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Research Paper

Importance of naturally occurring insect larvae and gutweed as complementary food for white shrimp (*Litopenaeus vannamei*) aquaculture

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ABSTRACT

The potential value of naturally occurring larvae of insects such as chironomids and mosquitoes, and of gutweed *Ulva intestinalis* (green alga), as food sources for juvenile white shrimp *Litopenaeus vannamei* was assessed. Shrimps (body weight 0.12 ± 0.03 g/individual, body length 2.7-2.8 cm) were fed for 3 weeks with either chironomid larvae, mosquito larvae or gutweed, and compared with shrimps fed with the usual pellets and with shrimps given no food, as controls. White shrimps consumed both animal and plant matter, but chironomid larvae were the most acceptable food and were most quickly digested. All the foods promoted shrimp growth but shrimp growth after 3 weeks with chironomid larvae and pellets was equally and significantly higher ($P < 0.05$) than growth of shrimps from the other treatments. The food conversion efficiency (FCE) of shrimps fed on chironomid larvae was highest and FCE was significantly different between treatments ($P < 0.05$). The survival rates of the shrimps given the three different foods were not significantly different and no evidence was seen of adverse effects on shrimp health. Chironomid larvae were most effective as the complementary food source, but mosquito larvae and gutweed were also promising as live food for juvenile white shrimps. The effective use of such insect larvae could reduce the requirement for feed pellets.

Key words: *Litopenaeus vannamei*, chironomid larvae, insect larvae, gutweed, natural foods

INTRODUCTION

White shrimp *Litopenaeus vannamei* is an economically important aquatic resource in many countries and is produced mainly by aquaculture. World production of white shrimp reached more than 3 million tons in 2012 (FAO 2012). In Thailand, for example, white shrimp accounts for more than 80% of total shrimp aquaculture production (Department of Fisheries of Thailand 2015).

The increase in shrimp aquaculture has led to increased demand for feed pellets that contain fishmeal as protein source. It is, therefore, of high commercial importance to find alternative protein sources and/or alternative food sources as a substitute or complement for the pellets. This is an urgent requirement to improve feeding practice for shrimp aquaculture (Hernández et al. 2004, McLean et al. 2006, Nunes et al. 2006, Amaya et al. 2007a, 2007b, Burford et al. 2004, Chookird et al. 2010).

In culture ponds, however, shrimps find and prefer natural foods. In ponds with enhanced natural productivity, they consume more natural food, and stomachs of shrimps from the enhanced ponds contained a higher proportion of natural food than of formulated feed (Porchas-Cornejo, et al. 2012). The data from these previous observations showed that

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shrimps had higher growth when reared in ponds with a high density of natural foods (Porchas-Cornejo et al. 2012). Several studies have shown the potential value of natural foods in the nutrition of shrimp (Martinez-Moss and Pruder 1995, Rubright et al. 1981, Tacon et al. 2002, Cordova et al. 2003, 2005, Gamboa-Delgado 2014). The main natural food materials consumed by shrimp are zooplankton and benthic organisms (Chiu and Chien 1992, Martinez-Cordova et al. 1998), though the main species of such organisms are different in different countries.

Gutweed *Ulva intestinalis* is a common green alga which is found in the natural waters and in shrimp culture ponds in Thailand. Muangyao et al. (2011a) described microcosm experiments containing many kinds of natural food sources including gutweed, insect larvae (chironomid and mosquito larvae) and zooplankton (copepods). They concluded that gutweed might play an important role as the shelter and habitat of chironomid larvae, mosquito larvae, and nauplii of copepods, especially harpacticoids.

It has been reported that the stomach contents of shrimp reared in different feeding regimes consisted of a variety of organisms and other organic matter (Muangyao et al. 2011b) and the correlation analysis from that work showed that shrimp growth was closely related to the amount of chironomid larvae in the nutrient material in the microcosms. Still, however, there are few reports on the feeding behavior and the digestion of dominant natural foods by shrimp. More investigations on a laboratory scale are necessary before new complementary food sources can be proposed for white shrimp aquaculture.

