USE OF HIGH PROTEIN DISTILLER'S DRIED GRAIN AS AN ALTERNATIVE INGREDIENT IN PRACTICAL DIETS OF DIFFERENT SPECIES (*Ictalurus punctatus, Litopenaeus vannamei* and *Oreochromis niloticus*)

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Abstract

Increasing worldwide competition and shifts in demand, technological advancements, and innovative research findings are triggering the ingredient manufacturing industry to include modified processing technologies and novel ingredients. New technologies have been introduced by the ethanol industry to improve the efficiency of ethanol production, resulting in new types of distiller dried grain with different nutrient profiles. One of the new processing techniques removes fibrous corn components before fermentation and removes the soluble fraction after fermentation to produce a high protein distiller grain with yeast (HP50Y) with 50% protein and a second product (HP40Y) that is 40% protein. The technique used to make these two ingredients was the same, however, performance of both products was needed to determine efficacy. High protein distillers dried grain with yeast is a relatively new product which is a variant of distillers dried grains that could be used as an improved protein source in animals feed formulations.

To evaluate the efficacy of HP40Y and HP50Y, numerous laboratory-based trials were conducted at the E.W. Shell Fisheries Center at Auburn University in Auburn, Alabama. A 10-week trial was conducted to evaluate the growth performance of juvenile catfish, *Ictalurus punctatus* (mean initial weight 1.80 ± 0.05g). 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 another 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%). Results 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 food conversion ratio (FCR) of catfish. In the poultry meal replacement series, complete replacement of PM with HP40Y 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 indicated that HP4Y is a good protein source when used at levels less than 30% of the diet.

Another growth trial was conducted to evaluate the efficacy of HP50Y and HP40Y as a replacement for corn protein concentrate (CPC) in diets of Pacific white shrimp, *Litopenaeus vannamei*. In this trial, graded levels of HP50Y (0.0, 5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (13.1, 10.0, 6.6, 3.5 and 0.2%). In the second series of diets, graded levels of HP40Y (5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (10.5, 8.0, 5.5 and 2.5%) which was evaluated over a 40-day growth trial (mean initial weight 0.54 ± 0.01 g; n=4). At the conclusion, no significant differences were detected in growth, FCR, survival and food consumption of shrimp ($p>0.05$). However, results from regression analysis revealed a significant increase in weight gain (%)of shrimp as the percentage inclusion level of HP50Y and HP40Y increased in shrimp diets. Results indicated that HP50Y and HP40Y are both good protein sources and can be used up to 20% inclusion level in the diets of shrimp.

A 10-week growth trial with tilapia was conducted, using nine diets formulated to contain 32% protein and 6% lipid. Each protein was included at various levels (0, 5, 10, 15 and 20%) replacing CPC on a protein basis. Juvenile tilapia (mean initial weight 5.23 ±0.20g) were evenly distributed in thirty-six, 75-L aquaria working as a recirculating system and fed twice daily to apparent satiation throughout the study. Tilapia exhibited no significant ($p>0.05$) differences in growth, FCR, survival, whole-body proximate composition, mineral composition, and hematological parameters when fed HP50Y and HP40Y supplemented diets compared to the control diet. Digestibility coefficients for the test ingredients were determined

in tilapia for dry matter, energy, crude protein, individual and total amino acids using 1% titanium oxide as the inert marker with 70:30 replacement strategies. All the values were found to be within an acceptable range for the distiller grains when compared to the literature. Results from this study revealed that HP50Y and HP40Y both are good alternative protein sources and can be used up to 20% inclusion level in the diets of tilapia.

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CHAPTER 1

GENERAL INTRODUCTION

Aquaculture, the farming of aquatic animals and plants, has continued to expand resulting in the production of 179 million tons in 2018. In response to increasing demand for seafood, aquaculture production has shown strong growth, increasing at an average annual rate of 3.3%. Seafood represents a high value and nutritious protein source for the human population. However, there are several needs that must be met if aquaculture continues to grow. Availability of land, clean water, and an adequate feed supply all influence aquaculture operations (FAO, 2020). Aquaculture production also depends upon the provision of nutrients as in other terrestrial farming practices (Tacon and Metian, 2008). Diets with proper nutrient balance are essential in enhancing fish health and higher fish production. Global fish feed production was estimated to increase up to 70,969 thousand tons by 2020, which is a nearly 10-fold increase from 1995 (Tacon, et al., 2011). With those rising figures, there is huge pressure with sourcing feed ingredients that are used to produce the aquatic animal feeds.

Feed costs are primarily driven by the cost of protein sources in the feed. Substitution of expensive protein sources with lower cost ingredients would potentially reduce feed cost. Fish meal is a good source of essential fatty acids (EFAs), minerals & vitamins, and digestible energy. However, because of the inadequate availability and high-cost feed, formulations have turned to increasing levels of alternative protein sources employing constrained amounts of animal proteins or fish meal (Tacon and Metian, 2008). In addition to economic concerns caused by limited supplies and increasing demand, the use of marine ingredients has received considerable attention by the public in terms of perceived sustainability issues. Hence, the

overall reduction in the use of fish meal and other marine ingredients is of considerable concern to the industry and should be viewed as a high priority.

Soybean meal is often considered the most reliable ingredient and cost-effective source in aquatic animal feed because of its high protein content, high digestibility, relatively well-balanced amino acid profile, reasonable price and steady supply (Davis and Arnold, 2000, Amaya et al., 2007). Usually, the use of plant proteins shows some limitation due to a variety of factors, including a deficiency or imbalance of essential amino acids (EAAs), presence of anti-nutritional factors or toxins, and decreased palatability. Many of these limitations can be overcome through the use of proper combinations of different types of plant proteins to balance essential nutrient profiles (e.g. amino acids and fatty acids); by developing specific processing procedures to inactivate, reduce, or eliminate anti-nutritional factors (e.g. heat treatment to inactivate heat labile components), and/or by limiting their inclusion in the diet to a level that does not influence animal performance (Li et al., 2000).

To concentrate the protein in cereal grains, several processes have been applied and are being studied to replace other protein sources. Corn protein concentrate (CPC) acquired from the wet-mill process is the dried protein portion of the corn mainly emerging from the endosperm after discarding the majority of the non-protein elements by enzymatic solubilization of the protein stream (Yu, et al., 2013). In the process of wet milling, corn is drenched in a solution to soften the kernel to ease the separation of the different component parts to produce a variety of co-products comprising corn gluten meal, corn oil, corn gluten feed (Rausch, et al., 2003; Singh, et al., 2006; Malumba, et al., 2015). In the aquaculture industry, CPC has great potential to be used in many diet formulations. It has been used in the diet of Atlantic salmon (*Salmo salar*) (Burr, et al., 2012), Pacific white shrimp (*Litopenaeus vannamei)* (Zhou, et al., 2014) and Florida Pompano (*Trachinotus carolinus*) (Cook, et al., 2016).

Distiller dried grains with solubles (DDGS) is a valuable feed ingredient and is one of the three co-products produced in dry grind ethanol plants, along with fuel ethanol and carbon dioxide (Shurson, 2003). DDGS in aquaculture feeds presents a substantial economic value as it is less expensive than other protein/energy sources like soybean meal. Corn DDGS are rich in vitamin A, niacin, and choline, and contain several minerals, including phosphorus (Lim et al., 2011). Lim and Yildirin-Aksoy (2008) reported that DDGS is a good source of vitamins, minerals, and essential amino acids. DDGS contain substantial amount of yeast. Yeast is also rich in protein, B-complex vitamins, and β-glucan.

DDGS has been used in fish diets since the late 1940s. Several studies have indicated that DDGS is a promising feed ingredient in a range of fish species, including rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), and tilapia (*Oreochromis niloticus*) (Tidwell et al., 1990, Webster et al., 1992, Webster et al. 1993, Cheng and Hardy 2004). Early studies demonstrated that up to 35% DDGS without lysine supplementation (Webster et al., 1992) could be used to partially replace soybean meal and fish meal in channel catfish diets without affecting fish growth. Webster et al. (1993) reported that 30% DDGS could be used as a replacement of a mixture of soybean meal and corn meal in channel catfish containing 8% fish meal. Li et al., (2011) found the same weight gain, feed efficiency ratio (FER), protein efficiency (PER) in fish fed diets with up to 30% wheat DDGS or up to 40% with lysine supplementation in Nile tilapia. Robinson and Li (2008) reported that up to 30-40% DDGS with supplemental lysine could be used in channel catfish.

As the technologies of the 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). To 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 be 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).

The high protein distiller's dried grain along with yeast (HP40Y and HP50Y) used in this research is a next step processing modification that made a high level of protein (40% and 50% crude protein) including spent yeast from the ethanol fermentation procedure. It is manufactured by separating corn fiber preceding to fermentation and gets rid of the solubles portion after fermentation to make a high-quality mixture of corn and yeast proteins. From the economic point of view these products are cheaper compared to fish meal/poultry meal as well as soybean meal. In summary, the purpose of this study is to investigate novel protein source (high protein distiller's dried grain with yeast) as replacements for conventional protein source (e.g., Poultry by-product meal, fish meal, soybean meal and corn protein concentrate) in the practical diets for catfish *Ictalurus punctatus,* Pacific white shrimp *Litopenaeus vannamei* and Tilapia *Oreochromis niloticus.* This study evaluated the efficacy of high protein distiller's dried grain with yeast, which has various levels of protein (40%, 42% and 50%) containing spent yeast from the ethanol fermentation process, in aquatic animal feeds of several commercial important aquaculture species.

1. To evaluate the efficacy of high protein distiller's dried grain with yeast HP42Y as a replacement with poultry meal as well as soybean meal to check growth indices

like final biomass, mean final weight, weight gain, weight gain percentage, FCR and net protein retention in catfish. The whole-body composition of channel catfish was also accessed.

2. To evaluate the efficacy of HP40Y and HP50Y as a replacement of corn protein concentrate to check growth indices like final biomass, mean final weight, weight gain, weight gain percentage, FCR, food consumption and net protein retention in shrimp.
3. Evaluation of growth parameters, nutrient digestibility, and hematological parameters of tilapia fed diets containing different levels of high protein distiller's dried grain (HP40Y and HP50Y) with yeast as a replacement for corn protein concentrate.

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CHAPTER II

USE OF HIGH PROTEIN DISTILLER'S DRIED GRAIN WITH YEAST IN PRACTICAL DIETS FOR THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS*

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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 ± 0.05g). 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.

KEYWORDS: Catfish, growth performance, High protein distillers dried grain, Poultry meal, soybean meal, alternative protein sources.

1. **Introduction**

Reducing feed cost is essential for the long-term sustainability of the 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 a 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 dry-mill 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 include numerous potential benefits to animals including moderate lipid and protein contents, along with phosphorus, vitamins, and 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 (ANDVantage^{TM} 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 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℃) overnight to attain a moisture content of less than 10%. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer (-20℃) 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 (Table 2 and 3).

2.2 Experimental Systems

Both growth trials were conducted in an indoor recirculation system. 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 m^2 media, 0.6 m × 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 minutes/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).

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±0.51 mg/L, 27.66±0.12°C, 4.15±0.22 g/L, 7.9±0.66, 0.25±0.26 mg/L, 0.05±0.06 mg/L, 50.0±1.7g/L, 36.3±4.7g/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±0.41 mg/L, 28.16±0.22°C, 4.55±0.24 g/L, 7.6±0.46, 0.15±0.16 mg/L, 0.02±0.01 mg/L, 60.0±1.8g/L, 35.3±4.7g/L, respectively.

2.3 Growth Trials

In the first growth trial, Twenty Juvenile fish (mean initial weight of 1.80 ± 0.05g) 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 initial dry matter)/ (protein in feed x amount of feed consumed). Five fish were taken randomly from each aquarium, packed in sealed bags and stored in a freezer (-20℃) 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 ±0.18g) 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℃) 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 Figures 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 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 (p-value=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 (26.94g) and in the restricted feeding group (25.00g) when compared to PDG9 (21.00g and 16.45g). 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, 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 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 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 technological 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 (LE-DDGS) 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 (Figure 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 (Figure 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).

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.

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	**Soybean 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

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/100g 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

[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/100g 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

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

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

Table 3: Amino acid profile (g/100g as is) of test diets fed to channel catfish

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

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

For Abbreviation of diets names see table 2

Table 4: Response of juvenile catfish (mean initial weight 1.80 ±0.05g) 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

Outcomes of ANCOVA (P-values)								
Model	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.31	<0.0001
Base	0.97	0.78	0.75	0.55	0.31	0.81	0.73	0.67
Inclusion level	<0.0001	<0.0001	<0.0001	0.00	<0.0001	<0.0001	0.12	<0.0001
Interaction (Base*Inclusion)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.41	<0.0001

Note: 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

[a]Weight gain= (final weight-initial weight)/initial weight × 100%

[b]FCR=Feed conversion ratio = feed offered/ (final weight-initial weight)

[c]PSE = Pooled standard Error

For Abbreviation of diets names see table 2

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 Regression				
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 Regression				
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.

