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Replacement of Fish Meal with Soybean Meal, Alone or in Combination with Distiller's Dried Grains with Solubles in Practical Diets for Pacific White Shrimp, *Litopenaeus vannamei*, Grown in a Clear-Water Culture System

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Abstract

The objective of this study was to evaluate inclusion of distiller's dried grains with solubles (DDGS) as partial replacement of commercial, solvent-extracted soybean meal (SBM) in fish meal-free diets for Pacific white shrimp, *Litopenaeus vannamei*. Aquaria connected to a recirculating biofiltration system were utilized to evaluate growth, survival, and feed conversion of shrimp during the 8-wk feeding trial. Each 110-L aquarium was stocked with 15 shrimp (mean individual weight 0.99 g) and fed one of five diets: a diet containing 20% fish meal (FM), which served as the control (Diet 1); a diet containing 0% FM and 52.5% SBM (Diet 2); and diets containing 0% FM and either 10, 20, or 30% DDGS as partial replacement of SBM (Diets 3, 4, and 5, respectively). Shrimp were fed according to a pre-determined feeding chart five times daily (0730, 1030, 1330, 1630, and 1930 h) and there were three replicates per dietary treatment. The results from the feeding trial demonstrated that final weight, weight gain (g), and percentage weight gain were significantly higher ($P < 0.05$) for shrimp fed Diet 1 (10.96 g, 10.01 g, and 1051%, respectively) compared to shrimp fed diets containing DDGS; however, shrimp fed diets containing DDGS had similar ($P > 0.05$) final weight, weight gain (g), and percentage weight gain as shrimp fed a diet containing 0% FM and 52.5% SBM (Diet 2). Feed conversion ratio (FCR) of shrimp fed Diet 1 (2.84) was significantly lower ($P < 0.05$) compared to shrimp fed any other diet. Survival (%) was not different ($P > 0.05$) among treatments and averaged 77.3% for the study. This study demonstrated that practical shrimp diets containing no FM had an adverse impact on growth performance of white shrimp when grown in a clear-water system and that further research is needed to refine diet formulations when culturing shrimp in these systems when attempting to feed a diet without FM.

Global aquaculture production of shrimp has grown dramatically within the last two decades. Specifically, the Pacific white shrimp, *Litopenaeus vannamei*, generated US$11 billion from a production volume of 2.7 million metric tons (m.t.) in 2010, accounts for 15% of the total value of internationally traded fishery products, and is currently the most valuable single aquaculture commodity (FAO 2012). Over 90% of farmed shrimp rely on high protein diets containing high percentages of marine fish meal (FM). FM is used as the primary protein source in shrimp diets because of its favorable nutrient profile, indispensible amino acid and fatty acid composition, palatability, and relatively high digestibility (Cruz-Suarez et al. 2007; Lemos et al. 2009; NRC 2011). However, a dichotomy exists between the rapid growth of the shrimp industry and declining production of FM.

FM is a finite resource with limited availability and high demand, and is the most expensive protein ingredient in shrimp diets. In 2006, 27%

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of the FM in the aquaculture sector was utilized in shrimp diets (Tacon and Metian 2008; Hardy 2010), and while advances have been made in FM substitution and reduction in the last decade, overall expansion of shrimp production has subsequently led to an increase in the quantity of FM utilized (Naylor et al. 2009; Hardy 2010). Typically, the inclusion of FM in shrimp diets is 20–50% of the total diet formulation, which results in higher diet and production costs. Diet costs can account for between 50 and 80% of a producer's operational costs, and thus directly influences producer profitability. Future growth and profitability within the shrimp aquaculture sector is dependent upon continued improvements in diet efficiency and formulation; specifically a reduction in the inclusion of expensive marine protein sources in shrimp diets (Hardy 2009). In addition, retailers and consumers have begun to consider both the environmental sustainability and health benefits of the foods they purchase, and there is growing concern regarding the sustainability of aquaculture practices that utilize high percentages of marine inputs, and/or deteriorate the natural environment.

