www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 14: 205-211 (2014) DOI: 10.4194/1303-2712-v14_1_22

Effects of Mannan Oligosaccharide and Serotonin on Molting, Growth, Body Composition and Hepatopancreas Histology of White Leg Shrimp *Litopenaeus vannamei* (Boone 1931)

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Received 30 October 2012
Accepted 26 February 2014

Abstract

The effects of dietary mannan oligosaccharide (MOS), serotonin (5-HT) and the combination of both on molting, growth, survival, body composition, and hepatopancreas histology of the Pacific white shrimp *Litopenaeus vannamei* were investigated for 75 days. Dietary inclusion level of MOS at 3 g kg^{-1}, serotonin at 20 mg kg^{-1}, and both were tested in triplicate groups against a control diet lacking MOS or serotonin. The shrimps having an average initial weight of 1.35±0.04 g attained to final weights an average of 10.71 control, 11.05 serotonin and 12.26 MOS respectively. Although survival rate was high (86%) in the shrimp fed MOS-supplemented diet, no statistical difference was found among the groups ($P>0.05$).

The results indicate that inclusion of 3 g kg^{-1} MOS into diet enhance shrimp survival, moulting rate, growth and FCR compared to the control. Serotonin inclusion at 20 mg/kg also improved the same parameters, but suppressed moulting rate in comparison to the other groups ($P<0.05$). Hepatopancreatic tissues were not affected by dietary treatments. Body composition (protein, lipid, ash and dry matter) of the shrimps were also similar in all the dietary groups. In conclusion, the current results have demonstrated that MOS at 3.0 g kg^{-1} inclusion level could be used as growth promoter in *L. vannamei* diets.

Keywords: *Litopenaeus vannamei*, mannan oligosaccharides, serotonin, molting, growth.

Mannan Oligosakkarit ve Seretonin'in Beyaz Bacaklı Karides *Litopenaeus vannamei* (Boone)'nin Kabuk Değişimi, Büyüme, Vücut Kompozisyonu ve Hepatopankreas Histolojisi Üzerine Etkileri

Özet

Mannan oligosakkarit, serotonin ve her ikisinin birlikte kullanımının Pasifik karidesi, Litopenaeus vannamei'nin kabuk değişimi, büyüme, yaşama oranı, et kompozisyonu ve hepatopankteas histolojisi üzerine etkileri 75 günlük bir çalışma sürecinde araştırılmıştır. 3 g kg^{-1} MOS, 20 mg kg^{-1} düzeyinde serotonin ve her ikisinin birlikte aynı düzeyde kullanımının etkileri kontrol grubu ile karşılaştırılarak test edilmiştir. 1.35±0.04 g başlangıç ağırlığına sahip karidesler 10,71(kontrol), 11,05 (serotonin) ve 12,26 gram (MOS) ağırlığa ulaşmışlardır. MOS destekli yemlerle beslenen grupta yaşama oranı en yüksek olmasına ragmen (%86) gruplar arası istatistiki fark bulunamamıştır ($P>0,05$).

3 g kg^{-1} düzeyinde MOS katkılı yemlerle beslenen karideslerin yaşama oranı, kabuk değiştirme sıklığı, büyüme oranı ve FCR'ı kontrol grubuna göre önemli ölçüde etkilemiştir. 20 mg/kg düzeyinde yemlere eklenen serotonin aynı parametreleri pozitif yönde etkilemesine rağmen kabuk değiştirme sıklığı üzerine olumsuz etkide bulunmuştur ($P<0,05$). Farklı yem katkıları hepatopankreatik dokuları etkilememiştir. Karideslerin et kompozisyonu (Protein, lipid, kül, kuru madde) tüm muamelelerde benzer bulunmuştur. Sonuç olarak yalnız 3 g kg^{-1} düzeyinde MOS ilavesi *L. vannamei* yemlerine büyümeyi teşvik edici madde olarak kullanılabilir.

Anahtar Kelimeler: *Litopenaeus vannamei*, mannan oligosakkarides, serotonin, kabuk değiştirme, büyüme.

