Kuroshio Science 13-1, 9-17, 2019

Research Paper

Evaluation of naturally occurring foods in aquaculture ponds as protein source and for partial replacement of pellets for white shrimp *Litopenaeus vannamei*

Pensri Muangyao[1]*, Kimio Fukami[2], Youngyut Predalumpaburt[1], and Putth Songsangjinda[3]

[1] Coastal Aquaculture Research and Development Regional Center 6 (Songkhla), Department of Fisheries, 1/19 Moo3 Kaosean Road, Kaorubchang, Muang, Songkhla 90000 Thailand

[2] Kochi University, Monobe, Nankoku, Kochi 783-8502, NIPPON Japan

[3] Senior Expert in Marine Shrimp Culture, Department of Fisheries, Chatuchak, Bangkok 10900 Thailand

Abstract

Growth and protein nutrition of juvenile white shrimp (*Litopenaeus vannamei*) were investigated by feeding naturally occurring larvae of chironomids and mosquitoes, and the green alga gutweed *Ulva intestinalis*. Shrimps with body weight 0.12 ± 0.03 g and body length 2.7 - 2.8 cm were fed for 3 weeks with one of these food materials, and compared with shrimps fed on the usual pellets or given no food, as controls. Net protein utilization by shrimps fed on chironomid larvae (36.6 ± 3.5%) was similar to that of shrimps fed on pellets (38.6 ± 2.1%), and higher ($P < 0.05$) than that of shrimps fed on mosquito larvae (18.6 ± 2.1%) or gutweed (20.4 ± 2.0%). Food conversion efficiency was highest for shrimps fed on chironomid larvae (147 ± 14.0%), followed by pellets (92.5 ± 5.02%); mosquito larvae and gutweed gave lower values ($P < 0.05$). Average growth rate (AGR) for shrimps fed chiromomid larvae or pellets was similar (21.1 ± 4.7%, and 19.5 ± 2.2%, respectively), and significantly higher ($P < 0.05$) than for shrimps fed mosquito larvae (8.4 ± 3.0%), gutweed (8.0 ± 0.8%) or no food (0.15 ± 0.5%). Feeding a combination of pellets and insect larvae showed higher ($P < 0.05$) AGR and protein efficiency ratio. As much as 75% of costly commercial pellets can be replaced by insect larvae without any negative effect on growth and survival of juvenile white shrimp in aquaculture ponds.

Key words: *Litopenaeus vannamei*, chironomid larvae, insect larvae, protein nutrition, replacement

INTRODUCTION

Shrimp aquaculture is a very important sector for world shrimp production. Since 1991, more than 50% of shrimp production has come from aquaculture, but production costs have been increasing and the industry tries to find ways to reduce these costs. Development of alternative protein sources may be one possibility to solve this problem. Many studies have reported the application of alternative protein sources from plants, poultry by-products, etc. (Amaya et al. 2007a,b, Suárez et al. 2009, Olmos et al. 2011).

The potential of naturally-occurring materials as additional food for shrimp aquaculture has been studied (Martinez-Cordova et al. 1998, Tacon et al. 2002, 2005, Martinez-Cordova et al. 2003, 2005,Gamboa-Delgado, 2014). Insect larvae such as chironimid and mosquito larvae are important as a natural source of food for aquatic animals (Habib et al. 2005, Habashy 2005). Interest in the importance of insect larvae as a protein source has recently increased more and more (Panini et al. 2017a, b, Henry et al. 2018, Iaconisi et al. 2018, Sankian et al. 2018). Some studies have shown the potential of insect larvae as a feed ingredient for fish (Lock et

Received 23 January 2019; Accepted 29 May 2019.

*Correspondence. E -mail: muangyaopensri@gmail.com

al. 2016, Belghit et al. 2018, Concetta Elia et al. 2018, Vargas-Abúndez et al. 2019) or shrimp (Panini et al. 2017a,b). The nutritional properties of insect larvae, however, depend on the species of insect (Alegbeleye et al. 2012, Barroso et al. 2014, Sánchez-Muros et al. 2014, Henry et al. 2015).

Martinez-Cordova et al. (1998) reported that supplementation with natural food seemed to be a better feeding strategy for white shrimp farmed in culture ponds and gave the lowest (i.e. best) FCR (food conversion ratio). It was already shown that naturally-occurring larvae of chironomids and mosquitoes, and the green alga gutweed (*Ulva intestinalis* Linnaeus) were easily digested by white shrimp; chironomid larvae, in particular, were the most preferred by shrimps (Muangyao et al. 2019). In that study, shrimp had eaten 98.5% of chironomid larvae within 6 hr, but only 58% of mosquito larvae and 38% of gutweed, respectively.

