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Original Article

Growth performance and nutrient utilization of glass catfish (*Kryptopterus lais*) larvae in response to varying dietary protein levels

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Abstract: This study evaluated the effects of varying dietary protein levels (35, 40, 45, and 50%) on growth, feed utilization, and body composition in Glass catfish, *Kryptopterus lais*, larvae over 30 days. Larvae with an initial average weight of 0.15±0.01 g were distributed into 12 aquaria at a density of 50 individuals per tank. Fish were fed four times daily to apparent satiation. The results indicated that weight gain (WG) increased with dietary protein levels up to 40%, beyond which WG declined. Similarly, the feed conversion ratio was lowest in fish fed the 40% protein diet. The protein efficiency ratio was significantly higher in *K. lais* larvae fed the 40% protein diet compared to those fed diets containing 45 and 50% protein. Body composition analysis revealed that larvae fed the 40% protein diet exhibited the highest protein content, while those fed the 35% protein diet had a significantly elevated lipid content. Broken-line regression analysis, based on the specific growth rate, estimated the optimal dietary protein requirement for maximal growth performance to be 42.89%. These findings provide a quantitative basis for formulating diets that effectively meet the protein requirements of *K. lais* larvae.

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Introduction

The glass catfish, *Kryptopterus lais*, is a freshwater species of ecological and economic significance, particularly in the Riau Province of Indonesia. Valued for its delicate taste and market demand, *K. lais* serves as an important food source while also supporting the livelihoods of rural fishing communities (Muchtar and Rosyadi, 2020). This species exhibits commercial value and has potential for development in the ornamental fish trade (Efrizal et al., 2023), while also contributing to native fish biodiversity in lakes and reservoirs (Muchtar and Rosyadi, 2020). However, the increasing market demand for *K. lais* has led to intensified fishing activity in the wild, raising serious concerns about over-exploitation and the potential risk of population decline or local extinction (Nurmayani et al., 2020; Thamrin, 2020). Aquaculture presents a sustainable solution to conserve this species. In addition to alleviating harvesting pressure on wild stocks, aquaculture offers the potential to enhance *K. lais* production while maintaining high-quality fish yields (Arisuryanti et al., 2020; Leksono et al., 2020).

The development of *K. lais* aquaculture continues to grow in response to increasing public demand. In this context, ensuring a consistent and reliable supply of larvae and juvenile fish is essential to support the sustainability of grow-out operations. However, many fish farmers lack awareness regarding the specific biological characteristics and dietary protein requirements of *K. lais* larvae. While commercially available artificial feeds are often used to meet the nutritional needs of larvae in mass production systems, their application is typically based on availability rather than suitability. According to Anizah et al. (2017), most commercial feeds are not formulated explicitly for *K. lais* larvae and fail to meet their essential protein and nutrient requirements. To achieve economically viable breeding and larval rearing of *K. lais*, feeding strategies must be tailored to support the nutritional needs of the larvae, thereby

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promoting optimal growth and survival. Among all dietary components, protein is widely recognized as the most critical nutrient, as it is required in the most significant proportion to support tissue development and overall growth. Notably, protein sources also represent the most expensive component in aquafeed formulations, underscoring the importance of accurately determining optimal dietary protein levels to balance growth performance with economic efficiency.

Therefore, understanding species-specific protein requirements is critical for formulating nutritionally balanced and cost-effective aquafeeds. Accurate determination of protein needs at various life stages and sizes is essential to optimize growth performance and feed efficiency in cultured fish. Moreover, the protein-to-energy (P/E) ratio must be specifically established for each species to ensure the formulation of an optimal diet. An appropriate P/E ratio not only promotes growth but also helps reduce production costs. Excess dietary protein, when not utilized for growth, is metabolized for energy, resulting in increased nitrogenous waste such as ammonia (Rahimnejad et al., 2021). Conversely, if dietary energy is insufficient, protein is diverted from growth to serve as an energy source, reducing feed efficiency. Thus, maintaining a balanced ratio of protein and energy in the diet is crucial to support optimal physiological function, growth, and environmental sustainability in aquaculture systems (Anizah et al., 2017).

