Effects of Water Temperature and Rations Size on Growth Performance, Feed Utilization and Carcass Biochemical Composition of Juvenile Benni (*Mesopotamichthys sharpeyi*)

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Received: November 2012  Accepted: April 2012

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Abstract

A study was conducted to determine the effects of water temperature and rations size on growth performance, feed utilization and carcass biochemical composition of juvenile *Benni*. Six treatments representing a combination of two water temperature (24 and 28 °C) and three feeding rations (1%, 3% and 5%) were tested with three replicates. Each replicate was stocked with 12 fish (30.66±0.81 g average weight). Growth performances were significantly affected (P<0.05) between water temperature and ration size. Mean weight gain and percentage of weight gain significantly increased with increasing water temperature from 24 to 28 °C and higher (P<0.05) at 3% ration size. Average daily growth and specific growth rate were significantly (P<0.05) affected only by ration size at 3% level. Increasing water temperature (24 to 28 °C) resulted in increasing feed conversion efficiency (FCE). Whereas, FCE significantly decreased with increasing rations size from 1 to 5% at each water temperatures. Protein efficiency ratio (PER) followed a trend similar to that in FCE. The highest protein content of carcass (41.17%) was observed at 3% feeding ration at 28 °C. Lipid content was significantly highest in group fed 5% at 24 °C (13.58%). Hepatosomatic index was not significantly (P>0.05) affected by water temperatures and feeding rations. Viscerosomatic index (VSI) was significantly affected (P<0.05) by with increasing water temperatures and ration size. Results showed the 3% BW/day ration was optimal for growth of *M. sharpeyi* juvenile at water temperature 28 °C. (P<0.05)

Keywords: Water temperature, Rations size, Growth performance, Carcass composition, *Mesopotamichthys sharpeyi* juvenile.

1. Introduction

*Benni* (*Mesopotamichthys sharpeyi* Güther, 1874), Actinopterygii, Cyprinidae, is an important rare native commercial fishery species in the Mesopotamia basin and highly valuable as freshwater food fish in the Karoon River region. Farm gate price for benni has fluctuated in the past decade and feed cost accounted for 40-60% of total production cost for this omnivore and detrivore feeder species. Productivity, economical viability and environmental sustainability of commercial benni culture operations is, therefore, highly dependent upon feed management.

Feed costs make up a large fraction of the total fish production expenses and optimizing growth rates and feed efficiency in fish depends on the way in which food is made available, the amount of food...
delivered, feeding method, feeding frequency, duration of each feeding period and the characteristics of the diet (Gelineau et al., 1998; Khan and Abidi, 2010). It is necessary to optimize feeding regimes to improve the economical and environmental sustainability of aquaculture. Optimal feeding ratio may vary depending on species, age, size, environmental factors, husbandry and feed quality (Goddard, 1995).

Water temperature and ration size are two of the most important factors influencing the growth of fish (Brett, 1979; Desai and Singh, 2009). The metabolic rate, growth, energy expenditure and feed intake of fish are highly influenced by water temperature. When temperature is low, growth rates, feeding rates, and metabolic rates are suppressed; whereas elevated temperatures correlate with an increase in growth up to an optimum point above which thermal stress occurs (Jobling, 1993; Baum et al., 2005; Fang et al., 2010), hence to determine the optimal feeding rate at specific water temperature is prerequisite to the success of aquaculture production (Desai and Singh, 2009). Therefore, it is important to investigate growth of benni in relation to temperature and ration so to provide useful knowledge for relevant farmers.

