***Original Research Article***

**Effect of Zinc Application on Phytoplankton Productivity, Survival and Growth of Fry and Fingerlings of Rohu (*Labeo rohita* H.)**

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ABSTRACT

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| **Aims:** The present study aims to evaluate the role of zinc (Zn) supplementation in enhancing plankton productivity and its subsequent effects on the survival, growth, haematological health, and innate immune response of *Labeo rohita* (rohu) fry and fingerlings.  **Study Design:** Laboratory-based microcosm and outdoor tank-based mesocosm experiments were conducted with varying Zn concentrations.  **Place and Duration of Study:** The experiments were carried out at ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha, India, over a multi-phase research period.  **Methodology:** The initial laboratory trials used different Zn concentrations (0.00 to 2.00 mg/l) to study plankton biomass and chlorophyll-a content under controlled photo-bioreactor conditions, with and without Zn top-dressing. Based on the outcomes, two follow-up studies were conducted to evaluate the impact of Zn on rohu fry and fingerlings using lower Zn concentrations (0.00 to 0.50 mg/l) in 100–1500 L FRP and concrete tanks. The study assessed survival, growth performance, blood parameters (Hb, PCV, RBC/WBC counts), and innate immunity (respiratory burst and myeloperoxidase activity).  **Results:** Zinc supplementation up to 1.00 mg/l significantly improved plankton productivity, particularly under top-dressed conditions. However, higher Zn concentrations negatively impacted survival and growth of rohu fry, indicating potential toxicity. Further, lower Zn levels (0.10 mg/l), enhanced survival, weight gain, and length in both fry and fingerlings. Moreover, Zn at 0.10 mg/l significantly improved haematological parameters and innate immune markers without adverse effects.  **Conclusion:** The findings clearly establish that Zn at an optimal concentration of 0.10 mg/l enhances primary productivity, promotes healthy growth, and strengthens the immune system of rohu, *Labeo rohita*. This study suggests that regulated Zn supplementation can be a sustainable strategy for improving freshwater aquaculture productivity while minimizing environmental and physiological risks. |

*Keywords: Zinc, Labeo rohita, plankton, chlorophyll, growth, innate immunity*

1. INTRODUCTION

For multiplication of plankton in fish ponds, several essential nutrients are required and deficiency of even one of these nutrients may lead to poor primary productivity. Plankton are the leading flora of the fish which utilize nutrients dissolved water. Most of the nutrients dissolved in the water come from inherent nutrient pool of the bottom soil. Since plankton and microalgae do not have any mechanism to extract essential nutrients from soil solution, they depend on bottom soil nutrients which are diffused through pore water into the overlying pond water (Boyd, 1995). Apart from some of the major nutrients, micronutrients are required at very low level for improving the plankton productivity as well as survival, growth and health of aquatic animals (Dawood, 2022). Specifically, the potential for micronutrients limitation in the aquaculture has got very low attention. Physiologically, when compared with macronutrients like C, N, P, and K etc., micronutrients (Zn, Fe, Mn, Cu, Ni, Co) are required in trace quantity to carry out metabolic processes within the cell. The nutritional imbalance in water can not only affect the carrying capacity and taxonomic composition of plankton, but also physiological characteristics of the cells thereby affect the growth of aquatic animal.

In all living organisms, zinc (Zn) is an essential part of several metallo-enzymes and act as catalysts for regulating the activity of enzymes like carbonic anhydrase (CA), alcohol dehydrogenase (ALH) and alkaline phosphatase (ALP) (Moore and Ramamoorthy, 1984). There are more than 300 proteins which require trace metals for their functions (Vallee and Falchuk, 1993). Moreover, Zn works as a cofactor for in excess of 200 enzymes in animal cells (Watanabe *et al.* 1997) and contributes in the antioxidation against reactive oxygen species and free radicals and forms an effective the antioxidant defense system (Feng *et al.*  2011). These proteins have direct role to play in metabolism of nucleic acids, carbohydrates and fatty acids *etc.* (Adhikari and Ayyappan, 2004). Carbonic anhydrase which plays a vital role in carbon use in photosynthesis, requires Zn as a co-factor (Goldman and Horne, 1983) and its deficiency leads to reduced plankton growth (Goldman, 1965).

