Evaluation of soybean meal from selected cultivars of soybeans for use in practical diets for *L.vannamei*.

by

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Abstract

This study was designed to evaluate the efficacy of twelve sources of soybean meal (SBM), which included new soy varieties in practical feed formulation for Pacific white shrimp, *Litopenaeus vannamei*, using both digestibility and growth trials. Apparent digestibility coefficients for dry matter (ADMD), energy (ADE) and protein (ADP) were determined in *L.vannamei* using chromic oxide as inert marker and the 70:30 replacement technique. Digestibility coefficients for ADMD, ADE and ADP ranged from 71.3% to 88.3%, 76.6% to 91.3% and 93.6% to 99.8%, respectively. In general, two distinct groups of high and moderate digestibility values were observed. Selected soybean meals were run in an 8-week growth trial with six replicate tanks per dietary treatment (10 shrimp per tank, initial weight 0.52 $\pm$0.04 g). A soybean meal-based reference diet was formulated using commercial soybean meal (52.7% diet), which was then completely replaced on iso-nitrogenous basis with other experimental soybean meals. Significant differences ($P > 0.05$) were observed in growth but not survival. The shrimps' final mean weights (5.73-7.60g), weight gains (4.90-6.81g), percent weight gains (508-864%), survivals (75.0-92.0%), and FCRs (1.7-2.4) were within typical limits. In general, the results and trend of digestibility data was similar to that of the growth trial with ingredients I-16, I-17, I-18, and I-19 demonstrating relatively higher values in digestibility and growth. These varieties have a potential to increase the nutritional values of soybean meal for shrimp feeds.

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1. Introduction

Pacific white shrimp *Litopenaeus vannamei* is presently the primary shrimp species cultured with a rapid expanding world production from 0.15 million tonnes in 2000 to 2.7 million tons in 2010 (FAO 2011). This rapid expansion of the industry has been paralleled with an increase in shrimp feed production and the ingredients used to make the feeds. Tacon and Metian (2008) reported that estimated production of commercial shrimp will increase from 0.9 million tonnes (mmt) in 1995 to 9.2 mmt in 2020. Typically, commercial shrimp feed contains 20 to 30% fishmeal; so, these estimates suggest that the global demand for fishmeal in marine shrimp feed is expected to continue to increase. However, the International Fishmeal and Fish oil Organization (IFFO, 2006) reported that fishmeal production has remained relatively constant since 1985 at about 7 million tonnes per year. The steady growth of aquaculture including shrimp production and the consequential increase in demand for fishmeal has resulted in a general increasing feed price over the last decade (Duarte et al., 2009; FAO, 2009).

Feed occupies as much as 40 to 60% of production costs with the cost of feed generally increasing due to the high price and limited supply of fishmeal (Hertrampf and Piedad-Pascual, 2000) in combination with rising prices of other feedstuffs. It is critical to minimize the cost of feeds relative to production output. Considering the ever-increasing demand for aquaculture products, using fishmeal in feed formulation will be neither an affordable, nor a sustainable practice (Tacon, 1996). Developing alternative sources to fishmeal, while reducing the feed cost and developing sustainable feed formulation is a primary issue to be solved.

Numerous studies have focused on using renewable plant proteins to replace fishmeal in commercial shrimp formulations. Plant proteins are an excellent choice as they have an acceptable protein level, suitable amino acid content, lower cost, and consistent quality. Plant protein sources and their products, such as soybean, pea, cotton seed, corn gluten, wheat gluten, and distiller's dried grains soluble (DDGS), have been successfully used in aquatic animal feeds (Alvarez et al., 2007; Boonyaratpalin et al., 1998; Carter and Hauler, 2000; Davis and Arnold., 2000; Fontainhas-Fernandes et al., 1999; Hernández et al., 2004; Kaushik et al., 1995; Kikuchi, 2007; Lim and Dominy, 1990; Mambrini et al., 1999; McGoogan and Gatlin, 1997; Samocha et al., 2004; Smith et al., 1999; Sudaryono et al., 1995, 1999; Watanabe et al., 1993; Webster et al., 1991, 1993, 1995). Among these plant protein sources, soybean meal (SBM) is usually considered an economically and nutritionally valuable substitute for fishmeal because of its reasonably well balanced amino acid profile, consistent composition, worldwide availability and lower price (Akiyama, 1989; Akiyam et al., 1991; Divakaran et al., 2000; Forter et al., 2002; Fox et al., 2004; Hardy, 1999; Lim and Dominy, 1990; Lim et al., 1998; Samoch a et al., 2004; Swick et al., 1995; Tacon, 2000).

Albeit soybean meal is a good choice it also has potential problems such as low levels of some essential amino acids (e.g. methionine), presence of anti-nutritional factors (e.g. trypsin inhibitors and saponins) and poor palatability, which limits its level of inclusion in some feeds. Soybean meal in combination with low levels of fishmeal or as a sole protein source in the feed has resulted in reduced performance of the shrimp (Lim and Dominy, 1990) and red drum (McGoogan and Gatlin, 1997). Supplementation of select amino acids, such as methionine, and the inclusion of attractants or palatability enhancers may improve the performance of animals offered soybean based diets by providing a better balanced diet and improved palatability (Akiyama, 1989). Anti-nutritional factors, such as trypsin inhibitors, lectins, oligosaccharides, antigens, and saponins, exist in raw soybeans and may affect the digestion and nutrient availability. These anti-nutrients can be eliminated or reduced through various manufacturing processes such as heat processing (Dersjant-Li, 2002, New, 1987).

As soybeans represent the largest protein sources for animals, improving the nutrient content of meals is a logical progression. Genetic modification can be used to develop new cultivars of soybean that in turn can be used to improve the soybean's quality. Soybean genetic modification can enhanc some essential amino acid such as methionine and lysine (Krishnan and Hari, 2005). However, in recent years, significant attention has been paid to the use of genetic modified crops in aquatic feed since the expression of a constituent in the GM plant may cause unintended changes and risks (Lehrer and Bannon 2005, Myhr and Dalmo, 2005). Recent advances of new cultivars using traditional breading practices have resulted in shifts in nutritional characteristics of soybeans, i.e. protein content, reducing levels of trypsin inhibitors, oligosaccharides (Dudley-Cash, 2003). The subsequent meals are of considerable interest in aquaculture as they may potentially provide a better nutrient source.

Given the potential of the new varieties and the lack of information, a systematic evaluation of these lines is in order. Consequently, the objective of the present study was to determine growth performance and digestibility coefficients (protein, energy and dry matter) for new varieties of soybean meal in diet of Pacific white shrimp juveniles *L. vannamei* and evaluate the efficacy of these soybean meals produced from various new cultivars as the primary protein source.

2. Materials and Methods

For this research, a digestibility and a growth trial were conducted with *L. vannamei* at Auburn University. These trials were primarily designed to compare select soybean meals produced by various cultivars and processing. The new soybean meal sources were obtained from Schillinger Genetics for evaluation in this research project (Table 1).

2.1 Diet preparation

The reference diets for the digestibility trial was formulated to contain 1% chromic oxide (Cr_2O_3) as an inter marker. Test diets were then made using a 70: 30 mixture of the basal diet and test ingredients. The test diets evaluated in the growth study were formulated to contain 36% protein and 8% lipid. All experimental diets were prepared in the Aquatic Animal Nutrition Laboratory at the Department of Fisheries and Allied Aquaculture, Auburn University (Auburn, Alabama, USA) using standard procedures for the laboratory production of shrimp feeds. Pre-ground dry ingredients and oil were mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Boiling water was then blended into the mixture to obtain a consistency appropriate for pelleting. Diets were pressure-pelleted using a meat grinder with a 3-mm die, dried in a forced air oven (<50℃) to moisture content of 8-10 %. After drying, pellets were crumbled, packed in sealed plastic bags and stored in a freezer until use. Dietary treatments were in order assigned and each experiment was run using a double blind experimental design.

2.2 Culture system

The digestibility trial and growth trial were conducted in a semi-closed recirculation system at separate times at the E.W. Shell Fisheries Research Station (EWS), Auburn, AL, U.S. The system consisted of 75L aquaria connected to a common reservoir tank (800L). Water quality was maintained by recirculation water through an Aquadine bead filter (0.185m^2 media, 0.61m x 1.07m) and vertical fluidized bed biological filter (600 L volume with 200 L of Kaldnes media) using a 0.25-hp centrifugal pump to circulate the water. During the feeding period, dissolved oxygen (DO), temperature and salinity were monitored twice daily (0830 and 1630) using an YSI 650 multi-parameter instrument (YSI, Yellow Springs, OH). Water samples were taken twice a week to measure TAN and nitrite levels. All the water quality parameters (DO, TAN, nitrite levels, temperature, salinity, pH) were maintained within acceptable ranges for pacific white shrimp.

2.3 Digestibility trial

Juvenile shrimps (approximately 7 g mean weight) were stocked at 8 shrimp/tank into the formetioned recirculation systems. Six groups of shrimp were offered each diet with fecal samples from every two tanks pooled to provide 3 replicate samples. The shrimp were allowed to acclimate to each diet for at least three days before starting the collection of feces. Feces were collected by siphon four times per day for a 4-5 day period. Each day, the first collection was discarded and the other three were rinsed with distilled water, oven-dried (90^{o}C) and stored in sealed plastic containers at -20^{o}C for subsequent analysis. Dry matter was determined by placing representative portions of each sample in an oven at 105 ℃ until a constant weight was obtained. Gross energy of diets and fecal samples was analyzed with a Semi micro-bomb calorimeter (Model 1425, Parr Instrument Co., Moline, IL, USA). Chromic oxide was determined by the method of McGinns & Kasting (1964) in which, after a colorimetric reaction, absorbance was read on a spectrophotometer (Spectronic Genesys 5, Milton Roy Co., Rochester, NY, USA) at 540 nm. Protein was determined by micro-Kjeldahl analysis (Ma & Zuazago 1942).

