Practical diets for Pacific white shrimp *Litopenaeus vannamei* utilizing alternative ingredients

by

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ABSTRACT

Global shrimp production has steadily increased and is currently one of the primary users of fishmeal (FM). At the same time, Global fishmeal supply has reached a plateau, with demand still continuing to increase resulting in reduced availability and increased prices. From an economic standpoint, it is important to look for cost -effective protein sources from a variety of plant and terrestrial animal sources as replacements for more expensive proteins such as FM. This study was dedicated to exploring the usage of alternative protein sources which included two conventional yeast products (BY50 and BY70), corn processing product (CPC: corn protein concentrate), enzyme-treated soybean meal products (SPC: soy protein concentrate), grain processing product (HPDDG: high protein distiller's dried grain) and by-products of salmon processing (SM and HSM: salmon meal and hydrolyzed salmon meal) as potential protein source to replace FM, CPC or SBM in practical shrimp diet. A series of 5 independent trials were conducted to evaluate those listed products. The first study results indicated that 180-240g/kg BY50 can be effectively used in shrimp diets as a replacement for FM, or up to 240g/kg when replacing SBM. Additionally, adding 20g/kg of BY70 does not cause impaired growth performance for the shrimp fed with low FM diet. Under green water conditions in the presence of natural foods, the second study results indicated that HPDDG is a good protein source and up to 20 or 15% HPDDG can be used to replace CPC or FM in practical shrimp diets. The third study demonstrated that total 92 or 138 g/kg of CPC and SPC (1:1 ratio) may be used in the diet of the Pacific white shrimp replacing 50 or 75% fishmeal in clear and green water under high stocking density and low salinity culture conditions, respectively. The fourth study results showed that up to 100 or 50% anchovy meal can be replaced by salmon meal or hydrolyzed salmon meal without causing impaired growth performance in both clear and green water conditions, respectively. The following up fifth study results indicated that the growth performance of shrimp

has not influenced by HSM up to 60 g/kg to replace 50% of the SM in practical diets; however, higher levels resulted in significant decrease in performance. Based on those results of those studies, potential protein ingredients like BY50, HPDDG, CPC, SPC, SM and HSM can be used in practical shrimp diet as a replacement for FM, CPC or SBM. With the shortage of FM and the expansion of aquaculture production, it is necessary for us to evaluate these alternative ingredients and determine the optimum inclusion level to promote sustainable and economically viable aquaculture production.

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CHAPTER I

Introduction

Pacific white shrimp *Litopenaeus vannamei* is one of the most important economic species that is widely cultured in the world. It has rapid growth, delicious taste, high survival and disease tolerance under a range of stoking densities (Amaya, Davis, & Rouse, 2007). It can also tolerance a wide range of salinity, which make it possible to culture in fresh to hyper-saline water as long as the ionic profile is appropriate (Roy et al., 2010). Recently, the culture of Pacific white shrimp was expanded to account for 80% of world shrimp production, which reach 3,879,786 in 2015 (FAO, 2018) and was estimated to increase by >10% in the next fifteen years (Bank, 2013).

With this rapid expansion of shrimp production, the demand for cost-effective protein sources continues to increase since there is considerable costs saving in shifting protein sources (X Qiu, H Tian, & DA Davis, 2018). The dietary protein requirement of the Pacific white shrimp is 30-36% to maintain optimal growth and the most ideal protein source is fishmeal. Fishmeal typically account for approximately 25% cost in the commercial shrimp feed formula, which is representing the primary and the most expensive protein ingredient. Fishmeal is not only an excellent source of dietary protein source, essential amino acid and indispensable fatty acid, but also a source of nucleotides, minerals, vitamins, cholesterol, and some unidentified growth factors (Samocha, Davis, Saoud, & DeBault, 2004). However, global supplies of fishmeal (FM) have reached a plateau; and fishmeal production has followed a fluctuating but declining trend. At the same time demand continues to increase, which makes it more expensive and less available (FAO, 2018). Thus, it is necessary to reduce or remove fishmeal and replace it with other more economical protein sources to reduce and stabilize feed costs.

There are a numbers of marine and non-marine ingredients originating from agriculture, fisheries and animal processing that can potentially serve as replacements for fishmeal in shrimp feeds (Guo et al., 2018; Guo, Qiu, Salze, & Davis, 2019; Guo, Reis, et al., 2019; X Qiu, Neori, et al., 2018; X Qiu, HY Tian, & DA Davis, 2018; Sookying & Davis, 2012). Soybean meal is usually regarded as the most nutritionally valuable protein and cost-effective protein source in both fish and shrimp feed (Guo et al., 2018), since its acceptable amino acid profile, consistent availability, high digestibility and reasonable price (Amaya et al., 2007). Even though SBM can be used at high levels, there are still some potential problem which can limit the utilization of SBM in aquatic feed, such as insufficient essential amino acid (Mai et al., 2006), poor palatability and presence of antinutritional factors (Suarez et al., 2009). Numerous studies have reported that soybean meal combined with other ingredient can be used to replace fishmeal in shrimp diets completely (Amaya et al., 2007; Lim & Dominy, 1990; Sookying & Davis, 2011). Meanwhile, there are still some aquatic species, which are less tolerant to the high levels of plant meals in the diets than others (Francis, Makkar, & Becker, 2001). Additionally, in the past decade, the price of SBM has tripled (https://www.indexmundi.com/commodities/). Thus, novel processing techniques must be applied SBM to improve availability of AAs or other nutrients in aquatic feed. Meanwhile, it is also important to find novel alternative protein sources to replace expensive protein sources, reduce feed cost and improve feed utilization in aquatic feed.

As the industry continues to develop, ingredient processing has improved to increase the nutritional value of SBM (Rossi Jr, Newcomb, & Gatlin III, 2017). This includes the production of protein concentrates using various separation and/or enzyme-based technologies, which can remove non-protein components and produce a range of products with elevated protein content (above 650g/kg) such as soy protein concentrate (SPC), corn protein concentrate (CPC), and corn gluten

meal (Barrows, Gaylord, Stone, & Smith, 2007; Tacon et al., 2002). Compared with traditional solvent-extracted SBM, these products have benefits such as good palatability, digestible protein, favorable AA profile and decreased anti-nutritional factors (Cruz-Suárez et al., 2009; Gatlin III et al., 2007). Further, high nutrient density means a lower inclusion level in the formulation, thereby opening more space (Fang, Yu, Buentello, Zeng, & Davis, 2016). Many studies showed that 40-100% fishmeal could be replaced by SPC without impairing growth (Dersjant-Li, 2002). There are many studies research on using independently CPC or SPC to replace fishmeal. However, there is limit information about a combination of CPC and SPC to replace fishmeal in practical shrimp feed which could provide a complimentary advantage. Using a varied combination of plant-based proteins helped maintain a balanced amino acid profile in practical shrimp diets, enhanced palatability, and simultaneously improved digestibility.

Other byproduct proteins include distiller's dried grain with solubles (DDGS) which is a co-product of the ethanol distillery industry, and has been suggested as a less expensive alternative to soybean meal (SBM) on a per unit protein basis (Roy et al., 2009). The composition of DDGS as a nutrient source offers protein, lipid, phosphorus, vitamin, yeast and glucans, which can improve growth performance and also immune function (Webster, Tidwell, Goodgame, & Johnsen, 1993). DDGS has been widely used to replace fishmeal (FM) or SBM in diets of several fish and crustacean species including tilapia *Oreochromis* spp. (Chatvijitkul, Davis, & Lim, 2016; Lim et al., 2007), channel catfish *Ictalurus punctatus* (M. Li, Robinson, Oberle, & Lucas, 2010; Lim, Yildirim-Aksoy, & Klesius, 2009; Robinson & Li, 2008), red claw crayfish *Cherax quadricarinatus* (Garza de Yta, Davis, Rouse, Ghanawi, & Saoud, 2012) and the Pacific white shrimp *L. vannamei* (Xuan Qiu, Tian, & Davis, 2017; Rhodes, Yu, Zhou, & Allen Davis, 2015; Roy et al., 2009; Sookying & Davis, 2011). Considerable research has demonstrated that replacement of FM or SBM with 10-40% DDGS in

shrimp diets can achieve acceptable growth, survival, and feed utilization (Adedeji et al., 2017; Rhodes et al., 2015; Roy et al., 2009). The high protein distiller's dried grain (HPDDG) used in this study is a processing variation the produced high protein (49% crude protein), low level of crude fiber (5.5%) and lipid (3.11%) product, which makes it a more valuable feedstuff. However, information about the efficacy of a HPDDG product to replace FM or corn protein concentrate in shrimp diets in green water is limited.

Besides, defatted microalgae biomass from bio-refineries, bacterial aggregates and yeasts from highly controlled production systems that employ agricultural byproducts as organic substrates have also become new alternative feed ingredients (Gamboa-Delgado, Fernández-Díaz, Nieto-López, & Cruz-Suárez, 2016). In general, they have good nutritional properties with high protein levels, consistent supply, and reasonable price. Yeast as one kind of single-cell protein source has become more affordable and has attracted extensive attention in recent years (Ferreira, Pinho, Vieira, & Tavarela, 2010; Gamboa-Delgado et al., 2016; P. Li et al., 2009; X Qiu & Davis, 2017; Zhao et al., 2017). However, only a few studies have evaluated brewer's yeast products as a protein source in shrimp feeds (Gamboa-Delgado et al., 2016; Qiu & Davis, 2017). Products (BY50 and BY70) are two kinds of high-protein brewer's yeast products designed to contain 500 and 700g/kg of crude protein, respectively derived by mixing brewer's yeast (*Saccharomyces cerevisiae*) with other plant proteins. Currently, there is limited or no data on these products nutritional value in shrimp diets.

Besides plant and single cell protein, some animal protein sources may be used as replacements for FM or SBM in aquatic feed. In 2016, almost all of the 90.9 MT fish produced and harvested in aquaculture were estimated to have been used for human consumption (FAO, 2018). Fish and shellfish are processed to different degrees before being sold, resulting in a substantial amount of by-products. These by-products cannot be used for human food, but can be repurposed to highly

nutritious ingredients for animal feeds.(Olsen & Toppe, 2017; Olsen, Toppe, & Karunasagar, 2014). Due to their protein content, amino acid profiles, and relative good palatability and digestibility, these alternative protein sources present excellent options for aquaculture producers (Samocha et al., 2004). Hence, one way to expand the availability of marine protein sources is to utilize the by-products from processing of marine fish species which are often discarded. Hence, the investigation of by-product meals (e.g. salmon by-product meals) is another alternative approach to reduce the cost of manufactured shrimp feeds, as the nutrient profiles of fish processing by product meals are similar regardless of origin (Barlow & Windsor, 1983). Fresh by-products also can be processed by protein hydrolysates technology, which can hydrolyzed the by-products to small peptides and free amino acids to a large extent (Espe et al., 2015). Multiple authors have suggested that fish by-products can be successfully used as a protein source to replace FM in the diet for a number of studies (Ali, Gheyasuddin, Zaher, Hossain, & Islam, 1994; Gallardo et al., 2012; Goosen, de Wet, & Görgens, 2016; Ramasubburayan et al., 2013; Ridwanudin & Sheen, 2014), which are demonstrated that added appropriate levels of fish by-products protein in diets have beneficial effects for the Aquatic animals. However, there is limit information about apparent digestibility coefficients for salmon meal (SM) products as compared to menhaden and anchovy meal. As leaching is seem like an issue for HSM, two differing processing methods (extruded and formed), were evaluated, using gelatin as binder and neutralize pH to improve the utilization of hydrolyzed salmon meal (HSM) in present study.

The long-term goal of this study is to develop a sustainable and environmentally beneficial feed formulation based on economical by product meals for pacific white shrimp *L. vannamei*. This purpose of this research is to evaluate the utilization of potential alternative ingredient in practical diets for Pacific white shrimp, *L. vannamei.* Five specific objectives were included to identify the response of shrimp to different diets:

1. To evaluate the efficacy of brewers' yeast protein sources in practical shrimp feeds.

2. To evaluate high protein Distiller's dried grain (HPDDG) to replace fishmeal and corn protein concentrate (CPC) in practical shrimp diet.

3. To evaluate CPC and soy protein concentrate (SPC) as protein source to replace fishmeal in practical shrimp diets.

4. To evaluate the efficacy of salmon meal or hydrolyzed salmon meal as new protein source in practical shrimp diets.

5. To determine the suitability of hydrolyzed salmon meal in practical feed formulations.

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CHAPTER II

USE OF HIGH-PROTEIN BREWER'S YEAST PRODUCTS IN PRACTICAL DIETS FOR THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Abstract

Two 6-week growth trials and a digestibility trial were conducted to evaluate the effects of brewer's yeast in practical shrimp feeds. In the first growth trial, graded levels (0, 60, 120, 180, and 240g/kg) of a brewer's yeast (BY50) were used to replace fishmeal and soybean meal, referred to as Diets DBY0, DBY6, DBY12, DBY18, DBY24, and Diets LFM0, LFM6, LFM12, DBY18 and LFM24, respectively. The results showed no significant differences in final biomass, survival, protein retention efficiency and feed conversion ratio; however, limited differences in final weight and weight gain were shown in the FM replacement series. There was no significant difference on the growth performance in the SBM replacement series. The second growth trial was conducted with Diet DBY0, DBY12, DBY18, DBY24, LFM0 and a low-FM diet containing 20g/kg of BY with 70% protein (Diet DBY70). Shrimp fed with Diet DBY0 exhibited significantly higher final mean weight and weight gain than those offered the Diet DBY24. Nutrient availability of BY50 and BY70 was similar to SBM and significantly higher than that of FM. Results indicated that 180-240g/kg BY50 can be effectively used in shrimp diets as a replacement for FM, or up to 240g/kg when replacing SBM.

Keywords: *Litopenaeus vannamei*, fishmeal replacement, soybean meal replacement, brewer's yeast product, growth trial, apparent digestibility coefficients

1.Introduction

Global supplies of fishmeal (FM) have reached a plateau; yet demand continues to increase, making it more expensive and less available (FAO, 2016). The use of solvent extracted soybean meal (SBM) as a protein source is the result of its well-balanced nutrient profile, high digestibility, expandable production, availability in large quantities, and reasonable price (Amaya, Davis, & Rouse, 2007; Samocha, Davis, Saoud, & DeBault, 2004). However, some disadvantages of SBM limit its use in shrimp and fish feed, including low levels of limiting amino acids (Mai et al., 2006), presence of anti-nutritional factors (Dias et al., 2009), and limited available phosphorus (Suárez et al., 2009). Thus, it is important to explore other protein sources which are cost-effective, sustainable, and environmentally friendly to reduce feed cost and support the rapidly expanding shrimp industry (Qiu & Davis, 2017).

Yeast biomass is not only a high-protein product but also a source of prebiotics, containing β-glucan, deacylated chitin, nucleic acid, oligosaccharidespolyamines, and a source of selenium and vitamins (Gatesoupe, 2007; Siwicki, Anderson, & Rumsey, 1994). Adding yeast to the diet has been shown to both improve growth performance and modulate immune response and metabolism in aquatic animals (Gatesoupe, 2007). A number of studies have demonstrated that yeast can be used as a protein source in diets for a variety of aquatic animal species, including the Pacific white shrimp *Litopenaeus vannamei* (Gamboa-Delgado, Fernández-Díaz, Nieto-López, & Cruz-Suárez, 2016; Qiu & Davis, 2017), Australian red claw crayfish *Cherax quadricarinatus* (Muzinic et al., 2004), juvenile sea bass *Dicentrarchus labrax* (Oliva-Teles & Gonçalves, 2001), and cobia *Rachycentron canadum* (Lunger, Craig, & McLean, 2006).

The nutrient composition of yeast varies considerably with different yeast strains and processing conditions. A conventional yeast product used in feed is brewer's yeast *Saccharomyces cerevisae*,

which contains 396-472g/kg of crude protein (Caballero-Córdoba & Sgarbieri, 2000; Yamada & Sgarbieri, 2005). BY50 and BY70 are two kinds of high-protein brewer's yeast products designed to contain 500 and 700g/kg of crude protein, respectively derived by mixing brewer's yeast (*Saccharomyces cerevisiae*) with other plant proteins.

Few studies have evaluated brewer's yeast products as a potential protein sources in shrimp feeds (Gamboa-Delgado et al., 2016; Qiu & Davis, 2017). Accordingly, there are limited or no data on the tested products nutritional value in shrimp diets. Generally, the price of yeast product (e.g.BY50, 1257$/ton) is cheaper than FM (1475$/ton) as reported by Ratanapariyanuch, Shim, Wiens, and Reaney (2018); hence it may be a viable alternative to fishmeal proteins. However, the replacement of soybean meal with yeast allows for a better comparison as there are fewer shifts in nutritional composition of the diet as compared to those occurring when replacing fishmeal. Although presently more expensive than soybean meal, yeast products do not contain antinutrients found in soybean meal and hence may be a better alternative. Therefore, the objective of this study was to evaluate the utilization of brewer's yeast as replacements for FM or SBM in the practical diets of Pacific white shrimp, *L. vannamei,* and to determine the apparent digestibility coefficients as compared to those of FM and SBM.

2. Materials and Methods

2.1. Experimental diets

Two brewers' by-products with yeast designed to contain 500 and 700g/kg protein (BY50 and BY70) were obtained from the F. L. Emmert Company, Cincinnati, OH, USA. The remaining ingredients were locally sourced. The proximate composition and amino acid profile (g/kg as-is) of the primary ingredients are shown in Table 1. Ten diets were formulated to be isolipidic (80g/kg crude

lipid) and isonitrogenous (350g/kg crude protein) on an as-is basis (Table 2). The formulations and proximate compositions of the experimental diets are presented in Table 2 and 3. In the first series of experimental diets, graded levels of BY50 (0, 60, 120, 180, and 240g/kg) were used to replace fishmeal, which were designated as DBY0, DBY6, DBY12, DBY18 and DBY24, respectively. A second series of experiment diets used graded levels of BY50 (0, 60, 120, 180, and 240g/kg) as a replacement for SBM, which were designated as LFM0, LFM6, LFM12, DBY18 and LFM24, respectively (note that DBY18 was used in both series). Finally, the tenth diet (DBY70) was produced by supplementing 20g/kg BY70 to replace SBM.

Diets were prepared by mixing pre-ground dry ingredients in a food mixer (Hobart, Troy, OH, USA) for 10–15 minutes. Boiling water (ca 40% by weight) was then blended into the mixture to obtain a consistency appropriate for pelleting. Diets were formed using a meat grinder with a 3-mm die. The moist pellets were then placed into a forced air oven (< 45 °C) overnight in order to attain a moisture content of less than 100g/kg. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer (-20°C) until needed. All the ingredients and diets were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate composition and amino acid profile (g/kg as-is, Table 1 and 3).

2.2. Experiment procedure

Two growth trials were conducted at the E.W. Shell Fisheries Center, Auburn, AL. Pacific white shrimp post-larvae were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and nursed in an indoor recirculating system using commercial feeds until they reached an appropriate size for research. In the first trial, the first nine diets were used (Table 2). To confirm the results of the first trial, a second trial was conducted with Diet DBY0, DBY12, DBY18, DBY24, LFM0, and DBY70. In each trial, juvenile shrimp (initial mean weight 0.17±0.01g and 0.82±0.01g, respectively)

were hand-sorted to uniform size and randomly stocked into 75-L aquaria or 800L circular tanks with 10 shrimp per aquarium or 30 shrimp per tank. Each diet was offered to four replicated tanks over a 42-day period.

During the growth trial, shrimp were hand-fed four times daily using a standardized feeding table that is based on historical results. In general, feed inputs are calculated assuming the shrimp will double their weight weekly up to one gram, then gain 0.8 g weekly with a feed conversion ratio (FCR) of 1.8. Shrimp were counted once a week to adjust the daily feed input. At the end of the growth trial, shrimp in each tank were counted and weighed to calculate survival, biomass, mean weight, FCR and weight gain. After weighing and counting the shrimp, 4-6 individuals per tank were randomly selected and frozen at −20°C for whole body samples to be utilized for later protein retention analysis. Crude protein content of whole body was determined by Dumas combustion method (Elemental Analyzer rapid N cube, Villeurbanne, France).

During the growth trials, dissolved oxygen (DO), water temperature and salinity were measured twice daily using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH, USA). The pH was tested twice weekly using a waterproof pHTestr30 (Oakton instrument, Vernon Hills, IL, USA). Total ammonia nitrogen (TAN) and nitrite were analyzed twice per week using the methods described by Solorzano (1969) and Spotte (1970), respectively. In trial 1, mean DO, temperature, salinity, pH, TAN, and nitrite were maintained at 5.15 ± 0.78 mg L^{-1}, 29.05 ± 0.45°C, 6.59 ± 0.32ppt, 7.34 ± 0.19, 0.16 ± 0.09mg L^{-1}, and 0.07 ± 0.04 mg L^{-1}, respectively. In trial 2, mean DO, temperature, salinity, pH, TAN, and nitrite were maintained at 6.19 ± 1.26 mg L^{-1}, 28.43 ± 0.9°C, 4.83 ± 0.14ppt, 7.32 ± 0.44, 0.39 ± 0.23 mg L^{-1}, and 0.11 ± 0.03 mg L^{-1}, respectively. Water quality conditions in both trials were suitable for normal growth and survival of this species.

2.3. Digestibility trial

The test diets were produced by supplementing 30% ingredients into 70% reference diet. The formulation of the reference diet and the proximate composition of primary ingredients are presented in Table 4 and 1, respectively. Chromic oxide was included at 10g/kg as an inert marker. The digestibility trial was conducted in the aforementioned recirculation system and utilized six shrimp (mean weight 7.19 ± 0.10g) per aquarium with six aquaria per dietary treatment. Feces collection started after a 3-day acclimation period to the test diets. To obtain fecal samples, the aquaria were cleaned by siphoning before each feeding. After cleaning, the shrimp were offered an excess of feed, and then about one hour later feed was removed and feces were collected by siphoning onto a 500μm mesh screen, with the first collection of the day discarded. Feces from two aquaria were pooled (n=3) and collected over a five-day period or until adequate sample quantities were obtained. Collected feces were rinsed with distilled water, dried at 95°C until a constant weight, and then stored in a freezer (-20°C) until analyzed.

Apparent digestibility coefficients for dry matter, protein and energy were determined by using chromic oxide (Cr_2O_3, 10g/kg) as an inert marker. Samples of diets and ingredients were dried to a constant weight at 95°C to determine dry weight. Crude protein of diets and fecal sample were determined by the micro-Kjeldahl method (Ma & Zuazaga, 1942). Chromium was determined by the method as described by McGinnis and Kasting (1964). Gross energy of diets and fecal samples were analyzed with a Semi-micro bomb calorimeter (Model 6725, Parr Instrument Co., Moline, IL, USA). Amino acids were analyzed by University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory.

The apparent digestibility coefficients (ADCs) of dry matter, protein, amino acids and energy were calculated according to Cho, Slinger, and Bayley (1982) as follows:

ADC of dry matter (%) =100 × [100× (Cr_2O_3in feed) / (Cr_2O_3 in feces)]

ADC of nutrients or energy (%) = [1− (dietary Cr_2O_3 / fecal Cr_2O_3) × fecal nutrient or energy /dietary nutrient or energy]] × 100

ADC of the test ingredients was calculated as follows (Bureau & Hua, 2006):

$$ADC\ (\%) = ADC_{TD} + (ADC_{TD} - ADC_{RD}) \times 0.7 \times Nutr_{RD} / 0.3 \times Nutr_{ING})$$

Where ADC_{TD} is the apparent digestibility of the nutrients or energy in the test diet (TD), ADC_{RD} is the apparent digestibility of nutrients or energy in the reference diet (RD), $Nutr_{RD}$ is the nutrients or energy concentration in the RD, and $Nutr_{ING}$ is the nutrients or the energy concentration in the test ingredient.

2.4. Statistical analysis

All data were analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). All data were subjected to one-way analysis of variance to determine significant differences ($P<0.05$) among treatments, followed by Tukey's multiple comparison test to determine differences between treatment means. Independent samples T-tests were performed to compare the Diet LFM0 with Diet DBY70 in trial 2. The pooled standard errors used across growth trials as the variance of each treatment were the same. Linear, second- or third-order polynomial regressions were performed to investigate the relationship between the dietary level of BY50 and the response variables of weight gain. To identify the most appropriate regression model, we compared the *P*-value of the model components, R^2 value, and adjusted R^2 values, and the sum of squares for error (SSE) with different regression models as well as according to the biological response.

3. Results

3.1. Growth trial

The growth performance of shrimp offered diets containing different levels of BY50 in trial 1 and 2 are presented in Table 5 and 6. After the first feeding in trial 1 (series 1), the results showed no significant differences in final biomass (34.58 to 40.78g), survival (82.5 to 92.5%), FCR (1.8-2.3), final mean weight (3.72-4.69g), weight gain (2028-2771%), protein retention efficiency (PRE) (20.7-25.3%) and weight gain (3.55-4.53g). Shrimp fed the 180g/kg BY50 diet exhibited significantly lower weight gain and final mean weight than that of those fed the diet with 120g/kg BY50. However, there was no significant difference between the diet supplemented with 120g/kg BY50 and the diet containing 240g/kg BY50. In trial 1 (the SBM replacement series 2), no significant differences in growth performances existed for shrimp reared on the various diets.

In the second growth trial, no significant differences were detected in final biomass (164.33-174.4g), survival (75.8-84%), PRE (25.8-28.2%) and FCR (1.7-1.9) among all the treatments (DBY0, DBY12, DBY18 and DBY24). Shrimp fed the DBY0 basal diet and diet DBY12 exhibited significantly higher final mean weight and weight gain than those offered the diet containing 240g/kg BY50. According to the results from t-test, there was also no significant difference in growth performance between Diet LFM0 and Diet DBY70.

3.2. Digestibility trial

Apparent dry matter (ADMD), apparent energy (AED) and apparent protein (APD) digestibility coefficients for the diets and ingredients using 70:30 replacement technique offered to Pacific white shrimp *L. vannamei* and are presented in Table 7.