In culture practices, feeding fish presents particular difficulties mainly ensuring that all the feed distributed in the water is effectively eaten (Desai and Singh, 2009). Under restricted feeding conditions, growth performance may change. Usually, the best feed conversion efficiencies (FCEs) are obtained below satiation (Meyer-Burgdorff et al., 1989; Zoccarato et al., 1994), and at temperatures slightly below that stimulating maximum growth rate (Imsland et al., 2000; Van Ham et al., 2003). Although, there are many published papers about the effects of ration and/or water temperature on growth, feed utilization and body biochemical composition of different fish species (Hung et al., 1993; Russell et al., 1996; Burel et al., 1996; Ruyet et al., 2004; Sun and Chen, 2009; Desai and Singh, 2009; Fang et al., 2010; Khan and Abidi, 2010), this study reports on the effect and interaction of water temperature and feed ration, on growth rate, feed utilization, hepatosomatic index and carcass biochemical composition of *Mesopotamichthys sharpeyi* juvenile in order to provide fundamental information for the adjustment of ration level and water temperature in the large-scale artificial culture of benni.

2. Materials and Methods

2.1. Fish Acclimation

Juvenile of benni, *M. sharpeyi* were obtained from Azadegan Fish Farm, Khozestan, Iran. The juvenile fish were kept in laboratory in a 1000 L fiberglass tank filled with freshwater and temperature of 22 °C for 3 days. Then, 216 juvenile with 30.66±0.81 g average weight and 15.1±0.77 cm average length were selected and randomly transferred into 2 experimental tanks (1000 L) of the same water temperature. Fish were held in the experimental tanks at the final temperature for 7 days to ensure full thermal adaptation. The fish were fed to satiation twice a day at 09:00 and 17:00 h with artificial pellets (Table 1, Beyza Technology Co., Ltd., Iran) during acclimation. At the end of the acclimation, all fish were starved for 24 h to evacuate the gut. Normal and healthy fish were selected to weigh and assigned to different aquarium. Before weighting, fish were anaesthetized using MS-222 (60 mg L$^{-1}$) to reduce stress.

2.2. Experimental System and Feeding Trail

The experiment consisted of six treatments with three replicates each and was conducted in glass aquaria (100 × 48 × 25 cm) equipment with separate thermostatic water heaters (MX-1017 Titanium heater, WEIPRO Aquarium Equipment Co. Ltd, China) were used in the experiment. Fish were held at water
temperatures of 24 and 28 °C and fed on three rations size of same diet (Commercial diet, Beyza Feed Mill, Iran) were tested at each temperature. Each aquarium was stocked with 12 fish by the mean initial weight depicted in Table 2 at the time of stocking in each treatment. The proximate composition of the experimental diet is shown in Table 1. Feed was provided at 1%, 3% and 5% of body weight per day (BW/day). Fish were hand-fed to satiation twice a day (about 09:00 and 17:00) with corresponding ration levels throughout for 56 days. Uneaten food in the tank of each replicate was collected 30 min after feeding by pipetting and then dried at 75 °C. After every 15 days, all fishes from each aquarium were weighed individually for recording weight. During the experimental period, 25% of water in each aquarium was changed once a day by the same volume and temperature. Aeration was provided continuously except during feeding to maintain dissolved oxygen above 6 mg/l. Water quality parameters were monitored daily. During this period, all fish were exposed to a natural photoperiod (light periods ranging from 12 h to 13 h) with similar light conditions for all tanks.

Table 1. Proximate composition of experimental diet (g 100 g dry diet )

<table>
<thead>
<tr>
<th>Moisture</th>
<th>Crude protein</th>
<th>Fat</th>
<th>Ash</th>
<th>Carbohydrate</th>
<th>Gross Energy (MJ g )</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.39±0.05</td>
<td>40.64±0.11</td>
<td>14.54±0.08</td>
<td>9.23±0.04</td>
<td>28.57±0.12</td>
<td>2.027</td>
</tr>
</tbody>
</table>

* Value are means of three replicate samples per diet.

Carbohydrate, calculated by difference.

C Gross energy, calculated based on 0.017, 0.0398 and 0.0237 MJ/g for carbohydrate, lipid and protein, respectively.

