Development and Application of Grain Distillers Dried Yeast for Pacific White Shrimp *Litopenaeus vannamei*

by

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Abstract

In recent years there has been an increasing interest in developing low-cost feeds for the Pacific white shrimp, *Litopenaeus vannamei*. High prices and limited supply are the main factors that have affected primarily feed manufacturing companies. Therefore, animal and plant-based proteins have been evaluated as protein sources in order to reduce commercial shrimp feed costs. Facing this scenario, the idea of replacing fish meal with alternative protein sources, has reduced the dependence on marine ingredients commonly utilized in shrimp formulations. Fish meal has been used in shrimp diets as an excellent protein source due to its high level of nutrients as well as palatability. Nevertheless, the supply of fish meal have become very limited and most importantly, fluctuations in the price has stimulated alternative feed ingredients to be considered. Generally, animal proteins are more expensive than plant ingredients resulting in a considerable interest to evaluate plant by-products as protein sources. Shrimp formulations have included soybean meal, an acceptable protein ingredient with suitable digestibility and steady supply. However, soy products have a lower nutrient content compared to fish meal with regards to protein, essential amino acids, fatty acids, and minerals. Toward this goal, grain distillers dried yeast (GDDY), a co-product obtained from the bioethanol industry, has been evaluated in sunshine bass and rainbow trout with positive results. Therefore, a series of growth trials were conducted to evaluate the substitution of soybean meal with GDDY in clear and green water systems as well as pond conditions for shrimp culture.

Results from these studies suggest that formulated diets containing increasing percentages of GDDY (20, and 30% of diet) with or without lysine supplementation can be utilized as a protein source. Positive results were demonstrated using GDDY up to 30% of diet without affecting growth, feed conversion ratio, and survival in clear water tanks. Consequently, GDDY was assessed up to 15% of diet in green water tanks as well as production ponds. Feed response was acceptable for all commercial shrimp feeds and production parameters were at suitable levels. There were no significant differences between any of the GDDY dietary treatments in either ponds or tank growth trials.

Overall, results from these studies reveal that GDDY can be utilized as a promising protein source in combination with soybean meal in formulated diets for Pacific white shrimp, as long as dietary nutrients are well-balanced to meet their requirements. In conclusion, diets formulated with GDDY (up to 30% of diet) as the main protein source, did not impair shrimp performance. Furthermore, utilizing GDDY in commercial feeds might reduce feed costs and most importantly, promotes optimum growth.

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CHAPTER I

INTRODUCTION

Aquaculture, the farming of aquatic animals and plants, has continued to expand resulting in the production of 154 million tonnes in 2011. Whereas over the last decade, wild capture has held steady at around 90 million tonnes. In response to increasing demand for seafood, aquaculture production has shown a strong growth, increasing at an average annual rate of 6.6%. For example, the 42 million tonnes of production in 2004 grew to nearly 64 million tonnes in 2011. Seafood represents a high value and nutritious protein source for the human population. However, there are numerous needs that must be met if aquaculture it to continues to grow. Availability of land, clean water, and an adequate feed supply all influence aquaculture operations. Traditionally, shrimp is one of the most important seafood commodities traded in value terms, accounting for 15% of the total value of internationally traded fisheries products. Other important commodities such as salmon and tuna account for 14 and 8%, respectively (FAO 2012).

Shrimp aquaculture production has expanded over the past decade from 1.6 million tonnes in 2001 to nearly 3.6 million tonnes in 2011. The most important shrimp species cultured is the Pacific white shrimp, *Litopenaeus vannamei*, because of its market acceptance and high profits relative to other commonly farm-raised shrimp. Unfortunately, the use of high levels of marine ingredients such as fish meal, has resulted in expensive feeds and an increasing fishing

pressure on wild fish. If fish meal prices rise, it is more challenging to manage a commercial shrimp farm due to high feed costs (Davis & Sookying 2009).

Feeds represent 40-60% of the production cost of farm-raised shrimp (Tan & Dominy 1997). This has resulted in improved feed manufacturing practices and evaluations of alternative protein sources in order to reduce feed costs. High-quality plant protein (e.g. soybean meal, cottonseed meal, distillers grain with solubles, and some legume meals) or animal protein (e.g. poultry meal, meat and bone meal, and blood meal) can be used in commercial feeds without negatively compromising shrimp production. The first procedure in reducing feed cost is to identify the primary cost in a compound feed formulation, generally protein (Akiyama 1992).

Proteins are important organic molecules, being considered essential nutrients of all living organisms. Fish meal has been considered a high quality protein for fish and crustaceans because of its high protein density, amino acid balance, essential fatty acids, and its general enhancement of palatability. The quality of fish meal depends on the source of material, freshness, and processing techniques. Due to the high cost and limited availability of fish meal, numerous studies have been carried out in order to develop new alternatives and/or combinations from animal and plant protein in aquatic feeds. The nutritional content of the feedstuff is an important factor when replacing fish meal or any marine ingredient. Studies conducted by Davis & Arnold 2000; Samocha *et al*. 2004; Amaya *et al*. 2007; Roy *et al*. 2009; Markey *et al*. 2010; Sookying & Davis 2011; have demonstrated the feasibility of a complete substitution of fish meal utilizing a combination of alternative protein sources (e.g. soybean meal, poultry by-product meal, distillers grain, and pea meal) is possible. Animal protein sources including meat and bone meal, blood meal, poultry by-product meal, feather meal (e.g. hydrolyzed or enzyme treated), and specialized protein blends can also be used as a substitute for fish meal. These

feedstuffs are produced in considerable volumes from rendering plants and are typically available at lower cost than fish meal, but they are of variable composition and quality. Additionally, these meals may have palatability problems as well as possible microbial contamination (Tacon & Forster 2000).

Various seeds and/or their co-products are also protein sources used to replace fish meal partially or completely in diets. There are numerous sources of vegetable proteins, being soybean meal the most widely-used plant protein source for many aquaculture species. Soy is popular due to its high protein content, well-balanced amino acid profile, reasonable price, consistent quality, and steady supply. Since plant proteins are generally cheaper than proteins derived from animal sources, replacement of animal proteins by plant-based proteins should lead to the reduction of feed costs and an increasing use of plant proteins from renewable resources. Other sources of plant proteins such as cottonseed meal, peanut meal, canola meal, and distillers grain with solubles, have been utilized in diets without adverse effects on production performance. Nevertheless, plant proteins typically have low levels of essential amino acids, potential anti-nutritional factors, and decreased palatability (Li *et al*. 2000).

Yeast-based products may provide another alternative source of protein for the aquaculture industry. Yeast has been utilized in terrestrial livestock diets as a supplement to increase growth, feed efficiency, and disease resistance in lactating sows (Jurgens *et al*. 1997). Although the use of yeast may not be a common ingredient in aquatic feeds, there have been several studies conducted with regards to its use. Similar to terrestrial diets, yeast-based products have been utilized as an immune enhancement supplement in fish diets. Used in low concentrations (1-4% of diet), *Saccharomyces cerevisiae* influenced growth, feed efficiency, and resistance to *Streptococcus iniae* infection in sunshine bass (Li & Gatlin 2003).

Yeast-based products have also been used as fish meal replacements in some fish species. Tilapia fry diets derived from a blend of plant proteins and yeast, with 30% of protein from yeast, exhibited higher growth rates than fry fed diets containing 100% of protein from fish meal (Olvera-Novoa *et al*. 2002). Distillers dried grain with solubles, a product containing yeast, is a promising feed ingredient in fish species, including channel catfish (Webster *et al*. 1993), rainbow trout (Cheng & Hardy 2004), and tilapia (Coyle *et al*. 2004). Yeast-based products are showing potential as feed ingredients in the aquaculture industry and a new source from this feedstuff may become available worldwide due to the expansion of the ethanol industry. This new protein source has been identified as grain distillers dried yeast (GDDY).

Grain distillers dried yeast is a co-product obtained from the bioethanol industry. It is produced via a wet milling process where corn kernel is fractioned and corn germ, gluten, and fiber are removed and dextrose fed into fermentation. Non-fermentative *Saccharomyces* yeast is then extracted from the liquid fraction after ethanol distillation. Currently, around twelve billion gallons of bioethanol are produced in the United States. The use of GDDY may have the benefit of increasing sustainability and profitability of aquaculture. However, increased biofuels production may also have a detrimental impact on aquaculture as demand for bioethanol production increases. The price of a bushel of corn, for example, rose from $2.34 in 2000 to $5.30 in 2010, primarily as a result of increasing demand for bioethanol production (Renewable Fuels Association 2010). It is likely that the price of corn as well as other grains will continue to raise leading to difficulties in developing cost effective diets and significant increases of food prices in developing countries where poverty and hunger are serious social problems.

According to Gause & Trushenski (2011), GDDY resulted in no significant differences in mean final weight, feed conversion ratio, and survival in diets containing increasing percentages

of GDDY (0, 13, 27, 40, and 50% of diet), added as needed to maintain balanced formulations in sunshine bass (female white bass *Morone chrysops* x male striped bass *M. saxatilis*). No studies, however, have been conducted to evaluate the possibility of including GDDY as the primary protein source in shrimp diets. The primary objective of this study is to evaluate GDDY as an alternative protein source in practical diets for the Pacific white shrimp reared under various culture conditions. Two specific objectives were included to identify the response of *L. vannamei* to different treatments:

1. To evaluate the response of *L. vannamei* to increasing percentages of GDDY reared under clear water conditions.
2. To evaluate the response of *L. vannamei* to the use of GDDY in commercial feed formulations and reared under pond conditions.