[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.

For Abbreviation of diets names see table 2

Table 6: Response of juvenile catfish (mean initial weight 6.34 ±0.18g) 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 %
[d]SF	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
[e]RF	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 × 100%

[b]FCR= Feed conversion ratio = feed offered/ (final weight-initial weight)

[c]PSE = Pooled standard Error

[d]SF= Satiation feeding

[e]RF= Restricted feeding

Table 7: Whole-body composition (on wet weight basis) of channel catfish, fed different levels of HP40Y for 6 weeks (Trial II)

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.

[a]Analysis of variance was used to determine significant differences ($P<0.05$) among treatment means (n= 4) Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons.

[b]Pooled standard error of treatment means.

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

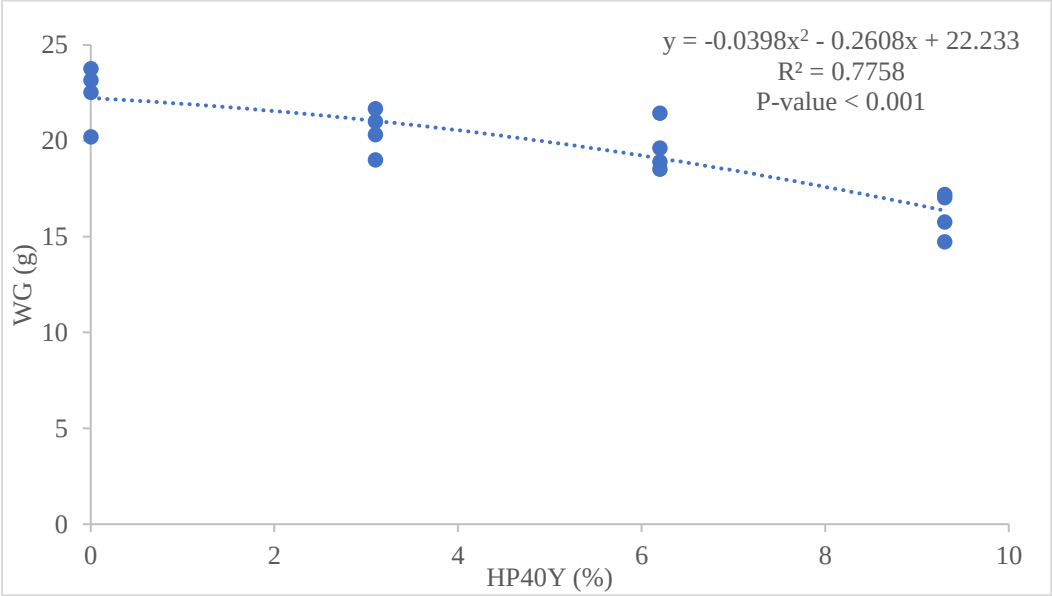
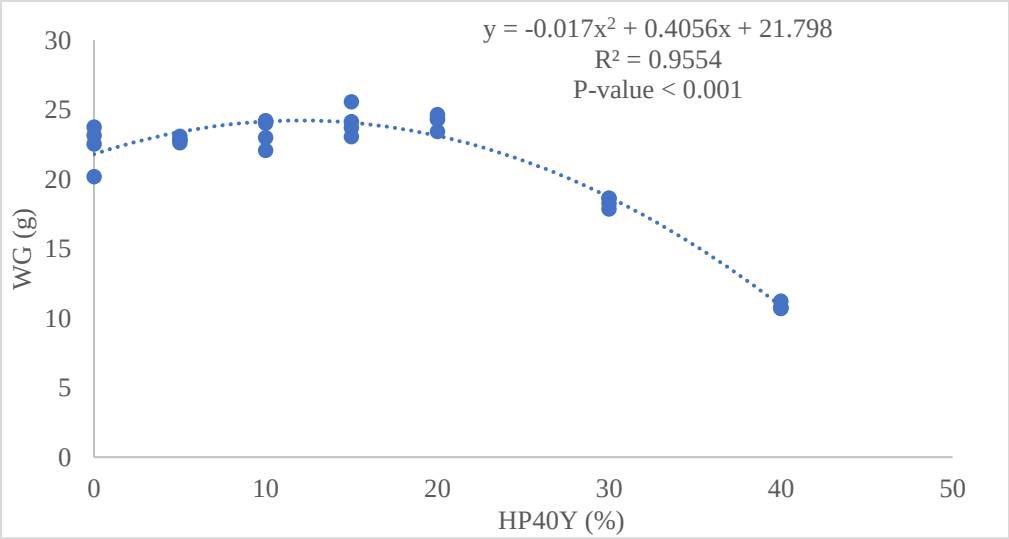


Fig 2: Relationship between weight gain and the inclusion level of high protein dried distillers' grains (HP40Y) in the diets replacing soybean meal.



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CHAPTER III

EVALUATION OF A HIGH PROTEIN DISTILLER'S DRIED GRAINS WITH YEAST AS A PROTEIN SOURCE IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *LITOPENAEUS VANNAMEI*

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Abstract

Increasing worldwide competition and shifts in demand, technological advancements, and innovative findings out of research are triggering the ingredient manufacturing industry to include modified processing technologies and novel ingredients. New technologies have been introduced by the ethanol industry to improve the efficiency of ethanol production, resulting in new types of distiller dried grain with different nutrient profiles. One of the new processing techniques removes fibrous corn components before fermentation and removing the soluble fraction after fermentation to produce a high protein distiller grain with yeast (HP50Y) 49% protein and (HP40Y) have 40% protein in it. The technique as described above to make these two ingredients was same but to keep two different level of proteins to check which one was more effective. The current study was conducted to evaluate the efficacy of HP50Y and HP40Y as a replacement for corn protein concentrate (CPC) in diets of pacific white shrimps, *Litopenaeus vannamei*. In the growth trial, graded levels of HP50Y (0.0, 5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (13.1, 10.0, 6.6, 3.5 and 0.2%) In the second series of diet, graded levels of HP40Y (5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (10.5, 8.0, 5.5 and 2.5%) which was evaluated over a 40 days growth

trial (initial weight 0.54 ± 0.01 g; n=4). At the conclusion, no significant differences were detected in growth, FCR, survival and food consumption of shrimp (P-value>0.05). However, results from regression analysis revealed that there was a significant increase in weight gain percentage of shrimp as the percentage inclusion level of HP50Y and HP40Y have increased in the diets of shrimp. Results indicate that HP50Y and HP40Y both are good protein source and can be used up to 20% inclusion level in the diets of shrimp.

KEY WORDS: High protein distillers dried grain, growth performance, corn protein concentrate alternative protein sources, *Litopenaeus vannamei*.

1. Introduction

The use of cost-effective feed formulations for *L. vannamei* is important to increasing profit margins by reducing feed expense. The choice of proper feed ingredients must focus not only on cost reduction but also increasing the nutritional quality of feeds and lessening metabolic waste as fulfilling all nutrient needs of the fast-growing shrimp. The advancement of commercial aquatic feeds has conventionally been relied on fish meal (FM) as the major protein source because of its balanced essential amino acid (EAA) profile and high protein content (Hardy, 1996). Fish meal is also a good source of essential fatty acids (EFAs), minerals & vitamins, and digestible energy. Though, because of the inadequate availability and high-cost feed; formulations have turned to increasing levels of alternative protein sources employing constrained amounts of animal proteins or fish meal (Amaya, et al., 2007; Tacon, et al., 2009).

As an alternative plant protein ingredient, soybean meal is an economical and a commonly accessible protein source with comparatively high digestible protein, good amino acid profile and high energy contents (Hertrampf and Piedad-Pascual, 2000). Numerous studies have been performed to assess the nutritional requirement of different soybean products in shrimp diets (Lim and Dominy, 1990; Sudaryono, et al., 1995; Cruz-Suarez, et al., 2001; Smith, et al., 2001; Samocha, et al., 2004; Roy, et al., 2009). Nevertheless, there are some limitations linked with the consumption of soybean meal as it contains adequate protein content and its somewhat low level of essential amino acids for example methionine. Therefore, high protein plant ingredients have been chosen as their nutrient density makes it more appropriate for different formulations and complement the amino acid profiles of the other protein sources.

To concentrate the protein in cereal grains, several processes have been applied and are being studied to replace other protein sources. Corn protein concentrate (CPC) acquired from the

wet-mill process is the dried protein portion of the corn mainly emerging from the endosperm after discarding majority of the non-protein elements by enzymatic solubilization of the protein stream (Yu, et al., 2013). In the current study, corn protein concentrate is high in protein (77.2%) and a rich source of methionine (1.94%). In the process of wet milling, corn is drenched in a solution to soften the kernel to ease the separation of the different component parts to produce a variety of co-product comprising corn gluten meal, corn oil, corn gluten feed (Rausch, et al., 2003; Singh, et al., 2006; Malumba, et al., 2015). In aquaculture industry, CPC has a great potential to be used in many diet formulations. It has been used in diet of Atlantic salmon (Burr, et al., 2012), Pacific white shrimp (Zhou, et al., 2014) and Florida Pompano (Cook, et al., 2016).

There has been a tremendous growth in ethanol production from corn, by either dry or wet milling processing, because of the huge demand for ethanol as a fuel additive (Rausch and Belyea, 2006). Distiller dried grains with soluble (DDGS) is a corn by-product of the dry-mill ethanol industry. The nutrient profile of DDGS differs greatly with processing conditions and grain sources. There are numerous grains from which DDGS can be produced for example wheat, sorghum, barley and other cereal grains but corn is more frequently used feedstock in the production of DDGS (Rhodes, et al., 2015a). As a nutrient source, the composition of DDGS offers yeast, phosphorus, vitamins, lipids, glucans and protein which can enhance growth performance and immune response (Tidwell, et al., 1993). Several studies have showed that DDGS can be effectively used as a protein source in the diets of multiple species for example channel catfish *Ictalurus punctatus*, (Webster, et al., 1992; Robinson and Li, 2008; Lim, et al., 2009; Li, et al., 2010) Pacific white shrimp *Litopenaeus vannamei* (Roy, et al., 2009; Sookying and Davis, 2011; Rhodes, et al., 2015b; Akinbode, et al., 2017), freshwater prawns *Macrobrachium rosenbergii*

(Tidwell, et al., 1993), tilapia *Oreochromis spp*. (Chatvijitkul, et al., 2016), redclaw crayfish *Cherax quadricarinatus* (Garza de Yta, et al., 2012) and yellow perch (Schaeffer, et al., 2011).

Typically, traditional corn DDGS comprises almost 10% fat, 28-32% crude protein, and about 11% fiber content which is high and restricts its use as an ingredient in aquaculture feed industry (Millamena, et al., 1996a; Singh, et al., 2005; Gatlin, et al., 2007). However, a lot of ethanol plants are applying an improved dry milling process known as fractionation. In this new technique, entire corn is milled, then arranged into individual fractions: bran, corn germ, and the endosperm. The two major co-products of the modified method are high-protein distiller's dried grains (HPDDG) and corn germ. In contrast to traditional DDGS, this HPDDG product has a protein level of 41-43% and lower levels of phosphorous and fat because it does not include the solubles part that would typically be added back to the distiller's dried grains (Tidwell, et al., 2017). Moreover, lipid-extracted DDGS (LE-DDG) which have reduced oil and of high quality, it can be processed to cut off the lipid and enhance the protein concentration of DDGS, which makes it a more valuable feed component. High protein and LE-DDGS products have been used in agricultural diets for cattle, swine and poultry (Rausch and Belyea, 2006).

The high protein distiller's dried grain along with yeast (HP40Y and HP50Y) used in this research is a next step processing modification that made a high level of protein (40% and 50% crude protein) including spent yeast from the ethanol fermentation procedure. It is manufactured by separating corn fiber preceding to fermentation and get rid of the solubles portion after fermentation to make a high-quality mixture of corn and yeast proteins. As from the economical point of view these products are cheaper as compared to fish meal/poultry meal as well as soybean meal. We previously evaluated the HP40Y as a replacement for soybean meal (SBM) and poultry meal (PM) in the practical diet of catfish (Nazeer, et al., 2022). However, there is little information

on HPDDG nutritional value as a replacement for corn protein concentrate (CPC), which is another essential plant protein source used in shrimp diets. Consequently, the purpose of this study is to evaluate the use of these products (HP40Y and HP50Y) in the practical diets of Pacific white shrimp, *L. vannamei* as a replacement for corn protein concentrate.

2. Materials and Methods

2.1 Diet Preparation

The test ingredients, high protein distiller's dried grain with yeast (HP40Y and HP50Y) was obtained from The Andersons, Maumee, OH, USA (ANDVantage™ 40Y, ANDVantage™ 50Y). Amino acid (AA) and proximate analysis of the main protein sources are given in Table 1. The proximate composition and formulation of the nine test diets are shown in Table 2. All test diets were made on an isolipidic and isonitrogenous basis to contain 8% lipid and 36% protein. To meet the nutritional requirements of the shrimps, basal and experimental diets were formulated. Two diet series were formulated. In the first series, graded levels of HP50Y (0.0, 5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (13.1, 10.0, 6.6, 3.5 and 0.2%) and the experimental diets were designated as DG50-0, DG50-5, DG50-10, DG50-15, and DG50-20. In the second diet series, graded levels of HP40Y (5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (10.5, 8.0, 5.5 and 2.5%) and were referred to as DG40-5, DG40-10, DG40-15 and DG40-20 (Table 2).