## Table 2. Growth performances and feed utilization of M. sharpeyi juvenile fed different size of rations and two water temperatures.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water temperature (°C)</th>
<th>Ration size (% BW/day)</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Mean weight gain (g)</th>
<th>Average daily growth (g)</th>
<th>Weight gain (%)</th>
<th>SGR (%/day)</th>
<th>FCEa</th>
<th>PERa</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 1</td>
<td>30.27±0.94</td>
<td>35.80±1.02</td>
<td>5.53±0.21</td>
<td>0.10±0.01</td>
<td>18.27±0.78</td>
<td>0.30±0.01</td>
<td>28.54±1.22</td>
<td>0.38±0.02</td>
<td>100±1.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 3</td>
<td>30.46±0.25</td>
<td>37.29±0.38</td>
<td>6.83±0.21</td>
<td>0.12±0.03</td>
<td>22.42±0.64</td>
<td>0.36±0.01</td>
<td>17.31±1.33</td>
<td>0.30±0.03</td>
<td>98±5.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 5</td>
<td>31.06±0.86</td>
<td>37.41±0.96</td>
<td>6.35±0.38</td>
<td>0.11±0.01</td>
<td>20.44±1.12</td>
<td>0.33±0.02</td>
<td>11.66±1.40</td>
<td>0.28±0.01</td>
<td>98±5.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 1</td>
<td>30.84±0.37</td>
<td>36.40±0.64</td>
<td>5.56±0.33</td>
<td>0.10±0.02</td>
<td>18.03±0.94</td>
<td>0.30±0.01</td>
<td>29.49±1.48</td>
<td>0.39±0.01</td>
<td>99±4.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 3</td>
<td>30.32±1.06</td>
<td>38.17±1.09</td>
<td>7.85±0.23</td>
<td>0.14±0.03</td>
<td>25.89±0.23</td>
<td>0.41±0.03</td>
<td>22.95±1.11</td>
<td>0.33±0.02</td>
<td>100±1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 5</td>
<td>31.02±0.45</td>
<td>37.51±1.06</td>
<td>6.49±0.32</td>
<td>0.12±0.03</td>
<td>20.92±1.22</td>
<td>0.34±0.02</td>
<td>11.56±1.19</td>
<td>0.29±0.01</td>
<td>99±3.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA (P-values) T ns 0.047 0.045 ns 0.039 ns 0.032 ns ns

Mean ± SE values (n=6) with different superscripts in each column are significantly different (P<0.05).

a SGR(Specific growth rate), FCE (Feed conversion efficiency), PER (Protein efficiency ratio).

b T, Water temperature; R, Ration size; T × R, Water temperature × Ration size interaction.
2.3. Proximate Analysis

At each temperature, initial body composition of fish was analyzed from 15 samples of fish and frozen at -20 °C prior to the commencement of trial. At the end of experiment, all fish were weighted. Four fishes from each replicate were sacrificed for the analysis of proximate composition of carcass and three fish specimen from each aquarium were dissected and liver and viscera weighed to determine hepatosomatic index (HSI) and viscerosomatic index (VSI), respectively. Proximate composition of experimental diets and whole body proximate composition were analyzed using standard methods (AOAC, 1997). Each analysis was conducted in triplicate. Moisture was determined by drying the samples in an oven at 105 °C for 24 h to a constant weight. Ash was determined by incineration of samples in a muffle furnace at 550 °C for 12 h. Crude protein (N×6.25) was measured by Auto kjeldahl unit (Buchi, German; model B-414, K-438, K-371 and K-370). Total lipid was extracted from samples by homogenization in chloroform and methanol (2:1, v/v) (Bligh and Dyer, 1959), methylated and transesterified with boron trifluoride in methanol (AOAC, 1997). Gross energy, was calculated based on 0.017, 0.0398 and 0.0237 MJ/g for carbohydrate, lipid and protein, respectively.

