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ARTICLE

Use of Lipid-Extracted Distillers Dried Grain with Solubles (DDGS) in Diets for Pacific White Shrimp

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Abstract

A series of trials (one growth trial and two digestibility trials) were conducted to evaluate the response of Pacific white shrimp *Litopenaeus vannamei* to the inclusion of lipid-extracted distillers dried grain with solubles (LE-DDGS). In the growth trial, a practical basal diet was developed for shrimp using soybean meal (56.76% diet) and fish meal (6% diet) as the primary protein sources. The LE-DDGS was substituted for soybean meal on an isonitrogenous basis at five levels of inclusion (0, 10, 20, 30, and 40%) with lysine supplemented to diets containing 30% and 40% LE-DDGS. A sixth diet containing 40% LE-DDGS but without a lysine supplement was also evaluated. The diets were offered to four replicate (15 shrimp/tank) groups of shrimp per treatment over an 8-week growth trial. At the end of the growth trial shrimp offered diets containing from 0% to 20% LE-DDGS performed similarly. Performance of shrimp fed higher levels of LE-DDGS was reduced but similar to each other. Removing the lysine supplement from the diet containing 40% LE-DDGS did not result in reduced performance of the shrimp, indicating lysine was not limiting in these feeds. In two digestibility trials, the LE-DDGS digestibility coefficients were determined in Pacific white shrimp for dry matter (ADMD), energy (AED), and crude protein (APD) using 1% chromic oxide as the inert marker with 70:30 replacement strategies. The ADMD, AED, and APD coefficient values for LE-DDGS were 53.77, 36.94, and 55.71 for digestibility trial 1 and 42.43, 44.65, and 20.87 for digestibility trial 2, respectively. Results from the digestibility data do not match the results of the growth trial, as poor digestibility would have resulted in more pronounced reduction in performance. Hence, this digestibility technique may not be appropriate for this type of ingredient. Based on the observed results the inclusion of LE-DDGS up to 20% of the diet is recommended.

As world shrimp production expands, the need for cost-effective protein sources continues to increase. There has been considerable effort to replace fish meal (FM) with a variety of proteins from terrestrial animals and plants (Tacon and Akiyama 1997). Demand for alternative proteins, including plant proteins such as soybean meal, is also increasing the cost. It is crucial to keep the feed cost down and use a combination of proteins to reduce costs and meet nutrient requirements. Using a variety of proteins helps to balance the amino acid profile, increase palatability, reduce the effects of antinutritional factors, and provide possible immunological benefits (Samocha et al. 2004). The aquaculture industry has become more interested in distillers

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dried grain with solubles (DDGS) as a protein source due to its inexpensive cost compared with other plant proteins. Due to the increased production of ethanol, the coproduct DDGS is currently less expensive than soybean meal. Distillers dried grain with solubles is a byproduct of the fuel ethanol industry and is produced in the fermentation process of cereal grain starch (Spiehs et al. 2002; Świątkiewicz and Koreleski 2008). There are several grains from which DDGS can be manufactured including corn, sorghum, wheat, and other cereal grains (the first word in the name is the predominating grain); the most common is corn DDGS.

Several studies have successfully used DDGS as a protein source in aquaculture diets for Channel Catfish *Ictalurus punctatus* (Webster et al. 1991, 1992, 1993; Robinson and Li 2008; Lim et al. 2009; Li et al. 2010; Zhou et al. 2010), sunshine bass (White Bass *Morone chrysops* × Striped Bass *M. saxatilis*) (Thompson et al. 2008; Trushenski and Gause 2013), Rainbow Trout *Oncorhynchus mykiss* (Cheng et al. 2003; Cheng and Hardy 2004a; Stone et al. 2005), tilapia *Oreochromis* spp. (Wu et al. 1994, 1996; Coyle et al. 2004a; Lim et al. 2007; Shelby et al. 2008; Schaeffer et al. 2009; Chatvijitkul 2013), Yellow Perch *Perca flavescens* (Schaeffer et al. 2011), freshwater prawns *Macrobrachium rosenbergii* (Tidwell et al. 1993; Coyle et al. 2004b), redclaw crayfish *Cherax quadricarinatus* (Thompson et al. 2006; Garza de Yta et al. 2012), and Pacific white shrimp *Litopenaeus vannamei* (Lemos et al. 2009; Lim et al. 2009; Roy et al. 2009; Sookying and Davis 2011; Zhou 2014). Based on these studies, there are recommendations for maximum inclusion levels of DDGS for Channel Catfish, Rainbow Trout, freshwater prawns, and tilapia. The recommendation for the maximum inclusion of DDGS in diets for Pacific white shrimp is still not available, as more than 10% DDGS has not been evaluated in practical shrimp diets. Therefore, more work to evaluate higher levels of DDGS inclusion in shrimp diets is warranted.

