EVALUATION OF SOYBEANMEAL QUALITY AS AN INGREDIENT IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

By

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Abstract

Of potential protein sources, conventional soybean meal produced through traditional solvent extraction procedures has received the most attention among terrestrial plant sources, considering its well-balanced amino acid profile, worldwide availability, low price and consistent composition. Solvent extracted soybean meal (SBM) is generated using different varieties of soybeans grown under a range of conditions and then processed at different crushing plants. Due to its competitive cost and availability, it is a popular plant based protein source for shrimp feed formulations. However, limited information exists about the effects of variations in the nutritional composition of soybean meal generated in different geographical regions of the world on growth performances of shrimp. Presence of anti-nutritional factors is often referenced as one of the major drawbacks of SBM, which may limit its inclusion level in animal feeds. In response, various processing strategies were developed over time to diminish the adverse characteristics of traditional SBM. Despite the higher manufacturing cost, inclusion levels of these new SBM products in to aquatic animal feed formulations can still be limited due to the different sensitivities of fish/shrimp and/or due to the secondary negative characteristics caused during the processing methods. Hence, the present study was designed with two objectives, 1) to determine the effects of different soy bean meals sourced from different geographical locations in the world and 2) differently processed SBM on growth performances of Pacific white shrimp (*Litopenaeus vannamei*).

Several growth trials were conducted with iso-nitrogenous (350 g/kg protein) and iso-lipidic (80 g/kg lipid) test diets formulated with twenty-four sources of soybean meal sourced from different geographical locations of the world (objective one), two sources of solvent extracted soybean meal (SBM44 and SBM49), enzyme treated soybean meal (ETSBM), fermented soybean meal (FSBM) and alcohol extracted soy protein concentrate (SPC) (objective two). Results from these studies demonstrated that the phosphorous, phosphorous in phytic acid and total phytic acid and raffinose are important components in SBM that may have significant effects on the growth performances of Pacific white shrimp. Furthermore, it was inferred that the traditional solvent extracted soybean meal performed equally with the enzyme treated SBM (ETSBM) while reduced performances of fermented SBM (FSBM) and alcohol extracted soy protein concentrate (SPC) might be due to the low nutrient digestibility and palatability in Pacific white shrimp.

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CHAPTER I

GENERAL INTRODUCTION

Pacific white shrimp, *Litopenaeus vannamei* is the most important cultured shrimp species (more than 90%) in Americas *(Cuzon et al. 2004)* due to its rapid growth rates, good survival in high-density, disease resistance (Cuzon et al. 2004), relatively low dietary protein requirements, and adaptability to wide ranges of salinity and temperature (Moss et al. 2007, Rocha et al. 2010, Lightner et al. 2009). Outside its native range (Eastern Pacific coast from Gulf of California, Mexico to Tumbes, North of Peru), Pacific white shrimp continues to be an important species for world aquaculture, accounting for 85% of total shrimp production in China (Li and Xiang 2013) and 80% of the farmed shrimp production in the world (Panini et al. 2017).

The aquaculture production of shrimp and most of the other species (70% cultured species) depends on the provision of nutrients in the form of industrially produced compounded feed. As this industry continues to expand so does the demand for feed production, which is currently growing at an average annual rate of 10.3% per year since 2000, and expected to grow to 65.4 million tonnes by 2020 and 87.1 million tonnes by 2025 (Tacon and Metian 2015). In general, commercial shrimp feeds contain 30–50% crude protein, which is the most expensive component of the diet (Lim and Dominy 1990, Mente et al. 2002) and one of the major nutrient required for maintenance and growth of shrimp (Shiau 1998). The minimum protein requirements for shrimp to maintain optimal performance varies depending on age or size of shrimp, quality of dietary protein (essential amino acid profiles and digestibility), availability of alternative food sources, water chemistry, environmental parameters, and culture management practices (D'Abramo and Sheen 1994, Venero 2006).

Fishmeal was the main protein source used in traditional aquaculture feed formulations, consuming approximately 68% of fishmeal production in world (Tacon and Metian 2015, Mallison 2013). This is not only due to its excellent amino acid profile, palatability and digestibility, but also because fish meal is a source of nucleotides, essential fatty acids, phospholipids, minerals, and fat soluble and water soluble vitamins (Tacon et al. 2009, Dersjant-Li 2002). Because of static supply, increasing demand, price and ethical issues, average dietary fish meal inclusion levels within compound feed for shrimp has been steadily declining (from around 28 to 7%) and it is expected that total usage will decrease by 37.7% from 2006 to 2020 (Tacon and Metian 2008). Fishmeal is no longer the primary protein source, but more of a strategic ingredient used in less price-sensitive phases in the culture cycle (Jackson 2012).

Of protein sources, solvent extracted soybean meal (SBM) received the most attention of terrestrial plant sources (Amaya et al. 2007b) considering its well-balanced amino acid profile, advantage of being resistant to oxidation and spoilage, worldwide availability, low price and consistent composition (Dersjant-Li 2002, Swick et al. 1995, Amaya et al. 2007c, Davis and Arnold 2000, Gatlin et al. 2007). Although SBM is available worldwide and widely used in shrimp and fish diet formulations, information on the complete nutritional profile of SBM sourced from different locations is limited and effects of differences in nutritional profile on production performances of shrimp or fish is not known. Palmer et al. (1996), Verma and Shoemaker (1996) and Van Kempen et al. (2002) indicated that the location of production could affect the growth characteristics, yield and nutritional value of SBM because of genetic variability among soybeans and several other factors, which are used to make the meal. However, all SBM follow the conventional solvent extraction procedure (cracking and dehulling followed by steam-conditioning, flaking, 3 cycles of hexane extraction, de-solventizing, toasting, cooking and milling), slight variations in procedure or processing specifications such as processing temperature, time, and moisture content could also add variation to the final nutritional quality of SBM (Balloun 1980, Van Kempen et al. 2002).

In addition to the inconsistencies in final nutritional quality, presence of anti-nutritional factors (ANFs) (trypsin inhibitors, antigens, lectins, saponins and oligosaccharides) within the carbohydrate fraction of solvent extracted soybean meal (SBM) is often referenced as one of the major drawback, which may limit its inclusion level in animal feeds (Dersjant-Li 2002, Gatlin et al. 2007). In addition to the allergenic and antinutritional effects, this could negatively influence on the palatability of the meal or could cause intestinal damage in fish (Dersjant-Li 2002, Conklin 2003). In response, different processing strategies such as thermal treatments, alcohol extractions, enzyme hydrolysis, fermentation, soaking, germination, etc. have been developed over the time to diminish the adverse quality characteristics while improving bioavailability of micro-nutrients and nutrient digestibility of traditional SESBM (Hotz and Gibson 2007, Qiu et al. 2018, Masumoto et al. 2001, Lim and Lee 2011, NRC 2011, Chou et al. 2004, Lim and Lee 2009, Refstie et al. 1998a).

As a consequence of the removal of carbohydrates, nutrient density of the ingredient also increase with resulting elevated protein contents in soybean meal (Conklin 2003). Sookying and Davis (2012) stated that the enhanced protein levels of these advance SBM provides more space in feed formulations to supplement the deficient nutrients and/or ingredients to enhance nutrition and pellet quality. Despite the advantages offered through different processing techniques, they are more costly than the production of traditional SBM (Lee et al. 2016). Furthermore, some of the differently processed SBM commodities possess product specific defects, which limits the inclusion level to the shrimp/fish diet formulations might due to the different sensitivities of fish/shrimp to SBM (Chou et al. 2004) and/or due to the secondary quality characteristics caused during the processing methods such as changes in texture, palatability, etc.

In practical applications, a clear understanding about effects of variation among sources of SBM and processing methods on growth of shrimp is needed. With the objective of filling research gaps, the current study was conducted with two major objectives;

1. To investigate the effect of different SBM sourced from different geographical locations in the world on growth performance of Pacific white shrimp (*Litopenaeus vannamei*).
2. To investigate the effect of differently processed SBM on growth performances of Pacific white shrimp (*Litopenaeus vannamei*).

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CHAPTER II

EVALUATION OF SOYBEAN MEAL FROM DIFFERENT SOURCES AS AN INGREDIENT IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP, *Litopenaeus vannamei*

Abstract

Solvent extracted soybean meal (SBM) is generated using different varieties of soybeans grown under a range of conditions and then processed at different crushing plants. Due to its competitive cost and availability, it is a popular plant-based protein source for shrimp feed formulations. However, there is limited information about the effects of variations in the nutritional composition of soybean meal can have on the performances of shrimp. Hence, the present study was designed to determine the effects of different soybean sources on the growth performances of *L. vannamei*. Two growth trials were conducted with iso-nitrogenous (350 g/kg protein) and iso-lipidic (80 g/kg lipid) test diets formulated with twenty-five sources of soybean meal. Trial one incorporated 14 treatments including a soy-based diet containing 517 g/kg SBM (eight replicates) and this soy-bean source was then replace with 13 different soybean sources (four replicates per treatment). The second trial used the same basal diet and 11 different sources of soybean meal (Total 12 diets) with five replicates per treatment. Both growth trials were conducted with a stocking density of 10 shrimp/aquarium (60L) in a semi-closed recirculating system. The initial mean weight of shrimp for trials 1 and 2 were 0.23 g ± 0.02 and 0.67 g ± 0.02 respectively. During the two trials, shrimp were fed four times/day assuming a FCR of 1.8, over 42 days for trial 1 and 35 days for trial 2. Results indicated significant differences among soybean meal sources for standardized percentage Thermal Growth Coefficients (TGC). Diet 21 containing SBM4550 had the largest value for TGC, whereas the lowest TGC was observed for shrimp fed diet 17 containing SBM45536. According to the statistical analysis on the chemical profile of SBM, phosphorous, phytate-phosphorous and total phytic acid levels had significantly positive correlations ($p<0.05$) with TGC whereas raffinose ($p= 0.086$) had a negative trend with TGC. Results of this work indicates phosphorous, phosphorous in phytic acid and total phytic acid and raffinose are important components in SBM that may have significant effects on the growth performances of Pacific white shrimp.

KEYWORDS: Soybean meal, production location, nutritional quality, shrimp growth

1. Introduction

The Pacific white shrimp, *Litopenaeus vannamei*, continues to be an important species for world aquaculture. This species accounts for 85% of total shrimp production in China (Li and Xiang 2013) and 80% of global farmed shrimp production (Panini et al. 2017). The aquaculture production of shrimp depends on the provision of nutrients in the form of industrially produced compounded feed. As this industry continues to expand so does the demand for key feed ingredients. Fishmeal was the main protein source used in aquaculture feed consuming approximately 68% of fishmeal production in the world (Tacon and Metian 2015, Mallison 2013). This is not only due to its excellent amino acids profile, palatability and digestibility, but also because fish meal is a source of nucleotides, essential fatty acids, phospholipids, minerals, and fat soluble and water soluble vitamins (Tacon et al. 2009). Because of static supply, increasing demand, price and ethical issues, average dietary fish meal inclusion levels within compounded shrimp feed has been steadily declining (from around 28 to 7%) and it is expected that total usage will decrease by 37.7% from 2006 to 2020 (Tacon and Metian 2008). Fishmeal is no longer the primary protein source , but more of a strategic ingredient used in less price-sensitive phases in the culture cycle (Jackson 2012).Of protein sources, solvent extracted soybean meal (SBM) received the most attention of terrestrial plant sources (Amaya et al. 2007b) considering its well-balanced amino acid profile, advantage of being resistant to oxidation and spoilage, worldwide availability, low price and consistent composition (Dersjant-Li 2002, Swick et al. 1995, Amaya et al. 2007c, Davis and Arnold 2000, Gatlin et al. 2007). However, the inclusion level of SBM in practical shrimp diets is restricted due to the presence of anti-nutritional factors (ANFs) (trypsin inhibitors, antigens, lectins, saponins and oligosaccharides), insufficient levels of essential amino acids (EAA) (methionine and lysine) and poor palatability, which negatively affects digestion and nutrient availability to shrimp (Dersjant-Li 2002, Qiu et al. 2018, Gatlin et al. 2007).

Although SBM is available worldwide and widely used in shrimp and fish diet formulations, information is limited on the complete nutritional profile of SBM sourced from different locations and the effects of nutritional profile differences on production performances of shrimp or fish. Palmer et al. (1996), Verma and Shoemaker (1996) and Van Kempen et al. (2002) indicated that the location of production could affect the growth characteristics, yield and nutritional value of SBM because of genetic variability among soybeans and some of the other environmental factors (such as climate, soil conditions, etc.), which are used to make the meal.

