Hindawi
Aquaculture Nutrition
Volume 2022, Article ID 1648747, 11 pages
https://doi.org/10.1155/2022/1648747

Research Article

Evaluation of a Commercial High-Protein Distiller's Dried Grain with Solubles (HP-DDGS) Product in the Diet of Juvenile Nile Tilapia (*Oreochromis niloticus*)

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Received 22 October 2021; Revised 17 December 2021; Accepted 4 January 2022; Published 31 January 2022

Academic Editor: Mansour Torfi Mozanzadeh

A comparative feeding trial and digestibility determination were conducted to evaluate the nutritional value of a commercial, high-protein distiller's dried grains with solubles (HP-DDGS) ingredient in the diet of Nile tilapia (*Oreochromis niloticus*). For the feeding trial, six diets were formulated to contain 360 g total dietary protein kg^{-1} and were prepared with incremental levels of protein from the HP-DDGS (0, 75, 150, 225, 300, and 375 g of dietary protein kg^{-1}) replacing protein from fishmeal and soybean meal. Juvenile tilapia (10.4 g ± 0.37; average initial weight ± SD) were evenly distributed in 24, 38-L aquaria operated as a recirculating system and fed twice daily to apparent satiation throughout the 8-week trial. Nile tilapia exhibited no significant ($P > 0.05$) differences in weight gain, feed efficiency, condition indices, whole-body proximate composition, or innate immunological responses when fed any DDGS-supplemented diets compared to the control diet. Percent apparent digestibility coefficients (ADCs) of the DDGS product for organic matter, crude protein, and gross energy were 74.8%, 83%, and 82.8%, respectively. Availability values for all amino acids were 89% or greater. Thus, this high-protein DDGS was established as a readily digestible protein feedstuff suitable for replacing soybean meal and fishmeal at up to 375 g of total dietary protein kg^{-1} in a practical diet for Nile tilapia.

1. Introduction

Aquaculture has reached an all-time peak, supplying over 100 million of the world's 180 million tons of fish and aquatic products for human consumption as of 2018, and aquacultural production is likely to continue expanding due to the demand of a growing human population and largely static harvest from capture fisheries [1–3]. The aquaculture sector is the fastest growing food production industry and has experienced an average growth rate of 5.8% from 2000-2016 [3] furthering the economic strain placed on providing valuable feed resources to support intensive fish production (e.g., fishmeal and fish oil) [4].

To meet this challenge, the aquaculture industry has invested in evaluating fishmeal and fish oil replacements to alleviate unprecedented demand for those resources and allow for continued growth to meet the goal of providing cost-effective seafood for the global population. Promising alternatives to fishmeal and fish oil are diverse in nature but generally must contain adequate protein and/or lipid concentrations. These alternative ingredients range from terrestrial plant-based proteins and lipids (e.g., soybean meal and oil), single-cell protein and oil (e.g., phototrophic and heterotrophic algae), rendered terrestrial animal products (e.g., poultry by-product meal), seafood by-products (e.g., seafood processing wastes), and even insect meals (e.g.,

Black soldier fly larvae meal) [5]. Specifically, plant protein feedstuffs have been identified as crucial to the sustainability of aquaculture [6]. However, researchers are mindful of potential antinutritional factors in some plant feedstuffs that may negatively affect the health of cultured organisms, such as trypsin inhibitors in soybean meal [7] and gossypol concentrations in cottonseed meal [8].

Distiller's dried grain with solubles (DDGS) is a maize-based coproduct of ethanol production via fermentation which is accomplished by a combination of various enzymes and yeasts [9]. Historically, DDGS is produced during dry grind ethanol production through the steps of liquification, saccharification, fermentation, distillation (dry coproduct DDGS is produced), and dehydration [10]. Once the DDGS is harvested, reported variability in physical properties (e.g., color and texture) and moisture content of DDGS products are primarily based on variances in steam treatment and screw speed throughput [11, 12]. The dry grind ethanol process creates an economically feasible and sustainable alternative protein feedstuff at approximately 10% the cost relative to commercial fishmeal and 45% to that of soybean meal [13]. When compared on a dry-matter, crude-protein (CP) basis, DDGS (USD0.12 kg^{-1} CP) is also substantially cheaper than fishmeal (USD0.58 kg^{-1} CP) and soybean meal (USD0.21 kg^{-1} CP) potentially reducing high costs associated with high-protein aquatic diets [13–15].

