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Original Article

Effects of dietary inclusion of fish blood by-product from canning industry on growth and digestive enzyme activity in Pacific white shrimp, *Litopenaeus vannamei* (Boone, 1931)

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Abstract

Dry fish blood (DFB), a by-product from the fish processing industry, is a rich source of nutrients, small protein molecules and iron. This study aimed to examine the effects of dietary inclusion of canning by-product fish blood on growth performance and activity of digestive enzymes in *L. vannamei* with mean initial weight of 4.79±0.12 g. Six diets were formulated: four diets having poultry meal and soybean meal as the main protein sources contained DFB at 0 (control), 4, 8, and 16% of diet and the reference diets5 and 6contained 4% tuna viscera hydrolysate (TVH) and 16% fish meal, respectively. Triplicate groups of shrimp (12 shrimp tank^{-1}) were fed with respective diets five times daily for six weeks. The results showed that growth of shrimp decreased with increasing level of dry fish blood. Growth performance of shrimp fed 4% DFB was not significantly different from those fed 4% TVH. Survival rate was not significantly different among treatments (P>0.05). In summary, dry fish blood could be used as a feed ingredient in shrimp diet at 4% of diet with good growth performance. The results demonstrated that the high levels of dry fish blood had an effect on feed utilization efficiency, alkaline phosphatase activity and shrimp growth reduction.

Keywords: *Litopenaeus vannamei*, dry fish blood, fish meal replacement, growth, digestive enzymes

1. Introduction

Pacific white shrimp, *Litopenaeus vannamei*, is one of the most important marine aquaculture species in the world (González-Félix *et al*., 2016; Yu *et al*., 2016), is distributed throughout Central and South America, and was introduced into Thailand in 1998. For the success of shrimp culture industry, feed is extremely important for farm feasibility and profit because its cost usually exceeds 50% of the variable production costs (Bragaa *et al*., 2016). Protein quality and composition is the most important and the expensive component of shrimp and other crustacean feed which ranges from 30-40% of diet depending on species and stage of life (Jatobá *et al*., 2014; Perera & Simon, 2015; Terrazas-Fierro *et al*., 2010).

Fish meal has been the most important source of protein in aquaculture feed because of its essential amino acid composition, essential fatty acids, mineral content and palatability factors (Sookying & Davis, 2011).According to estimates by the International Fishmeal and Fish Oil Organization, the aquaculture industry utilized 73% of the fishmeal produced in 2010 (Food and Agriculture Organization of the United Nations [FAO], 2014). However, the combination of high price and fluctuating supply of fish meal has led to a search for alternative protein sources and modification of aqua feed formulation including shrimp diets that require supplementation of essential amino acids methionine and lysine (Terrazas-Fierro *et al*., 2010).

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Nowadays, as people around the world are increasingly concerned about health and quality aging, consumption of fish products, a key component for healthy diets, has increased continuously. Globally, almost 70 million tons of fish are processed by filleting, freezing, canning or curing, resulting in by-products and waste (FAO, 2014). During the production of different products, fish may be processed by bleeding, gutting, beheading, filleting, skinning and trimming before being bought by consumers (Olsen *et al.*, 2014). A large quantity of by-products or waste output includes heads, bones, viscera, gills, dark muscle, skin, blood as well as vitamins and minerals making it available for other uses (Islam *et al*., 2004; Martínez-Alvarez *et al*., 2015; Sanmartín *et al*., 2012; Sila & Bougatef, 2016). Wastewater, especially from the filleting and trimming processes, contains fat, oil and grease, blood, small pieces of fish and protein. Seafood processing wastewater was also noted to sometimes contain high concentrations of chlorides from processing water and brine solutions, and organic nitrogen (0–300 mg L^{-1}) from processing water (Islam *et al*., 2004). Thailand is the world's largest producer of canned tuna and annually exports about half a million tons of it (FAO, 2016).The amount is only about 32–40% of the raw material input and the remaining is solid waste or by-products which could be as high as 65% of the original material (FAO, 2014).

