishes [1], such as many salmonids, require high levels of dietary protein [2]. Historically, the primary protein source in salmonid feeds has been fishmeal [3-5]. However, the limited supply of fishmeal and rapid growth of aquaculture has led to an increase in price of aquafeeds [6-8]. Therefore, there is a need for lower-cost, sustainable protein sources to replace fishmeal in salmonid diets [7].

Soybean *Glycine max* products are some of the leading alternatives to dietary fishmeal [9,10]. Soybeans are highly palatable [11-13], high in protein, and have a balanced amino acid profile [2,14]. However, there are antinutritional factors associated with soybeans which hinder fish digestion [2,15,16], and can also cause gastro-intestinal issues, such as enteritis [12,17-19]. Another aspect limiting soybean use in fish diets is a large concentration of non-digestible carbohydrates [14,20]. These antinutritional factors and carbohydrates limit the inclusion levels of soybeans in diets for many carnivorous species [2,21-23].

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 [14,24,25], as typically happens during the feed extrusion process. Other antinutritional factors such as phytic acid or oligosaccharides are heat stable, but phosphorous from phytic acid can be made available to fish by hydrolysis [14]. Another form of bioprocessing is fermentation, which has been shown to eliminate or reduce many antinutritional factors [26-29].

While many studies have examined the inclusion of soybean products in Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* diets [30], 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 [31] production, but there is still considerable production of brown trout for recreation and conservation [32].

Only two studies have examined replacement of fishmeal with plant-based protein sources in brown trout diets. Michl et al. [33] replaced fishmeal with a combination of numerous plant-based proteins, making comparison to other experiments difficult.

Sotoudeh et al. [34] 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 fed 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 brown trout growth, feed conversion, and other rearing performance attributes.

## 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<sub>3</sub>, 360 mg/L; alkalinity as CaCO<sub>3</sub>, 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 Flowwatch meter (JDC Electronic SA, Yverdon-les-Bains, Jura-Nord Vaudois, Vaud, Switzerland).

Four dietary treatments (four replicates per treatment) were used (Table 1), 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 (Extrudes model 325, Sabetha, KS, USA). Feed was analyzed according to AOAC [35] method 2001.11 for protein, 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and AACC [36] method 08-03 for ash content.

	Diet (%)			
Ingredients	1 (0)	2 (60)	3 (80)	4 (100)
Fishmeal <sup>a</sup>	35.3	14.0	4.7	0.0
Bioprocessed soybean meal <sup>b</sup>	0.0	21.0	30.3	34.9
Wheat midds <sup>c</sup>	7.9	7.2	7.2	7.8
Whole wheat <sup>c</sup>	16.4	13.7	13.2	13.4
Poultry byproduct meal <sup>d</sup>	21.9	19.9	19.9	19.9

Blood meal <sup>e</sup>	2.6	2.5	2.5	2.5
Feather meal <sup>d</sup>	1.2	1.2	1.2	1.2
Vitamin premix <sup>f</sup>	0.8	2.0	2.0	1.1
Mineral premix <sup>f</sup>	0.8	2.0	2.0	1.1
Micro-mineral premix <sup>f</sup>	0.0	0.8	0.8	0.8
Choline chloride <sup>g</sup>	0.0	0.7	0.7	0.7
L-Lysine <sup>h</sup>	1.2	1.7	1.7	1.7
L-Methionine <sup>i</sup>	0.0	0.5	0.5	0.5
Stay-C 35 <sup>j</sup>	0.0	0.3	0.3	0.3
Fish oil <sup>k</sup>	10.1	12.0	12.5	12.6
Total	100.0	100.0	100.0	100.0
<b>Chemical analysis (% dry basis)</b>				
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
<sup>a</sup> Special Select, Omega Protein, Houston, TX; <sup>b</sup> SDSU; <sup>c</sup> Consumer Supply, Sioux City, IA; <sup>d</sup> Tyson Foods, Springdale, AR; <sup>e</sup> Mason City Byproducts, Mason City, IA; <sup>f</sup> NutraBlend, Neosho, MO; <sup>g</sup> Balchem, New Hampton, NY; <sup>h</sup> CJ Bio America, Fort Dodge, IA; <sup>i</sup> Adisseo USA, Alpharreta, GA; <sup>j</sup> DSM Nutritional Products, Ames, IA; <sup>k</sup> Virginia Prime Gold, Omega Protein, Houston, TX.				

