Effect of Soy Protein Products and Gum Inclusion in Feed on Fecal Particle Size Profile of Rainbow Trout

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Abstract: Replacement of fishmeal (FM) with alternative plant proteins, especially soybean meal (SBM), can cause a diarrhea-like symptom in rainbow trout (RBT), characterized by very fine fecal particles. These fines do not settle out in raceway effluent for collection and can contribute to pollution of receiving waters. In this study, two experiments were conducted. Experiment 1 examined effects of nine protein sources (sardine meal, menhaden meal, soy protein concentrates (SPC) (three types), SBM (regular and high protein), corn protein concentrate (CPC), and poultry by-product meal (PBM)) on fecal particle size distribution. Results showed that all five soy-based diets produced feces in RBT having 75.7–89.3% fines and only about 1.0% large particles, while the remaining four diets yielded feces having a balanced particle size distribution. Oligosaccharides present naturally in soy products, thought to contribute fecal fines, were not correlated to fecal particle size classes. Instead, high crude fiber content in soy-based diets was found to be responsible for unbalanced fecal particle distribution in RBT. Experiment 2 examined if improvements in formulation could reduce the negative effect of soy-based ingredients. Eight practical diets (FM, SPC, SPC + 0.3% guar gum, PBM + CPC, PBM + CPC + 20 or 30% SPC, and PBM + CPC + 20 or 30% SPC + 0.3% guar gum) were formulated to contain 40% protein and 20% lipid. Results showed that diets containing mixtures of PBM, CPC, and 20% or 30% SPC plus guar gum produced trout feces with the highest percentage of large particles and lowest of fines, while the diet containing SPC alone (56%) plus guar gum resulted in trout feces having the highest content of mid-size particles. It was concluded that crude fiber in soy protein products (SBM and SPC) caused undesirable fecal particle profiles in RBT, and the addition of guar gum could significantly alleviate this negative effect.

Keywords: fecal particle size; feces; guar gum; soy protein concentrate; soybean meal; trout feed

1. Introduction

Fishmeal (FM) is the gold standard protein source for numerous fish species, including salmonids, due to its complete essential amino acid profile, high protein content, high digestibility, fatty acid profile, and palatability [1]. Many aquaculture feed formulations still have fishmeal included at levels of 50% or higher. However, there is a high risk associated with reliance on any one ingredient due to potential supply, price, and quality fluctuations [2]. As a strategy to reduce the risk, development of alternatives to fishmeal in aquaculture diets remains a high priority. Several animal proteins, including poultry byproduct meal (PBM), feather meal, blood meal, and others, have been utilized as a partial replacement of FM. However, they suffer from various downsides, such as low palatability, reduced digestibility, or inferior protein quality [1,3]. Oilsseeds, grains, and their processing co-products are important aquafeed ingredients due to their abundance. Among them,
soybean meal (SBM) is the number one alternative source of proteins in aquaculture [4,5]. However, the natural presence of anti-nutritional factors in soybeans and soy products, including protease inhibitors, phytic acid, oligosaccharides, etc., can reduce the nutritive value of soy products and cause undesirable health effects to fish [3,4,6]. For example, diets containing SBM can cause pathomorphological changes in the distal, intestinal epithelium of rainbow trout, which are also accompanied by diarrhea [4,7–9].

Waste production and its removal from the water of aquaculture facilities are major concerns [10]. They are compounded by the incorporation of SBM into rainbow trout (RBT) feeds [11]. Soy-induced gastroenteritis (diarrhea-like symptoms) in fish decreases fecal stability and promotes the formation of very fine particles that do not settle in the quiescent zone of raceways [12,13]. Fecal solids from trout fed soy-based feeds can breakdown and solubilize easily, causing an increase in release of dissolved nutrients, such as phosphorus and nitrogen [14,15]. In contrast, large and more stable fecal particles are resistant to hydromechanical stress in flow-through systems and more apt to drop rapidly out of suspension for removal from the effluent [16]. Therefore, an increase in fecal particle size (FPS) through diet manipulation can improve solid removal efficiency [17–19], reduce impact of effluent on the environment, and ensure compliance with environmental regulations.

Fecal particle size is considered a direct measure of the mechanical stability of feces [20]. The effect of diet on FPS distribution has not been definitively determined, but the smaller the fecal particles, the more difficult they are to remove by filtration or sedimentation. Trout fed soy-based diets can exhibit diarrhea-like symptoms [21], and it has been hypothesized that the natural presence of oligosaccharides in soybeans and soy products, which include mainly raffinose and stachyose, are responsible for the symptoms [4]. Processing soybean meal (SBM) into protein concentrates or isolates not only increases protein content (>60%) but also removes some oligosaccharides [22], which theoretically should reduce the occurrence of soy-induced diarrhea.

Modification of dietary formulations has been implemented to mitigate the negative impacts of SBM to fecal quality with varying degrees of success. Use of soy protein isolates or concentrates as partial or complete SBM replacement has been employed in formulations to alleviate diarrheal symptoms in rainbow trout without adequate confirmation of a substantive effect [4]. Other changes in formulation have proven more successful. For example, incorporation of dietary polysaccharides as binders has improved fecal durability and reduced fecal fines in RBT fed soy-based feeds [11,15,17,18,20,21,23]. Among them, guar gum, a galactomannan polysaccharide extracted from guar beans, has shown the most potential as a fecal stabilizer in fish feeds.

