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Aquaculture

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Use of high protein distiller's dried grain with yeast in practical diets for the channel catfish, *Ictalurus punctatus*

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ARTICLE INFO

Keywords: Catfish Growth performance High protein distillers dried grain Poultry meal Soybean meal Alternative protein sources

ABSTRACT

As the ethanol industry matures and adopts new technologies, the co-products produced are also changing with their composition adjusted to meet feed industry needs. High protein distillers dried grain with yeast (HP40Y) is a relatively new product which is a variant of distillers dried grains that could be used as an improved protein source in catfish feed formulations. To evaluate the efficacy of HP40Y, a 10-weeks growth trial was conducted on the growth performance of juvenile catfish, *Ictalurus punctatus* (mean initial weight 1.80 ± 0.05 g). In the growth trial, graded levels of HP40Y (0.00, 3.10, 6.20 and 9.30%) were used to replace poultry meal (PM: 6.0, 4.0, 2.0 and 0.0%) and other series of diets were used with HP40Y (5.0, 10.0, 15.0, 20.0, 30.0 and 40.0%) to replace soybean meal (SBM: 51.00, 46.49, 41.90, 37.40, 28.20, 19.20%). Analysis of Covariance (ANCOVA) indicated a significant interaction between replaced protein (PM and SBM) and the inclusion level of HP40Y (up to 20%) on biomass, mean final weight, weight gain, and FCR (P < 0.05) of catfish. Therefore, the two sets of diets were analyzed separately. One-way Analysis of Variance (ANOVA) followed by Tukey multiple comparison test was used to detect significant differences between treatment means while regression analysis was used to determine the relationship between HP40Y inclusion level in the diet and weight gain and whole-body protein level of fish. In PM replacement series, complete replacement of PM with HP40Y in diet PDG9 resulted in poor performance, indicating a possible nutritional deficiency when the animal protein was removed. As a replacement for SBM, increasing levels of HP40Y only resulted in reduced growth of catfish when included in the diet at 30 and 40%. Results indicate that HP4Y is a good protein source when used at levels less than 30% of the diet.

1. Introduction

Reducing feed cost is essential for the long-term sustainability of aquaculture industry. One approach to decrease feed cost is to steadily reduce or substitute the most costly components of the feed. This can be done in such a way as to reduce overall production costs while confirming that such replacement will not compromise fish performance. Towards this objective, several studies have been performed with the purpose of reducing fish meal-based protein with plant protein sources in feed formulations (Brinker and Reiter, 2011). In aquatic feeds, the use of plant-based proteins has increased as they are cost effective protein sources with reliable quality and worldwide accessibility (Watanabe,

2002). The use of plant-based proteins in aquaculture feeds dictates the presence of unique nutritional attributes of its composition for instance low levels of fiber and anti-nutritional compounds (NRC, 2011). It should also incorporate a comparatively high protein content, balanced amino acid profile, reasonable price, acceptable palatability, suitable supply, and high nutrient digestibility (Lim et al., 2008; NRC, 2011).

Distillers dried grains with solubles (DDGS), a co-product of the drymill ethanol industry, are the dried residue that remains after the fermentation of corn (or other grains) mash by selected yeasts and enzymes to produce ethanol and carbon dioxide. The characteristics of DDGS includes numerous potential benefits to animals including moderate lipid and protein contents, along with phosphorus, vitamins, and

https://doi.org/10.1016/j.aquaculture.2021.737387

Received 10 May 2021; Received in revised form 29 July 2021; Accepted 23 August 2021 Available online 26 August 2021 0044-8486/© 2021 Elsevier B.V. All rights reserved.







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trace minerals. Moreover, the other benefit of DDGS is that it does not have anti-nutritional factors found in different plant protein sources like trypsin inhibitor and phytate which are present in soybean meal (Wilson and Poe, 1985; Shiau et al., 1987) and gossypol which is present in cottonseed meal (Jauncey and Ross, 1982; Robinson, 1991). These compounds can produce negative effects to aquatic digestive system and may possibly affect feed palatability.

As the technologies of bioethanol fermentation industry advance so do the products that are produced. One of the more recent products, is a high-protein distiller dried grain (HPDDG). This can be accomplished by use of front-end fractionation technology to isolate the fermentable part of the corn kernel from the non-fermentable portion before fermentation in dry-grind ethanol plants. It has higher crude protein, and lower fat and fiber than conventional DDGS (Singh et al., 2005). At some extent, DDGS products are made up from yeast remnants. Yeast from Saccharomyces cerevisiae is commonly used in the fermentation stage. According to (Ingledew, 1999) the addition of yeast biomass to the weight of DDGS is estimated to 3.9%, and the percentage of yeast protein in the overall protein content of DDGS could be at least 5.3%. Recently, in aquaculture feeds, S. cerevisiae has been assessed as a potential protein source (Overland et al., 2013), and yeast cells are sources of, mannan oligosaccharides, nucleic acids and β -glucans that can also be used as immunostimulants in fish diets (Refstie et al., 2010).

