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# Preliminary Evaluation of Rainbow Trout Diets Containing PepSoyGen, a Fermented Soybean Meal Product, and Additional Amino Acids

## Abstract

Juvenile rainbow trout *Oncorhynchus mykiss* diets containing PepSoyGen, a commercially-available fermented soybean meal product, were supplemented with methionine and other amino acids. A fish meal-based control and four experimental diets were used in a 36-day feeding trial; two diets contained 40% PepSoyGen and 10% fish meal, and two diets contained 50% PepSoyGen and no fish meal. Each of these diets was supplemented with either methionine, or methionine and additional amino acids. One mortality was observed during the trial. Overall weight gain, percent gain, and feed conversion ratio were significantly greater for the fish meal control than for any of the PepSoyGen diets, although feed conversion ratios from all of the diets were still below 1. Apparent protein digestibility was significantly less in the fish receiving the control diet compared to any of the experimental diets, and significantly increased with increasing PepSoyGen concentrations. There was no significant difference in length, weight, condition factor, hepatosomatic index, viscerosomatic index, or any fish health responses among dietary treatments. Fillet composition, as determined by crude protein, crude lipid, water, and ash, was also not significantly different among fish reared on any of the diets. The supplementation of other amino acids in addition to methionine had no noticeable effect. The results from this study indicate that PepSoyGen with amino acid supplementation can completely replace fish meal in juvenile rainbow trout grower diets over a relatively short time-frame, albeit with some decrease in rearing performance.

## Keywords

Alternative proteins, diets, fermented soybean meal, fish meal, *Oncorhynchus mykiss*, PepSoyGen, rainbow trout

## Disciplines

Agriculture | Aquaculture and Fisheries | Bioresource and Agricultural Engineering

## Comments

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# Preliminary Evaluation of Rainbow Trout Diets Containing PepSoyGen, a Fermented Soybean Meal Product, and Additional Amino Acids

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**Abstract:** Juvenile rainbow trout *Oncorhynchus mykiss* diets containing PepSoyGen, a commercially-available fermented soybean meal product, were supplemented with methionine and other amino acids. A fish meal-based control and four experimental diets were used in a 36-day feeding trial; two diets contained 40% PepSoyGen and 10% fish meal, and two diets contained 50% PepSoyGen and no fish meal. Each of these diets was supplemented with either methionine, or methionine and additional amino acids. One mortality was observed during the trial. Overall weight gain, percent gain, and feed conversion ratio were significantly greater for the fish meal control than for any of the PepSoyGen diets, although feed conversion ratios from all of the diets were still below 1. Apparent protein digestibility was significantly less in the fish receiving the control diet compared to any of the experimental diets, and significantly increased with increasing PepSoyGen concentrations. There was no significant difference in length, weight, condition factor, hepatosomatic index, viscerosomatic index, or any fish health responses among dietary treatments. Fillet composition, as determined by crude protein, crude lipid, water, and ash, was also not significantly different among fish reared on any of the diets. The supplementation of other amino acids in addition to methionine had no noticeable effect. The results from this study indicate that PepSoyGen with amino acid supplementation can completely replace fish meal in juvenile rainbow trout grower diets over a relatively short time-frame, albeit with some decrease in rearing performance.

**Keywords:** Alternative proteins, diets, fermented soybean meal, fish meal, *Oncorhynchus mykiss*, PepSoyGen, rainbow trout.

## INTRODUCTION

There is an abundance of research on the use of various soybean meal products in rainbow trout diets [1]. However, primarily because of the presence of numerous anti-nutritional factors [2-5] and negative effects on intestinal form and function [5-12], soybean meal inclusion in trout diets is limited.

The use of heat, pressure, chemical treatments, and other processes on soybean meal has reduced or eliminated many of the undesirable effects and anti-nutritional factors [4, 13-16]. Biological fermentation may also improve the suitability of soybean meal as an alternative protein source in trout diets. Although fermented soybean meal has been used successfully in the diets of a variety of vertebrate animals [17-19], 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* [20], red sea bream *Pagrus major* [21], pompano *Trachinotus ovatus* [22], and Japanese flounder

*Paralichthys olivaceus* [23, 24]. Yamamoto *et al.* [25] noted that soybean meal fermented for 10 h with 30% water addition did not cause any changes in intestinal morphology when used in non-fish meal rainbow trout diets, whereas less-fermented soybean meal in the diet did cause the typically-observed intestinal morphological effects. Yamamoto *et al.* [25] stated that fermented soybean meal had the potential to be the predominant protein source in rainbow trout diets.