Yet, the effects of individual protein ingredients on FPS distribution and whether this effect can be shifted by gum addition have not been symmetrically investigated. There is also a lack of information documenting the direct effect of the gum on soy ingredient inclusion at varying levels. Therefore, the present study was conducted with multiple objectives, including (a) to determine the effect of commercially available protein ingredients from various sources on FPS distribution in rainbow trout fed the formulated diets, (b) to test the hypothesis that the oligosaccharides in soy protein products are responsible for causing high proportion of fecal fines when fed diets containing soy protein products, and (c) to determine whether addition of guar gum to feed can help alleviate the negative impact of soy protein ingredients on FPS distribution in rainbow trout. No doubt, knowledge gained from the research can help formulate plant-based diets for RBT with improved FPS distribution for effluent management.

2. Materials and Methods

2.1. Experimental Design

This study consisted of two experiments. Experiment 1 was conducted to determine the effect of specific protein sources on FPS distribution, while Experiment 2 evaluated the effect of practical diets containing ingredients known to cause or prevent diarrhea and determine if improved formulation can decrease the diarrheic effect of SPC inclusion.
In Experiment 1, nine ingredients were tested, including two fish meals (sardine and menhaden), three soy protein concentrates (SPC) (No. 1, 2, and 3), two SBM (regular and high-protein), corn protein concentrate (CPC), and poultry by-product meal (PBM) (Table 1). During feed formulation, all protein ingredients were added at the same amount, consisting of 52.53% (w/w) of all experimental diets. For Experiment 2, eight experimental diets were formulated to contain 40% protein and 20% lipid (Table 2), including fish meal (sardine), SPC (No. 1), SPC + guar gum (G), and five diets with PBM + CPC as a basis (without SPC, with 20% SPC, with 30% SPC, with 20% SPC + G, and with 30% SPC + G). Inclusion level of guar gum for the diets containing G was 0.3% of the diet by mass.

Table 1. Ingredient composition (g/100 g wet matter) of experimental diets in Experiment 1. The effects of various soy products and animal products on fecal particle size.

| Ingredient Composition          | Sardine Meal | Menhaden Meal | SPC 1    | SPC 2    | SPC 3    | Soybean Meal, Regular | Soybean Meal, High Protein | Poultry Meal | CPC 1 |
|---------------------------------|--------------|---------------|----------|----------|----------|-----------------------|---------------------------|--------------|-------|
| Sardine meal                    | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Menhaden meal                   | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Soy protein concentrate 1       | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Soy protein concentrate 2       | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Soy protein concentrate 3       | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Soybean meal, regular           | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Soybean meal, high protein      | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Poultry by-product meal         | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Corn protein concentrate        | 52.53        | 52.53         | 52.53    | 52.53    | 52.53    | 52.53                 | 52.53                     | 52.53        | 52.53 |
| Wheat flour                     | 26.6         | 24.85         | 20.6     | 20.2     | 20.4     | 20.28                 | 19.84                     | 31.76        | 20.31 |
| Menhaden oil                    | 14.5         | 15.5          | 19.55    | 19.2     | 18.7     | 19.52                 | 18.81                     | 9.89         | 18.00 |
| Lysine HCl                      | 0.6          | 1             | 1.7      | 1.75     | 1.35     | 1.35                  | 1.95                      | —            | 3.00  |
| Methionine                      | 0.2          | 0.35          | 0.75     | 0.8      | 0.95     | 0.65                  | 0.8                      | 0.25         | 0.30  |
| Threonine                       | 0.1          | 0.3           | 0.1      | 0.1      | 0.2      | 0.2                   | 0.6                      | —            | 0.39  |
| Taurine                         | 0.5          | 0.5           | 0.5      | 0.5      | 0.5      | 0.5                   | 0.5                      | 0.5          | 0.5   |
| Dicalcium phosphate             | 2            | 2             | 2        | 2        | 2        | 2                     | 2                        | 2            | 2     |
| Vitamin premix                  | 1            | 1             | 1        | 1        | 1        | 1                     | 1                        | 1             | 1     |
| Choline chloride                | 0.6          | 0.6           | 0.6      | 0.6      | 0.6      | 0.6                   | 0.6                      | 0.6           | 0.6   |
| Trace min premix                | 0.1          | 0.1           | 0.1      | 0.1      | 0.1      | 0.1                   | 0.1                      | 0.1           | 0.1   |
| Vitamin C                       | 0.3          | 0.3           | 0.3      | 0.3      | 0.3      | 0.3                   | 0.3                      | 0.3           | 0.3   |
| Potassium Chloride              | 0.56         | 0.56          | 0.56     | 0.56     | 0.56     | 0.56                  | 0.56                     | 0.56          | 0.56  |
| Sodium Chloride                 | 0.56         | 0.28          | 0.28     | 0.28     | 0.28     | 0.28                  | 0.28                     | 0.28          | 0.28  |
| Magnesium oxide                 | 0.56         | 0.05          | 0.05     | 0.05     | 0.05     | 0.05                  | 0.05                     | 0.05          | 0.05  |
| Astaxanthin                     | 0.08         | 0.08          | 0.08     | 0.08     | 0.08     | 0.08                  | 0.08                     | 0.08          | 0.08  |