Several studies have been reported on the use of DDGS in channel catfish feeds. The use of traditional DDGS as a replacement for soybean meal without lysine addition succeeded to reach 40-45% of diets, (Lim et al., 2009) and up to 40 to 60% with lysine addition without adverse effects on growth performance (Lim et al., 2007). It is noteworthy that, HPDDG has approximately twice the protein content of traditional DDGS (Webster et al., 2008; Prachom et al., 2013; Tidwell et al., 2017). The increase protein density makes it more appropriate ingredient for higher protein feed as compared to traditional DDGS. This high-protein 42 distillers dried grains with yeast (HP40Y) is a 42% protein product containing spent yeast from the ethanol fermentation process. This is an advance step in the evolution of the separation technologies. It is produced by separating corn fiber prior to fermentation and removing the solubles fraction after fermentation to produce a high-quality combination of corn and yeast proteins. Therefore, the present study was conducted to investigate the utilization of HP40Y product as a replacement for soybean meal and animal meal (Poultry meal) in the practical diets of juvenile channel catfish.

2. Materials and methods

2.1. Diet preparation

The test ingredient, high protein distiller's dried grain with yeast (HP40Y) was sourced from The Andersons, Maumee, OH, USA (AND-Vantage[™] 40Y). Proximate and amino acid (AA) compositions of the primary protein sources are presented in Table 1. The formulation and proximate composition of the ten test diets are presented in Table 2. All test diets were formulated on an isonitrogenous and isolipidic basis to contain 32% protein and 6.5% lipid. The basal and experimental diets were formulated to meet the nutritional requirements of the fish (NRC, 2011). Two series of diets were formulated, where HP40Y was used to incrementally replace poultry meal (PM) and soybean meal (SBM) (Table 2). In the PM replacement series, graded levels of HP40Y (0.0, 3.1, 6.2, and 9.3%) were used to replace PM (PM: 6.0, 4.0, 2.0, and 0.0%) and were referred to as Diets Basal, PDG3, PDG6, and PDG9. In the SBM replacement series, graded levels of HP40Y (5.0, 10.0, 15.0, 20.0, 30.0 and 40.0%) were used to replace SBM (SBM: 51.00, 46.49, 41.90, 37.40, 28.20, 19.20%) and were referred to as Diets SDG5, SDG10, SDG15, SDG20, SDG30 and SDG40.

The experimental diets were prepared at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University (Auburn, AL), using standard procedures Table 1

Proximate and amino acid composition (% as is) of poultry meal (PM), soybean meal (SBM) and high protein distiller's dried grain with yeast (HP40Y) used in growth trial.

	Poultry meal	Soyabean meal	HP40Y
Crude Protein	64.59	46.66	42.25
Moisture	8.95	11.48	9.14
Crude Fat	12.29	0.48	8.48
Crude Fiber	1.04	3.59	7.05
Ash	9.88	6.47	2.13
Alanine	4.05	2.04	3.19
Arginine	4.32	3.49	1.84
Aspartic Acid	5.29	5.31	2.86
Cysteine	0.77	0.69	0.83
Glutamic Acid	8.58	9.00	7.17
Glycine	5.54	2.00	1.56
Histidine	1.41	1.24	1.18
Hydroxylysine	0.23	0.02	0.00
Hydroxyproline	1.55	0.11	0.07
Isoleucine	2.64	2.27	1.88
Lanthionine	0.10	0.00	0.12
Leucine	4.55	3.64	5.48
Lysine	4.11	3.02	1.30
Methionine	1.22	0.61	0.86
Ornithine	0.06	0.03	0.03
Phenylalanine	2.57	2.38	2.34
Proline	3.59	2.21	3.44
Serine	2.53	2.26	1.82
Taurine	0.47	0.12	0.10
Threonine	2.55	1.83	1.58
Tryptophan	0.60	0.64	0.34
Tyrosine	2.15	1.73	1.79
Valine	3.21	2.31	2.30
Sum of AA	62.09	46.95	42.08

for fish feeds. In short, the pre-ground dry ingredients and oil were weighed and then mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Hot 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 moist pellets were then placed into a forced air oven (VWR Scientific E191047, PA, USA) (<45 °C) overnight to attain a moisture content of less than 10%. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer (-20 °C) until needed. All the diets were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO) for proximate composition and Amino Acid (AA) profile (Tables 2 and 3).

2.2. Experimental systems

Both growth trials were conducted in an indoor recirculation system. The growth trial I consisted of forty 75-L glass aquaria connected to a common reservoir tank (800-L) and growth trial II was conducted in sixteen tanks with same capacity of water. Water quality was maintained by recirculation through an Aquadyne bead filter $(0.2 \text{ m}^2 \text{ media}, 0.6 \text{ m})$ imes 1.1 m) and vertical fluidized bed biological filter (600-L volume with 200-L of Kaldnes media) using a 0.25-hp. centrifugal pump. Mean water flow for an aquarium was 4 L/min with an average turnover of ~ 21 min/tank. Dissolved oxygen was maintained near saturation using air stones in each culture tank and the sump tank using a common airline connected to a regenerative blower. During the trial, dissolved oxygen (DO), temperature and salinity were monitored twice daily using an YSI 55 multi-parameter instrument (YSI, Yellow Springs, OH) and total ammonia N (TAN) and nitrite-N were measured twice per week using YSI 9300 photometer (YSI, Yellow Springs, OH). The pH of the water was measured twice weekly during the experimental period using the pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA), while alkalinity, hardness and nitrate level of water was measured twice per month using WaterLink-Spin TouchFF photometer (LaMotte Company, Chestertown, MD).

