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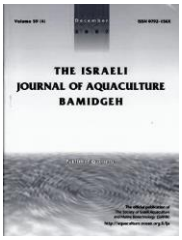

Optimum Replacement Value of Fish Silage for Protein Source in Pacific White Shrimp Diets

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Keywords: formic acid; diet attractability; *Vibrio challenge*; immunostimulant; *Litopenaeus vannamei*

Abstract

Chemical and biological evaluations were undertaken to determine nutritive value of acid fish silage (FS) as a dietary protein source in the diet of Pacific white shrimp (*Litopenaeus vannamei*). Proximate composition, and amino acid profile of FS were determined for chemical evaluation; amino acid profile of FS was employed to predict its nutritive value against shrimp requirements for essential amino acids (EAA). Three biological evaluations of FS were conducted: (1) the attractability of the FS diets were compared with diets containing no FS; (2) a growth trial of 60 days was performed to determine the effects of replacing protein sources in the diet with FS, on the growth performance of the shrimp; (3) shrimp from the growth trial in the second biological experiment were evaluated to determine whether or not FS in the diet resulted in enhanced immune response against *Vibrio parahemolyticus*. Experimental diets were prepared containing 5 levels of FS replacing the combined fish meal and *Acetes* meal (kept at a constant ratio of 1:3): 0% FS (control), 12.5%, 25%, 37.5% and 50% FS replacement. For results of the chemical evaluation, the computed EAAI of FS was 98.5 indicating a well-balanced profile. In the attractability test using a custom-made setup, results showed that shrimp (1-2 g) were significantly more attracted to the diet containing 50% FS replacement than any other diet; the control and the other experimental diets were statistically similar in attractability. In the growth trial experiment, shrimp (average weight =0.01g) were fed diets of the same composition as that used for the attractability test. Results showed that weight gain (WG) and specific growth rate (SGR) of the FS-supplemented groups were significantly higher than the control group; however, 25% FS group did not significantly differ from that of the 37.5% FS. Feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate did not differ significantly among experimental groups. In the *V. parahemolyticus* challenge test, optimum FS replacement for immune enhancement was estimated to be 24.5% FS using a broken line model. In conclusion, the findings revealed that FS could serve as a feeding stimulant at 50% FS replacement. Likewise, FS could serve as an immunostimulant against *V. parahaemolyticus* in *L. vannamei.*

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Introduction

Fishmeal is a feed ingredient produced from pelagic fish and is currently used as a protein source in various animal industries mainly in aquaculture. An estimate of 35% of the available fishmeal in 2012 was based on processed fish residue (FAO, 2014). Fishmeal processing requires large amounts of fresh raw materials and energy to process it (Naylor et al, 2009). An alternative to this costly ingredient is the immediate preservation of raw materials by means of silage technology using short-chain organic acids (Olsen and Toppe 2017) e.g. formic acid. The acid in the mixture accelerate protein hydrolysis, breakdown of bones and cartilage, and prevent the growth of spoilage bacteria (Goddard and Perret, 2005). Proteins in silage are hydrolysed by endogenous acid proteases to small peptides and free amino acids (Espe et al, 2015). Thus, silage can be considered a potential substitute for fishmeal in the feeds of fish and shrimp (Gallardo et al, 2012).

Fish silage has been used in some fish and shrimp species. Several results in fish under laboratory and practical conditions have shown that it is possible to replace fishmeal with fish silage (Haider et al, 2016; Güllü et al, 2014). The soluble protein fraction of fish silage is rich in essential amino acids and has high nutritional value for fish (Strøm and Eggum, 1981). Due to low average molecular weight, it is suitable as an easy digestible protein supplement in the feed for juvenile fish and domestic animals (Raghunath and Gopakumar, 2002). Feed attractability also plays an important role in facilitating feed ingestion and digestion and assimilation of these nutrients (Sae-alee and Tantikitti, 2008).

