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An initial investigation replacing fish meal with a commercial fermented soybean meal product in the diets of juvenile rainbow trout

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ABSTRACT

The inclusion of PepSoyGen (PSG), a commercially-available fermented soybean meal product, was evaluated with juvenile rainbow trout, *Oncorhynchus mykiss* in an initial 70-day feeding trial, with a supplemental trial involving a subset of the experimental diets continuing for an additional 40 d. Six diets containing 0%, 10%, 20%, 30%, 40%, or 50% PSG, with the PSG directly replacing fish meal, were used in the first trial. There were no significant differences in weight gain or feed conversion ratio between the fish meal-based control diet and diets containing up to 30% PSG. However, weight gain was significantly reduced and feed conversion ratio significantly increased with the 40% and 50% PSG diets. No health assessment differences were observed in fish receiving any of the diets, and no evidence of gross gut inflammation was evident. There were no significant differences in weight gain or feed conversion ratio among the four dietary treatments ranging from 0% to 30% PSG which were fed for an additional 40 d after the initial 70-d trial (110 days total). Based on these results, juvenile rainbow trout diets can contain up to 30% PSG without any loss of rearing performance, thereby replacing at least 60% of the fish meal.

Keywords: Rainbow Trout; Fermented Soybean Meal; PepSoyGen; *Oncorhynchus mykiss*; Diet; Alternative Proteins

1. INTRODUCTION

The primary protein source in hatchery feed for rainbow trout *Oncorhynchus mykiss* and other carnivorous salmonids is typically fish meal [1-3]. However, the limited supply of fish meal has not expanded in unison with the growth of global aquaculture, resulting in dramatic price increases [4-6]. Thus, there is an obvious need for lower-cost, sustainable protein sources to replace fish meal in salmonid diets [6].

A variety of plant proteins have been investigated as possible fish meal replacements for salmonids, with soybean meal most commonly used [7,8]. Inclusion rates for soybean meal in salmonid diets have typically been low because of the presence of trypsin inhibitors, lectins, saponins, poorly digestible carbohydrates (oligo- and polysaccharides), and other anti-nutritional factors [9-12]. Soybean meal in rainbow trout diets produces well-documented deleterious effects on the distal intestine, such as morphological changes and subacute enteritis [12-19], as well as changes to intestinal microbial communities [15,16,18], and hepatic morphology [12]. Similar effects have been reported in Atlantic salmon *Salmo salar* [20-23].

Many of the negative effects associated with soybean meal can be reduced or eliminated with further processing [11]. Anti-nutritional factors can be reduced during feed extrusion [24,25] and by infra-red micronization [24]. Processed (solvent and heat treated) soy protein concentrates have lower concentrations of many anti-nutritional factors [26]. Fermentation may also improve the suitability of soybean meal as an alternative protein source in fish diets [11], although few studies have been conducted with fish in general or rainbow trout specifically until very recently. Fermented soybean meal has been used in the diets of parrot fish *Oplegnathus fasciatus* [27], red sea bream *Pagrus major* [28], pompano *Trachinotus ovatus* [29], and Japanese flounder *Paralichthys olivaceus* [30,31]. Yamamoto *et al.* [32] noted that soybean meal fermented primarily by *Bacillus* spp. for 10 h with 30% water addition did not produce any changes in intestinal morphology when used in non-fish meal based rainbow trout diets. They also suggested that fermented soybean meal had the potential to become the dominant protein source in rainbow trout composite diets.

PepSoyGen® (PSG; Nutraferma Inc. of North Sioux City, SD, USA) is a soybean meal fermentation feed product manufactured via a proprietary process incorporating *Aspergillus spp*. and *Bacillus* spp. It has not previously been evaluated for use as a possible fish meal replacement in aquaculture diets. Thus, the objective of this study was to conduct an initial investigation into the incorporation of PSG into the diets of juvenile rainbow trout.

2. MATERIAL AND METHODS

2.1. Location and Fish Culture

The study was conducted at McNenny State Fish Hatchery, Spearfish, SD, USA, 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). Flow rates in each tank were set at 40 L/min.