For diet digestibility, the AED and APD of BY50 and SBM were significantly higher than those of BY70 and menhaden FM, while the ADMD of menhaden FM diet was significantly lower than that of the other diets. For ingredient digestibility, the results showed the same trend. Menhaden

FM had the lowest ADMD, AED and APD values. At the same time, APD of BY70 was significantly lower than that of SBM and BY50.

The apparent amino acid digestibility (AAD) values for BY50, BY70, menhaden fish meal (MFM) and the SBM using 70:30 replacement technique offered to Pacific white shrimp *L. vannamei* are presented in Table 8. The amino acid digestibility coefficients for BY50, BY70, MFM and SBM ranged from 81.8 to 94.45%, 59.08 to 87.8%, 44.08 to 69.22% and 84.59 to 101.42%, respectively. All the apparent amino acid digestibility coefficients and total amino acids availability of BY50 were similar to those of SBM but significantly higher than those of MFM. Most of the apparent amino acid digestibility coefficients (except hydroxy lysine and serine) and total amino acids availability of BY50 and all apparent amino acid digestibility coefficients and total amino acids availability of SBM were significantly higher than those of BY70. Apparent digestibility coefficients of arginine, cysteine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine and valine of BY70 were similar to those of MFM. Apparent digestibility coefficients of alanine, aspartic acid, glycine, hydroxylysine, proline, serine, threonine, tryptophan, tyrosine and total amino acids of BY70 were significantly higher to those of MFM.

4. Discussion

Recently, the specific use of yeast as an alternative protein source has been of interest in both fish (Hauptman et al., 2014; Kandaiah & Ramasamy, 2014; Sealey et al., 2015) and shrimp (J. Achupallas, Y. Zhou, & D. Davis, 2016; Gamboa-Delgado et al., 2016; Qiu & Davis, 2017; Zhao et al., 2017). Even though a number of studies have evaluated different yeast products in shrimp diets (Gamboa-Delgado et al., 2016; Li et al., 2009; Zhao et al., 2017), to our knowledge no research has been conducted to evaluate the use of these yeast products in shrimp diets.

Apparent digestibility coefficients are an important factor to determine the utilization of a feedstuff, to help to select ingredients that optimize the nutritional value, and to reduce the cost of formulated feed (Brunson, Romaire, & Reigh, 1997). However, data regarding apparent digestibility coefficients of yeast are scarce.

In the digestibility trial, the ADCs of energy and protein for SBM (80.94 and 91.85%, respectively) were similar to those of SBM reported by Siccardi III et al. (2006) (80.8 and 87.1%), Yang et al. (2009) (74.12-82% and 88.95-90.89%) and Divakaran, Velasco, Beyer, Forster, and Tacon (2000) (83.8-88% and 88.1-91.3%) for shrimp. The APD value of FM was 66.97%, which was also in line with previous studies reported by Terrazas-Fierro et al. (2010) (62.7-84.9%), Brunson et al. (1997) (76%) and Qiu, Nguyen, and Davis (2017) (65.78-69.77%). The results also showed that the ADCs of dietary dry matter, energy and protein for BY50 and BY70 were 73.07 and 62.5%, 80.77 and 65.52%, 92.53 and 75.54%, respectively. Similarly, Liu, Ye, Kong, Wang, and Wang (2013) indicated the energy and protein digestibility of brewer's yeast was 84.6% and 85.7% for Pacific white shrimp, respectively. The AED and APD values of ingredient BY50 were significantly higher than those of FM and similar to those of SBM. The APD of ingredient BY70 was significantly higher than that of FM and lower than that of SBM.

BY50 showed improved availability of protein and energy when compared to the results from Qiu and Davis (2017), who demonstrated that the protein and energy digestibility of flash-dried yeast (38.20 and 53.47%) in shrimp was significantly lower than that in FM and SBM. In terms of juvenile *Penaeus monodon*, the results with BY50 were higher than values reported by Chen, Liu, Xie, Zhang, and Niu (2016), who assessed the AED and APD of brewer's yeast was 64.51% and 82.18%, respectively. The higher APD of BY50 in *L. vannamei* was derived from the superior amino acid digestibility coefficients of BY50. Accordingly, individual amino acid digestibility in BY50 ranged

from 81.8% to 94.45%, which were superior to those in FM (44.08%- 69.22%) and similar to those in SBM (84.74% to 101.42%). The amino acid availability of BY50 was also higher than that of other yeast products for the same species reported by Qiu and Davis (2017) (34.8-72.85%) and Liu et al. (2013) (48.2-98.4%). The relatively high amino acid digestibility of BY50 can be attributed to its high protein content and relatively balanced amino acid profile (Table 1).

The AED represents the fractional sum of the ADC values including protein, lipids and carbohydrates (Siccardi, 2010), hence, the AED of a diet typically decreases as fiber content increases and vice versa (Fang, Yu, Buentello, Zeng, & Davis, 2016). The fiber content (41g/kg) of BY50 was relatively lower than previous results evaluated by Qiu and Davis (2017) (62.7g/kg) and Liu et al. (2013) (80g/kg). It also has similar fiber content as the solvent-extracted SBM (35g/kg) measured in this study and other SBM species (21-39g/kg) reported by Lech and Reigh (2012), which is consistent with the high AED value of BY50. By shifting the manufacturing process of brewer's yeast, considerable improvements can be made especially for the protein content (Blieck et al., 2007).

Improved digestibility can result in enhanced growth performance as higher levels of nutrients are available to the animals. However, improved digestion does not always equate with growth as this does not account for nutrient content or effects on palatability. In trial 1, as fishmeal was replaced at higher levels (>120g/kg inclusion of BY50) there was a decreasing trend in growth. This is apparent in both Tukey's multiple range test, as well as regression analysis (Table 5, Fig.1). In general, shrimp fed with 180g/kg BY50 diet exhibited significantly lower weight gain than those of shrimp fed the diets containing lower levels (Table 5). This is most likely due to uncontrolled shifts in nutrition and palatability of the diets which occur when using nutrient-rich and marine ingredients such as fishmeal that is replaced with ingredients which are not as rich in nutrients and palatability enhancers. To minimize such changes, we also evaluated the use of BY50 as a replacement for soybean meal.

When BY50 was used to replace soybean meal there were no significant difference in the shrimp's performance across all levels. This is likely due to BY and SBM being more similar in nutrient profiles and palatability. The present results showed that BY50 works fine as a replacement for SBM based diets; however, there are still some limits as a replacement for fishmeal. These results indicate an interaction of other nutrients or shifts in palatability when replacing fishmeal.

The second growth trial was initiated to demonstrate and confirm the results of trial 1 in fishmeal replacement series. As there was no effect at lower levels of replacement, the focus was on the higher levels of inclusion. Regression analysis confirmed that there was a significant decrease in growth as the inclusion level of BY50 was increased (Fig. 2). Results indicate that at least 180g/kg of BY50 can be used to replace fishmeal without reduced growth performance. Additionally, in both trials, the trend of PRE was consistent with the weight gain of the shrimp that were offered a different diet. However, PRE was not significantly affected even when BY50 was supplemented up to 240g/kg of the diet. To sum up, this present study suggests that 180-240g/kg of BY50 (equal to replacing 75-100g/kg FM) can be effectively used in shrimp practical diets to replace FM, and up to 240g/kg to replace SBM (equal to 510g/kg SBM) without significant reduction in growth performance and feed utilization.

These results indicate BY50 to be a good protein source for practical feed formulations. This result is similar to Gamboa-Delgado et al. (2016), who reported that the diet inclusion of torula yeast can improve the growth performance of shrimp, while also contributing high proportions of dietary nitrogen to growth when it is used to replace up to 60% FM in a basal diet containing 47.5% FM. Similar results also suggest that grain distillers dried yeast (GDDY) can be used up to 30% in shrimp practical diet without decreasing the growth performance (Achupallas, 2013). J. M. Achupallas, Y. Zhou, and D. A. Davis (2016) also demonstrated that GDDY can be used at up to 15% of diet without

causing significant differences in growth performance of Pacific white shrimp both in outdoor tanks and in green water pond conditions. Yeast protein can also be added to diets of other species without impairing growth performance. In catfish, yeast protein can be added up to 10-20% without impairing growth performance (Essa, Mabrouk, Mohamed, & Michael, 2011; Peterson, Booth, & Manning, 2012). Yeast can replace up to 50% in sea bass (Oliva-Teles & Gonçalves, 2001) and 50-75% in sunshine bass (Gause & Trushenski, 2011). For rainbow trout, GDDY can successfully replace FM up to 37.5% (Hauptman et al., 2014). The conditions of culture environment, aquatic animal's species, and the type of yeast product all have an effect on replacement level. Zhao et al. (2017) also documented that the suitable substitution of FM in aquatic animal feed was closely related to the dosage of FM in the basal diet.

In general, as the protein content of a yeast product increases so does the cost of the product. Hence, high protein yeast products e.g., 70% protein (BY70), which are typically targeted as specialty products often for their health enhancing properties. Hence, as a component of this work digestibility was determined and evaluated a low level of inclusion (20g/kg, BY70) in a low fishmeal diet (LFM0). As previously discussed both yeast products had good digestibility values although the BY50 in general had better digestibility values than that of the BY70 product. In the second growth trial, results demonstrated that adding 20g/kg of BY70 in a low-FM diet does not influence growth performance of the shrimp. A number of studies in fish have demonstrated that a low level of yeast-supplemented diets resulted in better growth performance than the control diet (Li & Gatlin, 2003, 2004; Ortuño, Cuesta, Rodríguez, Esteban, & Meseguer, 2002). Yeast biomass is not only a high-protein product but is also a potential source of prebiotics (Gatesoupe, 2007; Siwicki et al., 1994), and contain 120-200g/kg total nitrogen being composed of RNA nitrogen, mainly in the purine and pyrimidine bases of the nucleoproteins (Rumsey, Winfree, & Hughes, 1992). However, adding excessive levels of

nucleotide-rich ingredients in the diet may suppress growth performance, particularly in cases related to suppression of immunity (Burrells, Williams, & Forno, 2001). Presently, the potential of BY50 to stimulate immunity has not been evaluated. Shrimp offered Diet DBY24 had reduced growth. However, there was no significant reduction in growth of shrimp fed Diet LFM24, which would indicate it is more likely to have nutrient interaction as opposed to overstimulation of immunity. Hence, studies to evaluate possible immune-stimulating effects are worth pursuing.

5. Conclusion

Results of this study indicate that 180-240g/kg BY50 can be effectively used in practical diets as a replacement for FM, or up to 240g/kg when replacing SBM. Additionally, adding 20g/kg of BY70 does not cause impaired growth performance for shrimp fed low-FM diets.

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Table 1. Proximate and amino acid composition (g/kg as-is) of test ingredients used in digestibility trial. Analyses were conducted by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Nutrient	BY50	BY70	FM	SBM
Crude Protein	505.1	723.7	627.7	442.0
Moisture	47.4	84.9	96.0	120.1
Crude Fat	16.5	14.4	105.0	24.2
Crude Fiber	41.0	9.4	-	35.0
Ash	62.4	28.4	182.0	59.1
Amino Acid				
Alanine	21.4	34.4	39.8	19.2
Arginine	36.9	36.4	37.5	32.2
Aspartic Acid	55.4	87.4	56.0	49.2
Cysteine	7.2	10.4	5.1	6.6
Glutamic Acid	88.2	77.0	80.2	78.6
Histidine	12.9	14.9	13.1	11.5
Hydroxy lysine	1.1	1.4	2.4	0.8
Hydroxyproline	0.6	0.5	11.2	0.6
Isoleucine	24.3	41.5	23.9	22.3
Leucine	38.7	70.9	43.4	34.6
Lysine	31.4	52.2	46.8	28.8
Methionine	6.6	14.3	16.7	6.0
Ornithine	0.5	0.5	0.6	0.5
Phenylalanine	26.3	45.7	24.8	23.2
Proline	24.2	33.4	28.8	21.6
Serine	20.6	30.2	24.2	17.4
Taurine	1.3	0.5	7.1	1.4
Threonine	19.1	37.2	25.4	16.7
Tryptophan	7.0	9.2	6.2	7.0
Tyrosine	19.0	37.3	14.6	16.9
Valine	24.8	49.3	28.2	22.5
Total	488.6	719.7	581.6	436.4

Table 2. Formulation of test diets used to evaluate various yeast products (g/kg as-is).

	DBY0	DBY6	DBY12	DBY18	DBY24	LFM0	LFM6	LFM12	LFM24	DBY70
Menhaden fishmeal [a]	191.2	143.4	95.6	47.9		47.9	47.9	47.9	47.9	47.9
Soybean meal [b]	338.5	338.5	338.5	338.5	338.5	544.5	475.8	407.1	269.7	510.1
Menhaden fish Oil [c]	46.0	50.4	54.8	59.2	63.6	58.9	59.0	59.1	59.3	59.1
BY50 [d]		60.0	120.0	180.0	240.0		60.0	120.0	240.0	
BY70 [d]										20.0
Corn protein concentrate [e]	68.0	68.0	68.0	68.0	68.0	68.0	68.0	68.0	68.0	68.0
Lecithin [f]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cholesterol [g]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Corn Starch [g]	90.8	71.7	52.6	33.4	14.4	3.0	13.3	23.4	43.7	16.7
Whole wheat [g]	215.0	215.0	215.0	215.0	215.0	215.0	215.0	215.0	215.0	215.0
Mineral premix [h]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix [i]	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0
Choline chloride [g]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Stay-C [j]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CaP-dibasic [k]	14.0	16.5	19.0	21.5	24.0	26.2	24.5	23.0	19.9	26.7

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

[b] De-hulled solvent extract soybean meal, Bunge limited, Decatur, AL, USA.

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

[d] The F.L. Emmert Company, Cincinnati, OH, USA.

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

[f] The Solae Company, St. Louis, MO, USA.

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

[h] Mineral premix (g/100 g 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.

[i] Vitamin premix (g kg^{-1} premix): Thiamin. HCl, 4.95; Riboflavin, 3.83; Pyridoxine. HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 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.

[j] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[k] T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.

Table 3. Proximate and amino acid composition of experimental diet (g/kg as-is). Analyses were conducted by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

	DBY0	DBY6	DBY12	DBY18	DBY24	LFM0	LFM6	LFM12	LFM24	DBY70
Crude protein	380.3	381.5	377.8	376.3	377.8	386.0	382.5	382.9	374.6	389.2
Crude lipid	83.4	84.9	85.4	86.4	85.3	86.3	84.9	84.6	84.0	84.3
Crude Fiber	32.2	33.0	29.3	39.3	36.4	38.1	39.5	40.1	35.8	38.9
Moisture	48.6	48.6	50.8	51.7	51.3	49.9	52.4	50.3	59.8	46.0
Ash	78.8	74.5	71.4	66.2	60.9	71.0	68.8	67.3	62.5	70.5
Amino Acid										
Alanine	20.9	20.4	19.6	18.9	18.2	19.1	19.0	19.1	18.5	19.3
Arginine	22.0	22.7	22.6	23.5	23.4	24.0	23.4	23.3	22.7	23.5
Aspartic Acid	33.1	34.5	34.6	35.7	36.2	36.4	36.1	35.6	35.1	37.0
Cysteine	5.1	5.3	5.3	5.5	5.8	5.7	5.7	5.5	5.5	5.8
Glutamic Acid	65.2	68.0	68.3	70.3	71.4	71.1	71.0	70.1	69.1	70.5
Glycine	20.6	19.3	17.6	17.0	15.6	17.1	16.9	16.6	16.5	17.3
Histidine	9.0	9.2	9.1	9.2	9.2	9.3	9.2	9.2	9.0	9.4
Hydroxy lysine	1.2	1.2	1.1	1.0	1.1	1.1	1.1	1.1	0.9	0.9
Hydroxyproline	2.5	1.9	1.3	1.1	0.6	1.0	1.2	1.1	0.6	0.7
Isoleucine	16.5	16.9	17.0	17.1	17.3	17.4	17.2	17.2	17.0	17.8
Leucine	32.3	32.8	33.0	32.7	33.0	33.3	33.2	33.6	32.3	33.8
Lysine	21.3	21.2	20.5	20.2	19.6	20.8	20.5	20.1	19.9	21.1
Methionine	7.3	7.1	6.6	6.2	5.8	6.4	6.2	6.1	6.1	6.4
Ornithine	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Phenylalanine	18.5	19.0	19.3	19.6	19.9	19.9	19.9	19.9	19.1	20.1
Proline	23.9	25.2	24.1	23.9	23.2	24.0	23.8	24.7	24.1	24.5
Serine	14.4	14.8	15.0	15.5	15.8	16.0	15.9	15.5	14.8	15.7
Taurine	2.6	2.3	1.9	1.5	0.2	1.5	1.5	1.5	1.5	1.5
Threonine	13.4	13.5	13.4	13.4	13.4	13.7	13.6	13.5	13.1	13.8
Tryptophan	3.9	4.0	4.5	4.3	4.8	4.6	4.7	4.5	4.4	4.4
Tyrosine	13.4	13.8	13.3	13.9	13.7	14.3	14.2	14.1	13.2	14.1

Valine	18.0	18.3	18.2	18.1	18.1	18.4	18.1	18.2	18.1	19.0
Total amino acids	365.5	371.7	366.6	368.9	366.6	375.4	372.7	370.8	361.8	376.9

Table 4. Formulation of reference diet for the determination of digestibility coefficients (g/kg as-is).

Ingredients	(g/kg)
Menhaden fishmeal [a]	100.0
Soybean meal [b]	325.0
Menhaden fish oil [b]	32.0
Corn starch [c]	21.0
Whole wheat [c]	476.0
Mineral premix [d]	5.0
Vitamin premix [e]	18.0
Choline chloride [e]	2.0
Stay C [f]	1.0
Lecithin [g]	10.0
Chromic oxide [g]	10.0
Proximate analysis (g/kg)	
Crude protein	259.2
Cross energy (cal/g)	4278.0

[a] Menhaden fish meal, special select, Omega Protein Inc., Houston, TX, USA.

[b] De-hulled solvent extract soybean meal, Bunge limited, Decatur, AL, USA.

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

[d] Mineral premix (g/100 g 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.

[e] Vitamin premix (g kg^{-1} premix): Thiamin.HCl, 4.95; Riboflavin, 3.83; Pyridoxine.HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 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.

[f] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[g] The Solae Company, St. Louis, MO, US.

Table 5. Response of juvenile shrimp (0.17g mean initial weight) to diets with graded level of a yeast-based meal replacing fishmeal (DBY series) or soybean meal (LFM series) over a 42 days growth trial 1. Values within a column with different superscripts are significantly different based on Tukey's multiple range test. Each value represents the mean of four replicates.

Diet	Survival (%)	Weight gain (g)	Weight gain (%)[1]	Mean weight (g)	FCR[2]	Biomass	PRE (%)[3]
DBY0	90.0	4.30^{ab}	2497^{ab}	4.48^{ab}	1.9	40.20	24.1
DBY6	90.0	4.34^{ab}	2606^{b}	4.51^{ab}	1.9	40.78	24.9
DBY12	82.5	4.53^{b}	2771^{b}	4.69^{b}	1.8	38.65	25.3
DBY18	92.5	3.55^{a}	2028^{a}	3.72^{a}	2.3	34.58	20.7
DBY24	92.5	3.92^{ab}	2197^{ab}	4.10^{ab}	2.1	38.03	23.1
PSE[4]	6.86	0.19	132.21	0.19	0.11	3.72	5.08
P value	0.830	0.014	0.008	0.015	0.054	0.785	0.195
LFM0	82.5	4.28	2314	4.47	1.9	36.85	23.8
LFM6	87.5	4.25	2505	4.42	1.9	38.80	23.8
LFM12	90.0	4.24	2488	4.42	1.9	39.75	23.9
DBY18	92.5	3.55	2029	3.72	2.3	34.58	20.7
LFM24	87.5	4.25	2514	4.42	1.9	38.68	24.6
PSE[4]	5.00	0.20	176.71	0.20	0.11	3.05	3.05
P value	0.702	0.088	0.287	0.081	0.124	0.768	0.369

[1] Weight gain = (Final weight-initial weight) / initial weight × 100%.

[2] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).

[3] PRE: Protein retention efficiency = (final weight × final protein content) − (initial weight × initial protein content) × 100 / protein intake.

[4] PSE: Pooled standard error.

Table 6. Response of juvenile shrimp (0.82g mean initial weight) to diets with graded level of yeast products over a 42 days growth in trial 2. Values within a column with different superscripts are significantly different based on Tukey's multiple range test. Each value is mean of four replicates.

Diet	Biomass (g)	Mean weight (g)	Survival (%)	Weight gain (g)[1]	Weight gain (%)[2]	FCR[3]	PRE (%)[4]
DBY0	174.40	7.48^{b}	77.8	6.67^{b}	826^{b}	1.7	28.2
DBY12	165.43	7.28^{b}	75.8	6.46^{b}	790^{b}	1.8	26.9
DBY18	164.33	7.15^{ab}	76.8	6.33^{ab}	779^{ab}	1.8	26.8
DBY24	169.53	6.71^{a}	84.0	5.90^{a}	727^{a}	1.9	25.8
PSE[5]	11.98	0.12	5.08	0.12	14.07	0.05	1.82
P-value	0.931	0.004	0.667	0.004	0.003	0.081	0.385
LFM0	164.18	7.20	75.8	6.38	777	1.8	27.5
DBY70	171.03	7.11	80.0	6.29	765	1.8	26.1
T-test							
P-value	0.675	0.840	0.215	0.839	0.803	0.952	0.455

[1] Weight gain (g) = Final weight-initial weight.

[2] Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.

[3] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).

[4] PRE: Protein retention efficiency = (final weight × final protein content) − (initial weight × initial protein content) × 100 / protein intake.

[5] PSE: Pooled standard error.

Table 7. Apparent dry matter (ADMD), apparent energy (AED) and apparent protein (APD) digestibility values for the diet and ingredient using 70:30 replacement technique offered to Pacific white shrimp *L. vannamei.* Values within a column with different superscripts are significantly different based on Tukey's multiple range test. Each value is mean of three replicates.

	Diet			Ingredient		
	ADMD	AED	APD	ADMD	AED	APD
Basal	74.19^{b}	80.07^{bc}	90.48^{c}			
BY50	74.42^{b}	80.72^{c}	91.63^{c}	73.07^{b}	80.77^{b}	92.53^{c}
BY70	71.82^{b}	75.74^{ab}	81.89^{b}	62.50^{ab}	65.52^{ab}	75.54^{b}
Fishmeal	64.90^{a}	74.93^{a}	78.16^{a}	39.43^{a}	60.16^{a}	66.97^{a}
Soybean meal	74.20^{b}	81.19^{c}	91.39^{c}	70.45^{b}	80.94^{b}	91.85^{c}
PSE[1]	1.50	1.13	0.88	6.12	4.15	1.88
P-value	0.001	0.001	<0.0001	0.006	0.005	<0.0001

[1.]PSE: Pooled standard error.

Table 8. Percent apparent amino acid digestibility (AAD) value for BY50, BY70, Menhaden fish meal (MFM) and the soybean meal (SBM) using 70:30 replacement technique offered to Pacific white shrimp *L. vannamei*. Values within a row with different superscripts are significantly different based on Tukey's multiple range test. Each value is mean of three replicates.

	BY50	BY70	MFM	SBM	PSE[1]	P value
Alanine	84.38^{c}	65.73^{b}	54.59^{a}	84.74^{c}	2.10	<0.0001
Arginine	94.10^{b}	75.72^{a}	66.27^{a}	94.38^{b}	2.56	0.0003
Aspartic Acid	91.00^{c}	73.67^{b}	61.26^{a}	92.14^{c}	2.01	<0.0001
Cysteine	85.64^{b}	59.08^{a}	50.54^{a}	86.33^{b}	2.77	<0.0001
Glutamic Acid	92.44^{b}	70.65^{a}	65.03^{a}	93.34^{b}	2.06	<0.0001
Glycine	81.80^{c}	63.53^{b}	44.08^{a}	84.59^{c}	2.53	<0.0001
Histidine	90.67^{b}	72.20^{a}	67.29^{a}	91.64^{b}	2.02	0.0001
Hydroxy lysine	91.02^{bc}	87.80^{b}	56.12^{a}	101.42^{c}	2.53	<0.0001
Isoleucine	89.73^{b}	68.36^{a}	63.70^{a}	90.21^{b}	2.01	<0.0001
Leucine	88.24^{b}	71.57^{a}	66.28^{a}	88.93^{b}	1.85	0.0001
Lysine	90.78^{b}	73.66^{a}	69.22^{a}	91.94^{b}	1.40	<0.0001
Methionine	87.71^{b}	70.81^{a}	63.85^{a}	87.83^{b}	1.72	<0.0001
Phenylalanine	89.26^{b}	69.82^{a}	61.66^{a}	90.07^{b}	1.97	<0.0001
Proline	88.41^{c}	70.76^{b}	51.92^{a}	90.06^{c}	2.35	<0.0001
Serine	85.63^{bc}	75.38^{b}	54.46^{a}	88.05^{c}	2.02	<0.0001
Threonine	85.42^{c}	70.93^{b}	59.54^{a}	86.67^{c}	2.11	<0.0001
Tryptophan	94.45^{c}	78.64^{b}	67.96^{a}	93.83^{c}	1.34	<0.0001
Tyrosine	93.10^{c}	73.29^{b}	62.81^{a}	92.95^{c}	1.70	<0.0001
Valine	87.67^{b}	69.07^{a}	61.77^{a}	88.29^{b}	2.14	0.0001
Total amino acids	89.52^{c}	71.00^{b}	60.28^{a}	90.41^{c}	1.86	<0.0001

[1].PSE: Pooled standard error.

Figure 1. Response of juvenile shrimp (0.17 g mean initial weight) to diets with graded level of a dried brewer's yeast meal (BY50) replacing fishmeal over 42 days in growth trial 1. The relationship between weight gain (y) of shrimp and the inclusion level of BY50 (x) in the diets.