CHAPTER II

USE OF GRAIN DISTIILERS DRIED YEAST IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) REARED UNDER CLEAR WATER CONDITIONS

Abstract

Grain distillers dried yeast (GDDY) is a yeast-based protein source which is obtained from the bioethanol industry. Increasing economic concerns regarding the use of expensive feed ingredients in diets for marine shrimp have led to develop new alternatives or combinations from animal as well as plant protein in aquatic feeds. Numerous studies have been conducted utilizing promising protein sources under different culture conditions. Most yeast-based products have been evaluated as a nutritional supplement in fish diets. There is limited information regarding yeast products as an alternative protein source in shrimp diets. Therefore, the objective of this study was to evaluate the production response of *Litopenaeus vannamei,* fed diets containing increasing percentages of GDDY. The first series of five diets were evaluated under laboratory conditions with juvenile shrimp (1.90 ± 0.10 g, initial weight) designed to contain GDDY up to 30% of diet. In the second growth trial, juvenile shrimp (0.40 ± 0.01 g, initial weight) were fed diets similar to those in the first experiment with the following modification, lysine supplementation at high levels of GDDY (20, and 30% of diet). Both growth trials were conducted over an 8-wk period. The results from this study demonstrated that GDDY up to 30% of diet can be used in feed formulations for *L. vannamei* without causing negative effect on

biomass, mean final weight, feed conversion ratio (FCR), and survival. Furthermore, the addition of lysine to diets with high levels of GDDY (20, and 30% of diet) did not enhance growth performance under the reported conditions.

Introduction

World shrimp production has been rapidly increasing as aquaculture production has increased. Pacific white shrimp, *Litopenaeus vannamei*, is the major cultured shrimp species, accounting for over 3.5 million tonnes in 2011 compared to 186,113 tonnes in 1999. At this time, commercially farmed-raised shrimp have become one of the world's top aquaculture commodities in value terms, which account for 15% of the total value of internationally traded fisheries products (FAO 2012). The expanded production has resulted in an increasing demand for shrimp feeds as well as the ingredients utilized to make the feeds. Shrimp feeds have been generally formulated with fish meal as the primary protein source in shrimp diets because of its excellent source of nutrients, e.g., balanced amino acid profile, essential fatty acids, and mineral content (Tacon & Akiyama 1997). Therefore, it is a considerable concern to identify and make use of less expensive protein sources within crustacean feeds.

The first step in reducing feed costs is identifying the most expensive component in shrimp feeds, which is generally protein. The actual demand for protein sources depend primarily upon price, protein level, amino acid profile, and presence of anti-nutritional factors as well as processing considerations. Since fish meal is of limited supply and more expensive than most other protein sources, it is often the target for replacement. Animal by-products, primarily from rendering plants (e.g. poultry by-product meal, meat and bone meal, and blood meal) are less expensive ingredients and provide indispensable amino acids that can be used to replace all or part of the fish meal in shrimp diets resulting in considerable savings in terms of feed costs (Dominy & Ako 1988; Cheng *et al*. 2002; Forster *et al*. 2003; Cruz-Suárez *et al*. 2007). The qualities of these feedstuffs depend on both the quality of raw ingredients and the processing systems. Animal-based proteins have several advantages over plant-based proteins. They have a

high percentage of crude protein, are low in carbohydrates, provide a source of vitamins and minerals, and do not contain anti-nutritional factors as well as phytic acid which depresses the bioavailability of phosphorus and zinc in shrimp diets (Davis *et al*. 1993).

Other sources of protein for aquatic feeds are plant proteins. From a nutritional perspective, marine meals such as fish meal can be replaced totally or partially by a single or a combination of plant protein sources (Lim *et al*. 1997; Cruz-Suárez *et al*. 2001; Davis *et al*. 2002; Suárez *et al*. 2009). Among these plant-based proteins, soybean meal is one of the most ingredients widely used as protein sources for fish and shrimp feeds because of its protein level, acceptable amino acid profile, suitable availability, and affordable prices. Considerable research has been conducted with soybean meal in combination with other ingredients as a substitution of fish meal in shrimp feeds (Lim & Dominy 1990; Alvarez *et al*. 2007; Sookying & Davis 2011). However, soybean meal demand is expected to continue to increase, leading to future problems in terms of cost and availability.

Although soybean meal is a less expensive feedstuff, there are some constraints that affect when balancing amino acids, e.g., soy products are low in lysine, methionine, and arginine as well as lack of n-3 highly unsaturated fatty acids (HUFAs), e.g., eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA) which can have negative effects on growth performance of shrimp (Fox *et al*. 2004). Furthermore, plant-based proteins contain phytate, making certain minerals unavailable such as zinc and iron (Richardson *et al*. 1985; Liener 1994). Diet palatability may become an important limitation in feeds formulation, especially when oilseed-derived proteins are added to diets.

The use of soybean meal in shrimp feed is recommended in order to reduce feed costs. Nevertheless, there are several sources of plant proteins that can be used as an alternative

ingredient when non-fish meal is added to feeds. Grain distillers dried yeast is a co-product obtained from the bioethanol industry via a wet milling process where corn kernel is fractioned and corn germ, gluten, and fiber are removed and dextrose fed into fermentation. Non-fermentative *Saccharomyces* yeast is then extracted from the liquid fraction after ethanol distillation. Although the use of yeast-based products have been reported in rainbow trout (Sealey *et al*. 2007), channel catfish (Peterson *et al*. 2012), African catfish (Essa *et al*. 2011), tilapia (Schaeffer *et al*. 2012), pacu (Ozório *et al*. 2009), European sea bass (Tovar-Ramírez *et al*. 2004), and shrimp (Li *et al*. 2009), there is limited information with regards to shrimp performance fed GDDY.

The objective of this study was to evaluate the response of *L*. *vannamei* to increasing percentages of GDDY as replacement of solvent extracted soybean meal (SE-SBM) in practical diets for *L*. *vannamei* reared in tank-based systems.

Materials and methods

A series of experiments were conducted at E. W. Shell Fisheries Research Station in Auburn, AL. Two growth trials were carried out to evaluate the response of *L*. *vannamei* to increasing percentages of GDDY as replacement of SE-SBM.

Experimental diets

Diets were manufactured at Auburn University, Department of Fisheries and Allied Aquacultures, Auburn, AL. For the first trial (Trial I), five experimental diets were formulated to contain increasing percentages of GDDY (0, 5, 10, 15, and 20% of diet) as replacement of SE-SBM (Table 1). For the second trial (Trial II), five experimental diets were formulated to contain

increasing percentages of GDDY (0, 20, 30, 20+Lys, and 30%+Lys) with or without the addition of lysine in order to determine if lysine was limiting (Table 2).

Diets were prepared by mixing the ingredients in a mixer (Hobart, Troy, OH, USA) for 15 minutes. Subsequently, boiling water was added to the mixture until obtaining an appropriate consistency for pelleting. Diets were then passed through a 2.5-mm die in a meat grinder. Wet pellets were then placed into a drying oven (<45 °C) overnight in order to attain a moisture content of less than 10%. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer until use. All experimental diets were formulated to be isonitrogenous (approximately 35% crude protein with 8% lipid), replacing SE-SBM by GDDY as an alternative protein source. The nutrient composition and amino acid profile of experimental diets are listed in Table 3 and 4, respectively.

Growth trials

Juvenile shrimp were obtained from the nursery system and selected by hand-sorting to a uniform size. Then juvenile shrimp (1.90 ± 0.10 g, and 0.40 ± 0.01 g, initial weight Trials I and II, respectively) were stocked into 20 square tanks (0.6 x 0.6 x 0.5 m, 180-L volume) under a recirculating system at a density of 10 shrimp per tank. Five experimental diets were randomly assigned with four replications (tanks) per treatment. Test diets were applied four times daily at 0800, 0900, 1600, and 1700 h for an 8-wk period. Feed input was calculated on a weekly basis using an estimated FCR of 1.8:1 and a doubling in size until individual shrimp reached one gram. Thereafter, an individual growth rate of 1.0 g wk^{-1} was assumed. Each tank was covered with polystyrene foam lid and was continuously aerated with one air stone. Shrimp were counted

every week to readjust daily feed input. At the conclusion of the 8-wk period, shrimp were counted and group weighed. Biomass, mean final weight, FCR, and survival were determined.

Water quality monitoring

For both experiments, dissolved oxygen (DO), temperature, salinity, and pH were measured twice daily in one of the rearing tanks at 0800 and 1600 h using a YSI 556 MPS meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Water samples were taken in one of the tanks to determine total ammonia-nitrogen (TAN) on a weekly basis.

Statistical analysis

All data were statistically analyzed using one-way analysis of variance to determine significant differences ($P<0.05$) which was followed by the Student-Neuman-Keuls multiple comparison test to determine significant differences among treatment means if a significant treatment effect was observed. All statistical analysis were carried out using SAS (V9.1. SAS Institute, Cary, NC, USA).

Results

The laboratory trials were conducted without any noticeable problems with water quality or diseases occurrences. Water quality parameters were within suitable ranges for the culture of this species (Table 5).

For Trial I, results indicated that there were no significant differences ($P>0.05$) in biomass, mean final weight, FCR, and survival of *L. vannamei*, fed diets containing increasing percentages of GDDY in substitution to SE-SBM as a protein source (Table 6). Mean final

weight ranged between 8.64 and 8.85 g, with the 0% GDDY treatment having the numerically largest mean final weight and the 10% GDDY treatment having the lowest. Biomass ranged from 84.35 to 87.73 g tank^{-1}. FCR ranged between 1.65 and 1.69. Survival ranged from 95.0 to 100.0%. The 20% GDDY treatment had the lowest survival of the five dietary treatments.

Results of Trial I were confirmed in Trial II in which there were also no statistically significant differences (P>0.05) observed among any of the treatments for the production parameters evaluated (Table 6). Shrimp fed the diet containing the highest level of GDDY (30% of diet) showed the poorest performance numerically for most parameters evaluated, though these were not significantly different from any other treatment. Mean final weight ranged between 6.03 and 6.78 g, with the highest mean value for shrimp fed the 20% GDDY treatment. Biomass ranged from 58.13 to 67.00 g tank^{-1}. FCR and survival values ranged between 1.91 to 2.17 and 96.6 to 100.0%, respectively. Lysine supplementation (0.69% of diet) in diets with 30% GDDY, did not improve biomass, mean final weight, and FCR compared to diets with the same level of GDDY containing no lysine. Similar results were found in diets with 20% GDDY supplemented with lysine (0.45% of diet).