Using seafood processing waste as animal feed ingredients has been intensely studied during the past decades in order to improve the fisheries resource utilization and minimize the pollutants from the discards. Fish processing by-products are increasingly used as ingredients in fish meal production and it is expected that this resource will account for 40% of the produced fish meal by 2020 (Shepherd & Jackson, 2013). Mamauag and Ragaza (2016) included milkfish offal with and without processing as hydrolysate at 0, 5, 15, and 25% in the diets for juvenile *Epinephelus fuscoguttatus*. The milkfish offal processed as hydrolysate could be utilized in grouper diets and promoted growth when supplied at 10–15%. Fukada *et al*. (2016) used crude tuna broth, and wastewater from boiling, as ingredients in the diets for two farmed species, juvenile yellowtail (*Seriola quinqueradiata*) and red sea bream (*Pagrus major*).They found that the crude tuna broth enhanced juvenile growth at minimal cost, while the concentrated tuna broth required processing, but proved to be a valuable fish feed supplement because of its high palatability and growth-promoting effect.

Blood meal, a slaughterhouse by-product, is a feedstuff that is commonly used in livestock diets as it is a rich source of leucine and lysine though a poor source of isoleucine, cysteine and methionine compared to whole chicken egg protein blood meal (Hertrampf & Piedad-Pascual, 2000; National Research Council [NRC], 2011; Ofori & Hsieh, 2013). In aquaculture diets, blood meal should not exceed 5.0% for young animals and 10% for older animals which is in the range of 15-20% of the total protein content for practical conditions (Hertrampf & Piedad-Pascual, 2000). Fish blood is similar to that of any other vertebrate which carries nutrient material, glucose, amino acids and fatty acids, vitamins, electrolytes and trace elements from the alimentary canal to the tissues (Piska & Naik, 1972). In a similar approach, fish blood by-product from the fish processing industry should therefore be able to serve as an ingredient in aqua feed. Accordingly, the aim of this study was to examine the effects of dietary inclusion of fish blood by-product on growth performance and digestive enzyme activity in *L. vannamei*.

2. Materials and Methods

2.1 Experimental diets and pellet stability tests

Six isoprotein and isolipidic diets were formulated to contain 40% crude protein and 8% lipid. Diet1, without dry fish blood, is the control diet and had poultry and soybean meal as the main protein sources. Diets 2-4 were supplemented with dry fish blood at 4, 8 and 16% of diet, respectively. Two reference diets were included in the study, diets 5 and 6 which contained tuna viscera hydrolysate at 4% and fish meal at 16%, respectively (Table 1). The diets were processed by mixing the ingredients for 20 minutes in a Hobart mixer (Legacy HL200, USA).The feed mixture was passed through a meat grinder, broken into pellets, dried in an air-forced oven at 60^{o}C for 8 hrs. to reduce the moisture to approximately 5%. The prepared diets were packed into plastic bags and stored at -20^{o}C. The proximate composition of ingredients and experimental diets were determined using standard method of AOAC (1995).

Pellet stability assessed by leaching loss was performed in three replications according to the modified method from Cruz-Suarez *et al*. (2001). The leaching loss was then calculated as follows:

% leaching loss (% as-fed basis) = [(weight of pellet before immersion - weight of pellet after immersion, g)/ initial pellet weight, g] x100

2.2 Growth trial

L. vannamei juveniles were nursed in 1000 L fiberglass tank in order to acclimate them to the culture conditions, and were fed with a commercial diet for 4 weeks. Each dietary treatment was composed of three replicated 130 L glass aquarium tanks (48 cm x 70 cm x 41 cm) at water temperature of 27-29^{o}C and salinity of 22-24 ppt. Twelve shrimp with an initial weight of 4.79 ± 0.12 g shrimp^{-1} were weighed and stocked into each tank and fed with respective experimental diets five times daily to apparent satiation at 8.30, 11.30, 14.30, 17.30, and 20.30 hour for 42 days. The fresh seawater was replaced daily at 60% of the volume. The water quality parameters including temperature, salinity and dissolved oxygen were monitored periodically using thermometer, refracto salinometer (Master Refractometer, ATACO) and Hanna Dissolved Oxygen Meter, respectively.

2.3 Sample collection and chemical analysis

At the beginning of the feeding trial, 100 g of shrimp from the same population were randomly sampled for determination of initial whole body proximate composition. During the growth trial, feed intake, shrimp behavior and abnormality were recorded daily. At the end of the trial, shrimp were starved for 12 hrs, then individually weighed for the final weight and sampled for proximate composition analysis at 100 g tank^{-1}. Three shrimp per tank were randomly

Table 1. Composition of the experimental diets (g 100 g^{-1}, as-fed basis).