The nutrient content of traditional DDGS (approximately 306 g kg^{-1} CP and 100 g kg^{-1} lipid [15]) is suitable for moderate levels of dietary inclusion for some fish species. However, the carbohydrate (CHO) concentration of the ingredient could be a potential factor limiting inclusion when formulating diets for carnivorous fish which typically have lower ability to digest soluble CHOs. Total CHO content in DDGS has been measured upwards of 500 g kg^{-1} of dry weight, with nonstarch CHO (i.e., nondigestible fiber) composing approximately 341-420 g kg^{-1} of dry weight [15, 16]. Thus, DDGS would appear to be more efficiently supplemented in the diet of species capable of metabolizing higher levels of soluble CHO, such as Nile tilapia. Technological advancements have given rise to high-protein DDGS (HP-DDGS) created via further separation of indigestible fiber, as well as refinement of the dry grind process, thereby increasing crude protein concentration to 390-480 g kg^{-1} dry weight, but only moderately enhancing lysine and methionine concentrations [9, 17, 18]. Another obstacle inhibiting aggressive DDGS inclusion in diets of fish is its limited lysine and methionine concentrations (the most limiting amino acids in aquatic diets when replacing fishmeal) [19]. On average, lysine and methionine represent 10.0 and 6.0 g kg^{-1} dry weight of DDGS, respectively (~36.0 and 22.0 g kg^{-1} CP) [20] compared to 52.9 and 20.2 g kg^{-1} dry weight (~83.0 and 32.0 g kg^{-1} of CP) in menhaden fishmeal [14]. Deficiencies in these indispensable amino acids may result in decreased metabolic function of the organism, including impairment of growth performance, development, and overall health [21]. Nonetheless, adequate growth of cultured species can be supported through supplementation of limiting amino acids, thereby increasing the potential inclusion of HP-DDGS or other alternative protein ingredients [14]. Despite relatively high amounts of nondigestible CHO and limiting lysine and methionine concentrations, DDGS (traditional and HP-DDGS) are promising plant-protein ingredients due to a lack of antinutritional factors which may be present in other plant feedstuffs [6, 22].

A new commercial HP-DDGS ingredient, ProCap Gold (Marquis Energy, Hennepin, IL, USA), has been developed that separates protein- and lipid-rich fermented corn and yeast fractions from nondigestible fiber fractions in the dry grind process (Cristobal et al., 2020). Coupled with an increase in analyzed crude protein concentration (542.0 g kg^{-1} dry weight) and lysine concentration (15.2 g kg^{-1} dry weight) and decrease in nondigestible fiber (65.6 g kg^{-1}), the digestibility of the HP-DDGS ingredient could potentially be enhanced, particularly in energy and organic matter, thus potentially improving growth of cultured fish and allowing greater dietary levels of inclusion. This abundant and patented HP-DDGS ingredient presents a desirable alternative to fishmeal and soybean meal, based on the increased protein and indispensable amino acid composition. With a global production of approximately 7 million tons in 2020 and expected to increase in the near future [3], Nile tilapia represents a model species in which ProCap Gold HP-DDGS is desirable to spare dietary fishmeal inclusion and lessen the global demand.

Therefore, an 8-week comparative feeding trial was conducted to evaluate growth performance, body condition indices, whole-body composition, and immunological responses of juvenile Nile tilapia fed increasing levels of ProCap Gold DDGS in place of soybean and fishmeal protein in practical diets. Subsequently, a digestibility trial was conducted to assess apparent digestibility coefficients (ADCs) of this new HP-DDGS, Procap Gold, ingredient for Nile tilapia.

2. Methods

2.1. Feeding Trial

2.1.1. Diets, Cultured Fish, and System. The comparative feeding trial was conducted at the Texas A&M University (TAMU) Aquacultural Research and Teaching Facility (College Station, TX) using juvenile Nile tilapia. Initially, six practical isonitrogenous, isolipidic, and isoenergetic diets were formulated to contain 360 g crude protein (CP) kg^{-1}, 65 g crude fat kg^{-1}, and ~2910 Kcal gross energy (GE) kg^{-1} (Table 1). Nutrient proximate composition of the HP-DDGS experimental ingredient was also assessed to determine efficacy (Table 1). To further verify the viability of the HP-DDGS ingredient, amino acid content of the experiment ingredient was evaluated (Table 2) by MassTrak AAA HPLC Program (Waters Corporation, Milford, MA). Dietary AA composition was also assessed to ensure homogeneity of the dietary treatments and integrity of the comparative feeding trial using identical methodology (Table 3). All dietary treatments satisfied established nutrient requirements of Nile tilapia [15].

Of the 360 g kg^{-1} CP in the basal diet, soy-based protein (soybean meal and soy protein concentrate) contributed 306 g kg^{-1} with 36 g kg^{-1} of total protein from wheat flour

Table 1: Formulation and proximate composition (g kg^{-1} dry diet weight) of the control diet and experimental diets containing graded levels of ProCap Gold HP-DDGS ingredient which were fed to Nile tilapia. Diet labels indicate total protein contributed by HP-DDGS in g kg^{-1}.

Ingredient	Control	ProCap 75	ProCap 150	ProCap 225	ProCap 300	ProCap 375
HP-DDGS[a] (54% CP)	0.0	49.0	98.0	147.0	196.0	244.5
Soy protein concentrate[b]	76.0	76.0	76.0	76.0	76.0	76.0
Menhaden meal[c]	29.0	26.0	23.0	20.0	17.5	14.5
Soybean meal[d]	482.0	434.0	385.5	337.5	289.0	241.0
Wheat flour[e]	198.0	198.0	198.0	198.0	198.0	198.0
Dextrinized corn starch[f]	60.0	60.0	60.0	60.0	60.0	60.0
Carboxylmethyl cellulose[f]	20.0	20.0	20.0	20.0	20.0	20.0
Soybean oil	48.5	39.0	29.5	19.5	10.0	0.5
Vitamin premix[g]	30.0	30.0	30.0	30.0	30.0	30.0
Mineral premix[g]	40.0	40.0	40.0	40.0	40.0	40.0
DL-methionine[h]	5.0	5.0	5.0	5.0	5.0	5.0
Celufil[f]	11.5	23.0	35.0	47.0	58.5	70.5
Analyzed proximate composition of diet[i]						
Dry matter	888.7	886.3	885.9	903.7	904.4	905.9
Crude protein	370.0	368.2	371.1	373.2	371.8	372.0
Crude fat	65.2	61.4	66.9	67.2	68.6	70.8
Ash	76.1	73.9	71.7	69.9	67.2	64.8
Gross energy (Kcal kg^{-1})	2913.3	2914.5	2914.6	2911.4	2913.3	2912.4
Analyzed proximate composition of ProCap Gold ingredient[i]						
Dry matter	934.5					
Crude protein	541.6					
Crude fat	223.8					
∑saturated fatty acids	29.6					
∑monounsaturated fatty acids	69.2					
∑polyunsaturated fatty acids	99.2					
∑n-3 fatty acids	2.30					
∑n-6 fatty acids	97.8					
Total carbohydrate (CHO)	137.7					
Crude fiber	65.6					
Neutral detergent fiber (NDF)	380.2					
Acid detergent fiber (ADF)	140.2					
Ash	31.4					
Organic matter	968.6					
Gross energy (Kcal KJ^{-1})	5758.0					