According to the findings of Howell and Collins (1957) and Rennie and Tanner (1989), soybeans grown under warmer temperature conditions consist with higher oil content, whereas those grown at cooler temperatures yielded oils with higher levels of linoleic and linolenic acids. Meanwhile, Maestri et al. (1998) observed negative correlations of both protein and oil contents with total precipitation during the growing season in Argentina while protein content nor fatty acid composition were affected by temperatures during seed maturation at production locations. Soybean data collected over 11 years in four locations in Minnesota and Illinois, USA had a significant effects of year on oil content and effects of location on yield and oil content, but little effect of either on protein content (Breene et al. 1988). Conversely, Hurburgh et al. (1990) stated that the soybeans from northern and western soybean-growing states (North Dakota, South Dakota, Minnesota, Iowa, Wisconsin) contained 1.5–2% less protein and 0.2–0.5% more oil than soybeans from southern states like Texas, Arkansas, Louisiana, Mississippi, Tennessee, Kentucky, Alabama, Georgia, South Carolina and North Carolina. Furthermore, it was recorded that the protein content of soybean is inversely correlated with latitude while positive correlation of protein and oil contents were noted with altitude (Maestri et al. 1998). Though the information is scarce, it is clear that different environmental conditions in different geographical locations affect SBM quality (Natarajan et al. 2016). A study conducted by Van Kempen et al., (2002) revealed that the SBM collected from four regions within the United States varied some in nutrient quality over SBM sampled from Netherlands, which had comparatively less amino acid content causing negative effects on digestibility of pigs. As per the evaluation of Baize (1999) soybean meal samples collected from Europe, Turkey, Venezuela, Columbia, Mexico, Indonesia, Thailand, Philippines, Korea, China, Japan, and the United States had numerical differences in percent protein, lipid, fiber, potassium hydroxide solubility, amino acids, and urease pH. Therefore, sufficient evidence proves the variations in nutrient quality of soybean grown in different environmental conditions in different geographical locations might result in differences in production performance of shrimp or fish as well (Natarajan et al. 2016).

Even if the compositions of the raw soybeans are similar, variable processing methodologies and processing conditions at the processing plant could result in differences in chemical composition (both carbohydrates and amino acids) among the resultant soybean meals. Balloun (1980) and Van Kempen et al., (2002) stated that the processing temperature, time, and moisture content may add variation to the final nutritional quality of SBM. Other factors such as efficiency of oil removal and hull removal may also could affect the SBM value as an animal feed (Karr-Lilienthal et al. 2006). In practical applications, a clear understanding about the effects of variation among sources of SBM on growth of shrimp is needed. With the objective of filling research gaps, the current study, investigated the effect of different SBM sourced from different geographical locations in the world on growth performance of Pacific white shrimp (*Litopenaeus vannamei*).

2. Materials and Methods

2.1 Diet preparation

Twenty four sources of solvent-extracted soybean meal (SBM) along with data for proximate composition, indispensable and dispensable amino acid profiles, sugars (fructose, sucrose, raffinose, stachyose, etc.), fibers (acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin), macro and micro minerals (Tables 1b-f) for each source were obtained from the Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign (Lagos and Stein 2017). Each source of SBM was fed to Pacific white shrimp and the hypothesis of growth performances of Pacific white shrimp could be predicted from the nutrition profile of SBM was tested. Twenty-five soybean-based grow-out diets were formulated to be iso-nitrogenous (350 g/kg protein) and iso-lipidic (80 g/kg lipid). Twenty-four of the diets contained the aforementioned SBM from Illinois and a reference diet (Diet 1) was prepared using a local SBM (Tables 1a, 2a, 2b). The test diets were prepared in the feed laboratory at Auburn University, Auburn, AL, USA, using standard practices. Pre-ground dry ingredients and oil were weighted and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Hot water (~30% by weight) was then blended into the mixture to attain a consistency appropriate for pelleting. Finally, all diets were pressure-pelleted using a meat grinder with a 3-mm die, dried in a forced air oven (50 °C) to a moisture content of less than 10% and stored at 4^0C. All were analyzed for proximate composition, amino acid profile, pepsin digestibility and trypsin inhibitor levels at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) (Tables 2c, 2d 2e).

2.2 Culture system

The semi-closed recirculation system used for growth trials consisted of a series of 60-L aquaria connected to a common reservoir tank (800-L). Water quality was maintained by recirculation through an Aquadine bead filter (0.2 m^2 media, 0.6 m × 1.1 m) and a vertical fluidized bed biological filter (600-L volume with 200-L of Kaldnes media) using a 0.25-hp. centrifugal pump. Mean water flow for an aquarium was 3 L/min with an average turnover of 20 minutes/tank. Salt water was prepared by mixing artificial crystal sea salt (Crystal Sea Marinemix, Baltimore, MD, USA) with freshwater and maintained at around 7ppt during the each growth trial. Aquariums were covered with styrofoam sheets during the each growth trial (except during the weekly counting) to avoid any possible variation could cause due to different light conditions. Dissolved oxygen was maintained near saturation using air stones in each culture tank and the sump tank using a common airline connected to a regenerative blower. Dissolved oxygen, salinity and water temperature in the sump tank were measured twice daily using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA). Total ammonia-N (TAN) and nitrite-N were measured twice per week according to the methods described by Solorzano (1969) and Spotte (1979), respectively. The pH of the water was measured twice weekly during the experimental period using the pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA). All water quality parameters measured during the study are presented in Table 3d.

2.3 Growth trials

Dietary treatments were randomly assigned to tanks and each trial was conducted using a double blind experimental design. Growth trials were conducted in two phases. The first growth trial was conducted with 14 treatments and 4 replicates for diets 2 to 14, whereas 8 replicates were assigned to the control diet (Diet 1). Twelve treatments were tested during the second growth trial, each with five replicates including the control diet and diets 15 to 25. In each trial, ten shrimp were stocked per tank with an average initial weight of 0.23±0.02 g in trial one and 0.67±0.02 g in trial two. Shrimp were offered test diets four times daily. The daily ration of feed was calculated based on an estimated weight gain from previous trials and an expected feed conversion ratio (FCR) of 1.8. Shrimp were counted weekly and the feed was adjusted each week based on survival and observations of feeding responses of shrimp. Growth trial-1 was conducted for 6-weeks, whereas trial-2 was conducted for 5 weeks. At the conclusion, shrimp were counted and group-weighed. The average final weight, final biomass, percent survival, and feed conversion ratio were determined.

2.4 Statistical analysis

All data were analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA). Data from individual growth trials were analyzed separately using one-way ANOVA followed by Tukey pairwise comparison test to evaluate significant differences ($p < 0.05$) among treatment means (Tables 3a and 3b). The Thermal Growth Coefficients (TGC) for the shrimp were calculated with the objective of combining the growth data from trial 1 and 2. The TGC values of different SBM were standardized by calculating the “percentage TGC” reference to the TGC of the control diet for that trial. Standardized TGC values were analyzed using one-way ANOVA followed by Tukey pairwise comparison test to evaluate significant differences among treatment means (Table 3c; Figure 1). With the objective of reducing the dimensions and grouping different SBM sources, principle component analysis (PCA) and a cluster analysis were performed using the chemical characteristics of SBM (Table 4; Figure 2). For the PCA and cluster analysis, the entire data set was standardized by calculating z scores (standard scores) to avoid the different units and scales of measurements while some of the variables, which were balanced during the formulations (such as protein and its fundamental units such as essential and non-essential amino acids), were excluded from the analysis. Furthermore, ingredient data of SBM were adjusted based on the inclusion ratio as the diets were formulated to be iso-nitrogenous by adjusting the SBM inclusion in the diet. Multiple linear regression was performed to identify the relationships between TGC with principle components selected from PCA (Table 5). A correlation coefficient analysis was conducted to identify the relationships between TGC and major variables representing the principle components, which had a significant impact on TGC (Table 6).

3. Results

3.1 Growth performances

At the conclusion of the culture period of trial 1, no significant differences were detected in average final weight, weight gain, percentage weight gain, and TGC among shrimp fed the different diets, whereas FCR differed ($P < 0.05$) among diets (Table 3a). Diet-8, which contained SBM45537 resulted significantly largest FCR (1.97) compared to the FCR values of diets 4 and 5 (1.60 and 1.64, respectively). Mean survival, final weight and weight gain ranged from 80 to 98%, 5.1to 5.9g, and 4.8 to 5.7g respectively. At the end of trial 2, significant differences ($P < 0.05$) were detected among average final weight, weight gain, percentage weight gain, survival and TGC for shrimp fed experimental diets (Table 3b). Diet-21, which contained SBM45550 resulted in the largest average final weight, weight gain, and percentage weight gain, respectively, with 6.33g, 5.66g and 851%. According to the statistical analysis among percentage TGC values of all the experimental SBM, significant differences ($P < 0.05$) were observed among the sources of SBM (Table 3c, Figure 1). Diet 21, which contained SBM45550 resulted in the largest mean value for TGC, whereas the lowest mean value for TGC was noted from diet 17, which contained SBM45536.

3.2 Grouping information base on cluster analysis

According to the dendrogram generated through the cluster analysis, the 24 sources of SBM were separated in to five major groups, which were clearly observed in the score plot of PCA as well (Figure 2). The SBM used in diets 2 to 11 and in diets 14 to 19 were grouped together, whereas SBM used in diets 12, 13, 23, 24, 25 were clustered into another group. Three individual points were observed for the SBM used in diets 20, 21, and 22.

3.3 Principle component analysis

The PCA of chemical characteristics of SBM sources and their loadings are presented in Table 4. Collectively, the first five PCs explained 83% of the total sample variance. According to the loading values, PC1 was represented by sucrose (-0.31) and iron (0.33) and PC2 was represented by sodium (0.42), sulphur (0.38), non-phytate phosphorus (0.37), zinc (0.31), and phosphorus (0.29). Phosphorus in phytic acid (0.35), total phytic acid (0.35), Acid detergent fiber (ADF) (0.29), Neutral detergent fiber (NDF) (0.31), fructose (0.31), phosphorus (0.30) and raffinose (-0.30) were the components in PC3.

3.4 Multiple linear regression

The results of multiple linear regression of TGC on the first five PCs are presented in Table 5. The p-value for the entire model was less than 0.05, but only PC2 and PC3 had positive ($P < 0.05$) impacts on TGC. Combining the results of PCA and multiple linear regression, it was concluded that the phosphorus, non-phytate phosphorus, sodium, sulfur, zinc, phosphorus in phytic acid, total phytic acid, fructose, ADF and NDF had positive attributes for TGC, whereas raffinose had a negative impact on TGC.

3.5 Pearson correlation coefficients

Pearson correlation coefficients of TGC with raffinose, ADF, NDF, phosphorus, phosphorus in phytic acid, total phytic acid, non-phytate phosphorus, sodium, sulfur and zinc are presented in Table 6. Only phosphorus, phosphorus in phytic acid and total phytic acid levels were positively correlated with TGC, whereas raffinose ($p= 0.086$) appeared as the only negative correlation with TGC of the selected variables representing PC2 and PC3.

Except for the variables from PCA, Pearson correlation coefficients were calculated for the protein level of SBM, pepsin digestibility, and trypsin activity of diets against the mean TGCs of shrimp. A negative correlation was detected with protein in SBM ($p=0.001$, $R^2= 0.37$) and a positive correlation was observed with trypsin inhibitor level in diets ($p=0.042$, $R^2= 0.18$). There tended to be a negative trend with pepsin digestibility of diets against TGC ($p=0.152$, $R^2= 0.09$), and a positive correlation was observed with SBM inclusion level in the diet ($p=0.001$, $R^2= 0.40$)

4. Discussion

Historically, fishmeal has been the primary protein source used in shrimp feed formulations. However, as the aquaculture industry expands so does demand resulting in increases in the price of fishmeal, which then results in reduced concentrations of protein in the diets and use of alternative protein sources (Davis et al. 2008). Hardy (2010) argued that the fish meal demands for the production of feed may eventually exceed the world production of fish meal based on the expected growth rates of aquaculture and rates of fish meal utilization. As an alternative to the use of fish meal in fish feed formulations, a variety of plant- based dietary ingredients have been tested (NRC 2011). Soybean meal attracted most of the attention due to its comparable amino acid profile, worldwide availability, low price, and consistent composition (Amaya et al. 2007c, Dersjant-Li 2002, Davis and Arnold 2000).

SBM is available worldwide and is used as a primary protein source in shrimp and fish diet formulations, but information about the complete nutritional profile of SBM sourced from different locations and how differences among sources of SBM may affect production performance of shrimp or fish are scarce. Palmer et al. (1996), Verma and Shoemaker (1996) and Van Kempen et al. (2002) clearly stated that the location of production might affect the growth characteristics, yield, and nutritional value. Maestri et al., (1998) observed negative correlations between protein and oil contents in soybeans and total precipitation during the growing season in Argentina, whereas neither protein content nor fatty acid composition were affected by temperatures during seed maturation at production locations. The protein content of soybeans is inversely correlated with latitude, and positively correlated between protein and oil contents in soybeans and growing altitude (Maestri et al. 1998). A study conducted by Van Kempen et al., (2002) revealed that SBM collected from four regions within the United States varied a little in nutrient quality compared with SBM sampled from the Netherlands, which had reduced amino acid content causing negative effects on digestibility of amino acids by pigs. Therefore, there is evidence indicating that variations in nutrient quality of soybeans grown in different environmental conditions in different geographical locations (Natarajan et al. 2016) may also result in differences in production performances of shrimp or fish.