Recently, a soybean meal fermentation product manufactured via a proprietary process incorporating *Aspergillus* spp. and *Bacillus* sp. has been developed (PepSoyGen, Nutraferma Inc., North Sioux City, South Dakota, USA). Although it may not contain enough methionine to meet the dietary requirements of rainbow trout [26], it may be suitable as a protein source with amino acid supplementation. It may also have potential advantages with the inclusion of probiotic bacteria as part of the manufacturing process [12]. Thus, the objective of this preliminary study was to examine the use of PepSoyGen with amino acid supplementation in the diets of juvenile rainbow trout.

## MATERIALS AND METHODOLOGY

The trial occurred at McNenny State Fish Hatchery, Spearfish, South Dakota, USA, using degassed and aerated

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**Table 1. Percent Composition and Chemical Analysis of the Diets Used in the Trial**

| Diet  | 1     | 2          | 3        | 4          | 5        |
|---|-------|------------|----------|------------|----------|
| PepSoyGen (%)                                       | 0     | 40         | 40       | 50         | 50       |
| Amino acid supplementation                          | none  | methionine | multiple | methionine | multiple |
| <b>Ingredients</b>                                  |       |            |          |            |          |
| Menhaden meal <sup>a</sup>                          | 40.0  | 10.0       | 10.0     | 0.0        | 0.0      |
| PepSoyGen <sup>b</sup>                              | 0.0   | 40.0       | 40.0     | 50.0       | 50.0     |
| Whole wheat <sup>c</sup>                            | 20.0  | 9.0        | 10.0     | 10.0       | 9.0      |
| Yellow corn gluten <sup>d</sup>                     | 25.0  | 21.0       | 19.0     | 20.0       | 19.0     |
| Menhaden oil <sup>e</sup>                           | 11.5  | 14.1       | 14.1     | 14.9       | 14.9     |
| CMC <sup>f</sup>                                    | 0.6   | 0.0        | 0.0      | 0.0        | 0.6      |
| Vitamin premix <sup>g</sup>                         | 0.5   | 0.5        | 0.5      | 0.5        | 0.5      |
| Mineral premix <sup>h</sup>                         | 0.0   | 0.2        | 0.2      | 0.2        | 0.2      |
| Vitamin C (Stay-C) <sup>i</sup>                     | 0.5   | 0.5        | 0.5      | 0.5        | 0.5      |
| Yeast <sup>j</sup>                                  | 0.125 | 0.125      | 0.125    | 0.125      | 0.125    |
| L-Lysine <sup>k</sup>                               | 0.0   | 0.0        | 0.5      | 0.0        | 0.5      |
| L-Isoleucine <sup>k</sup>                           | 0.0   | 0.0        | 0.3      | 0.0        | 0.3      |
| L-Histidine <sup>k</sup>                            | 0.0   | 0.0        | 0.1      | 0.0        | 0.1      |
| L-Methionine <sup>k</sup>                           | 0.0   | 0.5        | 0.5      | 0.5        | 0.5      |
| Sodium chloride                                     | 0.5   | 0.9        | 0.9      | 0.9        | 0.9      |
| Potassium chloride                                  | 0.6   | 0.7        | 0.7      | 0.7        | 0.7      |
| Magnesium oxide                                     | 0.0   | 0.1        | 0.1      | 0.1        | 0.1      |
| Calcium phosphate                                   | 0.0   | 2.3        | 2.3      | 2.3        | 2.3      |
| <b>Chemical analysis (% dry basis) <sup>l</sup></b> |       |            |          |            |          |
| Crude protein                                       | 45.3  | 44.9       | 42.4     | 44.1       | 46.8     |
| Crude lipid   | 10.7  | 9.5        | 12.0     | 9.9        | 10.2     |
| Crude fiber   | 1.3   | 1.4        | 1.6      | 2.8        | 1.8      |
| Ash   | 10.5  | 10.0       | 9.8      | 8.9        | 8.8      |
| DE (MJ/kg dry matter)                               | 14.92 | 14.35      | 14.75    | 14.32      | 15.07    |

<sup>a</sup> IPC 740, Scoular, Minneapolis, Minnesota, USA.