Numerous studies have investigated protein requirements in various fish species, demonstrating that optimal levels vary depending on species-specific nutritional needs. For example, Mir et al. (2020) found that a dietary protein level of 55% supported optimal growth and survival in catfish *Clarias magur* larvae. Khan et al. (1993) reported an optimal protein requirement of 42% for *Mystus nemurus* larvae, while Chou et al. (2001) determined that 44.5% protein was ideal for cobia *Rachycentron canadum*. Similarly, Wang et al. (2023) identified 40.87% as the optimal protein level for loach, *Paramisgurnus dabryanus*, and Jang et al. (2022) reported that 54% protein supported the best growth performance in grouper, *Sebastes schlegeli*, larvae. However, information on the dietary protein requirements of *K. lais* at the larval stage remains unavailable. Therefore, the present study aimed to evaluate the effects of different dietary protein levels on the growth performance, feed utilization, and body composition of *K. lais* larvae.

Materials and Methods

Experimental diets: Four isoenergetic experimental diets were formulated with varying protein levels of 35, 40, 45, and 50%. The artificial feed, prepared in paste form, was made by mixing fish meal, shrimp by-product meal, soybean meal, wheat flour, fish oil, and a vitamin-mineral premix, according to the specific formulation for each treatment (Table 1). Water was gradually added to the dry ingredients until a uniform paste was achieved, following the method described by Hadi et al. (2024). The resulting paste feeds were analyzed for proximate composition (Table 1) and amino acid profiles (Table 2) using standard procedures outlined by AOAC (2005). To maintain quality, the feeds were stored in a freezer and thawed at room temperature for approximately 15 minutes before feeding (Hadi et al., 2024).

Experimental design and feeding trial: Two-day-old *K. lais* larvae (initial weight: 0.01±0.00 g) were obtained from artificial breeding at the Fish Seed Center of the Integrated Agricultural Unit, Universitas Islam Riau, located in Kubang Raya Village, Kampar Regency, Riau, Indonesia. The larvae were transported to the Microalgae and Fish Nutrition Laboratory, Faculty of Agriculture, Universitas Islam Riau, for the experiment. Upon arrival, the larvae were acclimated for 30 minutes in a holding tank under laboratory conditions. A total of twelve glass aquaria (30×15×15 cm) were used as experimental units. Each aquarium was filled with 5 liters of dechlorinated water and stocked with 50 *K. lais* larvae. For the first 10 days, *K. lais* larvae were fed a natural diet of finely chopped *Tubifex* sp. The transition to artificial feed (weaning period) commenced on day 11. During the experimental period, fish were fed to apparent satiation four times daily at 08:00, 12:00, 16:00, and

Table 1. Formulation and proximate composition of the experimental diets.

Ingredients	Protein content in feed (% dry matter)			
	35	40	45	50
Fish meal	25	35	48	61
Shrimp by-product meal	24	23	24	25
Soybean meal	22	20	13	5
Wheat flour	23	16	9	3
Fish oil	2	2	2	2
Vitamin mix[1]	2	2	2	2
Mineral mix[2]	2	2	2	2
Total	100	100	100	100
Proximate analysis				
Crude protein	35.14	40.28	45.22	49.70
Crude lipid	10.12	9.99	9.42	8.09
Ash	8.05	9.20	10.74	10.93
Crude fiber	1.79	1.84	1.96	2.21
NFE[3]	44.90	38.69	32.66	29.07
Energy GE (kcal/100 g)[4]	476.01	479.28	477.90	476.45
Digestible energy/DE (kcal/100 g)[5]	357.01	359.46	358.43	357.34
Ratio DE/P (kcal/100 g)[6]	102.00	89.87	79.65	71.47

[1]Vitamin mix (mg/100 g diet): thiamine 5.0; riboflavin 5.0; Ca-pantothenate 10.0; niacin 2.0; pyridoxine 4.0; biotin 0.6; folic acid 1.5; cyanocobalamin 0.01; inositol 200; ρ-aminobenzoic acid 5.0; menadion 4.0; vitamin A palmitate 15.0; chole-calciferol 1.9; α-tocopherol 20.0; choline chloride 900.0.
[2]Mineral mix (mg/100 g diet): KH2PO4 412; CaCO3 282; Ca (H2PO4) 618; FeCl3.4H2O 166; ZnSO4 9,99; MnSO4 6,3; CuSO4 2; CuSO4.7H2O) 0,05; KJ 0,15; Dekstrin 450; Selulosa 553,51.
[3]NFE is calculated based on the difference of (100 - protein - fat - ash - crude fiber)
[4]Gross energy was calculated according to Chandan et al. (2021) as 5.7, 9.5, and 4.0 kcal/g for protein, fat, and NFE, respectively.
[5]Digestible energy (DE) = 75% × GE (Hepher, 1988)
[6]Energy-protein ratio (DE/P) = DE / (10 × % protein) (Hepher, 1988)

20:00 hours.

