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Effects of Dietary Yeast Extract Levels on Growth Performance, Digestibility and Antioxidant Capacity of the Taiwan Loach

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Abstract

An 8-week feeding trial was conducted to determine the effects of different levels of dietary Yeast Extract (YE) on growth performance, digestibility and antioxidant capacity of the Taiwan loach. The trial contained 6 diets with different levels of wet YE, namely 0%, 1%, 2%, 3%, 4% and 5% (groups T1-T6). Each group contained 8 replicates with 12 loaches. Results indicated that the Specific Growth Rate (SGR) of loaches that were fed T5 diet were significantly higher than those of the other groups, whereas the feeding rate and feed conversion rate were just the opposite. The association of the regression analysis between different dietary YE and the loach SGR were: SGR=−9244YE3+622.03YE2-3.7768YE+3.9325($p<0.05$). The apparent digestibility coefficient of the dry matter and the Amylase activity levels of the loaches fed with the YE were higher than those that were fed the T1 diet. The liver SOD activity was increased significantly following the increase in the YE supplementation ($p<0.05$). In conclusion, YE supplementation in the loach diet could promote growth, improve feed digestion and utilization, and enhance antioxidant capacity. The optimum levels of wet YE required for feeding the Taiwan loach species were estimated to 4.16%.

Keywords: Yeast extract; Taiwan loach; Growth performance; Digestibility; Antioxidant capacity

Introduction

Taiwan loach belongs to a modified loach variety. In recent years, Taiwan loach has been widely farmed in China. Intensive high-density aquaculture has resulted in high requirements for feeding (increased feed coefficient), high incidence of diseases and increased costs. Therefore, recent studies have mainly focused on developing feeds that can improve the disease resistance of the Taiwan loach and reduce its feed coefficient [1,2]. YE is rich in nucleotides, β-glucan, mannooligosaccharides and small peptides. Certain studies have shown that nucleotides and small peptides can increase the umami and sweetness of the feed, which provides a strong attracting effect for fish [3,4]. Nucleotides can further accelerate the differentiation, growth and repair of intestinal cells [5], which can reduce the feed coefficient and improve growth performance [6,7]. In addition, it provides sufficient nucleotide supply for fish immune system [8]. Mannanoligosaccharides can prevent the adsorption and colonization of intestinal pathogens, and maintain intestinal health [9,10]. They further promote the absorption of nutrients, and improve the phagocytic rate of white blood cells and lysozyme activity. These processes play a major role in body immunity [11]. β-glucan can trigger an immune response by stimulating lymphocyte proliferation and activating cellular and humoral immunity [12].

It has been shown that YE can improve the digestive function, reduce the feed coefficient and improve the growth performance of fish. A previous study demonstrated that 1%, 2% and 4% of YE group significantly improved the specific growth rate of the rohu, *Labeo rohita* and reduced its feed coefficient compared with the corresponding parameters of the control group [13]. Furthermore, the increase in the YE can significantly enhance the feeding rate of Nile tilapia *Oreochromis niloticus* L. resulting in a linear weight gain rate [14]. An increase in the parameter specific growth rate was observed in the pikeperch (*Sander lucioperca* L.), which was fed with YE. Group fed with 6% of YE exhibited significantly higher growth rate compared with that of the control group [15]. The dry matter and crude protein digestibility of each test group were significantly higher than those of the control group when the fish meal was replaced by YE in the diet of the shrimp *Litopenaeus vannamei* [16].

Currently, the applications of YE have not been demonstrated in loach breeding. The objective of the present study was to assess the dietary supplementation of YE on the growth, nutrient digestibility and antioxidant capacity of Taiwan loach.

Materials and Methods

Experimental design

A total of 6 treatment groups with different levels of YE were designed in each experiment. Each treatment group was tested in 8 culture tanks and each culture tank had 12 loaches, resulting in a total number of 576 fishes.

YE

YE used in the present study was produced by the Guangzhou Xintun Aquatic Technology Co., Ltd. YE is a product of autolysis and enzymatic hydrolysis that is produced by cell wall decomposition of yeast. The percentages of moisture content, crude protein and glucan of the product were 57.30%, 20.18% and 8.5%, respectively. The extract contained 7.99% of small peptide content (<1000 D), 5.2% of mannooligosaccharide, 3.2% of total nucleic acid and 1.1/100 g of amino nitrogen.