<sup>b</sup> Nutra-flo Protein and Biotech Products, Sioux City, Iowa, USA.

<sup>c</sup> Bob's Red Mill Natural Foods, Milwaukie, Oregon, USA.

<sup>d</sup> Consumers Supply Distributing, Sioux City, Iowa, USA.

<sup>e</sup> Omega Protein, Inc., Houston, Texas, USA.

<sup>f</sup> Carboxymethyl cellulose, USB Corporation, Cleveland, Ohio, USA.

<sup>g</sup> ARS 702, Barrows *et al.* 2008, Nelson and Sons, Inc., Murray, Utah, USA.

<sup>h</sup> ARS 640, Barrows *et al.* 2008, Nelson and Sons, Inc., Murray, Utah, USA.

<sup>i</sup> DSM Nutritional Products France SAS, Village-Neuf, France.

<sup>j</sup> Diamond V, Cedar Rapids, Iowa, USA.

<sup>k</sup> PureBulk, Roseburg, Oregon, USA

<sup>l</sup> Analysis conducted on post-extrusion pellets.

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). Flows in each tank were set at 40 L/min. Shasta strain rainbow trout (initial weight 33.6 ± 1.5 g, length 146.7 ± 2.1 mm, mean ± SE) were placed into each of 15 fiberglass circular tanks (1.8 m diameter, 0.6 m depth) on September 2, 2010. Tanks were each loaded with 40 fish, and total tank weights were recorded to the nearest gram. Feeding commenced the following day and continued for 36 days until the end of the trial. Feeding amounts for the tanks were determined by the

hatchery constant method [27], with a planned feed conversion of 1.1 and a maximum growth rate of 0.066 cm/day, which was determined from the historical performance of the Shasta strain at McNenny State Fish Hatchery. Ration amounts were updated daily. Fish were hand fed once per day. All feed fed and fish deaths were recorded daily for each tank.

The 15 tanks were randomly assigned to one of five different diets (Table 1), with three replicate tanks per treatment group. In addition to a fish meal-based control, four other

**Table 2. Amino Acid Composition (% Dry Weight) of the Diets, and the Fermented Soybean Meal Product (PepSoyGen), Used in the Trial**

| Diet                            | 1    | 2          | 3        | 4          | 5        | PepSoyGen |
|---------------------------------|------|------------|----------|------------|----------|-----------|
| PepSoyGen                       | 0    | 40         | 40       | 50         | 50       |           |
| Amino acids added               | None | methionine | multiple | methionine | multiple |           |
| <b>Essential Amino Acids</b>    |      |            |          |            |          |           |
| Arginine                        | 2.19 | 2.30       | 2.34     | 2.24       | 2.09     | 3.36      |
| Histidine                       | 1.05 | 1.03       | 1.05     | 0.94       | 1.00     | 1.29      |
| Isoleucine                      | 1.83 | 2.01       | 2.20     | 1.96       | 2.06     | 2.41      |
| Leucine                         | 4.79 | 4.59       | 4.50     | 4.41       | 4.12     | 4.00      |
| Lysine                          | 2.32 | 2.08       | 2.38     | 1.85       | 2.10     | 2.90      |
| Methionine                      | 1.04 | 1.12       | 1.16     | 1.01       | 0.92     | 0.74      |
| Phenylalanine                   | 2.15 | 2.31       | 2.32     | 2.30       | 2.15     | 2.54      |
| Threonine                       | 1.57 | 1.51       | 1.56     | 1.44       | 1.36     | 1.96      |
| Tryptophan                      | 0.40 | 0.46       | 0.51     | 0.55       | 0.52     | 0.73      |
| Valine                          | 2.13 | 2.21       | 2.15     | 2.09       | 1.97     | 2.59      |
| <b>Nonessential Amino Acids</b> |      |            |          |            |          |           |
| Alanine                         | 3.10 | 2.66       | 2.59     | 2.39       | 2.26     | 2.26      |
| Aspartic acid                   | 3.24 | 3.78       | 3.92     | 3.76       | 3.49     | 5.67      |
| Cysteine                        | 0.52 | 0.58       | 0.58     | 0.58       | 0.52     | 0.78      |
| Glutamic acid                   | 7.91 | 8.02       | 7.97     | 8.22       | 7.53     | 8.22      |
| Glycine                         | 2.36 | 1.96       | 1.90     | 1.62       | 1.56     | 2.17      |
| Hydroxylysine                   | 0.08 | 0.05       | 0.06     | 0.03       | 0.04     | 0.02      |
| Hydroxyproline                  | 0.37 | 0.20       | 0.13     | 0.04       | 0.05     | 0.07      |
| Lanthionine                     | 0.12 | 0.00       | 0.00     | 0.00       | 0.00     | 0.21      |
| Orthonine                       | 0.03 | 0.04       | 0.04     | 0.03       | 0.04     | 0.04      |
| Proline                         | 3.11 | 2.82       | 2.58     | 2.72       | 2.56     | 2.44      |
| Serine                          | 1.75 | 1.67       | 1.80     | 1.81       | 1.65     | 2.16      |
| Taurine                         | 0.18 | 0.07       | 0.07     | 0.02       | 0.04     | 0.04      |
| Tyrosine                        | 1.66 | 1.67       | 1.67     | 1.60       | 1.55     | 1.81      |