[a]Marquis Energy (ProCap Gold), Hennepin, IL, USA. [b]Solae LLC, St. Louis, MO, USA. [c]Omega Protein Corporation, Abbeville, LA, USA. [d]Producers Cooperative Association, Bryan, TX, USA. [e]Rangen Inc., Angleton, TX, USA. [f]MP Biomedicals, Solon, OH, USA. [g]Same as in Moon and Gatlin III (1991). [h]Ajinomoto North America Inc., Itasca, IL, USA. [i]Means of three replicate analyses.

and the remaining 18 g kg^{-1} contributed by menhaden fishmeal. ProCap HP-DDGS was supplemented to the basal diet at five incremental levels replacing soybean meal and fishmeal such that the total protein contributed by HP-DDGS equaled 75, 150, 225, 300, or 375 g kg^{-1}. As HP-DDGS was supplemented to the diet, inclusion ratio of soybean products and fishmeal progressively and equally decreased until contributing 350 and 25 g kg^{-1} of dietary protein, respectively, in the diet with the highest level of HP-DDGS. Identification of experimental treatments is based on a measure of total protein contribution.

All diets were produced on site at the Texas A&M Aquacultural Research and Teaching Facility. Accurately weighed dry ingredients were homogenized by industrial grade V-mixer for 30 minutes prior to oil and water addition and mixing followed by pelleting into 3 mm strands, as previously described by Yamamoto et al. [23]. Proximate composition of the dried diets was measured in accordance with Association of Official Analytical Chemists (AOAC) [24] methods. Briefly, crude protein was measured by a LECO (St. Joseph, MI, USA) 828 Nitrogen and Protein Determinator, crude lipid content was determined by chloroform-

Table 2: Amino acid (AA) composition of ProCap Gold HP-DDGS experimental ingredient (g of AA kg^{-1} dry diet).

g of AA kg^{-1} dry diet	ProCap Gold
Indispensable AA	
Arg	22.9
His	12.1
Ile	15.9
Leu	50.8
Lys	15.2
Met	8.40
Phe	24.8
Thr	20.3
Val	25.0
Dispensable AA	
Ala	31.4
Asn/Asp	31.3
Cys	7.40
Gln/Glu	64.8
Gly	20.1
Pro	34.1
Ser	23.6
Tyr	20.0

Table 3: Amino acid (AA) composition of experimental diets supplemented with ProCap Gold HP-DDGS ingredient (g of AA kg^{-1}dry diet).

g of AA kg dry diet	Control	75	150	225	300	375
Indispensable AA						
Arg	26.6	28.1	25.0	26.3	24.9	23.8
His	9.40	9.90	9.20	10.2	9.90	9.40
Ile	17.3	18.0	16.6	17.5	17.1	17.0
Leu	27.0	29.7	27.9	30.3	31.1	32.3
Lys	18.6	19.6	16.7	16.3	16.1	16.3
Met	7.20	6.50	7.70	9.00	8.70	8.80
Phe	19.1	19.9	18.8	21.0	20.2	19.0
Thr	15.9	17.5	15.5	16.4	16.2	16.2
Val	17.4	19.	17.6	18.8	19.0	19.4
Dispensable AA						
Ala	17.8	20.1	18.8	20.3	21.3	22.6
Asn/Asp	30.8	32.7	28.9	28.8	28.4	28.3
Gln/Glu	78.6	85.0	76.3	77.7	78.0	79.0
Gly	17.7	18.5	17.1	18.6	18.0	17.5
Pro	22.6	24.2	23.0	25.0	25.4	26.1
Ser	39.1	42.1	38.3	40.6	40.0	39.5
Tyr	11.7	12.2	11.7	13.1	12.2	11.5

methanol extraction (Folch et al., 1957), and ash/organic matter content was determined by combustion in a furnace at 650°C for a 3.5-hour duration.