Protein is of greatest nutritional importance and also is responsible for the greatest cost component in any diet (New 1980). Commercially available pellets are produced with an optimized composition of nutrient sources to give a high protein content as well as amino acid and fatty acid profiles that are suitable for aquaculture. Chironomid larvae have a high content of protein and essential amino acids, and of lipid, including essential fatty acids (Habib et al. 1997, Habashy, 2005), vitamins, and minerals (McLamey et al. 1974, Habib et al. 1997). Habib et al. (1997) stated that, in aquaculture practice, minerals might be supplemented to fish and shrimps by feeding chironomid larvae. Shrimps feeding on insect larvae, therefore, should not be deficient in any other nutrients. The value of the naturally occurring foods as protein sources should still be assessed more, however. It is also fundamental to develop alternative food and protein sources and to evaluate the nutritional value of the protein in these sources.

The objectives of this study, therefore, are (i) to evaluate the protein content and the amino acid composition of the larvae of chironomids and mosquitoes and gutweed, (ii) to evaluate the effectiveness of the protein for white shrimps fed on these natural foods, and finally (iii) to evaluate the effects of combinations of two different foods, replacing, in part, feed pellets with the natural food.

MATERIALS AND METHODS

Materials used and the experimental design

Larvae of chironomids and mosquitoes used in this study were collected from culture ponds in the finfish hatchery of Coastal Aquaculture Research and Development Regional Center 6 (CARDRC6, Songkhla) in Thailand. Gutweed samples were collected from a pond in a shrimp farm in Trang Province, Thailand. All these materials were kept in the refrigerator (4°C) until they were used for feeding experiments. The pellets used were commercially available ones for white shrimp and were obtained from Charoen Pokphand Foods PCL. Post-larval white shrimps were provided by Blue Gen Solution Hatchery, Songkhla Province, Thailand. The shrimp post-larvae were maintained in the hatchery of the CARDRC6 until they were used in the experiments.

Experiment 1: Evaluation of individual food items

The experiment was conducted with a completely randomized design (CRD) of five treatments, with three replicates for each. The five treatments were as follows: shrimps fed with chironomid larvae (T1), fed with mosquito larvae (T2), fed with gutweed (T3), and, as controls, fed with commercial pellets (T4) or given no food (T5). Preparation of shrimps and feeding experimental design were similar to those described in a previous paper (Muangyao et al. 2019). In brief, twenty shrimps (initial body weight 0.12 ± 0.03 g and body length 2.8 ± 0.2 cm) were cultured in a 35 l glass tank containing seawater of 20 psu. Shrimps were fed with the selected food and the feeding rate was adjusted to provide 46.4g dietary protein/kg shrimp/day, which corresponds to the recommended protein requirement for maximum growth of juvenile white shrimp (Kureshy and Davis 2002). This feeding rate was calculated from the initial weight of shrimps at the beginning of each week and the rate was fixed for the week until the shrimps were weighed again.

Body weights of shrimps were measured every week. Shrimps caught in a hand net were placed on a sheet of paper towel to remove the surrounding water before weighing. The number of shrimps was also counted every week for three weeks. After weighing, living shrimps were returned to the container. Uneaten food was collected and weighed to calculate food intake rate and protein intake. The organic nitrogen content and amino acid composition of the shrimps were determined at the end of the feeding experiments. Average growth rate (AGR), food conversion ratio (FCR), food conversion efficiency (FCE), net protein utilization (NPU) and protein efficiency ratio (PER) were calculated.

Experiment 2: Evaluation of the effects of food combinations

The experiment was conducted with a completely randomized design (CRD) of five treatments, with three replicates for each. The five treatments were as follows: shrimps fed on pellets (T6), fed on 50% of pellets and 50% of chironomid larvae (T7), fed on 25% of pellets and 75% of chironomid larvae (T8), fed on 50% of pellets and 50% of mosquito larvae (T9) and fed on 25% of pellets and 75% of mosquito larvae (T10).

Twenty shrimps, with initial body weight of 0.28 ± 0.00 g, were cultured in a 35 l glass tank containing water at salinity 20 psu. The water temperature was 28-29°C. About 50% of the water was exchanged every afternoon before the shrimps were fed. Shrimps were fed with the selected food at 08:00, 12:00, 17:00 and 22:00 each day (corresponding to the feeding time in local shrimp farms). The feeding rate was adjusted as described for experiment 1. Pellets and insect larvae (wet matter) were given separately but at the same time. Uneaten food was collected and weighed to calculated protein intake. The body weights of shrimps were measured and numbers of living shrimps were counted every week. The organic nitrogen content and amino acid composition of the shrimps were determined at the end of the feeding experiments. The survival rate, PER and AGR were calculated.