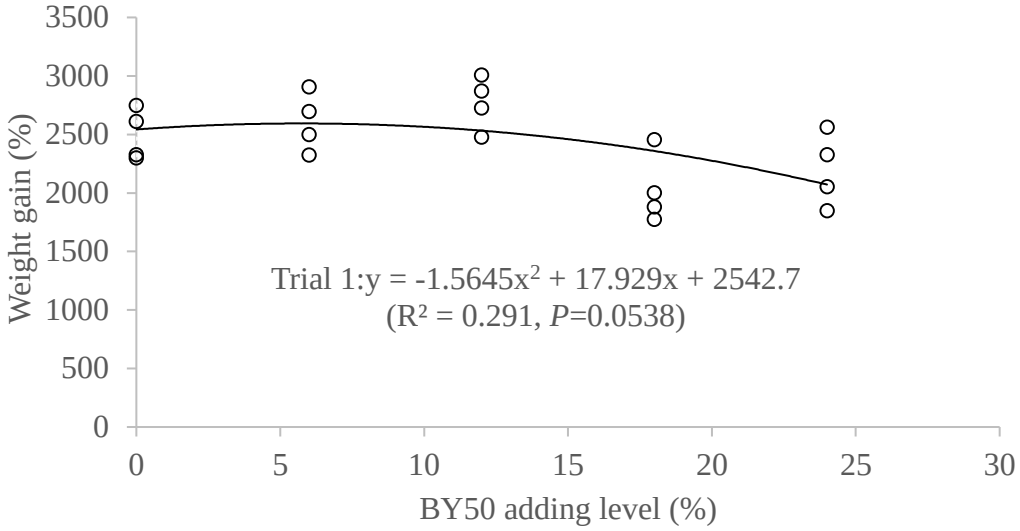
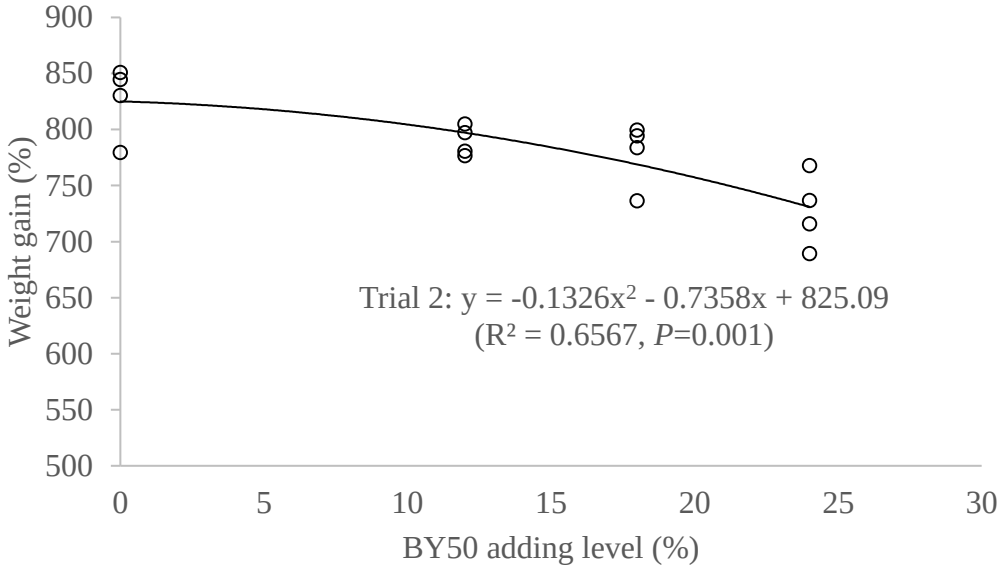


Figure 2. Response of juvenile shrimp (0.82 g mean initial weight) to diets with graded level of a dried brewer's yeast meal (BY50) replacing fishmeal over 42 days in growth trial 2. The relationship between weight gain (y) of shrimp and the inclusion level of BY50 (x) in the diets.



CHAPTER III

USING HIGH PROTEIN DISTILLER'S DRIED GRAIN PRODUCT TO REPLACE CORN PROTEIN CONCENTRATE AND FISHMEAL IN PRACTICAL DIETS FOR THE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Abstract

An 8-week growth trial was conducted to evaluate the use of high protein distiller's dried grain (HPDDG, NexPro®, Flint Hills Resources, LP, Wichita, KS, USA) on the growth performance of juvenile Pacific white shrimp *Litopenaeus vannamei.* In the growth trial, graded level of HPDDG (0, 10, 15, and 20%) were used to replace corn protein concentrate (CPC:12.60, 6.30, 3.15, and 0%) or fishmeal (FM:17.40, 9.79, 6.00 and 2.21%), respectively. A commercially produced formulation was also included in the trial as a reference. Each diet was randomly assigned to four replicate groups of 30 shrimp stocked into 0.8 m^3 culture tanks. Under green water conditions in the presence of natural foods, the results indicated that growth performance and feed conversion ratio were not significantly different by increasing levels of HPDDG when used to replace CPC. The FM replacement series trial results showed that shrimp fed the diet with 20% HPDDG exhibited significantly decreased trend on biomass. Results of this study demonstrated that HPDDG is a good protein source and 20% HPDDG can be used to replace CPC in shrimp diets, or up to 15% when replacing FM.

Keywords: High protein distiller's dried grains; growth trial; fishmeal; *Litopenaeus vannamei*

1.Introduction

As the world's shrimp production expands, considerable effort to replace fishmeal (FM) using a variety of plant proteins or terrestrial animal byproducts in shrimp diets has gained momentum. It is important to look for steady supply, consistent quality, and cost-effective protein sources to keep the feed cost down. As the U.S. ethanol industry continues to develop, it modifies its processes to produce large quantities (34.4 million tonnes in 2012) of maize co-products from dry-grind ethanol production (Licht & Association, 2014). Thus, it is very important to looking for new approach to consume those by-products.

Distiller's dried grain with solubles (DDGS) is a co-product of the ethanol prodution distillery industry and has been suggested as a less expensive alternative to soybean meal (SBM) on a per unit protein basis. The nutrient quality of DDGS varies with grain sources and the type of processing conditions. Typically, traditional corn DDGS contains about 28-32% crude protein, 10% fat, and high fiber content (about 11%), which limits its use as an ingredient in aquafeed (Bonnardeaux, 2007; Gatlin III et al., 2007; Singh et al., 2005). High-quality, lipid-extracted DDGS processing can increase the protein concentration and reduce the fiber content. The high protein distiller's dried grain (HPDDG) used in this study is a processing variation the produced high protein (49% crude protein), low level of crude fiber (5.5%) and lipid (3.11%) product, which makes it a more nutrient dense feedstuff. A previous study demonstrated that 30% HPDDG can be used to replace SBM, and 18% HPDDG can be utilized to replace a combination of SBM and FM in clear water condition (Qiu, Tian, & Davis, 2017).

However, information about the efficacy of a HPDDG product to replace FM in shrimp diets in green water is limited. HPDDG can reduce diet cost compared to conventional plant protein ingredients, like SBM or corn protein concentrate (CPC) (Tyner, 2015). Furthermore, HPDDG

contains fewer antinutritional factors (e.g., phytic acid) after going through the fermentation and hydrothermal process than most plant proteins (Chatvijitkul, Davis, & Lim, 2016). We previously evaluated the HPDDG as a replacement for SBM, however, there is no data on HPDDG's nutritional value as a replacement for CPC, which is another important plant protein source used in shrimp diets. Therefore, the objective of this study is to evaluate the utilization of HPDDG products as replacement for CPC or FM in the practical diets of Pacific white shrimp, *L. vannamei* in a green water system.

2. Materials and Methods

2.1. Experimental diets

HPDDG was obtained from Flint Hills Resources, Wichita, KS, USA. The remaining ingredients were locally sourced. Proximate and amino acid compositions for each of the main ingredients are presented in Table 1. The formulations and proximate compositions of the experimental diets (Diet 1-7) are presented in Table 2 and 3, respectively. In the growth trial, all test diets were formulated on an iso-nitrogenous and iso-lipidic basis to contain 35% protein and 8% lipid. Two series of diets were formulated (Diet 1-7), where HPDDG was used to gradually replace CPC or FM (Table 2). In the CPC replacement series, graded levels of HPDDG (0.00, 10.00, 15.00 and 20.00%) were used to replace CPC (CPC:12.60, 6.30, 3.15, and 0.00%) referred to Diets 1, 2, 3 and 4, respectively. In the FM replacement series, graded levels of HPDDG (0, 10, 15 and 20%) were used to replace FM (FM:17.40, 9.79, 6.00 and 2.21%) referred to Diets 5, 6, 3 and 7, respectively. Note that Diet 3 was formulated to be used in both series. In addition, another reference was added, the FM-free shrimp diet produced by Zeigler Bros, Inc. as a benchmarking

treatment (Diet 8), which has an equivalent formulation and proximate composition reported by Ullman, Rhodes, Hanson, Cline, and Davis (2017).

The experimental diets were produced at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University (Auburn, AL, USA), using standard procedures for shrimp feeds. Diets were prepared by mixing the pre-ground dry ingredients in a food mixer (Hobart, Troy, OH, USA) for 10–15 minutes. 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 (< 45 °C) overnight to attain a moisture content of less than 10%. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer (-20 °C) until needed. All the ingredients and diets were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate composition and amino acid (AA) profile (% as-is, Table 1 and 3).

2.2. Experiment procedure

The growth trial was conducted in a green water (outdoor) recirculating aquaculture system (RAS) at Claude Peteet Mariculture Center (Gulf Shores, AL USA), which was managed to have natural productivity present. The research system consists of a central reservoir (~1,000 L), a 1.0 hp circulation pump, 32 circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter) and supplemental aeration. In this green water system, a second sump pump is used to move unfiltered water from a shrimp production pond to the central reservoir at a rate of ~8 L min^{-1} for 2 h between 08:00 h and 12:00 h daily (5% daily water exchange). This results in the replacement of system water every few days, replenishing natural productivity to mimic a production pond setting. Each tank and central reservoir were equipped with an air stone

connected to a 1 hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA) to supply aeration.

Pacific white shrimp post-larvae were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and nursed in an indoor recirculating system using commercial feeds until they reached an appropriate size for research. Juvenile shrimp (initial mean weight: 0.36±0.01g) were randomly selected and stocked at 30 shrimp per tank in green water.

During the growth trial, four replicate tanks were assigned per treatment, and shrimp were fed two times per day (8AM and 4PM). In general, feed inputs are calculated assuming the shrimp will double their weight weekly up to one gram, then gain 1.2 g weekly with FCR of 1.3 in green water. At the end of the growth trial, shrimp in each tank were counted and weighed to calculate survival, biomass, mean weight, FCR, weight gain, and weekly gain. After weighing and counting the shrimp, 6 shrimp per tank were randomly selected and frozen at −20°C for whole body samples to be utilized for later protein retention analysis.

Water temperature was maintained at around 29°C. Dissolved oxygen (DO) was maintained near saturation (6 mgL^{-1}) using air stones in each tank with a common airline connected to a regenerative blower. During the growth trial, DO, water temperature, pH, and salinity were measured twice daily using a YSI Proplus multi-parameter instrument (YSI, Yellow Springs, OH, USA). Total ammonia nitrogen was measured twice per week using a Thermo Orion ISE probe (Thermo Fisher Scientific Inc., Waltham, MA, USA), while nitrite and nitrate were analyzed once per week using LaMotte test kits (LaMotte Company, Chestertown, Maryland, USA). Over the 56 days of the trial, DO, temperature, salinity, pH, total ammonia nitrogen, nitrite, and nitrate were maintained at 6.53±0.63 mgL^{-1}, 29.42±1.38 °C, 11.61±2.33 ppt, 7.90±0.38, 0.01±0.03 mgL^{-}

^{1}, 0.33±0.21 mgL^{-1}, and 9.24±4.90 mgL^{-1}, respectively. Water quality conditions in the trial were suitable for normal growth and survival of this species.

2.3. Statistical analysis

All data were analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). Two replacement series data were subjected to run ANCOVA to evaluate the correlation between HPDDG adding level and replaced protein source (CPC or FM). One-way ANOVA analysis of variance was run to determine significant differences ($P<0.05$) among each replacement series (CPC or FM) and all the treatments, followed by Tukey's multiple comparison test to determine differences between treatment means. The pooled standard error used across all the data as the variance of each treatment is the same. Dunnett's t-test was performed to compare growth performance of shrimp fed with commercial reference Diet 8 with the other diets as post hoc test.

3. Results

3.1. Growth trial

The growth performance of shrimp offered diets containing various levels of HPDDG to replace CPC or FM is shown in Table 4. From the ANCOVA analysis presented in Table 4, the results show that there is a significant interaction between replaced protein (FM or CPC) with HPDDG level on the biomass of shrimp. From the ANOVA result in Table 4, there was a slight decrease in the FM replacement series on the biomass and the feed utilization of the shrimp with the replacement level increased. There was no significant difference in CPC replacement on the growth performance and feed utilization for shrimp. The growth performance of shrimp offered Diet 8 compared to the growth performance of shrimp offered Diets 1-7 is shown in Table 5. There was no significant difference among all the diets in the final biomass (221.2 to 239.5 g), survival

(97.5 to 100%), FCR (1.4-1.6), final mean weight (7.49-8.08 g), weight gain (1920-2106%), weight gain (7.13-7.71 g), and weekly gain (0.89-0.96g).

Dunnett's t-test was performed to compare the growth performance of shrimp fed the commercial reference Diet 8 with those fed the other test diets (Table 5). The shrimp fed Diet 7 have significantly lower biomass compared to those fed Diet 8.

4. Discussion

DDGS is an inexpensive protein ingredient included in many aquafeeds in combination with other plant protein sources to decrease cost and balance nutrient content. The composition of DDGS varies with processing, batches, and producers. Many studies have demonstrated that DDGS can be successfully used as a protein source in aquafeed for a variety of species (Gause & Trushenski, 2011; Blake Stewart Hauptman, 2012; Blake S Hauptman et al., 2014) including the Pacific white shrimp (Adedeji et al., 2017; Qiu et al., 2017; Rhodes, Yu, Zhou, & Allen Davis, 2015; Roy et al., 2009; Sookying & Davis, 2011). The HPDDG product used in this study is developed to have a higher protein concentration (>49%) with a lower concentration of fat (3%) and fiber (5.5%) compared to conventional DDGS which contains about 28-32% crude protein and 10% lipid (Gatlin III et al., 2007). The HPDDG can meet the shrimp's dietary protein requriment at a lower inclusion level, therefore, opening more space in the diet formulation (Fang, Yu, Buentello, Zeng, & Davis, 2016). However, no research has been conducted to evalute the use of HPDDG in shrimp diets to replace CPC or FM under practical outdoor conditions.

Given the previous study results using the same product, up to 30% HPDDG can be used to replace SBM in shrimp diets, and up to 18% HPDDG can be used to replace a combination of

the SBM and FM in the diet without affecting growth performance of shrimp in clear water (Qiu et al., 2017). In this follow up study, graded levels of HPDDG are used to replace CPC or FM, as this results in more shifts in nutrient composition and possible palatability changes of the diets in FM replacement series than those of diets in CPC replacement series. Firstly, the results show that there was a significant interaction between replaced protein (FM or CPC) on the biomass of shrimp. This indicates that the effect on growth performance will be different with the kind of protein source we replaced. There was no significant trend when the HPDDG replaced CPC. However, there was a significant decreased trend on the biomass as the HPDDG level increased in the FM replacement series; the mean weight, weekly gain, and weight gain (*P=0.079*) also mirrored this trend although no statistically significant differences were found. To allow the comparison to a commercially produced feed Diet 8 was included in the trial. Dunnett's t-test as post hoc test was used to compare commercial Diet 8 with each test diet. Results also showed that the biomass of shrimp fed Diet 7 was significantly lower compared to that of those fed the commercial diet. The results showed that 20% HPDDG can be used to replace CPC, or up to 15% when replacing FM in practical shrimp diets.

The FCR ranged from 1.40-1.56, which is consistent with Rhodes et al. (2015) who reported that the FCR ranged from 1.5 to 1.8 for shrimp fed graded levels of lipid extracted DDGS in clear water conditions. It was lower than Qiu et al. (2017) who demonstrated that the FCR ranged from 1.64 to 2.14 for shrimp fed with the same product (HPDDG) in clear water. In general, the FCR observed in all the diets for this study was reasonable for shrimp reared in green water. The shrimp that were fed the reference Diet 8 had a higher biomass and lower FCR than shrimp fed test Diet 7. This is likely due to processing conditions which can improve pellet stability and digestibility for shrimp. The primary aim in adding the commercially-produced diet was to have

an independent reference point to compare growth performance and feed utilization although the commercial diet has a totally different set of ingredients, nutrient composition, and processing conditions (Achupallas, Zhou, & Davis, 2016). The final biomass and FCR of the shrimp fed the high replacement level for FM were lower and higher than both the commercial Diet 8 and basal Diet 1, respectively. In all, those results indicate that though HPDDG works as a replacement for CPC, there are limitations when replacing FM.

The growth performance results demonstrated HPDDG to be a good protein source for practical shrimp feed formulations. Our results with HPDDG showed similar replacement levels demonstrated by Rhodes et al. (2015) who determined that up to 20% lipid extracted DDGS can be used in practical shrimp diet containing 6% FM in green water. Further, this study showed improved results compared to those reported by Qiu et al. (2017) who demonstrated that 18% HPDDG can be used to replace a combination of SBM and FM in clear water. Additionally, the HPDDG showed a higher replacement level for CPC and FM when compared to the results observed by Sookying and Davis (2011) who tested shrimp diets containing high levels of SBM with 10% DDGS, which had no negative impact on the growth performance of the shrimp in outdoor tanks and ponds. Roy et al. (2009) also obtained a similar result to Sookying and Davis (2011) with the same dietary treatments, both in laboratory and outdoor conditions. A primary reason HPDDG can get so high replacement level and make a suitable protein source for shrimp reared in green water is due to environmental effects and the ability to obtain additional nutrients from natural sources, which could mask negative effects of DDGS as a protein replacement in practical shrimp diet

Methionine and lysine are usually the limiting amino acids in fish and shrimp diets, especially those containing higher levels of plant-protein sources (Espe, Lemme, Petri, & El-

Mowafi, 2006; Mai et al., 2006). HPDDG contains a low level of lysine compared to that of SBM (2.01 % vs 2.82 %, respectively), while HPDDG contains higher methionine content compared to that of SBM (1.01% vs 0.64%) and lower than that in FM (1.69%). Hence, lysine needs to be supplemented if those diets cannot meet the nutritional requirement of shrimp when higher inclusion level of DDGS is added in the diets. In the present study, the lysine content of all the diets satisfied the lysine requirement as reported by Fox, Lawrence, and Li-Chan (1995) and was not likely to be limiting.

5.Conclusion

In conclusion, 20% HPDDG can be effectively used to replace CPC in shrimp diets, or up to 15% when replacing FM. HPDDG proves to be a good protein source in shrimp diets. In addition, the HPDDG also contains an elevated level of yeast. Future work to evaluate the possible immune-stimulating effects are worth pursuing.

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Table 1 Proximate and amino acid composition (% as-is) of test ingredients used in growth trial. Analyses were conducted by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

	Fish Meal	SBM	CPC	HPDDG
Crude Protein	64.75	44.91	78.07	49.20
Moisture	6.28	10.35	8.16	8.00
Crude Fat	9.09	1.82	2.03	3.11
Crude Fiber	0.66	3.61	0.87	5.50
Ash	19.77	6.15	1.03	4.87
Alanine	4.01	1.94	6.42	3.36
Arginine	3.78	3.21	2.25	2.30
Aspartic Acid	5.49	5.04	4.29	4.05
Cysteine	0.54	0.68	1.30	0.87
Glutamic Acid	7.69	8.01	14.68	7.23
Glycine	4.97	1.91	1.95	1.95
Histidine	1.66	1.14	1.48	1.33
Hydroxy lysine	0.24	0.03	0.10	0.00
Hydroxyproline	1.11	0.07	0.00	0.01
Isoleucine	2.56	2.14	2.96	2.19
Leucine	4.31	3.42	12.97	5.57
Lysine	4.89	2.82	1.14	2.01
Methionine	1.69	0.64	1.80	1.01
Phenylalanine	2.45	2.28	4.80	2.57
Proline	3.00	2.15	7.31	3.33
Serine	2.21	1.93	3.80	2.08
Taurine	0.70	0.09	0.03	0.02
Threonine	2.50	1.74	2.48	2.02
Tryptophan	0.65	0.62	0.37	0.43
Tyrosine	1.92	1.68	4.24	2.01
Valine	2.97	2.19	3.23	2.87
Total	59.46	43.76	77.84	47.39

Table 2. Formulation of test diets used to evaluate the HPDDG products (% as- is).

	1	2	3	4	5	6	7
Menhaden fishmeal[a]	6.00	6.00	6.00	6.00	17.40	9.79	2.21
Soybean meal[b]	42.40	42.40	42.40	42.40	42.40	42.40	42.40
HPDDG - Flint Hills[c]	0.00	10.00	15.00	20.00	0.00	10.00	20.00
CPC - Empyreal 75[d]	12.60	6.30	3.15	0.00	3.15	3.15	3.15
Menhaden fish oil[e]	5.54	5.35	5.26	5.17	4.69	5.07	5.45
Lecithin (soy)[f]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol[g]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Corn Starch[g]	10.26	6.75	4.99	3.23	9.16	6.39	3.59
Whole wheat[g]	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Mineral premix[h]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[i]	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride[g]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Rovimix Stay-C[j]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
KP dibasic[k]	2.50	2.50	2.50	2.50	2.50	2.50	2.50

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

[b] De-hulled solvent extract soybean meal, Bunge limited, Decatur, AL, USA.

[c] NexPro, Flint Hills Resources, LP, Wichita, KS, USA.

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

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

[f] The Solae Company, St. Louis, MO, USA.

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

[h] Mineral premix (g/100 g 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.

[i] Vitamin premix (g kg^{-1} premix): Thiamin. HCl, 4.95; Riboflavin, 3.83; Pyridoxine. HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 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.

[j] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[k] T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.

Table 3. Proximate and amino acid composition of experimental diet (% as-is). Analyses were conducted by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Diet	1	2	3	4	5	6	7
Protein	35.55	35.52	36.39	36.27	35.78	35.92	36.13
Moisture	7.13	7.58	6.38	6.84	6.65	7.04	7.27
Fat	8.61	8.93	9.09	9.12	8.51	8.88	9.09
Fiber	3.17	3.56	3.51	3.22	2.40	3.35	3.89
Ash	6.21	6.62	6.87	7.06	8.44	7.12	6.49
Amino acid							
Alanine	2.05	2.00	2.02	1.95	1.88	1.93	1.99
Arginine	2.02	2.13	2.21	2.27	2.30	2.23	2.18
Aspartic acid	3.18	3.27	3.36	3.40	3.44	3.36	3.29
Cysteine	0.56	0.59	0.60	0.60	0.49	0.56	0.62
Glutamic acid	6.98	6.76	6.76	6.58	6.27	6.48	6.74
Glycine	1.48	1.54	1.61	1.64	1.87	1.69	1.50
Histidine	0.86	0.91	0.95	0.97	0.92	0.93	0.94
Hydroxy lysine	0.09	0.09	0.09	0.09	0.11	0.09	0.08
Hydroxyproline	0.07	0.08	0.08	0.09	0.19	0.12	0.05
Isoleucine	1.60	1.62	1.66	1.66	1.61	1.63	1.64
Lanthionine	0.18	0.20	0.21	0.21	0.16	0.19	0.20
Leucine	3.61	3.44	3.42	3.23	2.88	3.15	3.44
Lysine	1.72	1.85	1.95	2.02	2.23	2.05	1.86
Methionine	0.68	0.68	0.69	0.68	0.71	0.68	0.68
Ornithine	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phenylalanine	1.93	1.90	1.92	1.87	1.76	1.84	1.91
Proline	2.33	2.29	2.31	2.25	2.03	2.15	2.36
Serine	1.52	1.53	1.57	1.57	1.44	1.50	1.56
Taurine	0.15	0.16	0.15	0.15	0.24	0.18	0.14
Threonine	1.28	1.33	1.38	1.39	1.35	1.35	1.36
Tryptophan	0.42	0.46	0.45	0.45	0.46	0.45	0.46
Tyrosine	1.37	1.42	1.43	1.41	1.29	1.37	1.45
Valine	1.70	1.76	1.83	1.85	1.74	1.78	1.83
Total	35.80	36.03	36.67	36.35	35.39	35.73	36.30

Table 4. Response of juvenile shrimp to diets with graded levels of HPDDG to replace CPC or FM for one 56-day growth trial (n=4). ANCOVA results are presented below. One-way ANOVA was also run by diet type. No significant difference is across treatments for CPC diet type. However, biomass and FCR were significantly affected in the FM diets. Means with different was labeled with superscripts, which are significant difference.

Diet	Diet type	HPDDG Level (%)	Biomass (g)	Mean weight (g)	Survival (%)	Weight gain[1] (g)	Weight gain[2] (%)	FCR[3]	Weekly gain (g)
1	CPC	0	224.6	7.49	100.0	7.13	1997	1.5	0.89
2	CPC	10	227.3	7.64	99.2	7.28	2032	1.5	0.91
3	CPC	15	230.0	7.88	97.5	7.52	2106	1.5	0.94
4	CPC	20	221.2	7.50	98.3	7.14	1996	1.5	0.89
5	FM	0	239.5^{b}	8.05	99.2	7.68	2104	1.4^{a}	0.96
6	FM	10	234.9^{b}	7.90	99.2	7.54	2093	1.4^{a}	0.94
3	FM	15	229.9^{ab}	7.88	97.5	7.52	2106	1.5^{ab}	0.94
7	FM	20	215.6^{a}	7.38	97.5	7.02	1920	1.6^{b}	0.88
ANCOVA									
Model			0.025	0.126	0.689	0.130	0.377	0.039	0.130
Diet type			0.263	0.226	0.744	0.238	0.614	0.285	0.237
HPDDG level			0.029	0.248	0.251	0.249	0.361	0.033	0.248
Interaction			0.047	0.079	0.957	0.079	0.158	0.773	0.080

[1] Weight gain (g) = Final weight-initial weight.

[2] Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.

[3] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).

Table 5. One-way ANOVA result about comparing the response of juvenile shrimp to diets with graded levels of HPDDG to replace CPC or FM with the commercial Diet 8 for one 56-day growth trial (n=4).