diets contained the commercially-produced PepSoyGen product (51.3% protein, 1.2% fat, and 2.6% fiber). Two of these diets contained 10% fish meal and 40% PepSoyGen, and two diets contained only 50% PepSoyGen (Table 2). To compensate for an anticipated methionine-deficiency in the PepSoyGen diets, methionine was added to all of the experimental diets. In addition, lysine, isoleucine, and histidine were also added to one of the 40% and one of the 50% PepSoyGen diets to match the essential amino acid profile of the fish meal control. The amino acid composition of each of the diets and PepSoyGen is listed in Table 2. Experimental diets were analyzed according to AOAC [28] methodology for amino acids (method 982.30), protein (method 2001.11) and crude lipid (method 2003.5, modified by substituting petroleum ether for diethyl ether), and for ash content by AACC [29] method 08-03. The protein and lipid amounts obtained by these methods were multiplied by their respective physiological fuel values of 23.6 and 39.5 [26] to obtain estimated digestible energy values.

At the end of the trial, total tank weights were recorded to the nearest g, with weight gain calculated by subtracting the initial weight from the final weight for each tank. Feed conversion ratio for each tank was calculated by dividing the total amount of food fed by the total weight gain. In addition to total tank measurements, five fish from each tank were randomly selected from each tank and individually weighed to the nearest g and measured (total length) to the nearest mm. Fish health profiles for these sampled fish, based on a modification of Goede and Barton [30], Adams *et al.* [31], and Barton *et al.* [32], were completed using the score sheet described in Table 3. Liver weights were recorded to the nearest mg and the hepatosomatic index (HSI) determined using the formula:  $HSI (\%) = 100 \times (\text{liver weight}/\text{whole fish weight})$  [33]. Viscera weights were also recorded to the nearest mg and the viscerosomatic index (VSI) determined using the formula:  $VSI (\%) = 100 \times (\text{viscera weight}/\text{whole fish weight})$ .

**Table 3. Criteria Used at the End of the Study for Fish Health Observations [Based on Goede and Barton [30], Adams *et al.* [31], and Barton *et al.* [32]]**

| Structure or 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              |

Apparent protein digestibility was determined using a direct method [34]. Digesta was removed from five fish per tank at the end of the trial. Each fish was dissected and the last cm of the distal end of the intestine was gently squeezed to remove the contents. Digesta from five fish per tank was pooled and flash frozen on dry ice prior to analysis. Protein analysis was conducted using AOAC [28] method 990.03. Apparent protein digestibility was calculated using the formula: apparent protein digestibility = (protein in the diet – protein in the digesta) / protein in the diet.

At the end of the experiment, five whole fish per tank were euthanized; muscle fillets were then removed and flash frozen for determination of carcass 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, USA) using AOAC [28] method 992.15. AOAC [28] acid hydrolysis method 948.15 with a 50:50 mix of diethyl ether and petroleum ether for extraction was used for fat analysis, and moisture was determined by drying loss using AOAC [28] method 952.08.