Examination of protein content and amino acid composition of natural foods

Analyses of samples (naturally occurring foods and shrimps) were performed at the Chon Buri Aquatic Animal Feed Technology Research and Development Center. Two replicate samples of each naturally occurring food, pellets and shrimps were freeze-dried and ground before the organic nitrogen content was analysed by CHN analyser (Truspec CN, LECO). Protein contents were calculated by multiplying the amount of organic nitrogen by 6.25. Amino acid compositions were analysed for samples of each naturally occurring food, pellets and shrimps. The samples were hydrolysed with hydrochloric acid to give free amino acids and the compositions were then analysed by high-performance liquid chromatography (HPLC, Agilent 1100 series), with post-column derivatization (In-house method based on Bidlingmeyer et al. 1987). The moisture content was also measured by a standard oven drying method (AOAC, 2005), and dry weight was calculated.

Net protein utilization (NPU) and protein efficiency ratio (PER) were calculated as indicators of the nutritional value of proteins and used to evaluate the effectiveness of the protein sources.

Calculations

Food intake rate (g/g shrimp/day)
  = total food eaten (g wet weight) /total shrimp weight (g wet weight) / number of days
Food conversion ratio (FCR)
  = food intake (g wet weight) / weight gain (g wet weight)
Food conversion efficiency (FCE) (%)
  = weight gain (g wet weight) × 100 / food intake (g dry weight)
Protein intake (g/g shrimp/day)
  = total protein eaten (g dry weight) / total shrimp weight (g wet weight) / number of days
Average growth rate (AGR) (% body weight gain/day)
  = total weight gain (g wet weight) / initial weight (g wet weight) / days × 100
Survival rate (%)
  = (final number of shrimps / initial number of shrimps) × 100
Net protein utilization (NPU) (%)
  = total protein gain in the shrimps (g dry weight) / protein intake (g dry weight) × 100
Protein efficiency ratio (PER)
  = weight gain (g wet weight) / protein intake (g dry weight)

Statistical analysis

Food intake rate, protein intake, FCR, FCE, AGR, NPU, PER and protein content of shrimps in each feeding treatment were analysed statistically by analysis of variance (One-Way ANOVA) and Duncan's New Multiple Range Test (SPSS version 16.0).

RESULTS

Protein content and amino acid composition

Protein content of the three food items and pellets is shown in Table 1 as percentage of dry and wet weight. Chironomid and mosquito larvae had high protein content of ca. 62% dry matter and these values were higher than the protein content of pellets on a dry weight basis, but lower when based on wet weight because of the higher water content of the live larvae. The protein content of gutweed was lower than that of insect larvae and pellets, on both dry and wet matter basis.

The content and amino acid composition of each food are shown in Table 1. The content of essential amino acids (EAA) and non-essential amino acids (NEAA), and ratio of the essential to non-essential amino acids (EAA/NEAA) in larvae of both chironomid and mosquito were 30-31%, 34% and 0.9, respectively, and were approximately similar. These values were lower than in pellets but higher than in gutweed (Table 1). The sulphur amino acids methionine (essential) and cysteine (non-essential) and aspartic acid were higher in the larvae than in pellets.

The protein content of shrimps cultured with these foods is shown in Table 2. Shrimps fed on chironomid larvae had significantly higher protein content ($P < 0.05$) than those fed

Table 1. Protein content and amino acid composition of different foods for white shrimps.

List	Chironomid larvae (T1)	Mosquito larvae (T2)	Gutweed (T3)	Pellet (T4)
Protein content				
% dry matter	62.3 ± 0.18^a	62.4 ± 0.11^a	32.7 ± 0.08^b	37.6 ± 0.48^c
% wet matter	11.5 ± 0.03^a	12.1 ± 0.02^a	1.94 ± 0.00^b	33.5 ± 0.43^c
Essential amino acid: EAA (g/100 g protein)				
Arginine	3.61	4.14	2.83	7.03
Histidine	1.82	2.11	0.73	2.20
Threonine	3.06	3.16	2.17	3.33
Phenylalanine	3.73	3.20	2.18	4.03
Valine	3.07	3.19	2.08	3.62
Methionine	2.99	3.62	0.90	0.70
Isoleucine	2.96	2.61	1.44	3.38
Leucine	4.41	4.42	2.82	6.10
Lysine	4.54	4.51	1.77	8.37
Total EAA	30.19	30.96	16.91	38.75
Non-essential amino acid: NEAA (g/100 g protein)				
Aspartic acid	7.31	5.89	5.62	0.00
Glutamic acid	8.85	9.48	4.74	14.99
Serine	3.02	2.96	2.10	3.45
Glycine	2.85	2.75	2.20	5.83
Alanine	5.71	4.81	3.16	6.80
Proline	2.57	2.96	1.41	4.73
Tyrosine	2.67	3.61	1.29	4.28
Cysteine	0.67	0.69	0.55	0.10
Taurine	ND	0.83	ND	ND
Total NEAA	33.64	33.98	21.07	40.18
EAA/NEAA	0.90	0.91	0.80	0.96

Note: Numbers in the same row with different superscripts are significantly different ($P < 0.05$), (N = 2 for protein and moisture, N = 1 for amino acid), ND = Not detected

on mosquito larvae or pellets. Shrimps fed on gutweed or no food, however, had significantly lower protein content ($P < 0.05$) than those fed on any other food (Table 2).