a Sardine, Skretting USA (Tooele, UT), crude protein: 66.5 g/100 g. b Menhaden Special Select, Omega Protein, Inc. (Reedville, VA), crude protein: 61.0 g/100 g. c Solae (now known as Dupont Protein Products, St. Louis, MO), Pro-Fine VF, crude protein: 69.3 g/100 g, made by an aqueous ethanol leach method. d ARS soy protein concentrate, 64.5 g/100 g crude protein, made by an acid leaching method at our own lab. e Hamlet Protein (Horsens, Denmark), HP-300, 56.0 g/100 g crude protein, made by an enzymatic treatment of soy meal. f Soybean meal, ADM (Dekalb, IL), 47.0 g/100 g crude protein, made from conventional soybeans. g Navita Premium Feed Ingredients (West Des Moines, IA), high protein and low oligosaccharide soybean meal, 54.0 g/100 g crude protein, made from low oligosaccharide soybeans, e3010, formerly known as SG3010. h IDF, Inc. (Springfield, MO), 83.2 g/100 g protein. i Cargill, Inc. (Baltimore, MD), Empyreal 75, 75.6 g/100 g crude protein. j Manildra Milling, Inc. (Mission, KS), 12.0 g/100 g protein. k Virginia Prime menhaden oil, Omega Protein, Inc. (Reedville, VA, USA). l NB Group Co. LTD (Zouping County, Shandong Province, China). m ARS 702; contributed, per kg diet; vitamin A 9650 IU; vitamin D 6600 IU; vitamin E 132 IU; thiamin mono-nitrate 9.1 mg; riboflavin 9.6 mg; pyridoxine hydrochloride 13.7 mg; pantothenate DL-calcium 46.5 mg; cyanocobalamin 0.03 mg; nicotinic acid 21.8 mg; biotin 0.34 mg. n Stay-C, 35%, DSM Nutritional Products (Columbia, MD). o Carophyll Pink 10, DSM Nutritional Products. 1 SPC = soy protein concentrate; CPC = corn protein concentrate.

2.2. Feed Manufacture

All diets were formulated to satisfy the nutritional requirements of salmonid fish [24] and balanced with respect to lysine, methionine, and threonine. Specific formulations to evaluate the effect of diet ingredients on FPS for Experiments 1 and 2 are provided above. All diets for each experiment in both phases of the project were processed with a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with an approximate retention time of 28 s in the 6 extruder barrel sections, and an average temperature of 125°C in the three working sections. The temperature of the diet plate and steam venting...
was used to control density (floating) and expansion (oil coating). Pressure at the die head was between 20 to 40 bar depending on diet. Pellets were dried in a pulse bed drier (OTW, Buhler AG, Uzwil, Switzerland) for 25 min at 102 °C with a 10 min cooling period to provide a final moisture level of less than 10%. A top coating of fish oil was added to all feeds using a vacuum-assisted top-coater (A.J. Mixing, ON, Canada). Diets were stored in plastic lined paper bags at room temperature until fed and used within 2 months of manufacture.

Table 2. Ingredient composition (g/100 g wet matter) of experimental diets in Experiment 2 on the effect of adding guar gum to soy-based diets on fecal particle size.

| Grams/100 g | Fish meal a | Soy protein concentrate 1 b | Poultry meal c | Corn protein concentrate d | Guar gum | Wheat flour e | Menhaden oil f | Methionine | Threonine | Taurine g | Dicalcium phosphate | Vitamin premix h | Choline chloride i | Trace min premix j | Potassium Chloride | Sodium Chloride | Magnesium oxide | Astaxanthin l |
|-------------|-------------|---------------------------|----------------|---------------------------|----------|---------------|---------------|------------|-----------|-----------|-------------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|
| Fish meal   | 55.50       | —                         | —              | —                         | —        | 25.88          | 14.50          | 0.10       | 0.10      | 0.50      | —                  | 1.00            | 0.60            | 0.10            | 0.56            | 0.56            | 0.56            | 0.08            |
| Soy protein concentrate 1 b | 56.10 | 56.10 | 36.00 | 10.05 | — | 17.03 | 19.90 | 0.85 | — | 0.50 | 2.00 | 1.00 | 0.60 | 0.10 | 0.56 | 0.56 | 0.08 |
| Poultry meal c | — | — | 36.00 | — | — | 16.73 | 12.50 | 0.85 | — | 0.50 | 2.00 | 1.00 | 0.60 | 0.10 | 0.56 | 0.56 | 0.08 |
| Corn protein concentrate d | — | — | 10.05 | — | — | 26.46 | 15.90 | 0.35 | — | 0.50 | 2.80 | 1.00 | 0.60 | 0.10 | 0.56 | 0.56 | 0.08 |
| Guar gum | — | — | — | 0.30 | — | — | — | — | — | 0.80 | — | 0.30 | — | — | — | 0.80 |
| Wheat flour e | — | — | — | 0.80 | — | — | — | — | — | 0.14 | — | 0.30 | — | — | — | 0.14 |
| Menhaden oil f | — | — | — | 1.26 | — | — | — | — | — | 0.11 | — | 0.50 | — | — | — | 0.11 |
| Methionine | — | — | — | 1.32 | — | — | — | — | — | 0.14 | — | 0.50 | — | — | — | 0.14 |
| Threonine | — | — | — | 1.26 | — | — | — | — | — | 0.11 | — | 0.50 | — | — | — | 0.11 |
| Taurine g | — | — | — | 1.32 | — | — | — | — | — | 0.11 | — | 0.50 | — | — | — | 0.11 |
| Dicalcium phosphate | — | — | — | 2.00 | — | — | — | — | — | 0.80 | — | 2.00 | — | — | — | 2.00 |
| Vitamin premix h | — | — | — | 2.00 | — | — | — | — | — | 2.80 | — | 2.80 | — | — | — | 2.80 |
| Choline chloride i | — | — | — | 2.00 | — | — | — | — | — | 2.80 | — | 2.80 | — | — | — | 2.80 |
| Trace min premix j | 1.00 | 1.00 | 1.00 | 2.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 2.00 | 1.00 | 2.00 | 1.00 | 1.00 | 2.00 | 1.00 | 2.00 |
| Potassium Chloride | — | — | — | 0.60 | — | — | — | — | — | 0.60 | — | 0.60 | — | — | — | 0.60 |
| Sodium Chloride | — | — | — | 0.60 | — | — | — | — | — | 0.60 | — | 0.60 | — | — | — | 0.60 |
| Magnesium oxide | — | — | — | 0.56 | — | — | — | — | — | 0.56 | — | 0.56 | — | — | — | 0.56 |
| Astaxanthin l | — | — | — | 0.08 | — | — | — | — | — | 0.08 | — | 0.08 | — | — | — | 0.08 |

