The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from January 2010 The Israeli Journal of Aquaculture - Bamidgeh (IJA) has been published exclusively as an **online Open Access** scientific journal, accessible by all.
Please visit our IJA Website
http://www.aquaculturehub.org/group/israelijournalofaquaculturebamidgehija
for free publications and to enable you to submit your manuscripts.
This transformation from a subscription printed version to an online Open Access journal aims at supporting the concept that scientific peer-reviewed publications and thus the IJA publications should be made available to all for free.

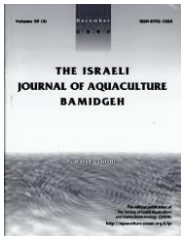
Published by the
The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)
in partnership with the
University of Hawaii at Manoa Library
and the
AquacultureHub
A non-profit organization 501c3
http://www.aquaculturehub.org

ISSN 0792 - 156X

PUBLISHER:

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

Published as an open-access journal by the Society of Israeli Aquaculture & Marine Biotechnology (SIAMB).
To read papers free of charge, please register online at http://www.aquaculturehub.org/group/israelijournalofaquaculture bamidgehija
The sale of *IJA* papers is strictly forbidden



Fermented Sweet Potato Meal, a Sustainable Dietary Protein Ingredient for Juvenile *Penaeus vannamei,* Boone 1931.

Traifalgar R F[1]*, Pagapulan J[1], Valdez M T[2], Ellamar J B[2], Ocampo E J C[2], Ilag L L. [3,4]

[1]*Institute of Aquaculture, College of Fisheries and Ocean Science, University of the Philippines Visayas, Miag-ao Iloilo Philippines*

[2]*Tarlac Agricultural University. Camiling Tarlac, Philippines.*

[3]*Plentex Philippine Inc. Tacloban, Philippines*

[4]*Xerion Limited, Brighton, Victoria, Australia*

Keywords: alternative Protein Source; *Penaeus vannamei*; vibrio; omega-6 Fatty acids; gut health

Abstract

Fermentation-biotechnology to produce high-protein agricultural biomass with potential as a feed ingredient is well-established. However, practical applicability of this technology in aquaculture has not been fully realized. The present work evaluates the nutritional and feed value of fermented sweet potato meal (ProEn-K^{TM}) to replace soybean meal in the diet of juvenile *Penaeus vannamei*. Four experimental diets containing graded levels of ProEn-K^{TM} replacing 0 (%), 25 (%), 50 (%) and 100 (%) of soybean meal were formulated and fed to *P. vannamei* for 8 weeks. Results showed that 100 (%) of soybean meal can be replaced by fermented sweet potato and 50 (%) replacement elicited growth promoting effects. Survival, feed conversion, and body composition were similar in all treatments. Dietary inclusion of fermented sweet potato promotes better ratio of the n-3/n-6 fatty acid and lowers the total gut bacteria as well as total *Vibrio*. Collectively these results suggest that fermented sweet potato meal could fully replace soybean meal in *P. vannamei* diet. The use of this feed ingredient is a practical approach to meet the increasing needs of proteins in feeds for the expansion and sustainability of *P. vannamei* aquaculture.

* Corresponding author. Tel:+63 033 3158090; email: rfmtraifalgar@upv.edu.ph

Introduction

Aquaculture of *Penaeus vannamei* in the Philippines is currently expanding due to the rising global market demands and the high profit gains of this farming system. (BFAR, 2016). The increase in production of cultured shrimp goes in tandem with the rising need for quality feed-protein ingredients, considered the most important and costly component of formulated feeds. The limited supply of feed-protein is considered the limiting factor in the sustainability and economic viability of this industry.

Significant research efforts have been directed to find alternative sources of feed protein for aquaculture since fish meal is a limited resource (Tacon et al., 2006). Use of soybean meal was found to lower the fishmeal inclusion in aquaculture feeds (Kaushik et al., 1995). However, livestock and aquaculture industries compete in the use of soybean meal which has resulted in the increase in prices and erratic supply of this ingredient. Feed prices and supply are therefore factors that dictate the sustainability and economic viability of aquaculture in the future (Tacon et al., 2006).

The application of biotechnology, specifically microbial-based solid state fermentation (SSF), has high potential in the production of cheap and sustainable feed ingredients for aquaculture. Technologies on SSF to convert agricultural biomass to a high-protein feed material are well-documented, feasible, and globally acknowledged (Apines-Amar et al. 2016, Zhang et al., 2018). In earlier reports it was shown that through SSF, the nutritional value and protein content of agricultural by-products such as copra meal was improved (Haryati et al., 2006; Dairo and Fasuyi, 2008; Hatta et al., 2014). However, information regarding the feed value and biological testing of these materials as a feed ingredient for aquatic animals is limited. In the present study we evaluated the feed value of SSF sweet potato as a replacement of soy bean meal in the diet of juvenile *P. vannamei*, a shrimp commonly cultured in industrial scale in the archipelagic countries of Southeast Asia and the Pacific region.