The concentration of EAA in shrimps fed on mosquito larvae or pellets was similar, and higher than in shrimps fed on chironomid larvae or given no food but lower than in shrimps fed on gutweed. The EAA/NEAA ratio of shrimp fed on chironomid larvae, gutweed and shrimp given no food was higher than for shrimp fed on mosquito larvae or pellets (Table 2).

Growth and protein nutrition of shrimps

Food intake rate, protein intake, FCR, FCE, AGR, NPU and PER of shrimps fed on different foods are shown in Table 3. Food intake rates of shrimps fed on larvae of chironomid and mosquito were approximately equal (Table 3). They were significantly different to those of Shrimps fed on gutweed and pellets ($P < 0.05$). Shrimps showed the highest food intake rate from gutweed and the lowest from pellets. Shrimps could not eat all of the gutweed they were fed, even though they ate all the time and food was always present in their gut. The protein intake of shrimps fed on mosquito larvae was similar to that of shrimps fed on chironomid larvae, but higher than for shrimps fed on pellets ($P < 0.05$). The protein intake of shrimps fed on gutweed was lowest ($P < 0.05$) (Table 3). The FCR of shrimps fed on pellets was lowest (1.22 ± 0.07). The FCR values for chironomid larvae (3.70 ± 0.36) were lower ($P < 0.05$) than those for mosquito larvae (7.09 ± 0.77) and gutweed (38.6 ± 4.00), and higher than those for pellets. Statistical analysis, however, showed that the difference between feeding chironomid larvae and pellets was not significant ($P > 0.05$) (Table 3). The FCE of shrimps fed on

Table 2. Protein content and amino acid composition of white shrimps fed with different foods.

List	Shrimp fed on chironomid larvae (T1)	Shrimp fed on mosquito larvae (T2)	Shrimp fed on gutweed (T3)	Shrimp fed on pellet (T4)	Shrimp with no food (T5)
Protein content					
% dry matter	71.4 ± 0.30^{a}	70.0 ± 0.00^{b}	68.8 ± 0.12^{c}	70.0 ± 0.25^{b}	58.9 ± 0.00^{d}
% wet matter	15.46 ± 0.06^{a}	15.82 ± 0.00^{b}	15.11 ± 0.03^{c}	15.70 ± 0.06^{d}	9.09 ± 0.00^{e}
Essential amino acid: EAA (g/100 g protein)					
Arginine	6.49	4.34	6.13	3.15	3.78
Histidine	1.78	1.97	1.89	2.13	1.8
Threonine	3.15	4.87	3.46	3.68	3.34
Phenylalanine	3.46	3.96	3.78	4.14	3.59
Valine	3.21	3.81	3.58	3.84	3.31
Methionine	4.72	2.22	5.40	2.16	2.06
Isoleucine	3.10	4.34	3.22	5.77	5.56
Leucine	5.40	7.97	5.81	7.10	5.67
Lysine	5.47	5.21	5.80	6.53	6.98
Total EAA	36.77	38.7	39.08	38.49	36.1
Non-essential amino acid: NEAA (g/100 g protein)					
Aspartic acid	7.28	9.21	8.30	9.22	7.25
Glutamic acid	11.89	13.36	12.79	14.16	10.42
Serine	3.28	3.83	3.50	3.86	3.44
Glycine	6.49	7.57	6.77	7.01	6.10
Alanine	5.34	6.50	5.28	6.33	4.30
Proline	2.97	6.99	3.17	3.85	2.83
Tyrosine	2.90	3.49	2.93	3.47	3.01
Cysteine	0.82	0.97	0.89	0.90	1.08
Taurine	1.22	1.43	1.30	1.08	2.16
Total NEAA	42.19	53.34	44.93	49.87	40.59
EAA/NEAA	0.87	0.73	0.87	0.77	0.89

Note: Numbers in the same row with different superscripts are significantly different ($P < 0.05$), (N = 2 for protein and moisture, N = 1 for amino acid), ND = Not detected

chironomid larvae was highest (147 ± 14.0%) followed by FCE of shrimps fed on pellets (92.5 ± 5.02%), mosquito larvae (73.1 ± 8.44%) and gutweed (44.0 ± 4.38%) (Table 3). The FCE of shrimps was significantly different between treatments ($P < 0.05$) (Table 3). The AGR of shrimps fed on chironomid larvae were significantly higher ($P < 0.05$) than for those fed mosquito larvae or gutweed, and comparable to AGR for shrimp fed on pellets (Table 3). Nevertheless, AGRs of shrimps fed on mosquito larvae or gutweed were similar and significantly higher ($P < 0.05$) than those of shrimp given no food (Table 3). The NPU and PER of shrimps fed on chironomid larvae or pellets was significantly ($P < 0.05$) higher than all the others. (Table 3).