Shrimp aquaculture is beset with some economically problematic diseases such as the acute hepatopancreatic necrosis disease (AHPND) caused by the bacteria *Vibrio parahaemolyticus* ubiquitous in shrimp farms (Li et al, 2017). There is a possibility that fish silage, consisting of short peptides and free amino acids, not only serves as a growth promoter in penaeid shrimp such as Pacific white shrimp but also in stimulating its immune system. The specific aim of the present investigation was to determine the optimal level of fishmeal substitution with fish silage for growth and immune enhancement in shrimps challenged with *Vibrio parahaemolyticus*.

Materials and Methods

Silage production

Muscle tissues including offal of commercially obtained skipjack tuna were sliced, chopped, and mechanically homogenized to obtain a fine and homogenous slurry. Formic acid (99.6%) and butylated hydroxytoluene (BHT) were added to the mixture at 3.0% and 250 mg/L; the latter served to inhibit lipid oxidation (Haider et al., 2016). The resulting pH was reduced to below 4 and maintained at this level to avoid petrification (Oetterer, 2002), inhibit growth of bacteria and fungi, as well as elimination of fish parasites and their eggs (Norman et al, 1979). The mixture was placed in a container and incubated at an ambient temperature (~30 °C) for a period of 30 days with daily mixing and pH monitoring.

To characterize the final silage preparation, FS was oven dried at 60^{o}C and proximate and amino acid (AA) composition of FS was analyzed. Proximate composition was expressed as both dry and wet weight basis while that of the AA was percent crude protein.

Experimental diets

Five experimental diets were prepared with varying proportions of FS replacing the combined dietary sources of fishmeal and *Acetes*; the ratio of two protein sources were maintained at 1:3*,* respectively (Table 1).

The pH of the FS slurry was maintained and adjusted to pH 4 using 6N sodium hydroxide (NaOH). Diets were prepared by thoroughly mixing the dry ingredients first followed by the liquid components such as oil, FS and water with the mixture resulting in a soft dough. The mixture was steam-cooked and oven-dried at 60^{o}C to a moisture content of 10% or less. Samples of the experimental diets from each treatment was then sent for proximate analysis.

Table 1. Composition of the experimental diets (g/100g diet).

Feed Ingredient	*Treatments (% Crude protein inclusion)*				
	Diet 1 (control)	*Diet 2 (12.5)*	*Diet 3 (25)*	*Diet 4 (37.5)*	*Diet 5 (50)*
Peruvian fishmeal	18.75	16.40	14.050	11.70	9.35
Acetes	56.25	49.20	42.15	35.10	28.05
Fish silage	0.00	9.40	18.80	28.20	37.60
CMC	5.48	5.48	5.48	5.48	5.48
Vitamin Mix	1.00	1.00	1.00	1.00	1.00
Mineral Mix	1.00	1.00	1.00	1.00	1.00
BHT	0.02	0.02	0.02	0.02	0.02
Lecithin	0.50	0.50	0.50	0.50	0.50
Cod Liver Oil	4.00	4.00	4.00	4.00	4.00
Starch	13.00	13.00	13.00	13.00	13.00
TOTAL	100.00	100.00	100.00	100.00	100.00
Determined proximate composition (dry weight basis)					
Moisture	7.55	7.28	8.17	7.88	7.70
Crude protein	50.23	50.24	51.27	53.27	55.51
Crude lipid	5.37	6.77	5.80	6.45	5.80
Crude fiber	3.56	3.50	3.01	2.46	1.98
Ash	15.91	15.24	14.01	12.01	10.48

Diet attractability test

The test was done using a custom-made chamber designed by Suresh et al (2011). Each glass tank (90 x 50 x 30 cm in length, width and height, respectively) consisted of an acclimatization chamber at one end and five feeding chambers at the other end separated by a shutter glass that could be lifted to start the test. The tanks were set up in an unlit room that only received diffused fluorescent light, and all assessments were conducted at similar time of the day commencing at 09:30 h. Diet attractability was performed on the five experimental diets in three simultaneous runs. Ten randomly selected shrimp (1-2g) were acclimatized for 1 h after which 2 g of each experimental diet was placed separately in each of the 5 feeding chambers. Following the lifting of the glass shutter, the number of shrimp that made it inside each of the feeding chambers was recorded. Diet attractability was expressed as percent of shrimp that were inside a particular feeding chamber containing a particular experimental diet after 1, 5, and 10 min of the start of the test.