Water quality analysis: The experimental aquaria were operated under static conditions, with 50% of the water replaced twice daily after feces and uneaten feed were removed by siphoning. Water quality parameters, including temperature, dissolved oxygen (DO), pH, and ammonia, were monitored every five days. Temperature and pH were measured using a digital pH tester (H198108, Romania), DO was assessed using a DO meter (Lutron PDO-519, Taiwan), and ammonia levels were determined using an ammonia meter (HI715, Romania). The measured water quality parameters remained within acceptable ranges throughout the experimental period: temperature ranged from 27.20 to 29.50°C, DO from 5.3 to 6.2 mg/L, pH from 5.54 to 6.50, and ammonia from 0.07 to 0.21 mg/L. These conditions were considered suitable to support the survival and growth of *K. lais* larvae.

Sample collection and calculation: On days 10, 20, and 30 of the experiment, 25 fish were randomly sampled from each aquarium to measure body weight. Whole-body composition analyses of the larvae were conducted at both the beginning and the end of the experimental period. Larval mortality was recorded daily, and the survival rate was calculated at the end of the experiment. Growth performance and feed utilization parameters were calculated using the following equations:

WG (g) = final weight (g) – initial weight (g)

SGR (%/day) = 100 × [ln final weight (g) - ln initial weight (g)] / time (days)

PER = body weight gain (g) / protein intake in the feed (g)

DFI (%/day) = 100 × total feed given per fish (g) / [(initial weight + final weight + weight of dead fish) ×

Table 2. Amino acid composition of experimental diets.

Amino acid (AA)	Protein level (%)			
	35	40	45	50
Essential AA				
Arginine	1.44	1.47	1.45	1.53
Histidine	0.27	0.33	0.55	0.70
Isoleucine	1.15	1.22	1.28	1.35
Leucine	2.30	2.39	2.46	2.48
Lysine	2.12	2.22	2.35	2.47
Methionine	0.44	0.50	0.58	0.64
Phenylalanine	1.02	1.18	1.27	1.34
Threonine	0.66	0.69	0.74	0.77
Valine	1.49	1.53	1.62	1.71
Non-essential AA				
Aspartic acid	1.95	2.11	2.52	2.74
Glutamic acid	3.02	3.68	4.06	4.66
Serine	1.33	1.46	1.54	1.62
Proline	1.03	1.11	1.23	1.32
Glycine	1.42	1.48	1.51	1.68
Alanine	1.37	1.45	1.48	1.55
Tyrosine	1.01	1.08	1.16	1.20
Cystine	0.53	0.51	0.57	0.59

days / 2]

FCR = total feed consumption (g) / [(final fish biomass (g) + weight of dead fish (g)) – initial fish biomass (g)]

CF (%) = 100 × [body weight (g) / body length^3 (cm)]

VSI (%) = 100 × viscera weight (g) / fish weight (g)

SR (%) = 100 × (total fish at final/total fish at initial)

Statistical Analysis: All data were analyzed using one-way analysis of variance (ANOVA) in SPSS version 24.0. When significant differences among treatment groups were detected, Duncan's Multiple Range Test (DMRT) was applied for post hoc comparisons. Statistical significance was considered at $P<0.05$. A second-order polynomial regression analysis was conducted to estimate the optimal dietary protein level.

Results

The growth performance, feed utilization, and survival of *K. lais* larvae fed diets with varying protein levels over 30 days are presented in Table 3. Over a 30-day period, the larvae exhibited variations in growth performance, feed utilization, and survival in response to differing dietary protein levels. A 40% protein diet resulted in significantly higher final weights and weight gain compared to the other experimental groups ($P<0.05$). The specific growth rate was significantly elevated in larvae fed the 40% protein diet compared to those fed 35 and 50% protein diets ($P<0.05$), with no significant difference observed when compared to the 45% protein group ($P>0.05$).