A total of 360 juvenile Nile tilapia were acquired from the stock maintained at the Aquacultural Research and Teaching Facility. Fish were evenly distributed into 24, 38-L aquaria operating as a recirculating aquaculture system (RAS), complete with settling chamber, biological filter, sand filter, and UV sterilizer. Photoperiod was regulated with fluorescent lights controlled with a timer to provide a 12 : 12 h light : dark cycle. The 15 fish per aquaria were conditioned and quarantined in the experimental system for 1 week prior to initiation of the trial during which time they were fed the formulated basal diet. After the conditioning period, quadruplicate groups of Nile tilapia (10.4 g ± 0.37; average initial weight ± SD) were then randomly assigned to each experimental diet and fed to apparent satiation for 8 weeks based on a set percentage of body weight per aquaria. Growth rate was monitored weekly by group weighing all fish in each aquarium, and the feeding ration, initially set at 4.0% body weight, was adjusted downward on a weekly basis to maintain a level close to apparent satiation without overfeeding. Water quality was consistently maintained at acceptable levels and measured twice weekly and reported as follows (average ± SD): temperature—27.8 ± 0.5°C, dissolved oxygen—5.7 ± 0.6 mg L^{-1}, total ammonia nitrogen—0.07 ± 0.04 mg L^{-1}, total nitrite nitrogen—0.04 ± 0.03 mg L^{-1}, pH—7.6 ± 0.1, and salinity—1.2 ± 0.4 g L^{-1}.

2.1.2. Sampling Procedures and Data Collection. An initial sample of 20 juvenile Nile tilapia was collected before the start of the feeding trial and stored at -20°C for analysis of whole-body proximate composition at a later date. At the end of the 8-week feeding trial, aquaria weight was measured for production performance parameters, including percentage weight gain ([g final weight – initial weight/g initial weight]. × 100), feed efficiency (g dry feed offered/g weight gain), protein conversion efficiency ([(final body wt.(g) × final body protein (%)) – (initial body wt.(g) × initial body protein (%))]/protein intake (g)] × 100), and survival ([#surviving fish/initial stocking density] × 100). After determination of total biomass weight per aquarium, six fish per aquarium were randomly collected for whole-body proximate composition analysis and determination of condition indices based on protocols described by Rossi Jr et al. [25]. Briefly, three of the six fish per aquarium were euthanized by an overdose (300 mg L^{-1}) of tricaine methanesulfonate (MS-222; Western Chemical, Ferndale, Washington) and stored at -20°C for whole-body proximate composition analyses, along with the initial sample of fish, according to [24] methods which were identical to procedures described for diet proximate composition analyses. The three fish per aquarium were combined and homogenized as composite samples of whole-body tissue.

The three remaining fish per aquarium were anaesthetized using tricaine methanesulfonate (100 mg L^{-1}) prior to blood sample collection via heparinized syringes through the caudal peduncle vasculature and then euthanized via a higher dose (300 mg L^{-1}) of tricaine methanesulfonate. Fish were bled prior to weighing to accurately measure innate immune responses as described below. Collected whole-blood samples were kept refrigerated at 4°C prior to centrifugation. After bleeding, fish were weighed and ventrally dissected for removal of intraperitoneal fat and liver for

computation of condition indices including intraperitoneal fat (IPF) ratio (100 × g IPF weight/g body weight) and hepatosomatic index (HSI) (100 × g liver weight/g body weight), as described by Castillo et al. [26]. Those three fish per aquarium also were filleted for determination of muscle ratio expressed as a percent of body weight (100 × g fillet weight/g body weight).

Collected whole-blood samples were aliquoted and stored separately in 2 ml Eppindorf tubes and then incubated with 2 mg of nitroblue tetrazolium (NBT, cat# 97061–412, VWR International) per mL^{-1} in a 96-well microplate for oxidative radical production analysis, first described by Siwicki et al. [27] with modifications according to Yamamoto et al. [28]. Remaining blood samples were centrifuged at 3,000 × g for a 15-minute duration for plasma separation and stored at -80°C for plasma immunological assays, including total protein, immunoglobulins, lysozyme activity, and antiprotease activity. Plasma total protein, immunoglobulin, and lysozyme activity was determined as described by Yamamoto et al. [28]. Plasma antiprotease activity measurement was performed according to the procedures of Ellis [29].

2.2. Digestibility Trial

2.2.1. Diets, Cultured Fish, and System. A nutritionally complete commercial diet for omnivorous fish (~360 g CP kg^{-1}, 55 g crude lipid kg^{-1}, and 4163 Kcal kg^{-1} dry-matter basis) was ground through a 1 mm hammermill screen and combined with 20 g kg^{-1} carboxymethyl cellulose (CMC) (MP Biomedicals, Solon, OH, USA) and 1 g kg^{-1} yttrium oxide (Y_2O_3, Sigma Aldrich Co.) and then pelleted by passing through a meat grinder and 5 mm diet to serve as the reference diet. The experimental diet (~410 g CP kg^{-1}, 100 g crude fat kg^{-1}, and 4835 Kcal kg^{-1} dry-matter basis) consisting of 730 g of the ground commercial reference diet kg^{-1}, 250 g of the ProCap DDGS ingredient kg^{-1}, 20 g CMC kg^{-1}, and 1 g yttrium oxide kg^{-1} was homogenized in a food mixer and pelleted similar to the reference diet. A total of 120 advanced stage Nile tilapia (ranging from 75 to 150 g), obtained from the stock maintained at the TAMU Aquacultural Research and Teaching Facility, were evenly distributed into four, 1,200-L round tanks operating as a RAS, complete with settling chamber, biological filter, sand filter, and UV sterilizer. Each tank was randomly assigned a digestibility diet, allowing for two replicates of each dietary treatment. Fish were fed to apparent satiation the assigned diets for a duration of 1-week prior to sample collections. After the initial fecal collection was completed, fish in all four tanks were then reconditioned for a period of 1 week using the commercial diet. Thereafter, each experimental digestibility diet was reassigned randomly to two tanks for an additional fecal collection. Thus, four independent replicate samples were obtained for the reference and experimental diets.