Discussion

Results from this study demonstrated that SE-SBM can be replaced by GDDY up to 30% of diet when formulating shrimp diets without compromising production performance of *L. vannamei*. Grain distillers dried yeast is an alternative protein source that may be an important yeast-based product suitable for use in marine shrimp feeds.

This study obtained no negative effect in performance of shrimp fed experimental diets containing high levels of GDDY (20, and 30% of diet) with or without lysine supplementation.

In a study conducted by Fox *et al*. (1995), they estimated lysine requirement at 1.8 and 2.1% of diet in shrimp formulations containing 35 and 45% protein, respectively. They concluded that lysine was probably the first-limiting amino acid as compared to methionine and arginine in shrimp diets. The diet formulated with 30% GDDY had the lowest lysine level (1.8% of diet). Lysine supplementation of this diet, however, did not produce a significant difference between treatments in the present study, confirming lysine was not limiting. The result of this study is in agreement with Ozório *et al*. (2009), who evaluated diets containing a dried yeast meal resulting from the sugar cane juice and molasses fermentation as a replacement of fish meal in diets for pacu (*Piaractus mesopotamicus*) cultured in indoor tanks. They found that 50% of the fish meal can be replaced in pacu diets without affecting performance when the diet contains a combination of dried yeast meal and soybean meal at 20 and 22% of diet, respectively. However, significant lower weight gain and higher FCR were observed in pacu fed diets where more than 70% of the fish meal had been replaced.

With rainbow trout, studies have shown that brewers dried yeast can be utilized up to 25% of diet without compromising fish performance (Rumsey *et al*. 1991). However, they did not obtain the same response when fish were fed brewers dried yeast at 50 and 70% of diet, lowering weight gain and feed intake. With the same species, Murray & Marchant (1986) reported similar negative effects on feed intake when a mixed biomass of yeasts were included in rainbow trout diets up to 50% of diet. These authors assumed a lower feed intake because of palatability problems found at high levels of brewers dried yeast during the trial period.

Studies have also reported beneficial effects of yeast in tilapia diets. Zerai *et al*. (2008) demonstrated that a combination of brewers yeast and soybean meal at 25 and 30% of diet, respectively; can substitute up to 50% of fish meal with no adverse effect on growth of tilapia.

Inclusion of brewers yeast above 25% of diet resulted in a significant decline in final weight and feed intake. According to Al-Hafedh & Siddiqui (1998), texture and palatability of diets change at high levels of plant proteins in which feed intake may decrease resulting in poor growth and FCR. Other studies of tilapia fed yeast have indicated that this ingredient can also be used as a probiotic. Yeast may reduce bacterial infection levels and enhance the activities on digestive enzymes on tilapia fry guts (Abdel-Tawwab 2011). Based on this research, yeast supplementation as a probiotic improved growth and resistance against *Aeoromonas hydrophila* infection on Nile tilapia fry at a level of 2.0 g kg^{-1}.

Yeast-based products have also been evaluated in crustaceans. Studies on Australian red claw crayfish (*Cherax quadricarinatus*) have been conducted by Muzinic *et al*. (2004). They determined no significant differences in mean final weight, percentage weight gain, and survival on juvenile (<1.0 g) red claw crayfish fed diets containing increasing percentages of brewers grains with yeast (0, 10, 20, and 30% of diet) as a replacement of fish meal. They found that brewers grains with yeast can be included in practical diets for red claw crayfish up to 30% of diet without causing any negative effect on growth and survival. In a study carried out with *L. vannamei*, Li *et al*. (2009) included two levels of brewers yeast (2, and 5% of diet) to shrimp diets. At the conclusion of the experiment, they suggested that brewers yeast can serve as an immunostimulant for shrimp. Survival increased slightly at both levels of brewers yeast compared to the basal diet formulated with fish meal and soy protein concentrate. Similar research was conducted with the same species by Scholz *et al*. (1999), who reported that yeast (*Saccharomyces cerevisiae*) increased survival and provided an immunostimulatory effect on shrimp at 1% of diet.

All these studies have been carried out with yeast-based products. Nevertheless, there is limited information about GDDY as a protein source in fish and shrimp diets. According to Gause & Trushenski (2011), GDDY was evaluated as an alternative protein source of fish meal in sunshine bass diets. Five experimental diets were formulated to contain increasing percentages of GDDY (0, 13, 27, 40, and 50% of diet). Results indicated that the performance of sunshine bass was not impaired by the inclusion of GDDY up to 40% of diet. However, complete replacement of fish meal (50% GDDY) resulted in a significant decrease in growth and high FCR. The low feed intake that caused the increase in FCR was probably due to palatability problems. In some cases, sunshine bass expelled the pellets several times or they were ignored completely. Reduced feed acceptability has been found when diets are formulated with high levels of yeast-based products (Murray & Marchant 1986; Rumsey *et al*. 1991; Zerai *et al*. 2008; Ozório *et al*. 2009; Gause & Trushenski 2011).

Results from the present study suggested that inclusion of GDDY up to 30% of diet in shrimp feeds will not compromise production performance. In addition, results also indicated that supplementation of lysine was not required in these formulations. The evaluation of GDDY in commercial production diets is encouraged.

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Table 1. Ingredient composition (g 100 g^{-1} as is) of five experimental diets used in Trial I containing increasing percentages of GDDY (0, 5, 10, 15, and 20% of diet) as a substitute for SE-SBM in diets for *L. vannamei*.

Ingredient	0% GDDY	5% GDDY	10% GDDY	15% GDDY	20% GDDY
Soybean meal solvent-extracted[1]	52.30	47.48	42.67	37.85	33.02
Grain distillers dried yeast[2]	0.00	5.00	10.00	15.00	20.00
Poultry by-product meal[3]	5.00	5.00	5.00	5.00	5.00
Menhaden fish oil[4]	5.49	5.39	5.29	5.19	5.09
Whole wheat[5]	25.00	25.00	25.00	25.00	25.00
Corn starch[5]	1.26	1.13	1.04	0.95	0.87
Trace mineral premix[6]	0.50	0.50	0.50	0.50	0.50
Vitamin premix[7]	1.80	1.80	1.80	1.80	1.80
Choline cloride[5]	0.20	0.20	0.20	0.20	0.20
Stay-C 25%[8]	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate[9]	2.50	2.55	2.55	2.56	2.57
Soy lecithin[10]	1.00	1.00	1.00	1.00	1.00
Cholesterol[11]	0.05	0.05	0.05	0.05	0.05
Corn gluten[12]	4.80	4.80	4.80	4.80	4.80

[1] Faithway Feed Co., Guntersville, AL, USA.
[2] ADM Co., Decatur, IL, USA.
[3] Griffin Industries Inc., Reedville, VA, USA.
[4] Omega Protein Inc., Reedville, VA, USA.
[5] Gold Medal, General Mills Inc., Minneapolis, MN, USA.
[6] Trace mineral premix (g 100 g^{-1}): cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 53.428.
[7] Vitamin premix (g kg^{-1}): thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0.
[8] Stay-C® (L-ascorbyl-2-polyphosphate), Roche Vitamins Inc., Parsippany, NJ, USA.
[9] MP Biochemicals Inc., Solon, OH, USA.
[10] Solae Company, St. Louis, MO, USA.
[11] USB Biochemicals, Cleveland, OH, USA.
[12] Grain Processing Corporation, Muscatine, IA, USA.

Table 2. Ingredient composition (g 100 g^{-1} as is) of five experimental diets used in Trial II containing increasing percentages of GDDY as well as lysine supplement in diets with high levels of GDDY (20, and 30% of diet) as a substitute for SE-SBM in diets for *L. vannamei*.

Ingredient	0% GDDY	20% GDDY	30% GDDY	20% GDDY+Lys	30% GDDY+Lys
Soybean meal solvent-extracted[1]	52.30	33.02	23.40	32.12	22.01
Grain distillers dried yeast[2]	0.00	20.00	30.00	20.00	30.00
Poultry by-product meal[3]	5.00	5.00	5.00	5.00	5.00
Menhaden fish oil[4]	5.49	5.09	4.89	5.10	4.91
Whole wheat[5]	25.00	25.00	25.00	25.00	25.00
Corn starch[5]	1.26	0.87	0.66	1.28	1.26
Trace mineral premix[6]	0.50	0.50	0.50	0.50	0.50
Vitamin premix[7]	1.80	1.80	1.80	1.80	1.80
Choline cloride[5]	0.20	0.20	0.20	0.20	0.20
Stay-C 25%[8]	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate[9]	2.50	2.57	2.60	2.60	2.68
Soy lecithin[10]	1.00	1.00	1.00	1.00	1.00
Cholesterol[11]	0.05	0.05	0.05	0.05	0.05
Corn gluten[12]	4.80	4.80	4.80	4.80	4.80
L-Lysine HCl[13]	0.00	0.00	0.00	0.45	0.69

[1] Faithway Feed Co., Guntersville, AL, USA.
[2] ADM Co., Decatur, IL, USA.
[3] Griffin Industries Inc., Reedville, VA, USA.
[4] Omega Protein Inc., Reedville, VA, USA.
[5] Gold Medal, General Mills Inc., Minneapolis, MN, USA.
[6] Trace mineral premix (g 100 g^{-1}): cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulphate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 53.428.
[7] Vitamin premix (g kg^{-1}): thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCl 1.0, DL Ca-pantothenate 5.0, nicotinic acid 5.0.
[8] Stay-C® (L-ascorbyl-2-polyphosphate), Roche Vitamins Inc., Parsippany, NJ, USA.
[9] MP Biochemicals Inc., Solon, OH, USA.
[10] Solae Company, St. Louis, MO, USA.
[11] USB Biochemicals, Cleveland, OH, USA.
[12] Grain Processing Corporation, Muscatine, IA, USA.

Table 3. Nutrient composition (dry matter basis) of experimental diets containing increasing percentages of GDDY as well as lysine supplement in diets with high levels of GDDY (20, and 30% of diet) as a protein source for *L. vannamei* growth trials reared under clear water condition systems.