Among plant-protein ingredients, soybean meal (SBM) has received the most attention and is the most widely used plant-protein ingredient in aquaculture diets because of its wide availability, nutritional consistency, balanced amino acid profile, and high digestibility (Akiyama et al. 1989; Gatlin et al. 2007; Lemos et al. 2009). Although SBM meal has several positive attributes that make it a good overall candidate ingredient for FM replacement, it also has negative attributes that may limit its use at high percentages or as the sole protein ingredient in commercial shrimp diets. Lim and Dominy (1990) reported reduced growth of shrimp fed levels of SBM greater than 28% and attributed the reduction of feed intake to diet palatability. When compared to FM, SBM have lower essential amino acid (EAA) concentrations and there are deficiencies in the EAA methionine, lysine, and threonine, as well as a lack of essential n-3 fatty acids EPA (eicopentaenoic acid) and DHA (docosohexaenoic acids) (Fox et al. 2004; Gatlin et al. 2007; NRC 2011). SBM is known to contain antinutritional factors such as trypsin inhibitors, lectins, phytic acid, saponins, antivitamins, and high levels of non-starch polysaccharides and oligosaccharides that may affect nutrient digestibility and/or availability to shrimp (Francis et al. 2001; Gatlin et al. 2007).

In addition, global demand for SBM is expected to increase and its price on world trade markets is projected to rise (Ash 2012). SBM prices have risen by 118% since 1998 from USD $197 to $431 per metric ton. In light of the rising cost of SBM, it has become imperative to evaluate suitable alternative ingredients that can be used to formulate shrimp diets that support adequate growth rates necessary for commercial production (Gatlin et al. 2007; Webster et al. 2008a; Sookying and Davis 2011).

A potential candidate ingredient for use in combination with SBM is distiller's dried grains with solubles (DDGS), a co-product of ethanol fuel production and the beverage distilling industry. Ethanol production in the USA has undergone significant expansion within the last 10 yr as a result of mandates for biofuel use in the US Energy Acts of 2005 and 2007. Increased ethanol production has led to increased production in the US which has more than doubled since 2005, from 10.4 million m.t. to 33.4 million m.t. in 2010 (Hoffman and Baker 2011). DDGS could be used as a protein source for shrimp due to its nutrient content, relative low cost per unit protein basis, lack of antinutritional factors found in other plant-based ingredients, and increasing availability. There have been studies to evaluate inclusion of DDGS in diets for tilapia (Coyle et al. 2004; Lim et al 2007); hybrid striped bass (Thompson et al. 2008); rainbow trout (Cheng and Hardy 2004); channel catfish (Webster et al. 1991, 1992, 1993; Robinson and Li 2008; Li et al. 2011); red claw crayfish (Thompson et al 2006; Garza de Yta et al. 2012); freshwater prawn (Tidwell et al. 1993a, 1993b); and Pacific white shrimp (Lemos et al 2009; Roy et al. 2009; Sookying and Davis 2011). However, no studies have been conducted to evaluate the partial replacement of SBM with increasing levels of DDGS in shrimp diets. Accordingly, the objective of this study is to evaluate the

ability of DDGS to partially replace SBM as the primary protein source in diets for juvenile Pacific white shrimp.

Materials and Methods

Diet Composition and Preparation

Experimental diets were formulated to evaluate the ability of DDGS to partially replace SBM as the primary protein source in shrimp diets. DDGS were obtained from Buffalo Trace Distillery (Frankfort, KY, USA). Five isonitrogenous (32% digestible protein basis) and isoenergetic diets were formulated and prepared to meet the known nutrient and energy requirements of penaeid shrimp (NRC 2011). Diet 1 (the control) was formulated similarly to a commercially available diet with 20% FM, and 24.5% SBM; Diet 2 completely replaced FM with SBM (52.5%); Diets 3, 4, and 5 evaluated partial replacement of SBM with DDGS at inclusion rates of 10, 20, and 30%, respectively. Supplemental L-lysine (Lys) and methionine (Met) were included in Diets 2–5 to meet the EAA profile (on a digestible amino acid basis) of the control diet (Table 1). Proximate analysis (Table 2) and amino acid composition (Table 3) of the experimental diets were determined by an independent commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA).