Diet	Biomass (g)	MW(g)	Survival (%)	Weight gain[1] (g)	Weight Gain[2] (%)	FCR[3]	Weekly gain (g)
8	238.1	8.08	98.3	7.71	2098	1.4	0.96
PSE[4]	11.297	0.437	1.796	0.216	62.520	0.038	0.027
P-value	0.064	0.180	0.951	0.177	0.275	0.070	0.176
Dunnett's t test							
D7 VS D8	0.026	0.390	0.233	0.405	0.926	0.064	0.405

[1] Weight gain (g) = Final weight-initial weight.

[2] Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.

[3] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).

CHAPTER IV

USE OF PLANT-BASED PROTEIN CONCENTRATES AS REPLACEMENT FOR FISHMEAL IN PRACTICAL DIETS FOR THE PACIFIC WHITE SHRIMP (*litopenaeus vannamei*) REARED UNDER HIGH STOCKING DENSITY AND LOW SALINITY CONDITIONS

Abstract

Two feeding trials were conducted to investigate the effect of replacing fishmeal with a combination of soy and corn protein concentrate (1:1 ratio) on growth performance of the Pacific white shrimp (*Litopenaeus vannamei*). A basal diet containing 200 g/kg fishmeal was reduced (200, 150, 100, 50, 0 g/kg) with the protein concentrate combination on an isonitrogenous basis. Additionally, two diets containing 0 or 50 g/kg fishmeal were supplemented with lysine and methionine to evaluate possible limitations in EAAs. Each diet was randomly allocated to five replicate tanks (15 shrimp per 75-L aquaria) reared in an indoor clear water system (Trial 1), or four replicate circular tanks (125 shrimp/m^2) reared in outdoor green water system (Trial 2). In trial 1, results indicated a slight decrease in shrimp performance as fishmeal was replaced at the highest levels. Meanwhile, the supplementation of lysine and methionine to the diets did not result in shifts in survival, growth or FCR. In trial 2, no significant differences in growth performance across the tested diets were found. This study demonstrated that plant-based protein concentrates may be used to replace fishmeal in practical shrimp diet in clear and green water under high stocking density and low salinity conditions.

Keywords: *Litopenaeus vannamei;* Fishmeal replacement; Corn protein concentrate; Soy protein concentrate; Growth trial; High stocking density

1. Introduction

Global supplies of fishmeal (FM) have reached a plateau yet demand continues to increase, making it more expensive and less available (FAO, 2016). Soybean meal (SBM) is often regarded as a cost-effective and nutritionally valuable protein source in shrimp and fish feeds (Guo et al., 2018) and is routinely added to commercial formulations for many species. Due to the low level of essential amino acids (Espe, Lemme, Petri, & El-Mowafi, 2006; Mai et al., 2006), limited available phosphorous (Yun et al., 2014) and the presence of anti-nutritional factors (Dias et al., 2009) that may have adverse effects on growth performance and feed utilization, which may discourage and limit the utilization of SBM in aquafeeds. Therefore, it is important to explore alternative protein sources that prosses a more optimal nutrient profile, and are available in sufficient quantities at a economical price.

More recently, enzyme-treated soybean meal products (ESBM, Nutrivance, TechMix, Stewart, MN) have entered the market as new specialty soybean protein ingredients, produced by a combination of non-alcohol extraction processes and enzymatic treatment to reduce ANFs (Jordan et al., 2014; Novriadi, Spangler, Rhodes, Hanson, & Davis, 2017). ESBM has recently been shown to effectively replace fishmeal for pompano (Novriadi, Spangler, & Allen Davis, 2018; Novriadi et al., 2017). Besides, corn protein concentrate (CPC) has been successfully used as a protein source to replace fishmeal in aquatic feeds in many fish and shrimp species such as Pacific white shrimp (Chen, Li, Xu, Sun, & Leng, 2017; Xie, Liu, Zeng, Niu, & Tian, 2016; Zhou et al., 2014), Nile tilapia *Oreochromis niloticus* (Khalifa, Belal, El‐Tarabily, Tariq, & Kassab, 2018), Florida pompano *Trachinotus carolinus* (Cook, 2014), and Atlantic salmon *Oncorhynchus mykiss* (Burr, Wolters, Barrows, & Hardy, 2012). All the studies above support the use of CPC and SPC

as suitable protein sources for fish or shrimp. Additionally, proper combinations of those plant protein sources could complement each other to provide optimal replacement levels (Amaya, Davis, & Rouse, 2007a, 2007b). However, practical information regarding practical diet formulations combining alternative protein sources (like CPC and SPC) as a replacement for FM in order to provide a better and balanced AA profile in practical shrimp diet is limited. Thus, the objectives of the present study were: (1) to determine the effect of utilizing combinations of CPC and SPC as replacement for fishmeal, in combination with and without CAA (Crystalline amino acids) supplementation in practical diets for the Pacific white shrimp in clear water; (2) to evaluate the effect of using of CPC and SPC for fishmeal replacement in practical shrimp diets in green water under high stocking density (125/m^2) and low salinity conditions.

2. Materials and Methods

2.1. Experimental diets

The main protein ingredients used in this research were locally sourced (Table 1) and the formulations as well as proximate compositions of experimental diets are presented in Table 2 and 3. The seven diets were formulated to be isolipidic (80g/kg crude lipid) and isonitrogenous (350g/kg crude protein) on an as-is basis (Table 2). In the experimental diets, combinations of graded levels (Total: 0, 46, 92, 138, and 184 g/kg) of SPC and CPC (1:1 ratio) were used to replace fishmeal (FM: 200, 150, 100, 50, and 0 g/kg), which were designated as D1, D2, D3, D4 and D5, respectively. Two additional diets, D6 and D7, had the same formulae as D4 and D5 respectively but supplemented with methionine and lysine.

Diets were prepared by mixing pre-ground dry ingredients in a food mixer (Hobart, Troy, OH, USA) for 10–15 minutes. Boiling water (ca 40% by weight) was then blended into the mixture

to obtain a consistency appropriate for pelleting. Diets were formed using a meat grinder with a 3-mm die. The moist pellets were then placed into a forced air oven (< 45 °C) overnight to attain a moisture content of less than 10g/kg. Dry pellets were crumbled, packed in sealed bags, and stored in a freezer (-20°C) until needed. All the ingredients and diets were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate composition and amino acid profile (g/kg as is) (Table 3).

2.2. Experimental procedures

Two separate growth trials were carried out including a 42-day trial in a clear water indoor system and a 56-day green water outdoor trial. Both experiments were conducted at E. W. Shell Fisheries Research Center in Auburn, Alabama. Pacific white shrimp post-larvae were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and nursed in an indoor recirculating system using commercial feeds until they attained an appropriate size. Each diet was randomly fed to 5 or 4 replicate groups of 15 and 100 shrimp per tank in the clear (indoor) (Trial 1 using diets D1-D7) and green (outdoor) water trials (Trial 2 using diets D1- D5), respectively.

In both the clear and green water trials, juvenile shrimp (initial mean weight 0.28±0.01g and 0.26±0.01 g) were hand-sorted to uniform size and then randomly stocked into 75-L aquaria or 800-L circular tanks, which were a component of a 2.5-m^3 or 21-m^3 recirculation system, respectively.

During the growth trials, shrimp were hand-fed four times daily using a standardized feeding table that is based on historical results. In general, feed inputs were determined assuming the shrimp would double their weight weekly up to one gram, then gain 0.8 g weekly with a feed conversation ratio (FCR) of 1.8 for clear water and gain 1.2 g weekly with FCR of 1.3 for the green water trial. Shrimp were counted once a week to adjust the daily feed input in clear water. At the

end of the growth trial, shrimp in each tank were counted and weighed to determine survival, biomass, mean weight, FCR and weight gain. After weighing and counting the shrimp, 4-6 shrimp per tank were randomly selected and frozen at −20°C for whole body protein retention analysis.

During growth trials, dissolved oxygen (DO), water temperature and salinity were measured twice daily using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH, USA). The pH was tested twice weekly using a waterproof pHTestr30 (Oakton instrument, Vernon Hills, IL, USA). Total ammonia nitrogen (TAN) and nitrite were analyzed once per week using the methods described by Solorzano (1969) and Spotte (1970), respectively. In trial 1, mean DO, temperature, salinity, pH, TAN, and nitrite were 6.20 ± 0.82 mg L^{-1}, 28.62 ± 0.51 °C, 7.42 ± 0.99 ppt, 7.58 ± 0.3, 0.12 ± 0.05 mg L^{-1}, and 0.09 ± 0.12 mg L^{-1}, respectively. In green (outdoor) water for trial 2, mean DO, temperature, salinity, pH, TAN, and nitrite were 6.94 ± 1.34 mg L^{-1}, 29.02 ± 2.43 °C, 4.59 ± 0.19ppt, 7.78 ± 0.2, 0.23 ± 0.21 mg L^{-1}, and 0.09 ± 0.09 mg L^{-1}, respectively. Water quality conditions in all the trials were suitable for normal growth and survival of this species.

2.3. Statistical analysis

All data were analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). All data were subjected to one-way analysis of variance to determine significant differences ($P<0.05$) among treatments (D1-5) in trial 1 and 2, followed by Tukey's multiple comparison test to determine differences between treatment means. Independent t-test was performed to compare D4 with D6, and D5 with D7 in terms of shrimp growth performance. Linear regressions were performed to investigate the relationship between the dietary fishmeal replacement with protein concentrate and the response variables of weight gain. The pooled standard errors were used across growth trials as the variances of each treatment were the same.

3. Results

3.1. Growth trial

Growth performance of shrimp offered diets containing different levels of CPC and SPC in trial 1 are presented in Table 4, respectively. In trial 1, results showed that shrimp fed with D5 exhibited significantly lower final biomass, final mean weight and weight gain than those of shrimp offered the basal diet (D1). Mean survival (76.0 to 86.7%) was not significantly different among shrimp offered the different diets. The FCR of the shrimp fed with D4 and D5 were significantly higher than those of shrimp fed diets D1, D2 and D3. Protein retention efficiencies (PRE) of shrimp fed with D4 and D5 were significantly lower than those diets D1, D2 and D3. An independent t-test was used to compare the results of shrimp performance offered D4 as compared to D6, and those fed D5 as compared to D7 (Table 4). The shrimp fed D6 and D7 has significantly higher PRE than those fed D4 and D5, respectively.

In trial 2, mean final biomass (926.4 to 1015.8g), survival (88.3 to 94.8%), final weight (10.09-10.54 g), weight gain (3772-3929%), protein retention efficiency (PRE) (42.3-44.7%) and weight gain (9.83-10.28 g) were not significantly different (Table 5). The mean FCR of shrimp fed diet D5 was significantly higher than that of shrimp fed D1 *($P<0.05$*).

In trial 1, the replacement of fishmeal with the protein concentrates significantly influenced growth of the shrimp particularly at the higher levels of inclusion (Table 4, Figure 1). In trial 2, there was no significant decrease in the trend for WG with increased fishmeal replacement level (Table 5, Figure 2). In trials 1 and 2, the regression lines are described by $y = -0.0395x^2 + 0.4178x + 1370.8$ ($R^2 = 0.5329$, *$p = 0.0002$*) and $y = -1.3669x + 4022.8$ ($R^2 = 0.1059$, *$P = 0.1746$*), respectively.

4. Discussion

Research has demonstrated that total fishmeal in practical diets for the Pacific white shrimp can be replaced by using a wide variety and different levels of alternative plant protein sources with high nutritional value (Amaya, Davis, & Rouse, 2007a, 2007b; Roy et al., 2009; D Sookying, Davis, & Soller Dias Da Silva, 2013; Daranee Sookying & Davis, 2011).

Soy protein concentrate (SPC) and corn protein concentrate (CPC) are considered suitable and promising protein substitutes for fishmeal (Khalifa, Belal, El‐Tarabily, Tariq, & Kassab, 2018; Daranee Sookying & Davis, 2012). These ingredients contain a similar amino acid profile to SBM but have a higher protein content and less anti-nutritional factors than SBM. In trial 1, there is a decreasing trend in growth performance as fishmeal was replaced at or below 50 g/kg fishmeal (75% replacement) in diet using a 1:1 ratio of SPC and CPC, which is apparent in Tukey's multiple range test (Table 4) as well as regression analysis (Fig. 1). In general, 92g/kg of SPC and CPC (1:1 ratio) in Diet 3 can be used to replace 50% fishmeal in practical diets containing high level of SBM for shrimp without adversely affecting growth and feed utilization in clear water. In the green water (outdoor) trial, there was no statistical differences in growth performance when 138g/kg of SPC and CPC (1:1 ratio) was used to replace 75% fishmeal under high stocking density and low salinity rearing conditions. Both trials indicated that high levels of CPC and SPC in shrimp diets with high level of SBM are feasible and result in acceptable growth and feed utilization by shrimp.

Higher replacement levels were achieved by shrimp reared in green water (even without indispensable amino acid supplementation) presumably due to the availability of additional nutrients from natural sources in green water. Hence, this could mask the true contribution of SPC and CPC as a protein replacement in practical shrimp diets. In zero water exchange systems, levels

of suspended particulate organic matter serve as a potential source of natural food for shrimp (Tacon et al., 2002). Likewise, under commercial conditions, satisfactory results can be attained using low fishmeal diets via the contribution of nutrients derived from primary production (Davis, Arnold, & McCallum, 2002). Thus, shrimp cultured in green water with natural food sources can be offered less complete diets and still achieve a similar performance to shrimp reared in clear water. Jory et al. (2001) reported that flocculated particles in the pond have high protein and contained some essential amino acids, like methionine (5g/kg) and lysine (21g/kg) that are beneficial to shrimp. This is also the reason why in our experiment we used a low fishmeal diet supplemented with AA (D6 and D7) in clear water but not in green water. These results parallel results achieved in our laboratory in both clear and green water (Zhou et al., 2014). Forster, Dominy, and Tacon (2002) also reported that 75% and 100% FM can be replaced by SPC in clear water (indoor) and green water (outdoor) in practical shrimp diets.

In this study, using a combination of CPC and SPC to replace fishmeal provided a complimentary advantage. This helped maintain a balanced amino acid profile in practical shrimp diets, enhanced palatability, and simultaneously improved digestibility (Hu et al., 2013; Quintero, Davis, & Rhodes, 2012). Similarly, Kissinger, García-Ortega, and Trushenski (2016) reported that a combination of algal and soy protein can account for more than half of the total protein in a low fishmeal diet. A level of 462.5g/kg SPC combined algal can replace up to 80% FM without significantly affecting performance of longfin yellowtail (Kissinger et al., 2016). These results were similar or even higher than other studied which used a single protein source such as CPC or SPC. Similarly, Daranee Sookying and Davis (2012) reported that up to 120g/kg SPC in a soybean-based diet can be used in commercial feed formulations for the Pacific white shrimp without a negative effect on growth performance and feed utilization under green water and field conditions.

If essential nutrients in practical diets are properly balanced to meet the growth requirements of shrimp, a high replacement level of plant protein may be viable. Thus, to evaluate possible limitations in essential amino acids, diets containing 50 and 0g/kg fishmeal were supplemented with synthetic crystalline lysine and methionine (Diets D6 and D7, respectively), which allowed for a similar level to those observed in high fishmeal diets. There was no significant improvement in mean growth, FCR or survival when these AA were supplemented to the diets. This is in contrast to observations by Yuan et al. (2011) and Sardar, Abid, Randhawa, and Prabhakar (2009). Gu, Zhang, Bai, Mai, and Xu (2013) who observed improvements in protein retention. These authors also reported some obstacles to using crystal amino acid (CAA) in shrimp feed, including potential leaching losses and desynchrony adsorption rate. Similarly, based on results from our laboratory, it is also questionable whether CAA are sufficiently utilized by shrimp (Unpublished data). On the other side, recently, our new experiment results even reported that the diet was limiting in methionine (3.5g/kg), lysine (4.5g/kg) or arginine (10g/kg), which is based on single deletion of each amino acid from the basal diet (Unpublished data). Therefore, the methionine requirement may also be as low as has been observed in all our study diets. Thus, the limitation of essential amino acids should not be the main problem causing growth depression in clear water. Digestibility or palatability shift may cause the negative effect on the growth performance for shrimp.

Quite often, nutrition studies are conducted at low culture densities to ensure maximum growth. However, there is considerable interest in commercial culture at high density and developing nutrition information under similar conditions. Producers using high density culture often indicate that these systems required high fishmeal diets. Yet, in the present trial, shrimp stocked at 125 shrimp/m^2 had adequate growth and feed utilization across all treatments. Results

of the present study were similar in terms of growth rates and better in terms of feed conversion as compared to results at 65 shrimp m^2 as reported by Daranee Sookying, Silva, Davis, and Hanson (2011). Additionally, the FCR was also decreased in the present experiment compared to the study by Daranee Sookying et al. (2011), which was conducted using a stocking density of 65 shrimp m^2 (1.35) in the pond and 65 shrimp m^2 (1.54) in an outdoor tank system. These data suggested that CPC and SPC served as adequate fishmeal replacement in the diets for shrimp, which also benefited from natural foods under green water culture conditions. The present study demonstrates that high-density culture of shrimp is feasible using plant-based protein concentrates in the diet. Meanwhile, shrimp were cultured under low salinity conditions in the present study, which also can offer reference data for inland shrimp aquaculture.

5. Conclusion

In conclusion, the present study demonstrated that 92 or 138 g/kg of CPC and SPC (1:1 ratio) can be used in the diet of the Pacific white shrimp replacing 50 or 75% fishmeal in clear and green water under high stocking density and low salinity culture conditions.

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Table 1. Proximate and amino acid composition (g/kg as is) of test ingredients used in the growth trials. Analyses were conducted by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

	FM	SBM	SPC	CPC
Crude Protein	647.5	449.1	625.5	780.7
Moisture	62.8	103.5	72.5	81.6
Crude Fat	90.9	18.2	14.8	20.3
Crude Fiber	6.6	36.1	44.8	8.7
Ash	197.7	61.5	43.1	10.3
Amino acid				
Alanine	40.1	19.4	27.2	64.2
Arginine	37.8	32.1	44.8	22.5
Aspartic Acid	54.9	50.4	70.1	42.9
Cysteine	5.4	6.8	8.4	13.0
Glutamic Acid	76.9	80.1	110.0	146.8
Glycine	49.7	19.1	26.5	19.5
Histidine	16.6	11.4	16.6	14.8
Hydroxy lysine	2.4	0.3	0.5	1.0
Hydroxyproline	11.1	0.7	0.0	0.0
Isoleucine	25.6	21.4	28.8	29.6
Lanthionine	0.3	0.0	0.0	1.7
Leucine	43.1	34.2	50.1	129.7
Lysine	48.9	28.2	40.0	11.4
Methionine	16.9	6.4	8.5	18.0
Ornithine	0.9	0.3	0.3	0.7
Phenylalanine	24.5	22.8	32.6	48.0
Proline	30.0	21.5	32.8	73.1
Serine	22.1	19.3	28.5	38.0
Taurine	7.0	0.9	0.9	0.3
Threonine	25.0	17.4	24.8	24.8
Tryptophan	6.5	6.2	8.6	3.7
Tyrosine	19.2	16.8	22.2	42.4
Valine	29.7	21.9	30.8	32.3
Total	594.6	437.6	613.0	778.4

Table 2. Formulation and proximate composition of experimental diets formulated to contain 350g/kg protein and 80g/kg lipids. (g/kg as is).

Diet number	D1	D2	D3	D4	D5	D6	D7
Menhaden fishmeal[a]	200.0	150.0	100.0	50.0		50.0	
Soybean meal[b]	420.0	420.0	420.0	420.0	420.0	409.0	406.0
SPC - Nutrivance[c]		23.0	46.0	69.0	92.0	69.0	92.0
CPC - Empareal 75[d]		23.0	46.0	69.0	92.0	69.0	92.0
Fish oil[e]	44.0	48.0	53.0	55.0	59.0	56.0	59.0
Lecithin soy[f]	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cholesterol[g]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Corn Starch[g]	49.0	44.0	38.0	35.0	30.0	39.0	36.0
Whole wheat[g]	240.0	240.0	240.0	240.0	240.0	240.0	240.0
Mineral premix[h]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix[i]	18.0	18.0	18.0	18.0	18.0	18.0	18.0
Choline chloride[g]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Stay-C 35%[j]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
CaP-dibasic[k]	10.0	15.0	20.0	25.0	30.0	25.0	30.0
Lysine (78.8%)[l]						5.0	7.0
Methionine[m]						1.0	1.0

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

[b] De-hulled solvent extract soybean meal, Bunge limited, Decatur, AL, USA.

[c] Nutrivance™, Midwest Ag Enterprises, Marshall, MN, USA.

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

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

[f.] The Solae Company, St. Louis, MO, USA.

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

[h] Mineral premix (g/100 g 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.

[i] Vitamin premix (g kg^{-1} premix): Thiamin. HCl, 4.95; Riboflavin, 3.83; Pyridoxine. HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 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.

[j] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[k] T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.

[l.] Ajinomoto Heartland Onc, Chicago, IL, USA.

[m.] Smartamine ®M, Kemin, Des Moines, IA, USA.

Table 3. Proximate composition of experimental diets 1 to 7 (g/kg as is). Diets were analyzed by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

	D1	D2	D3	D4	D5	D6	D7
Protein	361.6	365.5	361.4	361.3	366.1	363.6	363.3
Moisture	68.0	70.1	70.1	68.1	67.3	76.3	77.4
Lipid	82.4	82.8	85.6	84.8	84.2	83.7	83.8
Fiber	29.5	32.5	35.3	35.8	37.7	33.8	34.1
Ash	79.9	75.7	73.2	69.1	65.3	68.6	63.4

Table 4. Response of juvenile shrimp (0.28g mean initial weight) to the various test diets over a 42-day growth trial under clear water conditions, E. W. Shell Fisheries Research Station (n=5). Values within a column with different superscripts are significantly different based on Tukey's multiple range test. Independent T-test result for comparing growth performance results of shrimp performance offered D4 as compared to D6, and D5 as compared to D7.

Diet	Biomass (g)	Mean weight (g)	Survival (%)	Weight gain [1](g)	Weight gain[2] (%)	FCR[3]	PRE[4] (%)
1	51.1^{b}	3.94^{bc}	86.7	3.67^{bc}	1334^{c}	1.78^{a}	24.7^{b}
2	47.6^{ab}	4.20^{c}	76.0	3.92^{c}	1428^{c}	1.71^{a}	25.9^{b}
3	46.5^{ab}	3.96^{bc}	78.7	3.67^{bc}	1301^{bc}	1.76^{a}	24.8^{b}
4	40.4^{ab}	3.37^{ab}	81.3	3.08^{ab}	1099^{bc}	2.13^{b}	20.5^{a}
5	36.8^{a}	3.24^{a}	76.0	2.96^{a}	1058^{a}	2.21^{b}	20.2^{a}
PSE[5]	2.661	0.158	5.498	0.157	55.294	0.071	0.835
P-value	0.008	0.001	0.678	0.001	0.0004	<0.0001	0.0001
6	45.1	3.52	85.3	3.24	1130	2.04	21.3
7	42.2	3.46	81.3	3.18	1131	2.12	22.1
P-value for t-test							
D4 VS D6	1.000	0.495	0.548	0.474	0.277	1.000	<0.0001
D5 VS D7	0.596	0.558	0.467	0.553	0.508	0.700	<0.0001

[1.] Weight gain (g) = Final weight-initial weight.

[2.] Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.

[3.] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).

[4.] PRE: Protein retention efficiency = (final weight × final protein content) − (initial weight × initial protein content) × 100 / protein intake.

[5.] PSE: Pooled standard error.

Table 5. Response of juvenile shrimp (0.26g mean initial weight) to the various test diets over a 56-day growth trial under green water conditions at the outdoor blue tank, E. W. Shell Fisheries Research Station (n=4).

Diet	Biomass (g)	Mean weight (g)	Survival (%)	Weight gain[1](g)	Weight gain[2] (%)	FCR[3]	PRE[4] (%)
1	1015.8	10.09	93.7	9.83	3772	1.00[a]	42.9
2	997.2	10.53	94.8	10.27	3922	1.02[ab]	42.3
3	993.1	10.49	94.8	10.23	3905	1.02[ab]	44.4
4	929.0	10.53	88.3	10.26	3929	1.10[ab]	43.8
5	926.4	10.54	88.0	10.28	3921	1.12[b]	44.7
PSE[5]	20.161	0.384	1.995	0.374	170.661	0.023	1.431
P-value	0.024	0.888	0.058	0.903	0.959	0.015	0.746

[1.] Weight gain (g) = Final weight-initial weight.

[2.] Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.

[3.] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).

[4.] PRE: Protein retention efficiency = (final weight × final protein content) − (initial weight × initial protein content) × 100 / protein intake.

[5.] PSE: Pooled standard error.

Figure 1. Response of juvenile shrimp (0.28 g mean initial weight) to dietary fishmeal replacement with protein concentrates (%) over 42 days in trial 1. The relationship between weight gain (y) of shrimp and the replacement fishmeal level (x) in the diets with protein concentrates.