Treatment	Moisture	Protein	Lipid	Fiber
0% GDDY	7.19	38.16	8.37	2.84
5% GDDY	7.14	37.29	8.32	3.13
10% GDDY	7.52	36.90	8.58	3.06
15% GDDY	7.36	37.16	8.28	2.87
20% GDDY	9.10	35.86	8.30	2.97
30% GDDY	8.33	35.86	8.66	2.63
20% GDDY+Lys	8.45	36.28	8.39	2.10
30% GDDY+Lys	8.55	36.00	8.88	1.92

Diets were analyzed by Midwest Laboratories Inc., Omaha, NE, USA.

Table 4. Amino acid profile (as is) of experimental diets containing increasing percentages of GDDY as well as lysine supplement in diets with high levels of GDDY (20, and 30% of diet) as a protein source for *L. vannamei* growth trials reared under clear water condition systems.

Amino Acid	0% GDDY	5% GDDY	10% GDDY	15% GDDY	20% GDDY	30% GDDY	20% GDDY +Lys	30% GDDY +Lys
Alanine	1.68	1.72	1.81	1.86	1.86	2.03	1.85	1.95
Arginine	2.42	2.37	2.33	2.20	2.06	1.96	2.04	1.85
Aspartic Acid	3.55	3.49	3.50	3.36	3.17	3.09	3.15	2.92
Cysteine	0.51	0.50	0.50	0.49	0.49	0.49	0.50	0.46
Glutamic Acid	6.42	6.48	6.33	6.21	5.93	5.89	5.97	5.64
Glycine	1.61	1.59	1.63	1.59	1.57	1.57	1.55	1.50
Histidine	0.88	0.86	0.88	0.85	0.80	0.80	0.81	0.76
Isoleucine	1.65	1.64	1.67	1.65	1.61	1.65	1.63	1.60
Leucine	2.96	2.98	3.15	3.22	3.15	3.39	3.17	3.25
Lysine	2.02	2.00	1.99	1.94	1.81	1.82	2.17	2.25
Methionine	0.54	0.54	0.59	0.56	0.57	0.63	0.57	0.60
Phenylalanine	1.86	1.82	1.88	1.87	1.80	1.84	1.82	1.77
Proline	2.08	2.11	2.18	2.17	2.10	2.21	2.10	2.13
Serine	1.42	1.56	1.45	1.35	1.30	1.33	1.23	1.26
Threonine	1.27	1.27	1.32	1.28	1.24	1.28	1.21	1.22
Tryptophan	0.47	0.45	0.44	0.43	0.39	0.38	0.38	0.36
Tyrosine	1.30	1.28	1.37	1.36	1.32	1.40	1.35	1.31
Valine	1.76	1.77	1.80	1.84	1.80	1.88	1.83	1.80

Diets were analyzed by Midwest Laboratories Inc., Omaha, NE, USA.

Table 5. Water quality variables for *L. vannamei* growth trials reared under clear water condition systems over an 8-wk period for both trials.

Parameter	Trial I	Trial II
DO (mg L^{-1})[a]	6.29 ± 0.25	6.04 ± 0.42
	(5.90, 7.12)	(4.65, 7.31)
Temperature (°C)	27.74 ± 1.19	27.09 ± 1.18
	(22.00, 28.90)	(23.40, 29.30)
Salinity (ppt)	16.13 ± 0.10	20.60 ± 0.83
	(15.80, 16.30)	(19.10, 21.80)
pH	8.30 ± 0.19	7.91 ± 0.14
	(8.10, 8.47)	(7.71 ± 8.10)
TAN (mg L^{-1})[b]	0.47 ± 0.19	0.35 ± 0.11
	(0.38, 0.55)	(0.21 ± 0.55)

Values represent the mean ± standard deviation and values in parenthesis represent minimum and maximum readings.
[a]Dissolved oxygen.
[b]Total ammonia-nitrogen.

Table 6. Growth performance of *L. vannamei* in two trials to diets containing increasing percentages of GDDY as well as lysine supplement in diets with high levels of GDDY (20, and 30% of diet) conducted in clear water condition systems over an 8-wk period with an initial weight of 1.90 ± 0.10 g, and 0.40 ± 0.01 g, in Trials I and II, respectively.

Treatment	Biomass (g tank^{-1})	Mean final weight (g)	FCR[a]	Survival (%)
Trial I (n=4)				
0% GDDY	86.13	8.85	1.65	97.50
5% GDDY	87.73	8.76	1.67	100.00
10% GDDY	84.35	8.64	1.69	100.00
15% GDDY	86.65	8.68	1.68	100.00
20% GDDY	84.95	8.71	1.67	95.00
P-value[b]	0.968	0.965	0.960	0.544
PSE[c]	3.670	0.082	0.042	2.500
Trial II (n=4)				
0% GDDY	59.00	6.12	2.11	96.67
20% GDDY	65.57	6.78	1.91	96.67
30% GDDY	58.13	6.03	2.17	96.67
20% GDDY+Lys	67.00	6.70	1.93	100.00
30% GDDY+Lys	63.27	6.53	1.98	96.67
P-value[b]	0.411	0.467	0.498	0.903
PSE[c]	3.750	0.345	0.375	2.981

[a]Feed conversion ratio = Total feed offered / biomass increase.
[b]Analysis of variance was used to determine significant differences ($P<0.05$) among treatment means (n=4).
[c]Pooled standard error of treatment means.

CHAPTER III

USE OF GRAIN DISTILLERS DRIED YEAST IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) REARED UNDER FIELD CONDITIONS

Abstract

The objective of this study was to demonstrate the feasibility of diets formulated to contain increasing percentages of grain distillers dried yeast (GDDY) in production diets for *Litopenaeus vannamei*, reared in green water conditions and production ponds at Claude Peteet Mariculture Center in Gulf Shores, AL. Four practical diets containing increasing percentages of GDDY (0, 5, 10, and 15% of diet) were utilized to evaluate the response of shrimp over the culture period. The production pond trial was carried out in 16, 0.1-ha ponds using four replicates per diet. Juvenile shrimp (38 ± 4.26 mg, initial weight) were stocked at 30 shrimp m^{-2} and cultured under standardized pond conditions for a 16-wk period. The same four diets and a commercial reference diet (Rangen Shrimp Grower, 35% protein, 8% lipid) were offered to shrimp maintained in 750-L outdoor tanks over a 12-wk period. A total of 20 tanks were stocked with juvenile shrimp (3.05 ± 0.22 g, initial weight) obtained from production ponds at a density of 30 shrimp per tank. Daily feed input for both growth trials were calculated based upon an expected weight gain of 1.3 g wk^{-1} and an estimated feed conversion ratio (FCR) of 1.2. At the conclusion of these experiments, mean final weight, yield, FCR, and survival were evaluated. In the pond study, mean final weight ranged from 19.77 to 23.05 g, yield ranged between 4,760 and 5,606 kg ha^{-1}, survival ranged from 69.6 to 89.4%, and FCR was between 1.02 and 1.23.

Shrimp reared in the outdoor tanks confirmed the results of the pond trial. Mean final weight ranged between 18.12 and 18.97 g, survival ranged from 93.3 to 98.3%, and FCR was between 1.25 and 1.29. There were no significant differences in the outdoor tank study regarding mean final weight, FCR, and survival among dietary treatments, except for shrimp fed the commercial diet that had slightly higher mean final weight (20.00 g) and lower FCR (1.18). Based on the results from the previous study conducted in clear water conditions as well as of this study results, GDDY can be used up to 15% of diet in commercial feed formulations for *L. vannamei*.

Introduction

Based on the Food and Agriculture Organization of the United Nations (FAO), aquaculture has continued to expand resulting in the production of 154 million tonnes in 2011. The aquaculture industry provides a significant contribution to global production of fish, crustaceans, and molluscs, increasing from 5.2% in 1970 to nearly 42.0% in 2011. Pacific white shrimp, *Litopenaeus vannamei*, is considered the primary cultured shrimp species as well as the top aquaculture commodity, which accounts for 15% of the total value of internationally fisheries products. Therefore, world demand for shrimp is expected to continue to increase as aquaculture production increases (FAO 2012).

Since the shrimp industry has expanded as a profitable aquaculture business, production systems have generally shifted from traditional extensive to semi-intensive and intensive culture systems. The primary source of nutrients in shrimp culture is artificial feed that represents 50-60% of the total production cost mainly in semi-intensive and intensive systems. The use of inexpensive ingredients and nutritionally balanced feeds are fundamental for both feed manufacturing companies and shrimp commercial farms. Additionally, high-quality and water stable diets are needed in order to enhance production and reduce feed pollution to the environment (Tan & Dominy 1997). According to Aranyakananda & Lawrence (1993), protein is the most important nutrient that affects shrimp growth performance. Protein requirement of shrimp have been studied with levels varying from 30 to 55% for various shrimp species (Huang *et al*. 2003; Pérez-Velázquez *et al*. 2007; Hu *et al*. 2008). Shrimp cultured in intensive systems are typically offered feed containing 35% protein whereas, in semi-intensive systems, protein level varies from 25 to 35%. Studies conducted by Coloso & Cruz 1980; Kanazawa & Teshima 1981; Fox *et al*. 2006; reported that shrimp requires the same ten amino acids including arginine,

histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine as do fish. Furthermore, they also indicated that typically the three most limiting essential amino acids in shrimp culture are lysine, methionine, and arginine, respectively.

Current considerations with regards to price and social concerns over marine ingredients, centering around fish meal, have resulted in the development of alternative diets which use less expensive protein sources as a replacement for fish meal. One of the most common practices in aquaculture feed manufacturing is to reduce the quantity of fish meal by replacing it with a combination of alternative plant and animal proteins. Sources of terrestrial animal protein include rendered by-products such as poultry meal, meat and bone meal, and blood meal. These animal-based proteins generally contain 45-65% crude protein and are good sources of essential amino acids for fish and crustaceans. Several studies have been carried out assessing terrestrial protein sources in practical diets for shrimp resulting in acceptable growth, FCR, and survival (Davis & Arnold 2000; Forster *et al*. 2003).