The objectives of the present study are (i) to observe feeding behavior and the digestion of chironomid larvae, mosquito larvae, and gutweed by juvenile white shrimps, and (ii) to evaluate the growth and survival rates in each case. Knowledge of these factors will generate better understanding of how to promote shrimp growth by providing a specific kind of natural food. It will also be useful for improving feeding practice for shrimp aquaculture, and diminishing adverse effects of shrimp culture on the environment.

MATERIALS AND METHODS

Materials

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 was collected from an earthen pond in a shrimp farm in Trang province, Thailand. The pellets used were commercially available ones for white shrimp and were obtained from Charoen Pokphand Foods PCL. The chemical composition of the naturally occurring foods and pellets was measured. Analyses of samples were performed at the Chon Buri Aquatic Animal Feed Technology Research and Development Center. Two replicate samples of each naturally occurring food and pellets 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. The fat and ash content were measured (AOAC, 2016). The moisture content was also measured by a standard oven drying method (AOAC, 2005), and dry weight was calculated. Carbohydrate was calculated by Carbohydrate percentage = 100 - % protein - %fat -% ash. The chemical compositions are shown in Table 1.

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, Songkhla until they were used in the experiments.

Table 1. Chemical composition of different foods for white shrimps.

	Chironomid larvae (T1)	Mosquito larvae (T2)	Gutweed (T3)	Pellet (T4)
Moisture (%)	81.5 ± 0.04	80.5 ± 0.07	94.1 ± 0.02	10.7 ± 0.14
Protein (% dry matter)	62.3 ± 0.18[a]	62.4 ± 0.11[a]	32.7 ± 0.08[b]	37.6 ± 0.48[c]
Fat (% dry matter)	10.4 ± 0.01	14.6 ± 0.16	5.15 ± 0.08	8.11 ± 0.06
Ash (% dry matter)	5.40 ± 0.01	7.22 ± 0.08	20.0 ± 0.04	13.8 ± 0.03
Carbohydrate (% dry matter)	21.9 ± 0.26	15.7 ± 0.27	42.0 ± 0.19	40.5 ± 0.57

Note: Numbers in the same row with different superscripts are significantly different ($P < 0.05$), (N = 2)

Feeding behavior of shrimp

Thirty white shrimps *Litopenaeus vannamei* with initial body weight 0.12 ± 0.03 g and body length 2.7 ± 0.2 cm were starved for 24 h before testing. Each individual shrimp was held in a separate 2-liter container and was fed with 20 pieces, about 0.5 cm length, of one of the natural foods (larvae of chironomid or mosquito, or gutweed). Ten replicates for each were prepared. Numbers of pieces of natural food remaining in each container were counted after 10, 20, 30 min, and then every 30 min until 6 h. The average percentages of food pieces consumed by 30 shrimp replicates were calculated.

Digestion of chironomid larvae, mosquito larvae and gutweed

The time for digestion of each natural food was evaluated by stomach content analysis. Ten shrimps (as ten replicates) for each sampling occasion (7 times) and for each food (3 items) (210 individuals in total) were starved for 24 h before testing. They were fed on a natural food, either one chironomid larva, one mosquito larva or one piece of gutweed (each about 0.5 cm length). After 0.5, 1, 2, 3, 4, 5 and 6 h, shrimps that had eaten the natural food were collected and placed immediately into a salt solution (1 kg salt granules + 3 kg ice cubes + 3 l seawater) at -10ºC. The shrimps were then preserved in 10% formalin solution until they could be examined under a compound microscope. Then the foregut was dissected and the contents were washed onto a glass slide. Each food was observed as parts of the body of chironomid larva (head, body and jaw), those of mosquito larva (head, body, anal and siphon tube, and hair) and pieces of gutweed (Fig. 1). The digestion of each food was monitored; for insect larvae, the main body part was observed. The frequency of occurrence was then calculated according to the following equation:

$$\text{Frequency of occurrence (\%)} = Np/N' \times 100$$

Where Np = number of stomachs still containing the main part of each food item

N' = total number of shrimp individuals consuming the food

Growth of shrimps

1. Experimental design

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), fed with commercial pellets (T4; positive control) or given no food (T5; negative control).