Protein content of the SBM sources used in the present study was in the range of 44 to 51% and the 24 sources of SBM were separated in to five major groups based on the complete chemical profile through the cluster analysis, which was also indicated from the PCA. The limited groupings is in part due to the narrow variations and homogeneous chemical characteristics of the ingredients as well as specifications used in sourcing the materials. The three individual points that were observed for the SBM used in diets 20, 21, and 22 is likely due to the elevated levels of copper, sodium and iron in these meals compared with the other sources of SBM.

Differences in growth performances were not clearly overlaid through the SBM cluster analysis. However, the SBM used in diet 21, which was different from the other sources of SBM, resulted in the best growth of shrimp. No biological responses were observed in shrimp for trypsin inhibitor level, pepsin digestibility of the diets, or protein content of SBM (along with dispensable and indispensable amino acids of SBM), emphasizing the importance of considering the complete nutritional profile of an ingredient rather than individual variables (Francis et al. 2001). Biological responses to various meals are likely due to their combined interactions of nutrient level, digestion and absorption. The observed positive correlation of inclusion level on growth performances infer the augmented positive impact of another variable (or combination of several) in SBM which was natural occurrence during the diet formulation while balancing the protein content of the diet through inserting different SBM sources at variable levels to standardize protein. Simply, SBM sources added to the diet in greater quantities due to their lower protein value in general performed better over SBM with higher protein value, possibly overturning the individual biological effects of protein and trypsin activity on growth performances of Pacific white shrimp. This may also point to the fact that to maintain a higher protein level the meal may go through harsher processing resulting in poorer performance due to low protein quality.

Phosphorous is considered a critical element within the minerals required by penaeid shrimp due to its direct involvement in all energy-yielding reactions and the role as a structural material of nucleic acids, phospholipids, phosphoproteins, ATP and several key enzymes (Lovell 1989). According to the NRC (2011), different dietary requirements for phosphorous were mentioned for *Marsupenaeus japonicas (*Kanazawa et al. *1984), Penaeus monodon* (Peñaflorida 1999) and *Litopenaeus vannamei* (Davis et al. 1993b) while most of the researchers emphasized the interaction between calcium and phosphorous due to the elevated phosphorous requirements at presence of higher calcium levels. Therefore an optimal Ca : P ratios was suggested for different species, such as 1 : 1.7 for *F. chinensis* (Li et al. 1986) and 1 : 1 for *M. japonicas* (Kanazawa et al. 1984). According to Davis et al. (1993b), dietary levels of 0.5–1% and 1–2% phosphorus is required to maintain normal shrimp growth in the presence of 1 and 2% supplemental calcium, respectively and revealed a poor growth performance at higher calcium levels. Phosphorous levels of the SBM used during the present study varied from 0.57-0.81% showing a positive correlation with TGC while calcium levels ranged from 0.18-0.57% revealing a non-significant negative trend with TGC. Ca:P ratio of the SBM used during the study ranged from 1: 1.1-3.9 which showed a positive trend (p=0.097) with TGC of shrimp.

Though dietary phosphorous requirement is vital in shrimp nutrition, approximately two-thirds of total phosphorus in various grains is present as phytate or inositol hexaphosphate (1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) (Raboy 1997) which is less digestible to monogastric animals such as fish and shrimp. In addition, phytic acid has a potential to produce indigestible complexes with minerals such as Zn^{+2}, Fe^{+2}, Fe^{+3}, Ca^{+2}, Mg^{+2}, Mn^{+2}, Cu^{+2} and protein, restricting their availability as well (Chowdhury et al. 2015, NRC 2011, Liener 1989, Cosgrove and Irving 1980, Denstadli et al. 2006, Laining et al. 2010, Cheryan and Rackis 1980, D'Mello et al. 1991, Adeola and Sands 2003). According to Francis et al., (2001) commercial SBM contains 1.0–1.5 % phytate while Gatlin et al., (2007) stated the phytate fraction in SBM as 4%. Phosphorus bio-availability in SBM ranges from virtually nil (Riche and Brown 1996) to 22% in the rainbow trout (Sugiura et al. 1998). Reduced growth performance in cultured fish species such as carp, tilapia, trout and salmon due to phytate-containing ingredients in the diets were well documented, attributed to various factors such as reduced mineral bioavailability, impaired protein digestibility and depressed absorption of nutrients (Spinelli et al. 1983, Francis et al. 2001, NRC 2011). Davis et al., (1993a) and Qiu and Davis (2017), reported low bioavailability of phytate phosphorus to shrimp (*P. vannamei*) and emphasized the reductions in zinc bioavailability due to the effect of phytic acid. In addition to negative effects on growth performances of fish and shrimp, Kies et al., (2001) and Baruah et al., (2004) emphasized the potential environmental pollution due to high phosphorous concentration in the manure from animals fed with phytate containing diets which is one of the major concerns as well. During the current study, significantly positive correlations were observed for TGC with phytic acid and phytate phosphorous levels of the diets. Given the well-documented negative effects of phytate, the positive response is likely due a correlated effect from some other variable.

According to Snyder and Kwon (1987) and Refstie et al., (1999) raw soybeans contain approximately 100 g kg^{-1} di- and oligosaccharides including sucrose, raffinose and stachyose some of which are indigestible due to a lack of α-galactosidases in fish and shrimp (Gatlin et al. 2007). In fish, their negative effects may be either due to binding to bile acids or interfering with the uptake of nutrients through increasing the viscosity of the chime in the digestive tract (Storebakken et al. 1998, Refstie et al. 1998b). According to the present study, SBM raffinose levels ranged from 1.04-2.23%, which showed a negative trend (p=0.086) with TGC of shrimp. Thus confirming the negative effects of raffinose as were also observed by Zhou et al., (2015).

Most of the studies relevant to the anti-nutritional factors (ANFs) have been conducted using an ingredient rich in one particular factor and the observed effects have been attributed to the particular factor without considering the other anti-nutrients present in the ingredient, or interactions between them (Francis et al. 2001). For this research, holistic changes in antinutrients and nutrients occurred making it difficult to make firm conclusion about a specific culprit for the resultant growth performances of Pacific white shrimp and their threshold levels in shrimp diets might be due to their interaction effects. However based on the statistical outcomes from the present study, phosphorous, phosphorous in phytic acid and total phytic acid and Raffinose were screened with significant correlations, which could cause major effects on the growth performances of Pacific white shrimp.

5. Conclusions

It is difficult to make a firm conclusion about a specific culprit for the resulted fluctuations in growth performances of Pacific white shrimp and their threshold levels might be due to their interactive positive and negative effects. However, there was clear evidence that phosphorous,

phosphorous in phytic acid and total phytic acid and Raffinose were selected as vital chemical variables in SBM, which could cause significant effects on the growth performances of Pacific white shrimp. The results of this study demonstrate differences even in reasonably similar sources of soybean meal. Hence, to understand and predict the biological performance on animals, systematic research is needed to look at various processing and nutritional changes and how they influence performance of the animals.

Table 1a: Codes for different Soybean meal used in the experiment

Diet Number	Ingredient Code	Diet Number	Ingredient Code
1	AU Soy	14	45543
2	45531	15	45544
3	45532	16	45545
4	45533	17	45546
5	45534	18	45547
6	45535	19	45548
7	45536	20	45549
8	45537	21	45550
9	45538	22	45551
10	45539	23	45552
11	45540	24	45553
12	45541	25	45554
13	45542		

Table 1b: Chemical analysis[a] (proximate composition, gross energy and trypsin inhibitors) of the different soybean meal used in diets of Pacific white shrimp, *Litopenaus vannamei*

Soybean meal Sample key	g/100g as is					GE, kcal/kg	Trypsin Inhibitors/ mg (TIU)
	Dry Matter	Moisture	Ash	Crude Protein	Fat		
AU Soy	88.14	11.86	5.78	43.7	1.03	4394	
45531	89.37	10.63	6.44	45.85	1.25	4191	3.32
45532	89.77	10.23	6.58	46.40	1.53	4213	3.05
45533	89.42	10.58	6.42	45.35	1.39	4194	3.00
45534	89.70	10.30	6.36	45.78	1.10	4204	3.37
45535	89.40	10.60	6.48	45.92	1.07	4185	2.13
45536	88.93	11.07	6.99	47.50	0.86	4168	1.98
45537	88.85	11.15	6.96	46.62	1.40	4190	2.09
45538	89.51	10.49	7.06	47.87	1.37	4210	1.25
45539	89.01	10.99	7.01	47.16	1.38	4209	2.57
45540	89.43	10.57	6.90	47.43	3.47	4238	2.19
45541	88.19	11.81	6.77	47.31	1.45	4163	2.92
45542	88.26	11.74	6.39	48.02	2.13	4232	2.67
45543	90.01	9.99	7.45	51.08	0.83	4241	4.27
45544	88.08	11.92	6.42	50.29	2.55	4302	4.62
45545	87.55	12.45	6.46	51.02	1.55	4231	2.93
45546	88.59	11.41	6.45	47.70	1.55	4173	3.17
45547	88.66	11.34	6.12	47.79	1.88	4190	2.91
45548	89.68	10.32	6.41	49.94	2.00	4254	1.25
45549	87.83	12.17	7.34	47.02	1.44	4075	2.70
45550	87.77	12.23	7.43	45.48	1.51	4042	3.47
45551	88.53	11.47	8.60	48.06	1.47	4113	4.37
45552	88.82	11.18	6.84	49.07	1.83	4189	5.27
45553	87.23	12.77	5.60	50.96	0.87	4146	2.9
45554	88.72	11.28	6.59	50.63	0.63	4175	3.95

[a] Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos and Stein 2017).

Table 1c: Indispensable Amino acid profile[a] (as is basis) of the Soybean meal used diets of Pacific white shrimp, *Litopenaus vannamei*

Soybean meal Sample key	Indispensable Amino Acids (%)										
	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl alanine	Threonine	Tryptophan	Valine	Total
AU[2]	3.39	1.23	2.19	3.60	2.94	0.68	2.37	1.79	0.69	2.39	21.27
45531	3.31	1.26	2.07	3.33	2.86	0.61	2.22	1.58	0.65	2.13	20.02
45532	3.38	1.30	2.15	3.45	2.94	0.63	2.30	1.67	0.66	2.23	20.71
45533	3.33	1.28	2.11	3.40	2.88	0.61	2.25	1.63	0.64	2.17	20.30
45534	3.24	1.28	2.09	3.36	2.91	0.63	2.20	1.66	0.65	2.16	20.18
45535	3.36	1.24	2.08	3.39	2.91	0.64	2.23	1.68	0.68	2.16	20.37
45536	3.31	1.35	2.19	3.62	3.04	0.65	2.41	1.82	0.70	2.29	21.38
45537	3.23	1.33	2.18	3.61	2.97	0.63	2.40	1.78	0.69	2.27	21.09
45538	3.32	1.34	2.13	3.56	2.88	0.62	2.39	1.76	0.69	2.23	20.92
45539	3.33	1.36	2.16	3.64	3.04	0.65	2.42	1.83	0.71	2.24	21.38
45540	3.36	1.36	2.26	3.66	3.04	0.64	2.43	1.78	0.70	2.36	21.59
45541	3.22	1.30	2.23	3.59	2.91	0.61	2.41	1.72	0.66	2.30	20.95
45542	3.30	1.34	2.25	3.61	2.97	0.63	2.42	1.74	0.69	2.32	21.27
45543	3.56	1.41	2.39	3.83	3.14	0.66	2.60	1.86	0.68	2.48	22.61
45544	3.52	1.36	2.41	3.89	3.14	0.65	2.57	1.87	0.73	2.47	22.61
45545	3.55	1.41	2.46	3.96	3.15	0.68	2.68	1.87	0.72	2.51	22.99
45546	3.45	1.40	2.32	3.75	3.15	0.67	2.49	1.84	0.72	2.41	22.20
45547	3.40	1.38	2.24	3.68	3.06	0.64	2.42	1.77	0.70	2.34	21.63
45548	3.63	1.44	2.30	3.79	3.21	0.69	2.51	1.85	0.76	2.40	22.58
45549	3.40	1.38	2.26	3.68	3.05	0.66	2.43	1.78	0.68	2.31	21.63
45550	3.30	1.32	2.14	3.52	2.96	0.62	2.31	1.72	0.68	2.24	20.81
45551	3.42	1.39	2.29	3.73	3.08	0.66	2.42	1.82	0.65	2.38	21.84
45552	3.42	1.38	2.21	3.58	3.03	0.62	2.39	1.73	0.66	2.28	21.30
45553	3.71	1.46	2.41	3.92	3.25	0.68	2.62	1.90	0.70	2.49	23.14
45554	3.63	1.44	2.35	3.82	3.18	0.67	2.55	1.85	0.69	2.44	22.62

[a] Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos and Stein 2017).