Data were analyzed using the SPSS (9.0) statistical analysis program (SPSS, Chicago, Illinois, USA) with significance predetermined at  $P < 0.05$ . One-way analysis of variance (ANOVA) was conducted and if the treatments

were significantly different, mean comparisons were performed using the Tukey HSD test [35]. All percentage data were arcsine transformed prior to analysis to stabilize the variances [35].

## RESULTS

Overall weight gain and percent weight gain were significantly greater in the control diet compared to any of the diets containing PepSoyGen ( $P = 0.001$  and  $0.007$ , respectively, Table 4). Feed conversion ratio was significantly less in the control diet compared to any of the PepSoyGen diets ( $P = 0.001$ ), but the feed conversion ratios for all of the diets were below 1. Apparent protein digestibility was significantly lower ( $P < 0.001$ ) for the control diet (91.4%), than the 93.2% and 93.3% values in the fish receiving either of the diets containing 40% PepSoyGen, which in turn were significantly less than that observed in fish fed either of the 50% PepSoyGen diets. Only one mortality was observed during the trial.

Individual fish measurements were very similar among the diets (Table 5). There were no significant differences in individual fish lengths, weights, or condition factors among the treatment groups. Although liver weights were significantly less ( $P = 0.018$ ) in the fish receiving any of the

**Table 4. Total Tank Rearing Data (mean  $\pm$  SE), Including Feed Conversion Ratio and Apparent Protein Digestibility, for Tanks of Rainbow Trout Receiving one of Five Different Diets Containing Either 40 or 50% PepSoyGen, with or without Methionine and Additional Amino Acids. Means in a Row with Different Letters are Significantly Different ( $N = 3, P < 0.05$ ).**

| Diet                           | 1                 | 2                 | 3                 | 4                 | 5                 |
|--------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| PepSoyGen (%)                  | 0                 | 40                | 40                | 50                | 50                |
| Amino acid supplementation     | none              | methionine        | multiple          | methionine        | multiple          |
| Start weight (g)               | 1,218 $\pm$ 45    | 1,240 $\pm$ 68    | 1,276 $\pm$ 49    | 1,235 $\pm$ 51    | 1,285 $\pm$ 32    |
| End weight (g)                 | 2,229 $\pm$ 44    | 2,150 $\pm$ 90    | 2,185 $\pm$ 76    | 2,084 $\pm$ 54    | 2,171 $\pm$ 36    |
| Gain (g)                       | 1,011 $\pm$ 10 z  | 910 $\pm$ 23 y    | 909 $\pm$ 28 y    | 849 $\pm$ 4 y     | 886 $\pm$ 8 y     |
| Gain (%)                       | 83.2 $\pm$ 3.2 z  | 73.7 $\pm$ 2.5 y  | 71.3 $\pm$ 0.9 y  | 69.0 $\pm$ 2.6 y  | 69.0 $\pm$ 1.5 y  |
| Food fed (g)                   | 834               | 834               | 834               | 834               | 834               |
| Feed conversion ratio          | 0.82 $\pm$ 0.01 z | 0.92 $\pm$ 0.01 y | 0.92 $\pm$ 0.03 y | 0.98 $\pm$ 0.01 y | 0.94 $\pm$ 0.01 y |
| Mortality (%)                  | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.3 $\pm$ 0.3     | 0.0 $\pm$ 0.0     |
| Apparent protein digestibility | 91.4 $\pm$ 0.2 z  | 93.3 $\pm$ 0.4 y  | 93.2 $\pm$ 0.4 y  | 94.7 $\pm$ 0.1 x  | 95.2 $\pm$ 0.3 x  |