2. Determination of growth

Twenty shrimps, with 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 water at salinity 20 psu. The water temperature was 28-29ºC and 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 to provide 46.4 g dietary protein/kg shrimp/day, which corresponds to the recommended protein requirement for maximum growth of juvenile white shrimp (Kureshy and Davis 2002). Uneaten food was collected and weighed, and food intake calculated.

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. Numbers of living shrimps were counted every week for three weeks. After their weights were measured, living shrimps were returned to the glass tank. The average body weight, food

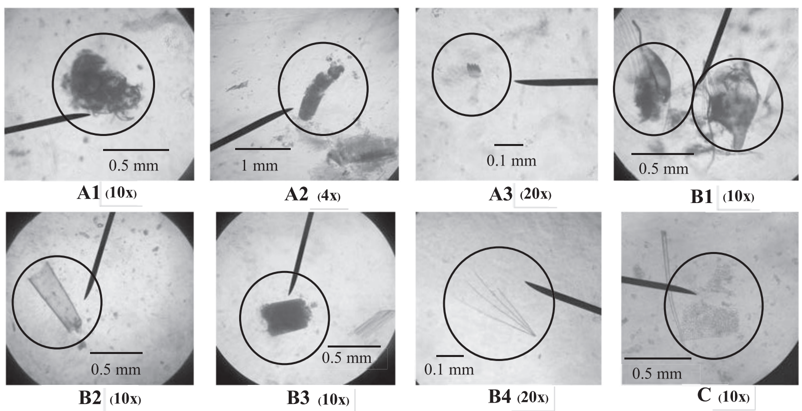

Fig. 1. Body parts of chironomid larvae (A1 = head, A2 = body, A3 = jaw); body parts of mosquito larvae (B1 = head, B2 = siphon, B3 = body, B4 = hair); piece of gutweed (C) in the stomach of white shrimps, as observed under the microscope.

conversion efficiency (FCE) and survival rate were calculated at the end of the experiment.

3. Calculations

The following equations were used for calculations.

3.1 Average body weight (g/individual)

= Total wet weight of shrimps / Number of shrimps

3.2 Food conversion efficiency (FCE) (%)

= weight gain (g wet weight) $\times$ 100 / food intake (g dry weight)

3.3 Survival rate (%)

= (Final number of shrimps / Initial number of shrimps) $\times$ 100

3.4 Statistical analysis

Average body weight, FCE and survival rate 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).

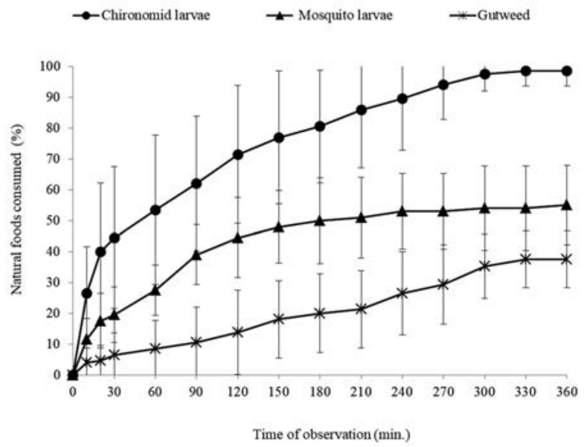

Fig. 2. Rate of consumption of three different natural food items (chironomid larvae, mosquito larvae and gutweed) by white shrimp over 6 h.