During the primary trial, approximately 200 McConaughy strain juvenile rainbow trout (initial weight 6.1 ± 0.5 g, length 85 ± 2 mm, mean ± SE) were randomly selected and placed into each of 24 fiberglass circular tanks (1.8 m diameter, 0.6 m depth) on July 21, 2009. Feeding commenced the following day and continued for 70 days until the end of the trial. Tanks were each loaded based on weight (as-is, or wet, basis) to the nearest 1.0 g, and fish numbers were estimated. Feeding amounts for the tanks were determined by the hatchery constant (HC) method [33], with a planned feed conversion of 1.1 and a maximum growth rate of 0.065 cm/day, which was based on the maximum growth rate of juvenile McConaughy strain rainbow trout historically observed at McNenny State Fish Hatchery [34]. Feed amounts were updated daily. Feed was uniformly dispensed from 07:00 to 19:00 in each tank using automatic EWOS 505 feeders (Norco-plast AB, Sweden), which were electronically programmed to release small amounts of feed for a duration of 2 min at 20-min intervals. All feed dispensed and fish mortalities were recorded daily for each tank. Percent mortality was determined by dividing the number of fish that died during the trial by the total number of fish (n = 200) initially present in each tank.

2.2. Diets and Chemical Analysis

The 24 tanks were randomly assigned to one of six different diets (**Table 1**); with four replicate tanks receiving the same diet. These six diets contained 0%, 10%, 20%, 30%, 40%, or 50% PSG, with the PSG incrementally replacing fish meal as the primary protein source. Feeds were manufactured via extrusion. Because the objective of this study was to evaluate PSG as a direct fishmeal replacement, the diets were not formulated to be isonitrogenous or isocaloric. The resulting pellets were analyzed according to AOAC [35] method 2001.11 for protein, method 2003.5 (modified by substituting petroleum ether for diethyl ether) for crude lipid, and AACC [36] method 08-03 for ash content. The protein and lipid amounts obtained by these methods were multiplied by their respective physiological fuel values of 23.6 J and 39.5 J [37], respectively, to obtain estimated gross energy values.

2.3. Data Collection

At the end of the primary feeding trial, total tank weights were recorded to the nearest 1.0 g, with weight gain calculated by subtracting the initial weight from the final weight for each tank. Feed conversion ratio for each

Table 1. Ingredients composition (%) and chemical analysis of the diets used.

Ingredients	Diet (%)					
	1	2	3	4	5	6
Herring meal[a]	50	40	30	20	10	0
PepSoyGen[b]	0	10	20	30	40	50
Whole weat flour[c]	15	15	15	15	15	15
Corn gluten meal[d]	15	15	15	15	15	15
Menhaden oil[e]	12.0	12.8	13.5	14.3	15.0	15.8
Celufil[f]	5.5	4.7	4.0	3.2	2.5	1.7
Vitamin and mineral mix[g]	2	2	2	2	2	2
Vitamin C (Stay-C)[h]	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Chemical analysis (% dry basis)[i]						
Crude protein	52.0	50.5	48.1	46.3	44.1	43.0
Crude lipid	15.3	15.5	15.8	15.8	16.0	14.2
Ash	9.3	8.6	7.8	7.0	6.1	5.6
Gross energy (kJ/g)	18.32	18.04	17.59	17.17	16.73	15.76

[a]Lortscher Agri Service, Inc., Bern, Kansas, USA; [b]Nutraferma, North Sioux City, South Dakota, USA; [c]Bob's Red Mill Natural Foods, Inc., Milwaukie, Oregon, USA; [d]Consumers Supply Distributing Company, Sioux City, Iowa, USA; [e]Omega Protein, Inc., Houston, Texas, USA; [f]USB Corporation, Cleveland, Ohio, USA; [g]Lasi Fish Premix, NB-8055, Lortscher Agri Service, Inc., Bern, Kansas, USA; [h]DSM Nutritional Products France SAS, Village-Neuf, France; [i]Analysis conducted on post-extrusion feed pellets.

tank was calculated by dividing the total amount of food fed by the total weight gain. In addition to total tank measurements, five fish were randomly selected from each tank and individually weighed to the nearest 1.0 g and measured (total length) to the nearest 1.0 mm. Fish health profiles, based on a modification of Goede and Barton [38], Adams *et al*. [39], and Barton *et al*. [40], were completed using the score sheet described in **Table 2**. Liver weights were also recorded to the nearest 1.0 mg and the hepatosomatic index (HSI) was determined using the formula: HSI (%) = 100 × (liver weight/whole fish weight) [41].