Table 1d: Dispensable Amino acid profile[a] (as is basis) of the different Soybean meal used in diets of Pacific white shrimp, *Litopenaus vannamei*

Soybean meal Sample key	Dispensable Amino Acids (%)									Sum of Amino Acids (%)
	Alanine	Aspartic Acid	Cysteine	Glutamic Acid	Glycine	Proline	Serine	Tyrosine	Total	
AU[2]	2.03	5.33	0.63	8.53	1.98	2.40	2.00	1.59	24.49	45.76
45531	1.79	4.78	0.62	7.77	1.75	2.06	1.86	1.57	22.2	42.22
45532	1.91	4.96	0.65	8.01	1.87	2.16	1.95	1.6	23.11	43.82
45533	1.86	4.86	0.63	7.87	1.82	2.07	1.95	1.55	22.61	42.91
45534	1.90	4.94	0.63	7.95	1.87	2.10	2.01	1.26	22.66	42.84
45535	1.91	4.96	0.65	8.02	1.9	2.13	2.06	1.57	23.20	43.57
45536	2.05	5.12	0.62	8.21	1.97	2.26	2.15	1.72	24.10	45.48
45537	2.00	5.02	0.60	8.07	1.92	2.20	2.07	1.67	23.55	44.64
45538	1.99	5.03	0.61	8.05	1.95	2.24	2.09	1.69	23.65	44.57
45539	2.03	5.15	0.62	8.30	1.93	2.26	2.2	1.73	24.22	45.60
45540	2.04	5.16	0.60	8.30	1.99	2.19	2.08	1.72	24.08	45.67
45541	1.98	5.11	0.59	8.16	1.98	2.22	2.08	1.62	23.74	44.69
45542	2.02	5.17	0.62	8.20	2.00	2.24	2.04	1.68	23.97	45.24
45543	2.17	5.50	0.65	8.78	2.10	2.37	2.18	1.80	25.55	48.16
45544	2.15	5.50	0.61	9.00	2.07	2.36	2.35	1.76	25.80	48.41
45545	2.16	5.50	0.66	8.98	2.10	2.44	2.26	1.82	25.92	48.91
45546	2.09	5.35	0.64	8.60	2.04	2.39	2.13	1.78	25.02	47.22
45547	2.02	5.19	0.62	8.34	1.97	2.30	2.05	1.67	24.16	45.79
45548	2.11	5.43	0.66	8.92	2.04	2.40	2.17	1.74	25.47	48.05
45549	2.02	5.24	0.61	8.46	1.98	2.21	2.09	1.62	24.23	45.86
45550	1.95	5.03	0.61	8.10	1.92	2.19	2.03	1.62	23.45	44.26
45551	2.05	5.33	0.64	8.61	2.07	2.36	2.21	1.66	24.93	46.77
45552	1.98	5.22	0.61	8.31	1.99	2.27	2.06	1.69	24.13	45.43

45553	2.14	5.66	0.64	9.11	2.12	2.51	2.27	1.74	26.19	49.33
45554	2.10	5.53	0.64	8.88	2.08	2.45	2.21	1.75	25.64	48.26

[a] Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos and Stein 2017).

Table 1e: Percentage composition of sugars & fiber[a] of the different Soybean meal used in diets of Pacific white shrimp, *Litopenaus vannamei*

Soybean meal Sample key	Sugars, %						Fiber, %		
	Fructose	Glucose	Sucrose	Maltose	Raffinose	Stachyose	ADF	NDF	Lignin
AU Soy									
45531	0.07	0.00	8.87	0.00	1.16	5.51	7.17	11.92	0.24
45532	0.07	0.00	9.54	0.00	1.12	5.75	4.37	7.79	0.07
45533	0.07	0.00	9.07	0.00	1.24	5.59	5.44	9.03	0.25
45534	0.07	0.00	8.97	0.00	1.13	5.66	5.85	9.94	0.21
45535	0.07	0.00	8.90	0.00	1.33	5.72	5.65	9.41	0.17
45536	0.06	0.00	8.05	0.00	1.34	5.50	3.3	6.27	0.08
45537	0.07	0.00	7.87	0.00	1.44	5.66	3.84	7.12	0.81
45538	0.12	0.07	7.50	0.00	1.66	4.77	4.41	9.37	0.28
45539	0.06	0.00	8.12	0.00	1.41	5.58	3.21	6.36	0.17
45540	0.07	0.00	6.77	0.00	1.60	4.96	3.92	7.28	1.14
45541	0.07	0.00	4.86	0.00	1.48	4.08	7.66	12.44	0.74
45542	0.08	0.00	4.81	0.00	1.47	3.58	5.68	9.69	0.30
45543	0.06	0.00	6.32	0.00	1.45	4.90	4.45	8	0.16
45544	0.07	0.00	6.20	0.00	1.88	4.69	3.04	4.88	0.13
45545	0.08	0.00	5.53	0.00	1.47	5.19	4.02	7.49	0.28
45546	0.08	0.00	8.29	0.00	1.93	6.46	3.39	6.72	0.09
45547	0.10	0.08	9.52	0.00	1.04	6.32	3.14	6.56	0.25
45548	0.07	0.00	8.52	0.00	1.12	6.69	3.12	6.88	0.33
45549	0.07	0.00	8.18	0.00	1.68	6.34	4.12	7.76	0.25
45550	0.06	0.00	8.71	0.00	1.51	6.72	4.74	8.49	0.09
45551	0.42	0.31	1.80	0.00	1.44	3.28	8.26	12.45	0.25
45552	0.00	0.00	5.09	0.00	2.15	5.66	6.35	10.04	0.38

45553	0.00	0.00	5.81	0.00	2.12	6.05	4.95	7.94	0.19
45554	0.00	0.00	6.10	0.00	2.23	5.43	6.18	9.58	0.20

[a] Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos and Stein 2017).

Table 1f: Composition of minerals[a] in the different Soybean meal used in diets of Pacific white shrimp, *Litopenaus vannamei*

Soybean meal Sample key	Minerals																
	Ca, %	P, %	P in PA, %	Total PA, %	Non-phytate P, %	Cr, ppm	Cobalt , ppm	Cu, ppm	Fe, ppm	Mg, %	Mn, ppm	Molybdenum, ppm	K, %	Se, ppm	Na, ppm	S, %	Zn, ppm
AU Soy	0.32	0.64						9.7		0.24							46.8
45531	0.20	0.66	0.52	1.85	0.14	19.8	< 0.2	7.74	120	0.25	31.1	2.72	2.08	< 4	9.45	0.42	44.6
45532	0.18	0.70	0.54	1.9	0.17	< 0.1	< 0.2	7.96	114	0.25	33.2	3.24	2.07	< 4	7.80	0.43	45.3
45533	0.18	0.68	0.55	1.96	0.13	< 0.1	< 0.2	7.41	105	0.25	31.2	2.23	2.13	< 4	5.32	0.42	44.2
45534	0.18	0.70	0.55	1.96	0.15	< 0.1	< 0.2	7.65	111	0.25	31.3	2.38	2.08	< 4	5.32	0.43	44.5
45535	0.19	0.69	0.54	1.9	0.15	< 0.1	< 0.2	7.38	106	0.25	30.9	2.54	2.07	< 4	< 0.2	0.42	43.5
45536	0.25	0.68	0.53	1.87	0.15	2.41	< 0.2	11.3	90.3	0.28	44.9	9.93	2.30	< 4	4.64	0.42	41.3
45537	0.24	0.67	0.52	1.86	0.15	< 0.1	< 0.2	11.3	78.5	0.28	41.3	8.24	2.28	< 4	2.27	0.41	40.7
45538	0.26	0.69	0.50	1.77	0.19	< 0.1	< 0.2	11.4	130	0.30	42.5	7.90	2.30	< 4	117	0.41	43.0
45539	0.24	0.70	0.53	1.89	0.17	< 0.1	< 0.2	11.0	68.1	0.29	39.8	8.42	2.31	< 4	6.38	0.42	39.6
45540	0.29	0.63	0.45	1.58	0.18	< 0.1	< 0.2	11.7	105	0.29	38.1	6.64	2.25	< 4	11.6	0.40	45.4
45541	0.28	0.61	0.43	1.53	0.18	< 0.1	< 0.2	8.22	172	0.32	26.9	4.14	2.11	< 4	7.67	0.39	50.3
45542	0.32	0.59	0.40	1.42	0.19	< 0.1	< 0.2	10.5	256	0.30	34.8	2.42	2.08	< 4	43.8	0.42	50.9
45543	0.28	0.62	0.46	1.62	0.16	< 0.1	< 0.2	7.42	141	0.32	30.5	5.34	2.27	< 4	< 0.2	0.43	49.3
45544	0.30	0.64	0.46	1.64	0.17	< 0.1	< 0.2	9.49	79.5	0.31	27.8	4.15	2.20	< 4	19.5	0.42	49.0
45545	0.33	0.65	0.47	1.67	0.18	< 0.1	< 0.2	9.74	110	0.33	29.0	3.73	2.17	< 4	2.97	0.43	53.9

45546	0.32	0.64	0.47	1.67	0.17	< 0.1	< 0.2	10.6	82.8	0.27	31.5	2.76	2.20	< 4	3.55	0.43	41.0
45547	0.24	0.63	0.47	1.65	0.16	< 0.1	< 0.2	12.5	101	0.28	39.4	3.49	2.12	< 4	53.6	0.43	47.1
45548	0.26	0.61	0.43	1.54	0.17	< 0.1	< 0.2	11.6	109	0.26	26.7	11.5	2.15	< 4	8.66	0.44	48.1
45549	0.57	0.64	0.45	1.58	0.20	< 0.1	< 0.2	44.1	167	0.28	61.3	4.13	2.14	< 4	371	0.42	153
45550	0.48	0.81	0.51	1.80	0.30	< 0.1	< 0.2	14.8	331	0.42	71.2	2.96	2.17	< 4	1470	0.52	97.1
45551	0.53	0.61	0.44	1.57	0.17	< 0.1	< 0.2	14.1	1590	0.35	78.0	0.187	2.01	< 4	22.6	0.41	54.7
45552	0.43	0.59	0.43	1.54	0.16	< 0.1	< 0.2	15.0	713	0.34	48.2	2.03	2.00	< 4	12.2	0.40	56.3
45553	0.34	0.57	0.41	1.45	0.16	< 0.1	< 0.2	16.2	395	0.32	46.3	3.29	2.03	< 4	9.59	0.43	59.2
45554	0.35	0.60	0.43	1.52	0.18	< 0.1	< 0.2	17.0	695	0.34	53.7	1.88	2.07	< 4	11.1	0.43	58.9

[a] Monogastric Nutrition Laboratory, Division of Nutritional Sciences, University of Illinois at Urban-Champaign. Results are expressed on an "as is" basis unless otherwise indicated (Lagos and Stein 2017).

Table 2a: Composition (% as is) of the basal diets used in the growth trials.

Ingredient (As basis g/kg feed)	Basal diet for growth trial
Fishmeal[a]	6.00
Soybean meal[b]	51.70[1]
Corn protein concentrate[c]	7.00
Menhaden fish oil[a]	5.76[1]
Lecithin[d]	1.00
Cholesterol[e]	0.05
Whole wheat[f]	23.0
Corn Starch[e]	0.39[1]
Mineral premix[g]	0.50
Vitamin premix[h]	1.80
Choline chloride[i]	0.20
Stay C 35% active[j]	0.10
CaP-dibasic[i]	2.50

[1] See Table 2b for adjustments for test diets.

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

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

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

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

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

[f] Bob's red mill, Milwaukie, OR, USA.

[g] Trace mineral premix (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664.

[h] Vitamin premix (g/kg premix): Thiamin HCl, 4.95; Riboflavin, 3.83; Pyridoxine HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81.

[i] VWR Amresco, Suwanee, GA, USA.