2.4. Data Analysis

Growth performance was determined and feed utilization was calculated as followings:

1. Daily weight gain (g) = [mean final body weight (g) - mean initial body weight (g)]/number of days.
2. Weight gain (%) = [(final body weight (g)-initial body weight (g))/initial body weight (g)] ×100.
3. Specific growth rate (SGR)(%/day)= [(In final weight - In initial weight) x100]/ duration in days.
4. Feed conversion efficiency (FCE) (%) = [wet weight gain (g)/dry weight of feed (g)] ×100.
5. Protein efficiency ratio (PER) = Increment in body weight (g)/ protein intake (g).
6. Hepatosomatic index = [weight of liver (g)/total weight of fish (g)] ×100.
7. Viscerosomatic index = [weight of viscera (g)/total weight of fish (g)] ×100.

Data are expressed as mean ± standard error. A two-way analysis of variance (ANOVA) was used to determine differences among water temperature and ration size (2×3 factorial), using SPSS 11.5 statistical software. Differences were considered significant at 0.05 (P<0.05). Tukey HSD multiple comparison test was used to determine significant differences among means.

3. Results

3.1. Growth Performances and Feed Utilization

Results of growth performances and feed utilization of M. sharpeyi juvenile fed different size of rations and two water temperatures are given in Table 2. Growth performance of M. sharpeyi were significantly affected (P<0.05) among the water temperature and ration size. Mean weight gain and percentage of weight gain significantly increased with increasing water temperature from 24 to 28 °C at each ration levels. In addition, these parameters were significantly higher at 3% ration size at two water temperatures (3%> 5%> 1%). Average daily growth (ADG) and specific growth rate (SGR) were significantly affected by ration size at 3% level (0.12 g and 0.36 % BW/day at 24 °C and 0.14 g and 0.41 % BW/day at 28 °C, respectively). At any given ration levels, ADG and SGR did not vary significantly with water temperatures. Increasing water temperature (24 to 28 °C) resulted in increasing feed conversion efficiency (FCE) regardless of the feeding levels; whereas, FCE significantly decreased with increasing rations size from 1 to 5% at each water temperatures. Protein efficiency ratio (PER) followed a trend similar to that in FCE.
3.2. Carcass Proximate Composition, Hepatosomatic Index and Viscerosomatic Index

Proximate composition (% wet weight) of carcass, hepatosomatic index (HSI) and viscerosomatic index (VSI) of *M. sharpeyi* juvenile fed different rations and two water temperatures is given in Table 3. Carcass protein content showed a linear relationship with water temperature at each feeding levels. At any given water temperatures, protein content of carcass did not vary significantly with ration size. The highest protein content (41.17%) was observed at 3% feeding ration at 28 °C. Lipid content of carcass showed an inverse relationship with water temperature at each rations size. Lipid content was found to be highest at a feeding ration of 3% at each water temperature. Lipid content was significantly highest in group fed 5% at 24 °C (13.58%), while fish fed 1% at 28 °C resulted in lowest lipid content (12.22%). However, there was no significant difference in lipid content by ration level 5% and 3%. Hepatosomatic index (HSI) of *M. sharpeyi* juvenile was not significantly (P>0.05) affected by water temperatures or feeding rations, and there was no significant water temperature-ration size interaction. Viscerosomatic index (VSI) was significantly affected (P<0.05) by water increasing temperatures and ration size.