**Table 1:** Diet formulation and composition analyses of the diets used in the 121-day trial. Analysis conducted on post-extrusion feed pellets.

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 [37], 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 [38]. 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 methane sulfonate, Sydel USA, Ferndale, Washington). On day 121, fish were euthanized using a lethal dose of 250 mg/L MS-222 [39]. 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) [40], splenosomatic index (SSI) [41], and viscerosomatic index (VSI) [41] 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. After dissection, the intestinal tissue was immediately put into 10% buffered formalin, and stained with hematoxylin and eosin using standard histological techniques [42,43]. 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 [44-46].

Score	Appearance
<b>Lamina propria of simple folds</b>	
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.
<b>Connective tissue between base of folds and stratum compactum</b>	
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.
<b>Vacuoles</b>	
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 and present in most epithelial cells.

**Table 2:** Histological scoring system used on brown trout fed fishmeal or incremental amounts of bioprocessed soybean meal in diets (Barnes et al. [46], modified from Geode and Barton [41], and Barton et al.[78]).

Data were 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 treatments in gain, percent gain, food fed, or

FCR (Table 3). However, significant differences were observed during specific rearing periods (time between fish data collection; approximately every 30 days). During the first rearing period the tanks of fish receiving the reference, fishmeal based, diet received 192 ( $\pm 5$ ) g (mean  $\pm$  SE) of food, which was significantly greater than the 144 ( $\pm 8$ ) g, 147 ( $\pm 6$ ) g, and 150 ( $\pm 9$ ) g fed for the tanks receiving diets with 60, 80, and 100% BSM replacement, respectively. Fish receiving the fishmeal diet had a negative FCR, indicating weight loss during this first rearing period.

Diet		1	2	3	4
BSM (%)		0	60	80	100
<b>Initial</b>					
Start weight (g)		489.6 $\pm$ 20.1	435.2 $\pm$ 15.0	464.0 $\pm$ 63.9	477.2 $\pm$ 9.6
<b>Days 1-35</b>					
	End weight (g)	511.6 $\pm$ 16.4	458.6 $\pm$ 21.4	477.1 $\pm$ 54.7	505.9 $\pm$ 14.5
	Gain (g)	22.0 $\pm$ 10.4	23.4 $\pm$ 8.9	13.1 $\pm$ 9.9	36.0 $\pm$ 10.2
	Gain (%)	4.7 $\pm$ 2.2	5.3 $\pm$ 2.0	3.6 $\pm$ 2.1	47.0 $\pm$ 40.9
	Food fed (g)	192 $\pm$ 5 z	144 $\pm$ 8 y	147 $\pm$ 6 y	150 $\pm$ 9 y
	FCR	-21.83 $\pm$ 30.30	11.03 $\pm$ 4.75	2.24 $\pm$ 4.20	5.39 $\pm$ 1.48
	SGR	0.13 $\pm$ 0.06	0.15 $\pm$ 0.06	0.10 $\pm$ 0.06	0.17 $\pm$ 0.04
<b>Days 36-61</b>					
	End weight (g)	553.2 $\pm$ 16.1	499.4 $\pm$ 34.3	519.4 $\pm$ 53.7	540.1 $\pm$ 16.1
	Gain (g)	41.7 $\pm$ 6.6	40.8 $\pm$ 14.6	42.2 $\pm$ 9.3	34.2 $\pm$ 12.7
	Gain (%)	8.2 $\pm$ 1.4	8.6 $\pm$ 2.7	9.3 $\pm$ 2.7	6.8 $\pm$ 2.6
	Food fed (g)	112 $\pm$ 6	81 $\pm$ 17	90 $\pm$ 13	82 $\pm$ 5
	FCR	2.85 $\pm$ 0.33	2.96 $\pm$ 1.16	2.34 $\pm$ 0.39	3.85 $\pm$ 1.31
	SGR	0.29 $\pm$ 0.05	0.30 $\pm$ 0.09	0.33 $\pm$ 0.09	0.24 $\pm$ 0.09
<b>Days 62-92</b>					
	End weight (g)	669.2 $\pm$ 32.6	565.5 $\pm$ 67.8	596.5 $\pm$ 67.9	601.8 $\pm$ 21.7
	Gain (g)	115.9 $\pm$ 24.3	66.0 $\pm$ 33.6	77.2 $\pm$ 19.0	61.7 $\pm$ 6.7
	Gain (%)	20.9 $\pm$ 4.4	12.1 $\pm$ 5.4	14.6 $\pm$ 3.2	11.4 $\pm$ 1.1
	Food fed (g)	197 $\pm$ 26	133 $\pm$ 33	120 $\pm$ 23	104 $\pm$ 7
	FCR	1.83 $\pm$ 0.22	3.18 $\pm$ 0.96	1.72 $\pm$ 0.26	1.73 $\pm$ 0.16
	SGR	0.61 $\pm$ 0.12	0.36 $\pm$ 0.15	0.44 $\pm$ 0.09	0.35 $\pm$ 0.03
<b>Days 93-121</b>					
	End weight (g)	794.2 $\pm$ 47.0	650.6 $\pm$ 96.1	663.0 $\pm$ 81.2	695.3 $\pm$ 41.5
	Gain (g)	125.0 $\pm$ 15.7	85.1 $\pm$ 28.7	66.4 $\pm$ 18.4	93.5 $\pm$ 20.2
	Gain (%)	18.5 $\pm$ 1.7	14.0 $\pm$ 3.14	10.8 $\pm$ 2.9	15.2 $\pm$ 2.9
	Food fed (g)	229 $\pm$ 31	144 $\pm$ 32	124 $\pm$ 34	127 $\pm$ 16
	FCR	1.83 $\pm$ 0.04	1.90 $\pm$ 0.21	2.08 $\pm$ 0.33	1.52 $\pm$ 0.26
	SGR	0.58 $\pm$ 0.05	0.45 $\pm$ 0.09	0.35 $\pm$ 0.09	0.49 $\pm$ 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
<sup>a</sup> FCR = feed conversion ratio = total food fed / total weight gain.					
<sup>b</sup> SGR = 100 x [(Ln (final weight) – Ln (initial weight)) / days]					