Effects of food combinations

Protein intake, PER, AGR, and survival rates of shrimps fed on different food combinations are shown in Table 4. The highest protein intake was seen for shrimp fed on 100% mosquito larvae, but there was no significant difference among all others ($P > 0.05$). The PER of shrimps fed on 100% pellets was 2.46. Feeding a combination of 50% pellets and 50% chironomid larvae gave the highest PER, the AGR being significantly higher ($P < 0.05$) than for shrimps fed on 100% pellets. Other feeding combinations, namely 25% pellets and 75% chironomid larvae or mosquito larvae gave similar ($P > 0.05$) PER and AGR to feeding pellets alone (Table 4). Survival rates were not significantly different among all the treatments ($P > 0.05$) (Table 4).

Table 3. Comparison of food intake rate, protein intake, food conversion ratio (FCR), food conversion efficiency (FCE), average growth rate (AGR), net protein utilization (NPU) and protein efficiency ratio (PER) of white shrimp fed with the different foods.

	Chironomid larvae (T1)	Mosquito larvae (T2)	Gutweed (T3)	Pellet (T4)	No food (T5)
Food intake rate (g wet weight/ g shrimp/day)	0.38 ± 0.00^{a}	0.38 ± 0.01^{a}	1.83 ± 0.02^{b}	0.12 ± 0.00^{c}	-
Protein intake (g wet weight/g shrimp/day)	0.044 ± 0.000^{ab}	0.046 ± 0.000^{b}	0.035 ± 0.000^{c}	0.042 ± 0.001^{a}	-
FCR	3.70 ± 0.36^{ab}	7.09 ± 0.77^{b}	38.6 ± 4.00^{c}	1.22 ± 0.07^{a}	-
FCE (%)	147 ± 14.0^{a}	73.1 ± 8.44^{b}	44.0 ± 4.38^{c}	92.5 ± 5.02^{d}	-
AGR (% body weight gain/day)	21.1 ± 4.70^{a}	8.40 ± 3.07^{b}	7.97 ± 0.77^{b}	19.5 ± 2.18^{a}	0.15 ± 0.51^{c}
NPU (%)	36.6 ± 3.47^{a}	18.6 ± 2.14^{b}	20.4 ± 2.03^{b}	38.6 ± 2.10^{a}	-
PER	2.37 ± 0.22^{a}	1.17 ± 0.14^{b}	1.35 ± 0.13^{c}	2.46 ± 0.13^{a}	-

Note: Numbers in the same row with different superscripts are significantly different ($P < 0.05$). Values are mean ± standard deviation of three replicates (N = 3)

Table 4. Protein intake, protein efficiency ratio (PER), average growth rate (AGR) and survival rate of shrimps fed with different food combinations for 3 weeks.

	100% pellets (Control) (T6)	50% pellets + 50% chironomid larvae (T7)	25% pellets + 75% chironomid larvae (T8)	50% pellets + 50% mosquito larvae (T9)	25% pellets + 75% mosquito larvae (T10)
Protein intake (g wet weight/g shrimp/day)	0.043 ± 0.000^{a}	0.043 ± 0.000^{a}	0.043 ± 0.000^{a}	0.043 ± 0.000^{a}	0.042 ± 0.000^{a}
PER	2.59 ± 0.21^{ab}	3.05 ± 0.30^{a}	2.88 ± 0.19^{ab}	2.79 ± 0.28^{ab}	2.55 ± 0.25^{b}
AGR (% body weight gain/day)	26.2 ± 1.97^{a}	34.1 ± 1.44^{b}	30.6 ± 3.43^{ab}	28.5 ± 3.26^{a}	25.3 ± 3.39^{a}
Survival rate (%)	86.7±2.89	83.3 ± 12.6	78.3 ± 5.77	85.0 ± 0.00	78.3 ± 5.77

Note: Numbers in the same row with different superscripts are significantly different ($P < 0.05$). Values are mean ± standard deviation of three replicates (N = 3)

DISCUSSION AND CONCLUSION

Chironomid larvae have a high protein content of 62% (Table 1), similar to that of fishmeal (60-72%) (Cho and Kim 2011), which is the main protein source in shrimp aquaculture pellets (Suárez et al. 2009, Olmos et al. 2011). This indicated that chironomid larvae could be used as a protein source. Compared to pellets, they have a higher protein content (dry matter basis), but the quality of protein from pellets is better. The concentrations of EAA and NEAA and the ratio of EAA/NEAA in the chironomid larvae and mosquito larvae were lower than in the pellets, but the quality of protein in pellets depends on the quality of the protein source used (Tantikitti et al. 2016). The concentrations of EAA and NEAA in the chironomid and mosquito larvae were higher than those reported in another study (Tantikitti et al. 2016) for pellets which were formulated with premium grade fishmeal. The EAA/NEAA ratios of those larvae were also higher than those for five diets containing fishmeal from different sources (Tantikitti et al. 2016).