The experimental diets were made at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University (Auburn, AL). The pre-ground dry ingredients and oil were weighed and then stir together in a food mixer (Hobart Corporation, Troy, OH, USA) for 15-20 min. Hot water was then added into the mixture to acquire a consistency suitable for pelleting. Diets were made using a meat grinder with a 3-mm die. The moist pellets were then put into a forced air oven (<45°C) overnight to accomplish a moisture

content of less than 10%. Dry pellets were crumbled, packed in sealed bags, and kept in a freezer (-20℃) till needed. All the diets were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO) for amino acid profile and proximate composition (Table 2 and 3).

2.2 Experimental Systems

The growth trial was done in an indoor recirculation system. The growth trial comprised of thirty-six 75-L glass aquaria linked to a common reservoir tank (800-L) at E.W. Shell Fisheries Center at Auburn University, Auburn, Alabama, in agreement with the Auburn University animal care policy. Water quality was maintained by recirculation through a vertical fluidized bed biological filter (600-L volume with 200-L of Kaldnes media) using a 0.25-hp. centrifugal pump and an Aquadyne bead filter (0.2 m^2 media, 0.6 m × 1.1 m) and average water flow for an aquarium was 4 L/min with an average turnover of around 20 minutes/tank. By using air stones, dissolved oxygen was maintained close to saturation in every culture tank and the sump tank using a common airline attached to a regenerative blower. Throughout the experiment, temperature, salinity, and dissolved oxygen (DO) were checked twice daily using an YSI 55 multi-parameter instrument (YSI, Yellow Springs, OH) and nitrite-N as well as total ammonia N (TAN) were measured twice per week by using YSI 9300 photometer (YSI, Yellow Springs, OH). During the experimental period, the pH of the water was measured twice weekly by using the pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA), whereas nitrate, alkalinity and hardness level of water was measured twice per month using WaterLink-Spin TouchFF photometer (LaMotte Company, Chestertown, MD).

All through the growth trial, DO, salinity, pH, temperature, total ammonia nitrogen (TAN), nitrite, nitrate and alkalinity were maintained within the normal ranges for Pacific white shrimp at

6.51±0.51 mg/L, 4.15±0.22 g/L, 7.9±0.66, 27.66±0.12℃, 0.25±0.26 mg/L, 0.05±0.06 mg/L, , 36.3±4.7g/L and 50.0±1.7g/L, respectively.

2.3 Growth Trial

For the experiment, Pacific white shrimp (~0.003 g), post larval (PL) were obtained from American Mariculture, Fort Meyers, Florida and reared in an indoor recirculating system. PLs were given a commercial feed (protein ≥ 50%, fiber ≤ 1%, fat ≥ 15% by Zeigler Bros. Inc. Gardners, PA, USA) for approximately 1 week and then shifted to crumbled commercial shrimp feed (protein ≥ 40%, fiber ≤ 3%, fat ≥ 9% by Zeigler Bros. Inc., Gardners, PA, USA) for around 1 week. Ten pacific white shrimp were stocked in each tank with average initial weight of 0.54±0.01 g and growth trial were carried out with four replicates. Test diets were given manually four times daily (8:00 am, 11:00 am, 2:00 pm and 5:00 pm) for the experimental period (40 days).

The feed ration was calculated on daily basis according to expected growth of shrimp assuming a feed conversion ratio of 1.8 and a doubling in size (nearly every 7 days) until the expected shrimp weight was more than 1 g. Afterward, a growth rate of 1 g/week was assumed. Daily ration of feed was adjusted based on observed feed consumption, weekly counts of the shrimp, and observed mortality. Upon termination, shrimps were group weighed and mean final weight, weight gain, final biomass, feed conversion ratio and survival were determined. After counting and weighing the shrimp, six shrimp per tank were randomly picked and frozen at -20℃ for whole-body samples to be used for later protein retention analysis. A portion of the shrimp were put together into a common holding tank and then reorganized for the consumption trials.

2.4 Food consumption

The system was set up by hand sorting shrimp to uniform size (mean weight 5.38±0.35 g) and stocking 10 shrimp per tank into a series of 36 glass aquaria and four replicates were used per

treatment. All the nine experimental diets were offered on a regular basis to the animals for two days prior to the food consumption trial started. The food consumption was determined as the quantity of food eaten by the shrimp (n=10) in a time of 30 minutes in each aquarium. The measurements were performed at (8:30 am) in the morning after an interval of 16 hours fasting. Before the test, every aquarium was siphoned to get rid of debris and after that 1.0 g (as is) of feed was given. The uneaten feed was accumulated onto pre-weighed dry cellulose filters (20 µ) by siphoning residuals after 30 minutes, which were then oven-dried (100º C) overnight and remaining dry feed was determined. Four measurements were collected for each treatment (20 samples/treatment) during five successive days. To calculate approximately the amount of dry matter lost in water, 1.0 g (as is) of feed was added to the aquaria without shrimp and retrieved by siphoning after 30 minutes as mentioned earlier. Four measurements were performed for each diet. Average food consumption was determined as follows: FC = Fo – (Fr x Fl)

where FC = food consumption (g); Fo = food offered (g); Fr = food recovered (g); Fl = food leaching, amount of food retrieval from aquaria without shrimp, calculated as Fl = (Fr/Fo). Results were computed on a dry matter basis.

2.5 Statistical analysis

All the data were analyzed using SAS (V9.4, SAS Institute, Cary, NC, USA). Analysis of Covariance (ANCOVA) was used to check the effect of base-protein source of the diet, inclusion level of HP40Y and HP50Y and the interaction ($P<0.05$) on variables tested throughout the study. Two diet series (HP40Y and HP50Y) were analyzed individually (as using the same basal diet in both sets of diets) using one-way ANOVA followed by the Tukey's multiple comparison test to estimate significant differences between treatment means. Moreover, regression analysis was used

to identify the relationship between weight gain of shrimp and the inclusion level of HP40Y and HP50Y in both series of diets

3. Results

Growth performances and food consumption of juvenile *L. vannamei* fed with diets containing different levels of HP50Y and HP40Y replacing corn protein concentrate (CPC) are presented in Table 4. Analysis of Covariance (ANCOVA) showed that there was no significant interaction between the inclusion level of HP40Y and HP50Y and replaced protein on biomass, mean final weight, weight gain, weight gain percentage, FCR, survival and food consumption (P-value>0.05) of shrimp in both diet series, which ranged from 17.7-27.0 g, 2.42-2.93g, 1.88-2.38 g, 348.7-443.8%, 3.21-4.33, 75-90% and 0.60-0.65g respectively (Table 4). One-way Analysis of Variance (ANOVA) followed by Tukey multiple comparison test was also used to test significant differences between treatment means of the tested variables for each diet type, but no significant differences (P-value>0.05) between treatment were found (Table 4). Moreover, according to the results of linear regression, a significant positive association was observed between weight gain percentage of shrimp and the percentage inclusion level of HP50Y (p-value=0.04; r^2= 0.20) (Fig. 1) and HP40Y (p-value=0.02; r^2= 0.25) (Fig. 2) in the tested diets during the study. In case of net protein retention (NPR) there was no significant differences (P-value>0.05) between all treatments (Table 4). However, based on the linear regression, NPR increased with increasing inclusions levels of HP50Y (p-value= 0.05; r^2= 0.19) and HP40Y (p-value= 0.03; r^2= 0.23) increased in the diets (Fig. 3). The proximate whole-body composition of shrimp was shown in Table 5. The results revealed that there were no significant (P>0.05) differences between moisture, ash, protein and fat of shrimp body composition.

4. Discussion

The advancements in the ingredient manufacturing industry are progressing at an increasing rate comparable to the faster growth in aquaculture feed production. Therefore, various alternative plant-based protein sources have been used, and accessible in the marketplace with different advantages for instance efficacy of complete or partial replacement of animal protein, reasonable price, more availability, sustainability and prebiotic or probiotic effects. Distillers dried grain with solubles (DDGS) along with combination of different plant proteins is used in several aquaculture diets to reduce the expense of feed and balance nutrient content. It was reported that proximate composition of DDGS of about 119 samples, showing that lipid can vary from 8.8% to 12.4% and protein content of corn DDGS can range from 28.7% to 32.9%. As the only protein source, DDGS does not have a balanced essential amino acid (EAA) profile as it is particularly low in sulfur-containing amino acids (methionine and cysteine) and lysine. (Spiehs, et al., 2002).

With the modification of the manufacturing process of an ethanol plant, significant improvements to the byproduct yield can be done. Different technologies can raise the protein concentration and decrease the fiber content of DDGS, which makes it a more beneficial feed component. One of the recent products called high protein distillers dried grains (HPDDG) was manufactured to have a higher protein concentration (>49%) and decreased fiber (5.5%) and fat (3%) and compared to traditional DDGS (Guo, et al., 2019). Likewise, with the more developments in technology, the lipid extracted distiller dried grains (LE-DDGS) product have evolved which had lower lipid content (4.81%) and that is why it named as lipid-extracted form of DDGS and contained protein (29.8%) (Rhodes, et al., 2015).

In the present study, the high protein distiller dried grains with yeast (HP50Y and HP40Y) at two different protein levels were used. It is the most recent innovation towards distillers dried

grain industry. These products were formed under improved technology by separating corn fiber preceding to fermentation and get rid of the soluble portion after fermentation to manufacture a high-quality blend of corn and yeast proteins. The results of the current study demonstrated that by using HP50Y and HP40Y as a replacement of corn protein concentrate can be used up to 20% in shrimp diets with no significant effects on feed utilization or growth (Table 4). Likewise, Guo et al., (2019) found that high protein distiller grains (HPDDG) could be used up to 20% in the shrimp diet when replacing CPC without compromising the growth of shrimps. The similar product HPDDG also used in shrimp diets up to 30% to replace soybean meal, and up to 18% can be used to replace a combination of the soybean meal and fish meal without impacting the growth performance of shrimp in clear water (Qiu and Davis, 2017). In another study by Rhodes et al., (2015) demonstrated that lipid extracted distiller grains (LE-DDGS) can be used up to 20% in practical diets that include 6% FM devoid of any negative effect on growth performance of shrimp. So, in general these all-different types of distiller grains like HP50Y are proved to be effective while using in shrimp diets as the ingredient profile is nutritionally better than another plant-based ingredient like soybean meal (Table 1).

According to the outcomes of linear regression, a significant increase was found in weight gain percentage of shrimp respond to the inclusion level of both HP50Y (p-value=0.04; r^2= 0.20) (Fig. 1) and HP40Y (p-value=0.02; r^2= 0.25) (Fig. 2) used in shrimp diets. These high protein distiller grain with yeast in it, were manufactured to have decreased level of fiber and higher protein content, hence allowing for the inclusion of these ingredients at higher levels in practical shrimp feeds without instigating negative effects on the digestive physiology of shrimp, as increasing the addition of more insoluble fiber in shrimp diets would decrease the digestibility of other dietary components such as gross energy, dry matter, amino acids and minerals (Ray, et al.,

2022). Moreover, methionine, lysine, arginine are considered to be the most limiting essential amino acids in feed formulations for shrimp (Akiyama, et al., 1991). *L. vannamei* has requirement for lysine that is 2.1% of the diet if fed 45% protein diet and 1.6% of the diet fed 35% protein diet (Fox, et al., 1995). Lysine has been proved to be an EAA for the normal growth of shrimp and is commonly the most limiting amino acid in the ingredients used to prepare fish and shrimp feeds. Moreover, lysine is also efficiently catabolized into glutamine, which is considered as conditionally EAA that play important roles in promoting growth, feed utilization, and stress resistance. It is also present in higher amount in shrimp carcass (Li, et al., 2009). A requirement for methionine in *L. vannamei* has not been fully established, but the requirement for kuruma shrimp *Marsupenaeus japonicus,* black tiger shrimp *Penaeus monodon*, and Atlantic ditch shrimp *Palaemonetes varians* are 1.4%, 2.4-2.9%, 2.0-2.4%, of dietary protein, correspondingly (Millamena, et al., 1996b; Teshima, et al., 2002; Richard, et al., 2010). As methionine plays a major role in the structure and synthesis of various metabolites, neurotransmitters, hormones and required substances for the growth of shrimp. It serves as the precursor of carnitine, which carries fatty acids across mitochondrial membranes and facilitates their oxidation to produce energy (Li, et al., 2009). The HP50Y and HP40Y contained suitable levels of lysine (1.73% and 1.25%) as compared to lysine level in CPC (1.16%) (Table 1). Alternatively, CPC has good level of methionine in it (1.94%) in comparison to HP50Y (1.24%) and HP40Y (0.89%). Therefore, considering this in mind with replacement of CPC, all the diets were made according to the shrimp need of essential amino acids (Table 3). Protein metabolism has been recognized as a key to the understanding of energy requirements of shrimp because growth depends strictly on protein. The high protein requirement and the limited capacity of shrimp to store reserve substances like lipids and carbohydrates could be related to the capacity of shrimp to use proteins as a source of energy

as well as for growth. Shrimp can change the metabolic substrates (measured as oxygen: nitrogen ratio) according to the physiological or nutritional requirements, passing from protein-lipid-carbohydrate metabolism to protein metabolism, indicating that shrimp are well adapted to use proteins as a source of energy (Dall and Smith, 1986).