Introduction

The main purpose of aquaculture sector is to produce fish and crustacean species by the fastest growth rate and within a short period of time. Increasing the surface of production or intensification of production are the means of increasing production. Intensification of production can suppress the growth because of decreasing water and food quality, and increasing stress, bacterial, viral and parasitic infections. For these reasons, antibiotics are used generally to deal with the disease and maintain healthy organisms. Although preventive antibiotic treatment in farmed animals has numerous advantages including better production results and improvement in the general health condition, long-term low-dose

antibiotic therapy may lead to antibiotic resistance (Hillman, 2001). As a result, the application of antibiotics has been banned in Europe or their use has been restricted in the United States and some other countries. In addition, there is a growing public concern on the negative effects of the use of antibiotics (Cabello, 2006). Thus, drug-use policy and consumer attitudes have prompted interest in developing alternative strategies for disease control. One of the most encouraging paradigms in the recent year is to use functional feeds which extend the cultured organism's health and resistance of stress (Gatlin, 2002). Those compounds can be classified as immunonutrients and immunostimulants with the differences between the two relates to their mechanisms of action. One group of immunostimulants showing beneficial effects in terrestrial and aquatic animals is referred as prebiotics (Gibson and Roberfroid, 1995). Among the most common prebiotics, mannan oligosaccharide (MOS) has been recently receiving heightening application in aquaculture. The positive effects of MOS on growth, survival, gut health and bacterial resistant ability has been demonstrated in various fish and crustacean species (Li and Gatlin, 2004; Refstie *et al.*, 2006; Mahious *et al.*, 2006; Salze *et al.*, 2008; Staykov *et al.*, 2007; Genc *et al.*, 2007; Li *et al.*, 2007; Dimitroglou *et al.*, 2008; Dimitroglou *et al.*, 2009; Sang and Fotedar, 2010).

Pacific white shrimp, (*Litopenaeus vannamei*, Boone, 1931) is one of the most important cultured shrimp in all over the world. It was first introduced into Turkey for research purposes by the late 2007 (Kumlu *et al.*, 2011). Genc *et al.* (2007) found significant effects of MOS on growth performance and feed conversion rate in the green tiger shrimp *Penaeus semisulcatus*, when this substance was supplemented at a level of 3 g per kg diet. However, Chotikachinda *et al.* (2008) were unable to demonstrate any beneficial effects of inactive yeast cell wall on growth and survival of *L. vannamei* under their experimental conditions. They tested three dosages of inactive yeast cell wall (0, 1 and 2 g kg^{-1}) in three replicate groups of juvenile shrimps with an average initial weight of 7.15±0.05 g for four weeks. It was noticed that dosage, initial weight of shrimp and duration of the experiment of the above authors were insufficient to provoke any positive results. Therefore, we intended to test the effects of this growth promoter at higher dosage (3 g kg^{-1}) and experiment duration in the same species in order to see any improvements in a shrimp species other than *P. semisulcatus* (Genc *et al.*, 2007).

Serotonin or 5-Hydroxytryptamine (5-HT) is a neurotransmitter. This amine is discovered in the neurons of all major centres of the crustacean nervous systems (Elofsson *et al.*, 1982; Balzer *et al.*, 1997). It stimulates the release of several crustacean hormones including the hyperglycemic hormone (Keller *et al.*, 1985), the red pigment dispersing hormone (Rao and Fingerman, 1975), the neurodepressing hormone (Arechiga *et al.*, 1985), the molt inhibiting hormone (Mattson and Spaziani, 1985) and gonad stimulating hormone (Kullkarni *et al.*, 1992). It has been demonstrated that 5HT injection induced ovarian maturation in the crayfish Procambarus clarkii (Kullkarni *et al.*, 1992; Sarojini *et al.*, 1994) and the white Pacific shrimp *L. vannamei* (Vaca and Alfaro, 2000), the green tiger shrimp *P. semisulcatus* (Aktas and Kumlu, 2005). All the studies concerning serotonin have focused on broodstock maturation and endocrine system and that there is a clear lack of information on the effects of this hormone on moulting, growth and survival in other life stages of penaeid shrimps. The doses of serotonin used in this study were selected and modified for using orally based on previous kinetic studies in crustaceans (Aktaş and Kumlu, 2005; Genç *et al.*, 2007; Sainath and Reddy, 2011).

Therefore, the present study aimed at evaluating the effects of MOS, serotonin and the combination of both as dietary supplements on moulting, growth and survival of *L. vannamei* under controlled conditions.