The protein content and the concentrations of EAA and NEAA in the chironomid and mosquito larvae were higher than in gutweed (Table 1). Plant products usually have lower content of protein and amino acids than animal products (Hardy 2010, Tantikitti 2014)

The protein content of insects and their larvae depends on the species of insect. The adult variegated grasshopper (*Zonocerus variegatus*) had 61.5% crude protein (Alegbeleye et al. 2012). Other insect products such as black soldier fly larvae (*Hermetia illucens*) (42. 1%), housefly maggot meal (*Musca domestica*) (50.4%), adult of house cricket (*Acheta domesticus*) (63.3%), and silkworm pupae meal (*Bombyx mori* (Lepidoptera)) (60.7%) have different levels of crude protein (Tran et al. 2015). Results obtained in the present study showed that the protein content of larvae of chironomid and mosquito were higher than in most of these insects and larvae, almost reaching the level reported for the adult house cricket.

The NPU is directly related to shrimp growth. Shrimps were able to convert protein from the chironomid larva into that in the shrimp body at a higher level than from the other natural foods and at a similar level to that of protein from pellets (Table 3). Even though the NPU of shrimps fed on chironomid larvae was not significantly different to that of shrimps fed on pellets, the FCE in shrimps fed on chironomid larvae was higher. Pellets are produced to be a rich source of protein, and to contain other nutrient sources suitable for shrimp growth. The larvae also have high contents of essential amino acids, lipid, and essential fatty acids (Habib et al. 1997, Habashy 2005), vitamins and minerals (McLamey et al. 1974, Habib et al. 1997). The nutritional quality of the proteins in the pellets used in the present study is better than that of the protein in the chironimid larvae but, nevertheless, chironomid larvae are promising as a source of food to stimulate shrimp growth.

Mosquito larvae also have a high protein content (Table 1) and an amino acid profile like that of chironomid larvae (Table 2), although shrimps fed with mosquito larvae revealed lower growth and lower NPU. The FCE of mosquito larvae was lower than that of chironomid larvae, but Muangyao et al. (2019)(unpublished paper) reported that shrimp need a longer time to digest mosquito larvae than chironomid larvae, which are a more acceptable food. Shrimps fed on chironomid larvae had high growth, and their growth was comparable to that of shrimps fed on pellets.

Gutweed had the lowest protein content and concentration of EAA and NEAA (Tables 1 and 2), so shrimp have to eat more gutweed to obtain a high enough level of protein. Gutweed showed the highest food conversion ratio (FCR) and the lowest FCE (Table 3). In this case shrimps could not eat all of the gutweed fed, so the growth of shrimps fed gutweed was lowest.

Evaluation of the effect of feeding combinations of foods revealed that shrimp fed on the combination of chironomid larvae and pellets (50% or 75% of insect larvae + 50% or 25% of pellets) had higher AGR than shrimps fed on 100% pellets (Table 4). Feeding on 50% of chironomid larvae and 50% of pellets was particularly effective, even more so than feeding pellets alone (Table 4). These results show that feeding a combination of pellets and chironomid larvae can be more effective than feeding pellets or chironomid larvae alone; experiment 1 showed that pellets and chironomid larvae are similarly effective. The shrimps appeared to incorporate more protein from the combination and to have enhanced EAA content. Feeding mosquito larvae was also effective, but the shrimps prefer to eat chironomids. Pellets have higher concentrations of EAA and NEAA and higher ratios of EAA/NEAA but chironomid larvae have higher concentrations of methionine, aspartic acid and cysteine. So, the combination of pellets and chironomid larvae could provide a more suitable profile of amino acids than pellet or larvae alone. The combinations of pellets with up to 75% of chironomid or mosquito larvae gave similar growth to shrimps fed on pellets alone (Table 4). This indicated that up to 75% of the pellet feed could be replaced by these insect larvae, thus reducing the cost of providing feed for shrimp aquaculture.

The result obtained here does raise some questions, for example whether there may be some as yet unidentified factor in the chironomid larvae that stimulates the uptake of protein from pellets, or whether feeding the combination of pellets and insect larvae may give a more suitable nutritional balance for juvenile white shrimp. In any case, up to 75% of pellet feed could be replaced by the insect larvae without any negative effect on shrimp growth. The observation reported in this study may, therefore, be important for future improvements.

It can be concluded that stimulating the occurrence of chironomid larvae in a shrimp pond will support more shrimp growth than simply feeding pellets alone. The way to stimulate the occurrence of these insect larvae in ponds should be investigated. Knowledge of this would be of benefit by improving pond preparation practice in a way that would support shrimp growth and reduce the need for pellets, and thus reduce the production cost of shrimp culture.