Studies have also shown that replacing animal protein sources in commercial shrimp formulations using plant proteins have not compromised growth performance (Conklin 2003; Cheng & Hardy 2004; Samocha *et al*. 2004; Amaya *et al*. 2007a,b). Sookying & Davis (2011a,b) found no significant differences regarding shrimp performance utilizing soybean meal as a plant protein source in combination with poultry by-product meal, fish meal, distillers dried grain with solubles, and pea meal in practical diets for *L. vannamei*. Moreover, they also indicated that soy protein concentrate can be included in shrimp feeds up to 12% of diet without affecting growth and survival.

Grain distillers dried yeast is another alternative protein source that can be utilized in combination with soybean meal. This feed ingredient is a co-product obtained from the

bioethanol industry and has been suggested as a less expensive alternative to soybean meal. Studies have shown that GDDY is a promising protein source which has been assessed in sunshine bass and rainbow trout resulting in acceptable fish performance (Gause & Trushenski 2011; Stewart 2012). Nevertheless, there is limited information regarding the response of shrimp to diets with increasing percentages of GDDY. Therefore, the objective of this study was to evaluate the use of GDDY as a protein source in feeds for *L. vannamei*, reared in green water conditions and production ponds.

Materials and methods

A series of experiments were conducted at the Alabama Department of Conservation and Natural Resources, Marine Resources Division, Claude Peteet Mariculture Center in Gulf Shores, AL. Two growth trials were carried out in parallel, one a pond trial and the other an outdoor tank trial, during June through September 2011.

Experimental animals

Shrimp post larvae (PL) were received from a commercial hatchery, Shrimp Improvement System, Key West, FL. Dissolved oxygen (DO), temperature, salinity, and pH were randomly measured among the shipping bags using a YSI 556 MPS meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Two water samples were collected in order to determine total ammonia-nitrogen (TAN) using the Orion ammonia electrode probe (Thermo Fisher Scientific Inc., Waltham, MA, USA).

An oxygen tank with one air stone was operated to provide oxygen during the acclimation period. Newly hatched brine shrimp, *Artemia salina* (INVE Americas Inc., Salt Lake City, UT, USA), were prepared one night before PL reception and fed to PL in the acclimation

tank. Shrimp post larvae were slowly acclimated and released into the acclimation tank. Water quality parameters were measured to control temperature and salinity change in order to have acclimation rates in the range of 4 °C hr^{-1} and 2 ppt hr^{-1}, respectively. After acclimation, PL were concentrated by drain harvesting into a 57-L concentration tank and quantified volumetrically. Six sub-samples were taken using a 60 ml beaker while the water in the concentration tank was mixed vigorously. Average numbers of PL per ml were then determined. Sixty shrimp were randomly collected and individually weighed to determine average weight and predict biomass for determination of feed inputs.

The shrimp were divided equally into six nursery tanks (6,000-L). The nursery system was stocked at a density of 28 PL L^{-1}. Each nursery tank was equipped with three air lifts and two air stones to help with water circulation and aeration was provided by a common regenerative blower. The recirculating nursery system was composed of the six nursery tanks, a biological filter, a pressurized sand filter, and a 1.5-hp circulation pump (Aquatic Eco-Systems, Apopka, FL, USA). Feeds were applied on daily basis four times per day (Table 1). Uneaten feed was siphoned out to maintain the water quality in the nursery tanks. Shrimp post larvae were raised in the nursery system for a 16-day period. Water quality was monitored two times per day at 800 and 1600 h and TAN was measured twice weekly. Every three days, 60 PL per tank were randomly collected and sub-samples of 10 shrimp were pooled weighed in order to evaluate growth and readjust daily feed input.

At the end of the 16-day period, each tank was partially drained to concentrate the shrimp. The shrimp were then collected by dip nets. Six sub-samples were taken from each nursery tank to determine average weight. Once the number of shrimp per unit weight was determined, shrimp were divided evenly into 16 buckets, each representing one production pond.

Shrimp from each nursery tank were distributed across all ponds to minimize any bias from variation in the nursery tanks. The shrimp were then transferred to the corresponding pond and stocked. Mean final weight, weight gain (g), FCR, and survival for each nursery tank was determined.

Experimental diets

Four experimental diets were formulated to contain solvent extracted soybean meal (SE-SBM) as the primary protein source with increasing percentages of GDDY (0, 5, 10, and 15% of diet). To further improve the amino acid balance of the plant-based diets, poultry by-product meal and corn gluten meal were included at 8.0 and 4.8% of diet, respectively (Table 4). The experimental diets were manufactured by Rangen Inc., under commercial feed manufacturing condition as a sinking extruded pellet. All experimental diets contained approximately 36% protein and 8% lipid with lysine and methionine plus cystine contents of 5 and 3% of protein, respectively (Table 5, Table 6). The experimental diets were evaluated in an outdoor tank-based system and production ponds.

Production pond trial

Ponds used for the trial were approximately 0.1 ha in surface area (rectangular 46 x 20 m) with a 1.0 m average depth. Each pond was equipped with a 20-cm diameter standpipe, a concrete catch basin (3.0 x 2.0 x 0.5 m), and lined with 1.52 mm thick high-density polyethylene lining (HDPE). The bottom of each pond was covered with a 25-cm deep layer of sandy-loam soil. After each culture cycle, ponds were dried and tilled in order to allow oxidation and mineralization of organic matter. Approximately three weeks before stocking, ponds were filled

with brackish water from the intra-coastal waterway canal between Mobile and Perdido Bay. Incoming water was filtered through a three foot 250 μm-mesh nylon filter sock (Micron Domestic Lace Mfg., Inc) to prevent the introduction of predators, as fish larvae, crab, or other relatively large planktonic organisms, but allow the introduction of primary productivity.

Two weeks before stocking juvenile Pacific white shrimp, a combination of inorganic liquid fertilizers (32-0-0 mixed with 10-34-0) was applied at a rate of 1,697 and 303 ml, respectively, per pond which provided 5.73 kg of N and 1.03 kg of P_2O_5 ha^{-1} in order to promote natural productivity in all ponds. A second fertilization was made at half of the initial rate when the Secchi disk reading was greater than 40 cm. All ponds were provided aeration about 10 hp ha^{-1} using 1-hp paddlewheel aerators (Little John Aerator, Southern Machine Welding Inc., Quinton, AL, USA) and either 1-hp or 2-hp Aire-O2 aerators (Aire-O2, Aeration Industries International Inc., Minneapolis, MN, USA) to maintain DO above 3 mg L^{-1}, additional aerators were provided as needed when DO fell below 3 mg L^{-1}. This study was designed to be a sustainable semi-intensive system with well-managed feeding and minimal water exchange.

Juvenile *L. vannamei* (38 ± 4.26 mg, initial weight) were collected from the nursery system and stocked in the production ponds at a rate of 30 shrimp m^{-2}. Four test diets were randomly assigned to 16 production ponds using four replicates per diet. Rations were divided to two feedings per day, early morning 800 h and late afternoon 1600 h. Feeding strategy for the first four weeks was based on previous studies that assumed that PL fed on primary productivity. Thus, during this period, a small amount of feed was applied to promote natural productivity in the ponds. Thereafter, feed input was calculated based upon an expected weight gain of 1.3 g wk^{-1}, an estimated FCR of 1.2, and a survival rate of 75% during the pond culture period (Table 7).

Dissolved oxygen (DO), temperature, salinity, and pH were monitored three times per day, at sunrise (0500 - 0530 h), during the afternoon (1500 - 1530 h), and at night (2000 - 2200 h), using a YSI 556 MPS meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Secchi disk reading and TAN were monitored once weekly. Water samples were taken in all ponds at the depth of 80 cm and TAN was determined using a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc., Rochester, NY, USA) following the Nesslerization method (APHA 1989). Each week, sixty shrimp were sampled using two 5-foot cast nets (monofilament net, 1.22 m radius and 0.95 cm opening) to determine average weight.

The shrimp were harvested at the end of the 16-wk period. Feed was withheld 36 h before harvest in order to clear the shrimp gut. To harvest, about two thirds of the water was drained from the ponds the night before harvest. The following day, the remaining water was drained and the shrimp were pump harvested from the catch basin using a fish pump equipped with a 25-cm diameter suction pipe (Aqua-Life Pump, Magic Valley Heli-Arc & Mfg., Twin Falls, ID, USA), dewatered and collected into a hauling tank. The shrimp were rinsed and weighed, then approximately 120 shrimp from each pond were randomly selected to measure individual weight. Mean final weight, yield, FCR, size distribution, and survival were determined.

Outdoor tank trial

The outdoor tank system was a semi-closed recirculating system which consisted of a central reservoir (800-L) with a biological filter, a 0.33-hp sump pump, and 20 circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter). Another sump pump was used to move water from one of the production ponds to the central reservoir at

a rate of 8 L min^{-1} during the study period between 800 and 1400 h to mimic a production pond setting.

Juvenile *L. vannamei* (3.05 ± 0.22 g, initial weight) were collected from the production ponds and size-sorted by hand. Juvenile shrimp were randomly selected and stocked at a rate of 30 shrimp per 750-L tank. Each tank and the central reservoir were provided with two air stones connected to a 0.5-hp regenerative blower (Sweetwater Aquaculture Inc., Lapwai, ID, USA) to supply aeration. All tanks were covered by netting to prevent shrimp from jumping out.

Five test diets were randomly assigned amongst the 20 tanks. The four experimental diets used in the production ponds were tested using four replicates per diet. The fifth diet, a reference commercial diet (Rangen Shrimp Grower, 35% protein, 8% lipid), was also tested using four replicates. Juvenile shrimp were offered test diets twice per day at 0800 and 1600 h. Daily feed input was calculated based upon an expected weight gain of 1.3 g wk^{-1} and an estimated FCR of 1.2. Dissolved oxygen (DO), temperature, salinity, and pH were monitored twice per day at 600 and 1600 h using a YSI 556 MPS meter. Water samples were taken bi-weekly from central reservoir and two tanks to measure TAN with a spectrophotometer according to previously described methods. At the conclusion of the 12-wk period, shrimp were counted and group weighed. Mean final weight, biomass, FCR, and survival were determined.