Table 2

Formulation and Proximate composition of test diets used to evaluate the efficacy of HP40Y (% as is) in the diets of channel catfish.

Ingredient	Basal	PDG3	PDG6	PDG9	SDG5	SDG10	SDG15	SDG20	SDG30	SDG40
Poultry meal ^a	6.00	4.00	2.00	0.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal ^b	55.50	55.50	55.50	55.50	51.00	46.49	41.90	37.40	28.20	19.20
HP40Y ^c	0.00	3.10	6.20	9.30	5.00	10.00	15.00	20.00	30.00	40.00
Menhaden fish oil ^d	3.59	3.55	3.51	3.47	3.15	2.72	2.28	1.84	0.96	0.09
Corn Starch ^e	3.46	2.40	1.34	0.28	3.40	3.34	3.37	3.31	3.39	3.26
Corn ^f	28.00	28.00	28.00	28.00	28.00	28.00	28.00	28.00	28.00	28.00
Mineral premix ^g	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix ^h	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Choline chloride ⁱ	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Rovimix Stay-C ^j	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
CaP-dibasic ^k	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Proximate composition ¹ (g	/100 g as is)									
Crude protein	33.7	33.58	33.32	34.00	32.75	33.69	32.94	32.48	33.64	33.53
Moisture	6.57	6.22	6.31	6.11	7.86	6.13	7.41	7.69	8.25	6.10
Crude Fat	4.85	5.03	4.96	4.82	4.85	5.03	4.86	4.75	4.50	5.10
Crude Fiber	4.24	4.64	4.66	4.89	4.39	4.71	4.67	4.68	4.93	5.43
Ash	6.63	6.37	6.24	6.22	6.23	6.12	5.83	5.55	5.23	4.93

Abbreviations used: Poultry meal replacement distillers' grain (PDG3, PDG6 and PDG9) and Soybean meal replacement distillers' grain (SDG5, SDG10, SDG15, SDG20, SDG30 and SDG40.

^a Tyson Foods, Inc., Springdale, AR, USA.

^b De-hulled Solvent Extracted Soybean Meal, Bunge Limited, Decatur, AL, USA.

^c The Andersons, Maumee, OH, USA.

^d Omega Protein Inc., Houston, TX, USA.

^e MP Biomedicals Inc., Solon, OH, USA.

^f Faithway Feed Co., Gunterville, AL, USA.

^g Trace mineral premix (g/100 g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.250; Ferrous sulfate, 4.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 67.964.

^h Vitamin premix (g/kg premix): Thiamin HCl, 0.438; Riboflavin, 0.632; Pyridoxine HCl, 0.908; Ca-Pantothenate, 1.724; Nicotinic acid, 4.583; Biotin, 0.211; folic acid, 0.549; Cyanocobalamin, 0.001; Inositol, 21.053; Vitamin A acetate, 0.677; Vitamin D3, 0.116; Menadione, 0.889; dL-alpha-tocoperol acetate, 12.632; Alpha-cellulose, 955.589.

ⁱ VWR Amresco, Suwanee, GA, USA.

^j Stay-C® (L-ascorbyl-2-polyphosphate 35% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

^k VWR Amresco, Suwanee, GA, USA.

¹ Analysis conducted by University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA) (Results are expressed on g/100 g of feed as is, unless otherwise indicated).