Materials and Methods

Diet Formulation

Fermented sweet potato meal (ProEn-K^{TM}) was produced and obtained from Agricultural Biomass Fermentation Laboratory of the Tarlac Agricultural University, Philippines. This ingredient was produced by solid state fermentation (SSF) of sweet potato with mixed consortium of microbes and fungi following a process described by Hatta et al, (2014) and Haryati et al., (2006). All other ingredients were purchased from the South East Asian Fisheries Development Center, Aquaculture Department (SEAFDEC-AQD) Feed mill Laboratory Tigbauan, Iloilo Philippines.

Four experimental diets were formulated containing increasing dietary inclusion levels of ProEn-K^{TM} to replace soybean meal by weight at 0% (TC), 25% (T25), 50% (T50), and 100% (T100) respectively (Table 1). Prior to diet formulation all the dry ingredients were sieved through a 100 µm mesh to standardize the ingredient particle size. These dry feed ingredients including the ProEn-K^{TM}, vitamins, and mineral mix were weighed and thoroughly mixed in a mechanical food mixer (Hobart, USA). The wet ingredients including lecithin, fish oil including oil soluble vitamins, were prepared and gradually added and mixed with the dry ingredients. An adequate amount of water was then added to the compounded dry ingredients to form a moist dough. The resulting dough was pelleted by cold extrusion using a laboratory pelletizer (Hobart, USA). The pellets were collected, oven-dried at 60^{0}C, cut to appropriate size, and stored at 8^{0}C until use. Composition and nutrient contents of the experimental diets are presented in Table 1.

Table 1. Composition and Biochemical analyses of the experimental diets.

Ingredients	*Soybean meal Replacement Level*			
	TC (0 %)	T25 (25%)	T50 (50%)	T100 (100%)
Fish Meal	15.00	15.00	15.00	15.00
Plankton meal (mysids)	5.00	5.00	5.00	5.00
Soybean meal (defatted)	45.00	33.75	22.50	0.00
ProEn-K	0.00	11.25	22.50	45.00
Cod liver oil	2.00	2.00	2.00	2.00
Soybean oil	1.00	1.00	1.00	1.00
Lecithin	1.00	1.00	1.00	1.00
Wheat Flour	25.00	25.00	25.00	25.00
Vitamin mix [a]	1.00	1.00	1.00	1.00
Mineral mix [b]	2.00	2.00	2.00	2.00
Gluten (Binder)	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00
Proximate Composition (g/100g diet, Dry Weight)				
Crude Protein	40.18	38.81	38.68	38.87
Crude lipid	9.18	11.01	9.85	11.04
Crude fiber	5.14	5.34	5.62	5.57
Ash	6.96	6.98	6.97	7.01
NFE	38.54	37.86	38.88	37.51
Total	100.00	100.00	100.00	100.00

[a] Vitamin premix (mg.kg^{-1} of diet): B-carotene, 36; cholecalciferol, 3; thiamin, 72 ; riboflavin, 144; pyridoxine, 132 ; cyanocobalamin, 0.4 ; alpha-tocopherol, 330;menadione, 48 ; niacin, 288 ; pantothenic acid,80; biotin, 0.4 ; folic acid, 24 ;inositol, 600; stay C, 2000.
[b]Mineral premix (mg.kg^{-1} of diet):Mg, 300; Fe, 30; Zn, 84; Cu, 42; K, 1500; Co, 22; Mn, 32; Se, 0.02; Mo,0.01; Al, 0.5; I, 8.

Feeding Trial and Growth Evaluation

P. vannamei juveniles were obtained from a private shrimp hatchery at Car-car City, Philippines. The experimental animals were stocked in holding tanks (5-ton capacity), fed with commercial shrimp pellets and acclimated to laboratory conditions for 2 weeks. Prior to the experiment, random samples of shrimp were collected and sent to Fish Health Department of SEAFDEC-AQD to check for the presence of shrimp pathogens and to ensure that the experimental animals were in prime condition. Molecular analysis (PCR) and examination indicate that the shrimp proved negative for white spot syndrome virus (WSSV) and other *Vibrio* pathogens.

Three hundred and sixty shrimps weighing 3.48 ± 0.17 g were randomly assigned to twelve 75L capacity polyethylene aquaria (12 shrimp/aquaria), equipped with individual aeration in a closed recirculating system. The treatment groups were arranged following a Complete Randomized Design. Each experimental diet was allocated to each treatment group, applied at a feeding rate of 3% body weight. Feed was given daily at 08:00, 11:00, 14:00, and 17:00 h for 8 weeks. Water parameters were ensured to be optimum for requirements of the shrimp throughout the experimental period. Water temperature, salinity, dissolved oxygen and pH was monitored daily at 8:00 and 16:00 h.