The apparent digestibility coefficients for dry matter (ADMD), protein (ADP) and energy (ADE) were calculated according to Mainard and Loosli (1969) and Hardy and Barrows (2002), as follow:

$$\text{ADMD (\%)} = 100 - \left[100 \times \left(\frac{\text{\%Cr2O3 in feed}}{\text{\% Cr2O3 in feces}}\right)\right]$$

$$\text{ADP and ADE (\%)} = 100 - \left[100 \times \left(\frac{\text{\% Cr2O3 in feed}}{\text{\% Cr2O3 in feces}} \times \frac{\text{\% nutrient feces}}{\text{\%nutrient feed}}\right)\right]$$

2.4 Growth trial

Enhanced soybean meal cultivars were obtained for the evaluation of their potential as an ingredient in aquaculture feeds for *L. vannamei*. Each ingredient was characterized and then used in the growth trial. This growth trial utilized ten treatments with six replicates. Ten Pacific white shrimp were stocked per tank with mean initial weight of 0.8 $\pm$ 0.013g. Shrimp were offered test diets four times daily. Daily ration of feed was calculated based upon an estimated weight gain and expected 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 of the 8-week growth trial shrimp were counted and group weighed. Mean final weight, final biomass, percent survival, and feed conversion ratio were determined.

3 Statistical analysis

All data were analyzed using one-way ANOVA followed by the Student–Neuman–Keuls multiple comparison test to evaluate significant differences among treatment means. Correlation coefficient analysis, multiple linear regression with backward selection, principle component analysis or partial least square regression were selected as analysis method based on each experimental data. All statistical analyzes were carried out using statistical analysis system, SAS (V9.3 SAS Institute, Cary, NC, USA).

3.1 Statistical analysis of digestibility trial

Digestibility coefficients (ADMD, ADE and ADP) were statistically analyzed using one-way analysis of variance to determine significant differences ($P < 0.05$) among treatments, followed by the Student–Neuman–Keuls multiple comparison test to determine significant differences among treatment means (Montgomery 1997). Multivariate data analysis was performed to identify the association among digestibility coefficients (ADMD, ADE, ADP), and biochemical contents (amino acid profiles), and chemical contents of ingredients. Relationship between digestibility coefficients of ingredients (ADMD, ADE, ADP), and chemical contents of ingredients (TIU, raffinose, starchyose, protein, lipid, fiber, dry matter) were initially examined with linear regressions. Backward stepwise multiple regression was then used to study the relationship between digestibility coefficients of ingredients and chemical contents of ingredients. In this process, all the chemical content variables were included in the initial regression. Variables that did not contribute significantly to the model fit were removed prior to running the next regression. Principle component analysis (PCA) was performed to identify each principal trend in biochemical (amino acid) and chemical contents of ingredients. Partial least squares regression model was performed to identify relationships between digestibility coefficients and amino acid (AA) profiles after principle component analysis (PCA) and multiple linear regression.

3.2 Statistical analysis of growth trial

Growth trial data were statistically analyzed using one-way analysis of variance to determine significant differences among treatments, followed by the Student–Neuman–Keuls multiple comparison test to determine significant differences between treatment means (Montgomery 1997). Correlation coefficient analysis was also performed to identify the association among growth performance (final biomass, final mean weight, weight gain and FCR) and the chemical contents of ingredients as well as digestibility coefficients (ADMD, ADE, ADP). Relationships between growth performance (final biomass, final mean weight, weight gain, FCR) and the variables which had high correlation with growth performance, were examined by linear regressions with backward stepwise.

4. Results

4.1 Digestibility trial

Apparent digestibility coefficients for the reference diet, test diets and tested ingredients are presented in Table 5. Apparent digestibility of dry matter ranged from 63.4% to 95.4%, energy ranged from 72.5 % to 96.1%, and protein ranged from 80.3% to 98.5%, and significant differences existed among treatments. The experimental ingredient, I-19, appears the largest value in ADMD (95.4%) and ADE (96.1%), and the lowest ADMD and ADE were found in I-13

63.4%, 72.5% respectively. For ADP, I-19 has the highest ADP value（98.0 %）compared to others but the lowest ADP was found in I-14 (80.3%).

4.2 Growth trial

At the conclusion of the 8-week culture period, the growth performance of the shrimp: finial mean weight, weight gain, feed conversion ratio (FCR), and survival rate were summarized in Table 4 Significant differences existed in finial mean weight, weight gain, weight gain percent and FCR among the FF, I-13, I-16, I-17, I-18, I-19 treatments and I-Triveca, I-3010, I-10, I-11 treatments. The I-17 treatment establishes the numerically largest value in final mean weight (7.6g), weight gain (6.81g), while the I- Triveca has the lowest final mean weight (5.73g), weight gain (4.9g). Survival ranged from 75 to 92%. No significant differences were observed in terms of shrimp survival among dietary treatments. Feed conversion ratio ranged from 1.7 to 2.4. The lowest FCR was found in shrimp offered diets containing I-17, and the highest FCR in the diet containing I-Triveca.

4.3 Statistic analysis

4.3.1 Digestibility trial

Multiple linear regression

The summary of multiple linear regression with backward elimination between the responses, apparent digestibility coefficients (ADMD, ADE, ADP) and the predicting variables, chemical contents (trypsin inhibitor, raffinose, starchyose, protein, lipid, fiber, dry matter) were presented in Table 6. In the regression with ADMD as response, protein was left in the model and the removed order was dry matter, lipid, starchyose, fiber, trypsin inhibitor (TIU), raffinose. In the regression with ADE as response, protein was also left in the model and dry matter, starchyose, lipid, fiber trypsin inhibitor, raffinose were removed step by step. In the regression with ADP as response, trypsin inhibitor (TIU), raffinose, starchyose were left in the model and lipid, dry matter, fiber, protein were removed step by step.

Principle component analysis

Eigenvalues of the correlation matrix for chemical composition and amino acids were summarized in Table 7. The first principle component (PC) explained 74.53% of total sample variance. The first four principle components, collectively, explained 94.43% of total sample variance.

The component pattern of the first and second component was present in Fig 2. The variables spread evenly. Protein, amino acids and dry matter had high positive correlation with the first component. Trypsin inhibitor (TIU) also had relatively high correlation but negative with the first component. Almost all variables except stachyose and raffinose have relatively low correlation with the second component but stachyose and raffinose had high and positive correlation with the second component.

The component scores of the first and second components are presented in Fig 3. The observations spread evenly in the first component except observation 6 (ingredient 13) and 7 (ingredient 14), which were potentially outliers of the first component (Fig 3a). Observation 3 (ingredient 10) and 5 (ingredient 12) stand out in the component scores of the second component, which were potentially outliers of the second component.

Partial least square regression

Based on the Correlation loading plot (Fig 5), the content of amino acids of test ingredients exhibit high correlation with ADE and ADMD but ADP has relatively low correlation with amino acids. The X-scores are plotted as numbers for each observation. This plot appears to establish the whole of the observations close together with larger negative X-scores for factor 1. There are no clear grouping patterns, but observation 4 (I-11), 6 (I-14) and 7 (I-15) stand out. The loadings exhibited how much variation in each variable was accounted for by the first two factors, jointly by the distance of the corresponding point from the origin and individually by the distance for the projections of this point onto the horizontal and vertical axes. That the dependent variable was well explained by the model was reflected in the fact that the points for ADE and ADMD are near the 75% circle but all amino acids had relatively low correlation with ADP since it was on the 25% correlation circle.

4.3.2 Growth trial

Correlation coefficients analysis

The results of correlation coefficients analysis are summarized in Table 9. P-values of predictors with less than 0.15 are selected in later multiple regression. In the present study, trypsin inhibitor (TIU), dry matter, and ADP demonstrated a higher correlation with growth performance (final biomass, final mean weight, weight gain and FCR). The P-value of trypsin inhibitor (TIU), dry matter, ADP with weight gain were 0.052, 0.011 and 0.058, respectively. The P-values of trypsin inhibitor (TIU), dry matter, ADP with final biomass were 0.0041, 0.002 and 0.101, respectively. The P-values of trypsin inhibitor (TIU), dry matter, ADP with final mean weight were 0.0099, 0.006 and 0.058, respectively. The P-values of trypsin inhibitor (TIU), dry matter, ADP and FCR were 0.005, 0.009 and 0.0913, respectively. Overall, trypsin inhibitor had negative correlation with each growth performance but dry matter and ADP presented a positive correlation.

The results of correlation coefficients analysis between growth performance and eight essential amino acids (EAA) are summarized in Table 10. Methionine had the highest correlation with growth performance than any other EAAs and it had positive effect on growth performance and a negative effect on FCR.

Multiple linear regression

The summary of multiple linear regression with backward elimination between growth performances (final biomass, final mean, FCR, weight gain) and the predicting variables with high correlated variables (TIU, ADP, dry matter) are presented in Table 11. In the regression with FCR as the response variable, only TIU is left in the model. TIU, ADP are both kept in the model when final mean and weight gain are utilized as the response variables. .