2.3. Fish Feeding Trials

Both feeding trials were conducted in 400 L semi-square polytanks each equipped with 18-inch radial flow separators (RFS; Pentair, Inc., Cary, NC, USA) for fecal collection. Tanks were stocked with 30, approximately 150 g RBT (TroutLodge, Inc., Bonney Lake, WA, USA; Mention of products used in this study does not indicate endorsement by the USDA) receiving 24 L/min of 15 °C water with DO above 6 mg/L. Water quality (ammonia, oxygen, nitrates) was measured weekly. Fish were hand fed twice a day at 0730 h and 0430 h to apparent satiation. In both studies, each of the experimental diets (n = 9 in Experiment 1 and n = 8 in Experiment 2) was randomly assigned to three tanks. Trout in each tank were fed the experimental diets for four weeks, before feces were collected from the RFS. All experiments were conducted under the “Guidelines for Use of Fishes in Research” [25].
2.4. Fecal Collection System and Particle Size Determination

Feces were collected overnight from each of the 30 tanks for use in FPS classification. The fecal collection system and particle size determination method are described elsewhere [26]. Briefly, after the last feeding of the day prior to fecal collection, tanks and radial flow separators (RFS) were thoroughly cleaned at 1600 h. Effluent water was then diverted through the RFS overnight. The following morning effluent was diverted away from the fecal collectors, and feces were allowed to settle in the RFS for 1 h. Feces were removed via a ball valve at the bottom of the RFS, and the collected feces were then transferred to an Imhoff cone and allowed to settle for 1 h, producing three layers or fractions. A panel of three observers visually assigned volume to each particle size class (fraction). The total collected fecal volume and the volume of each fraction were averaged and recorded. The three fractions corresponded to 3 particle size classes: >1.2 mm (large), 0.6 to 1.2 mm (mid), and >0.6 mm (small or fines). Size distribution for each class was expressed as a percentage of the total feces collected.

2.5. Chemical Analysis

In Experiments 1 and 2, three samples (10 g) from each experimental feed were retained and frozen for analysis of proximate composition (moisture, crude protein, crude fat, and ash) and carbohydrate profile (total CHO, starch, oligosaccharides, and crude fiber). Moisture and ash contents were determined by official Association of Official Analytical Chemists methods [27]. The protein content was measured by a published combustion method [27] using a nitrogen-to-protein analyzer (Model FP-528, Leco Corp. St. Joseph, MI, USA), and a factor of 6.25 was used for converting total nitrogen to crude protein content (% crude protein = % N × 6.25). Crude fat content was determined by AOCS Approved Procedure Am 5-04 [28] using a fat analyzer (Model XT 10, Ankom Technology, Macedon, NY, USA). However, hexane was used as the extracting solvent instead of petroleum ether. Crude fiber was measured based on AOCS Approved Procedure Ba 6a-05 [28] using a fiber analyzer (Model A2000, Ankom Technology). This method determined crude fiber as the organic residue remaining after digesting with 0.255 N H$_2$SO$_4$ and 0.313 N NaOH. Starch content was measured according to Liu and Han [29]. Sucrose and oligosaccharide contents were measured using Raffinose/Sucrose/D-Glucose Assay Kit (K-RAFGL; Megazyme Ltd., Bray, Ireland). Oligosaccharide content was expressed as raffinose equivalent. Total CHO content was calculated as the difference in percentage between 100 and the sum of protein, oil, and ash contents, all expressed as % DM.

2.6. Statistical Analysis

A one-way analysis of variance (ANOVA) design was used with the experimental diet as the main effect in both studies. The PROC ANOVA procedure (SAS Software Version 9.4) (SAS, 2012) was used to conduct the analysis of variance, and each tank of fish was the experimental unit. Differences among treatment means ($n = 3$ tanks/experimental diet) for FPS classes were determined using the Tukey-Kramer procedure for pair-wise comparisons [30]. To determine the influence of dietary carbohydrate (starch, crude fiber, sucrose, and raffinose) content on fecal fines production, standard regression and correlation analysis using Pearson correlation coefficient ($r$) was conducted [30] for diets used in Experiment 1 only, since binders were added to some diets in Experiment 2. All percentages were arc-sin transformed prior to analysis. Treatment effects in all statistical analyses in this project were considered different when probabilities were less than 0.05.