**Table 5. Ending mean ( $\pm$  SE) Length, Weights, Condition Factors (K)<sup>a</sup>, Viscera Weight, Viscerosomatic Index<sup>b</sup>, Liver Weight, Hepatosomatic Index Values<sup>c</sup>, and Fish Health Assessments<sup>d</sup> for Rainbow Trout Fed Diets Containing Either 40 or 50% PepSoyGen with Methionine and Additional Amino Acids. Means with Different Letters Across a Row are Significantly Different ( $N=3, P < 0.05$ )**

| Diet                       | 1                 | 2                 | 3                 | 4                 | 5                 |
|----------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| PepSoyGen (%)              | 0                 | 40                | 40                | 50                | 50                |
| Amino acid supplementation | none              | methionine        | multiple          | methionine        | multiple          |
| Length (mm)                | 179 $\pm$ 3       | 176 $\pm$ 4       | 172 $\pm$ 7       | 171 $\pm$ 4       | 171 $\pm$ 4       |
| Weight (g)                 | 59.6 $\pm$ 3.6    | 56.7 $\pm$ 2.5    | 52.8 $\pm$ 6.1    | 49.7 $\pm$ 3.3    | 50.8 $\pm$ 3.2    |
| K                          | 1.00 $\pm$ 0.01   | 1.07 $\pm$ 0.10   | 0.99 $\pm$ 0.01   | 0.95 $\pm$ 0.01   | 0.99 $\pm$ 0.02   |
| Viscera weight (g)         | 6.12 $\pm$ 0.36   | 5.23 $\pm$ 0.19   | 4.92 $\pm$ 0.40   | 4.78 $\pm$ 0.27   | 4.86 $\pm$ 0.11   |
| Viscerosomatic index       | 10.32 $\pm$ 0.24  | 9.30 $\pm$ 0.08   | 9.45 $\pm$ 0.27   | 11.14 $\pm$ 1.94  | 9.68 $\pm$ 0.58   |
| Liver weight (g)           | 0.73 $\pm$ 0.01 z | 0.61 $\pm$ 0.01 y | 0.56 $\pm$ 0.08 y | 0.56 $\pm$ 0.02 y | 0.53 $\pm$ 0.06 y |
| HSI                        | 1.26 $\pm$ 0.06   | 1.09 $\pm$ 0.04   | 1.05 $\pm$ 0.02   | 1.31 $\pm$ 0.18   | 1.07 $\pm$ 0.05   |
| Eyes                       | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     |
| Fat                        | 1.6 $\pm$ 0.1     | 1.6 $\pm$ 0.1     | 1.8 $\pm$ 0.1     | 1.6 $\pm$ 0.1     | 1.8 $\pm$ 0.1     |
| Fins                       | 1.2 $\pm$ 0.2     | 1.1 $\pm$ 0.1     | 1.1 $\pm$ 0.2     | 1.2 $\pm$ 0.1     | 1.2 $\pm$ 0.1     |
| Gills                      | 0.3 $\pm$ 0.1     | 0.2 $\pm$ 0.1     | 0.3 $\pm$ 0.1     | 0.1 $\pm$ 0.1     | 0.2 $\pm$ 0.1     |
| Gut                        | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     |
| Kidney                     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     |
| Liver                      | 0.0 $\pm$ 0.0     | 0.1 $\pm$ 0.1     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     |
| Pseudobranchs              | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     |
| Opercles                   | 0.2 $\pm$ 0.1     | 0.2 $\pm$ 0.1     | 0.2 $\pm$ 0.1     | 0.3 $\pm$ 0.1     | 0.2 $\pm$ 0.1     |
| Spleen                     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     | 0.0 $\pm$ 0.0     |

<sup>a</sup> Condition factor (K) =  $10^5 \times (\text{weight})/(\text{length}^3)$

<sup>b</sup> Viscerosomatic index (VSI) =  $100 \times (\text{viscera weight}/\text{body weight})$

<sup>c</sup> Hepatosomatic index (HSI) =  $100 \times (\text{liver weight}/\text{body weight})$

<sup>d</sup> Fish health assessments rating system described in Table 2.

PepSoyGen diets, there was no significant difference in the hepatosomatic index. The viscerosomatic index was also not significantly different among the diets. None of the fish health values varied significantly among the fish receiving

any of the diets, and no gross visual gut inflammation was observed in any fish.