The quality of DDGS varies with grain source and processing conditions. However, high-quality reduced oil or lipid-extracted DDGS (LE-DDGS) processing can remove the lipid and increase the protein concentration of DDGS, which makes it a more valuable feed component (Rausch and Belyea 2006). High-protein and LE-DDGS products have been used in agricultural diets for poultry, swine, and cattle (USGC 2012). However, the data on LE-DDGS inclusion in aquaculture diets is very limited with the exception of use in hybrid tilapia (Nile Tilapia *O. niloticus* × Blue Tilapia *O. aureus*) (Chatvijitkul 2013). Consequently, the purpose of this study was to evaluate the growth response of Pacific white shrimp fed diets with LE-DDGS substituted for soybean meal on an isonitrogenous basis at five levels of inclusion (0, 10, 20, 30, and 40%).

METHODS

Growth trial.—A series of five diets were formulated substituting soybean meal with increasing levels of LE-DDGS—0, 10, 20, 30, and 40%—on an isonitrogenous basis. To maintain minimal levels of lysine, diets containing 30% and 40% LE-DDGS were supplemented with crystalline lysine. A sixth diet was formulated with 40% LE-DDGS but without a lysine supplement (Table 1). The nutrient composition (on an as-is basis) of LE-DDGS was determined by the University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia, Missouri, to contain 29.83% crude protein, 4.81% lipid, 6.78% crude fiber, 1.04% lysine, and 0.62% methionine. Practical diets were produced to have similar proximate analyses with 35% protein and 8% lipid. Diets were prepared in the Aquatic Animal Nutrition Laboratory at the School of Fisheries Aquaculture and Aquatic Sciences, Auburn University, Auburn, Alabama, using standard procedures for the laboratory production of shrimp feeds described by Zhou et al. (2015). In short, preground dry ingredients and oil were mixed in a food mixer (Hobart, Troy, Ohio) for approximately 10–15 min. Boiling water was then blended into the mixture to obtain a consistency appropriate for pelleting. Diets were pressure-pelleted using a meat grinder with a 3-mm die. The wet diets were dried overnight in a fan-ventilated oven (<50°C) to obtain a moisture content of 8–10%. Diets were stored at −20°C, and prior to use each diet was ground and sieved to an appropriate size. The diets were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories for proximate composition (Table 1) and amino acid profiles (Table 2).

Postlarval Pacific white shrimp were obtained from Shrimp Improvement Systems, Islamorada, Florida, and nursed in an indoor recirculating system at the Claude Peteet Mariculture Center, Gulf Shores, Alabama. Juveniles from the nursery system (initial mean weight, 0.49 g) were stocked into twenty-four 0.22-m^3 square tanks (0.6 × 0.6 × 0.6 m) with four replicates per treatment, at a density of 15 shrimp per tank. The recirculating system consisted of a water pump and supplemental aeration (using a central line, regenerative blower, and air diffusers) as well as mechanical and biological filtration. Culture systems were located in a greenhouse, which provided a natural light cycle of approximately 14 h light : 10 h dark. All tanks were covered by netting to prevent the shrimp from jumping out the tank. Based on the historic results in this facility, daily feed inputs were precalculated on a weekly basis using an expected feed conversion ratio (FCR) of 1.8:1, and shrimp would double their size each week up to 1 g then gain 0.8 g per week thereafter. Shrimp were counted weekly to adjust feed input throughout the trials based on survival. Shrimp were offered test diets four times per day. Water samples were collected biweekly to measure total ammonia nitrogen (TAN) using an ion selective electrode (Orion EA 940, Thermo Electron, Beverly, Massachusetts). Nitrite-nitrogen and nitrate-nitrogen levels were monitored utilizing test kits (3354-01, 3352-01, LaMotte, Chestertown, Maryland) once per week and converted to nitrite and nitrate. Water quality conditions including temperature, dissolved oxygen (DO), salinity, and pH were monitored twice daily using a multiprobe YSI ProPlus meter (YSI, Yellow Springs, Ohio). Survival,