Growth trial

Pacific white shrimp (*Litopenaeus vannamei*) post larvae (PL21) were purchased from a commercial hatchery in Cebu, Philippines, transported to the laboratory, and acclimatized for 3 days by feeding with the control diet. The study was conducted under controlled laboratory conditions in which 450 healthy shrimp post larvae (~0.01 g) were randomly distributed into 15 tanks in a closed recirculating system containing salt water (20 parts per thousand, ppt), flow rate of 19.4 L/h, and provided with continuous aeration. The shrimp were fed the experimental diets 4 times daily for 2 weeks and then 3 times daily after the 2 weeks. On the first day of the week, shrimp were fed near satiation and total amount of feed intake served as the basis for the feeding rate for the rest of the week. This cycle was conducted repeatedly until termination of the experiment.

Water temperature and pH were monitored twice a day before feeding, in the morning and 2 h before the last feeding in the afternoon. Ammonia and nitrite concentrations were analyzed once a week using commercial test kits. During the growth trial, water temperature was maintained at an average of 31.1°C, pH level at a range of 8.35-8.36, ammonia and nitrite values at 0.12-0.13 ppm, and 0.13-0.16 ppm, respectively. Uneaten feed and feces were siphoned off twice a day once before feeding in the morning and 2 h before the last feeding in the afternoon. Water in the reservoir was changed twice a week (~40% of the total volume) during the first 21 days of culture and three times thereafter.

Bulk weighing was conducted every 15 days after shrimp had been starved for a whole day prior to feeding which was resumed the following day. Feeding rate was

adjusted after every sampling in addition to consideration of the apparent feeding to satiation on the first day of every week.

Response parameters

During the experiment, shrimp in each tank were bulk weighed to determine average final body weight (ABW). Weight gain (WG), specific growth rate (SGR), survival rate (SR), protein intake (PI), protein efficiency ratio (PER), feed conversion ratio (FCR) were computed using the following formula:

a. ABW (g) = W_t (g)/n
   where: W_t – Total weight
   n- Total number of shrimp weighed

b. $WG\,(g) = W_f\,(g) - W_i\,(g)$ WG (g) = W_f - W_i
   where: W_f – Final weight
   W_i – Initial weight

c. SGR = 100*(ln W_f (g) – ln W_i (g))/days
   where: W_f – Final weight
   W_i – Initial weight

d. SR (%) = 100*final n/initial n

e. PER = wet WG (g)/protein intake (g)
   where: WG – Weight gain

f. FCR = Total feed given (g)/(wet WG (g) + wt of dead shrimp)

Bacterial challenge

Vibrio parahaemolyticus was isolated from a shrimp culture pond at the Institute of Aquaculture, Multi-species Hatchery, University of the Philippines Visayas using the differential media, thiosulphate-citrate-bile salt agar (TCBS) and *Vibrio* chromogenic agar. Biochemical characterization of the isolate was performed according to the Bacteriological Analytical Manual (Kaysner and Depaola, 2004). Molecular confirmation was done by targeting the thermolabile hemolysin (tlh) gene of *V. parahemolyticus*. Stock cultures were maintained in nutrient broth with 1% (w/v) sodium chloride (NaCl) (NA+) and 20% (v/v) glycerol and stored at room temperature (24°C) until needed.

In preparation for the *V. parahemolyticus* challenge, a bioassay was conducted to determine the median lethal concentration (LC_{50}). Each treatment had 3 replicates (10 shrimp/tank, ABW=8.13±0.79g). Every bioassay consisted of 5 different bacterial concentrations from 0.1 to 0.5 based on optical density at 600nm. Shrimp were immersed in the bacterial suspension for 1 h and were transferred to a new culture tank. where they were kept in a 10 L polypropylene aquarium filled with UV-treated seawater (20ppt). These were were fed three times a day (0800H, 1200H, 1600H), and water temperature and salinity were maintained at 24°C and 20ppt, respectively. Water exchange was kept at minimum to promote bacterial infection. Shrimp were considered dead when observed as immobile and opaque. Mortalities were recorded twice daily (0800H and 1600H) for 14 days, and dead shrimp were removed. The median lethal concentration (LC50) was calculated using Probit analysis at 95% confidence interval.