The protein efficiency ratio showed no significant difference between the 35 and 40% protein groups ($P>0.05$), but was significantly lower in the 45 and 50% protein groups ($P<0.05$). Daily feed intake was significantly higher in larvae fed the 40% protein diet compared to all other treatment groups ($P<0.05$). Feed conversion ratio was significantly lower in the 40% protein group ($P<0.05$). The condition factor and viscero-somatic index did not exhibit significant differences among the groups ($P>0.05$). The survival rate decreased significantly with increasing dietary protein levels ($P<0.05$). Statistical modeling using second-order polynomial regression estimated the optimal protein requirement for growth at 42.86%

Table 3. Growth performance, feed utilization, and survival of *Kryptopterus lais* fed diets with different protein levels.

Parameters	Protein level (%)			
	35	40	45	50
IW (g)	0.15±0.01	0.15±0.01	0.15±0.01	0.15±0.01
FW (g)	0.46±0.01[a]	0.58±0.02[c]	0.53±0.01[b]	0.50±0.01[ab]
WG (g)	0.31±0.01[a]	0.43±0.03[c]	0.38±0.01[b]	0.35±0.01[ab]
SGR (%/day)	3.76±0.06[a]	4.53±0.21[c]	4.28±0.09[bc]	3.97±0.15[ab]
PER	0.81±0.04[c]	0.84±0.03[c]	0.57±0.02[b]	0.38±0.01[a]
DFI (%/day)	1.83±0.03[b]	2.10±0.04[c]	1.76±0.05[b]	1.40±0.06[a]
FCR	3.05±0.12[b]	2.68±0.07[a]	3.26±0.11[bc]	3.56±0.15[c]
CF (%)	0.34±0.01	0.34±0.02	0.34±0.01	0.33±0.01
VSI (%)	2.37±0.08	2.62±0.14	2.51±0.15	2.39±0.14
SR (%)	80.00±1.15[c]	79.33±2.40[c]	67.33±2.91[b]	53.33±2.40[a]

The values presented are the averages of three replicates and are shown as mean ± SE; values in the same row with different superscripts indicate significant differences (P<0.05).

Table 4. Whole-body composition of *Kryptopterus lais* larvae fed experimental diets with different protein levels.

Parameters (%)	Protein level (%)			
	35	40	45	50
Moisture	78.27±0.01	78.24±0.01	78.21±0.01	78.24±0.03
Crude Protein	11.28±0.04[a]	14.03±0.08[d]	12.47±0.09[c]	12.13±0.02[b]
Crude lipid	3.89±0.04[a]	3.63±0.04[a]	4.19±0.02[b]	4.41±0.03[c]
Ash	2.39±0.08	2.29±0.01	2.43±0.01	2.44±0.01

The values presented are the averages of three replicates and are shown as mean ± SE; values in the same row with different superscripts indicate significant differences (P<0.05).

(Fig. 1).

Whole-body composition of *K. lais* larvae: No significant differences in whole-body composition, in terms of moisture and ash, were observed among the treatments (P>0.05, Table 4). Whole-body crude protein content was significantly higher in fish fed the 40% protein diet compared to those in other treatment groups (P<0.05). In contrast, whole-body crude lipid content was significantly lower in fish fed the 35 and 40% protein diets (P<0.05).

Discussions

Protein is one of the most essential nutrients for fish growth (Zhang et al., 2017; Yan et al., 2021), and it also plays a critical role in tissue renewal and repair (Deng et al., 2011). The essential amino acids required for fish growth are entirely supplied by dietary protein, which also serves as an energy source. When dietary protein levels are too low, fish growth is impaired. Conversely, excessive dietary protein may be used for energy metabolism, leading to increased nitrogen excretion and potentially hindering growth and development (Debnath et al., 2007; Zhang et al., 2022).