TABLE 1. Ingredient compositions (g/100 g, as is) of six experimental Pacific white shrimp diets designed to contain increasing percentages of LE-DDGS (0 [i.e., 0DDGS], 10, 20, 30, and 40% and 40% without lysine [–L]). Proximate compositions (%, as is) were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories.

	Experimental diet					
Ingredient	0DDGS	10DDGS	20DDGS	30DDGS	40DDGS	40DDGS-L
Menhaden fish meal[a]	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal[b]	56.76	49.89	43.00	35.99	28.91	29.25
DDGS-lipid extracted[c]	0.00	10.00	20.00	30.00	40.00	40.00
Menhaden fish oil[a]	5.51	5.03	4.56	4.08	3.61	3.60
Corn starch[d]	11.73	9.08	6.44	3.87	1.33	1.15
Whole wheat[d]	9.00	9.00	9.00	9.00	9.00	9.00
Trace mineral premix[e]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[f]	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride[g]	0.20	0.20	0.20	0.20	0.20	0.20
Stay C[h]	0.10	0.10	0.10	0.10	0.10	0.10
Mono-dicalcium phosphate[d]	2.35	2.35	2.35	2.35	2.35	2.35
Lecithin[i]	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol[d]	0.05	0.05	0.05	0.05	0.05	0.05
Corn protein concentrate[j]	5.00	5.00	5.00	5.00	5.00	5.00
L-lysine[d]	0.00	0.00	0.00	0.06	0.13	0.00
Proximate composition (%)						
Crude protein	36.87	37.04	36.56	35.97	35.81	36.01
Moisture	4.11	3.69	4.00	3.74	5.03	4.52
Crude fat	8.71	8.80	9.27	9.41	9.40	9.50
Crude fiber	3.53	4.24	4.23	4.99	5.40	5.71
Ash	7.12	7.22	7.29	7.38	7.53	7.63

[a]Omega Protein, Reedville, Virginia.
[b]Dehulled solvent extracted soybean meal, Bunge, Alabama.
[c]Dakota Gold BPX, Poet Nutrition, Sioux Falls, South Dakota.
[d]MP Biochemicals, Solon, Ohio.
[e]Trace mineral premix without Mg (g/100 g premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.250; ferrous sulfate, 4.0; manganous sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; filler, 81.826.
[f]Vitamin premix (g/kg premix): thiamin HCL, 4.95; riboflavin, 3.83; pyridoxine HCL, 4.00; Ca-pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; vitamin A acetate (500,000 IU/g), 0.32; vitamin D_3 (1,000,000 IU/g), 0.05; vitamin E acetate (250 IU/g), 80.00; menadione, 0.50; alpha-cellulose, 756.81. Choline chloride, 100 g/kg, was added separately.
[g]Fisher Scientific, Fair Lawn, New Jersey.
[h]Stay C (L-ascorbyl-2-polyphosphate, 35% Active C), Roche Vitamins, Parsippany, New Jersey.
[i]Enhance D-97, The Solae Company, St. Louis, Missouri.
[j]Empyreal75 Cargill, Blair, Nebraska.

final weight, and FCR were determined at the end of the 53-d experimental period.