3. Results

3.1. Experiment 1

The nine diets had varying chemical compositions (Table 3) due to the use of different protein ingredients at a fixed inclusion amount. Protein content varied from 32.92% to 46.80%, lipid 16.10–18.78%, and ash 4.25–15.57%. Within CHO, starch also showed variation among diets (19.36–25.05%), but crude fiber showed even greater variation (0.60–2.62%).
Considerable variations in concentrations of sucrose (0.06–4.44%) and oligosaccharides (the raffinose equivalent, 0.03–2.39%) among diets were also observed (Table 3). The diet made with regular SBM had the highest level of oligosaccharides, while the diets having CPC or fish meal as the primary protein source had the lowest levels. The diets made of SPC or high protein SBM had varying levels of oligosaccharides, but all were significantly lower than that of the diet made with regular SBM.

Table 3. Chemical composition of nine diets in Experiment 1, each made with a single protein ingredient.

| Diet                  | Lipid  | Crude Protein | Ash    | Starch | Crude Fiber | Total CHO 2  | Sucrose | Oligosaccharides 3 |
|-----------------------|--------|---------------|--------|--------|-------------|--------------|---------|-------------------|
| Sardine meal          | 16.10  | 43.08         | 10.41  | 23.06  | 0.69        | 30.41        | 0.20    | 0.07              |
| Menhaden meal         | 18.22  | 41.14         | 15.57  | 19.36  | 0.60        | 25.07        | 0.12    | 0.03              |
| Soy protein concentrate 1 | 18.05  | 41.78         | 6.31   | 16.21  | 1.90        | 33.86        | 0.70    | 0.99              |
| Soy protein concentrate 2 | 17.65  | 42.54         | 4.65   | 16.20  | 3.20        | 35.16        | 0.18    | 0.17              |
| Soy protein concentrate 3 | 18.78  | 36.34         | 7.26   | 16.90  | 2.62        | 37.42        | 0.29    | 0.11              |
| Soybean meal, high protein | 16.98  | 38.12         | 6.99   | 16.72  | 2.43        | 37.92        | 4.44    | 0.41              |
| Soybean meal, regular | 17.82  | 32.92         | 7.89   | 16.45  | 2.12        | 41.37        | 3.83    | 2.39              |
| Poultry by-product meal | 16.78  | 44.26         | 6.45   | 25.05  | 0.81        | 32.51        | 0.06    | 0.44              |
| Corn protein concentrate | 16.51  | 46.80         | 4.25   | 16.55  | 1.14        | 32.44        | 0.19    | 0.02              |

1 For diet formulations and sources for dietary ingredients, see Table 1; 2 CHO = carbohydrate. 3 The content of oligosaccharides (mainly raffinose and stachyose) was measured and expressed as raffinose equivalent.

There was a significant effect of protein source on the FPS distribution from RBT fed the diets, particularly on the percentage of both large and small fecal particles (Table 4). Trout fed diets containing primarily FM, PBM, or CPC had higher percentages of large fecal particles and less fecal fines than trout fed any of the soy products. Trout fed diets containing different types of SPC or SBM produced feces having significantly higher fines and significantly lower large particle contents. Among diets containing soy products, large particles were all approximately 1%. The diet containing regular SBM yielded the highest fines (89.3%) and lowest mid-size particles (9.7%).

Table 4. The effect of feed ingredients on fecal particle size (expressed as % of total collected) of rainbow trout in Experiment 1. Values for each diet are averaged by tank.

| Diet                  | Large    | Mid-Size | Fines    |
|-----------------------|----------|----------|----------|
| Sardine meal          | 25.1 ± 0.7 A | 23.8 ± 1.0 | 51.1 ± 1.2 CD |
| Menhaden meal         | 22.0 ± 0.2 A | 30.5 ± 0.8 | 47.5 ± 1.0 D |
| Soy protein concentrate 1 | 1.2 ± 0.1 B | 23.1 ± 0.6 | 75.7 ± 1.9 AB |
| Soy protein concentrate 2 | 1.4 ± 0.2 B | 15.9 ± 0.6 | 82.7 ± 1.7 A |
| Soy protein concentrate 3 | 1.2 ± 0.1 B | 16.1 ± 0.5 | 82.7 ± 2.1 A |
| Soybean meal, high protein | 1.0 ± 0.1 B | 16.8 ± 0.4 | 82.2 ± 1.6 A |
| Soybean meal, regular | 1.0 ± 0.1 B | 9.7 ± 0.1 | 89.3 ± 2.1 A |
| Poultry by-product meal | 21.3 ± 0.8 A | 18.4 ± 0.8 | 60.3 ± 1.0 BCD |
| Corn protein concentrate | 22.9 ± 0.4 A | 20.2 ± 0.2 | 56.9 ± 1.1 BCD |
| Probability of α > F value | 0.01 | 0.06 | 0.01 |

Significant differences between treatments are denoted by a different superscript letter. 1 For diet formulations and sources for dietary ingredients, see Table 1.