Dry ingredients were weighed (Mettler AT261 Delta Range, Mettler Instruments, Zurich, Switzerland) and mixed together for 1 h using a Hobart mixer (Hobart A-200, Troy, OH, USA) Warm tap water was added to obtain a 35% moisture level. Diets were double-extruded through a meat grinder with a 2-mm die to form spaghetti-like strands and air-dried on ventilated drying racks. After drying, diets were ground into pellets of appropriate size using a S.500 disk mill (Glen Mills Inc., Clifton, NJ, USA). Diets were sieved (2-mm opening mesh and 0.5-mm mesh) using a US standard testing sieve (Fisher Scientific, Pittsburg, PA, USA). After sieving, a combination of soybean oil and menhaden fish oil that had previously been mixed together was added to the diets until all pellets were uniformly coated. Lipid was added after diets had dried so as to prevent highly unsaturated fatty acids from possible loss during extrusion. Diets were stored in labeled plastic containers at −20 C until fed.

TABLE 1. *Ingredient composition of five experimental diets with different levels of distiller's dried grains with solubles (DDGS) as replacement for soybean meal (SBM) fed to juvenile* Litopenaeus vannamei.[a]

	Diets				
Ingredient	1	2	3	4	5
Soybean meal	24.5	52.5	47.5	43.0	38.5
Distiller's dried grains with solubles	0.0	0.0	10.0	20.0	30.0
Wheat flour	36.7	26.6	22.3	17.7	12.8
Wheat gluten	5.0	5.0	5.0	5.0	5.0
Poultry by-product meal	5.0	5.0	5.0	5.0	5.0
Menhaden fish meal	20.0	0.0	0.0	0.0	0.0
Menhaden fish oil	2.0	3.7	2.7	2.2	1.9
Soybean oil	2.5	2.5	2.7	2.2	1.9
Dicalcium phosphate	1.5	1.5	1.5	1.5	1.5
Magnesium sulfate	0.6	0.6	0.6	0.6	0.6
Potassium phosphate	0.8	0.8	0.8	0.8	0.8
Lecithin	0.5	0.5	0.5	0.5	0.5
Vitamin premix[b]	0.4	0.4	0.4	0.4	0.4
Lysine-HCL	0.0	0.1	0.2	0.3	0.4
Methionine	0.0	0.18	0.18	0.17	0.16
Cholesterol	0.2	0.2	0.2	0.2	0.2
Choline chloride	0.15	0.15	0.15	0.15	0.15
Mineral mix[c]	0.10	0.10	0.10	0.10	0.10
Vitamin C (Stay C)	0.08	0.08	0.08	0.08	0.08

[a]Values are expressed as percentages of the diet.

[b]Vitamin mix supplied the following (mg or IU/kg of diet): biotin, 0.64 mg; B_{12}, 0.06 mg; E (as alpha-tocopherol acetate), 363 IU; folacin, 9.5 mg; myo-inositol, 198 mg; K (as menadion sodium bisulfate complex), 3.7 mg; niacin, 280 mg; D-pantothenic acid, 117 mg; B_6, 31.6 mg; riboflavin, 57.4 mg; thiamin, 35.8 mg; D_1, 440 IU; A (as vitamin A palmitate), 6607 IU.

[c]Mineral mix supplied the following (g/kg of diet): zinc, 0.07 g; manganese, 0.02 g; copper, 0.002 g; iodine, 0.010 g.