5. Discussion

Dietary protein has a significant effect on growth performance of shrimp and feed cost (Hu et al., 2008). Consequently, ingredients with highly digestibility protein would be easier for shrimp to absorb and may promote a higher grow rate than low digestibility ingredients. Furthermore, easily digested protein would improve protein retention into tissues and decrease excretion of nitrogen metabolites into the environment. However, the presences of anti-nutritional factors may limit the digestibility of protein in some plant ingredients such as soybean meal. Therefore, reducing the level of anti-nutritional factors and enhancing protein digestibility in feed ingredients are important factors in improving feed quality. It is possible that selectively-bred soybean varieties with reduced levels of anti-nutritional factors and enhanced protein content could be included at higher levels and improve the bioavailability of nutrients to the shrimp. Compared with conventional commodity soybean meal, if new soybean varieties allow a higher inclusion level or if due to their higher content of protein they are included at a reduced rate for a given protein level in the feed they could reduce the overall cost of shrimp feed formulations. In the present study, the values of apparent digestibility coefficients and growth performance of conventional commodity soybean meal (FF) are intermediate in the new soybean varieties, which indicate that not all soybean varieties can successfully substitute for the conventional commodity soybean meal. But some experimental ingredients (I-16, I-17, I-18, I-19) made from soybean varieties, do have a better performance than the conventional commodity soybean meal (FF).

5.1 Digestibility trial

Apparent digestibility of dry matter (ADDM)

The digestibility of a feedstuff is considered an important criterion when determining the utilization of a feedstuff (Akiyama et al., 1989). Digestibility data reflects the percentage of a feedstuff that is absorbed from an animal's intestinal tract (Lin et al. 2004). Apparent dry matter digestibility (ADMD) offers a method of testing an ingredient's gross digestibility as no specific nutrient is measured. As components of a feedstuff are not digested equally, dry matter digestibility coefficients can offer an estimate of the quantity of indigestible material present in feedstuffs than digestibility coefficients for individual nutrients (Brunson et al., 1997). In the present study, the ADDM for all SBM's ranged from 63.43% to 95.45% in *Litopenaeus vannamei*, which are generally similar to other reports. Akiyama (1989) reported an ADMD value (55.9%) for soybean meal to *Litopenaeus vannamei*. Divakaran et al., (2000) evaluated ADMD values of de-hulled, solvent extracted, toasted soybean meal which ranged from 61.2% to 84.7%. Also Cruz-Suárez et al. (2009) mentioned four different soy products' ADMD ranging from 82.7% to 91.7% with *Litopenaeus vannamei*. ADMD values of the present study generally agree with the values observed in previous studies.

Apparent digestibility of energy (ADE)

Protein (amino acids), lipids, and carbohydrates all contribute energy to feeds. The digestibility of energy (ADE) is the fractional sum of apparent digestibility coefficient (ADC) values since feed's energy consists of protein, lipids, and carbohydrates (Siccardi, 2006). The values of ADE ranged from 72.57% to 96.15% (Table 5), which are similar with prior reported results. Brunson et al. (1997) reported apparent energy digestibility of soybean meal as 76.09% for *Litopenaeus vannamei*. Yang et al. (2009) reported the values of apparent energy digestibility of fermented soybean meal, extruded soybean meal and soybean meal ranged from 74.12% to 82% in *L. vannamei*. These results confirm that the digestible energy content of various lines of soybean meal are reasonable and that various lines will have different DE values.

Lipids, one of the main energy sources, are a complex class of materials that are composed of many different compounds with different structures and compositions (Sargent et al., 2002). In the present study, lipid content of the experimental soybean meal ranged from 0.5% to 4.96% with the exception of I-15, which was 13.6%. In the case of I-15, it did not exhibit a significantly lower ADE value even though it had significantly lower ADP. This response could be offset due to the high level of lipids. Carbohydrates, consisting of fiber, sugar and starch, are one of main energy source and influence ADE values, which have been reported in several studies. Fiber is essentially indigestible to many animals since they have limited cellulose enzymes for digesting fiber. Hence, ADE values of a diet typically decrease as fiber content increases. Fiber contents are inversely related to energy digestibility of plant products for aquatic animals but have no digestibility energy value to monogastric species (Brunson et al., 1997, Lech and Reigh, 2012). However, in the present study, fiber content of all experimental soybean meals ranged from 2.1% to 3.9% without significant difference among them and their fiber contents were lower than those of reported other plant ingredients', such as canola meal 9.7 % and distillers dried grains with solubles (DDGS) 5.8% (Lech and Reigh, 2012). A slight correlation was also found in the present study between ADE and fiber of these experimental soybean meals (Table 6). The relatively small shift in fiber likely reduced energy but was not a significant component of energy.

Due to the relatively high cost of protein, diets are typically formulated to minimize the use of protein as an energy source. Similar results were found in the present study (Table 6). Among chemical contents of these experimental soybean meals, protein was left in the backward stepwise regression model when ADE as response and chemical contents (protein, lipid, stachyose, raffinose, fiber) including anti-nutrients (trypsin inhibiter) of ingredients as predictors. The results indicate that in the present study, protein influeced ADE values more than other predicators. Amino acids, the building blocks of protein, are important in meeting the energy (metabolic) requirements of fish and shrimp and also generally an efficient metabolic fuel for most fish and crustacean species. Using partial least square regression (Fig 5), we found a high correlation between ADE and select amino acids with ingredients I-13 and I-14 standing out among the other ingredients (Fig 5) in partial least square (PLS) factor 1 and are significantly lower than the rest of the soybean meals (Table 5). Similar results are found in principle component analysis of contents of experimental ingredients. The experimental ingredient I-13 and I-14 also stand out in the first component, which factor explains 74.53% sample varies (Fig 3a). Those results indicate that various amino acids contents may explain the lower ADE values displayed in I-13 and I-14. The experimental ingredient I-11 only stands out in PLS factor 2 (Fig 5). But it has the lowest methionine and histidine of the test ingredients (Fig 6) even though it does not appear to be an outlier based on Fig 3a. Although there are no studies reporting that the particular amino acid content of an ingredient has a directly relationship with ADE values, it does make sense that balanced amino acids are helpful for animal to digest nutrients and provide energy on dietary metabolic activities.

Apparent digestibility of protein (ADP)

Apparent digestibility of protein (ADP), has been reported in several studies for soybean protein conducted with *L. vannamei*. Akiyama (1989) and Siccardi (2006) reported that ADP of soybean meal was 89.9% and 96.9% respectively. Ezquerra et al. (1997,1998) and Akiyama (1989) reported soybean protein ADPs as 91.0%, 85.9% and 96.4%, respectively. Divakaran et al. (2000) reported ADP of ingredients of dehulled, solvent extracted, and toasted soybean meals ranging from 91% to 102.2%. In this present study, the ADP values generally ranged from 80.39% to 98.05 %, which are intermediate of reported studies. The ADP of Ingredient 14 (80.39%) and 15 (83.12%) were significantly lower than the other soybean meals (90.4%-98.05%).

Observed differences in ADP could be attributed to several factors including various processing methods, presence of trypsin inhibitors and oligosaccharides (e.g. stachyose, raffinose). Compared to fishmeal with highly digestible protein, soybean meal is limited by the presence of a variety of anti-nutritional substances, presence of trypsin inhibitors and oligosaccharides (e.g. stachyose, raffinose). Several studies have reported that trypsin inhibitors had negative effect on protein digestibility of protein and lipid and growth rate in salmon (Olli and Berg-Lea, 1989), rainbow trout (Sandholm et al., 1976), carps (Abel et al., 1984;Viola et al., 1983), Nile tilapia (Wee and Shu, 1989) and channel catfish (Wilson and Poe, 1985). Alarcon et al. (1999) observed that sea bream alkaline digestive proteases were inhibited by 42.6% after incubation of extract with a solution containing raw soybean meal. Wilson and Poe (1985) observed that best growth of channel catfish occurred when 83% (e.g. 3.2 g TIU/g diet) of the trypsin inhibitor activity in the soybean meal had been destroyed. Olli et al. (1994) mentioned that trypsin inhibitor could reduce weight gain of Atlantic salmon by influencing trypsin activities. Francis et al. (2001) also reported that trypsin inhibitors have negative effects on protein digestibility since their binding to digestive enzyme. However, very few shrimp species like the Indian white shrimp, *Penaeus indicus*, have been reported to exhibit digestive sensitivity to soy-derived protease inhibitors (Osmondi, 2005). Soybean meal contains approximately 15% of oligosaccharides (sucrose, raffinose and stachyose). These carbohydrates are considered anti-nutritional components of soybean (Dersjant-Li, 2002). Information on the carbohydrate metabolism of crustaceans is limited. Typically, oligosaccharides have a negative correlation with nutrient utilization in fish. Significantly improved utilization efficiency of nutrients was found in salmon and rainbow trout by removing of oligosaccharides from soybean meal (Krogdahl, 1989). Arnesen et al. (1989) mentioned that alcohol soluble carbohydrate from soybean meal had a negative influence on digestibility of protein and lipid in salmon and in trout (tendency). In the present study, the anti-nutritional substances (trypsin inhibitor, stachyose, raffinose) have effect on ADP (Table 6) but there was no clear linear relationship between ADP values and stachyose and raffinose. Generally, experimental ingredients with low trypsin inhibiter, stachyose, raffniose (I-16, I-17, I-18, I-19), proved better ADP values except I-12. In general, experimental ingredients with any relatively higher anti-nutrient contents (I-Triveca, I-3010, I-10, I-11, I-13, I-14, I-15) could have contributed to lower ADP value.