Sampling for growth and adjustment of feed allocation were carried out every 15 days. During sampling, shrimp in a replicate tank were collected and bulked weighed. Complete change of the recirculating reservoir water and total cleanup of the tanks to prevent algal and bacterial biofilm growth were also conducted. At the end of the feeding trial, shrimp were collected, weighed, and counted. Overall growth performance in response to the dietary treatments was assessed in terms of biological response indices calculated as follows:

Specific Growth Rate (SGR) = $\frac{ln\,(final\ body\ weight) - ln\,(initial\ body\ weight)}{number\ of\ days} \times 100$

Feed Conversion Ratio (FCR) = $\frac{total\ feed\ intake\ (g)}{weight\ gain\ (g)}$

Protein Efficiency Ratio (PER) = $\frac{weight\ gain\ (g)}{protein\ intake\ (g)}$

Percent Weight Gain (WG %) = $\frac{final\ weight(g) - Initial\ weight(g)}{Initial\ weight\ (g)} \times 100$

Percent Survival (S %) = $\frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \times 100$

Protein Efficiency Ratio (PER) = $\frac{weight\ gain\ (g)}{total\ protein\ intake\ (g)} \times 100$

Protein Retention (PR) = $\frac{final\ body\ protein\ (g) - initial\ body\ protein\ (g)}{total\ protein\ intake\ (g)} \times 100$

Lipid Retention (LR) = $\frac{final\ body\ lipid\ (g) - initial\ body\ lipid\ (g)}{total\ lipid\ intake\ (g)} \times 100$

Biochemical Analyses

All analyses per sample were conducted in triplicate. Proximate composition analyses of the diets and carcass were conducted following the established methods of AOAC (1986). Crude protein was quantified by Kjeldahl total protein Nitrogen analysis (Foss Tecator™ Digestion and Foss Kjeltec ™ 8200 Auto Distillation). Total lipid was quantified by Soxhlet extraction with petroleum ether as solvent (Foss Soxtec ™ 2050 Automatic System) while total fiber was analyzed using Foss Fibertec ™ 2010 System employing the Ceramic Fiber Filter Method for crude fiber quantification. Moisture was analyzed using the infrared drying method (Mettler Toledo® Halogen Moisture Analyzer). Ash was quantified by furnace combustion method at 600°C (AOAC, 1996).

Total Amino acid profiling of ProEn-K™ was conducted using Prominence High Performance Liquid Chromatography Amino Acid Analysis System (Shimadzu, Japan), following the method detailed in the AOAC Official Method 994.12, Amino acids in feeds (Llames & Fontaine, 1994). Fatty acid profiling was only done in the control and in the treatment group exhibiting optimal growth responses in relation to the experimental treatment. Total fatty acid profiling of the experimental animals fed with the experimental dietary ingredient was performed using the Gas Chromatography/Mass Spectroscopy (GCMS) (Perkin Elmer Clarus 600) following the method described by Michael et al., (2006). Individual fatty acids were identified based on their retention times and equivalent chain length.

Antibacterial activity of fermented sweet potato extracts was conducted following the antibacterial disc assay described by Annie et al., (2009). The extract was prepared by soaking the dried fermented biomass with ethyl acetate for 24h and insoluble materials

removed through filtration. The collected solute was evaporated in a rotary evaporator. The residue was collected, dried, weighed, and dissolved in a similar solvent to prepare a 100 µg/ml solution. A 10-mm sterilized paper disc was prepared, added with 50 µl of the extract solution and dried at room temperature to remove the solvent. The control disc was prepared using only the solvent with no extract. The discs were then laid on the spread-plate culture of *Vibrio harveyi* (10^7 CFU/ml) in Luria-Bertani media containing 2% NaCl. Following the 24h incubation, diameters of the clear halo zones around the discs were measured as bactericidal zone of inhibition.

Similar to the fatty acid analysis, gut Vibrio and total bacterial loads were only quantified in the control and in the treatment group exhibiting optimal growth responses. To quantify the shrimp total gut *Vibrios* in response the test diet, the stomachs of shrimp were dissected aseptically collected and weighed. Sterile saline solution (1.5 % NaCl in distilled water) was added to the collected tissues which were then homogenized with a sterile tissue homogenizer. Ten-fold serial dilutions were prepared from the tissue homogenate and 100 µl aliquots were plated to the bacterial media, incubated at room temperature for 18-24 h and growing colonies were counted. Thiosulfate Citrate Bile Salt (TCBS) media was used to specifically quantify *Vibrio* colonies both the sucrose fermenters (yellow colonies) and the non-sucrose fermenters (green colonies). Total bacteria were counted using the general media Nutrient Agar (NA, Merck, Germany) containing 1.5% NaCl (Barcenal et al., 2015).