Statistical analysis

All data were statistically analyzed using one-way analysis of variance to determine significant differences ($P<0.05$) which was followed by the Student-Neuman-Keuls multiple comparison test to determine significant differences among treatment means if a significant

treatment effect was observed. All statistical analysis were carried out using SAS (V9.1. SAS Institute, Cary, NC, USA).

Results

Nursery system

Water quality parameters throughout the 16-day nursery period were within suitable ranges for the culture of this species (Table 2). At the conclusion of the shrimp nursery period, mean final weight 0.038 ± 4.26 g, weight gain 0.036 ± 4.26 g, survival 49.5 ± 14.4%, and FCR 2.05 ± 0.55 were observed (Table 3). The relatively low survival was probably due to high TAN concentrations reported in the nursery tanks (2.31 ± 2.18 mg L^{-1}).

Production pond trial

Water quality parameters were within an acceptable range for adequate growth and survival of shrimp (Table 8). One replicate pond corresponding to the 5% GDDY treatment was excluded from data analysis due to the presence of blue green algae that resulted in poor survival. Low DO levels (below 2.5 mg L^{-1}) were occasionally observed throughout the culture period when phytoplankton population collapsed. In contrast, high DO levels (above 10 mg L^{-1}) were observed typically in the afternoon due to photosynthetic activity.

Shrimp reared in production ponds, fed increasing percentages of GDDY (0, 5, 10, and 15% of diet) were not significantly different ($P>0.05$) with regards to mean final weight, yield, FCR, and survival (Table 9). At the conclusion of the production period, mean final weight ranged between 19.77 and 23.05 g. Yield ranged from 4,760 to 5,606 kg ha^{-1}. FCR ranged between 1.02 and 1.23 and survival ranged from 69.6 to 89.4%. The size distribution of shrimp

was determined as it is done commercially, where shrimp production is selected by size class based on the total number of head-on shrimp lb^{-1}. Similar size distribution was observed among dietary treatments (Figure 1).

Outdoor tank trial

Water quality parameters throughout the 12-wk period were maintained at suitable limits for adequate growth and survival of shrimp (Table 10). As expected because pond water was obtained from one of the production ponds in order to mimic a production pond setting at the outdoor tanks, temperature (from 21.01 to 33.31 °C) and salinity readings (from 9.48 to 17.08 ppt) were similar to those observed in the production ponds.

Results (Table 11) indicated that there were no significant differences ($P>0.05$) among treatments in mean final weight, biomass, FCR, and survival of *L. vannamei*, fed experimental diets at increasing percentages of GDDY (0, 5, 10, and 15% of diet). Mean final weight ranged between 18.12 and 18.97 g. Biomass ranged from 530.55 to 535.80 g $tank^{-1}$. FCR ranged between 1.25 and 1.29. Survival ranged from 93.3 to 98.3%. However, shrimp fed the commercial diet, was significant different ($P<0.05$) from the test diets. Reference treatment had slightly higher mean final weight (20.00 g), biomass (568.90 g $tank^{-1}$), and lower FCR (1.18).

Discussion

Yeast is a feed ingredient which can originate from a wide range of sources and is not yet commonly utilized in aquatic feeds, although several studies have been conducted to validate its inclusion either as a nutritional supplement (Burgents *et al*. 2004; Li & Gatlin 2004; Li *et al*. 2011; Vechklang *et al*. 2012) or protein source (Oliva-Teles & Gonçalves 2001; Olvera-Novoa *et*

al. 2002; Lunger *et al*. 2006). Grain distillers dried yeast is a promising protein source obtained from the bioethanol industry which has been evaluated in sunshine bass (Gause & Trushenski 2011) and rainbow trout (Stewart 2012). However, there is limited information about GDDY as a protein source in shrimp diets. Therefore, the present study assessed GDDY in commercial feeds and demonstrated that GDDY could be utilized in diets without causing adverse effects on growth performance of *L. vannamei,* reared under pond conditions as well as outdoor green water tanks. The results from this study showed that *L. vannamei,* fed increasing percentages of GDDY up to 15% of diet resulted in no significant differences regarding mean final weight, FCR, and survival on both culture conditions.

The result of this study is in agreement with Peterson *et al*. (2012), who found that a yeast-derived protein source (NuPro®), could be utilized in channel catfish diets without compromising fish growth up to 10% of diet. Essa *et al*. (2011) showed that African catfish, *Clarias gariepinus,* fed a diet formulated with brewers yeast (2% of diet) improved mean final weight and growth rate in floating cages. These experiments have confirmed the positive effect of yeast-based products either as a protein source or nutritional supplement in fish diets without affecting production performance.

Studies have reported that torula yeast (*Candida utilis*) can be utilized as an alternative protein source in tilapia fry diets. Olvera-Novoa *et al*. (2002) reported that acceptable growth was observed with torula yeast at 25% of diet. Furthermore, no significant differences were found among treatments when torula yeast was added up to 40% of diet. Studies have also been carried out with live yeast, *Saccharomyces cerevisiae,* as a dietary supplement in tilapia culture. Lara-Flores *et al*. (2003) suggested live yeast at 0.1% of diet as a growth-stimulating additive in tilapia fry. This study is supported by Abdel-Tawwab *et al*. (2010), who observed greater growth

performance with live yeast at a level of 10 g kg^{-1} regarding weight gain, feed efficiency, and FCR in Galilee tilapia, *Sarotherodon galilaeus*. Moreover, they also pointed out that live yeast may play an important role in reducing environmental copper toxicity.

With rainbow trout, Stewart (2012) found that GDDY could be used as an alternative protein source up to 11% of diet without causing adverse effects on weight gain, feed intake, and FCR. Grain distillers dried yeast levels above 11% of diet, however, resulted in poor growth performance. Palatability problems were discarded as a cause of this result as fish consumed more of the diets containing higher levels of GDDY. The decreased performance in this study, instead, was likely due to an amino acid deficiency. Additionally, yeast-based products have also shown beneficial effects as a supplement in the hatchery rearing of McConaughy strain rainbow trout based on the improvements of FCR and increased growth (Barnes *et al*. 2006).

Yeast products have also been evaluated in marine fish without affecting production performance. Oliva-Teles & Gonçalves (2001) indicated that brewers yeast could be used as a protein source up to 55% of diet without causing negative effects on fish growth and feed intake in juvenile sea bass (*Dicentrarchus labrax*) diets. With the same fish species, Alliot *et al*. (1979) did not find any adverse effects when fish meal (50% of diet) was replaced by an alkane yeast protein. Lunger *et al*. (2006) utilized a yeast-based, certified organic protein source (NuPro®) as a replacement of fish meal in cobia diets. They did not find any significant difference when the yeast-based protein was added up to 20% of diet with regards to weight gain and FCR. However, NuPro® levels above 20% of diet resulted in higher FCR and dramatically impaired survival when fish meal was completely replaced by the yeast protein (78% of diet). Yeast-based products have also been used as a feed supplement in hybrid striped bass. According to Li & Gatlin (2003, 2004, 2005), brewers yeast supplementation (2% of diet) influenced growth

performance and feed efficiency as well as resistance to bacterial challenges with *Streptococcus iniae* and *Mycobacterium marinum*. Similar results were obtained with Pacific white shrimp, *L. vannamei*, by Burgents *et al*. (2004), who evaluated a yeast culture feed supplement against the negative effects of live *Vibrio* sp. They reported that yeast supplementation (1% of diet) increased resistance to the bacterial infection in shrimp culture.

Yeast-based products, mainly brewers yeast, are an important source of beta-glucans. These polysaccharides are commonly found in the bran of cereal grains, cellulose in plants, and the cell wall of yeast. Studies have indicated that beta-glucans enhance disease resistance against pathogenic infections. They stimulate nonspecific components of the immune system providing antigens during the exposure to diseases. In crustaceans, beta-glucans induce the activation of the prophenoloxidase system, reducing microbial activity in plasma and superoxide production in hemocytes (Itami *et al*. 1994; Song & Hsieh 1994; Sung *et al*. 1996; Chang *et al*. 2000; Campa-Córdoba *et al*. 2002). Hence, these studies confirm the important role of beta-glucans as immunostimulants in aquatic organisms.

In conclusion, the results from this study reported that in well-formulated commercial feeds containing GDDY as an alternative protein source in combination with SE-SBM are feasible for *L. vannamei*, reared under a variety of research conditions. It is likely that the production responses were the result of an adequate formulated diet, well-manufactured feed, appropriate feed inputs, and suitable pond management.

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Table 1. Feeding regime for *L. vannamei* post larvae, based on mean shrimp weight determined every three days, percent body weight, and feed types utilized throughout a 16-day nursery period.

Day	Mean weight (mg)	Feed type	Feed (% body weight)
1 to 3	2.20	100 Artemia[a] / PL, PL redi[b] (1:2)	25.0
4 to 6	7.20	PL redi, PL raceway #0[c] (1:1)	25.0
7 to 9	8.50	PL raceway #0, PL raceway #1[d] (1:1)	25.0
10 to 12	13.90	PL raceway #0, PL raceway #1 (1:1)	25.0
13 to 16	25.20	PL raceway #1	15.0

[a]INVE Americas Inc., Salt Lake City, UT, USA.
[b]PL Redi-Reserve, 400-600 microns. 50% Protein, produced by Zeigler Bros Inc., Gardners, PA, USA.
[c]PL Raceway #0, 400-600 microns. 50% Protein, produced by Zeigler Bros Inc., Gardners, PA, USA.
[d]PL Raceway #1, 600-850 microns. 50% Protein, produced by Zeigler Bros Inc., Gardners, PA, USA.