Heat treatment, part of the processing of soybean meal, is usually used to enhance digestibility of plant proteins by reducing trypsin inhibitors (Anderson and Wolf, 1995) On the other hand, excessive heat treatment lowers protein digestibility by creating new linkages in proteins that are resistant to digestion (Arndt et al. 1999). Comparing I-13, I-14 and I-15, which are from the same soy source (lot59p22), I-14 and I-15 are boiled before processing into a meal and press cake, which means, compared to the regular processing method, these two ingredients have been processed under reduced heat. . The boiling preparation of I-14 would solubilize some carbohydrates and seems to have contributed to a higher protein content (67.7%) as compared to I-13 (56.88%), but lower ADP values. In this study, under-heating could be another reason that I-14 and I-15 exhibit significantly lower ADP values.

Significant differences exist among ingredients in each apparent digestibility coefficients (ADC) and the values of ADC of each experiment ingredient generally agree with prior observed researches. The experimental ingredient I-19 displayed the highest apparent digestibility coefficients (ADMD, ADE and ADP) compared to the other experimental ingredients. Ingredient 13, 14, 15, which are from same soy source but different preparing treatments and processing methods, showed significant difference with each other. Apparent digestibility of energy of I-13 and I-14 are lowest among the experimental ingredients even though they have relatively higher protein content and lower carbohydrates. The ADP of I-14 and I-15 which were boiled during preparation, show the lowest values. Inadequate heat treatment may result in this low ADP value. The experimental ingredients I-16, I-17 and I-18 with low anti-nutritional substances and appropriate heat treatment displayed higher apparent digestibility coefficients values than the experimental ingredients, which either had relatively higher anti-nutritional substances or inadequate heat treatment.

5.2 Growth trial

Soybean meal has been successfully used as a partial substitute for fishmeal without depression in growth. Lim and Dominy (1990) reported that fishmeal could be substituted with soybean meal up to 40% without any growth depression. Eight-week weight gains in the present study, ranged from 4.9 g to 6.77 g, are intermediate with the data reported by Lim and Dominy (1990). However, significant differences existed in mean finial weight, percent weight gain and FCR among shrimp fed diets containing the various sources of selected soybean meals. To evaluate such interactions in the present study, correlation analysis followed by regression analysis was utilized.

Based on correlation analysis, it was found that trypsin inhibitor and ADP have significant correlations with shrimp growth (Table 9). Ingredients with highly digestible protein and lower anti-nutritional substances would be easier for shrimp to absorb and have a higher growth rate. The experimental ingredients with lower trypsin inhibitor and higher ADP values had better growth performance even though they do not present clearly linear relationships with values of growth performances. It is well accepted that trypsin inhibitors have a negative effect on growth rate and protein digestibility in many species. Trypsin inhibitors that contribute approximately 6% of soybeans protein (Kakade et al. 1973) have been shown to produce growth depression and pancreatic hypertrophy in numerous experimental animals (Lim & Akiyama, 1991). Olli et al. (1994) reported some organisms are able to compensate for digestive proteases when challenged with inhibitors equivalent to 5mg/g feed by Atlantic salmon. Kaushik et al. (1995) mentioned that low levels of trypsin inhibiter activity (<3 mg/g) did not result in adverse effects on rainbow trout. The growth performances of I-Triveca, I-3010, I-10 and I-11 are lower than those of other ingredients and the content of trypsin inhibiter and PDI (with the exception of I-3010) is higher than the other ingredients. However, I-3010, which contained trypsin inhibiter

(442), showed significantly lower growth performance than the ingredients (I-16, I-17, I-18 and I-19) where trypsin inhibiters were analyzed to be higher than I-3010. This new soybean culture variety I-3010 did not show better growth performance even though its ADP value is high and anti-nutrients arelow.

Similarity, more highly digestibility protein can contribute to higher weight gain but it is not necessary that animal with higher weight gain must have higher digestibility of protein since the feed intake of shrimp does not depend on digestibility. That explains the response to I-13 with relatively lower ADP values but higher weight gain. Higher weight gain can be from higher intake of feed even though the ADP of ingredient is low. With the exception of I-13, in the present study, most experimental ingredients with higher protein digestibility produced larger shrimp.

Methionine is one of the ten essential amino acids that have been found to be critical for optimal growth and survival of shrimp (Coloso and Cruz, 1980; Kanazawa et al. 1981 Pascual and Kanazawa, 1986; Shewabatt, 1972. High correlation between methionine content and growth performances are found in Table 10. The experimental ingredients with relatively higher methionine content (I-13, I-16, I-17, I-18, I-19) supported better growth responses. I-11, which presents clearly the lowest point in Fig 6, does not have higher growth response. In addition to low methionine, other factors could lead to reduced growth responses. Therefore, other ingredients (I-Triveca, I-3010, I-10) did not have lower methionine but still had lower growth response and could have been influenced by other reasons such as previously mentioned anti-nutritional substances or various processing methods.

在 *6. Conclusion*

Results of the present study demonstrate significant differences among ingredients both in terms of digestibility and the ability to support good growth of shrimp. In the digestibility trial, the experimental ingredient I-19 displayed the highest apparent digestibility coefficients (ADMD, ADE and ADP) compared to the other experimental ingredients. ADE of I-13 and I-14 were significantly lower than that other experimental ingredients' even though they had relatively higher protein content and lower carbohydrates. Based on statistical analysis of amino acids of experiment ingredients, the ADE values of I-13 and I-14 were odd as compared to other ingredients. Many factors impact ADP values of ingredients such as anti-nutritional substances and various processing methods. Not appropriate heat treatment and/or the boiling used to prepare soybeans, may be the major reasons I-14 and I-15 had lower ADP values than other ingredients. The experimental ingredients (I-16, I-17, I-18, I-19) with lower anti-nutritional substances and no extra heat treatment did display higher ADP values than other experimental ingredients, which either had higher anti-nutritional substances or were treated by extra heat. The experimental ingredient, I-16, displayed the best growth performance not I-19 with the highest ADP value. Growth performances had positive correlation with ADP but negative correlation with trypsin inhibitor. Generally, the experimental ingredients (I-16, I-17, I-18, I-19) with relatively higher ADP and lower trypsin inhibitor still appeared to has better growth responses.

Based on these results, the experimental ingredients I-16, I-17, I-18, I-19 may hold a high potential as shrimp feed ingredient. The other experimental soy varieties still need to find the appropriate processing methods for increasing digestible protein density and reduce their anti-nutritional substances in shrimp feeds.

Table 1 Description and proximate analyses of the various test ingredients used in diets of Pacific white shrimp *Litopenaeus vannamei*.

Auburn ID	Description	Fraction	Protein	Ash	Lipid	Fiber	Dry matter	Raffinose	Stachyose
FF	Faithway Feed Co. Inc.	M	49.9	6.03	1.19	2.83	88.36	-	-
Triveca	Blended SBM	M	48.16	6.36	4.96	3.9	90.77	0.05	1.31
3010	Previous production line SBM	M	54.70	6.79	0.5	2.7	90.7	<0.5	0.7
I-10	Soy lot 129T755	WF	49.6	6.59	0.6	3.1	91.2	1.0	4.9
I-11	Soy lot 31TD735-ULT	WF	54.2	6.49	0.7	3.2	90.8	<0.5	0.9
I-12	Soy E3311	WF	50.9	6.63	0.8	2.7	90.5	1.0	5.0
I-13	Soy lot 59P22	M	66.6	7.60	0.6	2.8	93.8	<0.5	<0.5
I-14	Soy (boiled) lot 59p22	M	67.7	7.06	0.5	2.1	93.4	<0.5	3.0
I-15	Soy (boiled) lot 59p22	PC	55.4	5.92	13.6	3.5	90.7	<0.5	2.5
I-16	Soy Lot 27D616	M	52.1	7.44	0.9	3.8	94	<0.5	<0.5
I-17	Soy Lot 28D618	M	52.3	7.54	0.7	2.5	92	<0.5	<0.5

I-18	Soy lot 28D617	M	55.7	6.40	0.7	2.9	93.1	<0.5	<0.5
I-19	Soy lot 34D635	M	53.0	7.64	0.8	3.1	92.5	<0.5	<0.5

*Unless noted, ingredients were provided by Shillinger Genetics Inc. Des Moines, IA.

M- Meal, WF- White flake, PC- Press cake

Table 2 Proximate and amino acid composition and carbohydrate (%) of test ingredients used in diet of Pacific white shrimp and ingredients were analyzed by Schillinger Genetitcs, IA, U.S.