Ingredients	Experimental diets and level of dry fish blood					
	1 (0%)	2 (4%)	3 (8%)	4 (16%)	5 (Ref. 1)	6 (Ref. 2)
Dry fish blood (55% CP)	0.00	4.00	8.00	16.00	-	-
Fishmeal (60% CP)	-	-	-	-	-	16.00
TVH[1](71% CP)	-	-	-	-	4.00	-
Poultry meal (63% CP)	20.92	17.54	14.16	7.40	16.36	5.90
Soybean meal (48% CP)	30.00	30.00	30.00	30.00	30.00	30.00
Wheat gluten (76% CP)	6.00	6.00	6.00	6.00	6.00	6.00
Wheat flour	35.22	34.54	33.84	32.48	35.44	33.67
Fish oil	0.98	1.01	1.05	1.11	1.17	1.29
Vegetable oil	0.98	1.01	1.05	1.11	1.17	1.29
Lecithin	2.00	2.00	2.00	2.00	2.00	2.00
Cholesterol	0.55	0.55	0.55	0.55	0.55	0.55
Vitamin premix	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin C	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix	0.50	0.50	0.50	0.50	0.50	0.50
DCP[2]	0.20	0.20	0.20	0.20	0.20	0.20
BHT	0.02	0.02	0.02	0.02	0.02	0.02
DL-methionine (99%)	0.53	0.53	0.53	0.53	0.49	0.48
Proximate composition (as-fed basis)						
Protein	39.90	39.38	39.38	40.36	39.40	39.00
Lipid	7.59	7.50	7.82	8.43	7.62	7.65
Ash	7.93	7.53	7.98	8.06	7.67	8.91

[1] Tuna viscera hydrolysate
[2] Dicalciumphosphate

sampled for use in trypsin, alkaline phosphatase and leucine aminopeptidase assays.

Shrimp carcasses were analyzed for proximate composition using standard method of AOAC (1995). Crude protein was determined using the Kjeldahl method (Kjeltec TM8100, FOSS, Höganäs, Sweden), crude lipid by ether extraction using Soxhlet method (Soxtec System HT 1043 Extraction Unit, Lincoln, United States) and ash by combustion at 500°C for 3 hrs.

2.4 Trypsin, leucine aminopeptidase and alkaline phosphatase

2.4.1 Sample preparation

Hepatopancreases were dissected and kept immediately in liquid nitrogen before enzyme activity determination. Extraction of brush border enzyme was then performed and stored at -80°C for enzyme activity analysis. Crude protein content was determined by a modified Lowry method (Lowry *et al*., 1951) using bovine serum albumin as a standard. All steps in the sample preparation described above were conducted at low temperature by working on ice.

2.4.2 Trypsin, leucine aminopeptidase and alkaline phosphatase activity assays

Trypsin, leucine aminopeptidase (lap) and alkaline phosphatase (alp) activity was determined by a modified method of Srichanun *et al*. (2013), Buarque *et al*. (2009) and Puttige and Nooralabettu (2011), respectively. Benzoy-larginine p-nitroanilide (BAPNA**,** 0.01 M Sigma B4875), L-leucine p-nitroanilide (0.004 M; Sigma L9125) and p-nitrophenol-phosphate (0.054 M; Sigma P0757) were used as a substrate for trypsin, lap and alp activity determination, respectively. The measurements of each enzymatic activity was carried out at 25°C under optimum pH for Pacific white shrimp, pH 7.5 for trypsin, 8 for lap and 8.4 for alp. One unit of enzyme activity (U) was defined as 1 µmole of each product released per min and per mL of enzyme homogenate.

2.5 Statistical analysis

Differences in growth performance and feed efficiency parameters among diets were subjected to ANOVA and differences between means by Duncan's Multiple Range Test using SPSS 17 for Windows.

3. Results

3.1 Stability of diets

The stability of experimental diets was significantly different among treatments (P<0.05) which ranged from 12.09-84.47% (Figure 1). The reference and the control diets showed equivalently higher stability (81.07-84.47%) than those containing DFB. Diet 4 containing the highest level of DFB had the lowest stability (P<0.05).

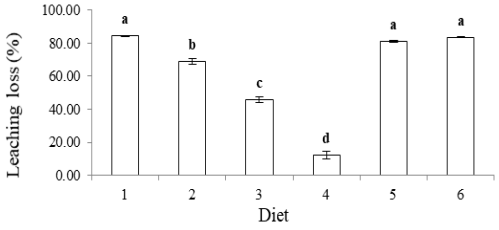

Figure1. Stability test of experimental diets for 1 hr.