Table 2. Water quality variables observed over a 16-day nursery period for *L. vannamei* post larvae, nursed in a recirculating system composed of six, 6,000-L nursery tanks.

	Temperature (°C)	DO (mg L^{-1})[a]	pH	Salinity (ppt)	TAN (mg L^{-1})[b]
am	26.27 ± 1.91 (22.48, 28.54)	4.84 ± 0.88 (2.59, 6.13)	7.87 ± 0.24 (7.18, 8.38)	16.46 ± 0.34 (15.56, 17.03)	2.31 ± 2.18 (0.05, 6.00)
pm	27.96 ± 1.65 (24.76, 30.22)	4.36 ± 0.81 (2.30, 6.58)	7.85 ± 0.26 (7.38, 8.43)	16.35 ± 0.61 (14.10, 17.25)	

Values represent the mean ± standard deviation and values in parenthesis represent minimum and maximum readings.
[a]Dissolved oxygen.
[b]Total ammonia-nitrogen.

Table 3. Mean production parameters for *L. vannamei* post larvae (initial weight of 2.20 mg), stocked at a density of 28 PL L^{-1} in a recirculating system composed of six, 6,000-L nursery tanks over a 16-day nursery period.

	Mean	Minimum	Maximum	Standard deviation	CV[a]
Final weight (mg)	38.07	31.59	44.07	4.26	11.19
Weight gain[b] (mg)	35.89	29.41	41.89	4.26	11.87
Survival (%)	49.55	30.20	73.44	14.40	29.09
FCR[c]	2.05	1.58	3.07	0.55	26.71
Yield (g $tank^{-1}$)	3,086.00	1,963.00	3,867.00	676.28	21.91
Standing crop[d] (kg m^{-3})	0.86	0.55	1.07	0.19	21.91

[a]CV = Standard deviation / mean * 100.
[b]Weight gain = Final weight - Initial weight.
[c]Feed conversion ratio = Total feed offered / biomass increase.
[d]Standing crop (kg m^{-3}) = (Yield/1,000) / 3.6 m^3.

Table 4. Ingredient composition (g 100 g^{-1} as is) of four experimental diets designed to contain increasing percentages of GDDY (0, 5, 10, and 15% of diet) as a protein source for *L. vannamei* growth trials reared under different culture conditions.

Ingredient	0% GDDY	5% GDDY	10% GDDY	15% GDDY
Soybean meal	53.56	48.17	42.80	37.51
Milo	22.37	22.84	23.31	23.68
Poultry by-product meal	8.00	8.00	8.00	8.00
Corn gluten meal	4.84	4.84	4.84	4.84
Grain distillers dried yeast	-	5.00	10.00	15.00
L-Lysine HCL	-	0.07	0.14	0.21
Dicalcium phosphate	3.13	3.13	3.13	3.13
Fish oil	5.00	4.85	4.68	4.53
Bentonite	1.50	1.50	1.50	1.50
Soy lecithin	1.00	1.00	1.00	1.00
Vitamin premix[a]	0.33	0.33	0.33	0.33
Mold inhibitor	0.15	0.15	0.15	0.15
Mineral premix[a]	0.09	0.09	0.09	0.09
Stay-C 35% (C)	0.02	0.02	0.02	0.02
Copper sulfate	0.01	0.01	0.01	0.01

Diets were formulated to contain 36% protein and 8% lipid.
Feed ingredients were sourced and diets were manufactured by Rangen® (Angleton, TX, USA) using extrusion processing.
[a]Vitamin and mineral premix are proprietary products, thus their composition is not listed.

Table 5. Nutrient composition (dry matter basis) of four experimental diets containing increasing percentages of GDDY (0, 5, 10, and 15% of diet) as a protein source for *L. vannamei* growth trials reared under different culture conditions.

Nutrient	0% GDDY	5% GDDY	10% GDDY	15% GDDY
Protein	36.60	37.10	36.50	37.80
Moisture	11.02	11.43	11.22	8.84
Lipid	9.95	9.83	9.71	11.50
Fiber	2.09	2.52	1.00	2.44
Ash	8.22	6.46	6.61	5.04
Lysine	1.98	1.82	2.01	2.06
Methionine + Cystine	1.04	1.10	1.01	1.07

Diets were analyzed by Midwest Laboratories Inc., Omaha, NE, USA.

Table 6. Amino acid profile (as is) of four experimental diets designed to contain increasing percentages of GDDY (0, 5, 10, and 15% of diet) as a protein source for *L. vannamei* growth trials reared under different culture conditions.

Amino acid	0% GDDY	5% GDDY	10% GDDY	15% GDDY
Alanine	1.31	1.20	1.48	1.23
Arginine	2.32	1.48	1.50	4.10
Aspartic Acid	3.23	3.47	3.29	3.23
Cystine	0.49	0.50	0.45	0.46
Glutamic Acid	5.82	6.22	6.54	6.36
Glycine	1.82	2.30	1.83	1.74
Histidine	0.90	0.95	0.99	1.28
Isoleucine	1.86	1.33	2.19	1.54
Leucine	3.68	3.15	3.77	3.21
Lysine	1.98	1.82	2.01	2.06
Methionine	0.55	0.60	0.56	0.61
Phenylalanine	1.01	1.82	1.61	1.27
Proline	1.94	2.33	2.71	2.26
Serine	1.66	2.07	2.16	1.40
Threonine	1.61	1.43	2.24	1.43
Tyrosine	1.62	1.80	1.43	2.51
Tryptophan	0.30	0.21	0.28	0.22
Valine	1.97	2.44	1.62	1.91

Diets were analyzed by Midwest Laboratories Inc., Omaha, NE, USA.

Table 7. Feeding regime for *L. vannamei*, reared in 0.1-ha ponds over a 16-wk period based on an expected growth rate of 1.3 g wk^{-1} and an assumed survival rate of 75%.

Week	Population	Feed input per day	
		kg $pond^{-1}$	kg ha^{-1}
1	30,000	1.50	15.0
2	29,500	3.00	30.0
3	29,000	6.00	60.0
4	28,500	6.30	63.0
5	28,000	6.20	62.0
6	27,500	6.10	61.0
7	27,000	6.00	60.0
8	26,500	5.90	59.0
9	26,000	5.80	58.0
10	25,500	5.70	57.0
11	25,000	5.60	56.0
12	24,500	5.50	55.0
13	24,000	5.40	54.0
14	23,500	5.30	53.0
15	23,000	5.30	53.0
16	22,500	5.30	53.0

Table 8. Summary of water quality variables monitored over a 16-wk period for *L. vannamei*, fed experimental diets containing increasing percentages of GDDY (0, 5, 10, and 15% of diet) as a substitute for SE-SBM.

Parameter	0% GDDY	5% GDDY	10% GDDY	15% GDDY
Temperature (°C)				
Am	29.99 ± 1.95 (23.43, 32.90)	30.12 ± 2.01 (23.57, 33.49)	29.85 ± 2.03 (23.09, 33.04)	30.11 ± 2.05 (23.56, 33.17)
Noon	31.69 ± 2.00 (25.00, 35.02)	31.83 ± 2.04 (25.26, 36.26)	31.57 ± 2.10 (24.22, 35.22)	31.94 ± 2.08 (25.03, 35.48)
Pm	31.13 ± 1.92 (25.22, 34.86)	31.25 ± 1.95 (24.79, 35.60)	31.07 ± 1.95 (24.52, 34.77)	31.39 ± 1.96 (25.24, 35.49)
DO (mg L^{-1})[a]				
Am	3.80 ± 0.90 (1.15, 6.82)	3.77 ± 0.98 (1.25, 7.10)	3.75 ± 0.87 (1.38, 6.56)	3.64 ± 0.95 (1.18, 6.40)
Noon	9.23 ± 2.70 (3.15, 17.45)	9.76 ± 3.27 (3.17, 18.77)	9.89 ± 3.53 (2.96, 19.72)	9.91 ± 3.38 (3.07, 19.73)
Pm	7.08 ± 2.33 (1.83, 14.85)	7.40 ± 2.74 (1.09, 18.49)	6.99 ± 2.18 (1.27, 16.90)	7.14 ± 2.43 (1.65, 15.07)
pH				
Am	8.07 ± 0.50 (7.02, 9.26)	8.18 ± 0.55 (7.21, 9.87)	8.11 ± 0.55 (7.14, 9.66)	8.01 ± 0.53 (7.03, 9.74)
Noon	8.77 ± 0.44 (7.28, 9.80)	8.71 ± 0.51 (7.11, 9.94)	8.73 ± 0.51 (7.11, 10.9)	8.66 ± 0.55 (7.05, 10.33)
Pm	8.57 ± 0.50 (7.09, 9.65)	8.51 ± 0.53 (7.02, 9.95)	8.67 ± 0.47 (7.35, 9.88)	8.52 ± 0.49 (7.20, 9.74)
Salinity (ppt)				
Am	13.22 ± 2.13 (9.01, 16.98)	13.79 ± 2.24 (9.02, 16.97)	13.68 ± 2.01 (9.40, 16.97)	13.50 ± 2.12 (9.10, 16.95)
Noon	13.32 ± 2.18 (8.53, 16.98)	13.86 ± 2.27 (8.04, 16.99)	13.72 ± 2.18 (7.93, 17.17)	13.68 ± 2.26 (8.07, 17.81)
Pm	13.33 ± 2.26 (8.59, 17.92)	13.97 ± 2.45 (8.07, 17.86)	13.76 ± 2.27 (8.01, 17.99)	13.67 ± 2.26 (8.15, 18.19)
Secchi (cm)	31.64 ± 23.77 (10, 100)	29.38 ± 21.09 (10, 120)	30.08 ± 20.46 (10, 100)	27.8 ± 18.85 (10, 100)
TAN (mg L^{-1})[b]	0.24 ± 0.51 (0.00, 3.00)	0.21 ± 0.39 (0.00, 2.00)	0.33 ± 0.74 (0.00, 5.00)	0.20 ± 0.27 (0.00, 1.00)

Values represent the mean ± standard deviation and values in parenthesis represent minimum and maximum readings.
[a]Dissolved oxygen.
[b]Total ammonia-nitrogen.