Table 3

	Amino acid prof	ile (g/100 g	as is) of test	diets fed to	channel catfish.
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Diets ^a	Basal	PDG3	PDG6	PDG9	SDG5	SDG10	SDG15	SDG20	SDG30	SDG40
Alanine	1.60	1.61	1.59	1.64	1.64	1.75	1.80	1.84	1.91	2.10
Arginine	2.34	2.30	2.28	2.19	2.25	2.23	2.14	2.05	1.95	1.74
Aspartic Acid	3.53	3.52	3.44	3.41	3.43	3.37	3.20	3.05	2.92	2.69
Cysteine	0.49	0.52	0.52	0.51	0.51	0.52	0.53	0.52	0.54	0.57
Glutamic Acid	5.77	5.80	5.83	5.91	5.70	5.82	5.69	5.59	5.55	5.55
Glycine	1.64	1.56	1.43	1.40	1.58	1.61	1.57	1.53	1.48	1.48
Histidine	0.86	0.87	0.87	0.88	0.85	0.87	0.87	0.86	0.86	0.86
Hydroxylysine	0.08	0.08	0.08	0.06	0.08	0.07	0.06	0.07	0.07	0.06
Hydroxyproline	0.25	0.19	0.16	0.10	0.18	0.26	0.22	0.21	0.19	0.22
Isoleucine	1.62	1.60	1.62	1.59	1.57	1.57	1.51	1.49	1.46	1.44
Lanthionine	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05
Leucine	2.63	2.69	2.79	2.85	2.71	2.86	2.91	3.02	3.15	3.43
Lysine	2.08	2.02	1.98	1.90	1.99	1.94	1.85	1.76	1.66	1.48
Methionine	0.52	0.52	0.51	0.50	0.54	0.56	0.57	0.56	0.58	0.64
Ornithine	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Phenylalanine	1.68	1.69	1.73	1.71	1.66	1.70	1.60	1.68	1.68	1.68
Proline	1.76	1.81	1.84	1.88	1.81	1.93	1.96	2.05	2.13	2.27
Serine	1.13	1.17	1.17	1.21	1.22	1.32	1.28	1.28	1.29	1.30
Taurine	0.17	0.17	0.14	0.13	0.15	0.17	0.16	0.16	0.15	0.15
Threonine	1.17	1.19	1.17	1.18	1.21	1.24	1.22	1.21	1.21	1.20
Tryptophan	0.42	0.41	0.41	0.41	0.39	0.38	0.38	0.36	0.35	0.32
Tyrosine	1.16	1.17	1.18	1.16	1.16	1.20	1.15	1.20	1.22	1.14
Valine	1.76	1.76	1.77	1.75	1.72	1.75	1.72	1.71	1.70	1.72

For Abbreviation of diets names see Table 2.

^a Analysis was conducted by University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA) (Results are expressed on g/100 g of feed as is, unless otherwise indicated).

During growth trial I, DO, temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite, alkalinity and nitrate were maintained within the acceptable ranges for channel catfish at 6.51 \pm 0.51 mg/L, 27.66 \pm 0.12 °C, 4.15 \pm 0.22 g/L, 7.9 \pm 0.66, 0.25 \pm 0.26 mg/L, 0.05 \pm 0.06 mg/ L, 50.0 \pm 1.7 g/L, 36.3 \pm 4.7 g/L, respectively.

During growth trial II, DO, temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite, alkalinity and nitrate were maintained within the acceptable ranges for channel catfish at 7.41 \pm 0.41 mg/L, 28.16 \pm 0.22 °C, 4.55 \pm 0.24 g/L, 7.6 \pm 0.46, 0.15 \pm 0.16 mg/L, 0.02 \pm 0.01 mg/L, 60.0 \pm 1.8 g/L, 35.3 \pm 4.7 g/L, respectively.

2.3. Growth trials

In the first growth trial, Twenty Juvenile fish (mean initial weight of 1.80 ± 0.05 g) sourced from USDA, Auburn, AL, USA were stocked into each aquarium in the experimental system. Each diet was randomly assigned to the aquaria and offered to fish in four replicates. Diets were offered to fish at 3.0-8.0% BW daily over two daily feedings, Fish were weighed every other week and the ration was adjusted each week based on growth and observation of the feeding response. At the end of the growth trial after 10 weeks, fish were counted, and group weighed to determine mean final biomass, final weight, survival, weight gain, feed conversion ratio (FCR). Net protein retention (NPR %) was calculated as: (final weight x final protein content x final dry matter) – (initial weight x initial protein content x final dry matter) – (initial weight x initial protein sealed bags and stored in a freezer (-20 °C) for further analysis.

A second growth trial was conducted with the purpose of confirming if the decreased performance of the fish fed the PDG9 was due to poor feed consumption (i.e. Poor palatability) or a nutrient deficiency. Fifteen juvenile fish (mean initial weight of 6.34 ± 0.18 g) were stocked into each aquarium in the experimental system. Two diets (Basal and PDG9) were offered at two feeding rates. One group was fed close to satiation (SF) while the other group was offered a restricted ration (RF). Fish were weighed every other week and the ration at 4.0–9.0% of BW was adjusted each week based on growth and observation of the feeding response. Feed for the restricted group was reduce by 1% of body weight as compared to that of the satiation group with all feed being quickly consumed. At the end of the six-week growth trial, fish were counted, and group weighed to determine mean final biomass, final weight, survival, weight gain and feed conversion ratio. Five fish were taken randomly from each aquarium, packed in sealed bags, and stored in a freezer (-20 °C) for further analysis.

2.4. Statistical analysis

All the data were analyzed using SAS (V9.4, SAS Institute, Cary, NC, USA). Analysis of Covariance (ANCOVA) was used to determine the effect of base-protein source of the diet (PM and SBM), inclusion level of HP40Y (up to 20% inclusion) and the interaction (P < 0.05) on variables tested during the study. In the presence of significant interaction, two diet series (PM and SBM) were analyzed separately (while using the same basal diet in both sets of diets) using one-way ANOVA followed by the Tukey's multiple comparison test to evaluate significant differences between treatment means. Additionally, regression analysis was performed to identify the relationship between weight gain and the inclusion level of HP40Y in both series of diets.