ACKNOWLEDGEMENTS

The authors thank the Agricultural Research Development Agency (Public Organization), ARDA, for providing financial support for this study. We are grateful to the Coastal Aquaculture Research and Development Regional Center 6 (Songkhla) and Chon Buri Aquatic Animal Feed Technology Research and Development Center, Department of Fisheries, Thailand, for laboratory facilities and technical support. Thanks also to the Faculty of Agriculture and Marine Science, Kochi University, Japan, for facilities during preparation of this manuscript and to Dr. George Britton for helping to improve the English of this manuscript. We acknowledge Blue Gen Solution Hatchery Mr. Paitoon Chuaymark and Mr. Wimala Janpakdee, for their supply of white shrimp larvae used in this study and Mr. Pirun Tongsiri for the supply of gutweed samples.

REFERENCES

Alegbeleye W.O., Obasa S.O., Olude O.O. and Otubu K. 2012. Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L.) for African catfish Clarias gariepinus (Burchell. 1822) fingerlings. Aquac. Res. 43: 412-420.

Amaya E.A., Davis D.A. and Rouse D.B. 2007a. Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. Aquaculture, 262: 393-401.

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

Association of Official Analytical Chemists (AOAC). 2005. Official Methods of Analysis. 18th Edition, AOAC INTERNATIONAL, Maryland, 20877-2417, USA.

Barroso F.G., De Haro C., Sánchez-Muros M.-J. and Venegas E. 2014. The potential of various insect species for use as food for fish. Aquaculture, 422-423: 193-201.

Bidlingmeyer B.A., Cohen S.A., Tarvin T.L. and Frost B. 1987. A new rapid, high sensitivity analysis of amino acids in food type samples. J. Assoc. Off. Anal. Chem. 70: 241-247.

Belghit I., Liland N.S., Waagbø R., Biancarosa I., Pelusioc N., Li Y., Krogdahl Å. and Lock E. 2018. Potential of insect-based diets for Atlantic salmon (*Salmo salar*). Aquaculture, 491: 72-81.

Cho J.H. and Kim I.H. 2011. Fish meal - nutritive value. J. Anim. Physiol. Anim. Nutr. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1439-0396.2010.01109.x (Last access 24 November 2018).

Concetta Elia A., Teresa Capucchio M., Caldaroni B., Magara G., Martin Dörr A. J., Biasato I., Biasibett, E., Righetti M., Pastorino P., Prearo M., Gai F., Schiavone A. and Gasco L. 2018. Influence of *Hermetia illucens* meal dietary inclusion on the histological traits, gut mucin composition and the oxidative stress biomarkers in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 496: 50-57.

Gamboa-Delgado J. 2014. Nutritional role of the natural productivity and formulated feed in semi-intensive shrimp farming as indicated by natural stable isotopes. Reviews in Aquaculture, 6: 36-47.

Habashy M.M. 2005. Culture of chironomid larvae (insect-diptera: chironomidae) under different feeding systems. Egypt J. Aquat. Res. 31: 403-418.

Habib M.A.B., Yusoff F.M., Phang S.M., Ang K.J. and Mohamed S. 1997. Nutritional values of chironomid larvae grown in palm oil mill effluent and algal culture. Aquaculture, 158: 95-105.

Habib M.A.B., Yusoff F.M., Phang S.M., Ang K.J. and Mohamed S. 2005. Experimental production and chemical composition of Culex cosquito larvae and lupae grown in agro-industrial effluent. Asian Fish. 18: 107-119.

Hardy R.M. 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aqua. Res. 41:770-776.

Henry M.A., Gai F., Enes P., Peréz-Jiménez A. and Gasco L. 2018. Effect of partial dietary replacement of fishmeal by yellow mealworm (*Tenebrio molitor*) larvae meal on the innate immune response and intestinal antioxidant enzymes of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 83: 308-313.

Henry M., Gasco L., Piccolo G. and Fountoulaki E. 2015. Review on the use of insects in the diet of farmed fish: past and future. Anim. Feed Sci. Technol. 203: 1-22.

Iaconisi V., Bonelli A., Pupino R., Gai F. and Parisi G. 2018. Mealworm as dietary protein source for rainbow trout: Body and fillet quality traits. Aquaculture, 484: 197-204.

Kureshy N. and Davis D.A. 2002. Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 204: 125-143.

Lock E., Arsiwalla T. and Waagbø R. 2016. Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (*Salmo salar*) postsmolt. Aquac. Nutr. 22: 1202-1213.

Martínez-Córdova L. R. and Peña-Messina E. 2005. Biotic communities and feeding habits of *Litopenaeus vannamei* (Boone 1931) and *Litopenaeus stylirostris* (Stimpson 1974) in monoculture and polyculture semi-intensive ponds. Aquacult. Res. 36: 1075-1084.