Materials and Methods

Shrimp and Experimental Design

This study was undertaken at the Marine Research Station of Faculty of Fisheries, Mustafa Kemal University, Iskenderun, Hatay, Turkey. The Pacific white shrimp post-larvae of *L. vannamei* were obtained from Faculty of Fisheries, Cukurova University, 100 km away from our research center. Twelve glass rectangular gray-coloured aquariums (80 x 40 x 40 cm, 100 L water capacity) were used for the growth trial in the study. The aquariums were covered with a Styrofoam plate to decrease illumination, external disturbance and to prevent jumping out of the aquariums. The animals were acclimated to final experimental conditions for a period of seven days in the aquariums prior to starting of the experiment. The post-larvae were stocked into the respective aquariums after being weighed individually on an electronic balance to the nearest 0.001 g (Scaltec, SBA 53, Denver, Colorado, USA). Three replicates were assigned to each treatment or the control group. The initial weight (mean ± SD) of the shrimps was 1.35±0.04 g and 15 shrimps were stocked into each aquarium (0.15 shrimp L^{-1}).

Salinity and temperature were measured with a digital salinometer (YSI 30 model saline and thermometer USA). Throughout the experiment, water temperature was constantly maintained at 24±1°C by placing a 150-W submersible aquarium heater into each aquarium. Constant aeration was supplied through a silicon rubber tube with a glass rod at the tip. Inflow of fresh seawater (35 g L^{-1} salinity) was maintained at 15 L/day throughout the experiment. Total ammonium, nitrate and nitrite

levels in seawater was measured with a spectrophotometer (4050 UV/visible, LKB Biochrom Ultrospec II) (Parsons *et al.*, 1985).

Feed and Feeding

Phosphorylated MOS (MOS; AQUA-MYCES, Vitomix, CO, USA) from the outer portion of the cell wall of the yeast (S*accharomyces cerevisiae*) and serotonin (5-hydroxytryptamine, 5-HT, Creatinine sulfate complex, Sigma, St. Louis, MO, USA) were used as feed additive. A commercial sea bass larval diet (produced by INVE Belgie nv, Baasrode, Belgium) was used as basal diet. Four experimental diets were prepared by supplementation of 3 g kg^{-1} MOS, 20 mg serotonin, 3 g kg^{-1} MOS plus 20 mg kg^{-1} serotonin, and without additive (Control). All the diets were treated in the same way during the preparation. The basal diet and supplements were blended in a kitchen blender for homogenous mix, and sufficient water (400 g kg^{-1}) was added to form soft dough. The resultant dough was then passed through a mincer with 2 mm diameter die. The pellets were air dried at 40°C in an oven and stored at 4°C throughout the experiment. Diets were delivered to the shrimp by hand at 6% of the total biomass (Villalon, 1991). The amount of diets was justified according to the total shrimp biomass calculated for each sampling period. All feed was delivered to aquariums twice a day at 8 and 16 hours during the 75 days experimental period. All the shrimps were collected, weighed individually and then returned to their respective aquarium at every twenty days to determine their growth performance, survival rates as well as to adjust feeding rates. Exuviate were collected daily by siphoning to determine molting rate during the experiment.

At harvest, all the animals were weighed individually, counted and final survival, molting rate, daily weight gain, (final weight−initial weight /days of rearing), specific growth rate (SGR, 100 x log final body weight - log initial body weight /days), final biomass (average shrimp final weight × number of remaining of shrimp), feed conversion ratio (FCR, feed consumption / weight gained) were calculated.

At the end of the study, three shrimp from each group were anaesthetized with 5 mg L^{-1} quinaldine sulphate (Sigma Chemical Company, St. Louis, Missouri, USA) and the coeloma was opened for hepatopancreas exposure, which was then removed for light microscopy studies. Hepatopancreas samples were taken from the carapax region of the proximal intestine area. The samples were fixed for 24 h in 4% buffered formaline. After dehydration by passing tissues through a series of alcohol solutions (70, 85 and 98%), the samples were vacuum embedded in paraffin. The histological sections (4-5 lm; Leica, Bensheim, Germany) were stained for general morphological purposes with haematoxylin and eosin (H&E), and analysed and documented photographically with an Olympus BX50 microscope (Japan) (Takashima and Hibiya 1995; Roberts and Smail 2004). And then each group pooled and stored for frozen for proximate analysis. Proximate compositions of shrimp were analyzed according to AOAC (1997) procedures as follows: moisture was determined by oven-drying at 105°C for 24h, crude protein (Nx 6.25) by the Kjeldahl method and crude ash by combustion in a muffle furnace at 550°C for 16 h. Total lipid concentration was determined by extract with the chloroform-methanol method described by Bligh and dyer (1959).