Experimental Conditions

This experiment was conducted at the Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky, USA in an indoor recirculating saltwater system comprised of 15 aquaria (110-L each). Crystal Marine® salt mix (Marine Enterprises International, Baltimore, MD, USA) was added to dechlorinated city (tap) water to obtain salinity levels equivalent to seawater (27–28 ppt). Saltwater recirculated through a 2000-L mechanical and biological filtration system containing vertical polyester screens and polyethylene bio-balls (Red Ewald, Karnes City, TX, USA) for solids removal

TABLE 2. *Nutrient composition (%) and water stability (mean ± SE) of five experimental diets containing increasing levels of distiller's dried grains with solubles (DDGS) to replace soybean meal (SBM).*[a]

	Diets				
	1	2	3	4	5
Moisture	89.70 ± 0.01	90.01 ± 0.14	89.69 ± 0.01	89.44 ± 0.01	91.54 ± 0.09
Protein[b]	42.54 ± 0.12	41.48 ± 0.11	40.55 ± 0.65	40.42 ± 0.24	40.16 ± 0.08
Lipid[b]	10.17 ± 0.04	9.44 ± 0.06	10.10 ± 0.31	10.53 ± 0.33	11.05 ± 0.17
Ash[b]	10.00 ± 0.05	8.04 ± 0.01	8.70 ± 0.01	8.65 ± 0.05	8.61 ± 0.04
Fiber[b]	0.67 ± 0.56	2.39 ± 0.28	2.73 ± 0.17	3.41 ± 0.05	3.39 ± 0.10
WS[c]	87.0 ± 0.0[a]	81.7 ± 0.9[b]	81.7 ± 0.3[b]	82.0 ± 0.0[b]	81.7 ± 0.9[b]

WS = water stability (% dry solids).

[a]Values are expressed as means (± SE) of two replicates per diet (nutrient composition) and three replicates per diet (water stability).

[b]Dry-matter basis.

[c]Means in the same row with different superscript letters are significantly different ($P < 0.05$).

TABLE 3. *Amino acid composition (% of diet) of five experimental diets fed to shrimp for 56-d.*

	Diets					
Amino acid	1	2	3	4	5	Mean
Cystine	0.45	0.49	0.48	0.49	0.50	0.48
Methionine	0.74	0.71	0.70	0.70	0.70	0.71
Aspartic acid	3.44	3.74	3.50	3.31	3.35	3.47
Threonine	1.47	1.36	1.31	1.28	1.32	1.35
Serine	1.81	1.97	1.89	1.82	1.92	1.88
Glutamic acid	7.98	8.49	8.11	7.79	8.02	8.08
Proline	2.58	2.52	2.52	2.57	2.70	2.58
Glycine	2.14	1.80	1.79	1.72	1.80	1.85
Alanine	1.86	1.58	1.61	1.64	1.80	1.70
Valine	1.81	1.74	1.66	1.65	1.69	1.71
Isoleucine	1.61	1.63	1.55	1.51	1.54	1.57
Leucine	2.87	2.87	2.86	2.91	3.12	2.93
Tyrosine	1.15	1.16	1.16	1.14	1.18	1.16
Phenylalanine	1.81	1.91	1.84	1.80	1.85	1.84
Total lysine	2.77	2.68	2.63	2.64	2.57	2.66
Histidine	0.94	0.95	0.91	0.89	0.93	0.92
Arginine	2.38	2.46	2.31	2.13	2.21	2.30
Tryptophan	0.43	0.45	0.41	0.40	0.40	0.42

and fixed-film nitrification. Water then passes through a propeller-washed bead filter (Aquaculture Systems Technologies, New Orleans, LA, USA), which provides substrate for nitrifying (*Nitrosomonas* and *Nitrobacter*) bacteria and aides in the removal of additional nitrogenous wastes before water circulates back to the aquaria. Water was supplied to each aquarium at a rate of 4.0 L/min. An immersion heater located in the biological and mechanical filtration system was used to maintain water temperature at 29–31 C. Continuous aeration was provided by a Rotron blower (Ametek, Kent, OH, USA), which supplies atmospheric oxygen to a single 4-inch airstone in each aquarium and additional airstones in the biological and mechanical filtration components. To account for water-loss from evaporation and routine maintenance (siphoning), approximately 5% of the total water volume was replaced daily using a combination of dechlorinated city (tap) water and high salinity water (40 ppt) held in separate 380-L storage tanks. The photoperiod was provided by overhead fluorescent ceiling lights with a 14:10 light : dark cycle. All aquaria were siphoned daily to remove uneaten diet and feces. Mortalities and molting were recorded daily, and molts were removed upon notice. All tanks were covered with polyethylene mesh to prevent shrimp access to adjacent aquaria and/or losses from jumping.