**Table 3:** Mean (± SE) gain, percent gain, food fed, feed conversion ratio (FCR<sup>a</sup>), specific growth rate (SGR<sup>b</sup>), 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).

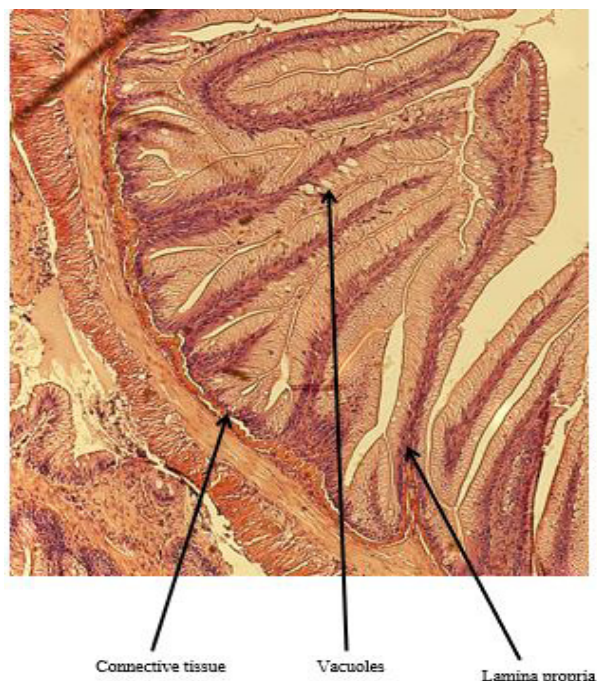
Individual fish weight, length, and condition factor were not significantly different between the dietary treatments at the end of the 121-day experiment or in any of the four rearing periods (Table 4). In addition, fin indices (pectoral, pelvic, and dorsal), organosomatic indices (HSI, SSI, and VSI), or gut histology scores were not significantly different among diets. Representative images of the distal intestines from fish fed each diet used for the scoring are shown in Figures 1-4.