2.4. Apparent Digestability

Apparent protein digestability was determined using a direct method [42]. Digesta was removed from five fish per tank at the end of the trial. Each fish was dissected and the last 1.0 cm of the distal end of the intestine was gently squeezed to remove the contents. Harvested digesta from the five fish per tank was pooled and flash frozen on dry ice prior to analysis. Protein analysis was conducted using AOAC [35] method 990.03. Apparent protein digestability was calculated using the formula: apparent protein digestability = (protein in the diet – protein in the digesta)/protein in the diet [37].

Table 2. Criteria used at the end of the study for visual fish health observations (based on Goede and Barton [38], Adams *et al*. [39], and Barton *et al*. [40]).

Structure of Tissues	Rating Criteria	Numeric Rating
Eyes	Normal	0
	Abnormal	1
Fat	None	0
	<50% of gut covered	1
	>50% of gut covered	2
	100% of gut covered	3
Fins	No erosion	0
	Light erosion	1
	Moderate erosion	2
	Severe erosion	3
Gills	Normal	0
	Clubbed, frayed, or discolored	1
Gut	Normal	0
	Slight inflammation	1
	Moderate inflammation	2
	Severe inflammation	3
Kidney	Normal	0
	Abnormal	1
Liver	Normal	0
	Abnormal	1
Pseudobranchs	Normal	0
	Abnormal	1
Opercles	Normal	0
	Short	1
Spleen	Normal	0
	Cysts or enlarged	1

2.5. Fillet Composition

At the end of the primary feeding trial, five fish per tank were also euthanized; muscle fillets were then removed and flash frozen for determination of fillet composition. The fillets from each tank were pooled and analyzed for crude protein levels with a TruSpec CNS combustion analyzer (LECO Corp., St. Joseph, Michigan) using AOAC [35] method 992.15. AOAC [35] method 948.15, which used acid hydrolysis with a 50:50 mix of diethyl ether and petroleum ether for extraction, was used for fat analysis. Moisture was determined by loss on drying using AOAC [35] method 952.08.

2.6. Secondary Continuing Trial

After data collection at the end of the initial 70-day trial, an unexpected opportunity occurred whereby a limited number of hatchery tanks became available for further experimentation. Only 12 tanks were used in this secondary trial, with each tank containing approximately 115 fish. The 12 tanks were assigned to one of four diets used in the initial trial (0%, 10%, 20%, or 30% PSG), with three replicate tanks receiving the same diet. Fish remained on the diet they had consumed previously in the primary trial. This secondary trial started on October 1, 2009, and ran for 40 days. Thus, in combination with the prior trial, the trout were on their respective diets for 110 days. Because this secondary trial was not initially planned, only rearing data were collected; no fish health data or fillet composition data was obtained.

2.7. Statistical Analysis of Data

Data were analyzed using the SPSS version 9.0 statistical analysis program (SPSS, Chicago, IL, USA) with significance predetermined at $P < 0.05$. One-way analysis of variance (ANOVA) was conducted, and if the treatments were significantly different, pairwise mean comparisons were performed using the Tukey HSD test [43]. All mortality percentage data were arcsine-square root transformed prior to analysis to stabilize the variances [43].

3. RESULTS

3.1. Primary Trial

There were no significant differences in total tank ending weights or weight gain among the tanks of rainbow trout receiving the fish meal control or diets containing 10%, 20%, or 30% PSG (**Table 3**). Total tank ending weights and weight gain did decrease significantly for fish receiving diets containing 40% PSG, with an additional significant decrease observed at the 50% inclusion level. Feed conversion ratio followed a similar pattern, except for that the results produced by the 40% PSG diet were not significantly different than those observed in any of the lower PSG concentrations. Mortality was minimal and not significantly different among any of the treatments.

Liver weights and the hepatosomatic index values were significantly different among the dietary treatments, with the smallest values observed in the fish receiving 50% dietary PSG (**Table 4**). None of the other fish health values varied significantly among the fish receiving any of the diets. There was no gross visible gut inflammation observed in any fish either.

Fillet protein percentages were significantly affected by the diets used in this study (**Table 5**). The fish fed diets with 50% PSG had 18.0% fillet protein content, which was significantly less than the 19.2 g% protein levels in the fillets from fish fed the control or 10% diets. There was no significant difference in fillet protein composition among fish receiving from 0% to 40% dietary PSG however. There were also no significant differences detected in fillet moisture, lipid, or ash contents among any diet treatments.

Table 3. Mean (+SE) rearing data values, including feed conversion ratio (FCR[a]), for tanks of rainbow trout receiving one of six different diets containing incremental amounts of PepSoyGen (PSG). Means with different letters in the same row differ significantly ($P < 0.05$, N = 4).