Dissolved oxygen, salinity, pH, and water temperature were measured daily using a Hydrolab Quanta Water Quality Monitoring System: Model QD 02152 (HACH, Loveland, CO, USA). Total ammonia nitrogen (TAN) and nitrite levels were recorded three times per week using a HACH DR 2800 spectrophotometer (HACH).

Juvenile *L. vannamei* were obtained from Shrimp Improvement Systems (Islamorada, FL, USA). Shrimp were acclimated to the salinity and temperature of the experimental system by gradually mixing the system water with the

holding water so that environmental changes did not exceed 1 C and 1 ppt salinity every 30 min. To ensure that all shrimp were nutritionally equivalent and that a nutritional baseline was established, a maintenance/conditioning diet was fed two times per day prior to the beginning of the study (Diet 2). Once acclimated, shrimp were randomly selected and batch-weighed to determine an average weight. Juvenile shrimp with an initial mean weight of 0.99 ± 0.05 g were stocked randomly into 15,110 L aquaria at a density of 15 shrimps per aquarium ($50/m^2$). Mortalities were replaced during the first week of the feeding trial.

Shrimp were hand-fed one of five diets, five times daily at 0730, 1030, 1330, 1630, and 1930 h for 56 d, with three replicates per dietary treatment. Amounts fed were based upon a pre-determined feeding schedule to ensure that growth of shrimp would not be limited by lack of diet and were (amounts fed based on percent biomass): wk 1, 20%; wk 2 16%; wk 3, 16%; wk 4, 14%; wk 5, 10%; wk 6 8%; wk 7, 6%; and wk 8, 4%. Aquaria biomass and daily feed rations were adjusted for mortalities when they occurred, and the amount of diet fed per day was recorded for each aquaria.

Pellet Stability

Dried experimental diets were evaluated for pellet stability in water. Ten grams of pelleted diet of equal length were uniformly distributed on a 2-mm mesh screen sieve (Fisher Scientific, Pittsburg, PA, USA). Samples were then lowered into static saltwater approximately 10 cm deep for 30 min, dried in an oven (60 C) for 24 h, and then cooled in a desiccator for 12 h prior to weighing. The residue left on the screen was recorded as dry solids not leached in water. The percentage of dry solids on the screen after 30 min in water to total solids in pellets was used as a comparative measure of pellet stability in water (Webster et al. 1994).

Data Analysis

At the conclusion of the 56 d feeding trial, shrimp were harvested, chill-killed in an ice-water bath to drastically reduce body temperature, weighed, placed into plastic storage bags, and frozen for subsequent amino acid, protein, moisture, lipid, fiber, and ash proximate analysis. Response parameters were recorded as:

Final individual weight (g/shrimp);
Weight gain (g) = mean individual final weight − mean initial individual weight;
Percent weight gain (%) = [(final weight − initial weight) × 100]/initial weight;
Protein efficiency ratio (PER) = wet weight gain/dry protein fed);
Feed conversion ratio (FCR) = dry weight feed intake/final weight gain; and
Percent survival (%) = (final number of shrimp/initial number of shrimp) × 100.

Data were analyzed by a mixed-model analysis of variance (PROC MIXED) with contrasts using Tukey's HSD test. Contrast statements were constructed to compare response variables of the experimental diets (2–5) with those of the control (Diet 1). Furthermore, regression analysis was also conducted on the data using PROC REG for corroboration. Statistical analysis was performed using SAS Systems. v. 9.1 (SAS Institute, Cary, NC, USA). All percentage and ratio data were log transformed prior to analysis (Zar 1984). Differences among mean responses were considered significant at the $P < 0.05$ probability level.

Results

Water quality parameters observed in the experimental system for the duration of the feeding trial were optimal for growth and survival of white shrimp and averaged: temperature (29.4 C), salinity (30.7 ppt), dissolved oxygen (4.69 mg/L), pH (7.71), TAN (0.12 mg/L), and nitrite (0.09 mg/L) for the duration of the study.