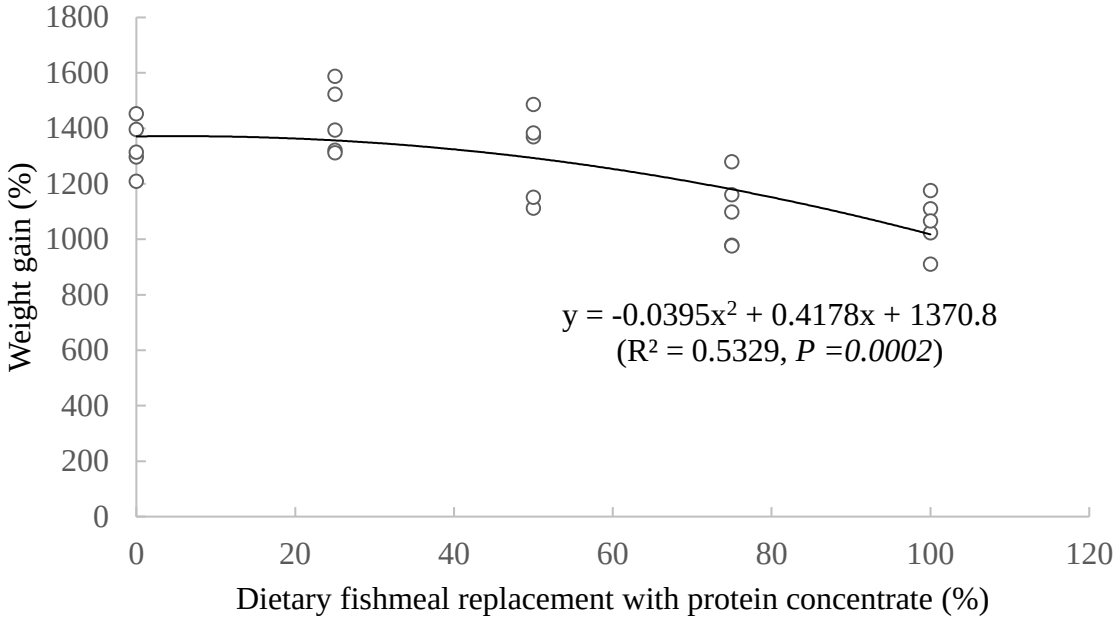
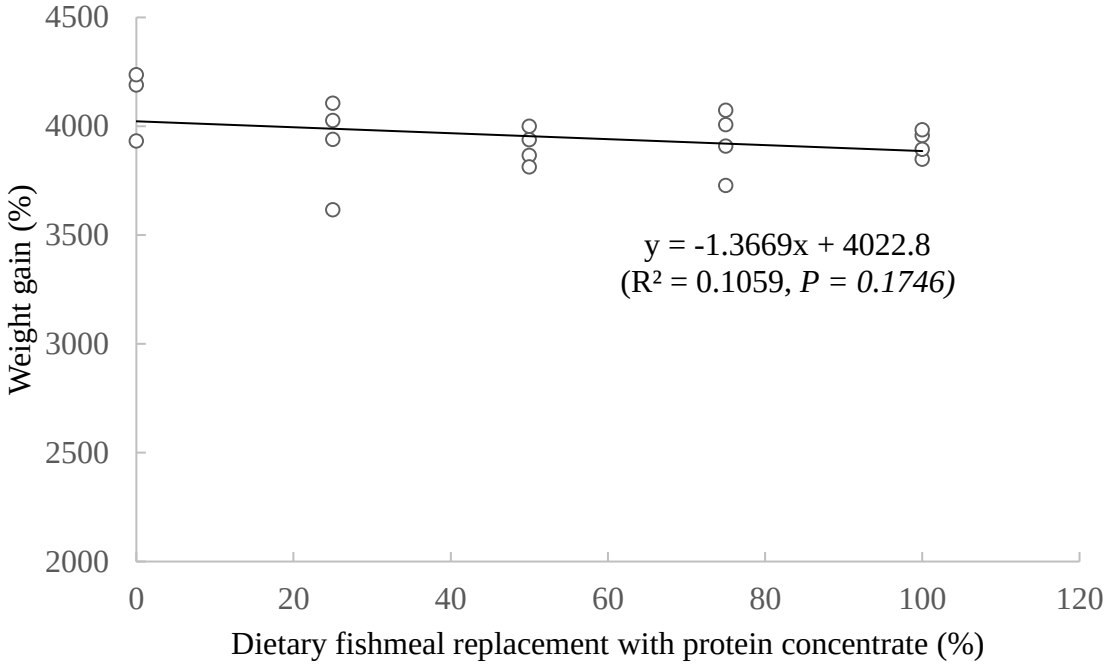


Figure 2. Response of juvenile shrimp (0.26 g mean initial weight) to dietary fishmeal replacement with protein concentrates (%) over 56 days in trial 2. The relationship between weight gain (y) of shrimp and the replacement fishmeal level (x) in the diets with protein concentrates.



CHAPTER V

USE OF SALMON BY-PRODUCT MEALS AS A REPLACEMENT FOR ANCHOVY MEAL IN PRACTICAL DIETS FOR THE PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Abstract

A series of trials were conducted with Pacific white shrimp *Litopenaeus vannamei*, to evaluate the efficiency of two salmon meals as compared to anchovy meal. The basal diet contained 200 g/kg anchovy meal, which was systematically replaced (0, 50, 75 and 100%) with salmon meal on an isonitrogenous basis. Additional diets were formulated with a hydrolyzed salmon meal to replace 50 and 100% anchovy meal. Each diet was randomly allocated to four replicate groups of 25 and 30 shrimp per tank in clear (indoor) and green (outdoor) water trials, respectively. The results suggested that growth performance and feed conversion ratio were not statistically different when salmon meal replaced anchovy meal in both trials. However, when hydrolyzed salmon meal was used to replace 100% of the anchovy meal, growth performance of the shrimp significantly decreased. The four kinds of fishmeal (anchovy, salmon by-product meals and menhaden) were evaluated in an ingredient digestibility trial using the 70:30 replacement technique. Overall, dry matter, energy, protein and individual amino acid digestibility of salmon meal were significantly higher than those of menhaden and anchovy meal. Results of this study demonstrated that salmon meals are a good protein sources which can replace anchovy meal.

Keywords: *Litopenaeus vannamei,* anchovy meal replacement, salmon meal, hydrolyzed salmon meal, growth trial, apparent digestibility coefficients

1. Introduction

The production of fishmeal (FM) and fish oil in many areas of the world is quite sustainable as these fisheries are well managed. However, the fishmeal production from natural fisheries is variable and is at or beyond maximum sustainable yields, which are well below demand, resulting in high price and providing an economic impetus to seek alternatives. There are a number of marine and non-marine ingredients originating from agriculture, fisheries and animal processing that can potentially serve as replacements for fishmeal in shrimp feeds (Amaya, Davis, & Rouse, 2007; Liu et al., 2012; Xuan Qiu, Tian, & Davis, 2017; Roy et al., 2009; Sookying & Davis, 2011; Suarez et al., 2009; Tan et al., 2005; Ye, Wang, Li, Sun, & Liu, 2011). However, many of these studies report significant reduction in growth when fishmeal is completely replaced with non-marine ingredients. One of the major reasons is that these non-marine ingredients typically contain less balanced nutrients (such as protein, amino acids, fatty acids, minerals profiles) and are less palatable which can result in reduced performance as compared to FM. Because of the nutritional quality of FM, many farmers still prefer to add fishmeal in the feed even if it is not required. Thus, it is still important to explore new protein sources which are cost-effective, sustainable, and environmentally friendly to reduce feed cost and support the rapidly expanding shrimp industry.

Salmonids are one of the most successful aquaculture species, and the annual average harvest of salmon has increased rapidly in the last two decades. The production of salmon has grown from a few thousand tonnes in 1980 to about 2.5 million tonnes in 2014 (Abolofia, Asche, & Wilen, 2017). Meanwhile, the industry is also looking for an alternative use of protein by-product of the salmon slaughtering process. Current estimates are that salmon meal could be 25% cheaper as a dietary source of protein than menhaden fish meal. Salmon meal and its by-products have shown their potential as fishmeal replacement in a number of studies. Fehringer, Hardy, and Cain (2014)

suggested that the utilization of pink salmon meal to replace 25% anchovy meal can stimulate some innate responses for rainbow trout (*Oncorhynchus mykiss*) without causing negative effects in growth. James et al. (2013) reported that salmon meal can replace 100% herring meal in the manufactured diet of red king crab (*Paralithodes camtschaticu)* without compromising its growth performance and economic benefit. In addition, Lee et al. (2015) demonstrated that supplementing 7% salmon by-product as a replacement for fishmeal can increase feed intake and metabolic efficiency for rainbow trout.

The successful utilization of salmon meal and its by-products as a replacement for traditional fishmeal (e.g menhaden and anchovy meal) in fish feeds indicates the possible application of these products in the diet for Pacific white shrimp, *Litopenaeus vannamei*. Hence, the objectives of this study were to evaluate the growth performance and feed utilization of Pacific white shrimp which were fed diets using salmon meal products to replace anchovy meal and to determine the apparent digestibility coefficients for salmon meal products as compared to menhaden and anchovy meal.

2. Materials and Methods

2.1. Experimental diets

The different fishmeals used in the research included menhaden fishmeal (Omega Protein Inc., Houston, TX, USA), anchovy meal (Fiordo Austral Company, Cardonal, Puerto Montt, Chile), salmon meal (SM, Salmo-Pet, Fiordo Austral Company, Cardonal, Puerto Montt, Chile) and hydrolyzed salmon meal (HSM, Amino Salmon P60, Fiordo Austral Company, Cardonal, Puerto Montt, Chile). Proximate and amino acid composition for each of the fishmeal ingredients are in Table 1. In the growth study, all test diets were formulated on an iso-nitrogenous and iso-lipidic basis to contain 350 g/kg protein and 80 g/kg lipid (Table 2). The basal diet was primarily

composed of 200 g/kg anchovy meal, 440 g/kg soybean meal, 230 g/kg whole wheat and corn starch. Four experimental diets were produced by supplementing the basal diet with graded levels of salmon meal (0, 105.5, 158.5 and 211 g/kg diet) to replace 0, 50, 75 and 100% of the anchovy meal, which were designated as Basal, SM50, SM75, and SM100, respectively. In addition, two experimental diets were formulated with two levels of a hydrolyzed salmon meal (HSM, 103 and 206 g/kg diet) to replace 50 and 100% anchovy meal, which were designated as HSM50 and HSM100, respectively. In addition, a reference diet for digestibility trial was utilized to determine digestibility coefficients in conjunction with 10 g/kg chromic oxide as an inert marker and 70:30 replacement technique.

The experimental diets were produced at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University (Auburn, AL, USA), using standard procedures for shrimp feeds. Diets were prepared by mixing the pre-ground dry ingredients in a food mixer (Hobart, Troy, OH, USA) for 10–15 minutes. 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 (< 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 (-20 °C) until needed. All the ingredients and diets were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate composition and amino acid (AA) profile (g/kg as is) (Table 1 and 3).

Processed pellets were then evaluated for pH and dry matter loss. To determine the pH of the feed, 5g samples of pellets were placed in beakers containing 80 ml deionized water which was stirred and allowed to set for one hour in triplicate. The water was then decanted and 40ml

supernatant sample was taken to measure diets' pH using a pre-calibrated pH meter. Dry matter loss was measured as the ratio of dry weight of pellet retained on a wire screen in a PVC tube after immersion in water for 45 minutes. For this analysis the dry weight of each screen using three replicates per diet was first determined. We placed 3 g of dry feed in each pre-weight PVC tube and placed in shaker bath (60 shakes/min) containing tap water for 45 minutes. We then removed each screen and dried them in the oven drying at 105^0 C to a constant weight. Dry matter loss was calculated by difference as described by Lim and Cuzon (1994).

Dry matter loss (%) = (dry weight of diet before immersion – dry weight of diet after immersion and dry)/ dry weight of diet before immersion *100

2.2. Experiment procedure

Two growth trials were conducted, one in a clear water (indoor) and one in a green water (outdoor) recirculating aquaculture system (RAS). The clear water system was maintained in an indoor building at E. W. Shell Fisheries Center (Auburn, AL, USA) and received limited natural light with lamp (24h) on and not natural food sources. It has identical tanks to that of the green waters system but includes a fluidized bed biological filter and bead filter for maintaining water quality. The green water trial was conducted in an outdoor system at Claude Peteet Mariculture Center (Gulf Shores, AL USA), which was managed to have natural productivity present. Both of the research systems consist of a central reservoir (~1,000 L), a 0.25 hp circulation pump, 24 circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter) and supplemental aeration. For the green water system, a second sump pump is used to move unfiltered water from a shrimp production pond to the central reservoir at a rate of ~8 L min^{-1} between 8:00 h and 12:00 h. This results in the replacement of system water every few days, replenishing natural productivity to mimic a production pond setting. Each tank and central reservoir are equipped with

two air stones connected to a 0.5 hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA) to supply aeration.

Pacific white shrimp post-larvae were obtained from Shrimp Improvement Systems (Islamorada, FL, USA) and nursed in an indoor recirculating system using commercial feeds until they reached an appropriate size for research. Juvenile shrimp (initial mean weight 0.63±0.02g and 0.98±0.05g) is randomly selected and stocked at 25 and 30 shrimp per tank in the clear and green water trials, respectively.

During the growth trial, four replicate groups per treatment were assigned and shrimp were fed two times per day in the green water system, and four times per day in the clear water system. In general, feed inputs are calculated assuming the shrimp will double their weight weekly up to one gram, then gain 0.8 g weekly with a feed conversation ratio (FCR) of 1.8 for clear water following the standard feeding strategy of Xuan Qiu et al. (2017) or gain 1.2 g weekly with FCR of 1.3 for green water following the standard feeding strategy of Sookying and Davis (2011).

Shrimp in the clear water system were counted once a week to adjust the daily feed input. At the end of the growth trial, shrimp in each tank were counted and weighed to calculate survival, biomass, mean weight, FCR, weight gain, and protein retention efficiency (PRE). After weighing and counting the shrimp, 4-6 shrimp per tank were randomly selected and frozen at −20°C for whole body samples to be utilized for later protein retention analysis. Crude protein content of whole body was determined by Dumas combustion method (Elemental Analyzer rapid N cube, Villeurbanne, France).

Water temperature was maintained at around 29 °C using a submerged 2,600 W heater (Aquatic Eco-Systems Inc., Apopka, Florida, USA). Dissolved oxygen was maintained near saturation (6 ppm) using air stones in each aquarium and the sump tank with a common airline

connected to a regenerative blower. During growth trials, dissolved oxygen, water temperature and salinity were measured twice daily using a YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH, USA), pH was measured twice weekly using a waterproof pH Testr30 (Oakton instrument, Vernon Hills, IL, USA), and total ammonia nitrogen and nitrite were analyzed once per week. Under clear water conditions, DO, temperature, salinity, pH, total ammonia nitrogen, and nitrite were maintained at 6.65±0.22 mg L^{-1}, 29.73±0.35 °C, 6.58±0.81 ppt, 7.62±0.44, 0.20±0.06 mg L^{-1}, and 0.12±0.03 mg L^{-1}, respectively. Under green water conditions, water quality including DO, temperature, salinity, pH, was measured twice daily using YSI Proplus multimeter (YSI, Yellow Springs, OH, USA) and total ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen measured once weekly parameters were maintained at 7.50±0.66 mg L^{-1}, 27.07±2.8 °C, 6.65±1.46 ppt, 7.94±0.28, 0.21±0.50 mg L^{-1}, and 0.52±0.64 mg L^{-1}, 12.10±9.33 mg L^{-1}, respectively. Water quality conditions in all trials were suitable for normal growth and survival of this specie.

2.3. Digestibility trial

The formulation of the reference diet is shown in Table 4. The digestibility diets were offered to groups of 8 shrimp (~ 6 g average weight). Shrimp were allowed to acclimate for three to four days to each test diet before starting the collection of feces. Prior to each feeding the tanks were cleaned. The shrimp were then offered a slight excess of feed. One hour after feeding, uneaten feed was removed, and the feces were collected by siphoning onto a 500 µm mesh screen. Shrimp were offered about five feedings per day with the feces obtained after the first feeding discarded. Collected feces were rinsed with distilled water, dried, and then stored in sealed plastic containers in a freezer for subsequent analysis. Samples were collected for four days or until a suitable

quantity for analyses was obtained. Daily samples were pooled by tank and three replicate aquaria (n=3) were utilized for each treatment.

Dry matter, crude protein, and energy were determined for the fecal, diet, and ingredient samples according to established procedures. Crude protein content of the sample was analyzed using the micro-Kjeldahl method (Ma & Zuazaga, 1942). Gross energy content was determined using a micro-calorimetric adiabatic bomb using benzoic acid as standard (Parr 6725, Moline, IL, USA). Amino acids were analyzed by University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory. Chromic oxide was analyzed following the McGinnis and Kasting (1964) procedures. Apparent digestibility coefficients (ADCs) of the dry matter, protein, and energy for each diet were then calculated by Cho, Slinger, and Bayley (1982) method as follows:

ADC of dry matter (%) =100 - [(100×Cr_2O_3in feed / Cr_2O_3 in feces) × 100]

ADC of nutrients or energy (%) = [1− (dietary Cr_2O_3 / fecal Cr_2O_3) × fecal nutrient or energy /dietary nutrient or energy]] × 100

ADC of the test ingredients were calculated as follows Bureau and Hua (2006):

ADC (%) =ADC_{TD}+ (ADC_{TD}−ADC_{RD}) ×(0.7×$Nutr_{RD}$/0.3×$Nutr_{ING}$)

Where ADC_{TD} is the apparent digestibility of the nutrients or energy in the test diet (TD), ADC_{RD} is the apparent digestibility of nutrients or energy in the reference diet (RD), $Nutr_{RD}$ is the nutrients or energy concentration in the RD, and $Nutr_{ING}$ is the nutrients or the energy concentration in the test ingredient.

2.4. Statistical analysis

All data were analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). All data was subjected to one-way analysis of variance to determine significant differences ($P<0.05$) among treatments, followed by Tukey's multiple comparison test to determine differences between

treatment means. The pooled standard error was used across all the data as the variance of each treatment is the same.

3. Results

3.1. Growth trial

The growth performance of shrimp offered diets containing different types and levels of salmon meal under clear water and green water condition is presented in Table 5 and 6. Under clear water conditions, the results showed no differences in final biomass (247.82 to 265.65g), survival (92 to 98%), FCR (1.6-1.7), final mean weight (FMW) (10.58-10.83 g), percent weight gain (1575-1621%) and PRE (30.0-32.2%) when up to 100% anchovy meal was replaced by SM. However, the growth of shrimp fed with the diet using hydrolyzed salmon meal to completely replace anchovy meal was significantly reduced compared to those fed with the basal diet and the diets containing different levels of SM. Under green water condition, the results showed the same trend. Under green water conditions, there was no significant difference in final biomass (307.05 to 333.05 g), survival (93 to 99%), FCR (1.2-1.3), final mean weight (FMW) (10.73-11.2 g), percent weight gain (988-1042%) and PRE (37.7-43.1%) when up to 100% anchovy meal was replaced by SM. Shrimp fed with diets using salmon meal to replace 50% and 75% anchovy meal had significantly higher growth performance than those fed with the diet using hydrolyzed salmon meal to replace 100% of the anchovy meal.

3.2. Digestibility trial

Apparent dry matter (ADMD), apparent energy (AED) and apparent protein (APD) digestibility coefficients for the diet (D) and ingredient (I) using 70:30 replacement technique offered to Pacific white shrimp are presented in Table 7. The ADMDD, AEDD and APDD for the

reference diet were 74.19%, 80.07% and 90.48%, respectively. Those ADMDD, AEDD and APDD values for the diets supplemented with menhaden fishmeal and anchovy meal were 64.9 and 64.86%, 74.93 and 75.81%, and 78.16 and 78.53% respectively. Those ADMDD, AEDD and APDD values for the diets supplemented with salmon meal and hydrolyzed salmon meal were 72.3 and 67.34%, 82.38 and 76.16%, and 88.24 and 90.95%, respectively. Those ADMDI, AEDI and APDI values for the ingredients of salmon meal and hydrolyzed salmon meal were 64.12 and 47.59%, 84.13 and 64.76%, and 85.75 and 90.85%, respectively. Those ADMDI, AEDI and APDI values for the ingredients menhaden fishmeal and anchovy meal were 39.43 and 39.32%, 60.16 and 65.49%, and 66.97 and 68.45%, respectively.

The results showed that the ADCs of dry matter, energy and protein for the salmon meal were significantly higher than those of menhaden fishmeal and anchovy meal. Besides, the ADCs of dry matter and energy of salmon meal were also higher than those in hydrolyzed salmon meal diet. The ADCs of protein for the hydrolyzed salmon meal were significantly higher than those of menhaden fishmeal and anchovy meal and similar to that of salmon meal. For the ingredient digestibility results, it also shows the same trend.

The apparent amino acid digestibility (AAD) value for menhaden fishmeal, anchovy meal, salmon meal and hydrolyzed salmon meal using 70:30 replacement technique offered to Pacific white shrimp are presented in Table 8. The amino acid digestibility coefficients for menhaden fishmeal, anchovy meal, salmon meal and hydrolyzed salmon meal were ranged from 44.08 to 82.2%, 54.69 to 85.49%, 61.81 to 95.01% and 75.69 to 95.45%, respectively. Most of the apparent amino acid digestibility coefficients (except cysteine) of menhaden fishmeal and most of apparent amino acid digestibility coefficients (except tryptophan and cysteine) of anchovy meal were significantly decreased when compared to those consuming diets including salmon meal.

Most of the apparent amino acid digestibility coefficients (except taurine) of menhaden fishmeal and anchovy meal were significantly decreased when compared to those provided hydrolyzed salmon meal. Apparent digestibility coefficients of threonine, phenylalanine, lysine, arginine, serine, glycine, alanine, cysteine, glutamic acid, proline and aspartic acid of hydrolyzed salmon meal were similar to those of salmon meal. Apparent digestibility coefficients of isoleucine, tryptophan, leucine, methionine, valine, histidine, tyrosine of hydrolyzed salmon meal was higher to those of SM. Apparent digestibility coefficients of taurine of salmon meal were increases compared to that in hydrolyzed salmon meal.

3.3 Dietary pH and dry matter loss

All the diets pH and dry matter loss were presented in Table 9. The pH in diet 5 and 6 were significantly lower than the other diets and the pH of diet 6 significant lower than that of diet 5. The dry matter loss in diet 3 and 4 were significantly lower than that in diet 1 and 2. However, the dry matter loss in diet 5 and 6 was significantly higher than that in diet 1.

4. Discussion

In an effort to reduce the cost of shrimp diets, manufacturers have continued to consider sustainable and cheaper protein sources. Salmon meal and its by-products have been proven to be a promising alternative ingredient for aquatic animal feeds (Deng, Ju, Dominy, Bechtel, & Smiley, 2013; James et al., 2013; Lee et al., 2015; Liang, Wang, Chang, & Mai, 2006). Nutritional quality is an important factor to take into consideration before new ingredients are incorporated into feeds (Ahlstrøm, Tjernsbekk, & Tauson, 2012). Protein levels of meals from the present study, salmon meal (646g/kg protein) and hydrolyzed salmon meal (662g/kg protein) have higher level of protein as compared to pink salmon hydrolysate meal (552g/kg), red salmon hydrolysate meal (514g/kg),

and salmon meal with crushed bones (511g/kg) as reported by Folador et al. (2006). The salmon meal and hydrolyzed salmon meal not only have high-quality crude protein but also have similar AA profiles as compared to menhaden fishmeal and anchovy meal (Table 1). Hence, these products have the potential to become reasonable protein ingredients in shrimp feed formulations.

The ADCs value can provide estimates of nutrient availability to select ingredients, which can then be used to help optimize the nutritional value and cost of formulated diets (Brunson, Romaire, & Reigh, 1997).The ingredient digestibility of menhaden fishmeal is variable, however, the APD and AED of fishmeal in the present study was 66.97% and 60.16%, which was also in line with previous studies reported by X Qiu, Nguyen, and Davis (2017) (menhaden: 65.78-69.77%, and 65.78-69.77%) and Brunson et al. (1997) (menhaden: 75.85% and 74.59%). In the digestibility trial, the protein and energy digestibility values of salmon meal were significantly higher than those of MFM and anchovy meal. For the HSM, the ADMD and AED for both the test diet and ingredient were significantly lower than those of salmon meal, and similar to those of menhaden fishmeal and anchovy meal. As one would expect, increased protein digestibility translated to amino acids digestibility values for salmon meal and hydrolyzed salmon meal to be higher than menhaden fishmeal and anchovy meal in the present study. From the digestibility results, salmon meal and hydrolyzed salmon meal are more digestible than menhaden fishmeal and anchovy meal for shrimp. Salmon meal products are not typically utilized as a protein source in shrimp practical diets; hence, information concerning digestibility of salmon meal in the Pacific white shrimp is limited in the literature. However, the higher ADCs of energy, protein and amino acid digestibility in salmon meal and hydrolyzed salmon meal were also similar to other protein ingredients for shrimp diets, such as soybean meal, Peruvian fishmeal, peanut meal, yeast, plasma protein meal and shrimp by-product meal (X Qiu & Davis, 2017; Yang et al., 2009; Zhou, Davis, & Buentello,

2015). In the case of same kind of ingredient and species, Deng et al. (2013) reported that using salmon testes meal protein to replace 91% fishmeal protein did not decrease the apparent digestibility coefficients for dry matter (54.1%), lipid (87%), protein (85.5%) or gross energy (64.1%) of the test diets for shrimp.

The high digestibility value of salmon meal and hydrolyzed salmon meal would indicate that they are good nutrient sources. Digestibility coefficients are a good indication of nutrient availability, but biological assays are required to confirm suitability as feed ingredients on growth performance. In this work, we evaluated those salmon meals to replace anchovy meal in shrimp diet in both clear water systems lacking natural foods as well as green water systems for which natural foods are available. The present study demonstrated that measured parameters for growth performance are not statistically different when salmon meal replaces 100% anchovy meal in either clear or green water conditions. The reason why the growth performance in SM100 did not show much better than basal diet though the APD of salmon meal is 17% points higher than the APD of anchovy meal, is that the digestible protein for SM100 is just 3-4% points higher than the basal with 20% anchovy meal supplement. In this study, salmon meal showed a much greater replacement level when compared to the results from Fehringer et al. (2014), which suggested that adding pink salmon meal to replace 25% anchovy meal has no negative effect on the growth performance of rainbow trout, and can also stimulate some innate responses. Deng et al. (2013) also demonstrated that the pink salmon testes meal can replace up to 46% fishmeal without impairing growth. Similarly, James et al. (2013) showed that up to 100% herring meal could be replaced by cheaper salmon meal in the manufactured diets for red king crab (*Paralithodes camtschaticu)* without negatively affecting growth performance and economic benefit.