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

Table 2b: Basal diet ingredient modification (g/100g as is) to create the test diets. All other ingredients are the same as that of the basal diet (Table 1b)

Diet #	Soybean meal	Corn starch	Fish oil
2	49.30	2.87	5.68
3	48.70	3.59	5.56
4	49.80	2.44	5.61
5	49.40	2.69	5.76
6	49.30	2.78	5.77
7	47.60	4.36	5.89
8	48.50	3.73	5.62
9	47.30	4.9	5.65
10	47.90	4.31	5.64
11	47.70	5.5	4.65
12	47.80	4.44	5.61
13	47.10	5.45	5.30
14	44.30	7.62	5.93
15	45.00	7.69	5.16
16	44.30	7.94	5.61
17	47.40	4.88	5.57
18	47.30	5.14	5.41
19	45.30	7.15	5.40
20	48.10	4.14	5.61
21	49.80	2.5	5.55
22	47.10	5.14	5.61
23	46.10	6.29	5.46
24	44.40	7.54	5.91
25	44.40	7.44	6.01

Table 2c: Chemical analyses[a] (proximate composition, pepsin digestibility and trypsin inhibitors) of different diets fed to the Pacific white shrimp, *Litopenaus vannamei*

Diet	Crude protein	Moisture	Crude Fat	Crude Fiber	Ash	Pepsin Digestibility	Trypsin Inhibitor/ TIU/g
1	36.41	10.41	8.27	4.29	6.63	93.65	1924
2	34.10	11.88	7.83	4.62	6.16	93.52	1034
3	34.34	9.09	7.94	5.20	6.41	90.29	1036
4	35.40	8.10	7.25	4.77	6.43	93.31	849
5	35.93	7.23	7.06	4.77	6.80	93.27	1085
6	35.85	7.05	11.11	4.97	6.63	90.60	1087
7	35.07	8.85	13.17	3.98	6.57	92.54	1129
8	35.21	9.23	10.58	3.85	6.56	93.40	1167
9	36.45	6.90	8.21	3.85	6.76	94.17	1041
10	36.53	6.20	8.81	3.30	6.70	93.83	535
11	36.35	6.43	7.89	3.80	6.64	94.25	524
12	36.66	6.10	8.05	5.09	6.66	94.57	738
13	36.45	6.24	10.46	4.25	6.51	94.13	842
14	36.56	6.30	11.75	3.61	6.55	93.97	819
15	36.46	6.02	16.41	3.25	6.45	95.25	861
16	36.95	6.30	13.37	3.49	6.46	92.80	303
17	36.37	6.78	6.60	3.71	6.53	94.97	284
18	36.01	7.92	8.43	3.93	6.36	95.86	435
19	36.20	6.99	8.62	3.22	6.28	94.57	625

20	36.35	6.83	8.67	3.89	6.77	95.78	767
21	36.43	6.65	8.74	4.27	7.40	93.97	821
22	36.41	6.51	7.29	6.47	7.68	91.96	1455
23	36.23	7.06	9.75	4.63	6.74	94.30	1059
24	36.32	7.07	13.37	4.07	6.36	94.03	887
25	36.58	5.62	9.22	4.19	6.56	94.51	867

[a] Diets were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Results are expressed on an "as is" basis unless otherwise indicated.

Table 2d: Indispensable Amino acid profile[a] (as is basis) of the different Soybean meal used in diets of Pacific white shrimp, *Litopenaus vannamei*

Diet	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine	Total
1	2.24	0.98	1.60	3.18	2.00	0.60	1.92	1.33	0.47	1.71	16.03
2	2.09	0.91	1.45	2.87	1.87	0.56	1.76	1.18	0.43	1.54	14.66
3	2.12	0.93	1.51	2.94	1.92	0.57	1.78	1.19	0.46	1.62	15.04
4	2.18	0.88	1.55	3.01	1.93	0.59	1.78	1.24	0.46	1.68	15.30
5	2.17	0.92	1.56	3.09	1.96	0.60	1.82	1.28	0.46	1.67	15.53
6	2.22	0.98	1.57	3.05	2.01	0.60	1.85	1.29	0.50	1.69	15.76
7	2.10	0.88	1.58	3.06	1.92	0.61	1.80	1.32	0.49	1.71	15.47
8	2.09	0.96	1.57	3.06	1.96	0.60	1.86	1.31	0.47	1.68	15.56
9	2.21	0.99	1.60	3.16	1.99	0.60	1.92	1.30	0.49	1.73	15.99
10	2.19	0.99	1.62	3.14	2.06	0.63	1.92	1.37	0.51	1.74	16.17
11	2.14	0.90	1.60	3.16	1.95	0.60	1.86	1.36	0.49	1.72	15.78
12	2.14	0.92	1.61	3.12	1.96	0.60	1.89	1.34	0.50	1.70	15.78
13	2.19	0.98	1.66	3.17	2.01	0.60	1.93	1.28	0.49	1.76	16.07
14	2.24	0.99	1.68	3.21	2.03	0.59	1.97	1.30	0.49	1.79	16.29
15	2.19	0.94	1.68	3.25	1.97	0.61	1.94	1.31	0.48	1.78	16.15
16	2.22	0.99	1.70	3.32	2.00	0.58	2.01	1.31	0.46	1.78	16.37
17	2.17	0.98	1.63	3.15	2.03	0.62	1.91	1.35	0.51	1.73	16.08
18	2.18	0.98	1.59	3.11	2.00	0.60	1.88	1.32	0.47	1.69	15.82
19	2.18	0.95	1.57	3.16	1.99	0.61	1.87	1.35	0.50	1.68	15.86
20	2.18	0.90	1.60	3.18	1.95	0.59	1.86	1.32	0.48	1.71	15.77
21	2.18	0.98	1.58	3.14	2.03	0.59	1.90	1.31	0.51	1.69	15.91
22	2.13	0.94	1.64	3.20	1.93	0.61	1.85	1.32	0.46	1.76	15.84
23	2.16	0.97	1.62	3.12	1.99	0.60	1.88	1.29	0.49	1.73	15.85
24	2.22	0.98	1.65	3.23	2.00	0.60	1.93	1.29	0.49	1.77	16.16
25	2.25	1.00	1.64	3.19	2.02	0.60	1.93	1.33	0.49	1.75	16.20

[a] Diets were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Results are expressed on an "as is" basis unless otherwise indicated.

Table 2e: Dispensable Amino acid profile[a] (as is basis) of the different Soybean meal used in diets of Pacific white shrimp, *Litopenaus vannamei*

Diet	Alanine	Aspartic Acid	Cysteine	Glutamic Acid	Glycine	Proline	Serine	Taurine	Hydroxy-proline	Tyrosine	Hydroxy-lysine	Total
1	1.81	3.45	0.52	6.71	1.57	2.20	1.52	0.16	0.07	1.41	0.20	19.62
2	1.64	3.03	0.49	6.04	1.45	1.73	1.36	0.15	0.09	1.27	0.21	17.46
3	1.71	3.13	0.51	6.27	1.52	1.83	1.37	0.17	0.12	1.26	0.19	18.08
4	1.77	3.27	0.53	6.49	1.59	1.89	1.43	0.17	0.12	1.27	0.10	18.63
5	1.82	3.31	0.54	6.77	1.57	1.96	1.59	0.17	0.14	1.29	0.13	19.35
6	1.77	3.35	0.54	6.56	1.57	2.05	1.47	0.18	0.09	1.32	0.19	19.09
7	1.78	3.33	0.53	6.53	1.55	1.92	1.48	0.17	0.07	1.31	0.11	18.78
8	1.78	3.30	0.51	6.48	1.56	2.09	1.48	0.16	0.08	1.30	0.20	19.00
9	1.83	3.34	0.51	6.69	1.60	1.98	1.51	0.18	0.11	1.38	0.20	19.40
10	1.83	3.44	0.53	6.72	1.57	2.15	1.57	0.17	0.09	1.35	0.20	19.68
11	1.83	3.40	0.49	6.72	1.55	2.11	1.55	0.18	0.07	1.34	0.12	19.36
12	1.80	3.43	0.52	6.65	1.61	1.97	1.56	0.17	0.11	1.34	0.17	19.33
13	1.83	3.37	0.52	6.68	1.61	2.17	1.46	0.17	0.09	1.36	0.21	19.47
14	1.86	3.44	0.52	6.81	1.62	2.04	1.49	0.18	0.09	1.39	0.20	19.64
15	1.80	3.43	0.51	6.85	1.49	2.08	1.51	0.17	0.09	1.38	0.15	19.46
16	1.88	3.48	0.50	6.90	1.58	2.09	1.54	0.18	0.07	1.44	0.20	19.86
17	1.82	3.49	0.53	6.71	1.60	2.41	1.52	0.18	0.09	1.36	0.20	19.98
18	1.79	3.36	0.52	6.61	1.57	1.97	1.52	0.18	0.08	1.35	0.19	19.14
19	1.85	3.46	0.54	6.91	1.60	2.01	1.64	0.18	0.10	1.35	0.13	19.81
20	1.82	3.36	0.48	6.74	1.59	1.97	1.53	0.17	0.09	1.36	0.12	19.23
21	1.81	3.32	0.52	6.64	1.58	2.01	1.54	0.18	0.09	1.36	0.20	19.25
22	1.83	3.41	0.52	6.73	1.62	1.98	1.48	0.20	0.11	1.34	0.08	19.33
23	1.78	3.41	0.50	6.68	1.57	2.00	1.46	0.19	0.08	1.34	0.14	19.15
24	1.84	3.41	0.51	6.85	1.60	2.09	1.47	0.18	0.09	1.39	0.17	19.60
25	1.82	3.44	0.52	6.82	1.59	2.03	1.50	0.19	0.08	1.39	0.19	19.57

[a] Diets were analyzed at University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA). Results are expressed on an "as is" basis unless otherwise indicated.

Table 3a: Response of juvenile shrimp (0.23 ± 0.02 g) fed with diets containing different sources of soybean meal over a 6-weeks experimental period (Trial 1). Values represented the mean of eight replicates for the basal diets and four replicates for the rest.

Trt.	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	TGC
1	5.69	5.46	2302	1.73^{ab}	85.0	0.98
2	5.78	5.54	2283	1.70^{ab}	90.0	0.99
3	5.54	5.31	2269	1.73^{ab}	90.0	0.97
4	5.94	5.71	2458	1.60^{b}	87.5	1.01
5	5.71	5.50	2602	1.64^{b}	85.0	1.01
6	5.61	5.38	2365	1.68^{ab}	85.0	0.98
7	5.58	5.36	2466	1.69^{ab}	95.0	0.99
8	5.06	4.84	2210	1.97^{a}	80.0	0.94
9	5.28	5.05	2231	1.78^{ab}	82.5	0.95
10	5.34	5.10	2152	1.73^{ab}	92.5	0.95
11	5.62	5.39	2371	1.71^{ab}	80.0	0.99
12	5.18	4.96	2259	1.75^{ab}	97.5	0.95
13	5.42	5.19	2290	1.70^{ab}	90.0	0.97
14	5.23	4.99	2165	1.80^{ab}	85.0	0.95
PSE	0.39	0.38	217.65	0.13	7.94	0.003
P-value	0.07	0.07	0.23	0.06	0.07	0.067

Values with different superscripts within the same column are significantly different based on Tukey pairwise comparisons

Feed conversion ratio: feed offered/ (final weight-initial weight)
Weight gain: (final weight-initial weight)/initial weight × 100%
Thermal Growth Coefficient (TGC): (Final weight$^{1/3}$-Intial weight$^{1/3}$)/Σ (Temp *days)*1000

Table 3b: Response of juvenile shrimp (0.67 ± 0.02 g) fed with diets containing different sources of soybean meal over 5-weeks experimental period (Trial 2). Values represented the means of five replicates.

Trt.	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)	TGC
1	6.07^{ab}	5.40^{ab}	811^{ab}	1.86^{ab}	86^{ab}	0.92^{ab}
15	5.53^{b}	4.86^{b}	731^{ab}	1.93^{ab}	92^{ab}	0.87^{ab}
16	5.36^{b}	4.70^{b}	712.2^{b}	2.02^{a}	96^{a}	0.85^{b}
17	5.44^{b}	4.76^{b}	697^{b}	2.04^{a}	90^{ab}	0.85^{b}
18	5.52^{b}	4.85^{b}	717^{b}	1.97^{ab}	96^{a}	0.86^{b}
19	6.02^{ab}	5.36^{ab}	807^{ab}	1.81^{ab}	88^{ab}	0.92^{ab}
20	5.97^{ab}	5.31^{ab}	807^{ab}	1.79^{ab}	96^{a}	0.91^{ab}
21	6.33^{a}	5.66^{a}	851^{a}	1.67^{b}	92^{ab}	0.95^{a}
22	5.89^{ab}	5.20^{ab}	749^{ab}	1.84^{ab}	90^{ab}	0.89^{ab}
23	6.08^{ab}	5.39^{ab}	791^{ab}	1.77^{ab}	92^{ab}	0.91^{ab}
24	5.85^{ab}	5.17^{ab}	764^{ab}	1.84^{ab}	92^{ab}	0.89^{ab}
25	5.55^{ab}	4.86^{b}	707^{b}	1.99^{a}	80^{b}	0.86^{b}
PSE	0.37	0.37	60.08	0.14	7.19	0.004
P-value	0.001	0.001	0.001	0.002	0.041	0.001

Values with different superscripts within the same column are significantly different based on Tukey pairwise comparisons

Table 3c: Total Growth Coefficients (TGC) of juvenile shrimp (as a percentage from TGC of basal diet) fed with diets containing different sources of soybean meal (Trials 1 & 2 combined data). PSE = 3.87 and P- value <0.001.