RESULTS

Feeding behavior of shrimps

Changes in the consumption of the three food items are illustrated in Fig. 2 as average percent for 30 shrimp replicates. When feeding, a shrimp held a larva or a food particle and ate it completely before moving to the next individual item. Of the three food items, chironomid larvae were consumed fastest, followed by mosquito larvae, and gutweed was the slowest. Shrimps consumed chironomid larvae very rapidly; 26.5% of chironomid larvae were eaten within 10 min and more than 50% were eaten within 1 h, whilst only 11.5% of mosquito larvae and 4.0% of gutweed pieces were eaten after 10 min. Thirty percent of shrimps within 3.5 h, and 90% of shrimps at the end of the observation (6 h), had eaten all the 20 chironomid larvae given (Fig. 2). None of the shrimps had eaten all 20 mosquito larvae or pieces of gutweed given. Shrimps had eaten 50% of the mosquito larvae within 3 h, whereas 50% of gutweed still remained after 6 h (Fig. 2). On average, 98. 5% of chironomid larvae were consumed per shrimp, compared with 55% of mosquito larvae and 38% of gutweed (Fig. 2).

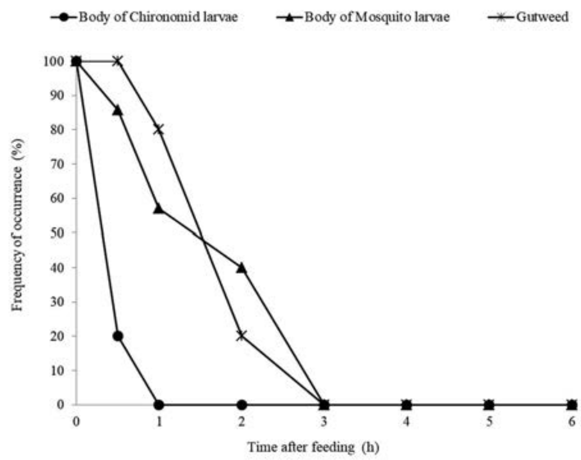

Fig. 3. Changes in the percentage of shrimp stomachs containing the main part of the natural foods chironomid larvae, mosquito larvae or gutweed, after 0.5, 1, 2, 3, 4, 5 and 6 h of feeding. Note: The main part of the body occupies 95% of the whole body in chironomid and 70% of the whole body in mosquito larvae.

Digestion of natural foods

When chironomid larvae, mosquito larvae or gutweed were eaten, parts of them remained in the stomach of every shrimp. The percent frequency of occurrence decreased after 0. 5 h (Fig. 3). The main body part made up 95% of the chironomid larva and 70% of the mosquito larva, by length (see Fig. 1, B2, C3). The main body part of the chironomid larva was consumed and digested very quickly, so that only 20% of the shrimps had any of the main body of the larva left 0.5 h after eating, and the chironomid larva was completely digested within 1 h. In contrast, the main body part of the mosquito larva and the gutweed pieces were more resistant to digestion, but they were digested completely after 3 h (Fig. 3). At the end of the observation period, however, 57.1% of the shrimp stomachs still retained the hair of mosquito larvae (Fig. 1, C4), whilst none had any part of chironomid larva or gutweed.

Growth, food conversion efficiency and survival rate of shrimps

Increase in body weight of shrimps fed with one of the three food items is illustrated in Fig. 4. Average body weight of shrimps fed with chironomid larvae was significantly higher ($P < 0.05$) than those of shrimps fed with mosquito larvae or gutweed and was comparable to that of shrimps that were fed with pellets. The average body weights of shrimps fed with mosquito larvae or gutweed were similarly but significantly higher ($P < 0.05$) than those of shrimps given no food, although they were lower than those fed with chironomid larvae or pellet feed (Fig. 4). The FCE of shrimps fed on 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%) (Fig. 5). The FCE of shrimps was significantly different between treatments ($P < 0.05$).

Survival rates of shrimps with each of the foods were more than 90% (Fig. 5), and were significantly higher than the survival rate of shrimps given no food (60.0 ± 22.9%).