Fillet composition was not significantly affected by any of the diets used in this study (Table 6). Fillet protein per-

**Table 6. Mean ( $\pm$  SE) Percent Water, Crude Protein, Crude Lipid, and ash Concentrations from Fillets of Rainbow Trout fed Diets Containing Either 40 or 50% PepSoyGen, with Methionine and Additional Amino acids (N = 3). There were no Significant Differences Among the Treatment Groups ( $P < 0.05$ )**

| Diet                       | 1              | 2              | 3              | 4              | 5              |
|----------------------------|----------------|----------------|----------------|----------------|----------------|
| PepSoyGen (%)              | 0              | 40             | 40             | 50             | 50             |
| Amino acid supplementation | none           | methionine     | multiple       | methionine     | multiple       |
| Water (%)                  | 77.3 $\pm$ 0.9 | 75.1 $\pm$ 0.1 | 76.1 $\pm$ 0.2 | 75.9 $\pm$ 0.2 | 75.9 $\pm$ 0.4 |
| Crude protein (%)          | 18.6 $\pm$ 0.3 | 18.6 $\pm$ 0.3 | 19.4 $\pm$ 0.3 | 19.2 $\pm$ 0.2 | 19.4 $\pm$ 0.1 |
| Crude lipid (%)            | 4.6 $\pm$ 0.1  | 5.3 $\pm$ 0.3  | 4.2 $\pm$ 0.4  | 4.4 $\pm$ 0.4  | 4.8 $\pm$ 0.3  |
| Ash (%)                    | 1.5 $\pm$ 0.1  | 1.5 $\pm$ 0.1  | 1.4 $\pm$ 0.1  | 1.4 $\pm$ 0.1  | 1.5 $\pm$ 0.1  |

centages ranged from a low of 18.6% in fish fed the fish meal control to 19.4% in fish receiving feed with 50% PepSoyGen. Mean fillet lipid contents were also very similar among the diets.

## DISCUSSION

The reductions in weight gain and increases in feed conversion ratio of fish fed diets containing the commercial PepSoyGen product in the present study cannot be directly compared to Yamamoto *et al.* [25], who noted no difference in final body weight and gain between their fish meal control and one of their PepSoyGen diets. Although the experimental diets in both studies were similar, Yamamoto *et al.* [25] fed fish to satiation, focused only on growth, and did not report feed conversion ratios. In contrast, this study used a production-based feeding model based on well-established feeding rates [36] in an attempt to eliminate the possible confounding effect of unregulated feeding [37]. It is possible that increasing feeding rates for the fish receiving PepSoyGen may have produced similar growth rates with the fish meal-based control diet, although feed conversion ratios would likely have been much greater. Yamamoto *et al.* [25] also supplemented their PepSoyGen diets with four amino acids (arginine, threonine, tryptophan, and valine) not included in this study. However, none of these four amino acids, nor any essential amino acids, were below the critical values listed by the National Research Council [26] in any of the diets used in the present study. The study by Yamamoto *et al.* [25] was also conducted in much warmer water (16.3°C) than this study. Other studies evaluating different dietary ingredients have indicated a possible influence of water temperature on feed conversion ratio [38, 39], weight gain [38, 39], and nutrient digestibility [40, 41].

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 [42]. In a study by de Francesco *et al.* [43] differences in trout rearing performance between fish meal and plant-based diets did not become apparent until after 12 weeks. The present study lasted only 36 days, but this was sufficient for significant differences in gain and feed conversion ratio to appear among the dietary treatments. It is unlikely that prolonging this trial for a longer period would have produced different results. However, given the small sample sizes used in this study, it is possible that additional replication may have produced different results.

At only 0.74%, the PepSoyGen used in this study is relative low in methionine in relation to the dietary requirements of rainbow trout diets [26]. This is not unexpected, as soybean meal typically does not contain enough methionine to meet the nutritional requirements of rainbow trout [1, 44]. Methionine has been supplemented in other studies using soybean meal or soy protein concentrates fed to rainbow trout [5, 9, 45]. Incorporating other amino acids in addition to methionine did lead to an increase in the percentage of these amino acids in the diets, but appeared to provide no noticeable improvement to any of the parameters studied.