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Experimental diets

A total of 6 isonitrogenous and isoenergetic experimental diets (Table 1) were performed using Thai fishmeal, soybean meal, peanut meal and fermented soybean meal as protein sources. The diets were prepared with 0%-5% of YE corresponding to each group (T1-T6 group), respectively. Y_2O_3 was added to the experimental diets (0.1%) as an inert marker for the digestibility trial.

Experimental procedure

The rearing experiment was carried out in the aquaculture laboratory of Hebei Normal University. The experimental fish species were purchased from a loach farm in the Luquan District (Shijiazhuang, China). After acclimatization, 576 loaches with an average initial body weight of 0.2 g were stocked into 96 cylindrical plastic tanks with a diameter of 50 cm and a height of 50 cm. The water depth was 35 cm. Water temperature was controlled at 28 (± 0.1). The 1/3 of the water volume was exchanged daily to maintain the water quality. Indoor natural light was used and the experimental process was performed in a quiet environment. The loaches were domesticated for 2 weeks with commercial feed to adapt to the culture environment and the feeding time during the experiment.

Following the acclimation period, loaches were fasted for 24 h and the initial body weight (W_0) was measured. Each group was fed different experimental diets. The feeding was performed three times a day (8:00, 13:00, 18:00) and the residual diets were drained by a siphon and collected in a residual bait bag after 20 min. The samples of residual diets were placed in an air dry oven, and kept at a constant temperature of 65°C. The feces were collected from the 7th day to the end of the formal experimental period and dried at 65°C in order to measure and record the dry weight of the residual diets. At the end of the 56 day experimental period, loaches were fasted for 24 h and weighted. One fish was collected from each aquarium and anesthetized with MS222 (Green Hengxing Biotech Co., Ltd., Beijing, China) at a concentration of 220 mg/L [17]. The body length (Lt) of the loach was measured with a vernier caliper (± 0.02 mm), and subsequently the fish was dissected in order to extract the liver and intestine organs. The tissues were washed with pre-cooled normal saline (0.65%), and placed in microtubules. All tissues were stored in liquid nitrogen and the liver samples were used to determine SOD activity and MDA content. The intestinal samples were used to determine the digestive enzyme activity. The remaining loach was used as a total fish sample to determine its body composition.

Ingredients	T1	T2	T3	T4	T5	T6
Fishmeal	200	200	200	200	200	200
Wheat middling	130	130	130	130	130	130
Soybean meal	115	115	115	115	115	115
Peanut meal	110	110	110	110	110	110
Wheat Bran	80	80	80	80	80	80
Fermented soybean meal	55	55	55	55	55	55
Cottonseed meal	45	45	45	45	45	45
Rapeseed meal	45	45	45	45	45	45
Beer yeast powder	28	22.4	16.8	11.2	5.6	0
Yeast extract	0	10	20	30	40	50
Zeolite powder	22	17.6	13.2	8.8	4.4	0
Lecithin	85	85	85	85	85	85
Mono-calcium phosphate	30	30	30	30	30	30
Calcium carbonate	15	15	15	15	15	15
Sodium chloride	10	10	10	10	10	10
Vitamin-mineral premix[a]	18	18	18	18	18	18
Others[b]	12	12	12	12	12	12
Total	1000	1000	1000	1000	1000	1000
Nutrient Content (%)						
Moisture	8.4	8.4	8.4	8.41	8.41	8.4
Crude protein	33.33	33.38	33.37	33.33	33.36	33.33
Crude lipid	7.16	7.15	7.16	7.17	7.16	7.16
Ash	17.58	17.72	17.5	17.67	17.7	17.6
Dietary energy (KJ/g)	16.44	16.45	16.45	16.44	16.45	16.45
[a]Vitamin-mineral premix: provided by Hebei Haitai Technology Corporation, Hebei, China.						
[b]Others: potassium chloride 1.0, methionine 1.0, lysine 3.0, taurine 3.7, choline chloride 2.0, mildew inhibitor 0.3, Y_2O_3 1.0.						

Table 1: The proximate compositions and nutrient content of the experimental diets (g kg^{-1}).