Table 9. Mean production parameters over a 16-wk period for juvenile Pacific white shrimp, *L. vannamei* (38 ± 4.26 mg, initial weight), fed experimental diets containing increasing percentages of GDDY (0, 5, 10, and 15% of diet) as a substitute for SE-SBM and cultured in 0.1-ha ponds.

Treatment	Yield (kg ha^{-1})	Mean final weight (g)	FCR[a]	Survival (%)
0% GDDY	5,526.75	21.38	1.03	86.54
5% GDDY	5,292.17	19.77	1.09	89.41
10% GDDY	4,760.30	23.05	1.23	69.65
15% GDDY	5,606.38	21.67	1.02	86.93
P-value[b]	0.183	0.213	0.150	0.114
PSE[c]	285.884	0.976	0.069	5.839

[a]Feed conversion ratio = Total feed offered / biomass increase.
[b]Analysis of variance was used to determine significant differences ($P<0.05$) among treatment means (n=4).
[c]Pooled standard error of treatment means.

Figure 1. Size distribution of head-on shrimp (% production) at the end of the 16-wk period for *L. vannamei*, fed diets containing increasing percentages of GDDY (0, 5, 10, and 15% of diet) and cultured in 0.1-ha ponds.

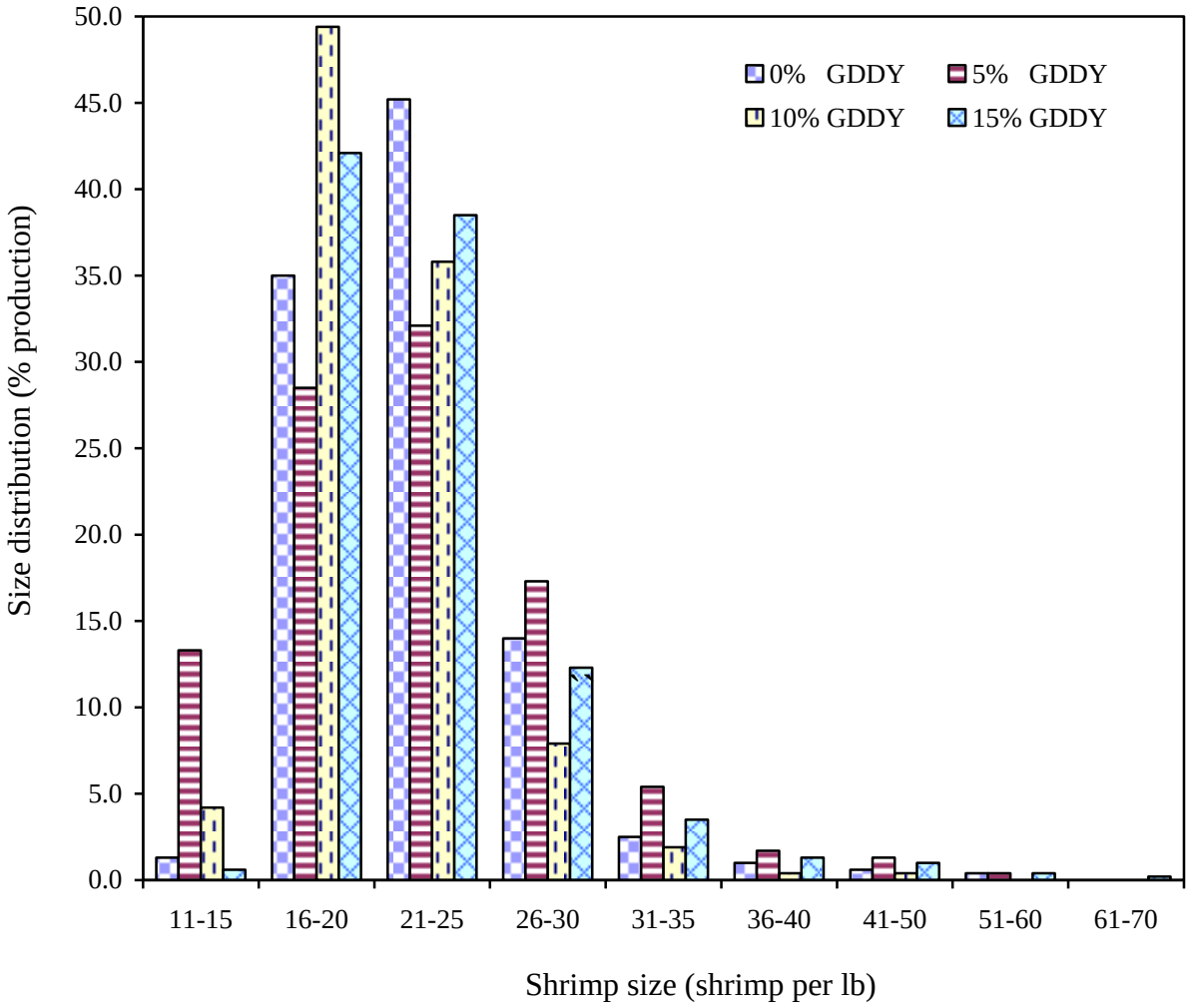

Table 10. Water quality variables monitored over a 12-wk period for juvenile *L. vannamei*, fed experimental diets containing increasing percentages of GDDY (0, 5, 10, and 15% of diet) as a substitute for SE-SBM and reared under an outdoor tank-based system.

	Temperature (°C)	DO (mg L^{-1})[a]	pH	Salinity (ppt)	TAN (mg L^{-1})[b]
am	28.13 ± 1.78	5.63 ± 0.64	8.16 ± 0.38	13.95 ± 1.79	0.33 ± 0.46
	(19.70, 30.55)	(4.11, 7.21)	(7.24, 9.63)	(9.16, 17.13)	(0.00, 2.00)
pm	30.20 ± 1.99	5.57 ± 0.71	8.25 ± 0.44	14.01 ± 1.73	
	(21.01, 33.31)	(4.17, 7.71)	(7.09, 9.50)	(9.48, 17.08)	

Values represent the mean ± standard deviation and values in parenthesis represent minimum and maximum readings.
[a]Dissolved oxygen.
[b]Total ammonia-nitrogen.

Table 11. Mean production parameters over a 12-wk period for *L. vannamei* (3.05 ± 0.22 g, initial weight), fed diets containing increasing percentages of GDDY (0, 5, 10, and 15% of diet) as a substitute for SE-SBM and a commercial feed used as a reference diet, reared under an outdoor tank-based system.

Treatment	Biomass (g tank^{-1})	Mean final weight (g)	FCR[a]	Survival (%)
0% GDDY	535.80a	18.47a	1.25a	96.7
5% GDDY	534.55a	18.12a	1.25a	98.3
10% GDDY	530.55a	18.97a	1.29a	93.3
15% GDDY	534.78a	18.29a	1.26a	97.5
Reference diet[b]	568.90b	20.00b	1.18b	95.0
P-value[c]	0.001	0.001	0.001	0.348
PSE[d]	6.201	0.249	0.012	1.341

[a]Feed conversion ratio = Total feed offered / biomass increase.
[b]Reference diet = 35% Protein, Rangen 35,0 (Buhl, ID, USA).
[c]Analysis of variance was used to determine significant differences ($P<0.05$) among treatment means (n=4).
[d]Pooled standard error of treatment means.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The primary objective of this study was to evaluate the substitution of soybean meal with grain distillers dried yeast, a promising protein source obtained from the bioethanol industry in commercially manufactured diets for *Litopenaeus vannamei*. Currently, there are economic and environmental concerns about feed ingredients utilized in aquatic feeds. High prices and limited supply are the main factors that have led to research in the area of aquaculture nutrition in order to develop low-cost feeds. Most research studies have been focused in assessing alternative protein sources, as fish-based protein is generally the most expensive nutrient commonly found in fish and crustacean diets. Plant-based proteins have been the target for most studies when developing feed formulations for aquatic species because they are less expensive feed ingredients than animal proteins. Moreover, plant proteins will reduce the dependence on wild fish population commonly utilized in fish meal processing plants. Towards this goal, soybean meal has been the primary plant protein source used in shrimp diets due to its reasonable price and steady supply compared to marine ingredient sources such as fish meal. Nevertheless, the use of soybean meal in shrimp feed formulations is limited because of its low level of essential amino acids such as methionine and lysine as well as potential anti-nutritional factors (e.g. trypsin inhibitors, lectins, antigens, and oligosaccharides) which may impair digestibility and palatability of feed. Therefore, various combinations of soybean meal and other protein sources, commonly

by-products, have been evaluated to provide a more balanced feed formulation for shrimp growth.

Based on the principle of evaluating alternative protein sources for shrimp diets, these studies were carried out in clear and green water systems as well as pond conditions. The first study assessed shrimp performance fed increasing percentages of GDDY (0, 5, 10, 15, 20, and 30% of diet) with or without the addition of lysine in diets with high levels of GDDY (20, and 30% of diet) in clear water tanks over an 8-wk period. In the second study, commercial shrimp diets designed to contain increasing percentages of GDDY (0, 5, 10, and 15% of diet) were evaluated in a green water system and production ponds for a 12 and 16-wk period, respectively. Furthermore, an additional commercial feed was used as a reference diet in the outdoor green water tanks. Results from both growth trials demonstrated that GDDY can be used as a protein source in shrimp diets at high levels of inclusion (15-30% of diet) without affecting growth, yield, FCR, and survival. Based on the observed feed response, inclusion of GDDY in shrimp feed formulation up to 30% of diet is acceptable and promotes good growth in Pacific white shrimp. In conclusion, GDDY is a promising protein source that can be successfully used as a partial replacement for soybean meal without compromising animal performance in commercial shrimp production. Moreover, reducing soybean meal levels would be profitable for both feed manufacturers and shrimp producers as soy grain prices continues to increase. Such diets should facilitate to develop low-cost feeds and promote the use of more sustainable plant protein sources in shrimp production.

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