Feed conversion ratios below 1.0 are not unusual for rainbow trout reared at production hatcheries in South Dakota [36] or elsewhere [46]. The 0.82 feed conversion ratio for fish fed the control diet was at the low end of that reported by Barrows *et al.* [47] for their fish meal-based control diets. Even though the feed conversion ratios for the PepSoyGen diets were higher than the control, they were still less than those reported by Cheng *et al.* [48, 49] for rainbow trout fed soybean-free, fish meal-based diets, as well as diets containing soybean meal. All of the feed conversion ratios from the fermented soybean-meal-containing diets were similar to a commercial fish meal-based control diet used in a study by Adelizi *et al.* [45]. The relatively low rearing densities used in this trial may also have influenced the observed feed conversion ratios [50, 51].

The apparent protein digestibilities observed in this study are very similar to those reported by Yamamoto *et al.* [25] for rainbow trout receiving diets with fermented soybean meal. The positive relationship between dietary PepSoyGen concentrations and protein digestibility in the current study does not follow the pattern of decreased digestibilities associated with soybean meal inclusion in rainbow trout diets [44]. The fermentation process of the PepSoyGen product used in this study hydrolyzes the long chain proteins into small chain proteins allowing them to be more digestible for young animals (relative to de-hulled soybean meal and other soy proteins). The fermentation process also reduces indigestible oligosaccharides (raffinose and stachyose) and trypsin inhibitor that can have a negative influence on nutrient digestibility. Overall, the protein digestibilities in the current study are much higher than that reported by Refstie *et al.* [52] for both fish meal control and 60% soybean meal diets, and similar to those from Cheng *et al.* [48] and Kaushik *et al.* [53] for rainbow trout receiving diets with low concentrations of soybean meal or other soybean-based products. It



can be difficult to compare digestibilities among studies [26], and it is possible that the different fecal collection methods used in these studies may have influenced the assessment of protein digestibility as well [34, 42, 54, 55].

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 PepSoyGen was observed in this study. However, microscopic examination did not occur. Yamamoto *et al.* [25] noted that lengthy and moist fermentation of soybean meal using *Bacillus* sp. could eliminate the occurrence of physiological abnormalities typically observed with the use of soybean meal in salmonid diets. The process by which PepSoyGen is manufactured reduces or eliminates a variety of anti-nutritional factors that can negatively impact fish health in general, and intestinal health specifically. It is also possible that as a result of the fermentation process, PepSoyGen may also contain immunomodulatory components [20, 23, 56].

Although HSI is positively related to dietary carbohydrate levels [57, 58], there was no difference in HSI among any of the diets. Because dietary phosphorus is inversely related to liver lipid levels and HSI [59], the lack of difference in HSI among the diets would appear to indicate no deficiencies in phosphorus availability from any of the diets. The HSI values in the current study were similar to those reported from rainbow trout receiving fish meal-based diets [45, 47] but lower than those reported from rainbow trout fed diets with relatively high concentrations of soybean meal or other soy products [9, 45, 53, 60].

Panserat *et al.* [61] observed higher VSI values in rainbow trout receiving a plant-based diet in comparison to a diet with fish meal as a primary protein source. In the current study, no differences in VSI occurred among the diets. The lack of difference in VSI may be due to the similar lipid levels among the diets, given the positive relationship between dietary lipid and VSI [62-64]. It could also be due to the small sample sizes used in this study.

Fillet protein concentrations for fish fed any of the diets was similar to that reported by Adelizi *et al.* [45] from trout fed a commercially-produced, fish meal-based diet. They were also similar to that reported by Yildiz [65], but less than that observed by Sealey *et al.* [66] for rainbow trout fed a diet containing 29% fish meal and 16% soybean meal.

## CONCLUSION

In conclusion, the PepSoyGen product used in this study with amino acid supplementation support the conclusion by Yamamoto *et al.* [25] that fermented soybean meal in general shows promise as the main source of protein in rainbow trout diets. Additional longer-term research is obviously needed to determine the dietary adjustments needed to produce rainbow trout diets wherein PepSoyGen completely replaces fish meal with no loss of rearing performance. Additionally, research is needed to determine if any changes in intestinal morphology and function are occurring as a result of high concentrations of dietary PepSoyGen, as well as the possible immunological benefits of this PepSoyGen product. These additional studies to determine if PepSoyGen can completely replace fish meal in salmonid diets should be

undertaken because of the rapid growth in global aquaculture [67] and the increased demand and market prices for limited fish meal stocks [68, 69].

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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