3. Results

During trial I, the growth performance of juvenile catfish fed diets containing various levels of HP40Y to replace SBM and PM are presented in Table 4 and graphically presented in Figs. 1 and 2. Analysis of Covariance (ANCOVA) revealed a significant interaction between replaced protein (PM and SBM) and the inclusion level of HP40Y on biomass, mean final weight, weight gain, and FCR (P < 0.05) of catfish (Table 4). Therefore, One-way Analysis of Variance (ANOVA) followed by Tukey multiple comparison test was used to test significant differences between treatment means of the tested variables for each diet

Table 4

Response of juvenile catfish (mean initial weight 1.80 ± 0.05 g) fed diets containing different levels of HP40Y to replace PM or SBM over a 10-weeks experimental period. Values represented the mean of four replicates (Trial I).

Diets	HP40Y level (%)	Final Biomass (g)	Final weight (g)	Weight Gain ^a (g)	Weight Gain (%)	Total dry Feed (g)	FCR ^b	Survival (%)	NPR (%)
Basal	0.00	478.78a	24.26a	22.41a	1214.66a	24.35a	1.09c	98.75	42.85a
PDG3	3.10	445.03ab	22.25ab	20.50ab	1168.83a	23.27a	1.14bc	100.0	40.43ab
PDG6	6.20	411.08b	21.40b	19.62b	1103.25a	23.52a	1.20ab	96.25	38.47ab
PDG9	9.30	351.28c	18.02c	16.18c	881.92b	20.50b	1.27a	97.50	33.90b
PSE ^c		20.43	1.28	1.29	82.60	0.73	0.04	3.06	3.71
p-value		0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.03
SDG5	5.00	492.83a	24.64a	22.85a	1277.36a	24.24bc	1.06c	100.0	42.11ab
SDG10	10.00	503.58a	25.18a	23.33a	1262.93a	24.83ab	1.07c	100.0	44.82a
SDG15	15.00	511.50a	25.92a	24.13a	1348.62a	25.30ab	1.05c	98.75	43.78a
SDG20	20.00	506.93a	26.00a	24.19a	1340.35a	25.65a	1.06c	100.0	43.65a
SDG30	30.00	402.90b	20.15b	18.35b	1019.81b	23.32c	1.27b	100.0	35.26b
SDG40	40.00	253.00c	12.65c	10.88c	612.99c	18.30d	1.68a	100.0	24.39c
PSE ^c		14.62	0.83	0.85	62.90	0.58	0.03	1.72	3.15
p-value		0.00	0.00	0.00	0.00	0.00	0.00	0.30	0.00
•									
Outcome	s of ANCOVA (P-value	es)							

Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.31	< 0.0001
Base Inclusion level Interaction (Base*Inclusion)	0.97 <0.0001 <0.0001	0.78 < 0.0001 < 0.0001	0.75 <0.0001 <0.0001	0.55 0.00 <0.0001	0.31 < 0.0001 < 0.0001	0.81 < 0.0001 < 0.0001	0.73 0.12 0.41	0.67 <0.0001 <0.0001

One-way ANOVA was run by both diet type. Diet 1 kept as basal diet for both the sets. Significant difference is noted across treatments for PM and SBM diet type. Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons. PM: Poultry meal; HP40Y: high protein distiller's dried grain with yeast; SBM: Soyabean meal.

For Abbreviation of diets names see Table 2.

^aWeight gain = (final weight-initial weight)/initial weight \times 100%.

^bFCR = Feed conversion ratio = feed offered/ (final weight-initial weight).

 $^{c}PSE = Pooled standard Error.$



Fig. 1. Relationship between weight gain and the inclusion level of high protein dried distillers' grains (HP40Y) in the diets replacing poultry meal.



Fig. 2. Relationship between weight gain and the inclusion level of high protein dried distillers' grains (HP40Y) in the diets replacing soybean meal.

type. In PM replacement series, there was a significant decrease (P <0.05) in fish performance as PM was replaced with HP40Y up to the level of 6% and 9% resulting in reduced levels of weight gain. In diet PDG9, complete replacement of PM resulted in poor growth performance, indicating a possible nutritional deficiency when the animal protein was removed. FCR ranged from 1.09 to 1.27 (Table 4). Moreover, quadratic regression showed that weight gain in fish were observed to be declined as the inclusion levels of HP40Y increased in the diets Basal-PDG9 (pvalue = 0.00; $r^2 = 0.77$) (Fig. 1). In regard to NPR, the control diet had the highest NPR (42%) of all the treatment and significantly higher from PDG9 (33.9%) (Table 4). The proximate whole-body chemical composition of growth trial I was demonstrated in Table 5. The results indicated that there were no significant (P > 0.05) differences between protein, fat and ash of fish body composition. However, there was a decreased tendency in protein and fat content as HP40Y increased in diets (Table 5).