4. Discussion

The growth–ration relationship and energy allocation are affected by many factors, such as experimental method (Tang et al., 2003), feed type or composition (Cui et al., 1992, 1994; Yang et al., 2003), fish body size (Niimi and Beamish, 1974; Staples and Nomura, 1976; Xie et al., 1997), water temperature (Elliott, 1976; Malloy and Targett, 1994; Sogard and Spencer, 2004; Fang et al., 2010), etc. As an endemic species in Iran, many factors that affect the growth performance and feed utilization of *M. sharpeyi* are still poorly understood.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Lipid (%)</th>
<th>Ash (%)</th>
<th>HSI</th>
<th>VSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water temperature (°C)</td>
<td>Ration size (%BW/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual treatment means</td>
<td>24</td>
<td>1</td>
<td>76.92±0.32</td>
<td>40.18±0.69bc</td>
<td>12.78±0.24b</td>
<td>9.02±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>76.52±0.66</td>
<td>40.47±0.60b</td>
<td>13.52±0.22a</td>
<td>9.11±0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>76.28±0.78</td>
<td>40.69±0.58b</td>
<td>13.58±0.19a</td>
<td>8.43±0.03</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1</td>
<td>75.95±0.70</td>
<td>40.76±0.52b</td>
<td>12.22±0.21bc</td>
<td>9.33±0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>75.81±0.61</td>
<td>41.17±0.58c</td>
<td>13.36±0.23ab</td>
<td>8.64±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>75.90±0.51</td>
<td>40.18±0.55bc</td>
<td>13.22±0.25ab</td>
<td>9.01±0.04</td>
</tr>
<tr>
<td>Initial</td>
<td>76.91±0.28</td>
<td>39.78±0.54</td>
<td>11.25±0.21c</td>
<td>8.89±0.04</td>
<td>1.78±0.13</td>
<td>4.85±0.29</td>
</tr>
</tbody>
</table>

Mean ± SE values (n=3) with different superscripts in each column are significantly different (P<0.05).

**ANOVA**

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Mean Square</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>1</td>
<td>8.99</td>
<td>5.61</td>
<td>0.037</td>
</tr>
<tr>
<td>R</td>
<td>1</td>
<td>8.99</td>
<td>5.61</td>
<td>0.037</td>
</tr>
<tr>
<td>T×R</td>
<td>1</td>
<td>8.99</td>
<td>5.61</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Mean ± SE values (n=3) with different superscripts in each column are significantly different (P<0.05).

a T, Water temperature; R, Ration size; T×R, Water temperature × Ration size interaction.
The treatment containing high (28 °C) water temperature and intermediate (3%BW/day) feeding ration produced greater growth performances in *M. sharpeyi* juvenile. In addition, growth performances were significantly affected by water temperature and ration size. However, the increase in ADG and SGR were comparable among increasing water temperature. A higher water temperature imposes two antagonistic effects on growth: increasing temperature has a negative effect on growth due to higher energy cost for maintenance metabolism, but also a positive effect due to higher efficiency of transforming food energy into net energy (Xiao-Jun and Ruyung, 1992; Van Ham et al., 2003). Within the temperature range tolerated by a fish species, growth rate increases with increasing temperature (Zanuy and Carrillo, 1985; Shimeno and Shikata, 1993; Parpoua, 1998; Van Ham et al., 2003), but when the experimental temperature reaches the upper extreme of the tolerated range, the opposite result is obtained (Williams and Caldwell, 1978; Hasan and MacIntoch, 1991; Van Ham et al., 2003). According to the results of this study, it can be concluded that, although a temperature of 28 °C has been considered as optimal for growth of *M. sharpeyi* juvenile, the highest weight gain was observed in the fish maintained on 28 °C at 3% ration level. Houlihan et al. (1993), Britz et al. (1997) and Azevedo et al. (1998) observed that fish were markedly influenced by the temperature of water in which they lived. Increased growth has also been reported in *Labeo rohita* reared in polyhouse at average temperature of 19 °C as compared with those in outdoor tanks at average temperature of 14.8 °C (Khan et al., 2004). Desai and Singh (2009), who reported that gradual increase in mean body weight of *Cyprinus carpio* was observed from 4% to 6% ration while it decreased at 7% ration at the 24 and 28 °C water temperatures. Similar findings were observed in *L. rohita* (Ahmed, 2007), *Acipenser transmontanus* (Hung et al., 1989) and *Cirrhinus mrigala* (Khan et al., 2004).