Statistics

Data were subjected to one-way analysis of variance performed in the Statistical Package for Windows version 9.0 (SPSS Inc., Chicago, Illinois, USA). When overall differences were significant ($P<0.05$), Duncan's Multiple Range Test was used to compare the means between the dietary treatments.

Results

During the culture period, the water quality parameters were found to be similar in all aquariums. Temperature ranged from 24 to 25°C, salinity was 34-35 ppt and total ammonium, nitrate and nitrite were below the detection levels. Final shrimp survivals were high (>82%) and similar in all the experimental groups ($P>0.05$), although MOS supplementation resulted in higher survival (86%) than those of the others (Table 1).

Total numbers of molts were found to be different statistically among the experimental groups (Table 1). The shrimps fed MOS- supplemented diet molted a total of 48.33±3.2 times, those fed serotonin-supplemented diet molted only 30.00±2.65 times during the study ($P<0.05$). Generally, the MOS-supplemented diet at 3.0 g kg^{-1} improved growth performance and feed conversion of the shrimps ($P<0.05$) (Table 1). Growth performance of the shrimps fed serotonin or both serotonin and MOS-supplemented diets performed better than the control group, but the results were insignificant.

The shrimps fed on the control and experimental groups attained final average mean weights between 10.71 and 12.26 ($P>0.05$; Figure 1). Weight gain, feed conversion rates and specific growth rates of the shrimp fed on the diet supplemented with 3 g kg^{-1} MOS were better than those of the other groups ($P<0.05$). Body proximate compositions of the shrimp were not affected by the diets (Table 2). The morphology of the hepatopancreas was similar in all the groups (Figure 2).

Discussion

The current results suggest that diets supplemented with 3 g kg^{-1} MOS has a beneficial

Table 1. Growth performances of *Litopenaeus vannamei* fed on diets containing MOS and Serotonin (Srt)