At the conclusion of the 56-d feeding trial, final weight, and weight gain (g) of shrimp fed Diet 1 (control) were significantly ($P < 0.05$) higher compared to shrimp fed diets containing DDGS as partial replacement of SBM (Diets 3–5), but not significantly different ($P > 0.05$) compared to shrimp fed a diet without FM and containing 52.5% SBM (Diet 2; Table 4).

Shrimp fed all diets containing DDGS had similar ($P > 0.05$) final weight, weight gain, and percent weight gain as shrimp fed Diet 2. Percent weight gain was significantly ($P < 0.05$) higher (1051%) in shrimp fed Diet 1 compared to all other dietary treatments, but no significant differences ($P > 0.05$) in percent weight gain among shrimp fed Diets 2–5. FCR of shrimp fed Diet 1 was significantly lower ($P < 0.05$) compared to all other dietary treatments (2.84) while shrimp fed a diet containing 10% DDGS (Diet 3) had the highest FCR (9.27) and was significantly different ($P < 0.05$) compared to shrimp fed Diet 1 (control) and Diet 2 (52.5% SBM), but was not different ($P > 0.05$) compared to shrimp fed diets containing 20% (Diet 4) and 30% (Diet 5) DDGS (Table 4). Percent survival was not significantly different ($P > 0.05$) among all treatments and averaged 77.3% for the feeding trial.

Discussion

This is the first study to investigate the replacement of FM and SBM in diets for white shrimp with DDGS and data indicate that complete replacement of FM by either SBM and/or DDGS resulted in reduced growth and increased FCR in shrimp. There has been limited success of totally replacing FM in shrimp diets depending upon the nutrient quality and composition of the substitution ingredient(s), diet formulation, and culture system utilized (clear-water versus green-water). Furthermore, several feeding trials have been conducted under varying experimental and production conditions that have shown that SBM, used in association with terrestrial plant and/or animal protein sources, can be used to partially or totally replace FM as the primary protein source in shrimp diets without adverse effects on growth performance or survival (Alvarez et al. 2007; Davis et al. 2008). Webster et al. (1992) stated that use of two or more complimentary protein sources is more appropriate as a strategy to replace FM in practical diets than using a single source of protein. This approach could compensate for possible nutrient deficiencies of the single ingredient, increase cost-effectiveness, reduce effects of antinutritional factors through reduction of ingredients that contain them, increase palatability, and increase pellet stability of diets without FM (Davis and Arnold 2000; Davis et al. 2004; Fox et al. 2004; Samocha et al. 2004; Davis et al. 2008; Webster et al. 2008b; Sookying and Davis 2011; Yue et al. 2012).

Use of complimentary protein sources in shrimp diets has been evaluated (Mendoza-Alfaro et al. 2001; Molina-Poveda and Morales 2004: Suarez et al. 2009; Ye et al. 2011; Yue et al. 2012), but growth reduction is often associated when shrimp are fed protein sources at high levels of FM replacement. In light of the rising cost of SBM, it has become imperative to evaluate suitable alternative ingredients that can be used to formulate shrimp diets that support growth rates acceptable for commercial production (Gatlin et al. 2007; Webster et al. 2008a; Sookying and Davis 2011). In this study, use of DDGS as partial replacement of SBM resulted in reduced growth compared to shrimp fed a diet containing 20% FM; however, final weight, weight gain of shrimp fed all diets containing DDGS were similar to shrimp fed a diet containing SBM as the major protein source (Diet 2).

Sookying and Davis (2011) fed white shrimp a diet containing 58% SBM and 10% DDGS as total replacement of FM and reported no difference in final weight when compared to shrimp fed a control diet containing 10% FM when grown in ponds or outdoor tanks supplied with pond water. These data are similar to Roy et al. (2009), who reported high growth rates when shrimp were fed the same diets as used in Sookying and Davis (2011) in either a clear-water system or a green-water system. However, it was reported that shrimp grown in the clear-water system had dramatically reduced growth compared to those grown in the green-water system (Roy et al. 2009).