Challenge test against *V. parahaemolyticus* was conducted for 25 days. Shrimp used in the feeding trial were collected from each tank and transferred into a static system consisting of 15 tanks of 15L capacity inside an air-conditioned room with a thermostat set at 25°C. Each tank was filled with 13 L seawater at 20 ppt salinity and continuous aeration was provided. There were three replications of the test with each tank containing 15 shrimp. The challenge test was carried out by immersing the shrimps in 3 L of water containing live *V. parahaemolyticus* at previously determined LC_{50} of 5.56×10^6 CFU/ml for 1 h. The shrimp were then transferred to clean and newly prepared containers. During the duration of the challenge test, shrimp were fed 3 times daily. Water temperature and salinity were maintained at 24°C and 20ppt, respectively. Water

exchange was kept to a minimum and uneaten feed and fecal waste were siphoned off twice a day. Average values of water quality parameters were as follows: temperature, pH, ammonia, and nitrite levels were 31.74±0.76°C, 8.3±0.08, 0.50±0.00 ppm and 0.34±0.19 ppm, respectively. Dead shrimp were removed and subjected to identification of the causative bacteria. The hepatopancreas of dead shrimp was collected, placed in a tube containing nutrient broth, and homogenized. The solution was spread on thiosulfate citrate bile sucrose (TCBS) which is a selective media for *Vibrio* spp., incubated at 35°C for 24 h to confirm the presence of *V. parahaemolyticus*.

Statistical analyses

Data on growth, feed utilization, survival rate and attractability were subjected to Levene's test for homogeneity of variances and Shapiro-Wilkins for the normality of distribution. Data that passed the test were subjected to one way Analysis of Variance (ANOVA) at P=0.05 using SPSS version 16.0. Percentage date were arc sine-transformed prior to the statistical analyses. Post hoc analyses were done using Duncan's Multiple Range Test (DMRT) to rank the means.

Results

Proximate composition and amino acid profile of FS

The proximate composition of the FS (wet basis) in the present study showed that the wet crude protein (CP) content is well below 20% (Table 2). For practical purposes, FS should be included in the formulated diet on a wet weight basis replacing water in making dough before pelletization.

The amino acid profile of the prepared FS (Table 3) showed that the total essential amino acids were composed of 28-33% of the dietary protein and based on this profile, the essential amino acid index (EAAI) was computed using the EAA requirements of a generalized penaeid shrimp (Table 4).

Table 2. Proximate composition of acid fish silage (wet and dry bases) used in the present study.

Composition (%)	*Fish Silage (Wet)**	*Fish Silage (Dry)*
Moisture	71.49	7.97
Crude protein	16.75	58.76
Crude lipid	1.21	4.24
Ash	1.53	5.36

*values as wet basis were re-estimated from the dry basis composition using the dry basis values.

Table 3. Amino acid composition (g/100g crude protein) and crude protein content of fish silage.

Amino Acid	*AA/100g crude protein*
Essential:	
Arginine	3.03
Histidine	4.88
Isoleucine	1.65
Leucine	4.87
Lysine	3.41
Phenylalanine	3.36
Methionine	2.04
Threonine	2.26
Tryptophan	0.09
Valine	1.96
Non-essential:	
Aspartic acid	5.69
Glutamic acid	5.69
Proline	1.25
Serine	2.07
Glycine	2.22
Tyrosine	2.15
Alanine	3.36
Cysteine	0.01
CP (g/100g)	49.96

Table 4. Essential amino acid index (EAAI), chemical score (CS) and A/E ratios of fish silage and EAA requirement levels for *L. vannamei.*