As mentioned above, the favorable response of the shrimp to salmon meals used in the present experiment is probably due to the high-quality protein content of the ingredients used in terms of both nutrient profile and relative improved digestibility. As different fish meals typically also have similar profiles and are considered an excellent nutrient source, there is considerable interest in these meals. Processing by-products also have generally been favored over plant sources of protein as alternatives to shrimp diets due to their relative good palatability. Furthermore, salmon meal also contains other functional nutrients which are not measured in this study, such as nucleotides (Plante et al., 2008) and steroid hormones (Borghetti, Iwamoto, Hardy, & Sower, 1989; Matty & Cheema, 1978; Matty & Lone, 1985; Pelissero & Sumpter, 1992), which can also increase growth performance, feed intake, as well as immune response. Some studies also demonstrated that salmon meal is not only a good fishmeal replacement source, but also a functional protein for feed (Deng et al., 2013; Lee et al., 2015).

For the hydrolyzed salmon meal evaluated in this study, results indicated that it can replace 50% anchovy meal without significantly altering the growth performance of shrimp. Similarly, other studies demonstrated that the fish protein hydrolysates can replace 10-15% in Japanese sea bass *Lateolabrax japonicus* (Liang et al., 2006), post-smolt Atlantic salmon *Salmo salar* (Refstie, Olli, & Standal, 2004), and juvenile and adult Atlantic salmon *Salmo salar* (Espe & Lied, 1999). The results of this study demonstrated that salmon meal is a good alternative protein source and fishmeal substitute for shrimp diets when used at lower levels (21.1%). Hence, 10.3% hydrolyzed salmon meal can also be used to replace fishmeal though not as efficiently as the salmon meal.

Although the ADC values for hydrolyzed salmon meal were similar to the other two kinds of fishmeal, the hydrolyzed salmon meal was still not as efficiently utilized for shrimp growth. The potential of an undesirable taste is one of the biggest shortcomings to successful implementation

of hydrolyzed protein (Rustad, Storrø, & Slizyte, 2011). On the one side, the pH in HSM50 and HSM100 diets were significantly lower than that in the basal diet. The degree of the bitter taste has been found in some instance to relate to the degree of the hydrolysis (Shahidi, 1994). An especially high degree of hydrolysis is between 4 to 40%. In the present study, total free AAs in the hydrolyzed salmon meal accounted for more than 31% of total protein. Based on the authors' opinion, palatability is not likely the key factor causing growth differences in this study, since all of the groups of shrimp were observed to consume all of the feed offered at least in the clear water trial. However, stability maybe another issue for the diets adding hydrolyzed salmon meal. In the present study, the dry matter loss (17% in the basal diet vs 21% in HSM50 and 26% in HSM100) has significant difference between the basal diet with the diets containing HSM. This is probably an issue in that hydrolyzed products do not contain texturizable proteins which may lead to reductions in stability of the diet and a subsequent reduced intake by the shrimp. Additionally, several studies have shown that hydrolyzed protein with AA is not as efficient as protein-bound AAs due to the high potential of leaching loss of AAs (Gu, Zhang, Bai, Mai, & Xu, 2013; Teshima, Ishikawa, Alam, Koshio, & Michael, 2004) and the difference in uptake of free AAs and AAs digested from intact proteins in the intestine (Ambardekar, Reigh, & Williams, 2009; Schuhmacher, Wax, & Gropp, 1997). Moreover, a diet with a high level of hydrolyzed protein could induce a burst of nutrients—AAs and peptides—in the intestine, causing a saturation of transporter mechanisms (Kotzamanis, Gisbert, Gatesoupe, Infante, & Cahu, 2007). The for mentioned reasons could explain in part the negative effects on growth performance when high levels of hydrolyzed protein are supplemented. Similarly, diets supplied with high inclusion of fish silage have repeatedly been shown to affect growth negatively (Hardy, Shearer, Stone, & Wieg, 1983; Heras,

McLeod, & Ackman, 1994; Niu et al., 2014; Stone, Hardy, Shearer, & Scott, 1989; Wei, Liang, Mu, Zheng, & Xu, 2016).

Most of the novel fishmeal replacement strategies have been carried out under only laboratory clear water conditions which differs greatly from production conditions. The practical diets of this study were evaluated under clear and green water conditions to improve the validity of the data. The growth performance results in both trials showed the same trends. Additionally, the best overall shrimp growth performance [e.g. survival (97 vs. 93%)] and feed utilization was observed in animals raised under green water conditions. The feed conversion ratio and protein retention efficiency for shrimp reared in green water were considerably improved as compared to those observed in the clear water system. The primary reason is due to environmental effects and the ability to obtain additional nutrients from natural sources including algae, bacteria and some invertebrates which will decrease the observed FCR and protein retention (Tacon et al., 2002). The data collected from the green water trial closely mimic farm production conditions, and therefore has greater reference value for farmers (Tacon, 1996).

5. Conclusion

Results of this study indicate that salmon meal can be used effectively in practical diets for Pacific white shrimp as a replacement for up to 100% anchovy meal without causing impaired growth performance in both clear and green water conditions. Hydrolyzed salmon meal has also been proven to be a suitable protein source for replacing fishmeal in shrimp diet. However, hydrolyzed salmon meal can be used to replace only 50% anchovy meal in the Pacific white shrimp practical diet. Under green water conditions, shrimp growth showed the same trend as those reared under clear water. Future studies regarding the improvement of the processing technologies of

hydrolyzed salmon meal and the demonstration of salmon meal in practical diets under pond culture conditions are warranted.

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Table 1. Proximate and amino acid composition (g/kg as is) of test ingredients used in these trials. Analyses were conducted at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Nutrient	Menhaden Fishmeal	Anchovy Meal	Salmon Meal	Hydrolyzed Salmon Meal
Crude Protein	627.7	683.0	646.0	662.0
Moisture	96.0	85.3	91.4	77.0
Crude Fat	105.0	75.7	106.0	30.4
Ash	182.0	158.0	160.3	173.0
Phosphorus	28.2	23.2	27.0	14.7
Amino acids				
Alanine	39.8	41.4	42.2	38.0
Arginine	37.5	38.1	38.1	35.1
Aspartic Acid	56.0	58.7	50.0	53.6
Cysteine	5.1	6.5	4.6	5.4
Glutamic Acid	80.2	80.1	70.6	73.6
Glycine	45.7	38.2	64.5	43.7
Histidine	13.1	17.8	14.0	15.2
Isoleucine	23.9	28.9	22.5	26.7
Leucine	43.4	48.3	37.9	41.4
Lysine	46.8	51.0	39.3	47.5
Methionine	16.7	16.5	14.9	15.1
Ornithine	0.6	0.6	1.9	0.7
Phenylalanine	24.8	26.8	21.7	23.0
Proline	28.8	23.6	34.1	25.4
Serine	24.2	22.8	23.1	22.6
Taurine	7.1	6.9	8.9	3.8
Threonine	25.4	27.6	23.8	25.3
Tryptophan	6.2	7.6	5.5	5.7
Tyrosine	14.6	20.5	18.8	23.4
Valine	28.2	34.7	28.7	30.8

Table 2. Formulation of test diets used to evaluate various salmon meal products. (g/kg as is).

Diet number	1	2	3	4	5	6
Diet name	Basal	SM50	SM75	SM100	HSM50	HSM100
Anchovy meal[a]	200.0	100.0	50.0	0.0	100.0	0.0
Soybean meal[b]	440.0	440.0	440.0	440.0	440.0	440.0
Salmon meal[c]	0.0	105.5	158.5	211.0	0.0	0.0
Hydrolyzed salmon meal[d]	0.0	0.0	0.0	0.0	103.0	206.0
Fish oil[e]	51.5	47.9	46.1	44.3	55.9	60.4
Lecithin soy[f]	10.0	10.0	10.0	10.0	10.0	10.0
Corn Starch[g]	27.5	26.6	26.4	26.7	15.1	4.6
Whole wheat[g]	230.0	230.0	230.0	230.0	230.0	230.0
Mineral premix[h]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix[i]	18.0	18.0	18.0	18.0	18.0	18.0
Choline chloride[g]	2.0	2.0	2.0	2.0	2.0	2.0
Stay-C 35%[j]	1.0	1.0	1.0	1.0	1.0	1.0
CaP-dibasic[k]	15.0	14.0	13.0	12.0	20.0	23.0

[a] Anchovy meal: Fiordo Austral Company, Cardonal, Puerto Montt, Chile.

[b] De-hulled solvent extract soybean meal, Bunge limited, Decatur, AL, USA.

[c] Salmon meal: salmo-Pet, Fiordo Austral Company, Cardonal, Puerto Montt, Chile.

[d] Hydrolized salmon meal: amino salmon P60, Fiordo Austral Company, Cardonal, Puerto Montt, Chile.

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

[f.] The Solae Company, St. Louis, MO, USA.

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

[h] Mineral premix (g/100 g 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.

[i] Vitamin premix (g kg^{-1} premix): Thiamin. HCl, 4.95; Riboflavin, 3.83; Pyridoxine. HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 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.

[j] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[k] T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.

Table 3. Proximate and amino acid composition of experimental diet (g/kg as is). Analyses were conducted by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Diet number	1	2	3	4	5	6
Diet name	Basal	SM50	SM75	SM100	HSM50	HSM100
Crude Protein	389.5	388.1	386.1	378.2	378	368.7
Moisture	62.1	65.6	62.5	65.1	59.5	77.5
Crude Fat	86.1	85.5	87.3	82.6	96.1	100.2
Ash	76.6	77.9	78	77.9	81.2	83.5
Amino acids						
Alanine	18.7	19.2	19.4	19	18.4	17.9
Arginine	24.6	24.8	24.5	24.2	23.7	23.2
Aspartic Acid	37.5	36.5	35.5	34.4	35.6	34.6
Cysteine	5.2	4.7	4.8	4.6	4.8	4.7
Glutamic Acid	65.6	64.3	63.5	62.5	63.3	61.7
Glycine	18.2	21.2	23	24.2	18.6	19.1
Histidine	10.9	10.1	9.7	9.3	9.9	9.5
Isoleucine	17.6	16.9	16.4	15.8	16.8	16.3
Leucine	28.5	27.5	26.6	25.7	27.2	26.1
Lysine	25.4	24.2	23.5	22.5	24.3	23.8
Methionine	6.9	6.8	6.8	6.6	6.6	6.4
Ornithine	0.3	0.4	0.5	0.5	0.3	0.3
Phenylalanine	18.3	17.7	16.8	16.2	17.3	16.7
Proline	18.4	19.3	19.8	20.3	18.2	17.6
Serine	15	15.1	14.9	14.8	14.6	14.4
Taurine	2.3	2.4	2.5	2.7	1.9	1.6
Threonine	14.7	14.3	14	13.7	14.2	13.8
Tryptophan	4.9	5	4.8	4.7	4.8	4.6
Tyrosine	12.5	12.6	11.9	11.9	12.7	13.1
Valine	19.3	18.8	18.5	17.7	18.7	18

Table 4. Composition of digestibility reference diet (g/kg as is).

Ingredients	g/kg
Menhaden fishmeal[a]	100.0
Soybean meal[b]	325.0
Fish oil[c]	32.0
Corn starch[d]	21.0
Whole wheat[d]	476.0
Mineral premix[e]	5.0
Vitamin premix[f]	18.0
Choline chloride[f]	2.0
Stay C[g]	1.0
Lecithin[h]	10.0
Chromic oxide[h]	10.0

[a] Menhaden fishmeal, special select: Omega Protein Inc., Houston, TX, USA.

[b] De-hulled solvent extract soybean meal, Bunge limited, Decatur, AL, USA.

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

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

[e] Mineral premix (g/100 g 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.

[f] Vitamin premix (g kg^{-1} premix): Thiamin.HCl, 4.95; Riboflavin, 3.83; Pyridoxine.HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 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.

[g] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[h] The Solae Company, St. Louis, MO, US.

Table 5. Response of juvenile shrimp (0.63±0.02g mean initial weight) to the various test diets over a 56 days growth trial under clear water conditions, E. W. Shell Research Station. Values within a column with different superscripts are significantly different based on Tukey's multiple range test. Each value represents the mean of four replicates.

Diet	Biomass (g)	Mean Weight[1](g)	Survival (%)	Weight gain[2] (g)	Weight gain[3] (%)	Feed conversion ratio[4]	Weekly Gain[5] (g)	PRE[6] (%)
Basal	248.5^{bc}	10.71^{b}	93	10.09^{b}	1621^{b}	1.6^{a}	1.26^{b}	30.0^{abc}
SM50	247.8^{bc}	10.79^{b}	92	10.16^{b}	1603^{b}	1.7^{a}	1.27^{b}	31.0^{bc}
SM75	265.6^{c}	10.83^{b}	98	10.19^{b}	1599^{b}	1.6^{a}	1.27^{b}	32.2^{c}
SM100	251.2^{bc}	10.58^{b}	95	9.95^{b}	1575^{b}	1.7^{a}	1.24^{b}	30.0^{bc}
HSM50	219.0^{ab}	9.73^{ab}	90	9.11^{ab}	1467^{ab}	1.8^{ab}	1.14^{ab}	27.6^{ab}
HSM100	193.0^{a}	8.58^{a}	90	7.96^{a}	1299^{a}	2.1^{b}	1.00^{a}	25.4^{a}
PSE[7]	9.52	0.32	2.60	0.32	48.88	0.06	0.04	5.17
P-value	0.0005	0.0005	0.27	0.0005	0.0008	<0.0001	0.0005	0.002

[1.] MW: Mean Weight.

[2.] Weight gain(g) = Final weight-initial weight.

[3.] Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.

[4.] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).

[5.] Weekly Gain = (Final weight − Initial weight) / weeks.

[6] PRE: Protein retention efficiency = (final weight × final protein content) − (initial weight × initial protein content) × 100 / protein intake.

[7.] PSE: Pooled standard error.

Table 6. Response of juvenile shrimp (0.98±0.05g mean initial weight) to the various test diets over a 56 days growth trial under green water conditions at the Claude Peteet Mariculture Center. Values within a column with different superscripts are significantly different based on Tukey's multiple range test.

Diet	Biomass (g)	Mean Weight (g)[1]	Survival (%)	Weight gain (g) [2]	Weight gain (%)[3]	Feed Conversion Ratio[4]	Weekly Gain (g) [5]	Protein Retention Efficiency (%)[6]
Basal	311.3^{abc}	10.73^{abc}	97	9.75^{abc}	988^{abc}	1.3^{ab}	1.22^{abc}	40.0^{ab}
SM50	333.1^{c}	11.20^{c}	99	10.22^{c}	1042^{c}	1.2^{a}	1.28^{c}	43.1^{b}
SM75	307.1^{abc}	10.98^{bc}	93	10.01^{bc}	1028^{c}	1.3^{ab}	1.25^{bc}	37.7^{ab}
SM100	315.4^{bc}	10.98^{bc}	96	9.99^{bc}	1015^{bc}	1.3^{ab}	1.25^{bc}	41.1^{b}
HSM50	277.2^{ab}	9.43^{ab}	98	8.44^{ab}	863^{ab}	1.5^{bc}	1.06^{ab}	34.3^{a}
HSM100	266.0^{a}	9.10^{a}	98	8.54^{a}	832^{a}	1.5^{c}	1.07^{a}	33.8^{a}
PSE[7]	10.24	0.39	1.63	0.38	35.64	0.05	0.05	11.51
P-value	0.002	0.0032	0.2084	0.0025	0.0013	0.0007	0.0022	0.0012

[1] Mean Weight = Biomass/number of shrimp.

[2] Weight gain (g)= Final weight-initial weight.

[3] Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.

[4.] Feed conversion ratio = Feed offered / (Final weight − Initial weight).

[5.] Weekly Gain = (Final weight − Initial weight) / weeks.

[6.] Protein retention efficiency = (final weight × final protein content) − (initial weight × initial protein content) × 100 / protein intake.

[7.] PSE: Pooled standard error.

Table 7. Apparent digestibility coefficient of dietary dry matter (ADMD), energy (AED) and protein (APD) for the diet and ingredient using 70:30 replacement technique offered to Pacific white shrimp. Values within a column with different superscripts are significantly different based on Tukey's multiple range test. Each value is mean of three replicates.

	Diet (D)			Ingredient (I)		
Diet	ADMD (%)	AED (%)	APD (%)	ADMD (%)	AED (%)	APD (%)
Basal Diet	74.19[b]	80.07[b]	90.48[b]			
Menhaden Fishmeal	64.90[a]	74.93[a]	78.16[a]	39.43[a]	60.16[a]	66.97[a]
Anchovy meal	64.86[a]	75.81[a]	78.53[a]	39.32[a]	65.49[a]	68.45[a]
Salmon meal	72.30[b]	82.38[b]	88.24[b]	64.12[b]	84.13[b]	85.75[b]
Hydrolyzed Salmon Meal	67.34[a]	76.16[a]	90.95[b]	47.59[a]	64.76[a]	90.85[b]
PSE[1]	1.02	0.81	1.00	3.24	2.36	2.04
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1]PSE: Pooled standard error.

Table 8. Apparent amino acid digestibility (AAD) value for menhaden fish meal (MFM), anchovy meal (AM), salmon meal (SM) and hydrolyzed salmon meal (HSM) using 70:30 replacement technique offered to Pacific white shrimp. Values within a column with different superscripts are significantly different based on Tukey's multiple range test.

	MFM	AM	SM	HSM	PSE[1]	P value
Alanine	54.59^{a}	65.60^{b}	84.67^{c}	87.94^{c}	1.41	<0.0001
Arginine	66.27^{a}	78.30^{b}	89.05^{c}	91.55^{c}	1.19	<0.0001
Aspartic Acid	61.26^{a}	67.94^{a}	81.00^{b}	89.07^{b}	1.68	<0.0001
Cysteine	50.54^{a}	54.69^{a}	61.81^{ab}	75.69^{b}	2.84	0.004
Glutamic Acid	65.03^{a}	71.11^{a}	85.59^{b}	90.76^{b}	1.36	<0.0001
Glycine	44.08^{a}	62.64^{b}	89.34^{c}	86.06^{c}	1.17	<0.0001
Histidine	67.29^{a}	73.44^{a}	81.26^{b}	90.84^{c}	1.41	<0.0001
Isoleucine	63.70^{a}	64.72^{a}	78.05^{b}	90.07^{c}	1.84	<0.0001
Leucine	66.28^{a}	67.88^{a}	79.97^{b}	89.25^{c}	1.8	0.0002
Lysine	69.22^{a}	73.66^{a}	85.57^{b}	92.73^{b}	1.46	<0.0001
Methionine	63.85^{a}	68.21^{a}	83.54^{b}	90.74^{c}	1.31	<0.0001
Phenylalanine	61.66^{a}	64.39^{a}	78.03^{b}	87.42^{b}	1.97	0.0002
Proline	51.92^{a}	65.40^{b}	88.34^{c}	85.39^{c}	1.34	<0.0001
Serine	54.46^{a}	61.44^{a}	80.42^{b}	82.89^{b}	1.19	<0.0001
Taurine	82.20^{b}	85.49^{b}	93.50^{c}	70.98^{a}	1.28	<0.0001
Threonine	59.54^{a}	65.61^{a}	79.56^{b}	87.11^{b}	1.71	<0.0001
Tryptophan	67.96^{a}	72.86^{ab}	79.85^{b}	93.69^{c}	1.5	<0.0001
Tyrosine	62.81^{a}	68.15^{a}	80.54^{b}	94.24^{c}	1.53	<0.0001
Valine	61.77^{a}	64.81^{a}	78.31^{b}	88.22^{c}	1.79	<0.0001

[1.]PSE: Pooled standard error.

Table 9. Diets' pH and dry matter loss data. Values within a row with different superscripts are significantly different based on Tukey's multiple range test.

	Basal	SM50	SM75	SM100	HSM50	HSM100	PSE[1]	P-value
pH	6.32^{c}	6.34^{c}	6.34^{c}	6.32^{c}	5.75^{b}	5.45^{a}	0.47	P<0.0001
Dry matter loss(%)	16.68^{b}	13.15^{b}	12.23^{a}	12.67^{a}	20.57^{c}	25.66^{d}	0.69	P<0.0001

[1.] PSE: Pooled standard error.

CHAPTER VI

HYDROLYZED SALMON MEAL AS A REPLACEMENT FOR SALMON MEAL IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

Abstract

A series of growth, feed stability and consumption trials were conducted to evaluate the efficacy of salmon by-product in practical diets for Pacific white shrimp, *Litopenaeus vannamei*. This included a salmon by-product meal (Salmon meal: SM) and a silage hydrolysate (Hydrolyzed salmon meal: HSM). The basal diet containing 120g/kg SM, this was incrementally replaced (0. 25, 50, 75, 100%) by HSM to produce five test diets used in two trials. A sixth diet was included which evaluated gelatin supplementation (Trial 1) or pH neutralization (Trial 2). In trial 1, each diet was produced using two processing conditions (laboratory extruded and formed with meat grinder) and offered to shrimp in a clear water system. The results demonstrate that up to 50% of the SM can be used to replace with HSM; however further increases resulted in reduced performance for shrimp. The addition of gelatin reduced leaching but there was limited effect of processing on leaching. There were no detectible effects of pH adjustment of the diets. Results indicated that the growth performance of shrimp has not influenced by HSM up to 60 g/kg to replace 50% of the SM in practical diets; however, higher levels resulted in significant decrease in performance.

Keywords: *Litopenaeus vannamei;* Salmon meal replacement; Hydrolyzed salmon meal; Salmon meal; Growth trial; Leaching of aromatic amino acids

1.Introduction

The Pacific white shrimp *Litopenaeus vannamei* is the most valuable species produced in aquaculture, accounting for 70% of the total shrimp production in the world. As shrimp feed production continues to expand, it is critical that feed manufacturers have access to a suite of protein sources that can be used to meet nutritional and cost restrictions of feed formulations. Considerable

work has been conducted on strategies to replace expensive ingredients such as fishmeal (FM) and squid meal (SQM), or moderately expensive protein ingredients originating from corn or soy with less expensive ingredients or combinations of ingredients (X Qiu et al., 2018; Xuan Qiu, Tian, & Davis, 2017). Studies like these are important for the aquaculture industry as it provides information on a portfolio of ingredients that can be used to keep the feed cost down and avoid nutrient limitations.

Fish silage has been shown to have beneficial effects; however, there are often poor responses at higher levels of inclusion (Hevrøy et al., 2005; Refstie, Olli, & Standal, 2004) and as such low inclusion level are often recommended. Hydrolyzed fish protein has high concentrations of free amino acid and low molecular peptides, which may cause an influence on nutrient utilization and growth performance (Olsen & Toppe, 2017). Free amino acid may have different absorption rates in the animal as compared with the intact proteins (Gu, Zhang, Bai, Mai, & Xu, 2013). Davis and Duan (2017) reported that there was no clear asynchronous uptake or clearance from the hemolymph for shrimp offered diets with high levels of free amino acids. Other effects of silage would include increased leaching as well as pH shifts of the diet.

High leaching rates constitute direct economic losses and may cause deterioration of water quality (Meyers, Butler, & Hastings, 1972). Being highly water-soluble silage may also destabilize the pellet. The water stability of the feed can be modified by limiting or adding specific ingredients and through the use of binding agents (Argüello-Guevara & Molina-Poveda, 2013; Dominy et al., 2004), adjustments of the pelleting process (Akinbode et al., 2017), and the particles size of the ingredients (Obaldo, Divakaran, & Tacon, 2002).

In previous work, our group has observed successful replacement of 100 and 50% anchovy meal with salmon meal (SM) and hydrolyzed salmon meal (HSM), respectively (data unpublished). Salmon meal has been shown to be a suitable replacement for anchovy meal in shrimp diets whereas there were clear limits to the use of HSM. Given that the nutrient profile of SM and HSM are similar this provides a good model to evaluate the use of silage products in feeds. Thus essentially allowing

the evaluation of partially digested protein vs intact protein as a nutrient source. As leaching is likely an issue, we evaluated two different processing methods (extruded and formed) and the use of gelatin as binder. A second growth trial was conducted in outdoor tanks to provide a demonstration under practical conditions and to evaluate the possible effect of reduced pH on performance.

2.Materials and Methods

Two salmon by-product meals were obtained from Fiordo Austral Company, Cardonal, Puerto Montt, Chile. One meal was produced under traditional processing (SM, Salmo-Pet), while the other is a hydrolyzed powder made from salmon silage (HSM, Amino Salmon P60). These meals were used on an isonitrogenous basis in two series of diets designed to evaluate the efficacy of hydrolyzed salmon meal as a replacement for salmon by-product meal. This work includes two growth trials, a leaching trial, and a palatability trial.

2.1 Experimental Diets

The experimental diets were produced at the Aquatic Animal Nutrition Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences, Auburn University (Auburn, AL, USA), using standard procedures for shrimp feeds. Proximate and amino acid composition for main ingredients are shown in Table 1. All diets were prepared by mixing the pre-ground ingredients in a food mixer (Hobart, Troy, OH, USA) for 10–15 minutes. Two different processing conditions were then used to form the diets. The first process, which is typical to laboratory research, will be referred to as form diets (FD). To produce these diets boiling water (ca 40% by weight) was blended into the mash to obtain a consistency appropriate for extrusion forming using a meat grinder and 3-mm die. The second process produced extruded diets (ED) with a laboratory extruder (EX30, Exteec, Riberaso Preto, Brazil) and 2 mm die to extrude the diets under high pressure and temperature. In both cases, the moist pellets were then placed into a forced air oven (< 45 °C) overnight to attain a moisture content of less than 10%. Dry pellets were crumbled, packed in sealed bags, and stored in

a freezer (-20 °C) until needed. All the ingredients and diets were analyzed at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO, USA) for proximate composition and amino acid (AA) profile.

In the first growth trial, six test diets were formulated on an iso-nitrogenous and iso-lipidic basis to contain 300 g/kg protein and 60 g/kg lipid (Table 2). Five experimental diets were produced by replacing the salmon meal in the basal diet (120 g/kg) with graded levels of HSM (0, 29.3, 58.5, 87.8, and 117.1 g/kg) to replace 0, 25, 50, 75 and 100 % of the salmon meal. A sixth diet was designed to contain 100% HSM with 30 g/kg gelatin with the intent of reducing leaching. To compare the effects of processing (ED vs FD), Diets 1, 3, 5, and 6 were extruded as well as formed. Diets 2 and 4 were only formed. Proximate and amino acid composition of these test diets are presented in Table 3.