Apparent net protein retention was determined by several factors including initial weight, final weight and feed intake of animals along with the initial and final protein contents of animals (Hardy and Barrows, 2002). In shrimp, the exact feed intake is difficult to measure so, the feed intake was assessed by using feed offered to shrimp. In the current study, there were no significant differences between all the treatments regarding net protein retention (Table 4). Moreover, according to the results from regression analysis it was indicated that net protein retention was increased as the inclusion level of HP50Y and HP40Y in the diets was increased (Fig 3). This could be the reason for the trend of increased growth of shrimps as the inclusion level of HP50Y (Fig 1) and HP40Y (Fig 2) increased the diets.

To investigate the more effects of HP50Y and HP40Y, the proximate whole-body composition of shrimp was analyzed. It was observed that there were no significant differences between moisture, fat, ash and crude protein of all the treatments (Table 5). Likewise, a study conducted by Qiu et al., (2017) also observed that there was no significant difference in proximate composition of shrimps when high protein distiller dried grains (HPDDG) was used at different levels 0, 6, 12, 18, and 24%.

Conclusion

The outcomes of the current study proposed that both HP50Y and HP40Y are a great plant protein source and can be added in the shrimp diets up to 20% to replace corn protein concentrate

without compromising growth. As the reduction in shrimp performance may be due to any nutrient digestibility or palatability shifts but overall, there was a significant increased trend of shrimp growth as the percentage inclusion level of HP50Y and HP40Y in the diets were increased. In addition, HP50Y and HP40Y also comprise a higher level of yeast. Hence, upcoming work to assess the potential immune-stimulating effects is worth pursuing.

Table 1: Proximate and amino acid composition (% as is) of poultry meal (PM), soybean meal (SBM), corn protein concentrate (CPC) and high protein distiller's dried grain with yeast (HP40Y) and (HP50Y) used in growth trial.

	Fish meal	Soybean meal	Corn protein concentrate	HP40Y	HP50Y
Crude Protein	64.75	46.45	77.26	40.83	49.32
Moisture	6.28	11.26	7.00	8.49	7.20
Crude Fat	9.09	1.02	2.05	6.12	8.56
Fiber	0.66	3.58	0.99	9.51	7.43
Ash	19.77	5.88	0.91	2.26	4.29
Alanine	4.01	2.09	6.69	2.98	3.60
Arginine	3.78	3.36	2.32	1.69	2.20
Aspartic Acid	5.49	5.34	4.46	2.69	3.36
Cysteine	0.54	0.67	1.41	0.79	1.05
Glutamic Acid	7.69	8.48	16.09	6.88	8.37
Glycine	4.97	2.01	2.06	1.44	1.82
Histidine	1.66	1.23	1.56	1.09	1.38
Hydroxylysine	0.24	0.03	0.10	0.16	0.00
Hydroxyproline	1.11	0.07	0.01	0.08	0.05
Isoleucine	2.56	2.00	3.28	1.79	2.19
Lanthionine	0.03	0.00	0.00	0.11	0.11
Leucine	4.31	3.53	12.60	5.30	6.18
Lysine	4.89	2.92	1.16	1.25	1.73
Methionine	1.69	0.63	1.94	0.89	1.24
Ornithine	0.09	0.03	0.15	0.03	0.04
Phenylalanine	2.45	2.38	4.94	2.25	2.65
Proline	3.00	2.26	6.75	3.38	3.99
Serine	2.21	2.40	3.03	1.69	2.09
Taurine	0.70	0.09	0.04	0.09	0.09
Threonine	2.50	1.84	2.41	1.49	1.83
Tryptophan	0.65	0.66	0.47	0.30	0.41
Tyrosine	1.92	1.37	4.07	1.69	2.06
Valine	2.97	2.10	3.66	2.14	2.66
Sum of AA	59.46	45.48	79.20	40.20	49.10

Table 2: Formulation and proximate composition of test diets used to evaluate the efficacy of HP40Y and HP50Y (% as is) in the diets of *Litopenaeus vannamei*

Ingredients	Basal	HP50Y-05	HP50Y-10	HP50Y-15	HP50Y-20	HP40Y-05	HP40Y-10	HP40Y-15	HP40Y-20
Menhaden fishmeal[a]	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Soybean meal[b]	43.0	43.0	43.0	43.0	43.0	43.0	43.0	43.0	43.0
Corn protein concentrate[c]	13.1	10.0	6.6	3.5	0.2	10.5	8.0	5.5	2.5
HP50Y[d]	0.0	5.0	10.0	15.0	20.0	0.0	0.0	0.0	0.0
HP40Y[d]	0.0	0.0	0.0	0.0	0.0	5.0	10.0	15.0	20.0
Menhaden fish oil[a]	5.8	5.4	5.1	4.7	4.3	5.5	5.3	5.0	4.7
Lecithin[e]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cholesterol[f]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Corn Starch[f]	10.8	9.3	8.0	6.5	5.2	8.6	6.4	4.2	2.5
Whole wheat[f]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Mineral premix[g]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[h]	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Choline chloride[i]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Rovimix Stay-C[j]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
CaP-dibasic[k]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Proximate composition[1] (g/100g as is)									
Crude protein	36.6	36.1	35.2	35.8	36.3	36.1	36.1	35.6	36.4
Moisture	7.2	7.6	8.9	8.0	7.0	7.4	6.8	7.0	6.4
Crude Fat	8.2	8.3	7.7	7.8	8.0	8.2	8.2	8.1	8.2
Crude Fiber	2.9	3.1	3.8	4.7	4.8	4.4	4.9	5.3	5.8
Ash	7.1	7.1	7.3	7.3	7.3	7.2	7.4	7.4	7.4

[a]Omega Protein Inc., Houston, TX, USA.

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

[c]Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

[d]The Andersons, Maumee, OH, USA.
[e]The Solae Company, St. Louis, MO, USA.

[f]MP Biomedicals Inc., Solon, OH, USA.

[g]Trace mineral premix (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; 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, 69.664.

[h]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 D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81.

[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

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

Table 3: Amino acid profile (g/100g as is) of test diets fed to *Litopenaeus vannamei*

Aminoacids[a]	Basal	HP50Y-05	HP50Y-10	HP50Y-15	HP50Y-20	HP40Y-05	HP40Y-10	HP40Y-15	HP40Y-20
Alanine	2.13	2.00	1.96	1.96	1.92	2.01	1.99	1.94	1.93
Arginine	2.10	2.10	2.06	2.16	2.16	2.04	2.09	2.08	2.13
Aspartic Acid	3.34	3.32	3.21	3.31	3.32	3.25	3.27	3.23	3.33
Cysteine	0.57	0.56	0.52	0.56	0.56	0.54	0.55	0.54	0.54
Glutamic Acid	6.99	6.70	6.48	6.52	6.44	6.63	6.57	6.38	6.45
Glycine	1.54	1.55	1.52	1.60	1.59	1.53	1.57	1.57	1.58
Histidine	0.87	0.87	0.86	0.90	0.91	0.85	0.86	0.87	0.90
Hydroxylysine	0.10	0.10	0.11	0.11	0.11	0.11	0.12	0.12	0.12
Hydroxyproline	0.10	0.09	0.09	0.10	0.11	0.10	0.11	0.14	0.10
Isoleucine	1.70	1.67	1.62	1.63	1.64	1.62	1.63	1.62	1.65
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Leucine	3.70	3.43	3.33	3.29	3.21	3.45	3.40	3.28	3.26
Lysine	1.81	1.84	1.81	1.90	1.93	1.79	1.82	1.83	1.90
Methionine	0.68	0.67	0.63	0.66	0.64	0.66	0.65	0.63	0.63
Ornithine	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.02
Phenylalanine	1.98	1.90	1.84	1.85	1.84	1.89	1.88	1.83	1.85
Proline	2.41	2.31	2.26	2.26	2.25	2.28	2.30	2.24	2.30
Serine	1.51	1.45	1.43	1.47	1.49	1.51	1.48	1.44	1.46
Taurine	0.21	0.21	0.20	0.20	0.20	0.20	0.20	0.19	0.20
Threonine	1.31	1.28	1.26	1.31	1.32	1.30	1.30	1.28	1.31
Tryptophan	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Tyrosine	1.42	1.30	1.30	1.35	1.32	1.26	1.37	1.29	1.31
Valine	1.81	1.78	1.75	1.78	1.79	1.73	1.74	1.74	1.78

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

Table 4: Response of juvenile shrimp (0.54 ± 0.01 g) fed with diets contained different levels of HP50Y and HP40Y over a experimental period of 40 days. Values represented the mean of four replicates.

Diets	HP50Y level (%)	Final Biomass (g)	Final weight (g)	Weight Gain (g)	Weight gain[a] (%)	FCR[b]	Survival (%)	Food consumption (g)	Net protein retention (%)
Basal	0	17.73	2.42	1.88	348.70	4.33	75.0	0.63	12.92
HP50Y-05	5	21.73	2.54	1.99	364.07	3.83	85.0	0.64	13.84
HP50Y-10	10	19.13	2.63	2.09	389.40	3.96	72.5	0.60	14.97
HP50Y-15	15	24.50	2.72	2.18	398.93	3.63	90.0	0.60	14.66
HP50Y-20	20	25.53	2.93	2.38	431.46	3.21	87.5	0.60	16.41
PSE[c]		3.42	0.36	0.36	63.80	0.60	10.1	0.07	2.58
P-value		0.06	0.39	0.40	0.43	0.16	0.10	0.16	0.42
HP40Y-05	5	23.88	2.64	2.09	377.90	3.73	90.0	0.62	15.58
HP40Y-10	10	19.58	2.67	2.13	401.41	3.74	75.0	0.62	14.00
HP40Y-15	15	22.13	2.70	2.17	408.65	3.55	82.5	0.65	16.11
HP40Y-20	20	27.03	2.88	2.35	443.80	3.22	92.5	0.64	16.91
PSE[c]		4.33	0.33	0.32	60.6	0.5	14.4	0.06	2.35
P-value		0.06	0.46	0.42	0.29	0.07	0.32	0.48	0.16
ANCOVA									
Model		0.005	0.044	0.05	0.019	0.002	0.28	0.25	0.001
Diet type		0.773	0.74	0.8	0.862	0.759	0.834	0.654	0.453
Inclusion level		0.0005	0.005	0.006	0.002	0.0002	0.055	0.057	0.001
Interaction		0.868	0.812	0.836	0.873	0.95	0.931	0.756	0.831
Diet type*inclusion									

Note: One-way ANOVA was run by both diet type. Diet 1 kept as basal diet for both the sets. Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons.

[a]Weight gain %= (final weight-initial weight)/initial weight × 100%

[b]FCR=Feed conversion ratio = feed offered/ (final weight-initial weight)

[c]PSE = Pooled standard Error

Table 5: Proximate composition (% wet weight basis) of the whole body of *Litopenaeus vannamei*, fed different levels of HP50Y and HP40Y over an experimental period of 40 days. Values represented the mean of four replicates.

Diets	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Basal	4.13	69.47	6.39	12.46
HP50Y-05	4.21	69.82	6.78	12.03
HP50Y-10	4.28	68.89	6.90	12.39
HP50Y-15	4.26	69.10	6.83	12.48
HP50Y-20	4.51	68.92	6.93	12.25
PSE[b]	0.48	1.15	0.64	0.29
p-value[a]	0.83	0.75	0.76	0.23
HP40Y-05	3.85	70.23	6.96	12.14
HP40Y-10	3.97	69.72	6.69	12.48
HP40Y-15	3.84	70.09	7.31	12.03
HP40Y-20	3.83	69.93	6.85	12.51
PSE[b]	0.49	0.89	0.54	0.49
p-value[a]	0.89	0.77	0.23	0.53

Shrimp whole body analysis were analyzed by University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA).

[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.

Fig 1: Relationship between weight gain (%) and the inclusion level of high protein dried distillers' grains (HP50Y) in the diets.