Growth performance of catfish in trial II is summarized in (Table 6). The response of juvenile catfish fed under satiation feeding (Basal vs. PDG9) and restricted feeding (Basal vs. PDG9) showed significant difference (P < 0.05) with regard to final biomass, mean final weight, weight gain, weight gain percentage and FCR. The results indicated that basal diet showed highest weight gain both in satiation feeding group (26.94 g) and in the restricted feeding group (25.00 g) when compared to PDG9 (21.00 g and 16.45 g). The whole-body proximate composition of fish from trial II was presented in Table 7. The results indicated that there were no significant (P > 0.05) differences between protein, fat and ash of fish body analysis either in the satiation feeding group or in restricted feeding group.

As a replacement for soybean meal, catfish were fed with increasing levels of HP40Y (SDG5, SDG10, SDG15, SDG20, SDG30 and SDG40). The diets SDG30 and SDG40 were significantly different (P < 0.05) from all other diets. The treatment SDG20 showed highest FW and WG in catfish (Table 4). The lowest FCR was found in the SDG15 treatment and the highest FCR in the DG40 treatment. Furthermore, as per the results of quadratic regression the diets (SDG5, SDG10, SDG10, SDG15 and SDG20) promoted good performance of catfish apart from diets (SDG30 and SDG40) where the decreased trend of growth was found (*p*-value = 0.00; $r^2 = 0.95$) (Fig. 2). No significant differences were found in survival

Table 5

Whole-body composition (on wet weight basis) of channel catfish, fed different levels of HP40Y for 10 weeks (Trial I).

Diet	Moisture %	Protein (crude) %	Fat%	Ash %
Basal	72.15	14.65	8.98	2.64
PDG3	72.15	14.40	9.16	3.17
PDG6	73.12	14.40	8.42	2.89
PDG9	75.13	13.80	7.78	2.49
PSE ^b	1.42	1.25	0.74	0.42
p-value ^a	0.03	0.82	0.08	0.17
Linear Regress	ion			
r-square	40.8	5.90	33.4	3.20
p-value	0.008	0.36	0.01	0.50
SDG5	72.47	13.57	9.31	3.15
SDG10	70.72	15.02	9.50	3.19
SDG15	71.65	14.05	9.52	3.31
SDG20	71.55	13.92	9.86	2.82
SDG30	73.37	13.90	8.78	2.96
SDG40	73.40	13.15	8.23	3.21
PSE ^b	1.70	0.84	0.71	0.54
p-value ^a	0.29	0.08	0.06	0.58
Linear Regress	ion			
r-square	9.80	16.1	12.7	1.62
p-value	0.10	0.03	0.06	0.51

Fish whole body analysis were analyzed by Midwest Laboratories, Inc., Omaha, NE, USA.

For Abbreviation of diets names see Table 2.

 a Analysis of variance was used to determine significant differences (P < 0.05) among treatment means (n = 4).

^b Pooled standard error of treatment means.

among all treatments which ranged from 96 to 100%. The lowest growth was found in SDG40 in overall experiment as the inclusion level of HP40Y was high in that diet. In case of NPR, there was no significant difference between treatments except for SDG30 and SDG40. The significant decrease occurred in NPR in treatment SDG30 (35%) and

SDG40 (40%) respectively (Table 4). At the conclusion of the experimental period, biomass ranged from 253.0 to 511.5 g, FW ranged from 12.6 to 26.0 g. WG ranged from 612.9 to 1348.6%, survival ranged from 96.0 to 100.0%, FCR ranged from 1.05 to 1.68, total dry feed used ranged from 18.3 to 25.6 g and NPR ranged from 24 to 44% respectively.

4. Discussion

Catfish has a great ability to utilize diets that have a high carbohydrate content and digest protein and energy from both animal and plant feed ingredients (Glencross et al., 2011; Phumee et al., 2011). The establishment of different feed ingredients in fish diets depends on numerous factors including nutrient content, cost, availability, and physical properties (Hardy and Barrows, 2002). One of the primary goals of the feed industry is to develop information on the efficacy of a wide range of ingredients of potential use in commercial feeds; thus, allowing for more choices during the feed formulation process.

Distillers dried grains with solubles (DDGS) has been used in aquatic feeds since 1940s; nevertheless, inclusion levels in diets were relatively low (Phillips et al., 1964). The efficacy of DDGS from ethanol industry have been well studied (Robinson and Li, 2008; Tidwell et al., 1990; Webster et al., 1991, 1992, 1993) and these meals are commonly used in catfish feed formulations. The nutrient composition of DDGS varies substantially with grain sources and processing conditions. Corn is the most commonly used feedstock in the manufacturing of DDGS followed by sorghum, wheat, barley, and other cereal grains (Rhodes et al., 2015). Conventional DDGS comprises 28-32% crude protein and is comparatively high in fiber content, which limits its use in aquaculture feeds (Gatlin et al., 2007). By shifting the manufacturing process of an ethanol plant, significant improvements to the byproduct output can be made. Various technologies can increase the protein concentration and reduce the fiber content of DDGS, which makes it a more valuable feed component. More recently, high protein distiller dried grains (HPDDG) which contains high protein (41-48%) has become available as protein

Table 6

Response of juvenile catfish (mean initial weight 6.34 ± 0.18 g) fed diets containing different levels of HP40Y to replace PM at two feeding rates (satiation feeding and restricted feeding) over a 6-weeks experimental period. Values represented the mean of four replicates (Trial II).