Biochemical analysis of samples

The feed and whole fish samples were analyzed as follows:

The determination of crude protein, crude fat content and energy value were conducted by a Foss automatic Kjeldahl nitrogen analyzer Kjeltec8420 (Foss, USA), a Soxhlet extraction method by petroleum ether and a Parr6300 automatic oxygen bomb calorimeter (Parr, USA), respectively. Following digestion of the sample based on the method of Refstie et al. [18], the content of yttrium in the feed and faeces was determined by the American Thermo Scientific X Series 2 ICP-MS (inductively coupled plasma mass spectrometry) and the digestibility coefficient was accordingly measured. The intestinal digestive enzymatic activity, liver Superoxide Dismutase SOD activity (U/mL) activity and Malondialdehyde (MDA, nmol/mL) content were determined using the relevant kits (Jiancheng Biological Co., Ltd., Nanjing, China).

Data processing and statistical analysis

The parameters were measured according to the following formulas:

1. Specific growth rate (%/d, SGR)=(ln W_t−lnW_0)/t×100

2. Relative growth rate (%/d, RGR)=(W_t−W_0)/W_0/t×100

3. Feeding rate (%/d, FR)=IT/[(W_t+W_0)/2×t]×100

4. Feed conversion rate (FCR)=IT/(W_t−W_0)

5. Viscerosomatic index (%,VSI)=100×W_v/W_t

6. Condition Factor (%CF)=100×W_t/L_{t3}

7. Apparent digestibility coefficient of dry matter (%, ADDM)=(1−B/B')×100

8. Apparent digestibility coefficient of protein (%, ADCP)=(1−A/A'×B/B')×100

Where W_t is the body weight (g) on the specific sampling day, W_0 is the initial body weight (g) at the beginning of the trial, t is feeding duration in days from the beginning of the trial until the specific sampling day, IT is the total dry diet intake (g) from the beginning of the trial to the specific sampling day, W_v is the liver weight (g) on the specific sampling day, L_t is fish length (cm) on the specific sampling day, A and A' is the percentage of crude protein in faeces and feed respectively, B and B' is the percentage of Y_2O_3 in feed and faeces respectively.

The experimental data were analyzed statistically with ANOVA and

nonlinear regression using the Statistical 10.0 software. p value lower than 0.05 ($p<0.05$) was considered for significant differences.

Results

Growth performance of the Taiwan loach

Growth performance parameters are listed in Table 2. W_t, RGR and SGR were significantly influenced by the addition of YE ($F(5,42)=2.7283$, $p=0.0323$; $F(5,42)=3.3446$, $p=0.0126$; $F(5,42)=3.4959$, $p=0.0101$). The highest values were noted in the T5 group and the values corresponding to the T5 and T6 groups were significantly higher than those of the T1 and T2 groups. In addition, YE exhibited significant effects on the FR and FCR of the Taiwan loach ($F(5,42)=2.8772$, $p=0.0257$; $F(5,42)=2.9759$, $p=0.0221$). The lowest values were noted in the T5 group, followed by the T6 group. The T5 and T6 groups exhibited significantly lower parameters than those of the T1 and T2 groups ($p<0.05$).

A positive nonlinear regression was observed, according to the following regression equation: SGR=−9244YE3+622.03YE2−3.7768 YE+3.9325 ($F(5,42)=5.5335$, $p=0.0027$) (Figure 1). The highest value on the Y-axis that corresponded to SGR was 4.16%.

Intestinal digestive enzyme activity and digestibility of Taiwan loach

Results about intestinal digestive enzyme activity and digestibility are displayed in Table 3. YE caused significant changes on amylase activity ($F(5,30)=6.4849$, $p=0.0003$). The change noted in the T5 group was the highest, followed by T3 and T4 groups, which exhibited significantly higher amylase values than those of the T1 group ($p<0.05$). YE exhibited no significant effects on trypsin and lipase activities ($F(5,30)=2.3387$, $p=0.0660$; $F(5,30)=0.7466$, $p=0.5949$). YE indicated a significant effect on ADDM of Taiwan loach ($F(5,18)=5.4594$, $p=0.0076$). The increase caused in YE was followed by a decreasing trend. The ADDM of T5 group was the highest compared with that of the other groups. Significant changes were noted for T1-T4 groups ($p<0.05$). The effects of YE on ADCP were not significant ($F(5,18)=2.7684$, $p=0.0689$).