Diet	1	2	3	4	5	6
PSG (%)	0	10	20	30	40	50
End weight (g)	5694 ± 88[a]	5965 ± 128[a]	5783 ± 97 [a]	5402 ± 39[a]	4656 ± 173[b]	3603 ± 49[c]
Gain (g)	4388 ± 88[a]	4659 ± 128[a]	4477 ± 97[a]	4096 ± 39[a]	3350 ± 173[b]	2297 ± 490[c]
Gain (%)	336 ± 7	357 ± 10	343 ± 7	314 ± 3	256 ± 13	176 ± 38
Food fed (g)	3838	3838	3838	3838	3838	3838
FCR	0.88 ± 0.02[a]	0.83 ± 0.02[a]	0.86 ± 0.02[a]	0.94 ± 0.01[a]	1.15 ± 0.06[ab]	2.06 ± 0.62[c]
Mortality (%)	0.13 ± 0.13	0	0.25 ± 0.14	0.63 ± 0.32	0.50 ± 0.20	1.88 ± 1.39

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

Table 4. Mean (±SE) Individual fish lengths, weights, condition factors (K)[a], liver weights, hepatosomatic index values (HSI)[b], and fish health assessments[c] of rainbow trout fed diets containing incremental amounts of PepSoyGen (PSG). Means with different letters in the same row differ significantly ($P < 0.05$, N = 4).

Diet	1	2	3	4	5	6
PSG (%)	0	10	20	30	40	50
Length (mm)	121 ± 5	130 ± 1	133 ± 3	130 ± 4	125 ± 5	115 ± 4
Weight (g)	21 ± 2	26 ± 1	28 ± 2	26 ± 2	24 ± 3	18 ± 2
K[1]	1.16 ± 0.06	1.15 ± 0.02	1.14 ± 0.04	1.12 ± 0.02	1.15 ± 0.03	1.08 ± 0.02
Liver Weight (g)	0.23 + 0.04[a]	0.28 + 0.03[a]	0.29 + 0.04[a]	0.27 + 0.03[a]	0.23 + 0.04[ab]	0.16 + 0.04[b]
HSI[2]	1.07 + 0.09[a]	1.02 + 0.12[ab]	1.01 + 0.09[ab]	0.98 + 0.07[av]	0.91 + 0.04[ab]	0.83 + 0.15[b]
Health Assessment						
Eyes	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fat	3.0 ± 0.0	3.0 ± 0.1	2.9 ± 0.2	2.9 ± 0.1	3.0 ± 0.0	2.5 ± 0.3
Fins	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	1.0 ± 0.1	1.0 ± 0.1
Gills	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.6 ± 0.1
Gut	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Kidney	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Liver	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.1 ± 0.1
Pseudobranchs	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Opercles	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.2
Spleen	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0

[a]Condition factor (K) = 105 × (weight)/(length^3); [b]Hepatosomatic index (HSI) = 100 × (liver weight/body weight); [c]Fish health assessments rating system described in **Table 2**.

Table 5. Fillet composition of rainbow trout fed one of six diets containing incremental amounts of PepSoyGen (PSG). Means with different letters in the same row differ significantly ($P < 0.05$, N = 4).

Diet	1	2	3	4	5	6
PSG (%)	0	10	20	30	40	50
Water (%)	74.7 ± 0.1	72.1 ± 2.2	72.6 ± 0.6	73.1 ± 1.1	73.2 ± 0.1	73.9 ± 0.7
Crude protein (%)	19.2 ± 0.2^{a}	19.2 ± 0.1^{a}	18.4 ± 0.2^{ab}	18.4 ± 0.1^{ab}	18.9 ± 0.3^{ab}	18.0 ± 0.4^{b}
Crude lipid (%)	6.2 ± 0.3	6.6 ± 0.3	7.4 ± 0.2	5.8 ± 0.4	6.3 ± 0.2	6.2 ± 0.5
Ash (%)	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.7 ± 0.2

3.2. Secondary Continuing Trial

There were no significant differences in gain, feed conversion, or mortality among any of the diets evaluated (**Table 6**). There was also no difference in individual fish length, weight, or conversion factor (**Table 7**). None of the fish health index components were different among the fish receiving the four diets as well.