2.2.2. Fecal Collection and Calculated Apparent Digestibility Coefficients. Feces from each tank were collected on two separate and independent occasions and considered replicate samples, as previously described. Prior to collection, tanks were cleaned and siphoned with a partial water exchange to prevent contamination of the samples. For each collection, fish were fed to apparent satiation in the morning, and feces was physically harvested by netting every 30 minutes for a 6-hour duration [30]. Composite samples of feces from each tank were dried at 60°C overnight and finely ground with mortar and pestle. Then, fecal and diet samples were analyzed for proximate composition as previously described. In addition, samples were digested and analyzed for amino acid composition as previously described [26] and gross energy content by adiabatic calorimetry. Apparent digestibility coefficient (ADC) values of the reference and experimental diets were calculated according to the equation described by Amirkolaie et al. [31] and NRC [15] which also allowed calculation of the ADC values of the ProCap ingredient; $\text{diet ADC of nutrient} = (1 - [\text{yttirum}_{\text{diet}}/\text{yttrium}_{\text{feces}} \times \text{Nutr}_{\text{feces/}}\text{Nutr}_{\text{diet}}] \times 100$; $\text{ingredient ADC of nutrient} = (\text{ADC}_{\text{exp.diet}} + [(\text{ADC}_{\text{exp.diet}} - \text{ADC}_{\text{reference diet}}) \times (0.75 \times \text{Nutrient Comp.\%}_{\text{ref.diet}})/(0.25 \times \text{Nutrient Comp.\%}_{\text{exp.ingredient}})] \times 100$.

2.3. Statistical Analysis. Growth performance, condition indices, whole-body proximate composition, and immunological assays of Nile tilapia fed graded levels of HP-DDGS were evaluated by linear and quadratic regression using the JMP Pro 15 software (SAS Institute Cary, NC). The lesser P value was used to determine best fit of the model for significance determination. Homogeneity of variances was assessed by the Brown-Forsythe test, while data normality was assessed by the Shapiro-Wilk test. ADC values of the digestibility diets were assessed by one-way analysis of variance (ANOVA) using the JMP Pro 15 software. If significance ($P < 0.05$) was detected, data were subjected to Tukey's honestly significant difference. Significance was set at $\alpha = 0.05$ for all statistical analyses.

3. Results

3.1. Feeding Trial

3.1.1. Growth Performance and Condition Indices. Juvenile Nile tilapia did not exhibit any significant ($P > 0.05$) differences in measured parameters of growth performance, including percentage weight gain, feed efficiency (FE), protein conversion efficiency (PCE), fillet yield, or survival in response to increasing levels of HP-DDGS in the diet (Table 4). Additionally, no significant ($P > 0.05$) differences were identified for evaluated condition indices (HSI, IPF ratio, and muscle yield) as these parameters did not exhibit significance in either linear or quadratic regression models (Table 4).

3.1.2. Whole-Body Proximate Composition. Juvenile Nile tilapia fed diets containing from 0 up to 375 g of protein kg^{-1} from HP-DDGS were not significantly ($P > 0.05$) different in dry matter, crude protein, crude fat, or ash concentrations in whole-body tissues (Table 5), when evaluated as a linear or quadratic regression model. However, whole-body fat composition of tilapia fed the diet in which HP-DDGS

Table 4: Production performance of juvenile Nile tilapia fed diets supplemented with graded levels of HP-DDGS for 8 weeks.

	Initial weight (g)	Final weight (g)	Weight gain[a] (%)	FE[b]	PCE (%)[c]	Muscle ratio (%)[d]	HSI[e] (%)	IPF[f] (%)	Survival[g] (%)
Control	10.3	49.9	383	0.85	44.2	27.7	3.09	1.10	100
ProCap 75	11.3	47.6	321	0.79	40.0	27.3	2.73	1.33	97.8
ProCap 150	10.3	46.8	353	0.82	42.4	28.5	3.26	0.99	91.1
ProCap 225	10.7	50.4	376	0.87	43.4	26.1	2.92	1.13	100
ProCap 300	10.5	47.4	354	0.81	40.1	26.7	3.30	0.94	88.9
ProCap 375	10.5	48.6	364	0.83	42.5	26.7	3.32	1.40	95.6
PSE	0.34	2.32	28.33	0.04	0.025	0.011	0.002	0.003	0.036
Linear									
Pr > F	0.68	0.87	0.85	0.94	0.73	0.30	0.14	0.53	0.24
R^2	0.011	0.002	0.022	0.000	0.008	0.06	0.12	0.02	0.08
Quadratic									
Pr > F	0.81	0.91	0.89	0.988	0.85	0.59	0.27	0.54	0.39
R^2	0.028	0.013	0.001	0.002	0.02	0.067	0.157	0.079	0.117

Abbreviations: FE: feed efficiency; PCE: protein conversion efficiency; HSI: hepatosomatic index; IPF: intraperitoneal fat ratio; PSE: pooled standard error; Pr > F: probability associated with the F statistic; R^2: regression model goodness of fit. [a]Percentage weight gain ([g final weight – initial weight/g initial weight] × 100). [b]Feed efficiency (FE) (g dry feed offered/g weight gain). [c]Protein conversion efficiency (PCE) ([(final body wt.(g) × final body protein (%)) – (initial body wt.(g) × initial body protein (%))]/protein intake (g)]× 100). [d]Muscle ratio (100 × g fillet weight/g body weight). [e]Hepatosomatic index (HSI) (100 × g liver weight/g body weight). [f]Intraperitoneal fat (IPF) ratio (100 × g IPF weight/g body weight). [g]Survival (%) (#surviving fish/initial stocking density) × 100.

provided 375 g of protein kg^{-1} was numerically larger (82.9 g kg^{-1}) than all other experimental groups.