In India, culture of wide variety of fish species is practiced, however, Indian Major Carps (IMCs) rohu *Labeo rohita*, catla *Catla catla* and mrigal *Cirrhinus cirrhosus* are the most popular varieties (Uddin *et al.*, 1994, FAO, 1997; Miah *et al.*, 1997; Kanak *et al.*, 1999). Amongst IMCs, Rohu (*Labeo rohita*) prefers to feed along the water column and prefers plankton (Das and Moitra, 1955; Dewan *et al.*, 1977; Jhingran and Pullin, 1985; Wahab *et al.*, 1994).

Direct estimation of effect of Zn deficiency on plankton production is very difficult however, correlation of Zn:C ratios with mesozooplankton has been reported by a number of aquatic ecosystems (Twining *et al.*, 2011; Baines *et al.*, 2016). Further, the extent of micronutrients accumulation in plankton is influenced by quantity of nutrient in water, plankton species as well as metal content of the bottom soil (Elmaci *et al.* 2007). For example, enhanced accumulation of metals was observed in phytoplankton collected from sewage-fed lake of Bhopal, India (Shrivastava *et al.* 2003). While in Kolleru Lake, the bottom soil contained higher amounts of trace metals compared to water and plankton (Adhikari *et al.* 2009) which can be attributed to the deposition of plankton as well as sedimentation of suspended particles.

Rampant occurrence of Zn and other micronutrients deficiencies in soils of India has been a great cause of concern. For example, around 43% of agricultural soils analysed across different states of the country under the aegis of the All India Coordinated Research Project on Micro- and Secondary Nutrients and Pollutant Elements, Bhopal has been found to be deficient in Zn (Shukla *et al.* 2014). The doses of the nutrients are usually decided considering the ability of their levels in sediment and water (Hickling, 1971; Chaudhuri *et al.*, 1975; Boyd, 1984). Though, in limited soil base or soil less systems, it becomes a challenge to maintain the optimum level of the nutrients required for growth of plankton which are primary food for the fish being cultured. Therefore, application of inorganic fertilizers become critical for successful management of aquaculture system. The exogenous application of the nutrients influences the water quality and encourage the growth of plankton, which further impact the pond environment and food composition fish (Zhang 2021). Zinc fertilizers available in different forms like inorganic, organic, and nano formulations. Among widely used inorganic Zn fertilizers like zinc sulphate, zinc oxide, and zinc chloride, ZnSO4 is commonly applied in soil and water under different aquaculture systems (Reilly 2004). Therefore, an investigation was conducted to assess the optimum dose of Zn (through ZnSO4) for freshwater plankton production, survival and growth of *Labeo rohita* (Hamilton) fry and fingerlings under microcosm and mesocosms systems.

2. material and methods

2.1 Plankton Study

In order to find out the optimum dose of Zn for enhanced plankton growth under laboratory conditions, an experiment was set up with varying doses of Zn. Prototype photo-bioreactors (15 liters’ glass aquarium with provision for requisite controlled illumination) were used for the study by simulating natural photoperiod for growing photosynthetic organisms. The water (pH: 7.8; total alkalinity: 135 mg/l as CaCO3; total hardness: 78 mg/l as CaCO3; total ammoniacal nitrogen: 0.05 mg/l; dissolved phosphate: 0.012 mg/l; dissolved Zn: 146 µg/L) collected from a properly-managed culture pond was used for the study. Different doses of Zn: 0.00, 0.25, 0.50, 0.75, 1.00 and 2.00 mg/l through zinc sulphate (ZnSO4.7H2O) were applied in water under 6 different treatments with 2 replications each. Other essential nutrients like N, P, K *etc*. were applied through laboratory grade chemicals. The experiment was conducted for a period of 21 days. Keeping the depletion of nutrients in view, nutrients other than Zn were applied at weekly interval. Further, a similar experiment under same photo-bioreactors set-up was also conducted wherein besides the doses of Zn given at 1st day, corresponding equal amount of Zn was added to each treatment (top-dressing) on 14th day. For both the experiments, wet weight of plankton biomass was measured by collecting filtrate from plankton net (15 liters of water). For analysis of chlorophyll-a content, exactly 500 mL of water samples were filtered using vacuum filter across Millipore filters (pore size: 0.45 µm) which were pre-washed and buffered with magnesium carbonate. The filtrate was wrapped in aluminium foils and kept in a refrigerator at 4 0C for 24 hours before being extracted with acetone and estimated through spectrophotometric method (Eaton *et al.*, 1995). The various quality parameters of water used across the study was analysed as per the standard procedure (APHA, 1995).