Diet code Analysis (% as is)	Triveca	3010	I-10	I-11	I-12	I-13	I-14	I-15	I-16	I-17	I-18	I-19
Tryptophan	0.75	0.80	0.70	0.80	0.7	0.90	0.9	0.8	0.80	0.80	0.80	0.80
Cysteine	0.74	0.80	0.60	0.70	0.7	1.00	1.0	0.8	0.80	0.80	0.70	0.80
Methionine	0.69	0.80	0.70	0.70	0.7	1.00	1.0	0.8	0.80	0.80	0.80	0.80
Aspartic acid	5.60	6.50	5.60	6.20	5.8	8.10	7.9	6.6	6.10	6.50	6.10	6.30
Threonine	1.79	2.10	1.90	2.00	2.0	2.60	2.5	2.1	2.00	2.10	2.00	2.10
Serine	2.60	2.90	2.40	2.80	2.6	3.70	3.5	3.0	2.80	3.00	2.80	2.90
Glutamic acid	9.12	10.50	9.30	10.30	9.6	13.40	13.0	10.8	9.90	10.70	10.00	10.20

Proline	2.46	2.90	2.50	2.70	2.6	3.50	3.5	2.9	2.70	2.80	2.70	2.70
Glycine	2.03	2.30	2.10	2.20	2.1	2.90	2.8	2.3	2.20	2.40	2.20	2.30
Alanine	2.09	2.30	2.10	2.20	2.2	2.90	2.8	2.3	2.20	2.40	2.20	2.30
Valine	2.25	2.60	2.40	2.50	2.4	3.10	3.1	2.5	2.40	2.60	2.40	2.50
Isoleucine	2.14	2.50	2.20	2.40	2.3	3.10	3.0	2.5	2.40	2.50	2.40	2.40
Leucine	3.71	4.30	3.80	4.00	3.9	5.20	5.1	4.2	4.00	4.20	4.00	4.10
Tyrosine	1.58	1.80	1.70	1.70	1.7	2.30	2.2	1.9	1.80	1.80	1.70	1.80
Phenylalanine	2.42	2.80	2.60	2.70	2.6	3.30	3.4	2.8	2.70	2.80	2.70	2.70
Lysine	3.21	3.70	3.30	3.30	3.2	4.00	4.1	3.4	3.30	3.60	3.20	3.30
Histidine	1.28	1.50	1.30	1.10	1.4	1.70	1.7	1.4	1.30	1.40	1.40	1.40
Arginine	3.64	4.10	3.70	4.30	3.9	5.20	5.0	4.2	3.80	4.40	4.10	3.90
TIU/g	20500	5800	10400	17150	11800	1600	1900	2000	2000	6800	5400	7000
Raff	<0.05	<0.5	1.00	<0.5	1.1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Stach	1.31	0.70	4.90	0.90	5.0	<0.5	3.0	2.5	<0.5	<0.5	<0.5	<0.5

Table 3 Composition (as is basis) of diets formulated to contain equivalent amounts of protein from various soybean meal sources. Diets were formulated to contain 35% protein and 8% lipid.

Ingredient	FF[1]	Triveca	3010	I-10	I-11	I-13	I-16	I-17	I-18	I-19
Menhaden fishmeal [2]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal	45.30	46.94	41.63	44.75	41.69	37.69	43.39	40.22	43.59	41.98
Corn protein concentrate [3]	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70
Menhaden Fish Oil [2]	5.65	3.86	5.90	4.96	4.98	5.18	4.92	5.03	4.85	4.96
Corn Starch [4]	0.70	0.85	4.12	1.94	4.98	8.78	3.34	6.40	3.21	4.71
Whole wheat [4]	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00
Trace Mineral premix [5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix [6]	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Choline chloride [4]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 250 mg/kg [7]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

CaP-dibasic [8]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Lecithin [9]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol [4]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Analysis [10] (% as is)										
Protein (N x 6.25)	35.64	33.13	33.19	32.88	33.49	33.55	33.96	33.78	33.49	33.45
Moisture	6.75	7.16	7.51	8.03	6.21	6.86	5.46	5.61	6.94	6.56
Fat	8.66	7.63	8.54	6.78	7.18	7.57	7.74	7.67	7.66	7.93
Fiber	1.90	2.30	1.43	1.64	1.74	1.68	1.67	1.47	1.37	1.94
Ash	7.72	7.45	7.23	7.24	7.21	7.48	7.67	7.61	7.18	7.65
PDI [11]	16.75	40.02	21.12	42.85	34.88	24.47	23.12	24.36	22.48	24.16
Trypsin Inhibitor (Units/g)	13	4480	442	4000	4223	1119	1117	1109	846	2464

[1] De-hulled solvent extracted soybean meal, Faithway Feed Co. Inc., Guntersville, Alabama, USA.
[2] Omega Protein Inc., Huston TX, USA.
[3] Empyreal® 75, Cargill Corn Milling, Cargill, Inc., Blair, NE, USA
[4] MP Biomedicals Inc., Solon, Ohio, USA
[5] 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.
[6] 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.
[7] Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), DSM Nutritional Products., Parsippany, NJ, USA.
[8] J. T. Baker®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.
[9] The Solae Company, St. Louis, MO, USA.
[10] Analyses conducted by University of Missouri-Columbia, Agriculture Experiment Station Chemical Laboratory.
[11] Protein Dispersibility Index (PDI)

Table 4 Response of juvenile (0.79 ±0.031g mean weight ±SD) shrimp to test diets containing various soybean meals substituted on an iso-nitrogenous basis. Diets were formulated to contain 35% protein and 8% lipid and offered to six replicate groups of shrimp over and 8-week period.

Trt	Soy Source	Initial mean weight (g)	Final mean weight (g)	Weight gain (g)	Weight gain (%)	Survival (%)	FCR
1	FF	0.78	7.18 ^{a}	6.39 ^{a}	813.8^{ab}	75.0	1.8 ^{c}
2	Triveca	0.81	5.73^{b}	4.9^{b}	607.9^{d}	90.0	2.4 ^{a}
3	3010	0.77	6.39^{b}	5.62^{b}	729.6^{cd}	91.7	2.1 ^{b}
4	I-10	0.79	6.21^{b}	5.42^{b}	688.5^{cd}	88.3	2.2 ^{b}
5	I-11	0.79	6.28^{b}	5.48^{b}	693.1^{cd}	88.3	2.2 ^{b}
6	I-13	0.80	7.23^{a}	6.43^{a}	800.6^{ab}	92.0	1.8 ^{c}
7	I-16	0.78	7.55^{a}	6.77^{a}	864.6^{a}	86.7	1.8 ^{c}
8	I-17	0.80	7.6^{a}	6.81^{a}	852.4^{a}	86.7	1.7 ^{c}
9	I-18	0.81	7.40^{a}	6.59^{a}	815.1^{ab}	88.3	1.8 ^{c}

10	I-19	0.78	7.43[a]	6.64[a]	844.8[a]	86.7	1.8 [c]
*PSE		0.013	0.185	0.187	28.75	4.77	0.0644
P-value		0.5173	0.0001	0.0001	0.0001	0.4545	0.0001

*PSE: Predicted Square Error

Values with different superscripts within the same column are significantly different based on Student-Newman-Keuls (SNK) multiple range test.

Table 5 Mean (and standard deviations) of three replicate analyses used to determine digestibility values for juvenile L. vannamei offered a practical basal diet with chromic oxide as an inert marker. Apparent dry matter, energy and protein digestibility values for the diets (ADDM, ADE, ADP, respectively) and ingredients (ADMDI, ADEI, ADPI, respectively)

		Diet			Ingredient		
Diet	Ingredient	ADDM	ADE	ADP	ADDM	ADE	ADP
Basal I		73.71 ±3.13 [bc]	80.50 ±1.99 [bc]	92.11 ±0.88 [abc]			
10	I-10	73.65 ±1.10 [bc]	80.42 ±0.44 [bc]	90.90 ±1.51 [abc]	73.49 ±3.66 [bc]	80.26 ±1.39 [ab]	89.24 ±3.58 [a]
11	I-11	72.70 ±0.57 [bc]	79.25 ±1.29 [bc]	92.05 ±0.49 [abc]	70.32 ±1.91 [bc]	76.27 ±4.38 [b]	91.95 ±1.27 [a]
12	I-12	74.97 ±0.65 [b]	81.72 ±0.62 [ab]	93.84 ±0.68 [a]	77.90 ±2.17 [bc]	84.36 ±1.97 [ab]	96.37 ±1.67 [a]
13	I-13	70.63 ±1.63 [c]	78.28 ±1.25 [bcd]	91.39 ±1.48 [abc]	63.43 ±5.43 [c]	72.57 ±4.46 [b]	90.49 ±3.34 [a]
14	I-14	71.05 ±1.25 [bc]	78.21 ±0.70 [bcd]	86.25 ±1.07 [d]	64.84 ±4.16 [c]	73.05 ±2.28 [b]	80.39 ±2.13 [b]
15	I-15	74.60 ±2.31 [b]	81.88 ±2.00 [ab]	88.17 ±2.36 [bcd]	76.67 ±7.69 [bc]	84.43 ±5.72 [ab]	83.12 ±5.40 [b]
16	I-16	75.02 ±0.61 [b]	80.83 ±0.78 [bc]	92.79 ±0.65 [ab]	78.06 ±2.02 [bc]	81.67 ±2.74 [ab]	93.74 ±1.58 [a]

17	I-17	75.17 ±4.12 [b]	80.76 ±3.23 [bc]	92.37 ±0.80 [abc]	78.55 ±13.74 [bc]	81.42 ±11.58 [ab]	92.71 ±1.88 [a]
18	I-18	76.23 ±3.51 [b]	82.18 ±3.20 [ab]	93.84 ±1.46 [a]	82.10 ±11.69 [bc]	86.36 ±11.20 [ab]	96.38 ±3.59 [a]
19	I-19	80.24 ±1.33 [a]	85.39 ±0.82 [a]	94.36 ±0.26 [a]	95.45 ±4.43 [a]	96.15 ±2.63 [a]	98.05 ±0.69 [a]
Basal II		68.24 ±1.68 [c]	74.52 ±1.64 [d]	85.74 ±1.64 [d]			
2	FF	71.74 ±1.61 [bc]	76.84 ±1.08 [cd]	90.86 ±1.64 [abc]	79.89 ±5.38 [bc]	83.03 ±3.96 [ab]	95.13 ±2.94 [a*]
3	Triveca	71.13 ±1.88 [bc]	76.91 ±1.79 [cd]	87.55 ±1.64 [cd]	77.87 ±6.27 [bc]	83.37 ±6.64 [ab]	90.40 ±3.56 [a]
4	3010	73.73 ±0.81 [bc]	77.93 ±0.55 [bcd]	90.86 ±1.64 [abc]	86.54 ±2.69 [ab]	88.70 ±2.27 [ab]	91.77 ±4.79 [a*]
PSE		0.6262	0.4782	0.2801	1.807	1.580	0.840
P value		0.0001	0.0001	0.0001	0.0003	0.0025	0.0001