Statistical Analysis.

If applicable, data obtained were subjected to one-way analysis of variance (ANOVA). Significant differences observed among the treatment groups were resolved using Tukey's post hoc test. T-test was used to resolve the differences in comparing two treatment groups. Probability values in all test is set at a significance level of 0.05. Statistical analysis was carried out using the SPSS statistical package for windows version 18.

Results

Following the 8-week feeding trial, survival values among treatments were high and were not influenced by the dietary levels of fermented sweet potato meal. Significant improvement in weight gain in comparison to the control and the other treatment groups was exhibited in the T50 group. Weight gain of the other treatments, T25 and T100 were similar to the control group. Specific growth rate was also highest in the T50 group while TC, T25 and T100 groups exhibited similar values but were lower than those in T50. No significant treatment effects were observed in other biological growth indices including FCR, PER, and Nutrient Retentions (Table 2). Correspondingly, no treatment effects and significant changes were observed in terms of shrimp tissue biochemical composition even at the highest soybean meal replacement level (Table 3).

Table 2. Growth performance and Nutrient Utilization indices of *P. vannamei* fed experimental diets

Growth Indices	Soybean meal Replacement Levels			
	TC	T25	T50	T100
S (%)	80.00 ± 4.44	75.56 ± 3.22	91.7 ± 1.01	90.20 ± 2.01
WG (%)	332.00 ± 13.00^{a}	368.00 ± 2.30^{a}	446.00 ± 11.00^{b}	349.00 ± 3.00^{a}
SGR	2.44 ± 0.05^{a}	2.57 ± 0.03^{b}	2.85 ± 0.03^{c}	2.50 ± 0.01^{ab}
FCR	1.44 ± 0.02	1.47 ± 0.05	1.52 ± 0.02	1.40 ± 0.02
PER	1.86 ± 0.05	1.83 ± 0.07	1.78 ± 0.05	1.86 ± 0.03
PR	17.41 ± 1.37	22.17 ± 2.16	18.08 ± 2.89	16.67 ± 1.12
LR	9.99 ± 0.84	11.22 ± 1.03	9.77 ± 0.62	9.99 ± 0.84

***Where S(%) is the percent survival, WG(%) is the percent weight gain, SGR is the specific growth rate, FCR is the feed conversion ratio, PER is the protein efficiency ratio, PR is the protein retention and LR is the lipid retention*.**

Table 3. Whole body proximate compositions of *P. vannamei* after the 8-week feeding trial and the antibacterial activity of ProEn-K ethyl acetate extract with *V. harveyi* as test bacteria.

	Treatment Groups			
	TC	*T25*	*T50*	*T100*
Biochemical Composition (% Dry Weight)				
Total Protein	77.15±1.67	77.28±4.05	78.23±1.12	74.80 ±2.38
Total Lipid	11.22±1.71	10.51±2.23	9.98±1.52	10.91±1.11
Ash	10.72±0.08	10.17±0.18	8.81±0.38	9.81±0.21
ProEn-K Antibacterial Activity				
[a]Bacterial Zone of Inhibition (mm)				
[b]ProEn-K extract	23.00±1.2*			
Control (solvent only)	0.00			

[a] *Vibrio harveyi* (10^7CFU/ml) was used as the test bacteria.

[b] Ethyl acetate was used as the extraction solvent; Extract applied at a dose of 100µg/ml.

***Indicates significant statistical difference at α=0.05.**

Analysis of the nutritional composition showed that the fermented sweet potato meal had protein content of 40%, lipid content of 0.4%, ash content of 9%, fiber content of 4.2 %, and carbohydrate content of 46.4%. This ingredient also had complete content of essential amino acids. In comparison with the essential amino acid content of *P. vananmei* tissue protein, each amino acid comprising the fermented sweet potato protein, except for Lysine, exhibited greater than 90 chemical score value (Table 4).

Table 4. Essential amino acid profile *of P. vannam*ei muscle proteins, ProEn-K proteins and the essential amino acid chemical score index of this ingredient.