3.2 Growth and feed utilization

After the 6 week feeding period, there was no significant difference (P>0.05) in survival rate among treatments which ranged from 97.22-100% (Table 2). Shrimp fed the reference diet 6 containing 16% fish meal had the highest final weight, weight gain and specific growth rate followed by those fed TVH reference diet 5 as shown in Table 2.The growth performance of shrimp significantly decreased with increasing levels of DFB (P<0.05) particularly those fed diets fortified with 8% and 16% DFB.

Feed intake of shrimp fed the reference diet 6 was the highest followed by the control diet 1 and reference diet 5.The levels of feed intake significantly decreased as the DFB inclusion increased (P<0.05) and the group fed 16% DFB ate the least amount of only 1.77±0.02 g shrimp^{-1} for the 42-day rearing period (Table 3).The feed conversion ratio was in the range of 1.87±0.04 - 2.60±0.49. Good FCR was in the groups fed diets 3 and6 at 1.90±0.02 and 1.87±0.04, respectively while those of the control diet and diet 2 showed inferior FCR. Similar results were obtained for protein efficiency ratio which ranged between 1.00±0.18 and 1.37±0.03 and the shrimp fed diets 3 and 6 had the highest PER of 1.34±0.02 and 1.37±0.03, respectively. For productive protein value (PPV), the best results were in the shrimp fed diet 4 followed by those fed diets 3 and 6 of 29.93±0.58, 26.29±0.23 and 25.99±0.49, respectively. The significantly lower PPV were those fed the control diet, diet 2 and reference diet 5 (P<0.05).

3.3 Digestive enzyme activity

Shrimp fed diet 6 had the highest protein content in enzyme (11.43±0.44 mg mL^{-1}) followed by shrimp fed diets 1, 2, 5, 4, and 3 as shown in Table 4. Activity of trypsin in hepatopancreases was lowest in the control diet fed shrimp and highest in shrimp fed diet 3 (P<0.05) as shown in Table 4.The lap activity in hepatopancreases of shrimp fed reference diet 6 was the highest while that of reference diet 5 was the lowest. The hepatopancreatic activity of alp in the groups fed diet 2 and reference diets 5 and 6 was significantly higher than those fed the control diet 1 and the groups fed diets 3 and 4 had very low alp activity of only 0.86±0.32 and 2.76±1.57 unit mg^{-1} protein (P<0.05).

Table 2. Final weight, weight gain, specific growth rate (SGR), and survival rate of shrimp fed diets containing different levels of dry fish blood for 42 days.

Diet	Final weight (g shrimp^{-1})	Weight gain[1] (g shrimp^{-1})	SGR[2] (% day^{-1})	Survival (%)
1 (Control)	9.12 ± 0.43^{bc}	4.34 ± 0.46^{bc}	10.84 ± 1.15^{bc}	100.00 ± 0.00^{ns}
2 (4% DFB)	8.81 ± 0.68^{cd}	4.04 ± 0.72^{cd}	10.11 ± 1.80^{cd}	100.00 ± 0.00
3 (8% DFB)	8.32 ± 0.09^{d}	3.48 ± 0.05^{d}	8.70 ± 0.14^{d}	100.00 ± 0.00
4 (16% DFB)	5.73 ± 0.31^{e}	0.92 ± 0.24^{e}	2.29 ± 0.60^{e}	97.22 ± 4.81
5 (Ref.1)	9.67 ± 0.15^{b}	4.83 ± 0.28^{b}	12.07 ± 0.70^{b}	100.00 ± 0.00
6 (Ref.2)	10.50 ± 0.13^{a}	5.82 ± 0.11^{a}	14.55 ± 0.28^{a}	100.00 ± 0.00

Values are mean ± standard deviation (n=3).Means in the same column sharing same superscripts are not significantly different (P>0.05) by Duncan's Multiple Range Test and *ns* indicates no statistical difference among treatments. [1]Weight gain (g shrimp^{-1}) = final weight (g shrimp^{-1}) – initial weight (g shrimp^{-1}) [2]SGR (% day^{-1}) = $(\ln W_1 - \ln W_2/T_2\text{-}T_1) \times 100$; W_1 = initial weight, W_2 = final weight, T_2-T_1 = cultured period (days)

Table 3. Feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER) and productive protein value (PPV)of shrimp fed diets containing different levels of dry fish blood for 42 days.