* Mean of two replicates, one replicate was eliminated as the calculated value was over 100%

Table 6 Multiple linear regression with backward stepwise between apparent digestibility coefficients (ADMD, ADE, ADP) and chemical contents of ingredients

	ADMD		ADE		ADP	
	F	Pr(F)	F	Pr(F)	F	Pr(F)
TIU	0.18	0.6929	0.06	0.8222	*0.55******	*0.5004******
Raff	0.19	0.6828	0.11	0.7607	*1.67******	*0.2657******
Starch	0.07	0.7995	0.02	0.9064	*2.62******	*0.1811******
Protein	*3.64******	*0.1292******	*2.59******	*0.1827******	1.56	0.2795
Lipid	0	0.9799	0.03	0.8702	0.02	0.8970
Fiber	0.32	0.6018	0.26	0.6347	0.27	0.6316
Dry matter	0	0.9817	0.01	0.9400	0.35	0.5882

*Significant of regressions: *0.05<P<0.10; ***P=0.001. *Italics* indicates chemical contents retained after multiple backward stepwise regression

Table 7 Eigenvalues of the correlation matrix of chemical content and amino acids of ingredients in principle component analysis

	Eigenvalue	Difference	Proportion	Cumulative
1	18.6326677	16.5486997	0.7453	0.7453
2	2.0839680	0.3322515	0.0834	0.8287
3	1.7517165	0.6119943	0.0701	0.8987
4	1.1397222	0.6057523	0.0456	0.9443
5	0.5339699	0.1640363	0.0214	0.9657
6	0.3699335	0.1463613	0.0148	0.9805
7	0.2235722	0.1049307	0.0089	0.9894
8	0.1186416	0.0403238	0.0047	0.9942
9	0.0783177	0.0333294	0.0031	0.9973
10	0.0449883	0.0224859	0.0018	0.9991
11	0.0225024	0.0225024	0.0009	1.0000

Figure 1 Chemical contents and amino acids of ingredient's (a) scree plot of principle component with eigenvalue and (b) variance explained of proportion with principle component.

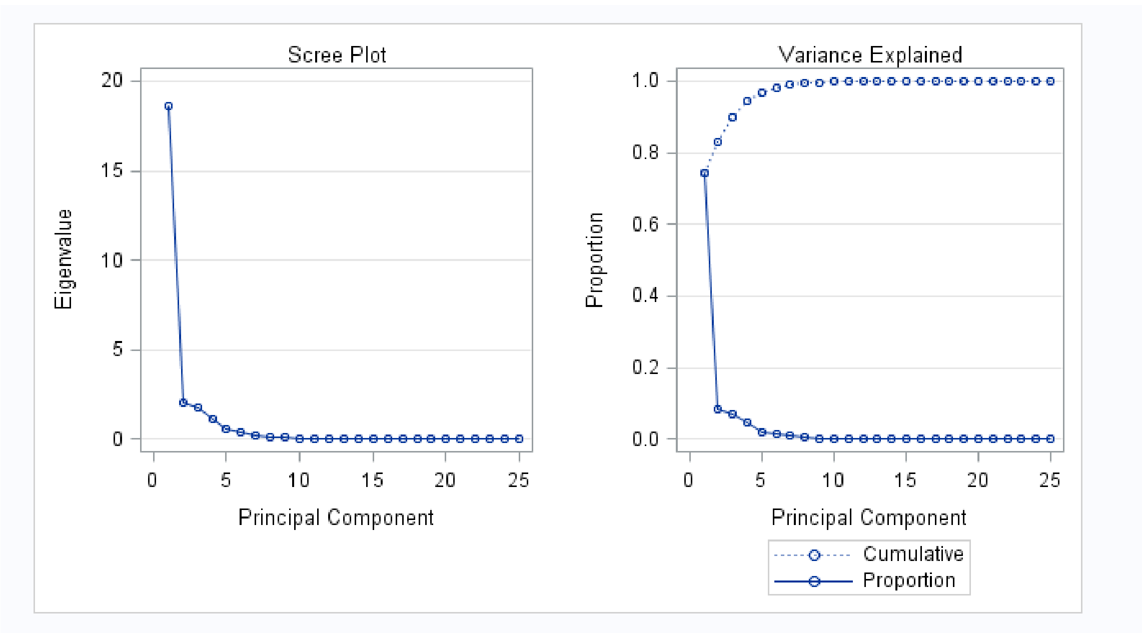
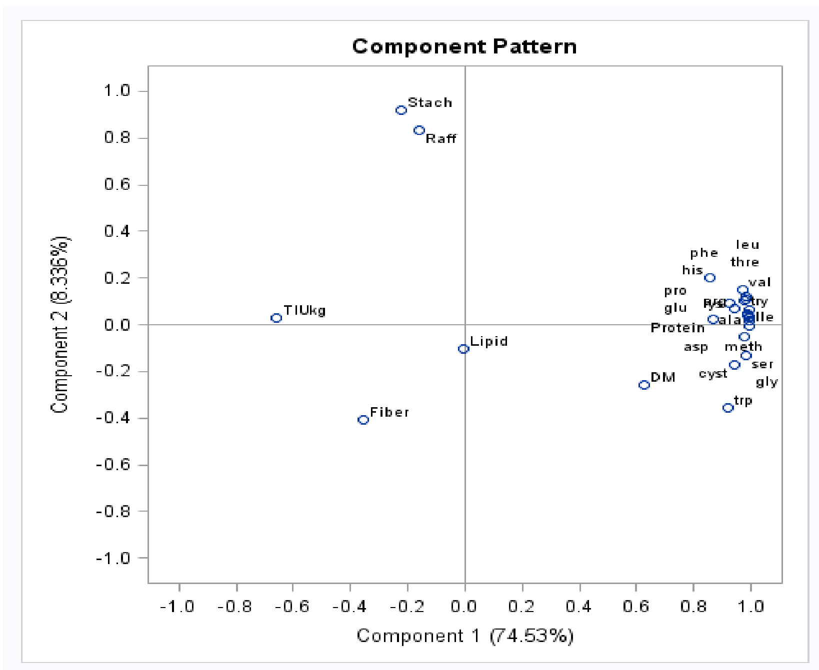


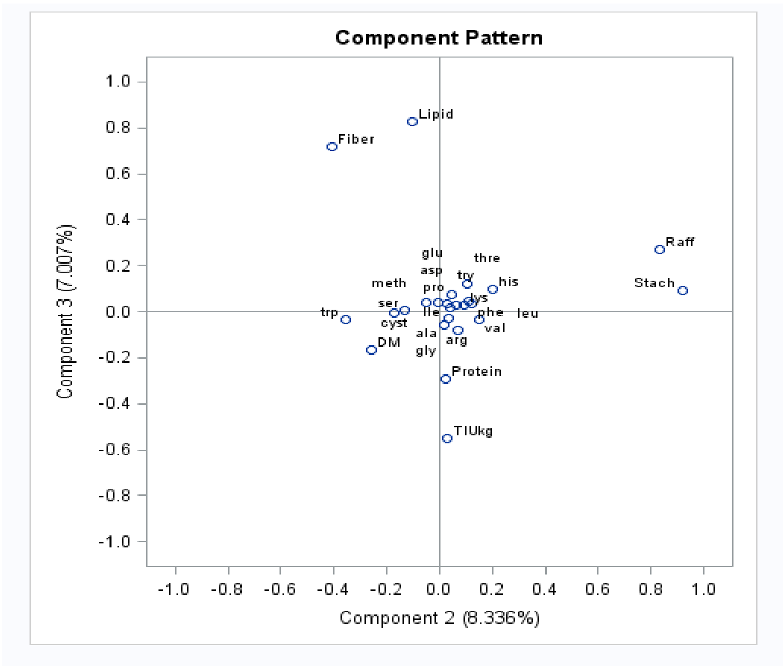
Figure 2 Component patterns (a) component 1 (74.53%) and component 2 (8.366%), (b) component 2 (8.366%) and component 3 (7.007%) of chemical and amino acids of ingredients.