Essential Amino Acids	*Fermented Sweet Potato Essential Amino acid[1] (% protein)*	*P. vannamei Essential Amino acid[2] (% protein)*	*Fermented Sweet Potato Essential Amino acid Chemical Score[3]*
Phenylalanine & Tryptophan	5.18	5.39	96.10
Valine	4.74	3.30	143.63
Threonine	2.63	2.52	104.36
Isoleucine	2.72	2.65	102.64
Methionine & Cystine	5.78	2.67	216.47
Histidine	3.6	1.62	222.22
Arginine	5.79	6.10	94.91
Leucine	5.18	4.69	110.44
Lysine	1.23	4.84	25.41
[4]Fermented Sweet Potato Chemical Score Index	25.41		

[1]Fermented Sweet Potato Essential Amino acid (% protein): Actual analyzed values.
[2]*P. vannamei* Essential Amino acid (% protein): from Forster et al., 2002.
[^3]Essential Amino Acid Chemical Score= {(Essential amino acid amount (g) in 100 g *PECM™* protein) /(Essential amino acid amount (g) in 100 g shrimp protein)} ×100.
[4]CSI (Protein Chemical Score Index) = It is the chemical score value of an amino acid exhibiting the lowest essential amino acid chemical score.

The overall chemical score of this ingredient is 25 with Lysine as the most limiting amino acid. Fatty acid composition of the experimental animals maintained with T50 exhibited a similar profile than in the control group. However in terms of n-3/n-6 fatty acid ratio the T50 group had higher values than that of the control group (Table 5).

Table 5. Tissue fatty acid profile of the shrimp fed the control diet and those fed diets containing 50% ProEn-K as a replacement of soy bean meal for 8 weeks.

	T0 (Control) (FAME)	*T50 (FAME)*
Fatty Acid Methyl Esters (FAME)	g/100g FAME	
Decenoic acid	0.29±0.03	0.00±0.00
Dodecanoic acid	0.10±0.00	0.07±0.00
Tetradecanoic acid,	0.53±0.03	0.72±0.06
Pentadecanoic acid	0.33±0.02	0.24±0.01
Hexadecenoic acid	1.49±0.17	1.55±0.32
Hexadecanoic acid	12.00±0.41	18.57±4.35
Heptadecanoic acid	1.90±0.29	0.90±0.09
Octadecadienoic acid (linoleic, N-6)	10.80±0.20	4.97±0.02
Octadecenoic acid	25.93±2.50	20.52±4.64
Octadecanoic acid	8.01±0.12	9.50±2.45
Eicosatetraenoic acid, (arachidonic, N-6)	2.71±0.04	1.66±0.04
Eicosapentaenoic acid (EPA, N-3)	12.20±0.41	11.47±0.64
Eicosadienoic	2.73±0.42	1.00±0.12
Eicosenoic acid,	3.50±0.12	3.53±0.08
Eicosanoic acid	0.51±0.02	0.25±0.00
Docosahexaenoic acid (DHA, N-3)	15.21±1.47	10.74±2.03
Docosenoic	1.37±0.08	14.14±1.04
Docosanoic	0.40±0.02	0.19±0.02
N-3 / N-6 ratio	2.02	3.35

Assessment of the antibacterial activity of the fermented sweet potato extracted with ethyl acetate showed that the extract at 100µg/ml exhibited an inhibition diameter zone of 23.00±1.2 mm with *Vibrio harveyi* as the test bacteria. No inhibition zone is observable in the control group (Table 3). Also, a 10-fold reduction in gut Vibrio was exhibited in the test group as compared to the control (Figure 1). Accordingly, the level of gut bacteria was found significantly lower in those receiving the test diet as compared to the control (Figure 2).

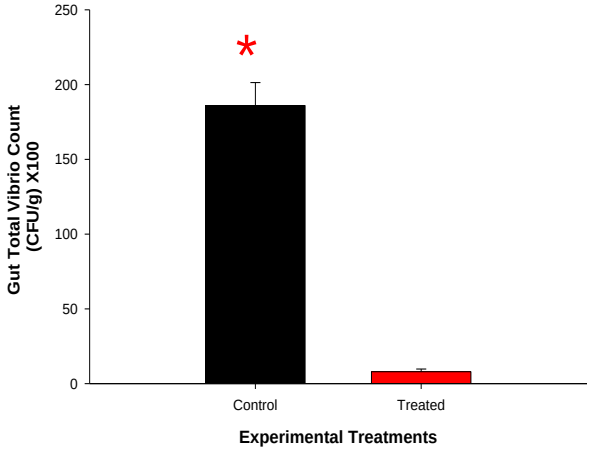


Figure 1. Total gut Vibrio load of the shrimp, *P. vannamei* fed with diets containing fermented sweet potato and the control. Values are mean ± standard error. Mean values with a star superscript are significantly different, T test, α=0.05.