DISCUSSION AND CONCLUSION

Results obtained in this study confirmed the omnivorous behavior of juvenile white shrimp in consuming both animal and plant matter, in agreement with previous reports (Dall 1968, Cockcroft and McLachlan 1986, Muangyao et al. 2011b). Shrimps, however, prefer animal-derived to plant-derived food. In the first hour of observation, shrimps consumed insect larvae more efficiently than gutweed, and chironomid larvae were the most preferred food for white shrimp. Nearly 100% of chironomid larvae were consumed (Fig. 2)

The results given in Fig. 3 show that shrimps needed only a short time to digest chironomid larvae, thus shrimps fed with chironomid larvae revealed the best growth (Fig. 4) and had the highest FCE (Fig. 5). Mosquito larvae have a refractory cuticle of chitin, a polymer of *N*-acetyl-*β*-D-glucosamine (Merzendorfer and Zimoch 2003) while chironomid larvae are worm-like and have soft skin. These results showed that chironomid larvae are a more suitable food.

Although mosquito larvae and gutweed could not promote shrimp growth rates so much as chironomid larvae or pellets did, they also gave survival rates 30% higher than those of the shrimp given no food (Fig. 6). The results clearly show

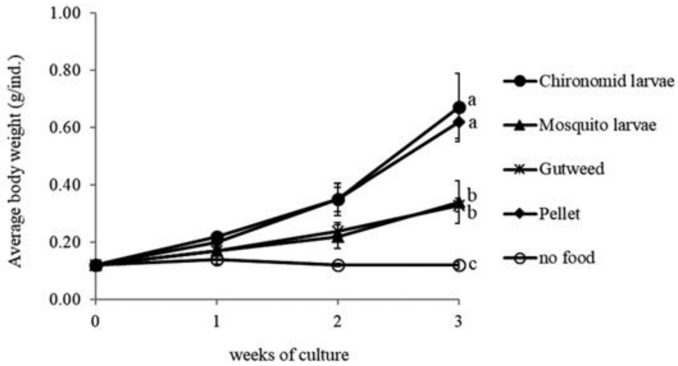

Fig. 4. Increase in the average body weight (g/ind.) of white shrimp fed with different foods.

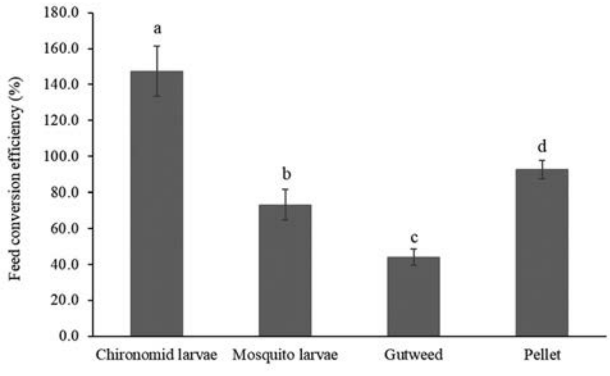

Fig. 5. Feed conversion efficiency of white shrimp fed with different foods. Different letters above columns indicate significant differences ($P < 0.05$).

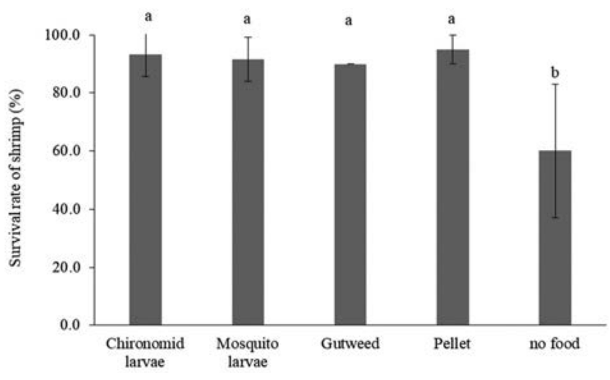

Fig. 6. Survival rate of white shrimp fed with different foods. Different letters above columns indicate significant differences ($P < 0.05$).

that chironomid larvae were the best among the three natural foods, but mosquito larvae and gutweed were also promising as a live food for white shrimp and could also be used. These results are useful in relation to providing naturally occurring food for white shrimp and show the potential of insect larvae, especially chironomid larvae, as complementary food for white shrimp.