Specification	*Fish Silage (FS)*	*Penaeid req.*	*A/E (%)*		*(A/E FS)/(A/E req.)(%)*
	AA (%CP)		*Fish Silage*	*Penaeid shrimp*	
Arginie	3.03	5.07	11.01	15.38	71.58
Histidine	4.88	2.20	17.73	6.67	265.64
Isoleucine	1.65	2.70	6.01	8.19	73.34
Leucine	4.87	4.30	17.67	13.04	135.48
Lysine	3.41	5.20	12.37	15.77	78.44
Phenylalanine	3.36	3.70	12.18	11.22	108.57
Mehtionine	2.04	2.40	7.41	7.28	101.77
Threonine	2.26	3.50	8.22	10.62	77.39
Tryptophan	0.09	0.50	0.31	1.52	20.43
Valine	1.96	3.40	7.10	10.31	68.89
Total	27.54	32.97	-	-	-
EAAI	-	-	-	-	98.50

Diet attractability

After 10 min of raising the shutter glass that separated the acclimatization chamber from the feeding chamber, shrimp were significantly attracted to the 50% FS diet only, while the rest of the diets exhibited attractiveness values statistically similar to the control diet (Table 5).

Table 5. Attractability to shrimp of the experimental diets after 10 min.

Protein replacement (%)	*Attractability (%)*
0.0 FS	15.0±0.6^{a}
12.5 FS	15.0±0.6^{ab}
25.0 FS	18.3±0.3^{ab}
37.5 FS	20.0±0.7^{ab}
50.0 FS	31.7±0.3^{b}

All values are expressed as mean±SEM.
Means in the same column sharing the same subscript are not significantly different (P>0.05).

Growth performance of L. vannamei during the growth trial

Shrimp from all dietary treatments exhibited statistically similar ABW during the first 15 days; significant differences were observed starting at Day 30 and beyond (Figure 1).

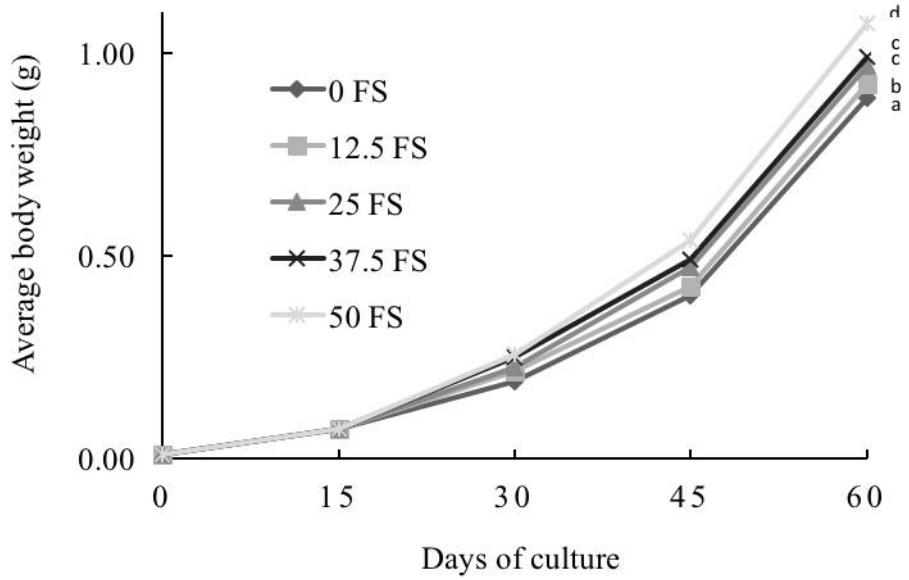

Figure 1: Cumulative average body weight of *L. vannamei* fed with the experimental diets for 60 days.