Diet	Feed intake (g shrimp^{-1})	FCR[1]	PER[2]	PPV[3] (%)
1 (Control)	10.75 ± 0.08^{a}	2.50 ± 0.25^{ab}	1.01 ± 0.10^{b}	20.70 ± 1.29^{d}
2 (4% DFB)	10.29 ± 0.12^{c}	2.60 ± 0.49^{a}	1.00 ± 0.18^{b}	20.91 ± 0.98^{d}
3 (8% DFB)	6.61 ± 0.04^{d}	1.90 ± 0.03^{c}	1.34 ± 0.02^{a}	26.29 ± 0.23^{b}
4 (16% DFB)	1.77 ± 0.02^{e}	2.01 ± 0.46^{bc}	1.28 ± 0.34^{ab}	29.93 ± 0.58^{a}
5 (Ref.1)	10.56 ± 0.16^{b}	2.19 ± 0.09^{abc}	1.16 ± 0.05^{ab}	22.85 ± 0.73^{c}
6 (Ref.2)	10.90 ± 0.02^{a}	1.87 ± 0.04^{c}	1.37 ± 0.03^{a}	25.99 ± 0.49^{b}

Values are mean ± standard deviation (n=3). Means in the same column sharing same superscripts are not significantly different (P>0.05) by Duncan's Multiple Range Test.[1] FCR = Feed intake (g shrimp^{-1}) / Weight gain (g shrimp^{-1})[2] PER = Weight gain (g) / Protein intake (g)[3] PPV (%) = (Protein gain (g) / Protein intake (g)) x 100.

Table 4. Specific enzyme activity of trypsin, leucine aminopeptidase (lap) and alkaline phosphatase (alp) measured in the pyloric caeca of fish fed diets containing different levels of dry fish blood for 42 days.

Diet	Protein (mg mL^{-1})	Enzyme activity (unit mg^{-1} protein)		
		Trypsin	lap	alp
1 (Control)	11.32 ± 0.57^{a}	1.34 ± 0.06^{b}	0.16 ± 0.05^{ab}	12.98 ± 0.28^{b}
2 (4% DFB)	10.45 ± 0.58^{ab}	1.77 ± 0.26^{ab}	0.18 ± 0.05^{ab}	16.52 ± 2.86^{a}
3 (8% DFB)	9.81 ± 0.07^{b}	1.95 ± 0.57^{a}	0.17 ± 0.05^{ab}	2.76 ± 1.57^{c}
4 (16% DFB)	10.10 ± 0.11^{b}	1.81 ± 0.63^{ab}	0.17 ± 0.04^{ab}	0.86 ± 0.32^{c}
5 (Ref.1)	10.29 ± 0.89^{ab}	1.61 ± 0.28^{ab}	0.13 ± 0.04^{b}	18.13 ± 1.59^{a}
6 (Ref.2)	11.43 ± 0.44^{a}	1.66 ± 0.26^{ab}	0.21 ± 0.06^{a}	17.81 ± 2.22^{a}

Values are mean ± standard deviation (n=3). Means in the same column sharing same superscripts are not significantly different (P>0.05) by Duncan's Multiple Range Test.

4. Discussion

In this study, fish blood was the by-product obtained from tuna canning industry from the processes of head and tail cutting and viscera removal. The dry matter content of fish blood by-product was low at 3.71%. This is less than the roughly 18% of slaughterhouse blood by-products such as from pigs and cows, whose blood is about 82% water (European Union, 2005). This is possibly due to fish blood by-product being diluted with water during the washing step of factory processing. The protein, lipid and ash content of dry fish blood were 61.32, 9.99, and 23.85% of dry matter, respectively. The protein content in DFB was lower than slaughterhouse blood meal from terrestrial animals which ranges between 87-89% (NRC, 2011; DeRouchey, 2013). However, the quality and quantity of protein content in blood meal depends on animal type and the drying process of raw the material.