In the second growth trial, six test diets (Table 4), were formulated as previously described albeit with the addition of corn protein concentrate to increase the protein to 350 g/kg. The sixth diet was equivalent to Diet 5 with pH adjusted with NaOH back to a pH equal to the basal diet. To adjust the pH, 10g of diet mash (without lipids) was put in a conical flask with 100 ml deionized water (3 replicates for each diet). The diet was stirred with a magnetic stir bar after which was allowed to stabilize for 30 minutes and pH recorded. A dilute solution of NaOH was then added to back titrate the pH of Diet 6 to that of Diet 1. The level of NaOH was then added to the mash prior to forming. Proximate and amino acid composition of these test diets are presented in Table 5.

2.2 Leaching of aromatic amino acids (AAA)

Leaching was determined by measuring the levels of AAA in the water for feed samples that have been immersed for a fixed amount of time. For this purpose, Diets 1, 3, 5, and 6, which were extruded and formed, were evaluated. To estimate AAA leaching, two grams of feed from each diet was mixed in 100 mL of deionized water in a 125mL conical flask which was then placed in a water bath shaker set at 140 rpm and 22°C. Water samples were taken after the feeds were in the water for 15, 30 and 60 min by using a 10mL syringe. The water sample was then filtered through a 1.6

μm glass fibre syringe filter (Whatman Inc., Florham Park, NJ, USA) and read on a spectrophotometer after zeroing with deionized water. Absorbance was then measured using a spectrophotometer (PerkinElmer Lambda 25 UV/vis, Germany) set at the following wavelengths: 257 nm for phenylalanine, 274 nm for tyrosine, and 280 nm for tryptophan.

2.3 Estimated feed intake

Feed intake was estimated using diets 1F and 5F (Formed diet) in trial 1 and all the diets in trial 2. For this work, 10 shrimp were stocked per tank (mean weight: 5g and 10g) with four replicates per treatment for trial 1 and 2, respectively. The shrimp for consumption of all the diets in growth trial 2 were divided and used twice to complete. Prior to feeding, 2g of each diet was weighed and settled solids removed by siphoning the tank bottom. Four feedings were offered each day to each aquarium with shrimp. To correct for leaching loss from the feed, 2g of each diet was placed in empty tanks which followed the same procedure as those fed to the shrimp. Diet not consumed by the shrimp after a period of 30 minutes was collected by siphoning and any non-food items removed by tweezers. Unconsumed diets were then dried, and feed intake determined by difference with the additional correction of dry matter loss.

2.4 Growth trial

The two growth trials were conducted, one is in a clear water indoor system (Trial 1) and another is in green water outdoor system (Trial 2) recirculating aquaculture system. The clear water system was maintained in an indoor building at E. W. Shell Fisheries Center (Auburn, AL, USA) and received limited natural light. It has identical tanks to that of the green water system but includes a fluidized bed biological filter and bead filter for maintaining water quality. The green water trial was conducted in an outdoor recirculation system at Claude Peteet Mariculture Center (Gulf Shores, AL USA), which was managed to have natural productivity present. Both the research systems consist of a central reservoir (~1,000 L), a 1 hp circulation pump, 36 (Trial 1) or 24 (Trial 2) circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter) and supplemental aeration. For the green water system, a second sump pump is used to move unfiltered

water from a shrimp production pond to the central reservoir at a rate of ~8 L min^{-1} between 8:00 h and 12:00 h. This results in the replacement of system water every few days, replenishing natural productivity to mimic a production pond setting. Each tank and central reservoir are equipped with an air stones connected to a 1 hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA) to supply aeration. In trials 1 and 2, juvenile shrimp (initial mean weight 0.17 g and 0.24g) were hand-sorted to uniform size and randomly stocked into 75-L aquaria or 800 L circular tanks which are a component of a 2.5 m^3 or 21 m^3 indoor recirculation system at 10 shrimp per aquarium or 30 shrimp per tank.

In the first growth trial, formed (Diets 1, 2, 3, 4, and 5) and extruded (Diets 1 and 5) diets were used over a 42-day period. The formed diets were used as 6 replicates per diet and the extruded as three replicates per diet. In the second growth trial, we assigned 4 replicated groups per treatment over a 56-day period. During the growth trial, shrimp were fed 2 times per day in the green water system and 4 times per day in the clear water system. In general, feed inputs are calculated assuming the shrimp will double their weight weekly up to one gram, then gain 0.8 g weekly with a feed conversation ratio (FCR) of 1.8 for clear water. Shrimp in the green water systems were assumed to gain 1.3 g weekly with FCR of 1.2. Shrimp in the clear water system were counted once a week to adjust the daily feed input. At the end of the growth trial, shrimp in each tank were counted and weighed to calculate survival, biomass, mean weight, FCR and weight gain. After weighing and counting the shrimp, 4-6 shrimp per tank were randomly selected and frozen at −20°C for whole body samples to be utilized for later protein retention analysis.

During growth trials, dissolved oxygen, water temperature, pH and salinity were measured twice daily using a YSI multi-parameter instrument (YSI, Yellow Springs, OH, USA). Total ammonia nitrogen and nitrite were analyzed once per week for clear water. Total ammonia nitrogen was measured twice per week using a Thermo Orion ISE probe, while nitrite and nitrate were analyzed once per week using Lamotte test kits for green water. Under clear water condition, DO, temperature, salinity, pH, total ammonia nitrogen, and nitrite were maintained at 6.44±0.30 mg L^{-1},

27.78±0.61 °C, 8.07±1.41 ppt, 8.38±0.22, 0.08±0.05 mg L^{-1}, and 0.05±0.08 mg L^{-1}, respectively. Under green water condition, water quality including DO, temperature, salinity, pH, total ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen measured once weekly parameters were maintained at 6.72±0.58 mg L^{-1}, 28.62±1.93 °C, 10.07±1.19 ppt, 8.32±0.31, 0.00±0.01 mg L^{-1}, 0.72±0.88 mg L^{-1}, and 6.60±6.09 mg L^{-1}, respectively. Water quality conditions in all trials were suitable for normal growth and survival.

2.5 Statistical analysis

All data were analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). The whole growth data set in trial 1 and 2, the growth data set fed formed diets in trial 1, the pH, dry matter loss and consumption (Cons) data of the second trial's diets were subjected to one-way analysis of variance to determine significant differences ($P<0.05$) among treatments , followed by Tukey's multiple comparison test to determine differences between treatment means.

Possible correlations between HSM level and diet form on the growth performance of shrimp fed with Diet 1 to 5 were assessed by ANCOVA analysis. ANCOVA analysis were used to access the possible correlations between HSM level and diet form on the AAA leaching of shrimp fed with Diet 1, 3, 5 and 6, and possible correlations between adding binder agent (gelatin) and diet form on the AAA leaching of shrimp fed with Diet 5 and 6. Independent T-test was performed to compare different for Diet 1F with Diet 5F in trial 1 on pH, consumption and dry matter loss in trial 1, and the shrimp growth fed with Diet 5 and Diet 6 which was pH adjusted in the second growth trial. Linear regressions were performed to investigate the relationship between the dietary SM replacement with HSM and the response variables of weight gain and mean weight. Levene's test was also used to test the equality of variances of weight gain and mean weight.

3. Results

3.1 Growth trial

The growth performance of shrimp offered diets containing various levels of HSM under clear and green water conditions are presented in Tables 6, 7 and 8, respectively. The diet form and HSM level has significantly difference on the growth performance and feed utilization of shrimp. There is no interaction between HSM addition level and diet form on the growth performance of shrimp fed with Diet 1 to 5 (Table 6). After the first feeding trial 1, the results showed that adding 58.5 g/kg HSM to replace SM in a practical shrimp diet had no significant effect on mean weight, survival, weight gain and FCR (Table 7). The results showed that shrimp fed the 58.5 g/kg HSM diet exhibited significantly lower biomass than those fed Diet 1.

Under the green water condition, the results showed that adding 60.8 g/kg HSM to replace SM in practical shrimp diets has no significant effect on growth performance (Table 8). When the supplement levels of HSM increased to 91.3 g/kg HSM, the final biomass, mean weight and weight gain were significantly decreased and the FCR was significantly increased. In the T-test, there was no significant difference between Diet 5 and Diet 6 with pH adjustment on the growth performance of the shrimp.

In both trials, the replacement of SM with the HSM influenced the growth of the shrimp particularly at the higher levels of inclusion. Two linear regression were used for each indicator in both trials; one is for D1 to D3 and another is forD3 to D5. In trial 1, the regression lines for WG (%) and MW (g) in the first three diets are described by y_{1a} = -18.162x + 3022.7(R^2 = 0.0295, *P=0.4953*) and y_{1c} = -0.0502x + 5.5331(R^2 = 0.0877, *P=0.1455*) , respectively. There no significant decrease on the WG and MW in the low SM replacement (Diet 1-3) in trial 1. The regression lines for WG (%) and MW (g) in the last three diets are described by y_{1b}= 65.744x + 3232.9 (R^2 = 0.4367, *P=0.0028*) and y_{1d} = -0.1164x + 5.9147 (R^2 = 0.364, *P=0.008*), respectively. There was significant decrease trend for WG and MW with increased HSM adding level in the last two diets (Table 7 and Fig. 1) in trial 1.

In trial 2, the regression lines for WG (%) and MW (g) in the first three diets are described by y_{2a} = 31.7x + 3906.8 (R^2 = 0.1173, *P=0.2759*) and y_{2c} = -0.0496x + 9.7283 (R^2 = 0.0542,

P=0.4666), respectively. The regression lines for WG (%) and MW (g) in the last three diets are described by y_{2b} = -85.081x + 4555.7 (R^2 = 0.3029, *P=0.0637*) and y_{2d}= -0.097x + 9.8086 (R^2 = 0.1529, *P=0.2088*), respectively. The WG in trial 2 also mirrored the same trend in trial 1 with increased HSM adding level, though there is no significant found (Table 8, Fig. 2).

We also use Levene's test to test the equality of variances of WG and MW. All the levene's test is negative (*P>0.05*).

3.2 pH, dry matter loss and feed Consumption

In trial 1 (Table 9), the dry matter loss and consumption of Diet 5F was significantly higher than that of Diet 1F. The pH in Diet 5F was significantly lower than that in Diet 1F. In trial 2 (Table 9), the pH of the diets decreased significantly as the HSM level increases. The dry matter loss in Diet 4, 5 and 6 was significantly higher than that in Diet 1 and 2. In consumption trial 2, there was no significant difference observed in consumption for shrimp fed Diet1 to Diet4. At the third collection of the consumption trial, the shrimp fed Diet 4, 5 and 6 had significantly higher consumption compared with shrimp fed with Diet 1.

3.3 Leaching trial

Leaching of the aromatic amino acids including Phenylalanine (Phe) (O.D. 257nm), Tyrosine (Tyr) (O.D. 274nm), and Tryptophan (Trp) (O.D. 280nm) at different times for Diet 1, 3, 5 and 6 were measured as a proxy for water soluble compounds. As results were very similar for each aromatic amino acids, only the data from Tyrosine is presented. ANCOVA P values results of leaching of Tyrosine at different time is presented in Table 10. The first column includes data on Diet 1, 3, 5 and 6 (Trial 1) which were used to evaluate the effect of Diet form and HSM level. The second column presents the results for the comparison of Diet 5 and 6 which were used to evaluate the effect of diet form and use of gelatin.

ANCOVA results, indicate that in both comparisons the P-value of the model at different times all have significant difference. In the first column, the diet form has significant difference in the leaching of Tyrosine at 15min. However, there is no significant difference at 30min and 60min.

On the contrary, the HSM level has no significant difference in leaching at the 15min; however, there is a significant difference in leaching at the 30min and 60min. The result also showed that there were significant interactions between diet form with HSM level in the Tyr leaching at 15min and 60min.

In the second column in Table 10, there is no significant interactions between use of gelatin with diet form in the Tyr leaching. The results indicated that adding 30 g/kg gelatin in Diet 6 had a significant effect on the leaching of Tyr at 30min and 60min.

4. Discussion

Based on the result of a previous unpublished study, dietary supplementation of salmon meal and hydrolyzed salmon meal in practical diet can replace 100% or 50% anchovy meal without causing impaired growth performance for shrimp both in clear and green water conditions, respectively. In this follow-up study, the objective was to evaluate the effect of using hydrolyzed salmon meal to replace salmon meal as a feed ingredient in shrimp diets. In both trials, there is no biological positive response for the low HSM adding level (up to 60g/kg) to replace SM in practical diets; however, higher levels resulted in significant decreases in performance.

To our knowledge, hydrolyzed salmon meal has not been studied in the Pacific white shrimp diets, however, the results were similar as compared to other studies on fish silage/hydrolysates for fish. With 250 g/kg fishmeal in the basal diet, Wei, Liang, Mu, Zheng, and Xu (2016) reported that the ultra-filtered hydrolyzed fish protein can replace 150 g/kg dietary protein in a high plant protein diet as a fishmeal replacement for juvenile turbot, *Scophthalmus maximus*. With 190 g/kg fishmeal in the basal diet, Zheng, Xu, Qian, Liang, and Wang (2014) showed that fish silage protein can be used to replace 11% fishmeal protein for Japanese flounder, *Paralichthys olivaceus*. With 150 g/kg fishmeal in the basal diet, Xu et al. (2016) also suggested that 10% of dietary protein can be replaced by fish silage protein without compromising the growth performance of juvenile turbot. In our study, we utilized a relatively low level of fishmeal (120 g/kg SM) in the basal diet and we were able to

utilize up to 60 g/kg HSM as a replacement this equals 11.1% dietary protein which is the level that is similar or higher than other studies.

In both trials, the addition of more than 60 g/kg HSM in shrimp diet, resulted in a significant decrease in growth performance. Similarly, other studies also have shown negative results with regards to using higher levels of fish silage protein in the diets (Hardy, Shearer, Stone, & Wieg, 1983; Stone, Hardy, Shearer, & Scott, 1989). Hydrolyzed fish meal have high amounts of free amino acid and small molecular peptides, which may be effecting the nutrient utilization and absorption (reviewed by Olsen and Toppe (2017)). Many studies reported that the free amino acid diet may cause potential loss of nutrients and there may be a difference between free amino acid and intact protein in intestinal uptake of AAs (Ambardekar, Reigh, & Williams, 2009; Schuhmacher, Wax, & Gropp, 1997). This desynchrony may shorten the time available for tissue protein synthesis and affect growth performance (Hardy et al., 1983). However, Davis and Duan, (2017) demonstrated that there was no clear asynchronous uptake or clearance from the hemolymph in shrimp fed with the high levels of free AA. Hence, a synchronous absorption is probably not the primary factor influencing poor performance.

Leaching of nutrients, changing feed intake or destabilization of the feed are other mechanisms that may influence shrimp performance. Results from the free amino acid leaching trials (Table 10) clearly indicated an effect of level, diet type and the use of gelatin as a binder. The increased level of leaching as HSM levels increased, point to leaching as the likely cause of reduced performance. Unfortunately, the effects of processing changes were minimal as compared to the changes that occurred over time (Fig.3). That is to say the change was much higher over time as compared to possible reductions in leaching due to processing or the presence of gelatin.

Another possibility is that the hydrolysate produced products that influenced palatability. In the feed consumption trials, the feed intake appeared to increase significantly with increases in HSM with both trials showing the same trend. Refstie et al. (2004) and Hevrøy et al. (2005) reported that the increased fish protein hydrolysates inclusion in the diet can increase the palatability of the

diet and produce improved growth. Fish silage may be a good attractant in fish or shrimp diets as a result of the free amino acids and small peptides inclusion which is a beneficial to the growth performance of fish or shrimp (Aksnes, Hope, Høstmark, & Albrektsen, 2006; Zheng, Liang, Yao, Wang, & Chang, 2012). It should be noted, that intake in shrimp is difficult to measure and that instability of the feed will add to this error. In our opinion, it is likely that there is multiple effect of instability of the diets and estimated increased consumption in this present study. Although there was an apparent increase in consumption, growth performance of the shrimp did not improve as HSM levels were increased. Leaching of AA from the diet would be considered a benefit from the point of view of attraction but a disadvantage from the standpoint of nutrient delivery (Yúfera, Kolkovski, Fernández-Dıaz, & Dabrowski, 2002).

Good feed stability is critical to the utilization of fish by-products where feed loss due to leaching can be decreased (Fagbenro & Jauncey, 1995). In this present study, two types of feed processing (formed and extruded) were evaluated for their effects on leaching and growth performance of the shrimp were evaluated in Trial 1. Those two diet processing methods are different with the degree of heat, moisture and pressure to produce the pellet. The feeds are processed into formed pellets, which aid in compression, improved water stability, and improve the feed efficiency (Hilton, Cho, & Slinger, 1981). Our results showed that the formed diet supported much better growth performance of the shrimp as compared to those offered the extruded diets. Based on AA leaching results (Table 10), the diet form only influenced leaching at 15 minutes where as HSM level influenced leaching at 30 and 60 minutes. Those results demonstrate that albeit diet form may have some effect, the primary factors are HSM level as well as time (Fig. 3). In general, the tested diet modifications did reduce leaching but not at a level that would be considered a major advantage. Slinger, Razzaque, and Cho (1979) also demonstrated that the leaching loss of vitamin from steam pellet was higher than the extruded pellet. Akinbode et al. (2017) reported that the greater porosity of extruded diets has allowed more potential interaction with the water, which

maybe lead to higher leaching loss. Additionally, it should be noted that the extruded diet did float a little and this may also have contributed to the poor performance.

Adding a binder in the diet is also important to improve water stability, feed consistency, and reduce nutrient leaching in the water system (Huang, 1989; Viola, Gur, & Zohar, 1985). We added 30 g/kg gelatin to the Diet 6 to potentially reduce leaching losses which was measured by determining the loss through the levels of aromatic amino acids (AAA) in the water for feed samples that had been leached. The results showed that the leaching loss significantly decreased for AAA in Diet 6 compared to Diet 5 which did not contain gelatin. This is in accordance with the results reported by Alam, Teshima, Koshio, and Ishikawa (2004). However, we did not evaluate the growth performance of Diet 6. Argüello-Guevara and Molina-Poveda (2013) and Pearce, Daggett, and Robinson (2002) reported that binder type and concentration can affect the feed ingestion, digestibility and palatability. Hence, further studies are recommended to evaluate possible biological effects of binders.

In addition to leaching losses, we also found that the pH of the diets decreases as higher inclusion rates of HSM were used (Table 9), which may be another factor affecting the palatability of the feed, and therefore affecting the growth performance of the shrimp. Fish silage are made by combining minced or parts of fish from trash fish or by-products of the fish processing industry with inorganic or organic acid preservation with added antioxidants (Ridwanudin & Sheen, 2014). One producer from Norway reported that about 40-50 g/kg formic acid is found in the protein hydrolysate from salmon by-product (B.Dulavik, Hordafor,Norway,per.comm.). Additionally, some fish silage is produced by anaerobic microbial fermentation processing, which produces lactic acid (Faid, Zouiten, Elmarrakchi, & Achkari-Begdouri, 1997). These acid sources could affect the pH of diets if silage is added as an ingredient in the formula. Such was the case, the pH of the diets decreased with increased HSM inclusion rates (Table 9) in our previous study and this present study including both trials. As the pH of diets with elevated inclusion rates of HSM were affected by the hydrolysate (e.g. Diet 5), the pH of D6 was adjusted to be equal to the basal diet (D1) in the second

growth trial. In the consumption trial, D6 with a pH adjustment had a higher feed consumption than that in D5. However, there was no effect of pH adjustment on the growth performance of the shrimp (Table 8). As growth was not influence, it would appear that the increased consumption we observed could be and artifact of reduced stability. Similarly, other studies reported that added formic acid or organic acid not only did not affect the growth performance, but also improved survival rate after the challenge test (Chuchird, Rorkwiree, & Rairat, 2015; Defoirdt, Halet, Sorgeloos, Bossier, & Verstraete, 2006; Koh, Romano, Zahrah, & Ng, 2016) and feed utilization (Koh et al., 2016). Those studies results indicated that pH is not the main factor in influencing the performance in high silage diet.

Given the complexity of the effects of fish silages on performance of fish and shrimp continued work to differentiate the effects of water-soluble compounds, specific biological active compounds and pH on the performance of animals should be continued.

5. Conclusion

Results indicate that the growth performance of shrimp has not influenced by the use of HSM up to 60 g/kg as a replacement of SM in practical diets; however, higher levels resulted in significant decreases in performance. The presence short peptides and free amino acids are likely to improve the attraction of the feed. Other studies have also shown nutritional advantages to the inclusion of hydrolysates but at the same time if used at high levels would result in a loss of nutrients and possible destabilizing effects on the feed. Based on aromatic amino acid leaching, there was less water-soluble compounds leaching from the formed feed and the feed containing 30g/kg gelatin. In all, though the amino salmon powder has an upper limit of inclusion, it appears to be a suitable protein source for shrimp feeds.

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Table 1. Proximate and amino acid composition of test ingredients used in these trials (g/kg as is).

Nutrient (g/kg)	Menhaden FM	Anchovy meal	Salmon meal	Hydrolyzed salmon meal
Crude Protein	627.7	683.0	646.0	662.0
Moisture	96.0	85.3	91.4	77.0
Crude Fat	105.0	75.7	106.0	30.4
Ash	182.0	158.0	160.3	173.0
Phosphorus	28.2	23.2	27.0	14.7
Essential AAs				
Isoleucine	23.9	28.9	22.5	26.7
Tryptophan	6.2	7.6	5.5	5.7
Threonine	25.4	27.6	23.8	25.3
Phenylalanine	24.8	26.8	21.7	23.0
Leucine	43.4	48.3	37.9	41.4
Lysine	46.8	51	39.3	47.5
Histidine	13.1	17.8	14	15.2
Arginine	37.5	38.1	38.1	35.1
Valine	28.2	34.7	28.7	30.8
Methionine	16.7	16.5	14.9	15.1
TEAA	266.0	297.3	246.4	265.8
Non-essential AA				
Serine	24.2	22.8	23.1	22.6
Tyrosine	14.6	20.5	18.8	23.4
Glycine	45.7	38.2	64.5	43.7
Alanine	39.8	41.4	42.2	38
Cysteine	5.1	6.5	4.6	5.4
Glutamic Acid	80.2	80.1	70.6	73.6
Proline	28.8	23.6	34.1	25.4
Aspartic Acid	56	58.7	50	53.6
TNEAA	294.4	291.8	307.9	285.7
Other				
Lanthionine	0	1.9	1.8	1.1
Taurine	7.1	6.9	8.9	3.8
Hydroxyproline	11.2	4.7	14.4	7.5
Hydroxylysine	2.4	1.2	3.7	4
Ornithine	0.6	0.6	1.9	0.7
TAA	581.7	604.4	585	568.6

Ingredients were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Table 2. Formulation of experimental diets (g/kg as is) formulated to contain 300g/kg protein and 60g/kg lipids. Diet 1, 3, 5 and 6 were extruded (E) as well as "formed" (F – cold formed on meat grinder). Diet 2 and 4 were only formed. Diets were used in growth trial 1 (clear water), palatability and leaching trials.

Diet number	1	2	3	4	5	6
Diet form	E/F	F	E/F	F	E/F	E/F
Salmon meal [a]	120.0	90.0	60.0	30.0	0.0	0.0
Hydrolyzed Salmon meal [b]	0.0	29.3	58.5	87.8	117.1	120.0
Gelatin [c]						30.0
Soybean meal [d]	396.6	396.6	396.6	396.6	396.6	331.9
Fish oil [e]	31.9	34.2	36.5	38.8	41.0	41.6
Lecithin soy [f]	10.0	10.0	10.0	10.0	10.0	10.0
Cholesterol [g]	0.5	0.5	0.5	0.5	0.5	0.5
Corn Starch [g]	22.5	20.9	19.4	17.8	16.3	47.5
Whole wheat [g]	360.0	360.0	360.0	360.0	360.0	360.0
Mineral premix [h]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin premix [i]	18.0	18.0	18.0	18.0	18.0	18.0
Choline chloride [g]	2.0	2.0	2.0	2.0	2.0	2.0
Stay-C 35% [j]	1.0	1.0	1.0	1.0	1.0	1.0
CaP-dibasic [k]	32.5	32.5	32.5	32.5	32.5	32.5

[a] Salmon meal: salmo-Pet, Fiordo Austral Company, Cardonal, Puerto Montt, Chile.

[b] Hydrolyzed salmon meal: amino salmon P60, Fiordo Austral Company, Cardonal, Puerto Montt, Chile.

[c] Rousselet Inc. Mukwonaga, WI, USA

[d] De-hulled solvent extract soybean meal, Bunge limited, Decatur, AL, USA.

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

[f] The Solae Company, St. Louis, MO, USA.

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

[h] Mineral premix (g/100 g 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.

[i] Vitamin premix (g kg^{-1} premix): Thiamin. HCl, 4.95; Riboflavin, 3.83; Pyridoxine. HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 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.

[j] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[k] T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.

Table 3. Proximate and amino acid composition (g/kg as is) of diets used in the first trial.