(a)



1. try-Tryptophan
2. cys-Cysteine
3. meth-Methionine
4. asp-Aspartic acid
5. thr-Threonine Serine
6. glu-Glutamic acid
7. pro-Proline Glycine
8. ala-Alanine
9. val-Valine
10. iso-Isoleucine
11. leu-Leucine
12. tyr-Tyrosine
13. phe-Phenylalanine
14. lys-Lysine
15. his-Histidine
16. arg-Arginine
17. TIU-trypsin inhibitor
18. raff-raffinose
19. stach-stachyo
20. DM-dry mat

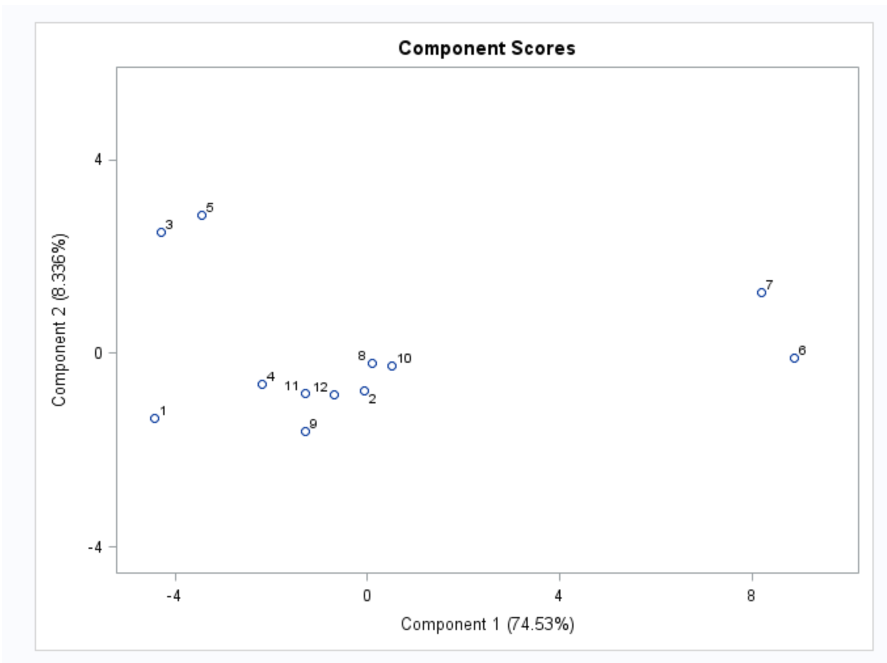
(b)



1. try-Tryptophan
2. cys-Cysteine
3. meth-Methionine
4. asp-Aspartic acid
5. thr-Threonine Serine
6. glu-Glutamic acid
7. pro-Proline Glycine
8. ala-Alanine
9. val-Valine
10. iso-Isoleucine
11. leu-Leucine
12. tyr-Tyrosine
13. phe-Phenylalanine
14. lys-Lysine
15. his-Histidine
16. arg-Arginine
17. TIU-trypsin inhibitor
18. raff-raffinose
19. stach-stachyo
20. DM-dry matter

Figure 3 Component scores (a) component 1 (74.53%) and component 2 (8.366%), (b) component 2 (8.366%) and component 3 (7.007%) of chemical and amino acids of ingredients.

(a)



*Unless noted, ingredients were provided by Shillinger Genetics Inc. Des Moines, IA.

[1]Ingredient-Triveca, blens soybean meal

[2]Ingredient-3010, previous production line of soybean meal

[3]Ingredient-10, Soy lot 129T755, white flake

[4]Ingredient-11, Soy lot 31TD735-ULT, white flake

[5]Ingredient-12, Soy E3311, white flake

[6]Ingredient-13, Soy lot 59P22, meal

[7]Ingredient-14, Soy (boiled) lot 59p22, meal

[8]Ingredient-15, Soy (boiled) lot 59p22, press cake

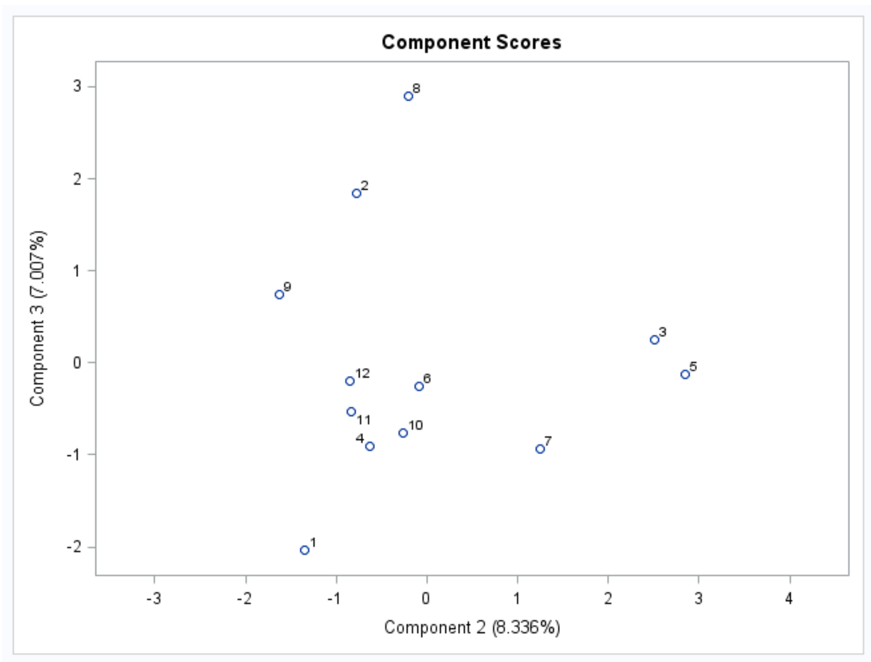
[9]Ingredient-16, Soy Lot 27D616, meal

[10]Ingredient-17, Soy lot 28D, meal

[11]Ingredient-18, Soy lot 28D617, meal

[12]Ingredient-19, Soy lot 34D635, meal

(b)



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[8]Ingredient-15, Soy (boiled) lot 59p22, press cake

[9]Ingredient-16, Soy Lot 27D616, meal

[10]Ingredient-17, Soy lot 28D, meal

[11]Ingredient-18, Soy lot 28D617, meal

[12]Ingredient-19, Soy lot 34D635, meal

Figure 4 R-Square analysis of model amino acids with digestibility coefficients in. partial least square regression

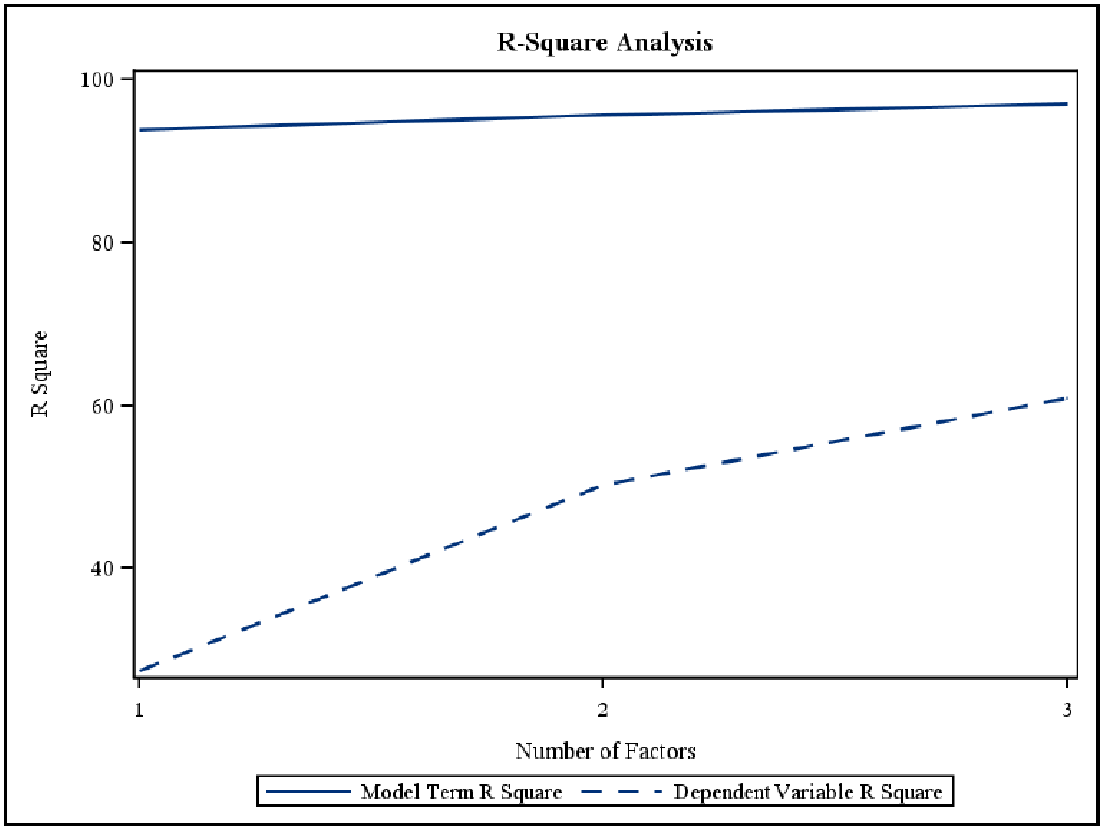
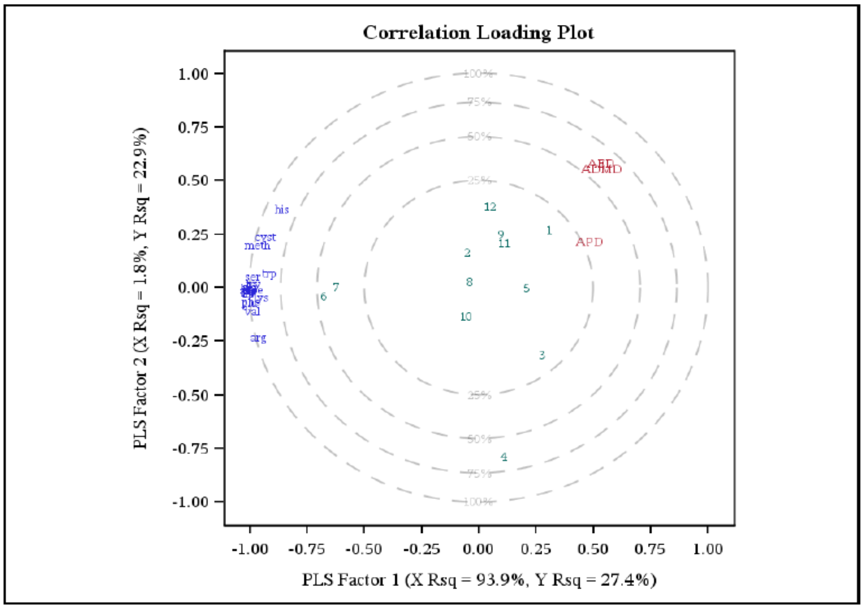