4. DISCUSSION

The results indicate that PSG can replace at least 60% of the fish meal without any negative repercussions on growth or feed conversion ratio. Unfortunately, these results cannot be directly compared to those described by Yamamoto *et al*. [32], who successfully eliminated fish meal from rainbow trout diets using fermented soybean meal. In addition to replacing the fish meal component with fermented soybean meal, Yamamoto *et al*. [32] also increased the corn gluten inclusion rate, while at the same time decreasing the percentage of wheat flour in the diets containing fermented soybean meal. Their control diet also contained 4.5% unfermented soybean meal. In contrast to Yamamoto *et al*. [32], during the current study both wheat flour and corn gluten were held constant at 15% in all of the diets; the control diet did not contain any soybean meal, and PSG directly replaced fish meal on a one-to-one basis. In addition, Yamamoto *et al*. [32] also supplemented their fermented soybean meal diets with multiple amino acids and conducted their study in much warmer water (16.3 C). Water temperature can have an effect on nutritional observations [44-47].

It is extremely difficult to compare the growth results from this study with the numerous other studies using either soybean meal or soy protein concentrates. In many of these other studies, fish meal-based control diets were not used, or the concentrations of other non-soy protein sources were altered in the experimental diets [14-16,24, 48-53]. However, Barrows *et al*. [54] suggested that soybean meal replacement for fish meal should be limited to less than 25% (or 10% to 15% dietary inclusion rates). Hardy [55] stated that a maximum of 20% soybean meal can be included in the overall diets of rainbow trout, although this amount may be too high for smaller trout [19].

The significant decrease in growth and feed conversion ratio from the fish receiving diets with either 80% or 100% of the fish meal replaced by PSG was likely due to a large degree because of the lower dietary protein concentrations, as well as inadequate amounts of certain essential amino acids [56]. In particular, soybean meal typically does not have enough methionine to meet the nutritional requirements of rainbow trout [8,57]. Although PSG has slightly more methionine than soybean meal [58], it is still relatively low in comparison to that required in the diets of rainbow trout [59]. Yamamoto *et al*. [60] found that amino acid supplementation was required for their experimental diet containing soybean meal. Yamamoto *et al*. [32] supplemented fermented soybean meal diets with a number of essential amino acids. Methionine supplementation was done in other studies using soybean meal or soy protein concentrates in rainbow trout diets [12,16,50].

Although specific feeding trial durations are not universally specified, they generally need to last long enough for any potential significant differences among the diets to materialize [61]. In a study by de Francesco *et al*. [62], differences in trout rearing performance between fish meal and plant-based diets did not become apparent until after 84 days. The initial trial in the present study lasted 70 days, but this was long enough for significant differences in gain and feed conversion ratio to become apparent at PSG inclusion levels over 40%. When combined with the subsequent 40-day trial, the juvenile rainbow trout receiving up to 30% dietary PSG were on their respective dietary treatments for 110 days, which should have been long enough for any differences in rearing performance to appear.

The feed conversion ratios obtained for the diets containing less than 40% PSG in this study are typical for rainbow trout production in public hatcheries within South Dakota [34]. Other studies investigating plant proteins in trout diets have produced similar results. The commercial control diet used by Adelizi *et al*. [50] led to a feed conversion ratio of 0.89, which was nearly identical

Table 6. Rearing data (mean ± SE), including feed conversion ratio (FCR[a]), for tanks of rainbow trout receiving one of four different diets containing incremental amounts of PepSoyGen (PSG) for an additional 40 days after the conclusion of the primary trial (N = 3).

Diets	1	2	3	4
PSG (%)	0	10	20	30
End weight (g)	5100 ± 38	5046 ± 101	5079 ± 73	5034 ± 21
Gain (g)	2380 ± 38	2326 ± 101	2359 ± 73	2314 ± 21
Gain (%)	87.5 + 1.4	85.5 + 3.7	86.7 + 2.7	85.1 + 0.8
Food fed (g)	2155	2155	2155	2155
FCR	0.91 ± 0.01	0.93 ± 0.04	0.92 ± 0.03	0.92 ± 0.01
Mortality (%)	0.00 ± 0.00	0.28 ± 0.28	0.00 ± 0.00	0.00 ± 0.00

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

Table 7. Mean (±SE) total lengths, weights, and condition factors (K[a]) for rainbow trout receiving one of four different diets containing incremental amounts of PepSoyGen (PSG) for 40 days after the conclusion of the primary trial (N = 3).