3.1.3. Immunological Assays. All measured immunological responses of Nile tilapia were not significantly ($P > 0.05$) affected by increasing levels of HP-DDGS inclusion based on linear or quadratic regression models (Table 6).

3.2. Digestibility Trial

3.2.1. Diet and Ingredient Apparent Digestibility Coefficients. Advanced stage Nile tilapia fed the experimental diet composed of 250 g kg^{-1} of the HP-DDGS ingredient exhibited no significant ($P > 0.05$) differences in all ADC values compared to the commercial reference diet (Table 7). Crude lipid ADC values were exceptionally low, indicating a potential limitation of the fecal collection method used in the current study, and therefore were excluded from results.

The HP-DDGS ingredient was readily digested by advanced stage Nile tilapia (Table 8). The ADCs for crude protein and digestible energy were high (83.1 and 82.8%, respectively), with marginally decreased crude lipid and organic matter ADC values (72.5 and 74.8%, respectively). Individual amino acid ADC values also were consistently high (Table 8).

4. Discussion

Traditional DDGS and HP-DDGS have been studied alternative protein ingredients in the diets of many aquacultured species with various degrees of success. Prior studies have evaluated the efficacy of dietary supplementation of both types of ingredients in the diets of freshwater omnivorous species such as Nile tilapia [32, 33] and channel catfish (*Ictalurus punctatus*) [19], as well as strictly carnivorous freshwater species, such as the hybrid striped bass (*Morone chrysops X M. saxitilis*) [34] and rainbow trout (*Oncorhynchus mykiss*) [35]. Successful supplementation of HP-DDGS products up to 50% of diet in place of soybean meal for omnivorous and carnivorous fish species attest to the potential use of this alternative protein feedstuff in diet formulations of various aquatic species [34, 36, 37]. While published literature often misconstrues traditional and HP-DDGS, it is understood that HP-DDGS (a total dietary fiber reduced ingredient) is more desirable when replacing fish-derived protein feedstuffs due to its higher protein content and more readily digested nutrients.

The present study observed no apparent effect of ProCap Gold HP-DDGS supplementation up to 375 g of total protein kg^{-1} (244.5 g of formulated diet dry weight kg^{-1}) on any growth performance parameters, condition indices, or whole-body proximate composition of juvenile Nile tilapia. It appears that ProCap Gold can replace soybean meal and fishmeal to comprise the majority of CP in a low-fishmeal diet. These results are mirrored by findings from numerous studies evaluating HP-DDGS and traditional DDGS supplementation in the diet of Nile tilapia. Coyle et al. [32] found that DDGS inclusion at 300 g dry weight kg^{-1} in combination with meat and bone meal (260 g kg^{-1}) and fishmeal (80 g kg^{-1}) protein produced similar growth responses compared to a control diet containing soybean meal and fishmeal at

Table 5: Proximate composition of the whole-body tissues (g kg^{-1} wet basis) of Nile tilapia after 8 weeks of feeding the experimental diets[a].

	Dry matter	Crude protein	Crude fat	Ash
Control	290.7	174.6	73.9	37.5
ProCap 75	272.3	168.3	65.0	37.1
ProCap 150	291.0	174.4	73.8	36.8
ProCap 225	291.7	171.8	77.5	38.9
ProCap 300	286.3	172.2	70.3	39.4
ProCap 375	301.3	174.2	82.9	39.1
PSE	0.007	0.002	0.004	0.002
Linear				
Pr > F	0.13	0.72	0.10	0.16
R^2	0.14	0.008	0.16	0.11
Quadratic				
Pr > F	0.19	0.65	0.15	0.38
R^2	0.19	0.05	0.22	0.12

Abbreviations: PSE: pooled standard error; Pr > F: probability associated with the F statistic; R^2: regression model goodness of fit. [a]Data represent means of triplicate groups ($n = 3$).

410 g kg^{-1} and 120 g kg^{-1}, respectively. Another experimental diet in that study was composed of 300 g DDGS kg^{-1} and 460 g soybean meal kg^{-1} but resulted in significantly ($P < 0.05$) reduced performance in all measured parameters [32]. Similarly, Herath et al. [37] observed no significant ($P > 0.05$) differences in mean weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio, and survival of tilapia when HP-DDGS were included in diets devoid of fishmeal at 524 and 332 g of dry weight kg^{-1}, respectively, in combination with soybean meal and poultry by-product meal when compared to a practical reference diet (343 g CP kg^{-1}). The current study and literature provide evidence of the capacity of HP-DDGS ingredients to provide the majority of CP in diets for Nile tilapia.