In the present study, the growth performance of *K. lais* larvae initially improved as dietary protein levels increased from 35 to 40%, but declined significantly with further increases. A similar phenomenon has been previously reported in several fish species, including red-spotted grouper (*Epinephelus akaara*) (Wang et al., 2016), common carp (*Cyprinus carpio*) (Ahmed and Maqbool, 2017), ide (*Leuciscus idus*) (Ren et al., 2017), Caspian trout (*Salmo caspius*) (Mohseni et al., 2019), white snapper (*Lateolabrax maculatus*) (Cai et al., 2020), rainbow trout (*Oncorhynchus mykiss*) (Ahmed and Ahmad, 2020), *Schizopygopsis younghusbandi* (Zeng et al., 2020), *Culter mongolicus* (Qian et al., 2022), and yellow grouper (*Larimichthys polyactis*) (Zhang et al., 2022). Park et al. (2022) reported that fish growth tends to plateau or decline when dietary protein levels exceed requirements, as the dietary protein is used to metabolize the excess amino acids. Conversely, excessive dietary protein intake can impair fish

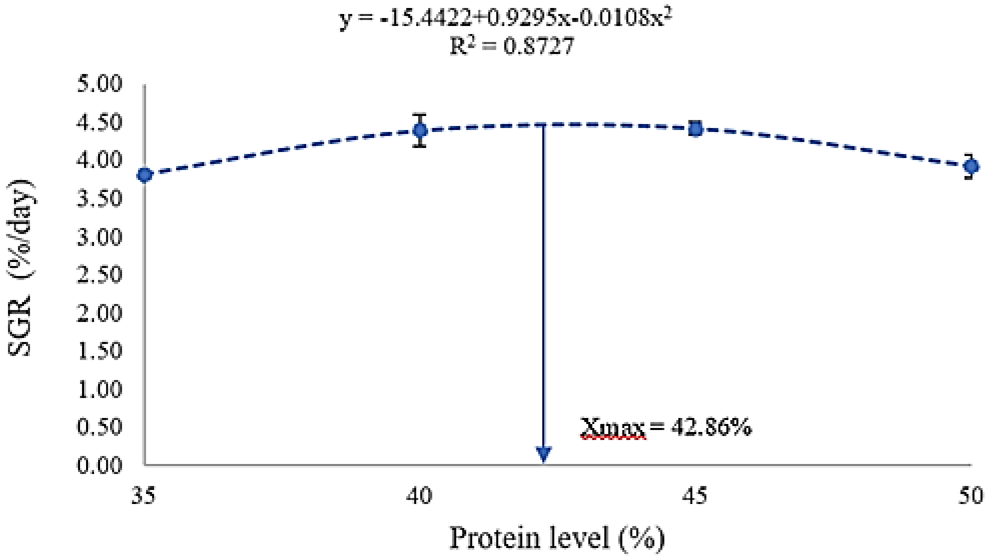

Figure 1. Dietary protein requirement for the growth of *Kryptopterus lais* larvae estimated using second-order polynomial regression.

growth performance, as a greater proportion of energy is diverted toward protein catabolism rather than protein deposition. The observed reduction in weight gain at elevated protein levels may be attributed to limited energy availability for growth, coupled with an insufficient supply of non-protein energy required for efficient deamination of surplus protein feed (Jauncey, 1982). The negative effects of surpassing optimal dietary protein thresholds, such as reduced growth rates and diminished protein utilization efficiency, have been well-documented in previous studies (Kim and Lee, 2005; Cho et al., 2005). Similar findings have also been reported in other species, such as striped catfish (*Pangasianodon hypophthalmus*) (Jayant et al., 2018) and silver catfish (*Rhamdia quelen*) (Rotili et al., 2018). The optimal protein range for cultured carnivorous fish has been reported in various studies to lie between 40 and 60% (Kpogue et al., 2013; Wang et al., 2017; Ye et al., 2017; Zhang et al., 2017; Wang et al., 2018; Liu et al., 2021; Yan et al., 2021; Cho et al., 2021; Ma et al., 2021). Dietary protein requirements can vary within a species due to individual differences in body size and feed formulations. Farmed animals have specific dietary protein levels, contingent on their farming environment, to ensure proper growth and development. Both excessive and insufficient dietary protein can negatively affect growth and development (Jiang et al., 2022).