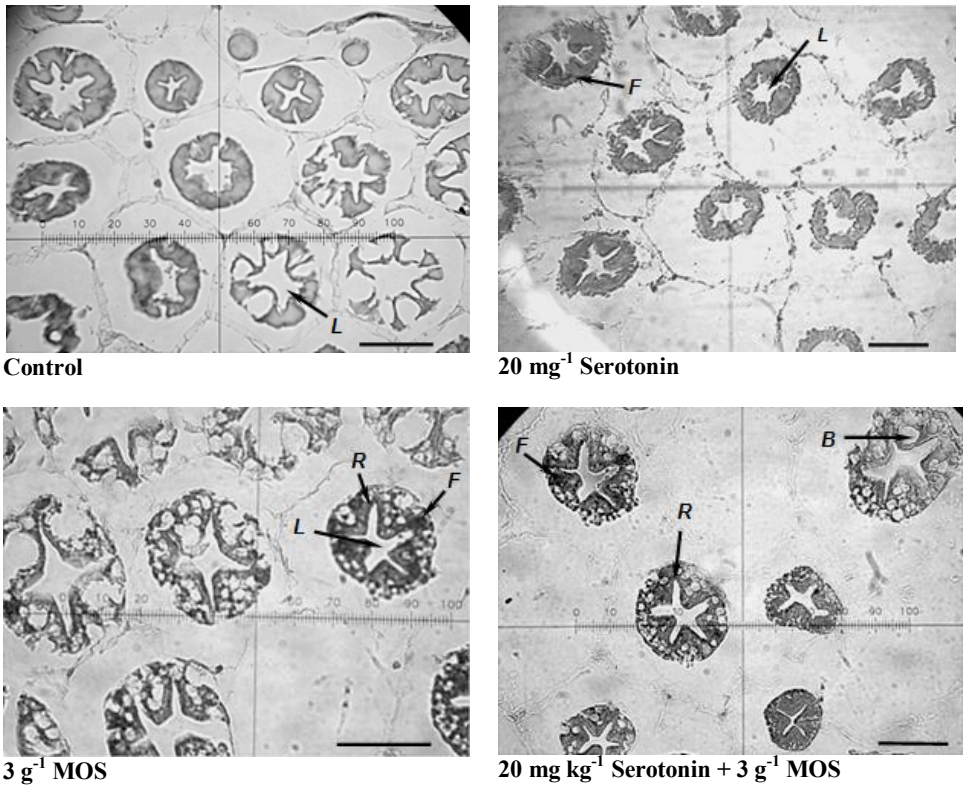
	Groups			
Parameters	Control	MOS (3 g kg^{-1})	Srt (20 mg kg^{-1})	MOS (3 g kg^{-1}) + Srt (20 mg kg^{-1})
Initial weight (g)	1.36±0.08[a*]	1.39±0.02[a]	1.31±0.03[a]	1.36±0.07[a]
Final weight (g)	10.71±1.04[a]	12.26±0.40[b]	11.05 ± 0.69[a]	10.87±0.17[a]
Live weight gain (g)	9.35±0.97[a]	10.87±0.40[b]	9.74 ± 0.68[ab]	9.51±0.16[ab]
Daily weight gain (g)	0.124±0.013[a]	0.144±0.005[b]	0.129 ± 0.009[ab]	0.126±0.002[a]
Survival (%)	83.00±3.46[a]	86.00±0.00[a]	82.33 ± 4.04[a]	84.00±3.46[a]
Number of molting	38.67±1.53[a]	48.33±3.21[b]	30.00 ± 2.65[c]	44.67±1.53[b]
Specific growth rate	2.75±0.07[a]	2.90±0.05[b]	2.84 ± 0.07[a]	2.78±0.06[a]
Feed conversion ratio	2.09±0.17[a]	1.91±0.17[b]	1.97 ± 0.11[b]	2.03±0.19[a]

*Mean values in rows with different superscripts are significantly different from each other (P<0.05).

Table 2. Chemical composition of whole *Litopenaeus vannamei* (g kg^{-1} of wet weight) fed on containing MOS and Serotonin (Srt)

Groups	Moisture	Crude protein	Lipid	Crude ash
Control	73.64	22.68 ± 2.10	1.79 ± 0.19	1.09 ± 0.00
MOS	74.66	22.55 ± 0.59	1.73 ± 0.13	1.06 ± 0.01
MOS+ Srt	74.00	22.80 ± 0.58	1.63 ± 0.13	1.57 ± 0.02
Srt	74.73	22.55 ± 2.47	1.65 ± 0.14	1.07 ± 0.02

*Values are the mean (± SEM) of triplicate composite samples of five shrimp.



Control **20 mg^{-1} Serotonin**

3 g^{-1} MOS **20 mg kg^{-1} Serotonin + 3 g^{-1} MOS**

Figure 1. Hepatopancreas transverse sections of *Litopenaeus vannamei* fed different supplemented-diets showing regular shaped tubular structures and types of epithelial cells.
*(L: lumen, R: small vacuolated cells, F: basophilic non-vacuolated cells, B: large vacuolated cells, Bar: 50 µm (H&E, ×40).

effect on growth performance of *L. vannamei*. Similar positive effects of dietary MOS supplementation on growth, survival and immune status of fish (Li and Gatlin, 2004; Staykov *et al.*, 2007; Genc *et al.*, 2007; Yılmaz *et al.*, 2007; Torrecillas *et al.*, 2007; Dimitroglou *et al.*, 2008; Nontawith, 2008; Gatlin and Burr, 2009), and crustaceans (Genc *et al.*, 2007; Li *et al.*, 2007; Sang *et al.*, 2009; Sang and Fotedar, 2010) have also been reported. The positive effect of prebiotics such as GroBiotic A, MOS and FOS were