Composition, Body index and antioxidant capacity of Taiwan loach

Results about composition, body Index and antioxidant capacity are displayed in Table 4. In addition, YE used in the feed exhibited a significant effect on the condition factor and the liver SOD activity ($F(5,42)=3.6375$, $p=0.0037$; $F(5,30)=5.5741$, $p=0.0020$). T4 group of the muddy Taiwan fertilizer in Taiwan exhibited significantly higher condition factor than that of the other 5 groups. The liver SOD activity levels of T4-T6 groups were significantly higher than those of the other three groups. The effect of adding YE to the feed did not cause significant changes on the other indicators ($p>0.05$).

Discussion and Conclusion

YE and feeding rate

The food intake of Nile tilapia *O. niloticus* was increased significantly with the increase of YE in the feed [14]. This finding could be attributed to the extract nucleotide contents, namely adenosine monophosphate, inosine monophosphate, uridine monophosphate and adenosine diphosphate that function as palatability enhancers and feed attractants [7]. The results of the present study differ to the findings reported previously. With the increase of the YE in the diets, the feeding rate of the Taiwan loach was decreased. The feeding rate corresponding to T5

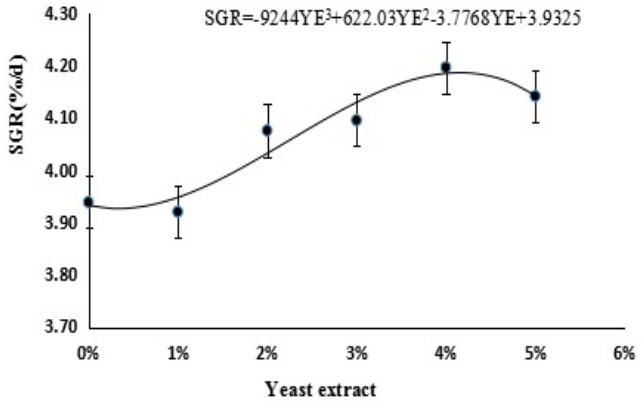

Figure 1: The relationship of the regression analysis between different dietary YE and the loach SGR. SGR=−9244YE3+622.03YE2−3.7768YE+3.9325 ($F(5,42)=5.5335$, $p=0.0027$). The highest value on the Y-axis that corresponded to SGR was 4.16%.

	W_0 (g/tail)	W_t (g/tail)	FR (%)	SGR (%/d)	RGR (%/d)	FCR
T1	0.35 ± 0.01	3.22 ± 0.25^a	5.33 ± 0.36^b	3.94 ± 0.12^a	5.13 ± 0.46^a	1.87 ± 0.15b
T2	0.35 ± 0.01	3.20 ± 0.40^a	5.34 ± 0.62^b	3.92 ± 0.21^a	5.08 ± 0.71^a	1.88 ± 0.27b
T3	0.35 ± 0.01	3.43 ± 0.45^{ab}	4.93 ± 0.68^{ab}	4.08 ± 0.23^{ab}	5.50 ± 0.80^{ab}	1.71 ± 0.29^{ab}
T4	0.35 ± 0.01	3.48 ± 0.40^{ab}	5.00 ± 0.56^{ab}	4.10 ± 0.19^{ab}	5.59 ± 0.71^{ab}	1.73 ± 0.23^{ab}
T5	0.35 ± 0.01	3.65 ± 0.08^b	4.65 ± 0.10^a	4.20 ± 0.06^b	5.90 ± 0.15^b	1.58 ± 0.04^a
T6	0.36 ± 0.01	3.64 ± 0.23^b	4.70 ± 0.27^a	4.14 ± 0.09^b	5.87 ± 0.40^b	1.61 ± 0.11^a
Different letters at the end of the figure on the same line indicate significant difference $p<0.05$ (Means ± SD).						

Table 2: Effects of dietary yeast extract levels on growth performance of Taiwan loach.