2.2 Fry Study

Consequent upon the results obtained under laboratory conditions, an experiment was set up under open conditions to assess the effect of varying doses of Zn on fish survival and growth. Different doses of Zn: 0.00, 0.50, 1.00, 1.50 and 2.00 mg/l each were applied in 100 liters FRP tanks, keeping all other nutrients constant. A pond water (pH: 7.1; total alkalinity: 56 mg/l as CaCO3; total hardness: 54 mg/l as CaCO3; total ammoniacal nitrogen (TAN): 0.20 mg/l; soluble orthophosphate: 0.03 mg/l; dissolved Zn: 0.06 mg/l) was used for the study. The experiment was conducted in triplicate for 5 weeks. Zinc was applied through zinc sulphate (ZnSO4.7H2O) whereas other essential nutrients were applied through conventional fertilizer sources as and when required. The ratio of N: P nutrients applied in all treatments was kept as 6.0:1.0 which was replenished at weekly interval. Fry of Rohu (mean initial weight 46.00±2.95 mg) were stocked in each treatment.

Another set of experiment was conducted with further reduced doses of Zn as the higher doses had shown negative impact on survival, growth, and development of fish in previous experiment. Under this experiment, lower doses of Zn were applied: control, 0.10, 0.20, 0.30 and 0.50 mg/l Zn. A pond water (pH: 7.20; total alkalinity: 90 mg/l as CaCO3; total hardness: 78 mg/l as CaCO3; total ammoniacal nitrogen (TAN): 0.18 mg/l; soluble orthophosphate: 0.025 mg/l; dissolved Zn: 0.06 mg/l) was used for the study. The fry (175.0±5.6 mg; 2.50±0.08 cm) were reared for 5 months in 100 litre FRP tanks under open conditions in three replications. N & P was applied in all treatments (N: 2.0 mg/l and P: 0.35 mg/l). Periodical fertilization was done while Zn was supplied through zinc sulphate (ZnSO4.7H2O).

2.3 Fingerlings Study

Consequent upon the results obtained under FRP tanks (fry rearing) under previous experiments under section, an experiment was set up in concrete tanks under open conditions to assess the effect of Zn on fish survival and growth. Two treatments were used in the experiment: T1: Control and T2: Zn 0.10 mg/l with three replicates in 1500 L capacity concrete tanks. The initial water quality parameters observed were: pH 8.10, total alkalinity 81 mg/l, and total hardness 72 mg/l (as CaCO3). The concentrations of total ammonia (TAN), soluble orthophosphate, and dissolved zinc were 0.19 mg/l, 0.03 mg/l, and 0.055 mg/l, respectively. Recommended dose of nitrogen and phosphorus fertilizers were applied in all treatments at regular interval. Zinc was applied through zinc sulphate. After tank preparation, healthy fingerlings of rohu (mean initial weight 21.60±2.81g/ 10.02±0.75 cm) were stocked after due acclimatization. After final harvest, fish samples were collected and processed for haematological and immunological analyses to study the changes in health and immune status of fingerlings.