Digestibility.—Two trials were conducted to determine apparent digestibility coefficients (ADC) for dry matter (ADMD), protein (APD), and energy (AED). The basal diet (approximately 28% crude protein; Table 3) and test diet (70:30 mixture of basal diet and test ingredient on a dry matter basis) were prepared as described previously for the growth trial diets. The first digestibility trial tested the LE-DDGS ingredient and second trial tested LE-DDGS and sorghum DDGS (unground and finely ground) ingredients determined using the 70:30 replacement strategies with chromic oxide (Cr_2O_3; 1%) as the inert marker. During the feeding period, DO, temperature, and salinity were monitored twice daily (0830 and 1630 hours) using an YSI 650 meter (YSI). Two independent digestibility trials were conducted with two different population of Pacific white shrimp, For both trials, shrimp (approximately 7 g mean weight, 8 shrimp per tank) were stocked in a closed recirculating system consisting of a series of 80-L (0.62 × 0.31 × 0.43 m) glass tanks, a biological filter, reservoir, circulation pump, and supplemental aeration. Each test diet was offered to shrimp in six tanks; for every pair of tanks the fecal sample was pooled to produce three replicate samples per diet. Shrimp were allowed to acclimate to each diet for at least 3 d before starting the collection of feces, after which feces were collected for 4–5 d. Prior to each feeding, the tanks were cleaned by siphoning. The shrimp were then offered an excess of feed. One hour after offering the feed, feces were collected by siphoning onto a 500-μm-mesh screen. Shrimp were offered four feedings per day. Feces obtained after the first feeding were discarded to

TABLE 2. Amino acid content (%) of practical diets formulated for Pacific white shrimp (see Table 1 for diet abbreviations and details) at Auburn University and analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories.

Amino acid component	Experimental diet					
	0DDGS	10DDGS	20DDGS	30DDGS	40DDGS	40DDGS-L
Alanine	1.84	1.94	2.00	2.00	2.11	2.10
Arginine	2.37	2.33	2.19	2.06	1.96	1.94
Aspartic acid	3.60	3.53	3.29	3.15	3.01	2.97
Cysteine	0.48	0.50	0.49	0.51	0.53	0.53
Glutamic acid	6.38	6.39	6.17	5.84	5.80	5.73
Glycine	1.65	1.64	1.60	1.52	1.55	1.53
Histidine	0.92	0.94	0.93	0.91	0.91	0.91
Hydroxylysine	0.07	0.04	0.07	0.08	0.09	0.09
Hydroxyproline	0.28	0.26	0.26	0.27	0.31	0.27
Isoleucine	1.58	1.58	1.58	1.50	1.48	1.49
Leucine	3.18	3.32	3.42	3.41	3.52	3.52
Lysine	2.10	2.08	1.96	1.88	1.88	1.73
Methionine	0.59	0.62	0.60	0.63	0.65	0.64
Ornithine	0.02	0.03	0.03	0.03	0.04	0.04
Phenylalanine	1.84	1.78	1.83	1.77	1.76	1.75
Proline	1.92	1.99	2.10	2.09	2.18	2.19
Serine	1.59	1.62	1.54	1.54	1.52	1.45
Taurine	0.08	0.08	0.08	0.07	0.08	0.07
Threonine	1.34	1.36	1.32	1.30	1.30	1.27
Tryptophan	0.45	0.44	0.43	0.42	0.38	0.36
Tyrosine	1.35	1.35	1.37	1.35	1.37	1.35
Valine	1.74	1.77	1.79	1.74	1.74	1.74

ensure the fecal strands were from the current day's feed. Collected feces were rinsed with distilled water and dried at 95°C and then stored in a freezer (−20°C). Gross energy of diets and fecal samples were analyzed with a semi microbomb calorimeter (model 1425, Parr Instrument, Moline, Illinois). Chromic oxide concentrations were determined by the method of McGinnis and Kasting (1964) in which, after a colorimetric reaction, absorbance was read on a spectrophotometer (Spectronic Genesys 5, Milton Roy, Rochester, New York) at 540 nm. Protein was determined by micro-Kjeldahl analysis (Ma and Zuazago 1942). The apparent digestibility coefficients for dry matter (ADMD), protein (APD), and energy (AED) were calculated according to Maynard et al. (1969) and Hardy and Barrows (2002), as follows:

$$\text{ADMD}\,(\%) = 100 - \left[100 \times \left(\frac{\%\,Cr_2O_3 \text{ in feed}}{\%\,Cr_2O_3 \text{ in feces}}\right)\right]$$

$$\text{APD and AED}\,(\%) = 100 - \left[100 \times \left(\frac{\%\,Cr_2O_3 \text{ in feed}}{\%\,Cr_2O_3 \text{ in feces}} \times \frac{\%\text{ nutrient feces}}{\%\text{ nutrient feed}}\right)\right]$$

Statistical analysis.—All data were subjected to a one-way ANOVA to determine significant ($P < 0.05$) differences among the treatment means. When appropriate, a Student–Neuman–Keuls multiple range test was used to distinguish significant differences between treatment means. All statistical analyses were conducted using SAS version 9.3 (SAS Institute, Cary, North Carolina).

RESULTS

Growth

Water quality variables remained within acceptable levels throughout the 53-d trial. The average ± SD for each variable was: temperature, 26.8 ± 1.6°C; salinity, 17.9 ± 0.9‰; DO, 6.26 ± 0.28 mg/L; pH, 7.81 ± 0.24; TAN, 0.04 ± 0.05 mg/L; nitrite, 1.00 ± 0.97 mg/L; nitrate, 40.22 ± 21.92 mg/L. At the end of the 53-d trial, no significant differences were observed in shrimp survival, which ranged from 95.0% to 100.0% ($P = 0.1469$). The performance of shrimp offered diets containing 10% LE-DDGS was significantly higher, as indicated by final mean weight ($P = 0.0129$), final biomass ($P = 0.0314$), and FCR ($P = 0.0152$), than that of shrimp offered diets containing 30% and 40% LE-DDGS and 40% LE-DDGS without lysine

TABLE 3. Ingredient formulation (g/100 g) of Pacific white shrimp reference diet for digestibility trials 1 and 2.

Ingredients	Reference diets
Menhaden fish meal[a]	10.00
Soybean meal[b]	32.50
Menhaden fish oil[a]	4.20
Whole wheat[c]	47.60
Trace mineral premix[d]	0.50
Vitamin premix[e]	1.80
Choline chloride[f]	0.20
Stay C[g]	0.10
CaP-dibasic[c]	1.00
Lecithin[h]	1.00
Cholesterol[c]	0.10
Chromic oxide[i]	1.00

[a]Omega Protein, Reedville, Virginia.
[b]Dehulled solvent extracted soybean meal, Bunge, Alabama.
[c]MP Biochemicals, Solon, Ohio.
[d]Trace mineral premix without Mg (g/100 g premix): cobalt chloride, 0.004; cupric sulfate pentahydrate, 0.250; ferrous sulfate, 4.0; manganous sulfate monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulfate heptahydrate, 13.193; filler 81.826.
[e]Vitamin premix (g/kg premix): thiamin. HCL, 4.95; riboflavin, 3.83; pyridoxine HCL, 4.00; Ca-pantothenate, 10.00; nicotinic acid, 10.00; biotin, 0.50; folic acid, 4.00; cyanocobalamin, 0.05; inositol, 25.00; vitamin A acetate (500,000 IU/g), 0.32; vitamin D_3 (1,000,000 IU/g), 0.05; vitamin E acetate (250 IU/g), 80.00; menadione, 0.50; alpha-cellulose, 756.81.
[f]Fisher Scientific, Fair Lawn, New Jersey.
[g]Stay C (L-ascorbyl-2-polyphosphate, 35%), Roche Vitamins, Parsippany, New Jersey.
[h]Enhance D-97, The Solae Company, St. Louis, Missouri.
[i]The Solae Company, St. Louis, Missouri.

supplementation (Table 4). However, growth performance indicators of the shrimp fed 40% LE-DDGS with lysine supplementation were not significantly different from those of shrimp fed 40% LE-DDGS diet without lysine supplementation.