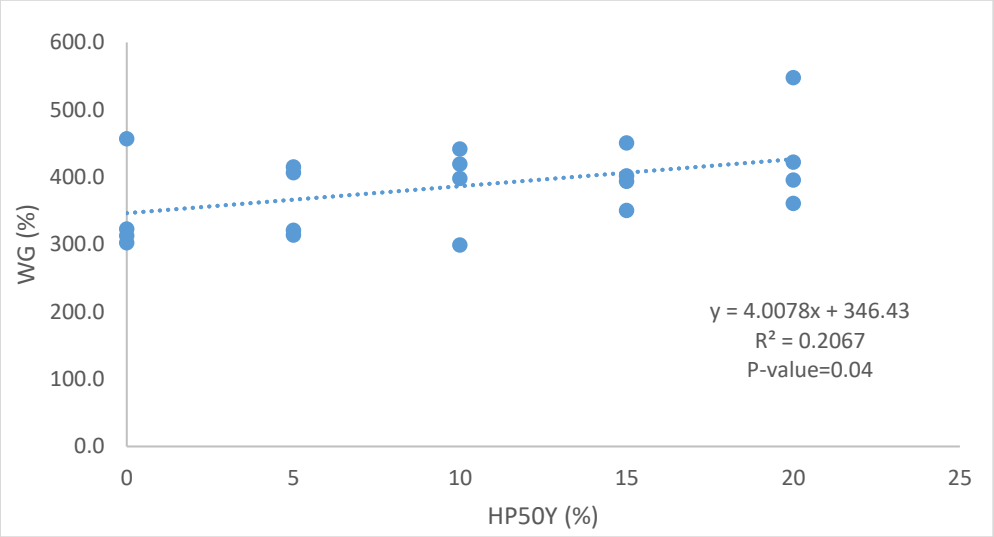


Fig 2: Relationship between weight gain (%) and the inclusion level of high protein dried distillers' grains (HP40Y) in the diets.

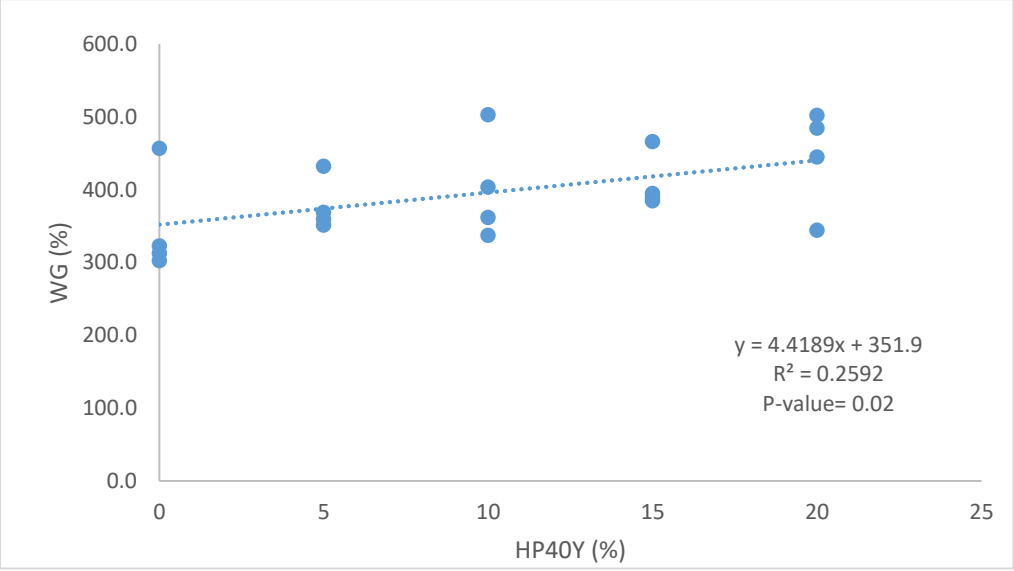
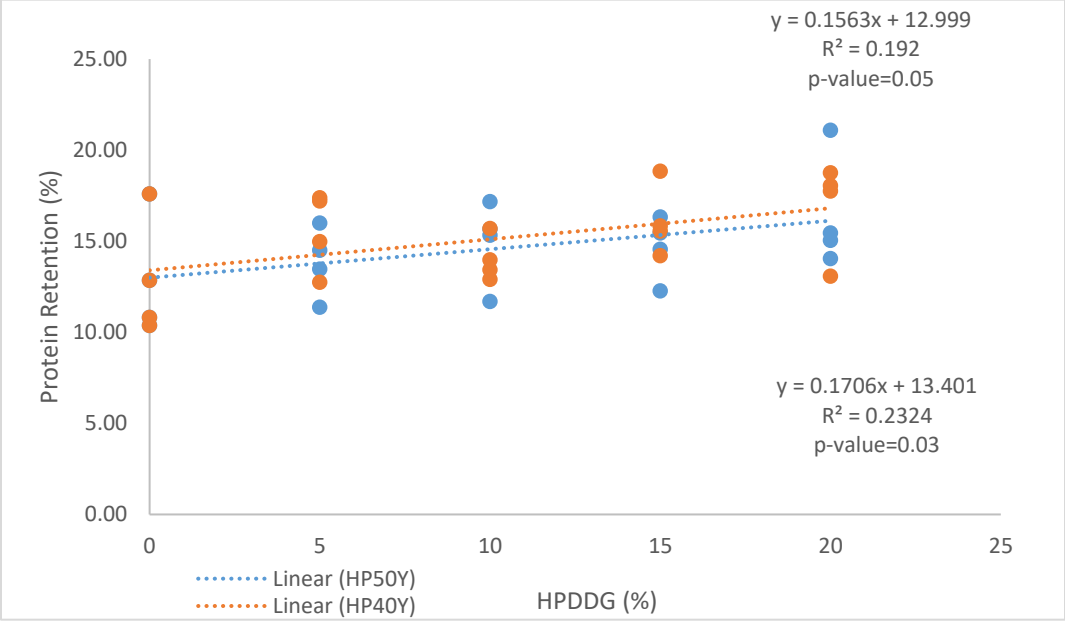


Fig 3: Relationship between protein retention (%) and the inclusion level of high protein dried distillers' grains (HP50Y and HP40Y) in the diets.



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CHAPTER IV

EVALUATION OF GROWTH, NUTRIENT DIGESTIBILITY, AND HEMATOLOGICAL PARAMETERS OF TILAPIA, *OREOCHROMIS NILOTICUS*, FED DIETS CONTAINING DIFFERENT LEVELS OF HIGH PROTEIN DISTILLER'S DRIED GRAIN WITH YEAST IN IT AS REPLACEMENT FOR CORN PROTEIN CONCENTRATE

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Abstract

Two novel technologically advanced high protein distiller dried grains with yeast in it (HP50Y & HP40Y) were evaluated for juvenile tilapia. This experiment assesses the nutritional potential of using high protein (40 and 50%) distillers dried grain to replace corn protein concentrate (CPC) in formulation of tilapia diets for more sustainable development of aquaculture. A 10-weeks growth trial was conducted, using nine diets formulated to contain 32% protein and 6% lipid. Each protein was included at levels (0, 5, 10, 15 and 20%) replacing CPC on protein basis. Juvenile tilapia (mean initial weight 5.23 ±0.20g) were evenly distributed in thirty-six, 75-L aquaria working as a recirculating system and fed twice daily to apparent satiation throughout the study. Tilapia exhibited no significant ($p>0.05$) differences in growth, FCR, survival, whole-body proximate composition, mineral composition, and hematological parameters when fed HP50Y and HP40Y supplemented diets compared to the control diet. Digestibility coefficients for the test ingredients were determined in tilapia for dry matter, energy, crude protein, individual and total amino acids using 1% titanium oxide as the inert marker with 70:30 replacement strategies. All the values were

found in acceptable range for the distiller grains when compared to literature. Results from this study revealed that HP50Y and HP40Y both are good alternative protein sources and can be used up to 20% inclusion level in the diets of tilapia.

KEYWORDS: Tilapia, growth performance, High protein distillers dried grain, Digestibility, corn protein concentrate.

1. Introduction

There have been substantial efforts to replace fish meal (FM) with different type of proteins from terrestrial plants and animals (Tacon et al., 2009). Hence, there is also increased demand for alternative proteins, which can to a lesser degree also help control rising ingredient costs. To keep feed costs down, it is typical to use a combination of proteins to reduce costs and meet nutrient requirements. Using a variety of proteins improves the balance of amino acid profile, increase palatability, and decrease the effects of antinutritional factors (Samocha et al., 2004). Consequently, there is a need to ensure we have a wider range of ingredients to select from when formulating feeds.

The aquaculture industry has become more receptive to distillers dried grain with solubles (DDGS) as a protein source because of its inexpensive cost as compared with other plant proteins. Due to the increased production of ethanol, the co-products of DDGS are presently less costly than soybean meal. Distillers dried grains with solubles is a co-product of the dry-mill ethanol industry, are the dried residue that persists after the fermentation of corn (or other grains) mash by certain yeasts and enzymes to make carbon dioxide and ethanol (Spiehs et al., 2002; Swiatkiewicz and Koreleski 2008). There are numerous grains from which DDGS can be produced containing one or more of the following: corn, wheat, barley, sorghum, and others but the most common and widely used in the U.S. is corn (Rhodes et al., 2015).

The DDGS are produced from dry grind ethanol production through the steps of liquification, saccharification, fermentation, distillation (dry co-product DDGS is produced), and dehydration. This conventional DDGS comprises 28–32% crude protein and is relatively high in fiber content, which limits its use in aquaculture feeds (Gatlin et al., 2007). Recent technological

developments have given rise to high-protein DDGS (HP-DDGS) formed via further separation of indigestible fiber, coupled with refinement of the dry grind process, thereby increasing crude protein concentration to 390-480 g kg-1 dry weight (Rho et al., 2017; Suehs and Gatlin 2021). Additionally, there has been another problem which limits excess use of DDGS inclusion in diets of fish is its inadequate methionine and lysine concentrations (the most limiting amino acids (AA) in aquatic diets when replacing fishmeal) (Lim et al., 2009). Deficiencies in these essential amino acids may result in decreased metabolic function of the organism, like impairment of growth performance, development, and overall health (Wu, 2013). However, by the supplementation of these limiting AA in animal diets, we can support adequate growth of cultured species; thus, increasing the potential inclusion of high protein distiller dried grains (HPDDGS), lipid extracted distiller dried grains (LEDDGS) or other alternative protein ingredients (Li et al., 2011).

Numerous studies have successfully used different types of traditional as well as novel high protein distiller dried grains 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), tilapia *Oreochromis* spp. (Wu et al. 1994, 1996; Coyle et al., 2004, Shelby et al., 2008; Schaeffer et al., 2009; Chatvijitkul 2013), sunshine bass (Thompson et al., 2008; Trushenski and Gause 2013), Yellow Perch *Perca flavescens* (Schaeffer et al., 2011), Rainbow Trout *Oncorhynchus mykiss* (Cheng et al., 2003; Cheng and Hardy 2004; Stone et al., 2005), redclaw crayfish *Cherax quadricarinatus* (Thompson et al., 2006; Garza de Yta et al., 2012), freshwater prawns *Macrobrachium rosenbergii* (Tidwell et al. 1993; Coyle et al. 2004), and Pacific white shrimp *Litopenaeus vannamei* (Lemos et al., 2009; Lim et al., 2009; Roy et al., 2009; Sookying and Davis 2011; Zhou 2014). But still there is a need to modify the manufacturing process of distiller grain with the help of advanced technology and to conduct research to introduce

novel alternative protein ingredients in industry. Therefore, this present study is conducted to introduce new high protein distillers dried grains with yeast HP50Y and HP40Y (50 and 40% crude protein) products containing spent yeast from the ethanol fermentation process. This is an advance step in the evolution of the separation technologies. It is manufactured by separating corn fiber preceding to fermentation after which the solubles portion is removed after fermentation to make a high-quality mixture of corn and yeast proteins. We previously evaluated the HP40Y as a replacement for soybean meal (SBM) and poultry meal (PM) in the practical diet of channel catfish *Ictalurus punctatus* (Nazeer et al, 2022) and in another study, HP50Y and HP40Y were used as a replacement for corn protein concentrate (CPC) in the practical diets of Pacific white shrimp, *L. vannamei*. As a result, the objective of this study is to evaluate the use of HP50Y and HP40Y products as a replacement for CPC in the practical diets of Tilapia.

2. Materials and Methods

2.1 Diet Preparation

In this trial, nine experimental diets were designed to meet the nutritional requirement of tilapia. All test diets were formulated on an isonitrogenous and isolipidic basis to contain 32% protein and 6% lipid. The test ingredients, high protein distiller's dried grain with yeast (HP40Y and HP50Y) were sourced from The Andersons, Inc., Maumee, OH, USA (ANDVantage^{TM} 40Y, ANDVantage^{TM} 50Y). Amino acid (AA) and proximate compositions of the primary protein sources were given in Table 1. The formulation and proximate composition of the nine test diets were presented in Table 2. Two sets of diets were formulated. In the first set, graded levels of HP50Y (0.0, 5.0, 10.0, 15.0 and 20.0%) were used to replace CPC (12.0, 9.0, 6.0, 2.5 and 0.0%) and the experimental diets were named as DG50-0, DG50-5, DG50-10, DG50-15, and DG50-20. In the second set of diet, graded levels of HP40Y (5.0, 10.0, 15.0 and 20.0%) were used to replace

CPC (10.0, 7.0, 4.5 and 2.0%) and were referred to as DG40-5, DG40-10, DG40-15 and DG40-20 (Table 2).