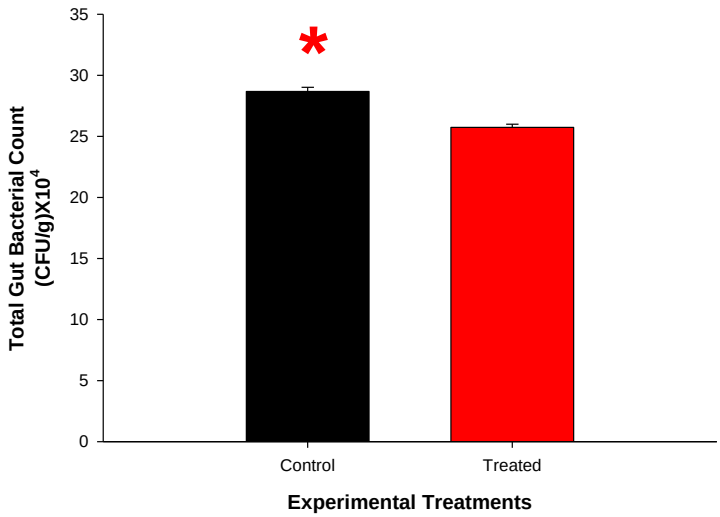


Figure 2. Total gut bacterial load of the shrimp, *P. vannamei* fed with diets containing fermented sweet potato and the control. Values are mean ± standard error. Mean values with a star superscript are significantly different, T test, α=0.05.

Discussion

Solid state fermentation aimed to improve the nutritional value of cellulosic and carbohydrate-rich agricultural biomass is considered a sustainable approach to meet the growing demands of feed ingredients for both the aquatic and the terrestrial animal-growing industries (Iyayi & Aderolu, 2004, Yousef & Alam, 2013, Ferreira et al., 2016). The enhancement of protein content of sweet potato to about 40% though SSF in the present study concurs with these earlier findings. Composition of the fermented material protein indicates complete and well-balanced essential amino acid content. All the essential amino acid exhibits a Chemical Score higher than 90 except for Lysine which scored lowest and is considered to be the most limiting essential amino acid. Similar to our results, improvement of the protein content and essential amino acid profile was also documented in wheat, soybean, and rice bran fermented with *Bacillus coagulans* and *Aspergillus niger* (Joseph et al., 2008). The amino acid profile of the fermented material is dictated by the microbial species and the biomass type used as substrate in the fermentation (Denardi-Souza et al., 2018). The low content of lysine in the present study could be attributed to the sweet potato used as substrate and the microbial species used in fermentation.

The feeding trial results confirm the viability of the fermented ingredient to completely replace soybean meal in the diet of juvenile *P. vannamei*. Growth response in treatments with complete replacement of soybean meal was found to be similar to that of the control. Moreover, significant growth enhancement was observed in the treatment receiving 50% soybean meal replacement. Earlier studies also indicate significant growth enhancement in broiler chickens fed diets containing yeast fermented products (Sulhattin et al., 2017), with fermented soybeans (Chah et al., 1975) and with fermented cereals (Sulhattin, 2015). In *P. vannamei*, feeding with fermented guar meal at 2.5% fish meal replacement was also reported to significantly enhance growth (Jannathulla et al., 2016). Growth promotion associated with fermented ingredients was attributed to the presence of small peptides in the fermented products, degradation of anti-nutritional factors and enhanced nutrient digestion due to the presence of residual microbial enzymes (Chah et al., 1975, Jannathulla et al., 2016, Sulhattin et al., 2017). Though not measured in the present study, these aspects may explain the growth enhancement effects of fermented sweet potato meal in *P. vannamei* as observed in this study.

The observed growth enhancement in the present study could also be attributed to improved gut health. Lower counts of gut associated bacteria and Vibrios were observed in the treated group (T50) as compared to the control group (TC). Furthermore, the ethyl acetate extract of the fermented material exhibits a potent antibacterial activity,

supporting the observed effects in lowering the gut microbial load of the treated shrimp groups. Similar to our findings, bacterial inhibitory activity of fermented feeds on gut microflora is well-documented in terrestrial animals including pigs (van Winsen et al, 2001) and broiler chickens (Missotten et al, 2013). Our present findings on the influence of fermented feeds on gut microflora are unprecedented in aquatic animals specifically in cultured shrimp.

Depressed growth in cultured shrimp is commonly attributed to the dominance of Vibrios in gut microflora. Gut infection of Vibrios in shrimp impairs digestive and absorptive processes and occasionally results to slow growth, infection, and eventually death (Kewadugama et al. 2017, Lavilla-Pitogo et al.1998). The decrease in the gut bacterial load may promote better nutrient absorption and assimilation, resulting to overall growth improvement as observed in the present study.