Digestibility

The water quality remained within acceptable levels throughout both trials. The ADCs for ADMD, APD, and AED in the reference diet, test diets, and ingredients LE-DDGS and sorghum DDGS are presented in Table 5. The LE-DDGS ingredient coefficients for trial 1 were: ADMD, 53.77 ± 5.77; APD, 36.94 ± 9.45; and AED, 55.71 ± 4.91; and for trial 2 were: ADMD, 42.43 ± 0.67; APD, 44.65 ± 4.06; and AED, 20.87 ± 0.73. The AED-I values of LE-DDGS for trial 2 was much lower than those in trial 1. In trial 2, the APD-I values for DDGS sorghum unground and DDGS sorghum ground were similar, but the AED-I value for DDGS sorghum unground was much lower than that for DDGS sorghum ground.

DISCUSSION

Distillers dried grain with solubles is included in many aquaculture diets in combination with other plant proteins to decrease the cost of feed and balance nutrient content. The quality and nutritional composition of DDGS varies from the grain source (e.g., corn, wheat, sorghum, and yeast), the processing methods, and the producer. Spiehs et al. (2002) reported the proximate composition from 119 samples of DDGS, indicating the protein content of corn DDGS can range from 28.7% to 32.9% and lipid can vary from 8.8% to 12.4%. As a single protein source DDGS does not have a balanced essential amino acid (EAA) profile as it is especially low in lysine and sulfur-containing amino acids (cysteine and methionine). To meet the nutritional requirements of shrimp, higher inclusion levels of DDGS in the diet may require supplementing lysine. Spiehs et al. (2002) reported that average EAA values for lysine, methionine, tryptophan, threonine, arginine, histidine, phenylalanine, isoleucine, leucine, and valine were 0.85, 0.55, 0.25, 1.13, 1.20, 0.76, 1.47, 1.12, 3.55, and 1.50%, respectively. The corn LE-DDGS product used in the current study (Dakota Gold BPX, Poet Nutrition, Sioux Falls, South Dakota) was within the reported range for protein (29.8%) but had a much lower lipid content (4.81%) as it is a lipid-extracted form of DDGS.

A low level of lysine in DDGS is a possible disadvantage. For example, Chatvijitkul (2013) documented that LE-DDGS

TABLE 4. Response of juvenile Pacific white shrimp (initial mean weight, 0.49 g) to diets containing incremental levels of LE-DDGS (0, 10, 20, 30, and 40% and 40% with lysine [L]; see Table 1 for diet abbreviations and details) over a 53-d culture period reared in square tanks. Values represent mean ± SD ($n = 4$). Values in the same column with different letters are significantly different ($P < 0.05$) based on ANOVA followed by Student–Newman–Keuls multiple range test.

Treatment	Final biomass (g/tank)	Final mean weight (g)	FCR[a]	Survival (%)
0DDGS	102.55 ± 14.31 y	7.19 ± 0.79 zy	1.64 ± 0.18 zy	95.0 ± 6.38
10DDGS	116.30 ± 4.27 z	7.76 ± 0.29 z	1.51 ± 0.06 y	100.0 ± 0.00
20DDGS	104.75 ± 4.33 y	7.11 ± 0.34 zy	1.65 ± 0.08 zy	98.33 ± 3.34
30DDGS	99.83 ± 6.45 y	6.66 ± 0.43 y	1.78 ± 0.13 z	100.0 ± 0.00
40DDGS	97.89 ± 3.38 y	6.53 ± 0.23 y	1.81 ± 0.06 z	100.0 ± 0.00
40DDGS-L	99.40 ± 7.06 y	6.53 ± 0.60 y	1.82 ± 0.19 z	100.0 ± 0.00
P-value	0.0314	0.0129	0.0152	0.1469
PSE[b]	3.79	0.24	0.06	1.47

[a]FCR = feed conversion ratio.
[b]PSE = pooled SE.