Lysine and methionine are essential amino acids that support key metabolic functions of fish, such as protein formation and energy production [38, 39]. As indicated earlier, lysine and methionine are typically the two most limiting amino acids in diets when replacing fishmeal [19]. Thus, lysine and methionine normally may need to be supplemented in formulated diets composed of high amounts of DDGS due to lower concentrations of those amino acids. Supplementation of lysine in diets with high inclusion of DDGS for Nile tilapia has been reported to range from approximately 2.0 to 6.0 g of dry diet weight kg^{-1} in published studies [33, 37, 40–42]. However, lysine composition of the HP-DDGS experimental ingredient used in the current study was analyzed at 15.2 g kg^{-1} dry weight (42.2 g kg^{-1} of protein), significantly higher than traditional DDGS and HP-DDGS (10.1 and 13.4 g kg^{-1} by weight and 33 and 34.4 g kg^{-1} of protein, respectively) [15]. Therefore, lysine supplementation was not necessary to meet the requirement of Nile tilapia in the current study, thus allowing increased HP-DDGS supplementation. Methionine is a limiting sulfur amino acid that is present abundantly in menhaden fishmeal (20.2 g kg^{-1}) compared to dehulled soybean meal (6.40 g kg^{-1}) [14]. As a result, methionine supplementation of alternative protein aquatic diets is generally understood. The HP-DDGS ingredient used in the current study contained ~8.40 g kg^{-1} of methionine, noticeably higher than dehulled soybean meal. Similar to lysine, excessive methionine supplementation in the present study was not necessary to meet the nutritional requirements of Nile tilapia.

Fish possess a highly evolved innate immune response, led by macrophages, leukocytes, and granulocytes [43]. Therefore, innate immune responses of whole-blood, plasma, and head-kidney-derived macrophages are consistently used for evaluation of immune capabilities of fish fed alternative ingredients. Once again, the current study observed no significant ($P > 0.05$) differences in measured innate immune response parameters. Similar to growth responses, this is consistent with literature that evaluated DDGS supplementation in Nile tilapia. While organismal variation in immune responses is evident between various trials, relative innate immune response parameters can be evaluated with confidence. Nile tilapia fed a diet supplemented with 400 g DDGS kg^{-1} exhibited no significant ($P > 0.05$) differences in white blood cell count, serum protein, or lysozyme activity compared to fish fed a practical control diet [41]. Lim et al. [41] also observed no significant differences in a disease challenge to test adaptive immune responses of these tilapias when exposed to *Streptococcus iniae*. Coupled with plasma/serum innate immune responses, whole-blood respiratory burst analysis also showed no significant ($P > 0.05$) differences in adult Nile tilapia [44]. Indirect analysis of immune function using circulating plasma cortisol revealed no apparent negative effects during acute stress challenge by short-term air exposure or confinement of Nile tilapia fed diets with up to 30% dry weight inclusion of DDGS [45]. Although the immunological effects of traditional DDGS are well documented, published knowledge of HP-DDGS on innate immune responses is somewhat lacking for Nile tilapia.

Conflicting reports of overstimulation, enhancement, or no effects on immune responses of fish fed high dietary levels of DDGS have led to contradictory conclusions on the effects of DDGS supplementation on immune function and health of various cultured species [34, 36, 40, 41, 46]. Specifically, hybrid striped bass fed experimental diets containing high protein ethanol yeast, an ingredient similar to HP-DDGS, exhibited abnormally elevated circulating peripheral cortisol levels in unstressed fish indicating immuno-overstimulation [34]. Conversely, Goda et al. [36] found that HP-DDGS improved overall immune function in European sea bass. Furthermore, differences observed could be resultant of physiological differences of fish between separate trials as well as discrepancies of the DDGS ingredient used (i.e., differences in production techniques or inconsistencies of the ingredient name (HP-DDGS and DDGS) in literature). HP-DDGS is composed of a high level

Table 6: Immunological responses of juvenile Nile Tilapia fed diets supplemented with graded levels of ProCap for 8 weeks[a].

	Blood neutrophil oxidative radical production (Abs at 545 nm)	Intracellular superoxide anion production (Abs at 620 nm)	Extracellular superoxide anion production (O_2^- nmol/well)	Plasma lysozyme activity (units/mL)	Plasma total protein (mg/mL)	Plasma total immunoglobulins (mg/mL)	Plasma antiprotease activity (%)
Control	0.649	0.163	0.381	483.3	36.4	5.01	81.1
ProCap 75	0.581	0.133	0.308	505.6	36.3	5.22	80.8
ProCap 150	0.647	0.313	0.914	433.3	33.9	4.10	81.8
ProCap 225	0.499	0.150	0.757	444.5	36.2	4.47	79.7
ProCap 300	0.522	0.165	0.665	444.5	36.7	5.72	81.0
ProCap 375	0.601	0.155	0.465	416.6	33.7	3.65	81.9
PSE	0.047	0.077	0.408	65.03	1.09	1.42	0.915
Linear							
Pr > *F*	0.20	0.86	0.68	0.31	0.33	0.65	0.70
R^2	0.099	0.002	0.011	0.065	0.060	0.013	0.009
Quadratic							
Pr > *F*	0.22	0.72	0.57	0.60	0.61	0.90	0.64
R^2	0.182	0.042	0.073	0.065	0.064	0.014	0.058

Abbreviations: Abs: absorbance; PSE: pooled standard error; Pr > *F*: probability associated with the *F* statistic; R^2: regression model goodness of fit. [a]Data represent means of triplicate groups ($n = 3$).

Table 7: Percent apparent digestibility coefficients (ADCs) of reference and experimental diets obtained with Nile tilapia[a]. The experimental digestibility diet was formulated on a 75 : 25 ratio of the reference diet to ProCap ingredient.