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)					

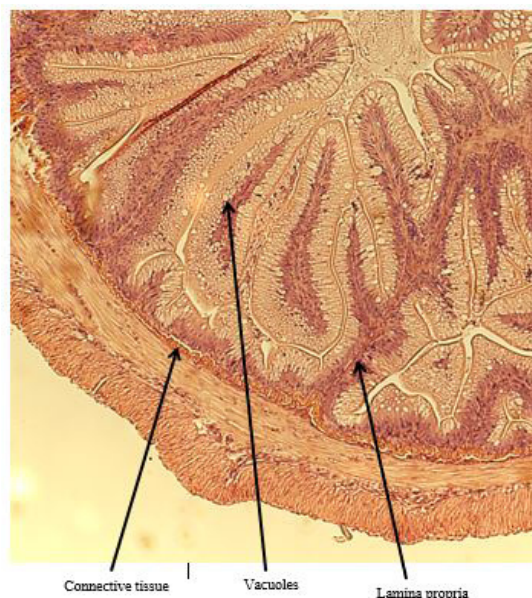


	End weight (g)	103.7 ± 7.3	90.2 ± 8.9	85.8 ± 8.7	94.0 ± 4.2
	End length (mm)	202.7 ± 6.6	192.7 ± 5.0	195.2 ± 6.7	201.7 ± 3.3
	K	1.23 ± 0.06	1.21 ± 0.04	1.11 ± 0.02	1.10 ± 0.01
	Pectoral index (%)	13.37 ± 0.35	13.54 ± 0.48	13.21 ± 0.49	13.51 ± 0.20
	Pelvic index (%)	11.14 ± 0.15	11.15 ± 0.21	10.70 ± 0.36	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
<sup>a</sup> K = 10 <sup>5</sup> x [weight / (length <sup>3</sup> )] <sup>b</sup> Fin indices = 100 x (fin length / fish length) <sup>c</sup> HSI = 100 x (liver weight / body weight) <sup>d</sup> SSI = 100 x (spleen weight / body weight) <sup>e</sup> VSI = 100 x (visceral weight / body weight)					

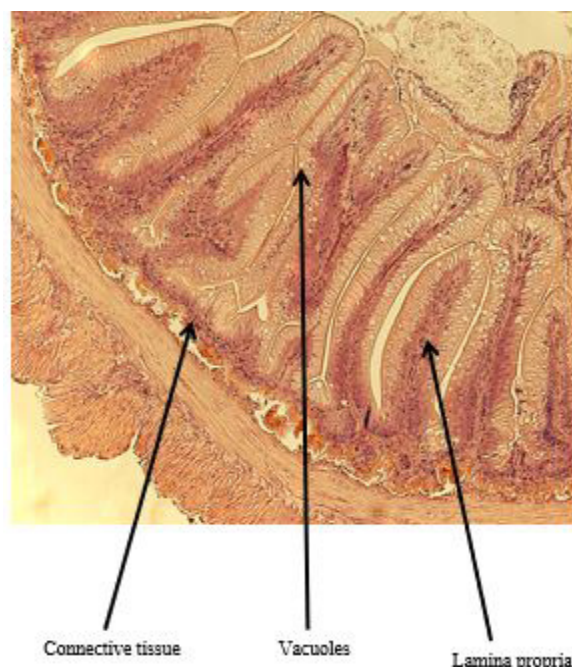
**Table 4:** Mean (± SE) condition factor (Ka), fin indices<sup>b</sup>, hepatosomatic index (HSI<sup>c</sup>), splenosomatic index (SSI<sup>d</sup>), viscerosomatic index (VSI<sup>e</sup>), and histology scores for lamina propria, connective tissue, and vacuoles of brown trout fed one of four diets 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).