Generally, the growth of the penaeid shrimp is affected by the level and quality of the protein and those fed with higher quality protein show higher protein digestibility, better growth, and less susceptibility to disease (Ezquerra *et al*., 1997; Pike & Hardy 1997). Results from the present study showed that fish blood by-products from the tuna canning industry were poorly utilized by shrimp. The increasing levels of DFB had an effect on reduced pellet stability, feed palatability and shrimp growth performance particularly at the highest inclusion level of (16%) of diet. The best inclusion level of DFB was at 4% of diet insofar as final weight, weight gain and specific growth rate were not significantly different from those of the control group. The similar results in shrimp were reported by Nunes (2012) who found that the feed conversion ratio of the shrimp that were fed diets containing animal blood meal from slaughterhouses as fish meal replacement was higher than those fed the control diet. Chookird *et al*. (2010) also found that white shrimp that were fed diets containing hemoglobin powder had poor growth performance. High levels of DFB possibly affected the amino imbalance of the diet because blood meal is a poor source of methionine as well as having an imbalance of branched chain amino acids, isoleucine, leucine and valine (Hertrampf & Piedad-Pascual, 2000). The best growth and feed utilization were achieved in the shrimp fed the reference diet 6, which contained a high level of fish meal with better amino acid profiles and protein quality. This explains the lower feed utilization and growth performance in those fed DFB fortified diets.

The pellet stability reduced with increasing inclusion levels of DFB that caused corresponding greater feed loss than the control diet and reference diets. During feeding, the feed would be further disintegrated by the shrimp feeding behavior that nibble the feed slowly using their pereiopods to catch and eat the feed particles. Feed intake of shrimp fed DFB containing diets also decreased with increasing DFB content to the significantly lowest intake of 1.77 g $shrimp^{-1}$ in the group fed 16% DFB, during the course of the 42-day study. The pellet stability and feed intake responses correlated well with the final weight which indicates that all experimental groups performed better than the diets containing 16% DFB. This implies that DFB or some components in the product caused pellet disintegration and was unpalatable to the shrimp, and so brought about reduction in feed intake and growth retardation. Suppression of feed intake and imbalance of essential amino acids were the main reasons for the reduced growth performance of shrimp as the dietary plant and animal by-product protein substitution of fish meal increased as documented by Forster *et al*. (2003) and Amaya *et al*. (2007).

In the present study, the digestive enzyme activities were determined using the hepatopancreas extract. This important organ functions in nutrient absorption, transport, secretion of digestive enzymes and storage of lipids, glycogen and minerals (Felgenhauer, 1992). A common characteristic of decapod digestive enzymes is the presence of enzymes with trypsin activity and chymotrypsin activity (Ezquerra *et al*., 1997), where the protein source seems to influence trypsin-specific activity (Lee *et al*. 1984). In comparison with shrimp fed the control diet, groups fed DFB diets showed increased trypsin activity. Alp activity, on the other hand, significantly decreased with increasing levels of DFB to the lowest level of 2.76±1.57 and 0.86±0.32unitmg^{-1} protein in the groups fed the diet with 8% and 16% DFB, respectively, while alp activity of the rest of experimental groups were in the similar range of 12.98±0.28 to18.13±1.59. In mammals (including humans), it is well known that the diet is a major factor affecting alp activity/expression alteration, which depends on the amount and type of ingredients in the diet, and that fasting dramatically decreases alp activity while re-feeding restores it (Lalles, 2010).The marked reduction in alp activity in the present study, in the shrimp fed diets containing 8% and 16% DFB, may be explained by the low feed consumption of only 1.77-6.61 g $shrimp^{-1}$ over the 42-day rearing period, and the nutritionally imbalanced diet. In rats, activity of intestinal alkaline phosphatase was low in the group fed a protein-free diet and legume proteins from soybean (*Glycine max*) reduced

it when they were substituted for casein in the diets, indicating that deficiency in essential amino acids decreases its activity (Lalles, 2010; Montoya *et al*., 2006).In white shrimp, Lee *et al*. (1984) suggested that protease activity responds to dietary protein quality. Later, Ezquerra *et al*. (1997) evaluated protease activities of the hepatopancreas extract from shrimp that were fed different fish meal replacement diets and found that a small amount of specific protein replacer (15%) in white shrimp diet could influence protease activity of the hepatopancreas.

5. Conclusions

Dry fish blood has high protein content which can be used as a feed ingredient in shrimp diet at the highest level of 4% of diet with good growth performance. The results demonstrated that high levels of dry fish blood by-product had an effect on reducing feed utilization efficiency, alkaline phosphatase activity, and shrimp growth. These effects were possibly due to imbalanced amino acid profiles and unfavorable factors that caused pellet disintegration and an unpalatable diet.

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References

Association of Official Analytical Chemists, (1995). *Official methods of analysis* (16th ed.). Arlington, VA: Author.

Amaya, E., Davis, D. A., & Rouse, D. B. (2007). Alternative diets for the Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture, 262*, 419-425.