Diet	Organic matter	Crude protein	Gross energy
Reference	61.3	70.8	68.5
Experimental	61.1	74.9	73.1
PSE[b]	4.28	1.29	3.03
One-way ANOVA			
P value	0.98	0.07	0.32

[a]Values were from duplicate tanks sampled at two distinct time points for a total of four replicate samples per treatment ($n = 4$). Data were subjected to one-way ANOVA, and if significant ($P < 0.05$) differences were detected, means were compared using Tukey-HSD test. Diet ADC of nutrient = (1 – [$\text{yttirum}_{\text{diet}}/\text{yttrium}_{\text{feces}} \times \text{Nutr}_{\text{feces}}/\text{Nutr}_{\text{diet}}$] × 100. [b]Pooled standard error.

of yeast that is used during dry grind ethanol production in the fermentation process, which is further increased when carbohydrates are filtered, concentrating the protein [10]. Yeast is recognized to contain appreciable levels of immunostimulants (e.g., β-glucans and nucleotides) that can positively impact the health of the organism, although this can vary considerably between DDGS and HP-DDGS production systems [47–49]. Additionally, traditional DDGS and HP-DDGS ingredients possess antioxidant chemicals, particularly ferulic acid, tocopherols, and xanthophylls, potentially increasing immune capabilities of various organisms [50]. Despite some reported immunoenhancements with either form of DDGS ingredient supplementation, various other publications and the present study are inconclusive on positive and/or negative effects of dietary HP-DDGS on immune capacity of fish.

Apparent digestibility coefficient measurements in the present study determined the HP-DDGS ingredient to be highly digestible by advanced stage Nile tilapia. Protein ADC for the test ingredient (83.1%) was similar to values reported in the literature for traditional DDGS (ranging from 87.0 to 89.2%) [51–53]. Interestingly, ProCap HP-DDGS resulted in higher organic matter and similar energy ADC values (82.8 and 74.8, respectively) than reported by Tran-Ngoc et al. (50.7 and 78.1%, respectively) (2019). Compared to traditional DDGS, the ProCap ingredient is composed of less nonstarch polysaccharides (NSP) potentially resulting in higher ADC values as NSP are less readily digested by monogastric animals including fish, thus lowering energy efficiency [51]. Coupled with a reduction in NDF compared to traditional DDGS [16], the composition of total carbohydrates in the HP-DDGS ingredient offers a credible explanation for higher ADC values for energy and organic matter. These observations are in accordance with the present study's hypothesis.

It is worth mentioning the authors discovered the lipid composition of the ProCap Gold HP-DDGS ingredient limited greater inclusion in a practical diet for Nile tilapia. Although it appears feasible, inclusion rates over 375 g of CP kg^{-1} led to increased dietary lipid values above industry standards. Nonetheless, the use of ProCap HP-DDGS as the primary protein feedstuff in low-fishmeal diets is supported by the current study.

Table 8: Apparent digestibility coefficients[a] (ADCs) of the ProCap ingredient for juvenile Nile tilapia (mean ± Std Dev).

	ProCap
Crude protein	83.1 ± 10.9
Crude fat	72.5 ± 2.70
Energy	82.8 ± 22.2
Organic matter	74.8 ± 25.8
His	93.7 ± 18.4
Ser	91.7 ± 20.3
Arg	92.9 ± 19.8
Gly	93.1 ± 16.4
Asp	92.5 ± 23.1
Glu	95.6 ± 12.6
Thr	85.0 ± 34.0
Ala	94.3 ± 15.6
Pro	94.8 ± 12.4
Cys	98.3 ± 9.70
Lys	89.1 ± 34.4
Tyr	91.9 ± 16.2
Met	89.0 ± 19.7
Val	92.1 ± 19.8
Ile	93.2 ± 20.4
Leu	93.6 ± 14.6
Phe	93.0 ± 17.8

[a]Values represent means of quadruplicate samples. Ingredient ADC of nutrient = ($ADC_{exp.\ diet}$ + [($ADC_{exp.\ diet}$ − $ADC_{reference\ diet}$) × (0.75 × Nutrient Comp. $\%_{ref.\ diet}$)/(0.25 × Nutrient Comp. $\%_{exp.\ ingredient}$)] × 100.

In conclusion, soybean meal and fishmeal supplementation with ProCap HP-DDGS up to 375 g CP kg^{-1} without lysine supplementation had no apparent negative effects on growth performance, immune responses, whole-body proximate composition, or condition indices of juvenile Nile tilapia. Additionally, ProCap HP-DDGS had a higher gross energy ADC value than traditional DDGS as reported in previous studies. Thus, inclusion of ProCap HP-DDGS up to 375 g CP kg^{-1} in the diets of juvenile Nile tilapia is supported in the current study.

Data Availability

The data that supports the findings of the present study are available upon request to the corresponding author.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

This study was performed at the Texas A&M University Aquacultural Research and Teaching Facility (College Station, TX, USA) and was funded by Marquis Energy LLC (Hennepin, IL, USA). Blaine Suehs is a Masters student in the Department of Wildlife and Fisheries Sciences and funded through Texas A&M Agrilife Research. The authors would like to thank the graduate students of the Texas A&M Fish Nutrition Laboratory for assistance in sampling and experimental procedures, as well as Mr. Brian Ray for logistical assistance.

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