No negative influence of the fermented ingredient on the tissue chemical composition of the shrimp was observed in the present study even at the highest inclusion level. However significant alterations in terms of N-3 and N-6 fatty acids were observed in groups receiving the fermented ingredient. The treated group exhibited a better profile of the N-3/N-6 ratio compared to the control group, indicating lower N-6 fatty acid tissue accumulation. To date, the significant decline in tissue N-6 fatty acids in animals as influenced by the fermented dietary ingredient has not been not previously documented in any other animal species and our work is the first report regarding this aspect. In vertebrates the heightened biosynthesis of N-6 fatty acids specifically arachidonic acid is known to be triggered by inflammatory responses due to infection (Eberhard et al, 2002). Similarly, in insects (Stanley-Samuelson et al, 1991**)** and in crustaceans (Heckmann et al, 2008) arachidonic acid (N-6) is also utilized as a precursor in the synthesis of eicosanoids an important immunity signaling molecule that plays a vital role during infections. In relation to the present findings, it is tempting to speculate that the lower N-6 fatty acid content of the treated group could be due to the decreased gut Vibrio content that reduces inflammatory responses leading to minimal synthesis and tissue accumulation of N-6 fatty acids. However, the mechanism on how the fermented ingredient influences the shrimp tissue fatty acid composition is not fully understood and this aspect requires additional thorough investigation.

Collectively our findings indicate that fermented sweet potato meal could completely substitute soybean meal and elicits a growth promoting effect if utilized as 50% substitution of soybean meal in the diet *P. vannamei*. Use of this fermented ingredient also lowers the gut Vibrio contents and improves the N-3/N-6 tissue fatty acid profile of the cultured shrimp. Utilization of this feed ingredient is a practical approach to improve the quality of farmed shrimp, lessen the risk of Vibriosis and promote the sustainability of available feed-protein supply for the shrimp culture industry.

Acknowledgements

UPV Foundation Inc., USAID Philippines, Plentex Philippines Inc., Tarlac Agricultural University, and UPV office for the Chancellor for Research and Extension are acknowledged for the Funding grant, for the technical help, use of facilities, provision of manpower and for all the supports that made this project possible.

References

Annie SS., Lipton A. and R. Paul Raj, 2009**.** Antibacterial activity of marine sponge extracts towards fish pathogenic bacteria. *Isr. J. Aquacult.-Bamidgeh, AquacultureHub,*60:170-174.

Apines-Amar M.J.S.*, Andrino-Felarca K.G.S., Cadiz R.E., Corre, Jr. V.L., Adelaida T. Calpe, 2016. Effects of Partial Replacement of Fish Meal by Fermented Copra Meal on the Growth and Feed Efficiency in Black Tiger Shrimp, 6 pages. *Isr. J. Aquacult.-Bamidgeh, AquacultureHub,* [IJA_68.2016.1244]

Barcenal AR., Traifalgar RFM. and VL. Corre Jr, 2015. Anti-*Vibrio harveyi* Property of *Micrococcus luteus* Isolated from Rearing Water under Biofloc Technology Culture System. *Curr Res Bacteriol.,* 8: 26-33.

BFAR, 2016. Philippine Fisheries Profile 2015. Bureau of Fisheries and Aquatic Resources, Diliman, Quezon City. pp 70.

Chah CC., Carlson CW., Semeniuk G., Palmer IS. and CW. Hesseltine, 1975**.** Growth promoting effects of fermented soybeans for broilers. *Poult. Sci*., 54:600–609.

Dairo, FAS. and AO. Fasuyi, 2008. Evaluation of fermented palm kernel meal and fermented copra meal proteins as substitute for soybean meal protein in laying hens diets. *J Central European Agricult.*, 9: 35-44.

Denardi-Souza T., Massarolo KC., Tralamazza SM. and E. Badiale-Furlong, 2018. Monitoring of fungal biomass changed by *Rhizopus oryzae* in relation to amino acid and essential fatty acids profile in soybean meal, wheat and rice. *CyTA - J Food*, 16:156-164.

Eberhard J., Jepsen S., Pohl L., Karl Albers H. and A. Yahya, 2002. Bacterial Challenge Stimulates Formation of Arachidonic Acid Metabolites by Human Keratinocytes and Neutrophils In Vitro. *Clin diagn lab Immunol.* 9:132-137.

Ferreira, J.A., Mahboubi A., Lennartsson P.R. and MJ. Taherzadeh, 2016. Waste biorefineries using filamentous ascomycetes fungi: Present status and future prospects. *Bioresour. Technol.,* 215:334–345.

Forster IP., Dominy W. and AGJ. Tacon, 2002. The use of concentrates and other soy products in shrimp feeds. In: Cruz-Suárez, L. E., Ricque-Marie, D., Tapia-Salazar, M., Gaxiola-Cortés, M. G., Simoes, N. (Eds.). *Avances en Nutrición Acuícola VI. Memoriasdel VI Simposium Internacional de Nutrición Acuícola.* 3 al 6 de Septiembre del 2002. Cancún, Quintana Roo, México.