Among plant-protein sources, SBM is considered one of the most nutritious and is the most widely used protein ingredient in shrimp diets because of its balanced amino acid composition, reliable availability, and consistent nutrient composition. However, use of SBM

TABLE 4. *Growth responses of juvenile Pacific white shrimp (initial weight 0.99 g ± 0.05 SE) fed five experimental diets with increasing levels of DDGS replacing SBM for 56-d.*[a]

	Diet				
Parameter	1	2	3	4	5
Final weight (g/shrimp)	10.96 ± 0.62^{a}	8.14 ± 1.56^{ab}	4.46 ± 0.53^{b}	6.54 ± 0.54^{b}	6.10 ± 0.30^{b}
Weight gain (g)	10.01 ± 0.63^{a}	7.09 ± 1.46^{ab}	3.64 ± 0.56^{b}	5.52 ± 0.52^{b}	5.01 ± 0.08^{b}
Weight gain (%)	1051 ± 82^{a}	670 ± 82^{b}	452 ± 82^{b}	547 ± 67^{b}	491 ± 81^{b}
FCR[b]	2.84 ± 0.26^{c}	5.06 ± 0.42^{b}	9.27 ± 1.78^{a}	6.57 ± 0.31^{ab}	7.66 ± 0.76^{ab}
Survival (%)	88.89 ± 4.44^{a}	73.33 ± 7.70^{a}	82.22 ± 2.22^{a}	71.11 ± 8.01^{a}	71.11 ± 4.44^{a}

[a]Values are represented as means of three replicates per dietary treatment (± SE). Numbers within the same row with different superscripts are significantly different ($P < 0.05$).

[b]Feed conversion ratio (FCR) = dry weight feed intake (g)/final weight gain (g).

as the sole protein source in aquafeeds has limitations due to lower levels of methionine and lysine compared to animal-source proteins, reduced palatability, presence of antinutritional factors, low levels of phosphorus, and lack of n-3 highly unsaturated fatty acids. When SBM has been used in combination with other protein sources in shrimp diets, acceptable growth and FCR have been reported (Amaya et al. 2007a, 2007b; Davis et al. 2004; Samocha et al. 2004; Roy et al. 2009; Sookying and Davis 2011); however, other studies have reported reduced growth in shrimp when SBM has been used as total replacement for FM (Lim and Dominy 1990; Molina-Poveda and Morales 2004).

In this study, growth performance of white shrimp significantly decreased when DDGS replaced SBM and while not statistically different, shrimp fed a diet without FM and in which SBM comprised the major protein source had reduced (26%) final weight compared to shrimp fed a diet containing FM and a significant reduction in percentage weight gain and higher FCR. It may be that differences in culture conditions among feeding studies results in the discrepancies found in the literature. Feeding studies that use green-water systems (ponds or tanks) where natural productivity contributes to the total nutrient intake of the shrimp, or studies where the control diet contains <15% FM, appear to show that total replacement of FM with SBM, either alone or in combination with other protein sources, is possible. Feeding studies that use clear-water systems, where there are no exogenous supplemental nutrient sources, seem to indicate that complete replacement of FM is more problematic. The presence of supplemental nutrients in a feeding study or lack of a true control diet (diet with >19% FM) fed during the study make valid FM replacement comparisons difficult (Amaya et al. 2007b; Roy et al. 2009; Sookying and Davis 2011).

Reasons for the reduced growth in shrimp fed increasing levels of DDGS are not readily apparent. All diets were formulated to contain similar levels of digestible protein and EAAs met nutrient requirements for white shrimp (NRC 2011). Shrimp fed Diet 1 (control diet) had growth rates of shrimp comparable to, or higher than, previous studies (Molina-Poveda and Morales 2004; Alvarez et al. 2007; Roy et al. 2009; Morris et al. 2011; Sookying and Davis 2011; Ye et al. 2011; Sanchez et al. 2012; Yue et al. 2012; Bulbul et al. 2013; Sa et al. 2013). Likewise, from visual observations of shrimp feeding, palatability of all diets appeared high. Upon feeding, shrimp actively and rapidly swam to diet pellets and began to consume them. One possibility could be pellet stability. While data may indicate to the contrary, it seemed that all diets containing DDGS rapidly disintegrated when shrimp began to manipulate and consume the pellets. While pellet stability data indicated that there were no differences among diets, the water stability test used was a static test. It may be that as shrimp began to consume the pellets, diets containing DDGS began to fall apart more rapidly. Visual observation supports this notion as it was