Bragaa, A., Magalhães, V., Hanson, T., Morris, T. C.,& Samocha, T. M. (2016). The effects of feeding commercial feed formulated for semi-intensive systems on *Litopenaeus vannamei* production and its profitability in a hyper-intensive biofloc-dominated system. *Aquaculture Reports, 3*, 172-177.

Buarque, D. S., Castro, P. F., Santos, F. M. S., Lemos, D., Carvalho, L. B., & Bezerra, R. S. (2009). Digestive peptidases and proteinases in the midgut gland of the pink shrimp *Farfantepenaeus paulensis* (Crustacea, Decapoda, Penaeidae). *Aquaculture Research, 40*, 861-870.

Chookird, D., Tantikitti, C., Pongdara, A., & Srichanun, M. (2010). Effect of hemoglobin powder substituted for fishmeal on growth performance, protein digestibility and trypsin gene expression in *Litopenaeus vannamei*. *Songklanakarin Journal of Science and Technology, 32*, 119-127.

Cruz-Suarez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Mc Callum, I. M., & Hickling, D. (2001). Assessment of differently processed feed pea (*Pisumsativum*) meals and canola meal (*Brassica* sp.) in diets for blue shrimp (*Litopenaeusstylirostris*). *Aquaculture, 196*, 87-104.

DeRouchey, J. M., Tokach, M. D., Nelssen, J. L., Goodband, R. D., Dritz, S. S., Woodworth, J. C., & James, B. W. (2013). Comparison of spray-dried blood meal and blood cells in diets for nursery pigs. *Journal of Animal Science, 80*, 2879-2886.

Ezquerra, J. M., Garcia-Carreño, F. L., & Haard, N. F.(1997). Effects of feed diets on digestive proteases from the hepatopancreas of white shrimp (*Penaeus vannamei*). *Journal of Food Biochemistry, 21*, 401-419.

European Commission. (2005). *Reference document on best available techniques in the slaughterhouses and animal by-products industries. BAT reference document (BREF).* Seville, Spain: European IPPC Bureau. Retrieved from http://eippcb.jrc.ec.europa.meu/reference/BREF/sa_bref_0505.pdf

Food and Agriculture Organization of the United Nations. (2014). *The state of world fisheries and aquaculture.* Rome, Italy: Author.

Food and Agriculture Organization of the United Nations. (2016). *GLOBEFISH monthly trade statistics-June 2016*. Rome, Italy: Author.

Felgenhauer, B. E.,(1992). Internal anatomy of the Decapoda: An overview. Decapod Crustacea 10. In F. W. Har rison, & A. G. Humes (Eds.), *Microscopic anatomy of invertebrates* (pp. 45-75). New York, NY: Willey Liss.

Forster, I. P., Dominy, W., Obaldo, L., & Tacon, A. G. J. (2003). Rendered meat and bone meals as ingredients of diets for shrimp *Litopenaeus vannamei* (Boone, 1931). *Aquaculture, 219*, 655-670.

Fukada, H.,Takahashi, N.,Hosomi, N., & Masumoto, T. (2016). Beneficial utilization of a tuna processing by-product as fish-feed additive. *Journal of Material Cycles and Waste Management, 18*, 231-238.

González-Félix, M., da Silva, F. S. D., Davis, D. A., Samocha, T. M., Morris, T. C., Wilkenfeld, J. S.,& Perez-Velazquez, M. P.(2010). Replacement of fish oil in plant based diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture, 309*, 152-158.

Hertrampf, J. W., & Piedad-Pascual, F.(2000). *Handbook on ingredients for aquaculture feed*. Dordrecht, The Natherlands: Kluwer Academic.

Islam, Md. S., Khan, S., & Tanaka, M. (2004). Waste loading in shrimp and fish processing effluents: Potential source of hazards to the coastal and near shore environments. *Marine Pollution Bulletin, 49*, 103-110.

Jatobá, A., da Silva, B. C., da Silva, J. S., Vieira, F. N., Mouriño, J. L. P., Seiffert, W. Q. and Toledo, T. M. (2014). Protein levels for *Litopenaeus vannamei* in semi-intensive and biofloc systems. *Aquaculture, 432*, 365-371.

Lee, P. G., Smith, L. L., & Lawrence, A. L. (1984). Digestive protease of *Penaeus vannamei* Boone: Relationship between enzyme activity, size and diet. *Aquaculture, 42*, 225-239.