	Trypsin (U/gprot)	Lipase (U/gprot)	Amylase (U/mgprot)	ADDM (%)	ADCP (%)
T1	0.868 ± 0.146	11.07 ± 2.23	0.3417 ± 0.0370^{a}	57.20 ± 2.27^{a}	76.19 ± 1.27
T2	0.911 ± 0.176	11.27 ± 1.42	0.3703 ± 0.0318^{ab}	61.44 ± 0.99^{ab}	77.69 ± 0.56
T3	1.004 ± 0.155	11.65 ± 1.64	0.4269 ± 0.0345^{c}	60.95 ± 2.34^{ab}	78.35 ± 3.65
T4	1.100 ± 0.136	12.51 ± 1.64	0.4203 ± 0.0413^{c}	61.53 ± 2.86^{ab}	78.14 ± 1.66
T5	1.125 ± 0.167	12.48 ± 1.89	0.4347 ± 0.0168^{c}	66.50 ± 3.16^{c}	81.52 ± 2.33
T6	1.067 ± 0.213	12.24 ± 1.74	0.4030 ± 0.0423^{bc}	63.89 ± 1.49^{bc}	80.52 ± 1.11
ADDM, apparent digestibility for dry matter; ADCP, apparent digestibility for crude protein; Different letters at the end of the figure on the same line indicate significant difference p<0.05 (Means ± SD).					

Table 3: Effects of dietary yeast extract levels on intestinal digestive enzyme activity and the digestibility of the Taiwan loach.

	Moisture (%)	Crude lipid (%)	Crude protein (%)	Ash (%)	Condition factor (%)	Visceroso-matic index (%)	SOD (U/ml)	MDA (nmol/mL)
T1	75.01 ± 3.82	4.27 ± 1.01	16.85 ± 2.37	3.15 ± 0.45	0.57 ± 0.04^{a}	5.26 ± 0.83	40.21 ± 5.87^{a}	2.44 ± 0.42
T2	75.41 ± 2.47	4.61 ± 0.81	16.75 ± 1.90	2.99 ± 0.27	0.57 ± 0.04^{a}	5.37 ± 0.95	42.01 ± 1.59^{a}	2.68 ± 0.32
T3	75.59 ± 3.36	4.21 ± 0.76	17.79 ± 2.39	3.06 ± 0.43	0.57 ± 0.05^{a}	5.22 ± 0.63	40.71 ± 4.89^{a}	2.83 ± 0.42
T4	74.40 ± 3.39	4.58 ± 0.96	17.47 ± 2.57	3.22 ± 0.41	0.61 ± 0.08^{b}	5.21 ± 0.78	49.52 ± 4.94^{b}	2.83 ± 0.33
T5	74.69 ± 0.92	4.57 ± 0.36	17.73 ± 1.18	3.17 ± 0.09	0.58 ± 0.04^{a}	5.52 ± 0.73	50.03 ± 3.57^{b}	2.42 ± 0.26
T6	73.63 ± 1.64	4.78 ± 0.48	16.66 ± 0.25	3.26 ± 0.13	0.57 ± 0.04^{a}	5.49 ± 0.95	51.01 ± 3.80^{b}	2.78 ± 0.80
Different letters at the end of figure on the same line indicate significant difference p<0.05 (Means ± SD).								

Table 4: Effects of dietary Yeast extract levels on Composition, Body Index and antioxidant capacity of Taiwan loach.

group was the lowest, which was 11.22% lower than that of T1 group ($p<0.05$). Reduced FR with increased doses of YE most likely resulted from the absence of YE palatability. On the other hand, the addition of YE increased the parameters ADDM, amylase activity and growth rate, and reduced the FCR. This resulted in an increased utilization efficiency of the loach on the feed, resulting in successful intake of sufficient nutrition components and energy supplies even in the presence of low feeding rate.