3.2. Experiment 2

In Experiment 2, the eight experimental diets used different protein ingredients but at varying inclusion levels based on a fixed protein content. Therefore, formulations for these diets were practical. Unlike Experiment 1, the diets in Experiment 2 had similar contents of protein, oil, ash, and total CHO (Table 5). Variation existed for starch and fiber contents. Concentrations of sucrose and raffinose equivalent also varied (Table 5), but overall, they were significantly lower than the diet containing regular SBM in Experiment 1 (Table 3). Results also showed significant variation in the FPS distribution in RBT fed different diets (Table 6) indicating a significant effect of feed formulation. As expected, trout
fed the FM diet produced feces with significantly more large and mid-size fecal particles and significantly less fines than fish fed the diets containing SPC. However, adding guar gum at 0.3% to the SPC diet significantly reduced fines in the feces from 85.1% to 27.6%. The diet containing PBM and CPC (Table 6) yielded an FPS profile less ideal than the diets containing either PBM or CPC alone (Table 4). Addition of 20% or 30% SPC to diets containing PBM and CPC caused significant changes in FPS distribution. There was a significant decrease in large and small fecal particles and a significant increase in mid-size particles (Table 6). More importantly, inclusion of guar gum at 0.3% to the diets containing mixtures of PBM, CPC, and SPC (20% or 30%) also caused significant changes in FPS distribution. There were significant increases in large particles and a significant decrease in fines, while mid-size particles remained relatively unchanged. These changes are favorable for better effluent management. Interestingly, in the all-SPC (56.1%) diet, the addition of guar gum increased mid-size but not large particle production, whereas for diets containing mixtures of PBM, CPC, and SPC (20% or 30%), the addition of guar gum caused an increase in large particles with little change in mid-sized particles. In both cases, fine particles decreased significantly as compared with the respective diets without guar gum (Table 6).

### Table 5. Chemical composition of eight diets in Experiment 2, each made with single or mixed protein ingredients.

| Diet 1 | Lipid | Crude Protein | Ash | Starch | Crude Fiber | Total CHO | Sucrose | Oligosaccharides 2 | Dry-Matter Basis, g/100 g |
|--------|-------|---------------|-----|--------|-------------|-----------|---------|-------------------|--------------------------|
| Fish meal | 18.39 | 44.76 | 9.28 | 20.98 | 0.58 | 27.57 | 0.11 | 0.00 |
| Soy protein concentrate (SPC) | 14.91 | 45.27 | 6.70 | 13.89 | 2.23 | 33.12 | 0.72 | 0.48 |
| Soy protein concentrate + guar | 13.84 | 45.87 | 6.58 | 13.56 | 2.38 | 33.71 | 0.30 | 0.96 |
| Poultry + CPC | 16.86 | 43.66 | 6.02 | 24.34 | 0.62 | 33.46 | 0.00 | 0.00 |
| Poultry + CPC + 20% SPC | 16.64 | 44.19 | 6.25 | 21.30 | 0.99 | 32.91 | 0.00 | 0.18 |
| Poultry + CPC + 30% SPC | 17.55 | 44.15 | 6.27 | 20.70 | 1.04 | 32.03 | 0.02 | 0.01 |
| Poultry + CPC + 20% SPC + guar | 16.42 | 44.87 | 6.44 | 21.14 | 0.99 | 32.28 | 0.26 | 0.30 |
| Poultry + CPC + 30% SPC + guar | 15.93 | 44.39 | 6.42 | 20.61 | 0.97 | 33.26 | 0.01 | 0.10 |

1 For diet formulations and sources for dietary ingredients, see Table 2. CHO = carbohydrate; SPC = soy protein concentrate; CPC = corn protein concentrate; Poultry = poultry by-product meal; guar = guar gum.

2 The content of oligosaccharides (mainly raffinose and stachyose) was measured and expressed as raffinose equivalent.

### Table 6. The effect of feed ingredients in eight practical diets on fecal particle size (expressed as % of total collected) of rainbow trout in Experiment 2. Values for each diet are averaged by tank.

| Diet 1 | Large | Mid-Size | Fines |
|--------|-------|----------|-------|
| Fish meal | 18.3 ± 0.4 B | 27.9 ± 0.6 B | 53.8 ± 1.2 B |
| Soy protein concentrate | 1.2 ± 0.2 C | 13.7 ± 0.4 B | 85.1 ± 2.3 A |
| Soy protein concentrate + guar | 8.0 ± 0.8 BC | 64.4 ± 2.2 A | 27.6 ± 0.8 C |
| Poultry + CPC | 12.4 ± 0.6 BC | 20.3 ± 0.4 B | 67.3 ± 1.4 AB |
| Poultry + CPC + 20% SPC | 5.4 ± 0.3 C | 31.6 ± 1.0 B | 63.0 ± 1.8 AB |
| Poultry + CPC + 30% SPC | 6.8 ± 0.1 C | 31.9 ± 0.6 B | 61.3 ± 1.0 AB |
| Poultry + CPC + 20% SPC + guar | 39.9 ± 0.7 A | 35.9 ± 1.8 B | 24.2 ± 0.5 C |
| Poultry + CPC + 30% SPC + guar | 51.1 ± 1.5 A | 30.4 ± 0.3 B | 18.5 ± 0.4 C |

Significant differences between treatments are denoted by a different superscript letter. 1 For diet formulations and sources for dietary ingredients, see Table 2. CHO = carbohydrate; SPC = soy protein concentrate; CPC = corn protein concentrate; Poultry = poultry by-product meal; guar = guar gum.