Insects have received attention in the past decade as protein sources for animals. Some studies showed the potential of insect larvae as a feed ingredient for fish such as rainbow trout (*Oncorhynchus mykiss*) (Borgogno et al. 2017, Concetta Elia et al. 2018), European seabass (*Dicentrarchus labrax*) (Magalhães et al. 2017), blackspot seabream (*Pagellus bogaraveo*) (Iaconisi et al. 2017), Jian carp (*Cyprinus carpio var. Jian*) (Li et al. 2017), Atlantic salmon (*Salmo salar*) (Lock et al. 2016, Belghit et al. 2018) and clownfish (*Amphiprion ocellaris*) (Vargas-Abúndez et al. 2019). Three promising insect species for fish feed purposes are the black soldier fly (BSF) (*Hermetia illucens*), the common housefly (*Musca domestica*) and the yellow mealworm (*Tenebrio molitor*) because the larvae of these species can grow well on organic waste and produce high-quality protein and fat (Cicková et al. 2015, Nguyen et al. 2015). Use of these species as food in the aquaculture industry would reduce the environmental impact. The studies cited above focused on the use of cultured insect larvae as a feed ingredient for fish.

For white shrimp culture, however, the naturally occurring larvae of chironomid and other insects in shrimp ponds were considered and provided a new concept for the aquaculture. Chironomids are benthic invertebrates which consume mainly sediment detritus (Henriques-Oliveira et al. 2003, Galizzi et al. 2012). The density of chironomid larvae depends mainly on the level of detritus available (Galizzi et al. 2012). The larvae can reach a high density and remove suspended and/or deposited organic matter, transferring it into the benthic webs (Hirabayashi and Wotton 1999, Malmqvist et al. 2001). Shrimp ponds accumulate organic matter in the bottom sediment (Funge-Smith and Briggs 1998, Avnimelech and Ritvo 2003). This is a source of nutrients that will enhance the density of chironomid larvae in the pond, and will also reduce the amount of organic sediment and thus the impact of waste from shrimp culture on the environment.

Chironomid larvae have all the attributes to be a natural food for juvenile shrimp. The naturally occurring larvae of chironomids in a shrimp pond are beneficial for shrimp culture and could reduce the requirement for pellet feed and help to maintain a suitable controlled environment for the shrimp. The reduction of pellet supply could reduce the accumulation of uneaten feed and thus help to maintain a good environment for shrimp culture throughout the day. There are some reports that chironomids can be parasitic or carry infection to other organisms, but this is specific to a few chironomids species and their host organisms such as insects, sponges, molluscs, bryozoans or fish (Sabine 2019). The species present in the ponds in the present study were not determined, but the most likely examples are species of genus *Chironomus*, and these have no reported risk of parasitic and other pathogenic infection of shrimp. The chironomid larvae are always present in the shrimp ponds and are a natural food eaten by the shrimp. The present study showed no adverse effects on growth and survival rate of the shrimp.

In conclusion, chironomid larvae were easily digestible and were the most acceptable of the natural foods provided. An abundance of chironomid larvae in a shrimp pond, therefore, can support growth of juvenile shrimp. An abundance of mosquito larvae and gutweed in the shrimp pond can also serve as additional food to help to support shrimp growth and survival rate. Moreover, gutweed can also provide shelter for natural food organisms in aquaculture ponds (Muangyao et al. 2011a). The results reported here can be of much benefit by improving feeding practice for shrimp culture. To provide a natural pond environment rich in larvae of insects such as chironomids is a promising way forward for shrimp aquaculture in tropical countries. Evaluation of the nutritional value and protein conversion efficiency of insect larvae such as chironimid larvae by white shrimp is needed and will be reported in another paper. This information will be of benefit for providing better understanding of shrimp growth and for application in shrimp culture systems.

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