All the experimental diets were made at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University (Auburn, AL). The pre-ground dry ingredients and oil were weighed and then stir together in a food mixer (Hobart Corporation, Troy, OH, USA) for 15-20 min. Hot water was added into the mixture to acquire a consistency suitable for pelleting. Diets were extruded through a 4-mm-diameter meat grinder, dried at 70 °C to a moisture content of less than 10%. Diets were crumbled, packed in sealed bags and stored in the freezer at -20 °C until used. All the diets were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO) for amino acid profile and proximate composition (Table 2 and 3).

2.2 Experimental Systems

The growth trial was conducted in an indoor recirculation system at E.W. Shell Fisheries Center at Auburn University, Auburn, Alabama, in agreement with the Auburn University animal care policy. The research system consisted of thirty-six 75-L glass aquaria connected to a common reservoir tank (800-L). Water quality was maintained by recirculation through an Aquadyne bead filter (0.2 m^2 media, 0.6 m × 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 approximately 20 minutes/tank. By using air stones, dissolved oxygen was maintained close to saturation in each culture tank and the sump tank using a common airline attached to a regenerative blower. Throughout the trial, temperature, salinity, and dissolved oxygen (DO) were checked twice daily using an YSI 55 multi-parameter instrument (YSI, Yellow Springs, OH) and nitrite-N as well as total ammonia N (TAN) were measured twice

per week by using YSI 9300 photometer (YSI, Yellow Springs, OH). During the experimental period, the pH of the water was measured twice weekly by using the pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA), whereas nitrate, alkalinity and hardness level of water was measured twice per month using WaterLink-Spin TouchFF photometer (LaMotte Company, Chestertown, MD).

During the growth trial, DO, temperature, salinity, pH, total ammonia nitrogen (TAN), nitrite, alkalinity and nitrate were maintained within the acceptable ranges for Tilapia at 7.61±0.31 mg/L, 28.55±0.21℃, 4.01±0.21 g/L, 7.8±0.45, 0.12±0.11 mg/L, 0.02±0.01 mg/L, 65.0±1.9g/L, 34.3±4.5g/L respectively.

2.3 Growth Trial

In this growth trial, fifteen juvenile fish (mean initial weight of 5.23 ±0.20g) were stocked into each aquarium in the experimental system. Each diet was randomly assigned to four replicate aquaria. Diets were offered to fish at 4.0-7.0% BW daily over two feedings daily. 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, weight gain percentage and feed conversion ratio (FCR). Net protein retention (NPR %) was calculated as: (final weight x final protein in fish x final dry matter) – (initial weight x initial protein in fish x initial dry matter)/ (protein in feed x amount of feed consumed). Four fish were taken randomly from each aquarium, packed in sealed bags and stored in a freezer (-20℃) for the determination of proximate whole body and mineral composition of fish.

2.4 Blood analysis

For the collection of blood, four fish per aquarium were randomly sampled and sedated using 100 ppm buffered tricane methanesulfonate (MS-222). The blood of the sampled fish was drawn from the caudal vein using a 21-gage needle and 1ml syringe. The blood samples were immediately centrifuged (Fisher Scientific: Marathon 16km, USA) at 3000 rpm for 15 minutes and the serum was collected in 1.5 ml microtubes and stored in a deep freezer at 80°C until analysis. The hematological parameters (alkaline phosphate, alanine aminotransferase, gamma glutamyl transferase, total bilirubin, bile acids, albumins, urea nitrogen and cholesterol) were determined using Vet Scan VS2 analyzer (Abaxis, Inc. Union City, CA). Moreover, to calculate the hematocrit, blood (500µl) was stored in a heparinized capillary tube and the sample was analyzed as described by Reitman and Frankel (1957).

2.5 Digestibility trial

Apparent digestibility coefficient for dry matter, protein, energy, and amino acids were determined by using Titanium oxide as an inert marker. 1% Titanium Oxide was added to a sub-sample of the basal diet (Table 2) used in the growth trial. Digestibility coefficients of test diets were determined using groups of 10 fish (~55 g weight). Fish were allowed to acclimate to the test diets before starting the collection of feces. Prior to each feeding the tanks and fecal settling chambers (FSC) were cleaned. Fish were offered two feeding and all feces collected using a settling system. Samples were collected for several days until a suitable quantity was obtained for analyses (~1 g dry weight). Daily samples were pooled by tank and three replicate aquaria (n=3) were utilized for each treatment. Feces were stored in sealed plastic containers and stored in a freezer. Dry matter, crude protein, total energy and amino acids was determined for the fecal and diet samples according with established procedures. Crude protein content was analyzed using the

micro-Kjeldahl method (Ma and Zuazaga, 1942). Total energy content using a micro-calorimetric adiabatic calorimeter bomb using benzoic acid as standard (Model 1425, Parr Instrument Co. Moline, IL, USA). Titanium oxide content analysis followed Short et al., (1996) procedures. Apparent digestibility coefficients of the dry matter, protein, and energy for each diet were calculated according to Cho et al., (1982) using the following formula:

$$ADMD_D\ (\%) = 100 - [100 \times (\%\ TiO_2 \text{ in feed} / \%\ TiO_2 \text{ in feces})]$$

$$APD_D \text{ or } AED_D\ (\%) = 100 - [100 \times ((\%\ TiO_2 \text{ in feed\%} / \ TiO_2 \text{ in feces}) \times (\%\ \text{nutrient feces} / \%\ \text{nutrient feed}))]$$

The apparent digestibility coefficients of dry matter ($ADMD_I$), protein (APD_I) and energy (AED_I) of the test ingredients (I) were calculated according to Bureau and Hua (2006) as follows:

$$ADMD_I = ADMD_D + [(ADMD_D - ADMD_{Dref}) \times (0.7 \times D_{ref} / 0.3 \times D_{ingr})]$$

$$APD_I = APD_D + [(APD_D - APD_{Dref}) \times (0.7 \times D_{ref} / 0.3 \times D_{ingr})]$$

$$AED_I = AED_D + [(AED_D - AED_{Dref}) \times (0.7 \times D_{ref} / 0.3 \times D_{ingr})]$$

Whereas,

D_{ref} =%nutrient (or KJ/g gross energy) of basal diet (dry weight)

D_{ingr} =%nutrients (or KJ/g gross energy) of test ingredient (dry weight)

2.6 Statistical analysis

All the data were analyzed using SAS (V9.4, SAS Institute, Cary, NC, USA). Growth performances, immunological parameters of blood, apparent digestibility coefficients, proximate whole-body and mineral composition of fish were subjected one-way to ANOVA followed by Tukey's multiple comparison test to evaluate significant differences among treatment means ($p < 0.05$). Analysis of Covariance (ANCOVA) was used to check the effect of base-protein source of the diet, inclusion level of HP50Y and HP40Y and the interaction ($P<0.05$) on variables tested throughout the study. Two diet series (HP40Y and HP50Y) were analyzed individually (while

using the same basal diet in both sets of diets) using one-way ANOVA followed by the Tukey's multiple comparison test to estimate significant differences between treatment means.

3. Results

Growth performances of Tilapia fed with diets containing different levels of HP50Y and HP40Y replacing corn protein concentrate (CPC) are presented in Table 4. Analysis of Covariance (ANCOVA) showed that there was no significant interaction between the inclusion level of HP50Y and HP40Y and replaced protein on biomass, mean final weight, weight gain, weight gain percentage, FCR, survival, total dry feed and net protein retention (p-value>0.05) of tilapia in both diet series, which ranged from 760.9-811.1 g, 54.9-59.6g, 49.8-54.4 g, 948.5-1055.6%, 1.16-1.25, 86.6-95.0%, 61.3-66.5g and 40.8-45.9% respectively (Table 4). One-way Analysis of Variance (ANOVA) followed by Tukey multiple comparison test was also used to test significant differences between treatment means of the tested variables for each diet type, but no significant differences between treatment were found (Table 4).

The proximate whole-body composition of fish was summarized in Table 5. The results revealed that there were no significant (P>0.05) differences between moisture, ash, protein and fat of tilapia body composition. Moreover, there were no significant differences found in all the mineral composition of fish, with sulfur (S), phosphorous (P), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) being evaluated (Table 6). In line with the statistical outcomes in growth performance of tilapia (based on one-way ANOVA), no significant differences were noted in hematocrit or any of the other hematological parameters tested during the study (Table 7).

Apparent digestibility coefficients of dry matter, protein, and energy of the test diets as well as ingredients are presented in Table 8. ADMDD of basal diet was significantly different (52.72%) from the diets containing HP50Y (46.69%) and HP40Y (45.74) respectively. Moreover, AEDD of basal diet and HP40Y diet was significantly different (67.08% and 67.32%) from the diet having HP50Y (64.88%). In case of APDD there was no significant difference was found between these three diets (Table 8). On the other hand, the apparent digestibility coefficients of dry matter, protein, and energy of the ingredients showed that ADMDI of HP50Y was significantly higher (32.62%) than HP40Y (29.45%) while AEDI of HP40Y was significantly greater (67.58%) than HP50Y (62.38%). There was no significant difference found in APDI for both ingredients (Table 8).

Amino acid (AA) profile (as is basis) of different digestibility diets formulated using 70:30 replacement technique was presented in Table 9. Apparent digestibility coefficients of individual and total amino acids of digestibility diets and ingredients were shown in Table 10. In case of AAADD, there was no significant difference found in all amino acids except aspartic acid and lysine which have significantly higher values in basal diet (87.90% and 88.10%) as compared to HP50Y (85.76% and 85.57%) and HP40Y (85.71% and 85.19%) respectively. For AAADI, there was no significant difference observed between all the amino acids except methionine, as HP50Y has significantly greater (88.20%) than HP40Y (84.92%) (Table 10).

4. Discussion

New technologies have been established and applied in ethanol plants to enhance production efficiency. The evolution of novel ingredients in feed manufacturing industry are progressing at an increasing rate corresponding to the accelerated growth in aquaculture feed production. Consequently, several alternative plant-based protein sources have been studied, and

accessible in the marketplace with varied benefits for instance efficacy of complete or partial replacement of fishmeal, higher availability, prebiotic and probiotic effects, competitive price, and sustainability. Corn gluten meal (CGM) and corn protein concentrate (CPC) are commonly used high protein co-products used as feed ingredients in aquaculture feed formulations produced from corn milling industry, which are under continuous improvements due to the advancement in processing technologies, research, and innovations. In the same way, by modifying the manufacturing process of an ethanol plant, different varieties of high protein distiller dried grains are being used in the diet of animals. In this study, we evaluated HP50Y and HP40Y which are the latest products to the sequence of corn-based feed ingredients.

During this study, graded levels (5, 10, 15 and 20%) of HP50Y as well as HP40Y were used to replace corn protein concentrate (Table 2). The results demonstrated that both HP50Y and HP40Y could be used up to 20% in tilapia diets with no significant effects on all growth performance parameters as well as on net protein retention (Table 4). Results from various studies showed that DDGS could be included in aquatic feeds up to 300 g kg−1 of diet. Coyle et al., (2004) concluded that a diet (300 g protein kg−1 of diet) containing DDGS at a level of 300 g kg−1 of diet in combination with soybean meal and meat and bone meal had no significant difference on growth performance of hybrid tilapia (*O. niloticus* × *O. aureus*). Likewise, Herath et al., (2016) observed no significant differences in mean weight gain, specific growth rate, feed conversion ratio, survival, and protein efficiency ratio of tilapia when HPDDGS were incorporated in diets lacking fishmeal at 524 and 332 g of dry weight kg−1 respectively, in combination with poultry by-product meal and soybean meal. In another study, lipid extracted distiller grains (LEDDGS) were used in hybrid tilapia diet at a level of 300 g kg−1 without causing any negative effects on fish growth (Chatvijitkul et al., 2016). Suehs and Gatlin, (2022) observed no apparent effect of

HPDDGS inclusion up to 375 g of total protein kg-1 on any growth performance parameters, and whole-body proximate composition of juvenile Nile tilapia when replaced with fish meal and soybean meal.

The results showed no significant difference on the proximate whole body composition analysis (moisture, protein, fat and ash) of tilapia (Table 5) as well as on mineral composition (micro and macro elements) (Table 6). Webster, et al., (2015) also found no significant difference in whole body composition of tilapia when fed DDGS up to 40% to replace fish meal. In another study, regarding mineral composition of tilapia; there was no significant difference between calcium, potassium, magnesium, sodium, phosphorus, copper and manganese with dietary inclusion of wheat-based distiller grain with yeast in it (Omar, et al., 2022). Likewise in another study by Omar, et al., (2021) on common carp revealed that mineral composition remained unaffected when wheat based DDGS with yeast was fed to carp at level of 30%. As fish may take minerals either from diet or the surrounding water. The characteristic concentration and functional forms of minerals and trace elements need to be maintained within narrow ranges for essential metabolic activities in cells, tissues, and organs of fish.