Lalles, J. P. (2010). Intestinal alkaline phosphatase: Multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. *Nutrition Reviews, 68*, 323-332.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *The Journal of Biological Chemistry, 193*, 265-275.

Mamauag, R. E. P., & Ragaza, J. A. (2016). Growth and feed performance, digestibility and acute stress response of juvenile grouper (*Epinephelus fuscoguttatus*) fed diets with hydrolysate from milkfish offal. *Aquaculture Research.*doi: 10.1111/are.12999

Martínez-Alverez, O., Chamorro, S., & Brenes, A. (2015). Protein hydrolysate from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A Review. *Food Research International, 73*, 204-212.

Montoya, C. A., Leterme, P., & Lalles, J. P. (2006). A protein-free diet alters small intestinal architecture and digestive enzyme activities in rats. *Reproduction Nutrition Development, 46*, 49–56.

National Research Council. (2011). *Nutrient requirements of fish and shrimp*. Washington, DC: The National Academy of Sciences.

Nunes, A. J. P. (2012). Animal protein meals reduce feed costs but don't improve shrimp performance. *Global Aquaculture Advocate, 15*, 34-36.

Ofori, J. A., & Hsieh, Y. H. P. (2012). *The use of blood and derived product as feed additive. Chapter 13: Feed additive.* Florida, FL: Agricultural and Biological Sciences, Florida State University.

Olsen, R. L., Toppe, J., & Karunasagar, I. (2014). Challenges and realistic opportunities in the use of by-products from processing of fish and shellfish. *Trends in Food Science and Technology, 36*, 144-151.

Perera, E., & Simon, C. (2015). Digestive physiology of spiny lobsters: Implications for formulated diet development. *Reviews in Aquaculture, 7*, 243-261.

Pike, I. H., & Hardy, R. W. (1997). Standard for assessing quality of feed ingredients. In L. R. D' Abramo, D. E. Conklin, & D. M. Akiyama (Eds.), *Crustacean nutrition, advances in world aquaculture* (Vol. 6, pp. 473-489). Louisiana, LA: World Aquaculture Society.

Piska, R. S., & Naik, S. J. K., (1972). *Fish biology and ecology. Chapter 7: Circulatory system of fish*. Hyderabad, India: University College of Science, Osmania University.

Puttige, K., & Nooralabettu, K. P. (2011). Alkaline phosphatase activity during homogenization of hepatopancreatic tissue of shrimps using sodium acetate, KCl solution, Tris-HCl and Glycine-NaOH buffer. *International Journal of Scientific and Engineering Research, 2*, 1-7.

Sanmartín, E., Arboleya, J. C., Iloro, I., Escuredo, K., Elortza, F., & Moreno, F. J. (2012). Proteomic analysis of processing by-products from canned and fresh tuna: Identification of potentially functional food proteins. *Food Chemistry, 134*, 1211-1219.

Shepherd, C. J., & Jackson, A. J. (2013).Global fishmeal and fish-oil supply: Inputs, outputs and markets. *Journal of Fish Biology, 83*, 1046-1066.

Sila, A., & Bougatef, A. (2016). Antioxidant peptides from marine by-products: Isolation, identification and application in food system: A review. *Journal of Functional Foods, 21*, 10-26.

Sookying, D., & Davis, D. A. (2011). Pond production of Pacific white shrimp (*Litopenaeusvannamei*) fed high levels of soybean meal in various combinations. *Aquaculture, 319*, 141-149.

Srichanun, M., Tantikitti, C., Utarabhand, P.,& Kortner, T. M. (2013).Gene expression and activity of digestive enzymes during the larval development of Asian seabass (*Latescalcarifer*). *Comparative Biochemistry and Physiology, Part B, 165*, 1-9.

Terrazas-Fierro, M., Civera-Cerecedo, R., Ibarra-Martínez, L., Goytortúa-Bores, E., Herrera-Andrade, M.,& Reyes -Becerra, A.(2010). Apparent digestibility of dry matter, protein and essential amino acid in marine feedstuffs for juvenile whiteleg shrimp *Litopenaeus vannamei*. *Aquaculture, 308*, 116-173.

Yu, Y. Y., Chen, W. D., Liu, Y. J., Niu, J., Chen, M., & Tian, L. X. (2016). Effect of different dietary levels of *Gracilaria lemaneiformis* dry power on growth performance, hematological parameter and intestine structure of juvenile Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture, 450*, 356-362.