FCE and PER were significantly affected by water temperatures and ration size in this study. Feeding parameters were gradually increased with increasing water temperature. The highest FCE and PER achieved at minimal ration level. These were decreased with increasing feeding rate at both temperatures in *M. sharpeyi* juvenile. Desai and Singh (2009), revealed that feed conversion efficiency of *Cyprinus carpio* differed significantly among the varying rations and decreased significantly when ration levels were increased to 7% at both 28 and 32 °C water temperature and noted higher FCE was recorded at 28 °C as compared with 32 °C. Zhen-Yu et al., (2006) and Ng. et al. (2000) reported that PER decreased with increasing feed ration in grass carp, *Ctenopharyngodon idella* and in bagrid catfish, *M. nemurus*, respectively. Similar patterns have been reported in *C. carpio* (Hasan and Macintosh, 1991), *C. mrigala* (Khan et al., 2004), *L. rohita* (Ahmed, 2007) and *Rachycentron canadum* (Sun et al., 2006). When the growth–ration relationship was asymptotic, food conversion efficiency usually showed a domed curve as ration increased so that growth and food conversion efficiency were not maximized at the same ration level (Xie et al., 1997; Fang et al., 2010). Under conditions of restricted feeding, fish tend to optimize their digestion in order to utilize nutrients in the feed more efficiently, thereby increasing the feed conversion (Meyer-Burgdorff et al., 1989; Zoccarato et al., 1994; Van Ham et al., 2003).

Carcass protein of fish after 56 days of experimental period exhibited no significant difference (P>0.05) for ration levels, but it was significantly different (P<0.05) for temperature indicating that there is a direct effect of ration on the proximate composition of fish at varying water temperatures. Maximum protein content was observed at 6% feeding ration at 28 °C (Desai and Singh, 2009). Khan et al. (2004) described at 4% and 6% ration levels in *C. mrigala*, highest protein content observed. While Van Ham et al. (2003) explained that whole body protein of *Scophthalmus maximus* juvenile increased significantly in all groups during the growth period, as a result of temperature, ration and
interaction effects. Carcass lipid content was significantly affected by water temperatures and feeding levels with different trend. The lipid content followed an inverse relationship with water temperature but effects of ration size showed irregular trend. Carcass lipid content of the fish fed at 3% ration level was higher than that of the fish fed at 1% and 5% ration levels. Similar result have been reported by Desai and Singh (2009) where the Body lipid contents of C. carpio at 28 and 32 °C increased up to 6% ration and thereafter decreased trend was found in both temperatures. In addition, Khan et al. (2004) in C. mrigala and Ahmed (2007) in L. rohita observed the same results. Generally, the proximate composition of cultured fish is affected by various endogenous and exogenous factors such as fish size, dietary energy input, metabolic energy demands, feeding strategy and environmental conditions (Shearer, 1994; Van Ham et al., 2003). The proximate composition results of this study on juvenile benni are generally in agreement with these conclusions.

Indices of condition, such as HSI and VSI, are often used to assess the nutritional status of fish because they can be determined easily and quickly, and may provide an indication of physiological condition (Cui and Wootton, 1988; Desai and Singh, 2009). HSI were not significantly affected (P>0.05) either by ration level or water temperature. Significant effect (P<0.05) of ration levels was observed on VSI at both the water temperatures. Desai and Singh (2009) reported that among the four rations i.e. 4%, 5%, 6% and 7% at two water temperature tested on C. carpio 4% ration at 28 and 32 °C had significantly lower VSI than fish fed higher ration levels where as HIS did not differ much among treatments. In conclusion, based on the results in the present study, it was found that water temperatures and rations size affected the growth of the juvenile of M. sharpeyi significantly. In this case, the optimum feeding strategy is an intermediate, not maximum ration, in order to obtain rapid growth at water temperature 28 °C. Thus, to obtain higher growth rate, commercial farmers could feed juvenile benni 3% BW/day ration size with artificial feed.

Acknowledgments

The authors thank director and staff at the Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology for providing the necessary facilities for the experiment. We would like to thank Khorramshahr University of Marine Science and Technology for supporting this work under research grant contract No 53.

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