Diets	1	2	3	4
PSG (%)	0	10	20	30
Start weight (g)	21 ± 8.8	26 ± 8.1	28 ± 10	26 ± 9.4
End weight (g)	74 ± 6.2	72 ± 3.4	70 ± 5.5	70 ± 6.3
Start length (mm)	120 ± 17	130 ± 13	133 ± 15	130 ± 16
End length (mm)	185 ± 5	186 ± 4	184 ± 6	185 ± 5
Start K	1.16 ± 0.30	1.15 ± 0.07	1.14 ± 0.10	1.12 ± 0.10
End K	1.13 ± 0.02	1.10 ± 0.02	1.10 ± 0.04	1.10 ± 0.02

[a]Condition factor (K) = $10^5 \times$ (weight)/(length^3).

to the 0.88 observed in this study. Barrows *et al.* [53] reported feed conversion ratios of 0.84 to 0.90 in a fish meal based diet containing 20% soybean meal. Higher feed conversion ratios of 0.99 and 1.02 from soy bean-free, fish meal based diets were observed by Cheng *et al.* [51] and Cheng *et al.* [63], respectively. Cheng *et al.* [63] also reported ratios from 1.08 to 1.18 in diets with 8% soybean meal, 15% fish meal, and 16% wheat gluten, which were comparable to the 1.15 observed in the diets containing 40% PSG and 10% fish meal in this study.

None of the diets in this study produced any observable deleterious effects on fish health. In particular, no gross inflammation of the distal intestines of the fish receiving dietary PSG was observed in this study. However, microscopic examination did not occur. Yamamoto *et al.* [32] noted that lengthy and moist fermentation of soybean meal using *Bacillus* spp. could eliminate the occurrence of physiological abnormalities typically observed with the use of soybean meal in salmonid diets. PepSoyGen is manufactured by solid state fermentation (low moisture) which incorporates a growth medium based on feed grains rather than an artificial growth medium. The fermentation process hydrolyzes the long chain proteins into small chain proteins allowing them to be more suitable for young animal diets (relative to de-hulled soybean meal and other soy proteins). The fermentation process also clears the product of indigestible oligosaccharides (raffinose and stachyose) and trypsin inhibitor that can have a negative influence on gut health and nutrient digestibility. Although growth was not affected by up to 30% dietary PSG, this may not indicate a lack of intestinal damage. Bureau *et al.* [64] stated that fish growth is not necessarily inhibited by the changes in distal intestinal morphology caused by soybean meal.

The observed hepatosomatic index of 0.98 to 1.07 in rainbow trout fed diets containing up to 30% PSG is similar to that reported by Barrows *et al.* [53] and Panserat *et al.* [65] for rainbow trout fed fish meal or other plant-based diets. However, these values are much lower than those from other studies which fed soybean meal or other soybean products [48,50,52,53]. The only significant decrease in HSI observed in the current study was in the fish which received all of their protein from PSG. There was no increase in HSI with increasing amounts of PSG, as might be expected due to the positive relationship between HSI and carbohydrate levels [66,67]. The lack of difference in HSI among the diets would also appear to indicate no phosphorus availability limitations, given that dietary phosphorus is inversely related to liver lipid levels and HSI [68].

At 19.2%, the fillet protein concentration from the control fish was identical to that from fish which received a commercial control diet in Adelizi *et al.* [50]. Among all of the diets evaluated in the current study, fillet protein was only significantly different in the trout receiving 50% dietary PSG. Adelizi *et al.* [51] noted that fillet protein levels dropped to as low as 17.8% when soybean products were included in rainbow trout diets, although these values were not statistically significantly different from those obtained from fish fed a commercially-produced, fish meal-based control diet. Fillet protein concentrations were also similar to that reported by Yildiz [69], but less than that reported by Sealey *et al.* [70] from rainbow trout receiving a diet of 29% fish meal and 16% soybean meal.

5. CONCLUSION

The results from this study support the supposition of Yamamoto *et al.* [32] that fermented soybean meal is a promising ingredient as the main source of protein in rainbow trout diets. Further investigation is obviously needed to determine the extent, if any, of intestinal morphological changes that may be occurring with the inclusion of PSG in rainbow trout feeds. Trials involving ad libitum feeding should be conducted, as well as experiments with diets containing high concentrations of PSG modified to become isonitrogenous and isoenergetic to fish meal controls. In addition, amino acid supplementation should be examined as a possible mechanism to increase the amount of fish meal that can be successfully replaced by PSG without sacrificing trout growth.

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