4. Discussion

SBM has been the primary protein source for FM replacement [31]. Feeds containing SBM can cause gastroenteritis and diarrhea-like symptoms and produce diffuse fecal material high in fine particles in RBT [4,6]. These fine fecal particles do not settle out in the quiescent zone and make fecal removal difficult with increased suspended solids loads and dissolved pollutants, such as phosphorus, in effluent [10,19]. Processing SBM into protein concentrates or isolates not only increases protein content (>60%), but also reduces the
levels of anti-nutritional factors [22]. Using these products in place of SBM, theoretically, should improve fecal stability and reduce fine fecal particle production.

In Experiment 1, diets containing any of three protein ingredients of animal origin, including two fish meals (sardine or menhaden) and PBM, as the primary protein source produced the greatest amounts of large and mid-size fecal particles (Table 4). Diets that contained CPC as the main protein source produced similar FPS profiles, even though it is of plant origin. However, when any of the soy protein products (two types of SBM and three types of SPC) were used as the primary protein source, the level of large fecal particles was significantly lower, but the level of fine fecal particles increased significantly compared with diets containing FM. An original hypothesis is that the natural presence of oligosaccharides in SBM is responsible for the diarrheal symptom in trout, which is characterized by high portion of fecal fines. Based on this hypothesis, the diets containing SPC 1, 2, and 3, which were made by aqueous ethanol leach (SPC 1), acid leach (SPC 2), or enzymatic treatment (SPC 3), or high-protein SBM produced from low oligosaccharide soybeans (Table 1), should not cause undesirable FPS distribution, due to oligosaccharide removal by processing or plant breeding. However, in a sharp contrast to our expectation, the diets containing these refined soy protein products (Table 1) produced FPS distribution similar to the diet containing regular SBM in RBT: significantly higher fine fecal particles and lower large particles (Table 4). Only a small increase in mid-size fecal particles and some decrease in fecal fines were observed when SBM was replaced by SPC (Table 4). Therefore, the present study rejected this hypothesis. The observations in Table 4 also indicate that production of feces having a large portion of fecal fines and very low amount of large fecal particles in RBT fed soy-based diets was caused by other components rather than soluble sugars (sucrose, raffinose, and stachyose) naturally present in soybeans.

In processing SBM into SPC, either by aqueous ethanol leaching, acid leaching, or enzymatic treatment, soluble CHO (mainly simple sugar and oligosaccharides) are removed, but insoluble CHO remain [32]. Breeding soybeans with low oligosaccharides also produces a soy protein product with reduced oligosaccharides. The insoluble CHO in soybeans include mainly cellulose, hemicellulose, and lignin. They are cell wall materials and are collectively known as fiber. Because it is difficult to measure each of these components, crude fiber is commonly measured and reported. Based on the principle and method used, crude fiber is the dried residue lost on ignition after digestion and solubilization of other materials present in a fat-extracted test sample with hot sulfuric acid and then sodium hydroxide [28,33]. It is a measure of the quantity of indigestible cellulose, pentosans, lignin, and other components of this type in plant-based food or feed. Although mature soybeans contain little starch, its content was also measured because all diets contained wheat flour, which served as a binder and a major source of starch.

Careful examination of Table 3 indicates that all soy-based diets contained crude fiber higher than other diets (1.90–3.20% for the five soy-based diets vs. 0.60–1.14% for the four non-soy diets). This suggests that crude fiber content could be the causative factor for the undesirable FPS profile of RBT fed soy-based diets. Indeed, there was a significant positive correlation between the percentage of fines in RBT feces and crude fiber content in diets of Experiment 1 (Table 7). Therefore, the higher the content of crude fiber in the diets, the higher the fecal fines produced by RBT. There were also significant negative correlations between the percentage of large particles in feces and crude fiber content in the diets and between mid-size particles and crude fiber content. In contrast, the oligosaccharide level in the diets did not correlate well with fecal particle size classes, nor did sucrose present in the diets (Table 7). Starch, on the other hand, had a significant positive correlation with large fecal particle production only. The observation that oligosaccharide content in the diets had little effect on fecal particle size of RBT fed the diets was a surprise. Therefore, the existing hypothesis that the large portion of fine fecal particles generated by RBT fed soy-based diets is attributed to soy oligosaccharides present in the diets was rejected. Furthermore, although processing SBM into SPC and breeding soybeans reduced oligosaccharides, these efforts did not reduce crude fiber content in the resulting soy protein products (Table 3).
Therefore, the diets containing these soy protein products did not increase large fecal particles production or reduce fecal fines significantly (Table 4). Although CPC is also a plant-based protein source, it contained significantly lower crude fiber than soy-based protein ingredients (Table 3). The observation that, unlike the soy-based diets, the CPC-based diet produced feces with a particle size profile as good as FM-based diets also supported our new hypothesis that crude fiber acts as a causative factor of a poor FPS profile.