Diets	HP40Y level (%)	Final Biomass (g)	Final weight (g)	Weight Gain ^a (g)	Weight Gain (%)	FCR ^b	Survival %
SF ^d	0.00	500.28a	33.35a	26.94a	420.4a	1.27b	100.0
SF	9.30	411.07b	27.40b	21.00b	328.4b	1.45a	100.0
RF ^e	0.00	471.57a	31.43a	25.00a	389.0a	1.07c	100.0
RF	9.30	338.97c	22.59c	16.45c	268.2c	1.34b	100.0
PSE ^c		15.89	1.05	1.00	15.19	0.03	0.35
p-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.5800

Note: Analysis of variance was used to determine significant differences (P < 0.05) among treatment means (n = 4). PM: Poultry meal; HP40Y: high protein distiller's dried grain with yeast.

^a Weight gain = (final weight-initial weight)/initial weight \times 100%.

^b FCR = Feed conversion ratio = feed offered/ (final weight-initial weight).

^c PSE = Pooled standard Error.

 $^{\rm d}~{\rm SF}={\rm Satiation}$ feeding.

 e RF = Restricted feeding.

Table 7

Whole-body	composition	(on wet w	eight basis)	of channel	catfish, f	fed different	levels of HP	40Y for 6	weeks ((Trial II).
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Diets	HP40Y level (%)	Moisture %	Protein (crude) %	Fat %	Ash %
SF	0	71.30b	14.50	9.40	3.19
SF	9.3	73.20ab	14.57	8.29	3.10
RF	0	73.67ab	14.50	9.12	2.87
RF	9.3	74.87a	14.95	7.37	2.44
PSE ^b		1.40	0.65	1.59	0.89
p-value ^a		0.02	0.73	0.31	0.65

Fish whole body analysis were analyzed by Midwest Laboratories, Inc., Omaha, NE, USA.

Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons.

^a Analysis of variance was used to determine significant differences (P < 0.05) among treatment means (n = 4).

^b Pooled standard error of treatment means.

source and use in aquaculture feed formulation (Tidwell et al., 2017; Goda et al., 2019). The HPDDG is a resulting product of biological ethanol fermentation that uses prefractionation technology (Prachom et al., 2013). This new ingredient revealed a promising composition as compared to prior ethanol industry by-products (DDGS) (Li et al., 2010; Lim et al., 2009; Robinson and Li, 2008; Webster et al., 1991).

Due to new developments and technology advancements, certain ethanol plants are modifying their processing and extracting lipid from DDGS resulting in an increased protein and reduced lipid contents. Results from numerous studies revealed that lipid extracted DDGS (LEDDGS) might be included in aquatic feeds up to 300 g kg - 1 of diet. Likewise, (Zhou et al., 2010) estimated fuel-based DDGS to substitute soybean meal and corn meal in juvenile hybrid catfish diet (320 g protein kg - 1 of diet). They recommended that diet comprising 300 g kg - 1 LE-DDGS provided good growth, and feed conversion in catfish.

In the present study, the HP40Y was processed under modified technology by separating corn fiber prior to fermentation and removing the soluble fraction after fermentation to produce a high-quality combination of corn and yeast proteins. This HP40Y product has a higher protein level of 41–43% than that in traditional DDGS (27–30%). In this study, graded levels of HP40Y are used to replace PM or SBM.

The basal diet was formulated to contain 6% poultry meal as the only animal protein source which was sequentially replaced by HP40Y resulting in a plant-based feed formulation. Results demonstrated (Table 4) that as PM was replaced there was a negative effect on weight gain (Fig. 1). Visual observations of feeding indicated a noticeable decrease in the feed intake which could have been due to poor palatability, dietary deficiencies and/or reduced size of the fish. Barnes et al. (2012) found decreased growth when 10% DDGS replaced fishmeal, wheat and corn gluten meal in diets for rainbow trout, even when the diets were supplemented with essential amino acids and phytase. With any protein replacement strategy alternative feedstuff might not be so palatable and consequently voluntary feed consumption may compromise, which in turn may influence growth performance.

Albeit plant-based diets have been used with channel catfish, such diet are more likely to have palatability or nutritional issues. To evaluate if palatability or nutrient limitations were responsible for this response a second trial was conducted. In this case our typical feed management was employed for one set of fish and a restriction of the ration was employed in the other set of fish. As the ration was restricted and all the feed was rapidly consumed, the issue of palatability was removed. Yet, in this case fish maintained on the basal diet outperformed fish offered the PDG9 diet irrespective of feeding strategy (Table 6). Confirming some nutrient limitation which is further supported by results in the diet series replacing soybean meal.