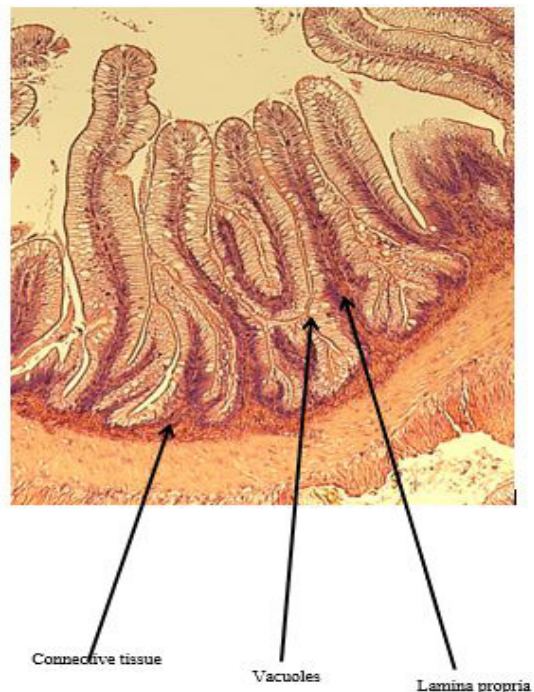
**Figure 1:** Distal intestine of brown trout receiving fishmeal.



**Figure 2:** Distal intestine of brown trout receiving 60% bioprocessed soybean meal.



**Figure 3:** Distal intestine of brown trout receiving 80% bioprocessed soybean meal.



**Figure 4:** Distal intestine of brown trout receiving 100% bioprocessed soybean meal.

## Discussion

The lack of significant differences in rearing performance among any of the four diets indicates BSM may be able to 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. [34] also indicated the suitability of fermented soybean meal. However, Sotoudeh et al. [34] 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. [28,29] 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 [46-50]. BSM has been evaluated in Atlantic salmon diets, but fishmeal replacement rates appear to be limited to 20% or less [27]. Other species with which a fermented, or other BSM, have been evaluated include Atlantic cod *Gadus morhua* [51,52], black sea bream *Acanthopagrus schlegeli* [53,54], Chinese sucker *Myxocyprinus asiaticus* [55], Florida pompano *Trachinotus carolinus* [56], gilthead sea bream *Sparus aurata* L. [57], Japanese flounder *Paralichthys olivaceus* [58], largemouth bass *Micropterus salmoides* [59], orange-spotted grouper *Epinephelus coioides* [60], white leg shrimp *Litopenaeus vannamei* [61,62], rockfish *Sebastes schlegeli* [63], white seabass *Atractosion nobilis* [64], and yellowtail jack *Seriola lalandi* [64].

At 121 days, the duration of this study should have met the Weathercup and McCracken [65] study length criteria to determine any differences in fish performance among the diets. It also met the NRC [2] 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 recommended by NRC [2]. Despite not reaching a 200% gain, this study still lasted longer than most soybean meal feeding trials, with few lasting over 100 days [19,23,46,47,66-68].

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 [49,69]. There are few published studies for brown trout that provide FCRs for comparison. Regost et al. [70] 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. [71] reported a feed conversion ratio of only 0.50. However, Kizak et al. [71] fed a restricted ration, which has been shown to improve feed



conversion ratio [1]. 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. [70] and Kizak et al. [71] 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 [67,72,73]. The BSM used in this study probably decreased or eliminated the saponins [18] and other antinutritional factors responsible for such enteritis [28,29,47,74]. 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. [46,48,75] for rainbow trout fed different diets.

The lack of any differences in HSI between the dietary 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 [76-78]. The HSI in this study (0.9-1.1) is similar to that reported by in Sotoudeh et al. [79] (0.9-1.4), but are lower than that reported for brown trout in other studies (1.4-1.8) [34,71,80]. 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, and lower values may indicate stress [78].

VSI indicates how lipids are being used or partitioned, and there is a positive relationship between lipid levels and VSI [81-83]. Thus, similar VSI values among the dietary treatments are likely due to similar dietary lipid levels. VSI values in this experiment (5.0-5.7) are similar to Sotoudeh et al. [34] (4.9-6.0), but are extremely low compared to Mambrini et al. [80] (8.9-10.6) or Kizak et al. [34] (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 [78] and antibody production mostly occurs in the spleen [84]. 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 [49,75,85,86].

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 [87], adequate feeding rates [88], nutritional differences [89,90], and good fish health [91]. Fin erosion can be due to several factors, including tank-induced abrasions [92], rearing unit size and type [93], aggressive behavior [87], feeding rates [88], rearing densities [94-96], and fish health [94]. Bosakowski and Wagner [93] 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 potential suitability of BSM as a complete replacement of dietary fishmeal in juvenile 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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