Trt.	TGC	Trt.	TGC
2	100.42 abcd	14	96.08 abcd
3	98.885 abcd	15	94.08 abcd
4	102.57 ab	16	92.45 cd
5	102.16 abc	17	92.34 d
6	99.94 abcd	18	93.67 abcd
7	100.43 abcd	19	99.62 abcd
8	95.39 abcd	20	99.27 abcd
9	96.97 abcd	21	102.74 a
10	96.57 abcd	22	96.9 abcd
11	100.11 abcd	23	99.36 abcd
12	96.49 abcd	24	97.14 abcd
13	98.3 abcd	25	93.4 bcd

Values with different superscripts within the same column are significantly different based on Tukey pairwise comparisons

Table 3d: Water quality data (mean ± SD[1]) of the growth trials, 1 and 2.

	Trial 1	Trial 2
Dissolved oxygen (mg/L)	6.02 ± 0.89	6.78 ± 0.30
Salinity (ppt)	7.51 ± 0.37	7.20 ± 0.49
Temperature (^{0}C)	28.19 ± 2.04	29.53 ± 0.60
pH	7.48 ± 0.48	7.45 ± 0.52
TAN[2] (mg/L)	0.11 ± 0.05	0.12 ± 0.08
Nitrite (mg/L)	0.07 ± 0.02	0.13 ± 0.02

[1] SD = Standard Deviation

[2] TAN = Total Ammonia Nitrogen

Table 4: Principle component analysis of chemical characteristics of SBM sources.

Variable	PC1	PC2	PC3	PC4	PC5
Trypsin Inhibitor	0.2138	-0.0050	0.0739	-0.3508	-0.1167
Fructose	0.1786	-0.0550	0.3096	0.3782	0.1568
Glucose	0.2267	-0.0594	0.2669	0.3216	0.1670
Sucrose	-0.3112	0.1642	0.0682	-0.1364	0.2045
Raffinose	0.1874	0.0245	-0.3020	-0.1670	-0.1315
Stachyose	-0.1868	0.2506	-0.1062	-0.2097	0.2323
ADF	0.2267	-0.1123	0.2917	-0.1822	-0.0175
NDF	0.1904	-0.0955	0.3059	-0.1194	-0.0022
Lignin	0.0105	-0.1362	-0.1243	0.2744	-0.1507
Ca	0.2973	0.2157	-0.1229	0.1022	0.1329
P	-0.1727	0.2926	0.2966	0.0502	-0.1050
P in PA	-0.2487	0.0980	0.3494	-0.0185	0.0911
Total PA	-0.2468	0.0900	0.3534	-0.0176	0.0833
Non-phytate P	0.1013	0.3683	-0.0315	0.0939	-0.2835
Cu	0.1345	0.2130	-0.2365	0.0576	0.5020
Fe	0.3297	-0.0279	0.1597	0.0647	0.0656
Mg	0.2447	0.2164	-0.0286	0.0541	-0.4240
Mn	0.2428	0.2542	0.0932	0.1746	0.1493
Mo	-0.2113	0.0006	-0.1997	0.4188	-0.0452
K	-0.2281	0.0951	-0.0520	0.4057	-0.1609
Na	0.0538	0.4160	0.0612	0.0040	-0.1989
S	-0.0456	0.3789	0.1278	-0.1142	-0.1657
Zn	0.1336	0.3068	-0.1470	-0.0292	0.3467
Eigen value	7.0844	5.0033	3.2787	2.0938	1.5463
% variance	30.8	21.8	14.3	9.1	6.7
Cumulative %	30.8	52.6	66.8	75.9	82.6

Table 5: Multiple linear regression of Thermal growth coefficient (TGC) with principle components (PC1, PC2, PC3, PC4, PC5)

Model p-value= 0.016 R^2 = 0.127	Parameter estimates	p- value for each variable
PC1	-0.1643	0.3108
PC2	0.4516	0.0195
PC3	0.5929	0.0142
PC4	-0.1286	0.6726
PC5	0.4413	0.2052

Table 6: Pearson correlation coefficients of TGC with raffinose, ADF, NDF, phosphorus, phosphorus in phytic acid, total phytic acid, non-phytate phosphorus, sodium, sulfur and zinc.

Variable	r-value	p- value	Variable	r-value	p- value
Raffinose	-0.358	0.086	Total phytic acid	0.426	0.038
ADF	0.256	0.228	Non-phytate phosphorus	0.140	0.514
NDF	0.298	0.157	Sodium	0.353	0.091
Phosphorus	0.469	0.021	Sulfur	0.327	0.119
Phosphorus in phytic acid	0.429	0.037	Zinc	0.199	0.351

Figure 1: Interval plot of standardized Total Growth Coefficients (TGC) of juvenile shrimp (as a percentage from TGC of basal diet) fed with diets contained different sources of soybean meal (Trials 1 & 2 combined data)

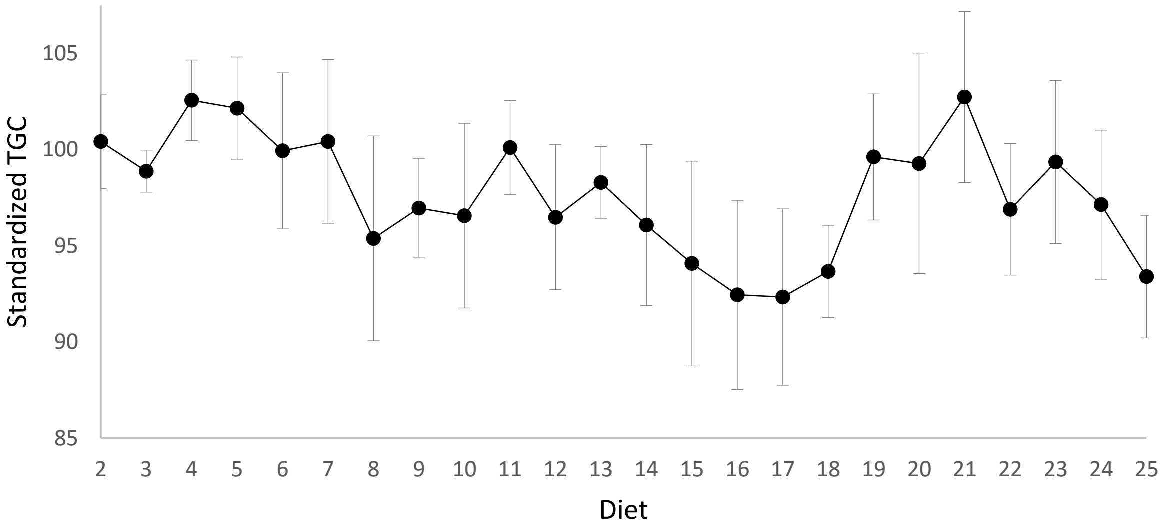
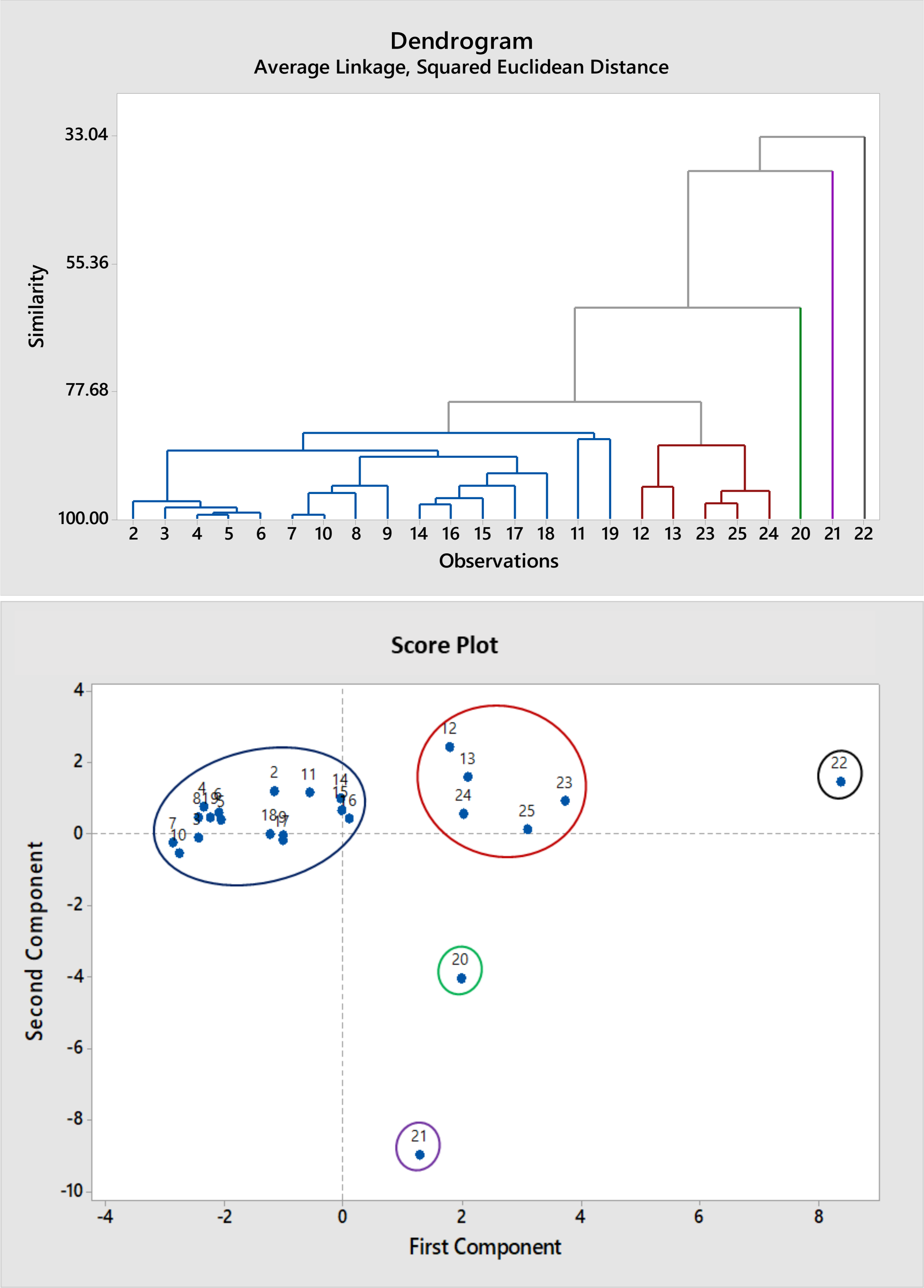


Figure 2: Dendrogram of Cluster analysis (grouping of SBM base on their chemical characteristics) and score plot of PCA (grouping of SBM base on their chemical characteristics over the component 1 (31% of variation) and component 2 (22% of variation) of PCA)



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CHAPTER III

DIFFERENTLY PROCESSED SOYBEAN AS AN INGREDIENT IN PRACTICAL DIETS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Abstract

Presence of anti-nutritional factors is often referenced as one of the major drawback of solvent extracted soybean meal (SBM) that may limit its inclusion level in animal feeds. In response, various processing strategies were developed over time to diminish the adverse quality characteristics of traditional SBM. Despite the higher manufacturing cost, inclusion levels of these new SBM products in to fish feed formulations can still be limited due to the different sensitivities of fish/shrimp and/or due to the secondary quality characteristics caused during the processing methods. Hence, the present study was designed to determine the effect of differently processed SBM on growth performances of Pacific white shrimp (*Litopenaeus vannamei*). The growth trial was conducted with iso-nitrogenous (350 g/kg protein) and iso-lipidic (80 g/kg lipid) test diets formulated with two sources of solvent extracted soybean meal (SBM44 and SBM49), enzyme treated soybean meal (ETSBM), fermented soybean meal (FSBM), alcohol-extracted soy protein concentrate (SPC). Growth trial was conducted with the stocking density of 10 shrimp/aquarium in a semi-recirculatory system and the initial weight of shrimp was 0.27±0.02 g. As per the statistical analyses, diets incorporated with SBM44, SBM49 and ETSBM yielded significantly higher mean final weights, weight gains and percentage weight gains and lower FCRs. Mean percentages survival of shrimp ranged from 81-95% with no significant differences among the treatments. Results of the present study infer that the traditional solvent extracted soybean meal performs equally with the enzyme treated SBM while shrimp fed with fermented SBM or alcohol extracted soy protein concentrate has reduced performances might be due to the lower nutrient digestibility or palatability in Pacific white shrimps.