TABLE 5. Apparent digestibility coefficients for dry matter (ADMD), energy (AED), and crude protein (APD) in the reference diet and test ingredients LE-DDGS and sorghum DDGS (ground and unground). Values represent mean ± SD ($n = 3$). Values in the same column with different letters are significantly different ($P < 0.05$) based on ANOVA followed by Student–Newman–Keuls multiple range test.

	Test diet			Ingredient		
Source	ADMD-D	APD-D	AED-D	ADMD-I	APD-I	AED-I
			Trial 1			
Reference diet	68.24 ± 1.68 z	85.74 ± 4.30 z	74.52 ± 1.64 z			
LE-DDGS	63.90 ± 1.73 y	74.30 ± 3.01 y	69.17 ± 1.40 y	53.77 ± 5.77	36.94 ± 9.45[a]	55.71 ± 4.91
P-value	0.0356	0.0127	0.0195			
			Trial 2			
Reference diet	73.15 ± 0.49 z	89.08 ± 3.46 z	78.07 ± 0.62 z			
LE-DDGS	62.60 ± 2.25 y	71.27 ± 1.63 y	65.15 ± 1.99 y	42.43 ± 0.67[a] z	44.65 ± 4.06 z	20.87 ± 0.73[a] z
DDGS sorghum	59.08 ± 0.24 x	67.17 ± 0.64 x	59.39 ± 0.69 x	26.24 ± 0.81 y	17.30 ± 1.65 y	3.77 ± 2.75 y
DDG sorghum ground	60.12 ± 1.91 yx	62.04 ± 1.56 x	60.78 ± 2.09 x	29.72 ± 6.35 y	19.65 ± 4.06 y	11.15 ± 8.10 zy
P-value	<0.0001	<0.0001	<0.0001	0.0177	0.0001	0.0471

[a] $n = 2$.

can be used up to 40% in hybrid tilapia diets when lysine is supplemented to the diets. The analyzed lysine value of the LE-DDGS used in the current trial was 1.04%, which resulted in reduced lysine levels as LE-DDGS was increased in the diet. The apparent lysine requirement for Pacific white shrimp is reported at 1.6% and 2.1% of diets (4.49% and 4.67% of the protein) when determined using a 35% or 45% protein diet, respectively (Fox et al. 1995). The analyzed percent of lysine in the 40% LE-DDGS diets was 1.88% and 1.73% (5.2% and 4.8% of the protein, respectively) for the supplemented and non-supplemented diets, respectively. Under the reported conditions, there was no notable difference in the performance of shrimp fed the 40% LE-DDGS diet with or without lysine supplementation indicating lysine was not limiting in these formulations. During previous Pacific white shrimp studies in green water tanks and pond production trials, no significant differences were observed using 10% corn DDGS compared with diets containing 10% poultry byproduct, fish meal, or pea meal (Roy et al. 2009; Sookying and Davis 2011). Grain distillers dried yeast is also acceptable for Pacific white shrimp feed up to a level of 15% inclusion as demonstrated in outdoor green water tanks and production ponds (Achupallas 2013). Zhou (2014) reported that sorghum DDGS can be used at an inclusion level up to 40% in practical diets in a clear-water system for Pacific white shrimp without adverse effect on growth, survival, and FCR. However, Cummins et al. (2013) reported significantly less growth and higher FCR in shrimp offered FM-free diets with 10–30% DDGS (from whiskey distillation) for Pacific white shrimp. This is most likely an example of the variable quality that can occur from the same grain DDGS but a different processing method. Based on the current results, the LE-DDGS used in this study can be included up to 20% in practical feeds that contain 6% FM. Feeds with higher levels of LE-DDGS (30% and 40% of the diet) resulted in shrimp with significantly lower final weights compared with the best-performing group, which were offered diets containing 10% LE-DDGS.

The FCR in the current study ranged from 1.5 to 1.8, which is appropriate for this species reared in the clear-water recirculating system. This is inconsistent with Cummins et al. (2013) who reported an FCR of 2.84 in Pacific white shrimp fed an FM-based diet in a clear-water system to 9.27 when soybean meal was replaced with DDGS in FM-free diets. The growth results of these studies cannot be directly compared since the diets containing DDGS were FM-free diets and the sources of DDGS were different. The DDGS in Cummins et al. (2013) was from a whiskey distillery whereas that in our study was a coproduct from ethanol-based fuel production. In general, the FCR observed in our trial was typical for shrimp reared in this type of system.