To further explore the effects of HP50Y and HP40Y, hematological parameters as well as hematocrit of tilapia were determined (Table 7). As fermented products may contain probiotic and anti-microbial characteristics to improve the immune responses and disease resistance against pathogens (Phongpaichit, et al., 2006). Yeast is known to contain considerable levels of immunostimulants (e.g., β-glucans and nucleotides) that can positively affect the health of the organism (Yamamoto et al., 2018). HP50Y and HP40Y are composed of high levels of yeast (approximately 22% and 16%, respectively) that is used during dry grind ethanol production in the fermentation process, which is further increased when carbohydrates are filtered, concentrating the

protein. Hematological parameters (RBC, WBC, Hb and Ht) were not affected by dietary inclusion of wheat DDGS or lysine supplementation (Omar et al., 2021). Similar findings were reported in Nile tilapia fed diets containing various levels of maize DDGS (Lim et al., 2007). In channel catfish, Lim et al. (2009) obtained significantly higher Hb and Ht in fish fed diets containing 100–400 g/kg maize DDGS. Welker et al., (2007) who observed no negative effects on the haematological parameters haematocrit (Htc) and haemoglobin (Hb) when Channel catfish (*Ictalurus punctatus)* fed on 0.2% dietary whole cell brewer's yeast compared with control. In the present study, there was no significant difference observed between any of the hematological parameters and hematocrit of tilapia (Table 7).

The nutrient digestibility of a feed ingredient is an essential factor to assess the total nutritive value of the ingredient as it is related to the quantity of the nutrient absorbed by the animals. Apparent digestibility coefficients of dry matter, energy, and protein, in the experimental diets, and test ingredients for tilapia are shown in Table 8. In this study, ADMDD of basal diet (52.7%) was significantly higher than diets containing HP50Y (46.6%) and HP40Y (45.7%). For AEDD, the basal diet (67.0%) and HP40Y (67.3%) diet were significantly higher than HP50Y (64.8%) diet. There was no significant difference found in APDD of all these three diets (84.7%, 85.2%, and 94.9%) (Table 8). In case of ingredient digestibility coefficients, it was observed that ADMDI of HP50Y (32.6%) was significantly higher than HP40Y (29.4%), AEDI of HP40Y (67.5%) was significantly higher than HP50Y (62.3%) respectively. There was no significant difference found in APDI of both ingredients (84.9% and 85.3%) (Table 8). For the digestibility value of DDGS in fish, various studies have been reported; for instance, the APD values of DDGS were 90.4% for Rainbow Trout (Cheng and Hardy 2004), 64.94% for sunshine bass (Thompson et al., 2008) 20.6% for Florida Pompano (Lech and Reigh 2012). In another study by Suehs and

Gatlin, (2021) found that ADC for the test ingredient (HPDDG) was 83.1%. These values suggest that the nutrient digestibility values reported for DDGS for fish can vary substantially among different species and methodology.

In this present study, amino acids profile of digestibility diets was presented in table 9. Based on this AA profile, apparent digestibility coefficient of amino acids of experimental diets as well as test diets of tilapia were calculated (Table 10). There were no significant differences were found between all the individual and total AA in the diets except lysine, as this was significantly higher in basal diet (88.1%) than HP50Y (85.5%) and HP40Y (85.1%) diets. In the same way in case of AAADI, there were no significant differences in all individual and total AA apart from methionine which was significantly higher in HP50Y (88.2%) than HP40Y (84.95) (Table 10).

Conclusion

The findings of the current study recommended that HP50Y and HP40Y are good plant protein source and can be supplemented in the tilapia diets up to 20% to replace CPC without compromising growth. In addition, these novel ingredients had no apparent negative effect on proximate whole-body composition, mineral composition, and hematological parameters of tilapia. The results of APD, AED, and AAAD of diets and ingredients were in the acceptable ranges. More studies should consider the supplementation of HP50Y and HP40Y as a valuable protein source for the diets of carnivorous aquaculture fish species, such as trout and other salmonids.

Table 1: Proximate and amino acid composition (% as is) of poultry meal (PM), soybean meal (SBM), corn protein concentrate (CPC) and high protein distiller's dried grain with yeast (HP40Y) and (HP50Y) used in growth trial.

	Fish meal	Soybean meal	Corn protein concentrate	HP40Y	HP50Y
Crude Protein	64.75	46.45	77.26	40.83	49.32
Moisture	6.28	11.26	7.00	8.49	7.20
Crude Fat	9.09	1.02	2.05	6.12	8.56
Fiber	0.66	3.58	0.99	9.51	7.43
Ash	19.77	5.88	0.91	2.26	4.29
Alanine	4.01	2.09	6.69	2.98	3.60
Arginine	3.78	3.36	2.32	1.69	2.20
Aspartic Acid	5.49	5.34	4.46	2.69	3.36
Cysteine	0.54	0.67	1.41	0.79	1.05
Glutamic Acid	7.69	8.48	16.09	6.88	8.37
Glycine	4.97	2.01	2.06	1.44	1.82
Histidine	1.66	1.23	1.56	1.09	1.38
Hydroxylysine	0.24	0.03	0.10	0.16	0.00
Hydroxyproline	1.11	0.07	0.01	0.08	0.05
Isoleucine	2.56	2.00	3.28	1.79	2.19
Lanthionine	0.03	0.00	0.00	0.11	0.11
Leucine	4.31	3.53	12.60	5.30	6.18
Lysine	4.89	2.92	1.16	1.25	1.73
Methionine	1.69	0.63	1.94	0.89	1.24
Ornithine	0.09	0.03	0.15	0.03	0.04
Phenylalanine	2.45	2.38	4.94	2.25	2.65
Proline	3.00	2.26	6.75	3.38	3.99
Serine	2.21	2.40	3.03	1.69	2.09
Taurine	0.70	0.09	0.04	0.09	0.09
Threonine	2.50	1.84	2.41	1.49	1.83
Tryptophan	0.65	0.66	0.47	0.30	0.41
Tyrosine	1.92	1.37	4.07	1.69	2.06
Valine	2.97	2.10	3.66	2.14	2.66
Sum of AA	59.46	45.48	79.20	40.20	49.10

Table 2: Formulation and proximate composition of test diets used to evaluate the efficacy of HP40Y and HP50Y (% as is) in the diets of Tilapia.

Ingredients	Basal	HP50Y-05	HP50Y-10	HP50Y-15	HP50Y-20	HP40Y-05	HP40Y-10	HP40Y-15	HP40Y-20
Menhaden fishmeal[a]	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Soybean meal[b]	37.80	37.80	37.80	37.80	37.80	37.80	37.80	37.80	37.80
Corn protein concentrate[c]	12.00	9.00	6.00	2.50	0.00	10.00	7.00	4.50	2.00
HP50Y[d]	0.0	5.0	10.0	15.0	20.0	0.0	0.0	0.0	0.0
HP40Y[d]	0.0	0.0	0.0	0.0	0.0	5.0	10.0	15.0	20.0
Menhaden fish oil[a]	3.10	2.73	2.37	2.01	1.64	2.84	2.59	2.34	2.15
Lysine[e]	0.20	0.13	0.08	0.02	0.00	0.16	0.11	0.06	0.02
Methionine[e]	0.00	0.00	0.01	0.03	0.02	0.00	0.01	0.01	0.03
Corn Starch[f]	8.45	6.89	5.29	4.19	2.09	5.75	4.04	1.84	0.55
Corn meal[f]	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00	30.00
Mineral premix[g]	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
Choline chloride[i]	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
CaP-dibasic[k]	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85	1.85
Proximate composition[1] (g/100g as is)									
Crude protein	33.48	32.28	33.01	32.68	33.25	33.61	34.12	33.66	33.91
Moisture	6.67	7.56	6.91	9.76	7.52	7.91	6.39	6.12	5.64
Crude Fat	5.12	5.32	4.77	4.28	4.66	4.81	4.82	4.41	4.58
Crude Fiber	3.72	4.23	4.63	4.71	5.31	4.26	4.66	5.49	5.84
Ash	5.96	5.73	5.78	5.82	5.94	5.81	5.92	5.98	6.01

[a]Omega Protein Inc., Houston, TX, USA.

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

[c]Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

[d]The Andersons, Maumee, OH, USA.
[e]The Solae Company, St. Louis, MO, USA.

[f]MP Biomedicals Inc., Solon, OH, USA.

[g]Trace mineral premix (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; 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, 69.664.

[h]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 D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81.

[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

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

Table 3: Amino acid profile (g/100g as is) of test diets fed to Tilapia

Amino acids[a]	Basal	HP50Y-05	HP50Y-10	HP50Y-15	HP50Y-20	HP40Y-05	HP40Y-10	HP40Y-15	HP40Y-20
Alanine	2.01	1.95	1.94	1.83	1.89	1.97	1.96	1.92	1.91
Arginine	1.91	1.88	1.96	1.91	2.02	1.86	1.94	1.92	2.01
Aspartic Acid	3.02	2.95	3.09	2.98	3.09	2.96	3.06	3.02	3.11
Cysteine	0.52	0.53	0.55	0.52	0.56	0.51	0.54	0.54	0.55
Glutamic Acid	6.18	5.98	6.03	5.63	5.81	6.00	6.04	5.90	5.92
Glycine	1.43	1.38	1.42	1.39	1.48	1.38	1.40	1.43	1.47
Histidine	0.79	0.78	0.83	0.82	0.87	0.79	0.82	0.82	0.86
Hydroxylysine	0.12	0.12	0.08	0.11	0.12	0.12	0.11	0.11	0.11
Hydroxyproline	0.11	0.14	0.15	0.15	0.11	0.16	0.14	0.16	0.16
Isoleucine	1.50	1.42	1.48	1.46	1.50	1.48	1.51	1.49	1.52
Lanthionine	0	0	0	0	0	0	0	0	0
Leucine	3.42	3.31	3.28	3.06	3.12	3.38	3.34	3.24	3.22
Lysine	1.78	1.68	1.78	1.71	1.78	1.71	1.77	1.73	1.77
Methionine	0.65	0.62	0.68	0.64	0.65	0.64	0.65	0.63	0.64
Ornithine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phenylalanine	1.81	1.74	1.70	1.69	1.73	1.77	1.79	1.76	1.78
Proline	2.35	2.37	2.50	2.36	2.46	2.48	2.45	2.43	2.40
Serine	1.42	1.41	1.46	1.33	1.40	1.36	1.41	1.39	1.41
Taurine	0.16	0.15	0.15	0.14	0.14	0.15	0.16	0.15	0.15
Threonine	1.24	1.22	1.28	1.23	1.28	1.21	1.26	1.25	1.28
Tryptophan	0.27	0.29	0.31	0.30	0.33	0.27	0.29	0.30	0.31
Tyrosine	1.32	1.29	1.26	1.23	1.26	1.27	1.30	1.26	1.27
Valine	1.60	1.54	1.62	1.61	1.69	1.61	1.63	1.63	1.68

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

Table 4: Response of juvenile tilapia (mean initial weight 5.23 ±0.20g) fed diets containing different levels of HP50Y and HP40Y over a 10-weeks experimental period. Values represented the mean of four replicates.

Diets	HP50Y level (%)	Final Biomass (g)	Final weight (g)	Weight Gain[a] (g)	Weight Gain (%)	FCR[b]	Survival (%)	Total dry feed (g)	NPR[d] (%)
Basal	0	783.25	54.93	49.90	992.37	1.23	95.0	61.54	45.90
50Y-5	5	760.92	56.34	51.08	972.98	1.24	90.0	63.63	45.80
50Y-10	10	787.25	56.10	50.75	948.53	1.25	93.33	63.18	42.70
50Y-15	15	772.27	55.16	49.85	939.01	1.23	93.33	61.35	42.80
50Y-20	20	773.47	59.63	54.46	1055.66	1.22	86.66	66.52	44.90
P-value		0.99	0.45	0.42	0.15	0.93	0.77	0.25	0.07
PSE[c]		102.21	3.81	3.72	66.20	0.05	10.0	3.39	1.91
40Y-5	5	779.67	56.02	50.93	1001.33	1.24	93.33	62.77	45.50
40Y-10	10	809.07	57.71	52.44	994.47	1.23	93.33	64.53	40.80
40Y-15	15	811.17	57.87	52.65	1006.45	1.19	93.33	62.66	43.10
40Y-20	20	795.52	58.75	53.47	1013.35	1.16	90.0	62.13	42.50
P-value		0.97	0.74	0.78	0.99	0.61	0.90	0.75	0.20
PSE		88.97	4.43	4.40	84.0	0.08	7.25	3.25	3.25
ANCOVA									
Model		0.99	0.75	0.76	0.65	0.80	0.94	0.52	0.09
Diet type		0.45	0.63	0.60	0.41	0.17	0.62	0.62	0.29
Inclusion level		0.98	0.36	0.38	0.47	0.63	0.62	0.41	0.01
Interaction Diet type*inclusion level		0.99	0.90	0.88	0.64	0.95	0.98	0.42	0.78

Note: One-way ANOVA was run by both diet type. Diet 1 kept as basal diet for both the sets. Values with different superscripts within the same column are significantly different based on Tukey Pairwise Comparisons.