KEY WORDS: Differently processed soybean meal, growth of shrimp

1. Introduction

Fishmeal has traditionally been the main protein source used in traditional aquaculture feed formulations increasing its demand and price parallel to the expansion of the industry (Davis et al. 2008). Supporting this, fishmeal utilization in feeds has increased steadily from approximately 15% to 65% over the last two decades (Tacon and Metian 2008). Hardy (2010) argued that the demand might exceed the world production of fish meal based upon the expected growth rates of aquaculture and rates of fishmeal utilization in feeds. As an alternative, a wide variety of plant- based dietary ingredients have been tested (NRC 2011, Amaya et al. 2007a) while solvent extracted soybean meal (SBM) attracted most of the attention due to its comparable amino acid profile, worldwide availability, low price and consistent composition (Dersjant-Li 2002, Swick et al. 1995, Amaya et al. 2007c, Davis and Arnold 2000, Gatlin et al. 2007). However, the inclusion level of SBM in practical shrimp diet may be restricted due to the presence of anti-nutritional factors (ANFs) (trypsin inhibitors, antigens, lectins, saponins and oligosaccharides), insufficient levels of essential amino acids (EAA) (methionine and lysine) and poor palatability. These factors may result in negative effects on growth, digestion and nutrient availability to shrimp (Dersjant-Li 2002, Gatlin et al. 2007).

Different processing strategies have been developed over time such as thermal treatments, alcohol extractions, enzyme hydrolysis, fermentation, soaking, germination, etc. with the objective of reducing or eliminating the antinutritional factors (ANFs), improving bioavailability of micro-nutrients and nutrient digestibility of traditional SBM (Hotz and Gibson 2007, Qiu et al. 2018, Masumoto et al. 2001, Lim and Lee 2011, NRC 2011, Chou et al. 2004, Lim and Lee 2009, Refstie et al. 1998a). In addition, Sookying and Davis (2012) stated that the enhanced protein levels of the differently processed SBM provide more space in feed formulations to supplement the deficient nutrients and/or ingredients to enhance nutrition and pellet quality. Despite the advantages offered through different processing techniques, they are more costly than the production of traditional SBM (Lee et al. 2016). Furthermore, some of the differently processed SBM commodities possess product specific defects which limits the inclusion level to the shrimp/fish diet formulations possibly due to the different sensitivities of fish/shrimp to SBM (Chou et al. 2004) and/or due to the secondary quality characteristics caused during the processing methods, such as changes in texture, palatability, etc. Therefore, the current study was conducted with the objective of investigating the effect of differently processed SBM on growth performances of Pacific white shrimp (*Litopenaeus vannamei*).

2. Materials and Methods

2.1 Diet preparation

Five iso-nitrogenous (350 g/kg protein) and iso-lipidic (80 g/kg lipid) test diets were formulated using differently processed soybean meal sources (Table 1). Solvent extracted soybean meals with 44 and 49% protein (SBM44, Bunge Limited, Decatur, AL, USA; SBM49, Faithway Feed Co., Guntersville, AL, respectively) were used in diets 1 and 5, respectively. While diets 2, 3 and 4 contained advanced soy products; enzyme treated soybean meal (ETSBM, Nutrivance™, Midwest Ag Enterprises, Marshall, MN, USA), fermented soybean meal (FSBM, PepsoyGen soybean meal, Nutraferma Inc., North Sioux City, SD, USA) and soy protein concentrate (SPC, Soycomil P, Archer Daniels Midland Company, Decatur, IL. USA). In addition to different sources of SBM, a fixed level of 6% of menhaden fish meal (Omega Protein Inc., Houston, TX, USA) and 7% corn protein concentrate (CPC Empyreal 75™, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA) were used as the dietary protein sources of the diets.

The test diets were prepared in the feed laboratory of Auburn University, Auburn, AL, USA using standard practices. Briefly, pre-ground dry ingredients and oil were weighted and mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Hot water (~30% by weight) was then blended into the mixture to attain a consistency appropriate for pelleting. Finally, all diets were pressure-pelleted using a meat grinder (Hobart Corporation, Troy, OH, USA) with a 3-mm die, dried in a forced air oven (50 °C) to a moisture content of <100g/kg. Dry pellets were crumbled and stored at 4 ^{0}C until used. The diets were analyzed at University of Missouri Agricultural Experimental Station Chemical Laboratories (Columbia, MO, USA) for proximate composition and amino acid profile (Table 1 & 2).

2.2 Experimental system

The growth trail was conducted in a semi-closed recirculation system consisted of a series of 60-L aquaria connected to a common reservoir tank (800-L). Water quality was maintained by recirculation through an Aquadine bead filter (0.2 m^2 media, 0.6 m × 1.1 m) and a vertical fluidized bed biological filter (600-L volume with 200-L of Kaldnes media) using a 0.25-hp. centrifugal pump. Mean water flow for an aquarium was 3 L/min with an average turnover of 20 minutes/tank. Salt water was prepared by mixing artificial crystal sea salt (Crystal Sea Marinemix, Baltimore, MD, USA) with freshwater and maintained at around 7ppt during the each growth trial. Dissolved oxygen was maintained near saturation using air stones in each culture tank and the sump tank using a common airline connected to a regenerative blower. Dissolved oxygen, salinity and water temperature in the reservoir tank (sump tank) were measured twice daily using a YSI-55 digital oxygen/temperature meter (YSI corporation, Yellow Springs, Ohio, USA). Total ammonia-N (TAN) and nitrite-N were measured twice per week according to the methods described by Solorzano (1969) and Spotte (1979), respectively. The pH of the water was measured twice weekly during the experimental period using the pHTestr30 (Oakton Instrument, Vernon Hills, IL, USA). During growth trial, DO, temperature, salinity, pH, TAN, and nitrite were maintained within the ranges of 5.15±1.23 mg/L, 29.6±0.6 ^{0}C, 7.7±0.4 ppt, 7.0±0.6, 0.23±0.18 mg/L, and 0.15±0.22 mg/L, respectively.

2.3 Growth trials

Five dietary treatments were randomly assigned to tanks and each trial was conducted using a double blind experimental design. Growth trial was conducted with eight replicates and ten Pacific white shrimp were stocked per tank with mean initial weight of 0.27±0.02 g. Test diets were offered four times daily for five weeks. Daily ration of feed was calculated based upon an estimated weight gain and expected feed conversion ratio (FCR) of 1.8. Shrimp were counted weekly and the feed was adjusted each week based on survival and observation of the feeding response. At the conclusion, shrimp were counted and group-weighed. Mean final weight, final biomass, survival, weight gain and feed conversion ratio (FCR) were determined (Table 3).

2.4 Statistical analysis

All the data were analyzed using SAS (V9.4, SAS Institute, Cary, NC, USA). Growth performances of shrimp was analyzed using one-way ANOVA to determine significant differences (p<.05) among treatments followed by the Tukey's multiple comparison test to evaluate significant differences between treatment means.

3. Results

Growth performances of juvenile *L. vannamei* treated with differently processed SBM products are presented in Table 3. At the conclusion of the five weeks culture period, diets incorporated with SBM44 (diet 1), ETSBM (diet 2) and SBM49 (diet 5) yielded significantly higher mean final weight, weight gain and percentage weight gain while significantly lower mean FCR values in shrimp. Mean survival of the shrimp ranged from 81-95% with no significant differences among the treatments.

4. Discussion

World aquaculture feed production is between 50 and 60 million metric tons (MMT). Based on industry estimates, 4.5 MMT of fishmeal and 15 MMT of SBM are used in aquaculture. Assuming 50 MMT of feeds, the inclusion of fishmeal averages only 9% and SBM 30% of the diet making soybean meal the dominant protein source in feeds. Due to the high popularity of SBM as an alternative to fishmeal, a fair amount of research work have been conducted with the objective of diminishing its anti-nutritional factors (ANFs) and to concentrate the essential amino acids (EAA) through removal or transformation of the carbohydrate portion from the particular ingredient. For this, different processing techniques such as thermal treatment, alcohol extraction, enzyme hydrolysis, and fermentation have been tested while achieving different success levels (Hotz and Gibson 2007, Qiu et al. 2018, Masumoto et al. 2001, Lim and Lee 2011, NRC 2011, Chou et al. 2004, Lim and Lee 2009, Refstie et al. 1998a). As a result, numerous scientific publications are available justifying the use of different SBM products replacing fishmeal in different quantities without compromising the growth and health of fish and shrimp. However, new SBM commodities produced through advanced processing methodologies are typically more costly than traditional SBM (Lee et al. 2016). Major limitations in inclusion levels are often reported presumably due to different sensitivities of fish/shrimp to anti-nutrients (Chou et al. 2004) and/or due to the secondary quality characteristics caused by processing methods.

All the different types of SBM used during the present study are commercial products already existing on the market which are manufactured through four main processing methodologies; traditional solvent extraction (SBM44 and SBM49), enzyme treated (ETSBM), fermentation processing (FSBM) and additional processing and alcohol extraction (SPC). The ETSBM, FSBM and SPC are often referred to as advanced soy products as they are produced by processing methodologies, which were modified or improved from the traditional solvent extraction procedure. These products contain higher levels for protein (62.55, 52.20 and 64.90% (as is), respectively) over SBM (43.70% and 48.80%). As the protein is simply condensed differences in essential and non-essential amino acid profiles between the ingredients and subsequently the diets is minimal (See table 2).

According to the outcomes of the present study, diets containing SBM (Diet 1 and 5) and ETSBM (Diet 2) resulted in significantly higher growth performances of Pacific white shrimp inferring the equal suitability of SBM produced via traditional solvent extraction method and through enzyme treatments. Given the similarity of EAA profiles of the feed, this is not likely due to difference in protein or amino acids in the meals. The different processing conditions did result in differences in trypsin inhibitor levels of the feed. However, based on regression analysis there was no response of the shrimp to trypsin levels of the diets (or protein content of the ingredients), emphasizing the importance of considering the complete nutritional profile of an ingredient rather than individual variables (Francis et al. 2001). The response is likely due to the combined effect of nutrients as well as interactions on nutrient digestibility and absorption.

Lower growth performance of fish fed fermented SBM (FSBM) is well documented in literature but it is quite limited for shrimp. Most of the studies claimed reduced levels of different antinutritional factors (mainly oligosaccharides) and improved protein and carbohydrate digestibility levels due to fermentation; however, very limited improvements in growth performances were detected in fish might be due to the effect of fermentation on overall nutritional value of soybean meal (Murashita et al. 2013, Yamamoto et al. 2010, Refstie et al. 2005, Shimeno et al. 1993, Trushenski et al. 2014). Relevant to shrimp, Sharawy et al., (2016) recorded that the solid state fermented SBM with yeast, *Saccharomyces cerevisiae* can replace dietary fishmeal up to 25% in diets of Indian white shrimp, *Fenneropenaeus indicus* while reduced growth performance over 25% replacement could be attributed to the presence of non-digestible oligosaccharides and lower protein digestibility. Thus, incomplete elimination of anti-nutritional factors (Yamamoto et al. 2010, Shiu *et al.* 2015), lower protein and amino acid digestibility (Xuan et al., 2017) and some of the other species-specific sensitivities of Pacific white shrimp might be the reason for the detected lower growth performances against FSBM.

Incurring the negative effects of higher inclusion levels of SPC in the diet of *L. vannamei*, Sookying (2010) observed a significantly lower weight gain and higher FCR in shrimp fed with diets containing SPC at 20% or greater and suggested a possibility of limited (10%) inclusion of SPC replacing SBM without compromising growth. Furthermore, Soares et al., (2015) reported a negative linear trend for total weight gain and feed intake as replacement of fishmeal by SPC. However, replacing up to 75% of fishmeal by SPC (27% inclusion) did not significantly impair the growth of shrimp (Soares et al. 2015). In contrast, Bauer et al., (2012), revealed the possibility of complete replacement of fishmeal with SPC (up to 28% as is) without causing significant differences in weight gain and FCR of *L. vannamei fed with diets supplemented with* DL-Methionine. However, in a similar kind of study conducted by Forster et al., (2002), a significant reduction of growth and feed intake of *L. vannamei was noted against* 100% replacement of fish meal by SPC while supplementing essential amino acids (methionine, arginine, and phenylalanine). This was further observed through the research of Sookying (2010), by having lower growth performances of shrimp at higher SPC inclusion level (40%) supplemented with DL-methionine or micro-encapsulated methionine to the diet. Thus, it could be a factor generate during the additional processing steps of SPC such as palatability (McGoogan and Gatlin 1997, Soares et al. 2015, Forster et al. 2002) which might limit intake and subsequently the growth of *L. vannamei* at higher inclusion levels.