In this study, the optimal dietary protein level for *K. lais* larvae with an initial body weight of 0.15 g was determined to be 42.86%. This finding aligns with protein requirements observed in other carnivorous fish species, such as giant snakehead (*Parachanna obscura*) at 42.5% (Kpogue et al., 2013), freshwater catfish (*Mystus nemurus*) at 42% (Khan et al., 1993), rockfish (*Sebastes schlegeli*) at 42% (Lee et al., 2002), and *P.dabryanus* at 40.87% (Wang et al., 2023). However, the estimated requirement in this study is higher than levels reported for other carnivorous species, including Indian butter catfish (*Ompok bimaculatus*) at 36.11% (Biswas et al., 2020), African catfish (*Clarias gariepinus*) at 35% (Ali et al., 2024), striped catfish at 37.1% (Jayant et al., 2018), and giant river catfish (*Sperata seenghala*) at 35% (Ali et al., 2014).

The feed conversion ratio of *K. lais* larvae exhibited a decreasing trend as dietary protein levels increased from 35 to 40%, beyond which it increased. This suggests that both insufficient and excessive dietary protein levels negatively impact growth and feed utilization efficiency. The protein efficiency ratio was significantly lower in larvae fed diets containing 45 or 50% protein compared to those fed 35 or 40% protein, indicating that excess dietary protein was likely catabolized for energy rather than being utilized for growth. Similar trends have been reported in other species, including Indian butter catfish fingerlings

(Biswas et al., 2020), yellow croaker (*Larimichthys polyactis*) (Zhang et al., 2022), and rainbow trout (Ahmed and Ahmad, 2020). In the present study, the FCR initially decreased and then increased as dietary protein levels rose from 35 to 50%, which may be attributed to imbalanced protein levels leading to overconsumption in an attempt to meet metabolic demands. Morphological indicators such as the viscero-somatic index (VSI) and condition factor (CF) are commonly used to assess the physiological response of fish under varying nutritional conditions (Chang et al., 2018). Moreover, both VSI and CF decreased with increasing dietary protein levels, a trend consistent with findings reported for red-spotted grouper (*Epinephelus akaara*) (Wang et al., 2016) and Asian catfish (*Clarias batrachus*) (Singh et al., 2009).

Whole-body protein content increased in correlation with dietary protein levels up to 40%, beyond which an inverse relationship was observed. The maximum protein content, achieved at a dietary protein level of 40%, can be attributed to the efficient utilization of available protein for growth at this concentration compared to other levels. Similar findings regarding body protein content in relation to protein requirements have been reported for various fish species, including tench (*Tinca tinca L.*) (Gonzalez-Rodríguez et al., 2014), rainbow trout (Ahmed and Ahmad, 2020), and hybrid pufferfish (*Takifugu obscurus* × *T. rubripes*) (Park et al., 2022). Kim and Lee (2009) noted that body protein content responds to dietary protein levels in a dose-dependent manner, with the maximum protein content occurring at the dietary protein level that also yields the highest growth rate. Similar trends have been reported in other fish species, such as mahseer (*Tor putitora*) (Islam and Tanaka, 2004) and Asian red-tailed catfish (*Hemibagrus wyckioides*). It is well established that body protein content increases until dietary protein requirements are met, after which excess dietary protein is metabolized for energy rather than used for tissue protein synthesis (Hardy and Gatlin, 2002). Whole-body lipid content, on the other hand, increased progressively with each elevation in dietary protein level. The maximum body lipid content was observed in fish fed the highest-protein diet (50% protein diet), which may be due to the deamination of surplus dietary protein, resulting in its deposition as body lipid (Deng et al., 2011) or its utilization as an energy source during metabolism (Wu and Gatlin, 2014).

Conclusions

The present study demonstrated that dietary protein levels significantly influence growth, feed utilization, and body composition in Glass catfish (*Kryptopterus lais*) larvae. A dietary protein level of 42.89% was identified as optimal for promoting growth and efficient feed utilization. These findings offer valuable insights for formulating nutritionally balanced diets to support the sustainable production of *K. lais* larvae.

Ethical clearance: All experimental procedures involving fish were conducted in accordance with the Syiah Kuala University Research and Ethics Guidelines, specifically under the Animal Care and Use in Research section, and adhered to national policies on the ethical treatment of fish in research.

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