In the case of using HP40Y as a replacement of soybean meal this is nutritionally a better comparison as profiles are more similar. The results of this portion of the study demonstrated that HP40Y could be used in juvenile catfish diets at a level of up to 20% to replace SBM with no significant effects on growth or feed utilization (Table 4). However, as the inclusion levels of HP40Y in the diets increased over 20% there was a trend of reduced growth performance of juvenile catfish (Fig. 2). Similarly, Tidwell et al. (2017) observed that 20% HPDDG of the total formulation can be included in the diet of channel catfish and increasing the inclusion level up to 40% showed a significant retardation in growth. Cheng and Hardy (2004) also reported that the inclusion level up to 20% dietary HPDDG with no negative effects on growth in rainbow trout. Similarly, Stone et al. (2005) found deficiencies in trout diets with conventional DDGS compared to a fish meal.

Methionine and lysine are generally the limiting amino acids in fish feeds, specifically those containing higher levels of plant protein sources (Mai et al., 2006). This HP40Y contains a lower level of lysine as compared to that of SBM (1.30 and 3.02%, respectively), whereas it contains higher methionine in comparison to that of SBM (0.86 vs. 0.64%) but lower than that of PM (1.22%). Depending on the ingredient matrix, it may be required to supplement lysine or other EAA to fulfill

the nutritional requirement of fish when higher levels of DDGS are included in the diets. The lysine requirement for fingerling channel catfish is approximately 1.5% of the dry diet or 5.0% of the dietary protein (Robinson et al., 1980). In our study, amino acid profile of all diets (Table 3) was analyzed and found that all diets fulfilled the lysine requirement except for a marginal decrease in diet SDG40 (1.48%) which showed decreased growth. Thus, possibly indicating a limitation in lysine or it could be any other nutrient which caused retardation in growth.

In this study, NPR of catfish significantly decreased with the increase of HP40Y in PM and SBM diets (Table 4). This might be the reason of reduced growth of fish at high inclusion level of HP40Y. The treatment PDG9 has 33% protein retention and treatment SDG30 and SDG40 has 35% and 24% respectively in regard of basal diet which contain 42% NPR. The other reasons could include smaller size fish which had less flesh and also may be due to imbalance of dietary EAA that could be lysine as indicated in our SDG40 treatment which has marginally low lysine level. Nguyen and Davis (2016) found that dietary lysine supplementation significantly increased protein retention in channel catfish. Similarly, Webster et al. (1992) observed that the protein content of channel catfish fed a diet with 90% corn distiller's dried grains with solubles (CDDGS) with added lysine was significantly higher in protein as compared to those fed the 90% CDDGS diets without lysine supplementation.

With regards to proximate composition of fish from trial I, higher levels of HP40Y substitution marginally affected the whole-body proximate composition. ANOVA indicated that both protein and lipid levels were not significantly different due to dietary levels of HP40Y. However, regression analysis demonstrates a small but significant decline in protein and lipids albeit both have poor r^2 values ($r^2 = 5.90$) ($r^2 = 33.4$) for PM diet series and $(r^2 = 16.1)$ $(r^2 = 12.7)$ for SBM diet series (Table 5). The lower lipid content may be due reduced digestible energy in the diets. Chatvijitkul et al. (2016) observed that there was a trend of reduced growth performance of juvenile tilapia as the inclusion levels of lipid extracted-DDGS in the diets increased. This might be due to the fact that LE-DDGS contains lower level of lipid than DDGS, thus it provides less energy to the diets. Tidwell et al. (2017) reported that channel catfish experienced a significant decrease in protein content with increasing HPDDG to 40% when compared to the control. Likewise, using 40% DDGS substitution for SBM significantly decreased body protein content, although, fish fed 40% with lysine supplementation did not show any significant differences in protein content (Lim et al., 2007).

5. Conclusion

The findings of the present study suggested that HP40Y is a good plant protein source and can be supplemented in the catfish diets up to 20% to replace SBM without compromising growth. In addition, the HP40Y also contains an elevated level of yeast, stimulating growth. In PM replacement series, complete replacement of PM with HP40Y in diet PDG9 resulted in poor performance, indicating a possible nutritional deficiency when the animal protein was removed. Further studies should contemplate the supplementation of HP40Y as an effective protein source for the diets of carnivorous aquaculture fish species, for instance trout and other salmonids.

Declaration of Competing Interest

Authors would like to confirm that there are no conflict of interest related to data and manuscript.

Acknowledgement

The authors would like to express gratitude and appreciation to those who have taken time to critically review this manuscript as well as those who helped to carry-out this research at the E.W. Shell Research Station, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University. This work was supported in part by the Hatch program (ALA016-08027) of the National Institute of Food and Agriculture, USDA and the Andersons, Maumee, OH, USA. Mention of trademark or proprietary products does not constitute an endorsement of the product by Auburn University and does not imply its approval to the exclusion of other products that may also be suitable. This study was part of the first author's Ph.D. dissertation, who had a fully funded scholarship from the Higher Education Commission, Pakistan.

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