De Carvalho et al., (2016) highlighted the importance of digestibility analysis to evaluate the overall nutritive value of the ingredients use in aqua-feeds ensuring the effectiveness of the formulation while reducing the negative impacts of aquaculture operations because of the waste by-products (Irvin and Tabrett 2005). Qiu et al., (2017) utilized same SBM sources (SBM44, ETSBM and FSBM) along with several other ingredients to evaluate the apparent digestibility on Pacific white shrimp, *L. vannamei* and revealed that the traditional soybean meal (SBM44) and enzyme treated soybean meal (ETSBM) perform better than fermented soybean meal (FSBM) considering the digestibility values of protein and amino acids. Therefore, higher growth performances of shrimp fed solvent extracted SBM (SBM44 & SBM49) and enzyme treated SBM (ETSBM) during the present study could be inferred through the higher digestibility values of particular ingredients. In vivo and in vitro digestibility studies conducted by Cruz-Suárez et al. (2009) recoded that the crude protein and amino acids digestibilities of SPC in *L. vannamei* was significantly lower compared to SBM, which could explain the discrepancies in growth performances between particular diets observed during the current study. Therefore, reduced performances of the shrimp observed when using fermented (FSBM) and alcohol extracted soybean meal (SPC) at high levels could be due to reduced nutrient digestibility or palatability of these ingredients for Pacific white shrimps and further research would be vital to apply appropriate remedies.

5. Conclusion

Results of the present study infer that when used at high levels of inclusion, the traditional solvent extracted soybean meal (SBM44 and SBM49) and enzyme treated soybean meal (ETSBM) support equivalent growth and feed conversion in juvenile shrimp. Whereas the reduced performances of the shrimp was observed when using fermented soybean meal (FSBM) and alcohol extracted soybean meal (SPC) at high levels which could be due to reduced nutrient digestibility or palatability of these ingredients for Pacific white shrimps.

Table 1: Formulation and chemical composition of test diets used in the growth trial (% as is).

Ingredient (g/100g as is)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Menhaden fishmeal[a]	6	6	6	6	6
SBM44[b]	51.7	-	-	-	-
SBM49[c]	-	-	-	-	46.3
SPC[d]	-	-	-	34.8	-
ETSBM[e]	-	36.2	-	-	-
FSBM[f]	-	-	43.3	-	-
CPC - Empareal 75[g]	7	7	7	7	7
Menhaden fish oil[a]	5.76	5.76	6.29	6.3	5.63
Lecithin[h]	1	1	1	1	1
Cholesterol[i]	0.05	0.05	0.05	0.05	0.05
Corn Starch[i]	0.39	15.89	8.26	16.75	5.92
Whole wheat[i]	23	23	23	23	23
Mineral premix[j]	0.5	0.5	0.5	0.5	0.5
Vitamin premix[k]	1.8	1.8	1.8	1.8	1.8
Choline chloride[l]	0.2	0.2	0.2	0.2	0.2
Stay C 35% active[m]	0.1	0.1	0.1	0.1	0.1
CaP-dibasic[l]	2.5	2.5	2.5	2.5	2.5
Proximate composition					
Pepsin Digestibility	92.23	93.01	93.46	94.45	94.21
Trypsin Inhibitor (TIU/g)	1237	0	151	53	153
Crude protein	37.51	34.89	36.05	35.74	36.21
Moisture	6.83	7.39	6.50	6.42	6.35
Crude Fat	9.37	8.21	8.29	8.33	8.85
Crude Fiber	4.34	3.61	3.92	3.49	4.04
Ash	6.71	5.00	6.72	5.68	6.42

Diet 1- Solvent extracted soybean meal (SBM44); Diet 2- Enzyme treated soybean meal (ETSBM); Diet 3- Fermented soybean meal (FSBM); Diet 4- Alcohol extracted soybean meal (SPC); Diet 4- Solvent extracted soybean meal (SBM49).

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

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

[c] Faithway Feed Co., LLC, Guntersville, AL, USA.

[d] Soycomil P, Archer Daniels Midland Company, Decatur, IL. USA.

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

[f] PepsoyGen soybean meal, Nutraferma Inc., North Sioux City, SD, USA.

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

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

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

[j] Trace mineral premix (g/100g premix): Cobalt chloride, 0.004; Cupric sulfate pentahydrate, 0.550; Ferrous sulfate, 2.000; Magnesium sulfate anhydrous, 13.862; Manganese sulfate monohydrate, 0.650; Potassium iodide, 0.067; Sodium selenite, 0.010; Zinc sulfate heptahydrate, 13.193; Alpha-cellulose, 69.664.

[k] Vitamin premix (g/kg premix): Thiamin HCl, 4.95; Riboflavin, 3.83; Pyridoxine HCl, 4.00; Ca-Pantothenate, 10.00; Nicotinic acid, 10.00; Biotin, 0.50; folic acid, 4.00; Cyanocobalamin, 0.05; Inositol, 25.00; Vitamin A acetate (500,000 IU/g), 0.32; Vitamin D3 (1,000,000 IU/g), 80.00; Menadione, 0.50; Alpha-cellulose, 856.81.

[l] VWR Amresco, Suwanee, GA, USA.

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

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

Table 2: Amino acid profile (as is basis) of test diets of Pacific white shrimp, *Litopenaus vannamei*

Amino Acid	Diet Code				
	1	2	3	4	5
Alanine	1.85	1.72	1.78	1.81	1.79
Arginine	2.26	2.05	2.07	2.18	2.19
Aspartic Acid	3.53	3.21	3.31	3.39	3.38
Cysteine	0.56	0.52	0.51	0.53	0.54
Glutamic Acid	7.10	6.42	6.62	6.84	6.74
Glycine	1.65	1.53	1.61	1.61	1.61
Histidine	0.89	0.82	0.84	0.86	0.86
Hydroxy lysine	0.05	0.03	0.04	0.03	0.05
Hydroxy proline	0.10	0.08	0.10	0.09	0.16
Isoleucine	1.71	1.59	1.68	1.73	1.71
Leucine	3.22	3.07	3.14	3.23	3.17
Lysine	2.05	1.83	1.82	1.90	1.93
Methionine	0.63	0.60	0.60	0.61	0.62
Ornithine	0.02	0.01	0.02	0.02	0.02
Phenylalanine	1.90	1.77	1.84	1.88	1.87
Proline	2.31	2.21	2.27	2.28	2.32
Serine	1.71	1.46	1.46	1.50	1.49
Taurine	0.00	0.18	0.18	0.18	0.16
Threonine	1.33	1.24	1.25	1.28	1.27
Tryptophan	0.45	0.45	0.45	0.47	0.45
Tyrosine	1.24	1.25	1.34	1.35	1.35
Valine	1.77	1.67	1.73	1.75	1.72
Sum of Amino Acids	36.33	33.71	34.66	35.52	35.40

Diet 1- Solvent extracted soybean meal (SBM44); Diet 2- Enzyme treated soybean meal (ETSBM); Diet 3- Fermented soybean meal (FSBM); Diet 4- Alcohol extracted soybean meal (SPC); Diet 4- Solvent extracted soybean meal (SBM49).

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

Table 3: Response of juvenile Pacific white shrimp, *Litopenaus vannamei* (0.27 ± 0.02 g) fed with diets containing differently processed of soybean meal over a 5-week experimental period stocked at 10 shrimp/ tank. Values represented the mean of eight replicates.

Diet	Final mean weight (g)	Weight gain (g)	Weight gain (%)	FCR	Survival (%)
1 (SBM44)	4.0^{a}	3.7^{a}	1460^{a}	1.9^{b}	88
2 (ETSBM)	3.6^{a}	3.4^{a}	1268^{a}	2.2^{b}	81
3 (FSBM)	2.9^{b}	2.6^{b}	983^{b}	2.7^{a}	90
4 (SPC)	2.9^{b}	2.7^{b}	1008^{b}	2.7^{a}	84
5 (SBM49)	3.8^{a}	3.5^{a}	1294^{a}	2.0^{b}	95
PSE	0.4	0.4	165.2	0.3	10.2
P-value	0.0	0.0	0.0	0.0	0.085

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

Diet 1- Solvent extracted soybean meal (SBM44); Diet 2- Enzyme treated soybean meal (ETSBM); Diet 3- Fermented soybean meal (FSBM); Diet 4- Alcohol extracted soybean meal (SPC); Diet 4- Solvent extracted soybean meal (SBM49).

Table 4: Water quality data (mean ± SD[1]) of the 5-weeks growth trial of Pacific white shrimp, *Litopenaus vannamei* fed with diets contained differently processed soybean meal.

DO (mg/L)	Temp (^{o}C)	Salinity (ppt)	TAN (mg/L)	Nitrite (mg/L)	pH
5.15±1.23	29.63±0.61	7.72±0.43	0.23±0.18	0.15±0.22	7.08±0.04

[1] SD = Standard Deviation

[2] TAN = Total Ammonia Nitrogen

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CHAPTER IV

SUMMARY AND CONCLUSION

Aquaculture industry is expanding rapidly over time exceeding the annual growth rates of poultry, pork, dairy and beef industries and it serves roughly half of the fish consumed globally. Although aquaculture contributes significantly to the global fish and shrimp production, its future growth is heavily depended on the effectiveness of sustainable feed formulations. In general, commercial fish and shrimp feeds contain 30–50% crude protein, which is the most expensive component of the diet, historically supplemented through fishmeal. However, as the aquaculture industry expands so does the demand resulting in increases in the price of fishmeal. Along with the rocketing prices, static supply and ethical issues, average dietary fishmeal inclusion levels within compounded feed have been steadily declining. As an alternative for the fishmeal in fish and shrimp feed formulations, wide variety of plant- based dietary ingredients have been tested, but soybean meal received the most attention considering its well-balanced amino acid profile, advantage of being resistant to oxidation and spoilage, worldwide availability, low price, consistent composition and sustainability. Therefore, the overall goal of this research was to evaluate the use of soybean meal in feed formulations for Pacific white shrimp. To be more specifically, the purpose was to investigate the effect of SBM sourced from different geographical locations in the world and differently processed SBM on growth performances of Pacific white shrimps (*Litopenaeus vannamei*).

Numerous factors affect the composition and quality of soybean meal. Therefore, it is vital that raw soybeans contain an optimal nutrient profile in order to produce the highest quality soybean meal. Because of the diversity of growing conditions throughout the world, it is expected that soybeans produced under different environmental conditions combined with the differences in varieties and agricultural practices would have varying nutrient compositions and qualities. Furthermore, the differences in meal processing methods and conditions such as moisture, temperature and drying time may add variations to the final nutritional quality of SBM. The results of this study demonstrate differences even in reasonably similar sources of soybean meal. As per the complete chemical profiles of the SBM sourced from different geographical locations in the world, phosphorous, phytate-phosphorous and total phytic acid levels had positive correlations ($p<0.005$) with Thermal growth coefficient (TGC); whereas raffinose ($p= 0.086$) had a negative trend with TGC. It is difficult to make firm conclusions about a specific culprit for the resulted growth performances and their threshold levels on Pacific white shrimp, might be due to the interactive effects of anti-nutrients and nutrients. However, phosphorous, phosphorous in phytic acid and total phytic acid and Raffinose were screened with significant correlations, which could cause major effects on the growth performances of Pacific white shrimp.

Presence of anti-nutritional factors (ANFs) such as trypsin inhibitors, antigens, lectins, saponins and oligosaccharides, etc. are often referenced as one of the major drawback of solvent extracted soybean meal (SBM) which may limit its inclusion level in animal feeds. In response, various processing strategies have been developed over time such as thermal treatments, alcohol extractions, enzyme hydrolysis, fermentation, soaking, germination, etc. with the objective of reducing or eliminating the antinutritional factors (ANFs), improving bioavailability of micro-nutrients and nutrient digestibility of traditional SBM. Despite the higher manufacturing cost, inclusion levels of these new SBM products in to fish feed formulations can still be limited due to the different sensitivities of fish/shrimp and/or due to the secondary quality characteristics caused during the processing methods such as changes in texture, palatability, etc. As per the present study, the traditional solvent extracted soybean meal and enzyme treated soybean meal support equivalent growth and feed conversion in juvenile shrimp at high level of inclusion. Whereas the reduced performance of the shrimp observed when using fermented and alcohol extracted soybean meal at high levels could be due to reduced nutrient digestibility or palatability of these ingredients for Pacific white shrimp.

These studies have indicated that the composition and quality of soybean meal could vary based on the production location and processing method. Based on the significant differences in growth responses of Pacific white shrimp, it proved that even in reasonably similar sources of soybean meal could cause those changes in growth might be due to an individual or interactive effect of nutrients and anti-nutrients or due to the differences in nutrient digestibility or palatability. Hence, to understand and predict the biological performance on animals, systematic research is needed to look at various processing and nutritional changes in SBM and how they influence on the performances of Pacific white shrimp.

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