Haryati T., Togatorop MH., Sinurat, A.P., Purwadaria T. and I. Murtiyen, 2006. Utilization off fermented copra meal with *A. niger* in broiler diet. *Indonesian J Anim Vet Sci* 11(3): 182-190.

Hatta U. and B. Sundu, 2009. Effects of copra meal fermented by Aspergillus niger and Trichoderma spp on performance of broiler. Paper presented at the The 1st International Seminar on Animal Industry Bogor, Nov 23-24, 2009, Bogor Indonesia.

Heckmann LH., Sibly R., Timmermans M., and A. Callaghan, 2008. Outlining eicosanoid biosynthesis in the crustacean Daphnia. *Frontiers in zoology*. 5. 11. 10.1186/1742-9994-5-11**.**

Iyayi EA. and ZA. Aderolu, 2004. Enhancement of the feeding value of some agro-industrial y-products for laying hens after their solid state fermentation with *Trichoderma viride*. *African J Biotechnol*, 3:182-185.

Joseph R., Paul R, and D. Bhatnagar, 2008. Effect of solid state fermentation on nutrient composition of selected feed Ingredients. *Indian J. Fish*., 55: 327-332.

Jannathulla R., Syama J., Ambasankar K., and M. Muralidhar, 2016. Evaluation of fungal fermented guar meal in the diet of *Penaeus vannamei.* In: Kaliwal, B. B., Pandey, B. N. and Kulkarni, G. K. (eds.), *Role of animal science in national development, Nanotechnology in aquaculture*. Today and Tomorrow's Printers and Publishers, New Delhi, India. p. 51-62.

Kaushik, SJ., Cravedi JP., Lall SP., Sumpter J., Fauconneau B. and M. Laroche. 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, Oncorhynchus mykiss. *Aquaculture* 133, 257– 27.

Kewadugama R., Prasanna R., Kumara S., and H. Mangalika. 2017. White faeces syndrome caused by Vibrio alginolyticus and Vibrio fluvialis in shrimp, Penaeus monodon (Fabricius 1798) -multimodal strategy to control the syndrome in Sri Lankan grow-out ponds. *Asian Fish Sci* 30:245–261.

Lavilla-Pitogo C., Leaño E. and M. Paner. 1998. Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent vibrios in the rearing environment. *Aquaculture* 164: 337–349.

Michael FR., Koshio S., Teshima S., Ishikawa M. and O. Uyan, 2006. Effect of choline and methionine as methyl group donors on juvenile Kuruma shrimp, *Marsupenaeus japonicus* Bate. *Aquaculture* 258: 521–528.

Missotten JA., Michiels J., Dierick N., Ovyn A., Akbarian A. and S. De Smet, 2013. Effect of fermented moist feed on performance, gut bacteria and gut histo-morphology in broilers. *British Poultry Sci*, 54:5, 627-634.

Stanley-Samuelson DW., Jensen E., Nickerson KW., Tiebel K, Ogg CL. and RW. Howard, 1991. Insect immune response to bacterial infection is mediated by eicosanoids. Proceedings of the National Academy of Sciences. 88:1064–1068.

Sulhattin Y., 2015. Solid-State fermented cereals promise a growth promoting effect in broiler chicken. 20th European Symposium on Poultry Nutrition, 24–27 August 2015, Prague, Czech Republic.

Sulhattin Y. and KY. Mustafa, 2017. Yeast fermented additive enhances broiler growth. *R. Bras. Zootec,* 46:814-820.

Tacon AGJ., Hasan MR. and RP. Subasinghe, 2006. Use of fishery resources as feed inputs for aquaculture development: trends and policy implications. *FAO Fisheries circular no*. 1018.99. Rome, Italy: FAO.

van Winsen R. L., Urling BAP., Lipman LJA., Snijders JMA., Keuzenkamp D., Verhejden JHM. and F. van Knapen, 2001. Effect of fermented feed on the microbial population of the gastrointestinal tracts of pigs. *Appl. Environ. Microbiol*. 67:3071–3076.

Yousef S., Al-Hafedh I. and A. Alam, 2013**.** Replacement of Fishmeal by Single Cell Protein Derived from Yeast Growth on Date (Phoenix dactylifera) Industry Waste in the Diet of Nile Tilapia (*Oreochromis niloticus*) Fingerlings. *J Appl Aquacult*, 25:346-358.

Zhang B., Li C., Wang X., Pi X., Wang Y., Wang X., Zhou H., Mai K., He G.*, 2018. Apparent Digestibilities of Selected Feed Ingredients Fermented by Host Bacteria in Juvenile Turbot (*Scophthalmus maxima* L.), 12 pages. *Isr. J. Aquacult.-Bamidgeh, AquacultureHub,* [IJA_70.2018.1501]