YE and growth performance

A previous study has shown that the increase in the amount of YE can increase the specific growth rate of the pikeperch *Sander lucioperca* L. up to 6% compared with that of the control group [15]. Similarly, the increase in the amount of YE caused a linear increase in the weight gain rate of Nile tilapia *O. niloticus* L. [14]. In the present study, the increase of YE caused an increase in the specific growth rate and relative growth rate of the Taiwan loach, followed by a subsequent decrease. The group that was fed 4% of YE exhibited the best growth performance compared with that of T1 group. The growth rate was increased by 15.01%. The rapid growth of the Taiwan loach in addition to the intake of mannooligosaccharides and β-glucan could improve the intestinal and physical health and the digestibility of the rainbow trout (*Oncorhynchus mykiss)*, Atlantic salmon (*Salmo salar*), Pacific white shrimp (*Litopenaeus vannamei)* [9,10,12]. A previous study has shown that the exogenous-supplemented nucleotides are essential for cell growth, renewal, and rapid organ development [19]. It can further promote red drum *Sciaenops ocellatus* growth [20]. According to the regression equation between the specific growth rate of the Taiwan loach and YE, the optimum dose of wet YE in the Taiwan loach feed was approximately 4.16%, which was equivalent to 1.78% of dry YE added to the Taiwan loach diet.

YE and feed digestibility

Digestibility, which is the ratio of nutrients absorbed by the fish to the total nutrient intake, is an important indicator for assessing the nutritional value of the feed [21]. The effects of YE on fish digestibility differ among studies. The digestibility of dry matter and crude protein of the shrimp *Litopenaeus vannamei* increased significantly with the increase of YE ($p<0.05$), whereas trypsin and amylase activities were significantly increased ($p<0.05$) and lipase activity was significantly decreased ($p<0.05$) [16]. The apparent digestibility of the dry matter of Nile tilapia *O. niloticus* was significantly decreased ($p<0.05$) following the increase in the YE of the feed, whereas the apparent digestibility of the crude protein decreased significantly ($p<0.01$) [22]. The results of the present study are different from the findings reported for tilapia. The addition of YE significantly increased the apparent digestibility of dry matter in the Taiwan loach to 16.26%, whereas the FCR was decreased by 15.51%. Furthermore, the addition of YE significantly increased the amylase activity levels of the Taiwan loach to 27.22%, whereas the trypsin and lipase activities were also increased. The aforementioned results indicated that the addition of YE could improve the digestion and utilization rate of the feed for the Taiwan loach and could increase the feed efficiency.

The mannan-oligosaccharides and nucleotides that were present in YE played a significant role in the improvement of the digestive function. Previous studies have found that YE rich in mannooligosaccharides can prevent the adsorption and colonization of intestinal pathogens [23,24] and improve the intestinal integrity [25,26]. This further improves gut digestive enzyme activity [27,28] and maintains intestinal health. It's was demonstrated that mannooligosaccharides could significantly increase the apparent digestibility of crude protein of the narrow clawed crayfish, (*Astacus leptodactylus*) *leptodactylus eschscholtz*, while amylase and lipase activities were also significantly improved [29]. Nucleotides play an important role in cell division, differentiation and development of intestinal cells and also promote the repair of intestinal mucosa [30]. Nucleotides can promote the number of Bifidobacterium in the intestines. The growth of Lactobacillus inhibits the proliferation of pathogenic bacteria such as *Escherichia coli* and *Clostridium perfringens*, and is beneficial to the gut of young animals [31]. In addition, YE used in the present study was a paste-like substance obtained by debittering, breaking and enzymatic hydrolysis. This processes results in concentrated brewer's yeast. Its total protein content has low molecular weight, 100% water solubility after enzymatic hydrolysis, and no cell wall, rendering it beneficial for absorption [32]. The cell wall has been suggested to cause the reduced nitrogen digestibility commonly found in single cell protein sources [33].

YE and antioxidant capacity

Superoxide Dismutase (SOD) is an important antioxidant enzyme [34], which can eliminate the harmful substances produced by fish during the metabolic process and can further improve the immune function of the fish body. The higher the enzyme activity, the better the health and metabolism of the animal [35]. The addition of YE to the feed can increase the activity of SOD in the liver of the Taiwan loach. SOD activities of the T4-T6 groups were significantly increased compared with that of the T1 group ($p<0.05$). The highest SOD activity increase noted was 26.86%. This finding indicated that YE could improve the antioxidant capacity of the Taiwan loach. Malondialdehyde (MDA) is an important product of membrane lipid peroxidation and can indirectly determine the degree of cellular membrane damage. The addition of YE did not significantly affect MDA in the liver of the Taiwan loach ($p>0.05$), indicating that the addition of YE to the feed improved the activity of the antioxidant enzymes, and could possibly boost the immunity of the Taiwan loach.

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