Martinez-Cordova L. R., Porchas-Cornejo M. A., Villarreal-Colemnares H., Calderon-Perez J. A. and Naranjo-Paramo J. 1998. Evaluation of three feeding strategies on the culture of white shrimp *Penaeus vannamei* Boone 1931 in low water exchange ponds. Aquacult. Eng. 17: 21-28.

Martinez-Cordova L. R., Torres A. C. and Porchas-Cornejo M. A. 2003. Dietary protein level and natural food management in the culture of blue (*Litopenaeus stylirostris*) and white shrimp (*Litopenaeus vannamei*) in microcosms. Aquacult. Nutr. 9: 155-160.

McLamey W.O., Henderson S. and Sherman M.M. 1974. A new method for culturing *Chironomus tentans* Fabricious. Aquaculture, 4: 267-276.

Muangyao P., Fukami K., Predalumpaburt Y. and Songsangjinda P. 2019. Importance of naturally occurring insect larvae and gutweed as a complementary diet for white shrimp (*Litopenaeus vannamei*) aquaculture. Kuroshio Sci. 13: 1-8.

New B.M. 1980. The diet of prawns. http://www.fao.org/docrep/field/003/AB915E/AB915E00.htm (Last access 24 December 2018).

Olmos J., Ochoa L., Paniagua-Michel J. and Contreras R. 2011. Functional feed assessment on *Litopenaeus vannamei* using 100% fish meal replacement by soybean meal, high levels of complex carbohydrates and bacillus probiotic strains. Mar. Drugs. 9: 1119-1132.

Panini R.L., Freitas L.E.L., Guimarães A.M., Rios C., da Silva M.F.O., Vieira F.N., Fracalossi D.M., Samuels R.I., Prudêncio E.S., Silva C.P. and Amboni R. D.M.C. 2017a. Potential use of mealworms as an alternative protein source for Pacific white shrimp: Digestibility and performance. Aquaculture, 473:115-120.

Panini R.L., Pinto S.S., Nóbrega R.O., Vieira F.N., Fracalossi D.M., Samuels R.I., Prudêncio E.S., Silva C.P. and Amboni R. D.M.C. 2017b. Effects of dietary replacement of fishmeal by mealworm meal on muscle quality of farmed shrimp *Litopenaeus vannamei.* Food Res. Int. 102: 445-450.

Sánchez-Muros M.-J., Barroso F.G. and Manzano-Agugliaro F. 2014. Insect meal as renewable source of food for animal feeding: a review. J. Cleaner Production, 65: 16-27.

Sankian Z., Khosravi S., Kim Y. and Lee S. 2018. Effects of dietary inclusion of yellow mealworm (Tenebrio molitor) meal on growth performance, feed utilization, body composition, plasma biochemical indices, selected immune parameters and antioxidant enzyme activities of mandarin fish (*Siniperca scherzeri*) juveniles. Aquaculture, 496: 79-87.

Suárez J.A., Gaxiola G., Mendoza R., Cadavid S., Garcia G., Alanis G., Suárez A., Faillace J. and Cuzon G. 2009. Substitution of fish meal with plant protein sources and energy budget for white shrimp *Litopenaeus vannamei* (Boone, 1931). Aquaculture, 289: 118-123.

Suriyaphan J., Kaewsuralikhit C., Ketma C., Limsuwan C., Chuchird N., Prasertsri S., Thongphitak D. and Hongrat P. 2008. Effect of gut weed (*Ulva intestinalis* Linnaeus) on benthos in pond-reared black tiger shrimp (*Penaeus monodon* Fabricius). Proceeding of the 46th Kasetsart University Annual Conference, Subject: Fisheries, Kasetsart, Bangkok, Thailand, 2008, pp. 210-219. (in Thai with English abstract)

Tacon A.G.J., Cody J.J., Conquest L.D., Divakaran S., Forster I.P. and Decamp O.E. 2002. Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. Aquacult. Nutr. 8: 121-137.

Tantikitti C. 2014. Feed palatability and the alternative protein sources in shrimp feed. Songklanakarin J. Sci. Technol. 36: 51-55.

Tantikitti C., Chookird D. and Phongdara A. 2016. Effect of fishmeal quality on growth performance, protein digestibility and trypsin gene expression in pacific white shrimp (*Litopenaeus vannamei*). Songklanakarin J. Sci. Technol. 38: 73-82.

Tran G., Heuzé V. and Makkar H.P.S. 2015. Insects in fish diets. Anim. Front. 5: 37-44.

Vargas-Abúndez A.J., Randazzo B., Foddai M., Sanchinia L., Truzzi C., Giorgini E., Gasco L. and Olivotto I. 2019. Insect meal based diets for clownfish: Biometric, histological, spectroscopic, biochemical and molecular implications. Aquaculture 498: 1-11.