After feeding the shrimp for 60 days, survival rates were not significantly different for all the dietary treatments and were relatively high at above 80%. Final average body weight (ABW), weight gain (WG) and specific growth rate (SGR) exhibited similar trends across dietary treatments (Table 6), i.e. the control group exhibited significantly the lowest values, the 12.5 FS groups significantly higher values than those of the control group. The 25 and 37.5 FS groups were both higher than that of the mentioned two groups while the 50 FS group exhibited significantly higher values. Feed conversion ratio (FCR) and protein efficiency ratio (PER) of shrimps did not differ significantly between dietary treatments. Feed intake increased as the proportion of FS was significanly increased in the 50% FS group

Table 6. Feeding and growth parameters after the 60-day feeding trial (mean ± standard error of the mean, SEM)

FS (%)	*Parameter*						
	ABW	*WG*	*SGR*	*FCR*	*PER*	*SR*	*FI*
0.0	0.40±0.00^{a}	0.39±0.00^{a}	8.55±0.01^{a}	1.89±0.01^{a}	1.05±0.00^{a}	82.22±1.11^{a}	1.66±0.01^{a}
12.5	0.42±0.01^{b}	0.42±0.01^{b}	8.67±0.02^{b}	1.88±0.04^{a}	1.06±0.02^{a}	81.11±1.11^{a}	1.72±0.05^{ab}
25.0	0.46±0.01^{c}	0.45±0.01^{c}	8.85±0.01^{c}	1.86±0.02^{a}	1.05±0.01^{a}	80.00±0.00^{a}	1.78±0.03^{bc}
37.5	0.48±0.01^{c}	0.47±0.01^{c}	8.96±0.01^{c}	1.89±0.02^{a}	1.01± 0.01^{a}	81.11±1.11^{a}	1.85±0.01^{c}
50.0	0.57±0.01^{d}	0.56±0.01^{d}	9.34±0.02^{d}	1.83±0.02^{a}	0.98±0.01^{a}	81.11±1.11^{a}	1.95±0.02^{d}

Average body weight (ABW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) survival rate (SR) and feed intake (FI) of L. vannamei after the 60-day feeding experiment.
Means in the same column sharing the same subscript are not significantly different (P>0.05).

Vibrio parahemolyticus challenge test

Cumulative mortality curve of infected shrimp during the 25 days of V. *parahaemolyticus* challenge test is presented in Figure 2. At the termination of the challenge test, the control group exhibited the lowest survival rate among the dietary treatments. The 50% FS group consistently exhibited the highest survival rate all throughout the test; it resulted in 271% increase in survival over that of the control group (Table 7).

Optimum FS replacement rate for immune enhancement was estimated using a broken line model in which the data on cumulative survival rate was used (Figure 3). It was estimated from the model that the minimum dietary inclusion rate of FS in the diet was 24.53 % FS for the shrimp to be protected against the pathogenic *Vibrio parahemolyticus*.

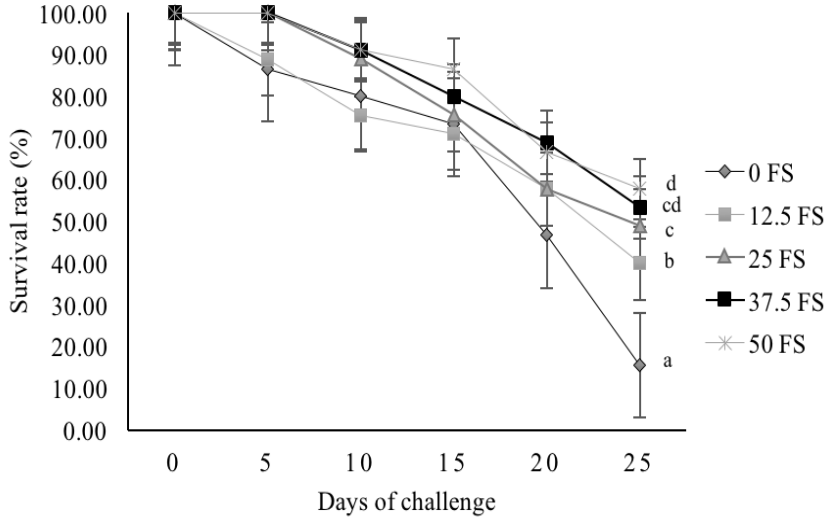

Figure 2: Cumulative mortality rate of *L. vannamei* fed the various diets and challenged with *Vibrio parahaemolyticus.*

Table 7. Survival of shrimp after 25 days of *Vibrio parahaemolyticus*) challenge.