Table 7. Regression analysis of dietary carbohydrate components versus fecal particle size classes in Experiment 1.

| Particle Size Class | Statistic | Starch | Crude Fiber | Sucrose | Oligosaccharides | Lipid | Crude Protein | Ash  |
|---------------------|-----------|--------|-------------|---------|------------------|-------|---------------|------|
| Fines               | \( r \)   | -0.63  | 0.91        | 0.6     | 0.56             | 0.46  | -0.65         | -0.52|
| p-value             | 0.06      | <0.001 | 0.09        | 0.12    | 0.22             | 0.08  | 0.15          |      |
| Effect              | N/I 2     | Positive| N/I         | N/I     | N/I              | N/I   | N/I           | N/I  |
| Mid-Size            | \( r \)   | 0.32   | -0.66       | -0.55   | -0.58            | -0.07 | 0.54          | 0.64 |
| p-value             | 0.4       | 0.05   | 0.12        | 0.1     | 0.87             | 0.13  | 0.07          |      |
| Effect              | N/I       | Negative| N/I         | N/I     | N/I              | N/I   | N/I           | N/I  |
| Large               | \( r \)   | 0.71   | -0.91       | -0.54   | -0.47            | -0.59 | 0.60          | 0.39 |
| p-value             | 0.03      | <0.001 | 0.14        | 0.2     | 0.09             | 0.08  | 0.30          |      |
| Effect              | Positive  | Negative| N/I         | N/I     | N/I              | N/I   | N/I           | N/I  |

1 The content of oligosaccharides (which included mainly raffinose and stachyose in soy products) was expressed as raffinose equivalent.
2 None/Inconclusive.

In Experiment 1, the objective was to examine the effect of nine protein ingredients on fecal quality in RBT by keeping the same inclusion level for all respective diets. Therefore, the nutritional composition varied among diets (Table 3). Yet, regarding non-carbohydrate components in the diet, our correlation analysis showed that the content of lipid, protein, and ash in the diets had inconclusive or no correlations with any of the three categories of fecal particle size (Table 7). This observation is consistent with the available literature, which did not show or suspect such an effect. Our finding was that FM, PBM, and CPC-based diets produced better FPS profiles compared with all tested soy-based diets. In order to determine if modification of feed formulation, such as the use of mixed-protein ingredients and addition of guar gum as a binder, can overcome the negative effect of soy-based diets on RBT fecal particle production, we conducted Experiment 2. Results showed that the addition of guar gum at 0.3% to the PBM + CPC + SPC diets or the diet containing SPC only significantly improved particle size distribution of RBT feces (Table 6). In other words, the inclusion of guar gum at 0.3% into feeds could effectively overcome the negative effect of soy-based diets on RBT fecal particle composition, whether SPC in the diets was the only protein source or as a part of mixed protein sources.

Previous researchers have shown that the addition of 0.3% of guar gum to traditional FM [17,20] or high-SBM feed [11] greatly improves the quality of feces. This is accomplished by improvement of the elastic modulus and viscosity of the feces causing fecal particles to have greater stability and durability in water [17,20]. The improved durability of the feces from guar gum addition significantly correlates with increased particle size (74–95% increase) and improvement in solids retention potential [10,20]. The present study not only confirmed the effect of guar gum inclusion into diets on FPS formation of fish, but also showed the effect of crude fiber in soy protein products on FPS distribution and how this effect could be alleviated by guar gum addition.

Based on data from Experiment 2, the addition of SPC to diets (made of either single-protein sources or mixed ones) increased crude fiber content but not oligosaccharide content (Table 5). An increase in crude fiber in the diets without guar gum corresponded to high fecal fines and a low portion of large fecal particles (Table 6). Therefore, our conclusion based on Experiment 1 is also applicable to Experiment 2, that is, it is crude fiber, not oligos!accharides, in soy protein products that caused undesirable fecal particle size distribution in trout. Interestingly, the addition of guar gum did not change crude fiber
content (Table 5), even though it caused a desirable change of fecal particle size distribution (Table 6).

Furthermore, the improvement in FPS distribution from guar gum addition in Experiment 2 depended upon the level of soy protein ingredients (such as SPC) in the diet. At 20% or 30% SPC in diet (with PBM and CPC providing the remaining protein), guar gum addition caused an increase in large particles from approximately 5–6% to 40–50% that was also 2–3 times higher than the FM diet, but guar gum had little effect on formation of mid-sized particles, which remained at 30–36% and similar to the diet made with FM. However, when SPC was used as the sole protein source (56% of the diet), upon the addition of guar gum, few large particles were produced. Almost all of the reduction in fine particles resulted in a large increase in mid-sized particles (64%). Brinker et al. [10] and Brinker [20] suggested that dietary inclusion levels of guar gum greater than 0.3% may not necessarily improve fecal properties, while very high levels of guar gum may interfere with digestion and absorption of some nutrients without improvement in fecal physical properties [10]. Nevertheless, it is possible that concentrations of guar gum greater than 0.3% are required to see improvement in diets containing very high levels of soy products, such as the 56% SPC diet. It is also possible that PBM and CPC in the diets work together with guar gum in suppressing the negative effect of SPC on fecal particle size distribution.

5. Conclusions

Soybean meal has been the primary FM replacement in fish feed due to its relative low cost, high protein content, and availability. However, rainbow trout fed diets having soy protein products (soy meal or soy protein concentrate) as the main protein source tend to produce feces with a poor particle size distribution featured by a very high proportion of fines and a very low proportion of large particles. This not only causes diarrhea-like symptoms in trout but also contributes to the pollution of effluent and receiving waters. The oligosaccharides in SBM have been hypothesized as a causative factor in the poor FPS profile of RBT fed feeds high in soy products. The present study is the first to show that crude fiber (not oligosaccharides) in soy protein products (such as SBM and SPC) was likely responsible for producing the undesirable fecal particle distribution in trout fed soy-based diets. Furthermore, a change in diet formulation, such as the addition of 0.3% guar gum and use of mixed protein ingredients, can alleviate the negative effect of soy protein products on fecal particle formation of trout feces. Such information can be valuable to expand soy protein use in feed of trout and other species.

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