For the digestibility value of DDGS in fish, some studies have been reported; for example, the APD values of DDGS were 90.4% for Rainbow Trout (Cheng and Hardy 2004b), 20.6% for Florida Pompano *Trachinotus carolinus* (Lech and Reigh 2012), and 64.94% for sunshine bass (Thompson et al. 2008). These values indicate that the nutrient digestibility values reported for DDGS for fish can vary considerably among different species and methodology.

There is a wide range of ADC values reported for shrimp comparing various plant and animal ingredients; however, very few studies have reported the nutrient digestibility of DDGS. In the current study (Table 5), the APD value of LE-DDGS (trial 1, 36.94 ± 9.45; trial 2, 44.65 ± 4.06) and sorghum DDGS (unground, 17.30 ± 1.65; ground, 19.65 ± 4.06) were much lower than APD value of 78.5 ± 1.4 for DDGS reported by Lemos et al. (2009) with the same species and similar protocol. Albeit similar protocols were used in the present study and Lemos et al.'s (2009) study, but the values from the present data are considerably lower. This difference could be due to source,

protein content of the meal, or differences in methods. Similarly, AED values reported in other studies for a range of ingredients ranged from 43.5% to 106.6% in white shrimp *Penaeus setiferus* (Brunson et al. 1997) and from 72.3% to 88.8% in Pacific white shrimp (Yang et al. 2009).

In the current study, AED values of LE-DDGS (trial 1, 55.71 ± 4.91; trial 2, 20.87 ± 0.73) and sorghum DDGS (unground, 3.77 ± 2.75; ground, 11.15 ± 8.10) in Pacific white shrimp are reported (Table 5). The poor ADC could be, in part, due to the technique or characteristics of the test ingredient. Numerous digestibility trials have been conducted using the same techniques with a range of ingredients. For example, at the time the reported samples were collected, other ingredients (e.g., soybean meal) were being tested and their digestibility values were reasonable. Zhou et al. (2015) used almost the exact same reference diet and reported values for ADMD (72.2%), APD (89.0%), and AED (77.9%) for the reference diet that were very similar to the values reported in our two trials. This indicates the technique is repeatable but may not be appropriate for all ingredients. We have noted low values with several ingredients including several distiller products. One theory was that the ingredient we used was not properly ground resulting in reduced digestion. To evaluate this, sorghum DDGS was used as it was received and as a finely ground flour. Yet little differences in APD and AED values between the two samples were observed (Table 5). We have also confirmed values for LE-DDGS over time by running the same sample in two different digestibility trials. Albeit, this technique appears to produce the reported results, they do not make sense biologically. Possibly another technique and/or inclusion levels will be required to determine ADCs for DDGS in shrimp.

Despite the low digestibility values for DDGS observed in the present study, the growth, survival, and FCR of Pacific white shrimp were not significantly different when we compared the response of shrimp offered the basal diet or a diet with 40% LE-DDGS. Similarly, Zhou (2014) studying the same species used the same source of sorghum DDGS and reported no significant differences in growth performance. In both studies LE-DDGS and sorghum DDGS accounted for 36% and 21% of the protein in the test diets. If the digestibility values of DDGS are correct, one would have expected a significant difference in growth due to reduced levels of digestible protein.

In conclusion, the current growth trial demonstrated that Pacific white shrimp can perform well with up to 40% LE-DDGS included in practical diets. There was no improvement in performance with lysine supplementation to the diet containing 40% LE-DDGS, indicating lysine was not limiting in the formulated diet. Results indicate that no significant impairment was observed at any inclusion of LE-DDGS up to 40%. Although the ADCs for LE-DDGS were lower than for the reference diet, the shrimp's growth and survival was acceptable, suggesting the nutrient utilization was adequate. Future work to evaluate other techniques measuring digestibility in shrimp would be beneficial.

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