Treatment (%)	*Survival (%)*
0.0 FS	15.6±2.2[a]
12.5 FS	40.0±0.0[b]
25.0 FS	48.9±2.2[c]
37.5 FS	53.3±3.9[cd]
50.0 FS	57.8±2.2[d]

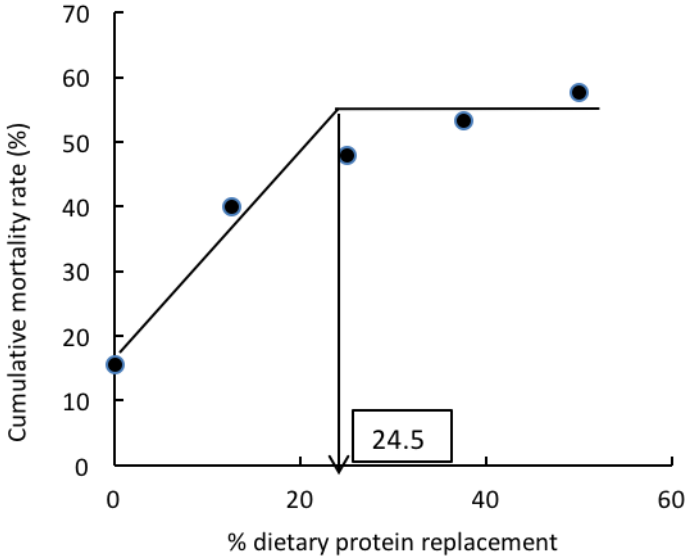

Figure 3. Broken line analysis to determine the optimum FS inclusion level to effect protection against *Vibrio parahemolyticus*.

Discussion

Normally, the quality of the total protein is evaluated by calculating the chemical score (CS) and the balance of the content of essential amino acids (EAAI) (Prado et al., 2016). The amino acid content of foods and feedstuffs is typically determined using acid hydrolysis to liberate the amino acids from the protein after which the liberated amino acids are separated and quantified. The acid hydrolysis conditions used to hydrolyze proteins are severe (typically 6 M HCl at 110 °C for 24 h in an oxygen-free environment); tryptophan is arguably the most acid labile amino acid and is largely destroyed during acid hydrolysis (Fountoulakis and Lahm 1998). Since tryptophan was remarkably low in the present study, it could be assumed that a large portion of it was lost in acid hydrolysis and a chemical score based on this was doubtful at best. The EAAI could likely represent the quality of the prepared FS and it is robust considering that it is based on geometric mean and anyone acid such as tryptophan could be disregarded in the estimation and the index remains near the precise index. The EAAI index of FS in the present study for *L. vannamei* was estimated to be 98.5 which put this ingredient as good quality protein source based on the criteria initially established by Oser (1959) for feedstuff which was later used by Peñaflorida (1989) in the penaeid shrimp *P. monodon*.

The positive effects of FS replacement by weight of the combined fishmeal and *Acetes* meal was apparent in the present study. Although nutrient utilization as reflected in the FCR and PER values were unchanged, growth performance was remarkably improved; in fact ABW, WG, and SGR did not reach their peaks and continued to improve up to 50% FS replacement. The superior growth demonstrated by shrimps in the FS groups, especially the 50% FS group, was had a superior FI with the 50% FS group exhibiting the highest FI significantly. The other FS diets and the control diet did not result in more attractive feeds and might be due to the already high baseline attractiveness of the combination of fishmeal and *Acetes* meal. Olsen and Troppe (2017) state that the mechanisms behind the positive effects of fish protein hydrolysate are still not fully

understood, but at least in diets containing a high content of plant proteins, a concentrated hydrolysate based on fish supplies free amino acids and non-amino acid nitrogen compounds that elicit feed attractant properties. In the present study, however, the basal diet used was consisted of predominantly animal proteins, and yet feed attractant properties emerged, although only between the control and the highest proportion of FS (i.e. 50%). Thus, FS concentration was an important factor for the attractability of FS diets especially if there was a high attractability baseline imparted by marine protein components in the diet (i.e. fishmeal and *Acetes* meal combined).