Diet number	1		2	3		4	5		6	
Diet form	E	F	E	E	F	E	E	F	E	F
Crude Protein	339.0	328.8	338.3	334.4	327.6	336.9	331.5	325.3	340.6	328.0
Moisture	56.0	84.8	56.7	61.5	86.1	49.1	58.9	87.7	57.9	89.1
Crude Fat	36.5	50.8	49.8	57.5	54.7	48.9	60.3	65.9	35.5	65.2
Crude Fiber	37.7	37.7	38.2	39.9	39.1	35.5	42.5	31.0	30.7	30.5
Ash	79.2	76.9	79.0	77.8	76.6	79.6	77.5	75.6	74.2	71.7
Alanine	15.4	14.8	15.1	15.1	14.6	15.1	14.5	14.3	16.2	15.5
Arginine	21.1	20.3	20.6	20.9	20.1	21.0	20.3	20.0	20.9	20.1
Aspartic Acid	30.6	29.7	30.2	30.9	29.8	31.5	30.3	30.0	29.4	28.2
Cysteine	4.7	4.5	4.7	4.7	4.6	4.9	4.5	4.6	4.4	4.3
Glutamic Acid	62.1	59.0	60.8	61.3	59.3	62.1	60.0	59.2	59.9	57.9
Glycine	18.6	17.8	17.8	17.4	16.6	16.7	15.6	15.3	21.5	20.3
Histidine	7.8	7.5	7.7	7.9	7.6	8.0	7.9	7.7	7.5	7.3
Hydroxylysine	0.8	1.1	0.9	0.9	1.0	0.9	0.9	0.9	1.2	1.2
Hydroxyproline	2.5	2.0	2.5	2.2	2.0	1.4	1.1	1.5	4.6	4.3
Isoleucine	13.9	13.7	14.0	14.4	13.9	14.7	14.3	14.2	13.7	13.2
Leucine	23.1	22.6	23.0	23.4	22.6	23.8	23.0	22.8	22.4	21.6
Lysine	19.5	18.8	19.3	19.8	19.1	20.2	19.7	19.4	19.4	18.6
Methionine	5.5	5.3	5.4	5.4	5.2	5.5	5.2	5.2	5.3	5.0
Phenylalanine	15.4	15.1	15.3	15.5	15.0	15.8	15.2	15.0	14.9	14.3
Proline	19.4	18.8	18.9	18.9	18.1	18.6	17.9	17.6	21.3	20.2
Serine	14.5	13.2	13.2	13.3	13.2	13.5	12.6	12.7	12.9	12.4
Taurine	2.5	2.5	2.3	2.2	2.0	2.0	1.9	2.0	1.9	1.9
Threonine	11.9	11.5	11.7	11.9	11.5	12.1	11.7	11.6	11.4	11.0

Tryptophan	4.4	4.5	4.3	4.3	4.5	4.8	3.6	4.1	3.8	3.6
Tyrosine	11.3	10.8	11.1	11.6	10.9	12.0	11.6	11.5	11.0	10.7
Valine	14.9	14.6	14.9	15.3	14.8	15.6	15.2	15.0	14.8	14.2
Total amino acids	316.1	304.3	309.9	313.5	302.4	315.9	303.9	301.0	319.5	307.2

Table 4. Diet formulation of experimental diets (g/kg as is) formulated to containing 350g/kg protein and 80g/kg lipids and offered to 0.24g shrimp over 56-day (Trial 2, green water).

Diet number	1	2	3	4	5	6
Salmon meal [a]	120.0	90.0	60.0	30.0		
Hydrolyzed salmon meal [b]		30.4	60.8	91.3	121.7	121.7
Soybean meal [c]	400.0	400.0	400.0	400.0	400.0	400.0
Corn protein concentrate [d]	78.0	78.0	78.0	78.0	78.0	78.0
Menhaden fish oil [e]	64.6	64.6	64.6	64.6	64.6	64.6
Lecithin [f]	10.0	10.0	10.0	10.0	10.0	10.0
Cholesterol [g]	0.5	0.5	0.5	0.5	0.5	0.5
Corn Starch [g]	2.4	2.0	1.6	1.1	0.7	0.7
Whole wheat [g]	273.0	273.0	273.0	273.0	273.0	273.0
Mineral premix [h]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix [i]	18.0	18.0	18.0	18.0	18.0	18.0
Choline chloride [g]	2.0	2.0	2.0	2.0	2.0	2.0
Stay-C 35% [j]	1.0	1.0	1.0	1.0	1.0	1.0
CaP-dibasic [k]	30.0	30.0	30.0	30.0	30.0	30.0

[a] Salmon meal: salmo-Pet, Fiordo Austral Company, Cardonal, Puerto Montt, Chile.

[b] Hydrolyzed salmon meal: amino salmon P60, Fiordo Austral Company, Cardonal, Puerto Montt, Chile.

[c] De-hulled solvent extract soybean meal, Bunge limited, Decatur, AL, USA.

[d] Corn protein concentrate, Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA.

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

[f] The Solae Company, St. Louis, MO, USA.

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

[h] Mineral premix (g/100 g 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.

[i] Vitamin premix (g kg^{-1} premix): Thiamin. HCl, 4.95; Riboflavin, 3.83; Pyridoxine. HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Bio n, 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.

[j] Stay C® (L-ascorbyl-2-polyphosphate 35% Active C), DSM Nutritional Products., Parsippany, NJ, USA.

[k] T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.

Table 5. Amino acid composition of experimental diets for the second trial (g/kg as is). Diets were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA).

Diet number	1	2	3	4	5	6
Protein	364.9	366.2	363.7	366.6	369.2	369.0
Moisture	72.6	81.4	80.3	76.5	69.2	72.0
Fat	98.7	98.8	101.2	101.2	104.4	103.4
Fiber	28.2	32.2	28.8	25.7	27.4	27.1
Ash	75.7	76.0	75.5	75.7	76.3	80.7
Amino acid						
Alanine	19.8	19.7	19.5	19.1	19.9	19.2
Arginine	21.4	21.3	21.1	21.0	21.4	21.1
Aspartic Acid	31.9	31.9	32.0	31.9	32.7	32.2
Cysteine	5.5	5.4	5.3	5.2	5.5	5.3
Glutamic Acid	68.8	68.4	70.3	67.9	70.4	67.9
Glycine	18.6	17.9	17.4	16.8	16.5	16.3
Histidine	8.6	8.6	8.5	8.6	8.9	8.7
Hydroxylysine	1.2	1.3	1.2	1.2	1.2	1.3
Hydroxyproline	1.7	1.5	1.5	1.2	1.0	1.0
Isoleucine	15.9	15.8	15.6	15.8	16.3	16.0
Leucine	32.1	32.3	31.6	31.3	33.0	31.5
Lysine	18.5	18.4	18.9	19.0	19.5	19.4
Methionine	7.1	7.0	6.7	6.7	6.9	6.9
Phenylalanine	18.4	18.4	18.0	18.0	18.7	18.1
Proline	23.4	23.1	22.9	22.1	23.0	22.0
Serine	15.0	14.9	16.5	14.8	15.2	14.8
Taurine	2.1	2.0	1.9	1.8	1.6	1.7
Threonine	12.9	12.9	13.1	12.9	13.3	13.0
Tryptophan	4.4	4.2	3.8	4.3	4.4	4.4
Tyrosine	13.3	14.0	13.7	13.5	14.2	13.6
Valine	17.0	16.9	17.0	16.9	17.5	17.1
Total	359.8	356.2	356.7	350.2	363.2	353.7

Table 6. Response of juvenile shrimp (0.63g mean initial weight) in trial 1 to the various test diets (Table 1) used to evaluate various salmon products over a 42 days growth trial under clear water conditions. ANOVA was used in to evaluate the whole data set followed by Tukey's multiple range test. ANCOVA is also presented to show the influence of the main effects. Each value represents the mean of six replicates for formed diets and three replicates extruded diets.

Diet Code	HSM level	Final Biomass	MW[1] (g)	Survival (%)	Weight gain[2](g)	Weight gain[3] (%)	FCR[4]
1E	0	54.2^{b}	5.61^{c}	96.7	5.43^{c}	3012^{ab}	1.82^{b}
1F	0	64.2^{c}	6.65^{d}	96.7	6.48^{d}	3743^{c}	1.48^{a}
2F	2.9	50.5^{ab}	5.33^{cd}	95.0	5.16^{bc}	2990^{ab}	1.87^{bc}
3F	5.8	43.5^{a}	5.26^{bcd}	83.3	5.08^{bcd}	2903^{ab}	1.96^{bcd}
4F	8.8	44.7^{ab}	4.82^{ab}	93.3	4.60^{ab}	2512^{a}	2.10^{bc}
5E	11.7	44.8^{ab}	4.56^{a}	98.3	4.38^{a}	2508^{a}	2.19^{d}
5F	11.7	54.2^{b}	5.59^{c}	96.7	5.42^{c}	3094^{b}	1.78^{b}
PSE[5]		1.893	0.152	3.362	0.151	106.9	0.057
P-value		<0.0001	0.0735	<0.0001	<0.0001	<0.0001	<0.0001
ANCOVA							
Model		<0.0001	<0.0001	0.879	<0.0001	<0.0001	<0.0001
HSM level		<0.0001	<0.0001	0.880	<0.0001	<0.0001	<0.0001
Diet form		<0.0001	<0.0001	0.430	<0.000	<0.0001	<0.0001
Interaction		0.979	0.970	0.919	0.985	0.820	0.443

[1]MW: Mean Weight.
[2]Weight gain (g) = Final weight-initial weight.
[3]Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.
[4]FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).
[5]PSE: Pooled standard error.

Table 7. Response of juvenile shrimp (0.63g mean initial weight) to increasing levels of HSM using only the formed diets. Values within a column with different superscripts are significantly different based on Tukey's multiple range test. Each value represents the mean of six replicates.

Diet number	Diet form	Final Biomass	MW[1] (g)	Survival (%)	Weight gain[2](g)	Weight gain[3] (%)	FCR[4]
1	F	54.2^{b}	5.61^{c}	96.7^{ab}	5.43^{c}	3012^{b}	1.82^{a}
2	F	50.5^{ab}	5.33^{bc}	95.0^{ab}	5.16^{bc}	2990^{b}	1.87^{a}
3	F	43.5^{a}	5.26^{bc}	83.3^{a}	5.08^{bc}	2903^{ab}	1.96^{ab}
4	F	44.7^{a}	4.82^{ab}	93.3^{ab}	4.60^{ab}	2512^{a}	2.10^{b}
5	F	44.8^{a}	4.56^{a}	98.3^{b}	4.38^{a}	2508^{a}	2.19^{b}
PSE[5]		1.834	0.146	3.496	0.144	96.51	0.057
P-value		0.0012	0.0002	0.0454	0.0001	0.0007	0.0003

[1.] MW: Mean Weight.
[2.] Weight gain (g) = Final weight-initial weight.
[3.] Weight gain (%) = (Final weight-initial weight) / initial weight × 100%.
[4.] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).
[5.] PSE: Pooled standard error.

Table 8. Response of juvenile shrimp (0.24g mean initial weight) to the various test diets over a 56-day growth trial under green water conditions at the Claude Peteet Mariculture Center. Values within a column with different superscripts are significantly different based on Tukey's multiple range test. Each value represents the mean of four replicates.

Diet	Final biomass (g)	Mean weight[1] (g)	Survival (%)	Weight gain[2] (g)	Weight gain[3] (%)	FCR[4]
1	296.0^{d}	9.79^{b}	100.8^{b}	9.55^{b}	3961^{b}	0.99^{a}
2	281.5^{bcd}	9.46^{ab}	99.2^{ab}	9.22^{ab}	3895^{ab}	1.05^{abc}
3	284.3^{cd}	9.48^{ab}	100.0^{ab}	9.26^{ab}	4154^{ab}	1.04^{ab}
4	249.4^{ab}	8.39^{a}	99.2^{ab}	8.16^{a}	3548^{ab}	1.18^{cd}
5	251.4^{abc}	8.90^{ab}	94.2^{a}	8.66^{ab}	3636^{ab}	1.17^{bcd}
6	241.4^{a}	8.40^{a}	95.8^{ab}	8.16^{a}	3382^{a}	1.22^{d}
PSE[5]	7.735	0.260	1.457	0.258	130.18	0.335
P-value	0.0003	0.0037	0.0307	0.0035	0.0116	0.0003
T-test (D5 and D6)						
P-value	0.301	0.127	0.594	0.125	0.299	0.409

[1.] MW: Mean Weight.
[2.] WG (g): Weight gain = Final weight-initial weight.
[3.] WG (%): Percent weight gain = (Final weight-initial weight) / initial weight × 100%.
[4.] FCR: Feed conversion ratio = Feed offered / (Final weight − Initial weight).
[5.] PSE: Pooled standard error.

Table 9. T- test for the pH, dry matter loss and consumption (Cons) data of the first trial's Diet 1F and 5F (formed). One-way ANOVA results for the pH, dry matter loss and consumption (Cons) data of the second trial's diets. Cons A, Cons B and Cons C were for the Diets 1F and 5F in trial 1, Diets 1 to 4 in trial 2, and Diets 1, 4, 5, 6 in trial 2, respectively. Values within a row with different superscripts are significantly different based on Tukey's multiple range test. Each value represents the mean of four replicates for dry matter loss and consumption data. Each value represents the mean of three replicates for pH.

	pH	Dry matter loss	Cons A	Cons B	Cons C
Diet 1F	5.91	0.09	5.00		
Diet 5F	5.38	0.29	6.82		
P-value	<0.0001	0.021	0.005		
Diet 1	6.43^{e}	0.18^{a}		3.99	4.09^{a}
Diet 2	6.21^{d}	0.18^{a}		4.11	
Diet 3	6.00^{c}	0.24^{ab}		4.25	
Diet 4	5.83^{b}	0.37^{bc}		4.52	6.33^{c}
Diet 5	5.66^{a}	0.36^{bc}			5.34^{b}
Diet 6	6.25^{d}	0.41^{c}			5.64^{bc}
PSE[1]	0.033	0.035		0.302	0.209
p-value	<0.0001	0.0004		0.6407	<0.0001

[1.]PSE: Pooled standard error.

Table 10. ANCOVA P values for leaching of Tyrosine (O.D. 274 nm) at different times. Diet 1, 3, 5 and 6 (Trial 1) were used to evaluate the effect of HSM level and diet form at the first column. Diets 5 and 6 were used to evaluate the effect of diet form and use of gelatin at the second column. Each value represents the mean of three replicates.

	Form and HSM Level		Form and Gelatin	
15min	Model	<0.0001	Model	<0.0001
	Form	<0.0001	Form	<0.0001
	HSM level	0.5975	Gelatin	0.3697
	Interaction	0.0203	Interaction	0.4566
30min	Model	0.002	Model	0.0004
	Form	0.1845	Form	0.0799
	HSM level	<0.0001	Gelatin	0.0082
	Interaction	0.1769	Interaction	0.3272
60min	Model	<.0001	Model	0.0213
	Form	0.0652	Form	0.0264
	HSM level	<0.0001	Gelatin	<0.0001
	Interaction	0.004	Interaction	0.8793

Figure 1 Response of juvenile shrimp (0.63 g mean initial weight) to diets with graded level of HSM replacing SM over 42 days in growth trial 1 (D1-D5). The relationship between weight gain (y_{1a} and y_{1b}) or mean weight (y_{1c} and y_{1d}) of shrimp and the inclusion level of HSM (x) in the diets.
Figure 2. Response of juvenile shrimp (0.24 g mean initial weight) to diets with graded level of HSM replacing SM over 56 days in growth trial 2 (D1-D5). The relationship between weight gain (y_{2a} and y_{2b}) or mean weight (y_{2c} and y_{2d}) of shrimp and the inclusion level of HSM (x) in the diets.

Fig 1

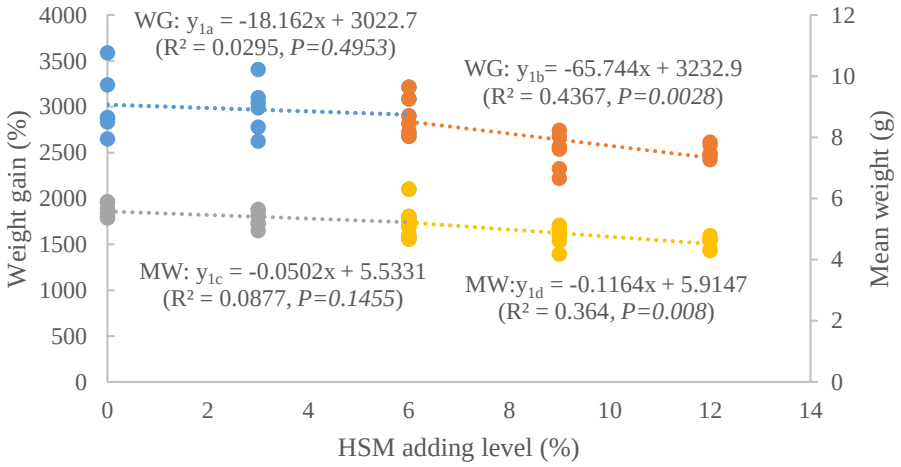


Fig 2

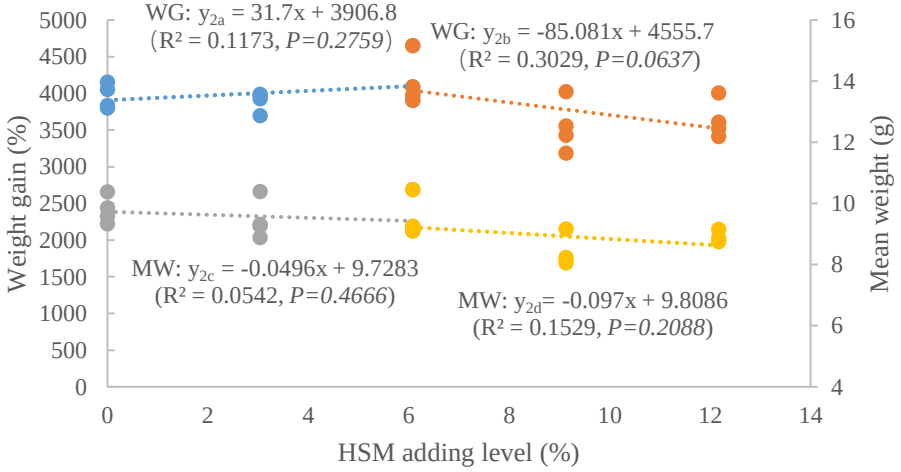
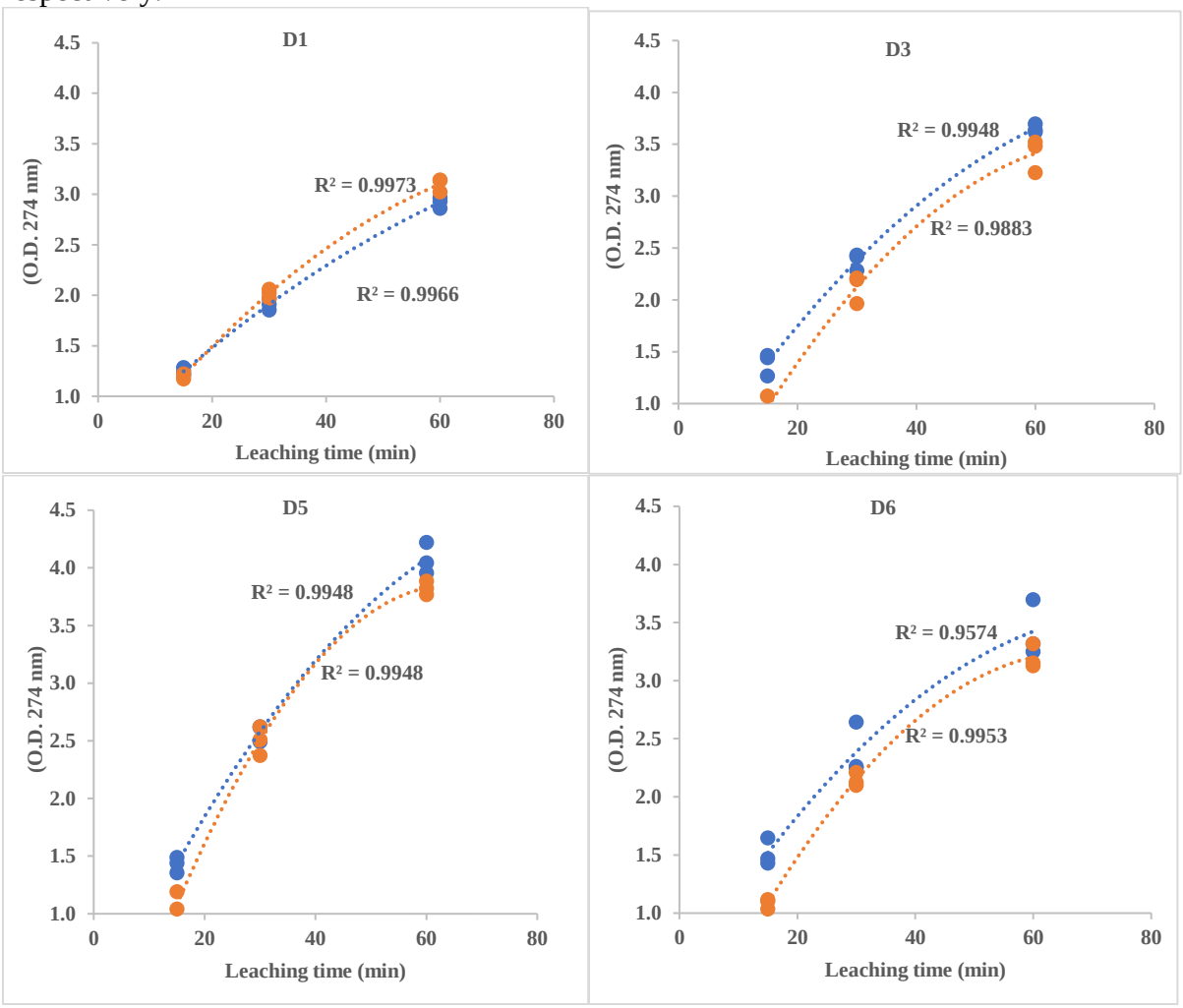


Figure 3. Comparison of leaching between two different processing methods for Diet 1, 3, 5 and 6 at 274 nm (tyrosine) in trial 1. The orange and blue colour represent formed and extruded diet, respectively.



CHAPTER VII

SUMMARY AND CONCLUSIONS

As the world's shrimp production expands, considerable effort to replace fishmeal (FM) using a variety of plant proteins or terrestrial animal byproducts in shrimp diets has gained momentum. It is important to look for adequate supply, consistent quality, and cost-effective protein sources to keep the feed cost reduced. In the present study, we want to explore some potential protein ingredients (BY50, corn protein concentrate, soy protein concentrate, high protein distiller's dried grain and salmon meals) as replacements of FM, corn protein concentrate or soybean meal in practical shrimp diets.

The first study was conducted to evaluate the effects of brewer's yeast in practical shrimp feeds, which includes two growth trials and one digestibility trial. The first growth trial results showed that there were no significant differences in final biomass, survival, protein retention efficiency and feed conversion ratio; however, limited differences in final weight and weight gain were shown in the FM replacement series. There was no significant difference on the growth performance in the SBM replacement series. In the second growth trial, the shrimp feed with Diet contain 24% BY50 exhibited significantly lower final weight and weight gain comparing with the basal diet. Nutrient availability of BY50 and BY70 was similar to SBM and significantly higher than FM. Results indicated that 180-240g/kg BY50 can be effectively used in shrimp diets as a replacement for FM, or up to 240g/kg when replacing SBM. Additionally, adding 20g/kg of BY70 does not cause impaired growth performance for shrimp fed low-FM diets.

The second study was conducted to evaluate the use of high protein distiller's dried grain (HPDDG) on the growth performance of juvenile Pacific white shrimp *L.vannamei*. Results of

previous study indicate that HPDDG is a good plant-based ingredient and can be included up to 30% as a replacement of SBM without compromising growth of shrimp. When HPDDG was utilized to replace a combination of SBM and FM, an upper limit of 18% of the diet should be recommended in shrimp feed formulation. Under green water conditions, the results indicated that growth performance and feed conversion ratio were not statistically influenced by increasing levels of HPDDG when used to replace corn protein concentrate. The FM replacement series trial results showed that shrimp fed the diet with 20% HPDDG has exhibited significantly decreased trend on biomass. Results of this study demonstrate that HPDDG is a good protein source and up to 20 or 15% HPDDG can be used to replace CPC or FM in practical shrimp diets under green water conditions.

The third study was conducted to investigate the effect of replacing fishmeal with a combination of soy and corn protein concentrate (SPC and CPC) (1:1 ratio) on growth performance of the Pacific white shrimp (*L.vannamei*). In indoor clear water trial, results indicated a slight decrease in shrimp performance as fishmeal was replaced at the highest levels. Meanwhile, the supplementation of lysine and methionine to the diets did not result in differences in survival, growth or FCR. In outdoor green water trial, there were no significant differences in growth performance across all the tested diets. In conclusion, the present study demonstrated that total 92 or 138 g/kg of CPC and SPC (1:1 ratio) can be used in the diet of the Pacific white shrimp replacing 50 or 75% fishmeal in clear and green water under high stocking density and low salinity culture conditions, respectively.

The fourth study was conducted with Pacific white shrimp *L.vannamei*, to evaluate the efficiency of two salmon meals as compared to anchovy meal. Results of this study indicate that salmon meal (SM) can be used effectively in practical diets for Pacific white shrimp as a

replacement for up to 100% anchovy meal without causing impaired growth performance in both clear and green water conditions. Hydrolyzed salmon meal (HSM) has also been proven to be a suitable protein source for replacing fishmeal in shrimp diet. However, hydrolyzed salmon meal can be used to replace only 50% anchovy meal in the Pacific white shrimp practical diet. Under green water conditions, shrimp growth showed the same trend as those reared under clear water. Future studies regarding the improvement of the processing technologies of hydrolyzed salmon meal and the demonstration of salmon meal in practical diets under pond culture conditions are warranted. A subsequent study was conducted to evaluate the efficacy of salmon by-product in practical diets for Pacific white shrimp, *L.vannamei*. Results indicate that the growth performance of shrimp has not influenced by the use of HSM up to 60 g/kg as a replacement of SM in practical diets; however, higher levels resulted in significant decreases in performance. The presence of short peptides and free amino acids are likely to improve the attraction of the feed. Other studies have also shown nutritional advantages to the inclusion of hydrolysates but at the same time if used at high levels would result in a loss of nutrients and possible destabilizing effects on the feed. Based on aromatic amino acid leaching, there was less water-soluble compounds leaching from the formed feed and the feed containing 30g/kg gelatin. There were no detectible effects of pH adjustment of the diets. In all, though the amino salmon powder has an upper limit of inclusion, it appears to be a suitable protein source for shrimp feeds.

In conclusion, BY50, CPC and SPC, HPDDGS, SM and HSM may be useful protein sources in the practical shrimp diet. The information developed in this research clearly demonstrates that it is possible for feed formulators to increase the usage of a variety of economical plant or animal-based protein sources in practical shrimp diets to reduce feed cost.