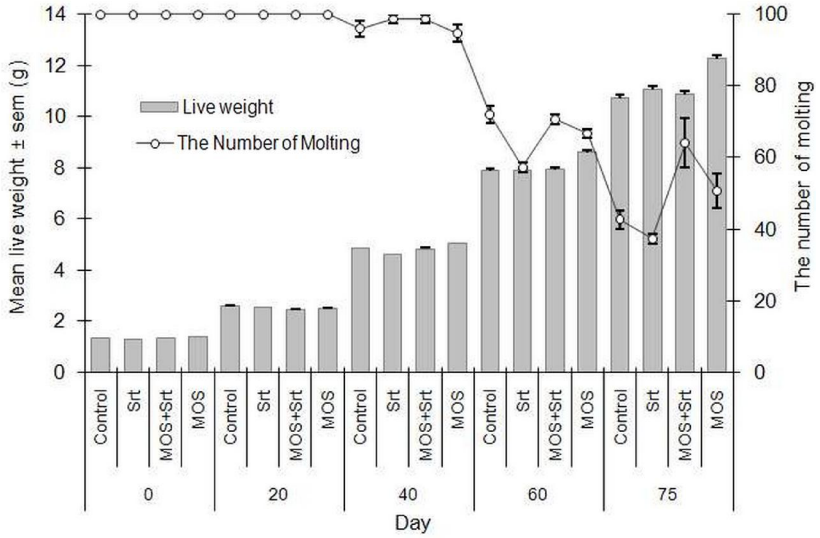


Figure 2. Molting and live weight gain of *Litopenaeus vannamei*, fed on diets containing MOS and serotonin (Srt).

attributed the increase in protein and organic matter digestibility (Gatlin *et al.*, 2006). On the other hand, Chotikachinda *et al.* (2008) reported an insignificant effect of 2 g kg^{-1} inactive yeast cell wall-supplementation on growth performance parameters of *L. vannamei* in a 28-day study period. It is obvious that the effect of MOS is related dosage, protein level of the diet and duration of feeding period. Based on our previous (Genc *et al.*, 2007) and the present results on shrimps, we recommend that a supplementation level of 3 g kg^{-1} MOS is suitable for the culture of shrimps.

Molting in crustaceans is under the control of both positively and negatively regulating hormones. Ecdysteroids and Methyl farnesoate is reported to induce precocious molting whereas molting is inhibited by molt inhibiting hormone secreted from X organ and sinus gland complex located in the eyestalks (Chang, 1993; Chang, 1997; Okumura and Aida, 2001). In the present study, the shrimps fed the diet supplemented with 3 g kg^{-1} MOS molted more frequently that the other groups. It is known that molting is repeated several times through life span of healthy shrimp in order to increase body size and mass for growth. It can be explain as one of the beneficial effect of MOS. The shrimps fed the diet supplemented with 20 mg kg^{-1} serotonin molted 62.5% less than those fed MOS-supplemented diet during the study ($P<0.05$). Sainath and Reddy (2011) also demonstrated that serotonin did not have an effect on the molting process in *O. senex senex*. Thus, it may be postulated that serotonin decelerate molting activity by stimulating the release of several crustacean hormones including the molt inhibiting hormone and mandibular organ inhibiting hormone.

Hepatopancreas is responsible for the production of some digestive enzymes, absorption of nutrients and deposition of lipids in shrimps (Dall *et al.*, 1990). It has been suggested that the R-cells of hepatopancreas could be used to investigate the nutritional value of prawn diets. So, Vogt *et al.* (1985) and Al Mohanna and Nott (1987) recommended applying histology in nutritional studies. In the present study, there were no apparent differences in the morphology of the R and F-cells between the experimental groups, nor were there any changes in the histological structures of the hepatopancreas (Figure 1). Similar results were also reported for *P. semisulcatus* (Genc *et al.*, 2007).

In conclusion, the present results indicate that inclusion of 3 g kg^{-1} MOS into diet enhance shrimp survival, moulting rate, growth and FCR compared to the control. Serotonin inclusion at 20 mg kg^{-1} also improved the same parameters, but suppressed moulting rate. Hence, it has been recommended that MOS at 3.0 g kg^{-1} inclusion level could be used as growth promoter in *L. vannamei* diets.

Acknowledgement

We thank Dr. Metin KUMLU from Department of Aquaculture, Fisheries Faculty, Cukurova University, Adana, Turkey for supplying the *L. vannamei* post larvae. This work was supported in part by grant from the Scientific Research Fund of Mustafa Kemal University (# 04.Y.0111).

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