Since FS in the present study was produced by adding formic acid, a short-chain organic acid, comparing the growth of shrimp fed the FS diets and that of the control largely boils down to the beneficial effects of this acid. Short-chain organic acids like formic acid are among the candidates that act as growth promoters in feed for poultry and pigs (Khan and Iqbal, 2016). Perhaps the main mechanism behind this phenomenon is the antimicrobial effects in the upper part of the gastrointestinal tract of animals (Olsen and Troppe, 2017). Formic acid could also improve absorption of certain minerals like calcium and phosphorus (Partanen and Mroz, 1999). Literature is scarce on the effects of organic acids on crustaceans. Pacific white shrimp did not exhibit improvement when fed a diet containing 0.3 or 0.6% formic acid (Chuchird et al, 2015). One study demonstrated the efficacy of coconut sap vinegar in promoting growth in *P. vannamei,* and of sugar cane vinegar in enhancing immune response against *V. parahemolyticus* (Jamis et al 2018). In *P. monodon* fed a diet with or without organic acids, similar growth was observed, but the organic acid group exhibited improved feed utilization (Ng et al, 2015). In contrast, during a 4 month feeding trial, growth performance improved significantly in mollusks such as the South African abalone, fed a diet which combined 1% formic acid and 1% acetic acid (Goosen et al, 2011). In a five week feeding trial using Pacific white shrimp, fish waste silage processed with formic acid improved growth when combined with soybean meal in a practical diet (Gallardo et al, 2012).

The protein breakdown during the FS preparation could also have a positive effect on the digestibility and therefore assimilation of nutrients (Plascencia-Jatomea et al., 2002). FS protein is highly digestible compared to regular fishmeal protein since fishmeal production requires high temperature which could enhance double links in protein structure (Gallardo et al., 2012). Several results on weight gain and digestibility of warm water species including tilapia *Oreochromis aureus* (Goddard et al., 2003; Goddard and Al-Yahyai, 2001) and *Oreochromis niloticus* (Fagbenro and Jauncey, 1993), pacu, *Piriactus mesapotamicus* (Vidotti et al., 2002), and Indian carp *Cirrhinus mrigala* (Ali et al, 1994) showed protein silages to be a good substitute for fishmeal in fish diets up to 75%.

Although there is limited information related to the effects of silage diets on the growth of shrimps, results obtained in the present study indicate that silage can be used as an ingredient to reduce fishmeal content in aquafeeds.

Comparison between shrimp fed with varying proportion of FS, boils down to differences in dietary levels of free amino acids, non-amino acid nitrogen compounds and short peptides concomitant with the increase in dietary FS. Peptides in general act as immunostimulants in animals. Although no effect on growth of *Litopenaeus vannamei* was observed when fed a diet containing 0.3 or 0.6% formic acid, they all demonstrated an improved survival rate when challenged with *Vibrio parahaemolyticus* (Chuchird et al, 2015). Tiger shrimp (*Penaeus monodon*) fed a feed containing 2% of a commercial organic acid mixture exhibited lower cumulative mortality than those fed the control diet after exposure to *Vibrio harveyi* (Ng et al, 2015). In brine shrimp *Artemia franciscana*, the addition of 20 mM of short-chain organic acids to the culture water resulted in significantly increased survival of infected nauplii, showing no difference between the different organic acids.

In conclusion, diets containing FS fed to Pacific white shrimp resulted in better growth performance due to improved feed intake but did not affect feed utilization. The replacement of dietary protein sources of combined fishmeal and *Acetes* meal by 50% FS resulted in a significant improvement (43%) in growth and 271% improvement in

survival of *Vibrio*-infected postlarvae. The estimated minimum level of FS inclusion that will elicit protection against *Vibrio parahaemolyticus* was estimated to be 24.5% FS replacement. FS served as a feed attractant, immunostimulant and growth promoter in the Pacific white shrimp and thus a very good candidate as feed additive in commercial diets for white shrimp. Its inclusion in commercial diets is very feasible even if it is in a slurry form since it could also replace part of the water requirement in the palletization process.

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