noticed that within a few seconds after shrimp began consuming the diet, much of the pellet was lying on the bottom of the aquarium with DDGS particles clearly visible. Thus, it may be that since shrimp could not consume the pellet in its entirety, they did not obtain the amounts of nutrients required for optimal growth.

A second possible reason for reduced growth could be DDGS quality. Nutrient composition and nutritional quality of DDGS varies among sources. There are several by-products from the corn ethanol industry, each with their own nutrient composition and quality. These include DDGS, distiller's solubles (DS), corn endosperm (CE), high-protein distiller's dried grains (HPDDG), and high-protein DDGS. The corn DDGS used in this study was from a distillery which may have had lower nutrient digestibility compared to DDGS from another source. It has been reported that there are large variations in nutrient composition of wheat DDGS among different production facilities (Cozannet et al. 2010; Reveco et al. 2011) as well as within the same facility (Bandegan et al. 2009). This has also been reported for corn DDGS (Spiehs et al. 2002; Kleinschmitt et al. 2007). Furthermore, large variations in nutritional value of corn DDGS in pigs and chickens has been reported, especially for lysine (Cromwell et al. 1993). The principal factors in these variations are the processing conditions, especially drying temperatures used. If drying temperatures are too high, it may result in Maillard reactions which decrease lysine digestibility.

It has been reported that shrimp diet intake has an inverse relationship as FM is replaced by plant-protein sources (Lim and Dominy 1990; Forster et al. 2003). In this study, as a predetermined feeding regime was used, it is not possible to make comparisons of diet intake among treatments. However, it was shown that FCR increased in all dietary treatments (Diets 2–5) compared to shrimp fed a diet with FM (Diet 1), which is probably due to overfeeding because of the reduced final weights of shrimp fed Diets 2–5. It is obvious that the percentages established *a priori* were too high as evidenced by the higher FCR values of shrimp compared to other published reports, but the calculated feeding percentages were reasonable. FCR of shrimp fed Diet 1 was somewhat higher compared to other reported values (Roy et al. 2009; Morris et al. 2011; Sookying and Davis 2011; Ye et al. 2011; Bulbul et al. 2013), but most of those studies used green-water conditions where natural productivity could increase growth and artificially decrease FCR values. This study was conducted in a closed, recirculating system where there was no possibility of exogenous nutrients. When compared to studies conducted in clear-water systems (Molina-Poveda and Morales 2004; Izquierdo et al. 2006; Alvarez et al. 2007; Sanchez et al. 2012; Sa et al. 2013), FCR reported in this study are similar. In a culture system where there are no exogenous sources of nutrients, it may be better to overfeed so as not to limit access to food and thereby artificially reduce growth rates. Feeding percentages can be reduced in green-water systems because there is continuous access to supplemental nutrients and FCR values have been reported to be much lower.

There was no difference in survival percentage in this study for any dietary treatments. All measured water quality parameters were within optimal ranges for white shrimp growth and health. While the average survival percentage in this study was lower than other reports (Molina-Poveda and Morales 2004; Roy et al. 2009; Morris et al. 2011; Ye et al. 2011), several of those studies used green-water culture systems where there was continuous access to supplemental nutrients. Percentage survival in this study, however, was similar to studies where clear-water culture systems were used (Izquierdo et al. 2006; Liu et al. 2012; Ye et al. 2012; Bulbul et al. 2013).

Under the conditions and diets fed in this study, it can be concluded that partial replacement of SBM with DDGS is not recommended until further refinement of diet formulations so as to determine the reason for the reduced growth observed. Further studies on diet stability, binder use and type, and DDGS particle size and their impact on shrimp growth should be conducted to improve economical, sustainable diet development for shrimp.

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