Figure 5 Correlation loading plot (PLS factor 1 and factor 2) of amino acids with digestibility coefficients in partial least square regression



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[8]Ingredient-15, Soy (boiled) lot 59p22, press cake

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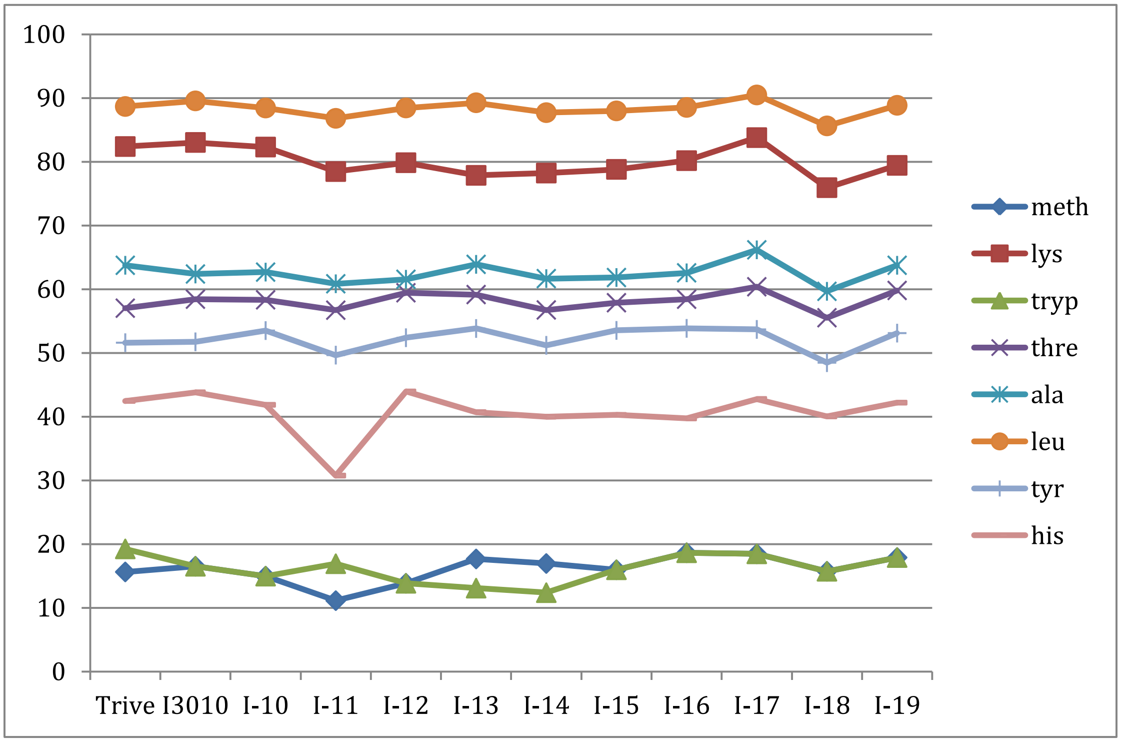
[10]Ingredient-17, Soy lot 28D, meal

[11]Ingredient-18, Soy lot 28D617, meal

[12]Ingredient-19, Soy lot 34D635, meal

Figure 6 Logarithm amino acid percent of each test new varieties soybean meal used in growth trial diet of pacific white shrimp



[1]Ingredient-Triveca, blens soybean meal

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[6]Ingredient-13, Soy lot 59P22, meal

[7]Ingredient-14, Soy (boiled) lot 59p22, meal

[8]Ingredient-15, Soy (boiled) lot 59p22, press cake

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[11]Ingredient-18, Soy lot 28D617, meal

[12]Ingredient-19, Soy lot 34D635, meal

Table 8 Parameter estimates for centered and scaled data of amino acids with digestibility coefficients in partial least square regression model.

	ADMD	ADE	ADP
Tryptophan	0.3869707237	0.3798242657	0.5226006620
Cysteine	0.2235901419	0.2471273351	-0.1097933418
Methionine	0.2854113537	0.2977684720	0.1230313934
Aspartic acid	-0.0491745372	-0.0506795830	-0.0327336473
Threonine	0.3298198162	0.3071128629	0.6918380725
Serine	0.2968691409	0.2864611504	0.4735470521
Glutamic acid	-0.2503114985	-0.2550310512	-0.2023494797
Proline	-0.3199044834	-0.3084336876	-0.5180638834
Glycine	0.0752451391	0.0628242072	0.2639023073
Alanine	-0.0382104975	-0.0440855540	0.0440584370
Valine	-0.2239491973	-0.2353516900	-0.0745665689
Isoleucine	-0.0476495182	-0.0592885458	0.1194731348
Leucine	-0.0799005543	-0.0813464211	-0.0668093709
Tyrosine	-0.3272100844	-0.3156864549	-0.5266964295
Phenylalanine	-0.3994685055	-0.3935473156	-0.5215991919
Lysine	-0.4995458686	-0.4788224373	-0.8495665563
Histidine	0.5971538884	0.6267527541	0.2040433939
Arginine	-0.4069832756	-0.4359172429	-0.0118828988

Table 9 Pearson correlation coefficients of growth performance (weight gain, final biomass, final mean, FCR), chemical contents of ingredients and digestibility coefficients (ADMD, ADE, and ADP). The first line of each cell is the value of correlation coefficient and the second line of each cell is P-value.

	Trypsin inhibitor TIU	Raffinose	Stachyose	Protein	Lipid	Fiber	Dry matter	Digestibility coefficients		
								ADMD	ADE	ADP
Weight gain	-0.833 *0.005**	0.189 0.625	-0.491 0.178	0.140 0.718	-0.09 0.817	0.069 0.858	0.497 *0.011**	0.261 0.497	0.202 0.602	0.648 *0.058**
Final biomass	-0.845 *0.0041**	0.103 0.791	-0.544 0.129	0.392 0.298	-0.163 0.675	-0.090 0.816	0.866 *0.002**	0.104 0.788	0.066 0.865	0.579 *0.101**
Final mean weight	-0.798 *0.0099**	0.131 0.735	-0.511 0.159	0.232 0.546	-0.193 0.618	-0.061 0.875	0.822 *0.006**	0.207 0.591	0.154 0.69	0.649 *0.058**
FCR	0.835 *0.005**	-0.166 0.668	0.501 0.168	-0.276 0.471	0.133 0.733	0.087 0.823	-0.801 *0.009**	-0.169 0.662	-0.120 0.7578	-0.594 *0.0913**

[*]significant of regressions: P<0.15; *Italics* indicates chemical contents and digestibility coefficients retained in multiple backward stepwise regression

Table 10 Pearson correlation coefficients of growth performance (weight gain, final biomass, final mean, FCR) and essential amino acid. The first line of each cell is the value of correlation coefficient and the second line of each cell is P-value.

	Tryptophan	Methionine	Threonine	Alanine	Leucine	Tyrosine	Lysine	Histidine
Final biomass	0.66149	0.74571	0.61949	0.58076	0.54754	0.5855	0.35847	0.54448
	0.0523	0.0211	0.0752	0.1010	0.1270	0.0976	0.3435	0.1296
Final mean weight	0.52892	0.60096	0.46874	0.42635	0.38364	0.43392	0.213	0.39453
	0.1432	0.0870	0.2031	0.2525	0.3081	0.02432	0.5822	0.2934
FCR	-0.58234	-0.67526	-0.55973	-0.51904	-0.4813	-0.51674	-0.32973	-0.49443
	0.0999	0.0459	0.1171	0.1522	0.1895	0.1543	0.3862	0.1761
Weight gain	0.5049	0.58253	0.45984	0.39942	0.37344	0.42725	0.22746	0.38279
	0.1657	0.0998	0.2130	0.2869	0.3222	0.2514	0.5561	0.3092

Table 11 Multiple linear regression with backward stepwise between growth performance (final biomass, final mean weight, FCR, weight gain) and TIU, ADP and dry matter

	Final biomass		Final mean weight		FCR		Weight gain	
	F	Pr(F)	F	Pr(F)	F	Pr(F)	F	Pr(F)
TIU	2.85	0.1524	*1.94****	*0.2365****	*3.26****	*0.1308****	*4.21****	*0.0955****
ADP	2.42	0.1806	*3.84****	*0.2221****	2.5	0.1750	*4.61****	*0.0847****
Dry matter	*2.55****	*0.1709****	1.51	0.2734	0.66	0.4540	0.55	0.4899

*Significant of regressions: *0.05<P<0.10; ***P=0.001. *Italics* indicates chemical contents retained after multiple backward stepwise regression

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