[a]Weight gain= (final weight-initial weight)/initial weight × 100%

[b]FCR=Feed conversion ratio = feed offered/ (final weight-initial weight)

[c]PSE = Pooled standard Error

[d]NPR= Net protein retention

Table 5: Proximate composition (% wet weight basis) of the whole body of tilapia, fed different levels of HP50Y and HP40Y for 10 weeks.

Diets	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Basal	73.12	16.67	6.76	3.13
HP50Y-05	73.55	16.42	6.49	3.24
HP50Y-10	73.15	15.95	6.78	3.74
HP50Y-15	73.35	16.02	7.12	3.53
HP50Y-20	72.82	16.35	6.85	3.35
p-value[a]	0.75	0.1	0.72	0.43
PSE[b]	0.79	0.39	0.62	0.48
HP40Y-05	73.55	16.85	6.43	3.34
HP40Y-10	72.75	15.75	6.50	3.89
HP40Y-15	72.85	15.87	6.53	3.62
HP40Y-20	73.67	15.52	5.97	3.97
p-value	0.78	0.06	0.46	0.36
PSE	1.24	0.81	0.59	0.66

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

[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.

Table 6: Mineral composition of the whole body of tilapia, fed different levels of HP50Y and HP40Y for 10 weeks.

	Marco-elements (% as is)						Micro-elements (mg/Kg as is)			
Diets	Sulfur	Phosphorous	Potassium	Magnesium	Calcium	Sodium	Iron	Manganese	Copper	Zinc
Basal	0.18	0.81	0.31	0.03	1.33	0.12	14.67	4.00	2.07	20.42
HP50Y-05	0.19	0.69	0.32	0.03	1.05	0.13	15.57	3.82	1.87	20.77
HP50Y-10	0.19	0.72	0.30	0.03	1.15	0.12	14.67	4.00	1.82	20.68
HP50Y-15	0.19	0.67	0.31	0.03	1.02	0.12	15.55	3.87	1.75	19.60
HP50Y-20	0.19	0.75	0.31	0.03	1.25	0.12	14.47	3.99	1.85	20.42
p-value[a]	0.32	0.43	0.40	0.95	0.22	0.81	0.87	0.11	0.69	0.73
PSE[b]	0.008	0.10	0.01	0.005	0.20	0.008	1.95	0.73	0.32	1.31
HP40Y-05	0.19	0.73	0.31	0.03	1.21	0.12	14.70	3.82	1.9	20.32
HP40Y-10	0.20	0.65	0.33	0.03	1.00	0.13	14.52	3.17	1.92	22.02
HP40Y-15	0.19	0.66	0.32	0.03	1.03	0.12	15.17	3.37	1.80	19.35
HP40Y-20	0.19	0.74	0.31	0.03	1.20	0.12	14.60	3.10	1.87	20.90
p-value	0.17	0.64	0.16	0.73	0.54	0.28	0.22	0.06	0.82	0.59
PSE	0.007	0.16	0.01	0.006	0.30	0.006	3.77	0.69	0.32	2.30

Mineral analysis were analyzed by Midwest Laboratories, Inc., Omaha, NE, USA.

[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.

Table 7: Hematological parameters of tilapia cultured during the trial. Values represented the mean of four replicates.

Diets	Alkaline phosphate (ALP)	Alanine aminotransferase (ALT)	Gamma glutamyl transferase (GGT)	Bile acids (BA)	Total bilirubin (TBIL)	Albumin (ALB)	Urea nitrogen (BUN)	Cholesterol (CHOL)	Hematocrit
Basal	8.67	37.70	0.00	15.33	1.23	3.26	1.00	201.33	36.38
HP50Y-05	8.56	36.30	0.66	22.33	1.03	3.40	1.33	201.00	35.58
HP50Y-10	8.00	35.50	0.25	20.25	0.65	3.52	1.25	198.30	32.55
HP50Y-15	8.33	32.67	0.66	9.33	0.46	3.80	1.00	217.00	33.23
HP50Y-20	8.24	29.25	0.25	16.75	0.60	3.17	1.25	205.00	35.47
p-value[a]	0.71	0.06	0.63	0.56	0.10	0.98	0.79	0.95	0.41
PSE[b]	14.10	19.60	0.63	10.03	0.36	1.54	0.42	32.00	3.23
HP40Y-05	27.00	36.50	0.25	20.00	1.15	2.40	1.25	197.00	32.32
HP40Y-10	34.00	45.00	0.50	22.50	1.25	2.65	1.50	246.50	36.92
HP40Y-15	20.70	47.00	0.00	25.30	1.23	2.40	1.33	187.70	32.85
HP40Y-20	13.67	44.33	0.00	18.67	1.20	2.10	1.33	185.67	36.97
p-value	0.20	0.90	0.46	0.78	0.57	0.27	0.84	0.06	0.42
PSE	12.20	16.90	0.35	9.96	0.08	0.62	0.50	20.80	4.54

[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.

Table 8: Apparent digestibility coefficients of dry matter (ADMD), protein (APD), energy (AED) of the diet (D) and ingredient (I) using 70:30 replacement technique offered to tilapia

Diets	$ADMD_D$	AED_D	APD_D	$ADMD_I$	AED_I	APD_I
Basal	52.72±0.68a	67.08± 0.59a	84.76± 1.06			
HP50Y	46.69±0.65b	64.88± 0.22b	85.21± 0.61	32.62± 2.17a	62.38± 0.47b	85.95± 1.65
HP40Y	45.74±1.96b	67.32± 1.12a	84.96± 0.39	29.45± 6.56b	67.58± 2.32a	85.31± 1.10

Note: Values from each diet/ingredient are means and *SD* of triplicate tanks. Values within column with different superscripts are significantly different ($p < .05$) based on one-way ANOVA followed by Tukey's multiple comparison test.

Table 9: Amino acid (AA) profile[a] (as is basis) of different digestibility diets formulated using 70:30 replacement technique

Amino acids	Basal	HP50Y	HP40Y
Alanine	1.99	2.35	2.24
Arginine	1.86	1.90	1.79
Aspartic Acid	3.02	3.01	2.90
Cysteine	0.52	0.63	0.58
Glutamic Acid	6.23	6.75	6.49
Glycine	1.37	1.46	1.38
Histidine	0.79	0.91	0.85
Hydroxylysine	0.07	0.08	0.08
Hydroxyproline	0.08	0.07	0.09
Isoleucine	1.55	1.65	1.59
Leucine	3.45	4.05	3.91
Lysine	1.76	1.70	1.60
Methionine	0.65	0.76	0.68
Ornithine	0.02	0.02	0.02
Phenylalanine	1.75	1.97	1.92
Proline	2.10	2.52	2.42
Serine	1.24	1.35	1.28
Taurine	0.17	0.15	0.16
Threonine	1.17	1.28	1.22
Tryptophan	0.28	0.32	0.30
Tyrosine	1.26	1.43	1.37
Valine	1.64	1.84	1.74

[a]Analysis conducted by Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri, USA

Table 10: Apparent amino acid (AA) digestibility for the diet (D) and ingredient (I) using 70:30 replacement technique offered to tilapia

AA	Basal	HP50Y (D)	HP40Y (D)	HP50Y (I)	HP40Y (I)
Alanine	85.83±1.30	86.00±1.01	85.78±0.64	86.25±2.48	85.70±1.70
Arginine	89.67±1.25	88.11±1.25	87.97±0.44	84.71±3.98	83.45±1.61
Aspartic Acid	87.90±0.59	85.76±0.86	85.71±0.63	80.73±2.89	79.84±2.33
Cysteine	88.19±1.40	88.71±1.05	88.77±0.40	89.42±2.48	89.75±1.09
Glutamic Acid	90.55±1.07	89.75±1.13	89.99±0.42	88.29±3.21	88.85±1.28
Glycine	79.08±1.85	77.47±2.32	75.65±0.73	74.39±6.77	67.85±2.39
Histidine	88.04±1.42	87.88±1.01	87.87±0.44	87.64±2.58	87.55±1.25
Isoleucine	84.96±1.28	83.84±0.67	83.72±1.01	81.68±1.98	81.05±3.20
Leucine	86.99±1.58	87.31±0.95	87.55±0.74	87.78±2.35	88.44±1.95
Lysine	88.10±0.94a	85.57±0.64b	85.19±0.63b	78.91±2.35	75.44±2.77
Methionine	88.37±1.28	88.30±0.49	87.23±0.46	88.20±1.22a	84.92±1.39b
Phenylalanine	85.06±1.57	84.75±0.97	84.92±0.71	84.23±2.58	84.67±1.96
Proline	86.35±1.69	86.94±1.73	87.38±0.04	87.76±4.15	88.96±0.10
Serine	87.17±0.60	86.17±0.78	86.02±0.56	84.38±2.18	83.60±1.74
Threonine	80.61±0.78	79.45±0.64	79.09±0.81	77.38±1.78	76.00±2.49
Tryptophan	87.07±1.75	87.22±1.04	87.34±0.45	87.46±2.69	87.84±1.32
Tyrosine	87.13±1.65	87.19±0.52	87.31±0.84	87.30±1.37	87.64±2.37
Valine	84.35±1.45	83.96±0.68	83.46±0.94	83.32±1.81	81.75±2.78
Sum AA	86.74±1.21	86.01±1.05	85.92±0.45	84.72±2.92	84.28±1.36

Note: Values from each diet/ingredient are means and ±*SD* of triplicate tanks. Values within column with different superscripts are significantly different ($p < .05$) based on one-way ANOVA followed by Tukey's multiple comparison test.

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CHAPTER V

SUMMARY AND CONCLUSION

Reducing feed cost is essential for the long-term sustainability of the 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 a replacement will not compromise fish performance. Towards this objective, several studies were performed with the purpose of reducing fish meal-based protein with plant protein sources in aquatic feed formulations. In aquatic feeds, the use of plant-based proteins has increased as they are cost effective protein sources with reliable quality and worldwide accessibility. 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. It should also incorporate a comparatively high protein content, balanced amino acid profile, reasonable price, acceptable palatability, suitable supply, and high nutrient digestibility. The advancements in the feed manufacturing industry are progressing at an increasing rate comparable to the faster growth in aquaculture feed production. Therefore, various alternative plant-based protein sources have been used and are accessible in the marketplace like distillers dried grain with solubles (DDGS), along with combinations of different plant proteins used in several aquaculture diets to reduce the expense of feed and balance nutrient content.

The current line of research was designed to evaluate the high protein distiller dried grains with yeast (HP50Y and HP40Y) at two different protein levels. With respect to aquatic feed formulations, it is the most recent innovation in the distillers dried grain industry. These products were formed under improved technology by separating corn fiber preceding fermentation and get

rid of the soluble portion after fermentation to manufacture a high-quality blend of corn and yeast proteins. We carried out several experiments on catfish, tilapia and shrimp to check the effectiveness of these products. The findings of the study conducted with catfish suggested that HP40Y is a good plant protein source and can be supplemented in catfish diets up to 20% to replace soybean meal without compromising growth. In addition, HP40Y contained an elevated level of yeast, which stimulated growth. In a poultry meal replacement series, complete replacement of poultry meal with HP40Y resulted in poor performance, indicating a possible nutritional deficiency when the animal protein was removed.

When HP40Y and HP50Y were evaluated in shrimp as a replacement of corn protein concentrate there was no significant interaction between the inclusion level of HP40Y and HP50Y and replaced protein on biomass, mean final weight, weight gain, weight gain percentage, FCR, survival and food consumption. Moreover, according to the results of linear regression, a significant positive association was observed between weight gain percentage of shrimp and the percentage inclusion level of HP50Y and HP40Y.

In the tilapia growth trial, in which corn protein concentrate was replaced with different levels of HP50Y and HP40Y, no significant interaction between the inclusion level of HP50Y and HP40Y and replaced protein on biomass, mean final weight, weight gain, weight gain percentage, FCR, survival, total dry feed and net protein retention was observed. Moreover, results revealed that there were no significant differences in whole body moisture, ash, protein and fat. We also evaluated the mineral composition, hematocrit and hematological parameters of tilapia and found no significant differences in all these parameters. Apparent digestibility coefficients of dry matter, protein, and energy and amino acids of the test diets as well as ingredients also showed no significant difference between them.

. Innovative feed ingredient products resulting from the manufacturing processes within ethanol plants can raise the protein concentration and decrease the fiber content of DDGS, which makes it a more beneficial component for aquatic feeds. More studies should consider the supplementation of HP50Y and HP40Y as a valuable protein source for the diets of carnivorous aquaculture fish species, such as trout and other salmonids.

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