3. results and discussion

3.1 Plankton Biomass and Chlorophyll Content

The plankton biomass, analysed after 21 days of experimental period was significantly influenced by Zn application under both the conditions: without and with top-dressing (Table 1). The plankton biomass was the lowest (16.20 mg/l) in no Zn treatment. With the increase in Zn concentration from 0.25 to 1.00 mg Zn/l, there was an incremental proliferation of plankton biomass, with the highest value recorded at 1.00 mg/l Zn was 33.30 mg/l without top-dressing and 37.40 mg/l with top-dressing, indicating a positive impact of Zn supplementation and top-dressing. On the other hand, at the highest Zn level (2.00 mg/l), a significant low biomass production was observed (26.20 mg/l without and 21.47 mg/l with top-dressing), suggesting potential Zn toxicity or inhibitory effects at higher concentrations. The results clearly indicated that Zn application up to 1.00 mg/l enhanced plankton productivity, and top-dressing further improved biomass yield.

**Table 1. Effect of different Zn treatments on plankton biomass in controlled conditions**

|  |  |  |
| --- | --- | --- |
| **Zn Treatments** | **Plankton biomass (mg/l)** | |
| **Without top-dressing** | **With top-dressing** |
| No Zn | 16.20d | 18.07d |
| Zn 0.25 mg/l | 18.60c | 23.67c |
| Zn 0.50 mg/l | 24.27b | 31.53b |
| Zn 0.75 mg/l | 32.40a | 33.07b |
| Zn 1.00 mg/l | 33.30a | 37.40a |
| Zn 2.00 mg/l | 26.20b | 21.47c |

The chlorophyll-a content of each treatment was estimated at weekly interval and results indicated that inclusion of Zn gradually enhanced chlorophyll-a content in Zn treatments till 1.00 mg/l of Zn, however, inhibition was observed beyond 1.00 mg/l Zn (reduction in chlorophyll content at 2.00 mg/l) (Fig.1). Further, the results also indicated that top-dressing at 14th day with equal dose of Zn further enhanced the chlorophyll-a content in lower doses of Zn however, at higher doses there was significant reduction (Fig. 2). The reduction was observed even at 1.00 mg/l Zn indicating 1.00 mg/l as optimum level in controlled soil less system.

**Fig 1: Effect of Zn treatments on Chlorophyll a concentration in plankton (without top dressing)**

**Fig. 2. Zn treatments vs Chlorophyll a concentration (top-dressing at 14th day)**

Zinc, an essential element for primary productivity has been reported as potential limitation for growth of dominant planktons (Morel *et al.* 1994; Chappell *et al.* 2016; Crawford *et al.* 2003) as low availability of free Zn2+ ion in water restricts phytoplankton growth (Brand *et al.* 1983). In low Zn waters, application of Zn even through soil enhanced plankton biomass in freshwater aquaculture (Adhikari & Ayyappan, 2004). Barlaya *et al.* (2023) have also reported that supplementation of micro-nutrients to the culture system affects the taxonomic quality of plankton produced. The supply of Zn seems to stimulate growth of smaller cells and increases the proportion of chlorophyll within the small fraction (Crawford *et al.* 2003).

3.2 Effect of Zn Application on Rohu Fry

**Higher Concentrations**

The results of 5 weeks’ study on effect of Zn application revealed a clear dose-dependent negative impact of Zn on survival and growth of rohu fry performance (Table 2). Survival rate (%) of fry significantly decreased (92.08% in the control group) with increase in Zn dose (36.88% at 2.00 mg/l Zn). Similarly, mean final weight (mg) and weight gain (mg) declined sharply with increasing Zn concentration. The weight gain recorded under control, 0.50, 1.00, 1.50 and 2.00 mg/l was 128.4±4.8, 107.9±3.8, 76.7±4.0, 32.3±6.2 and 20.0±1.7 mg, respectively. Likewise, fry length at the harvest also showed declining trend which reflects low somatic growth with raise in Zn concentration in the water.

**Table 2. Effect of different concentrations of Zn on growth attributes (Mean±SEm) of rohu *L. rohita* Fry(after 5 weeks)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Zn concentration (mg/l)** | **Initial weight (mg)** | **Final weight (mg)** | **Weight gain (mg)** | **Length at harvest (cm)** | **Survival (%)** |
| Control | 46.00±2.95 | 174.37±4.81a | 128.4±4.8a | 2.62±0.05a | 92.08±2.53a |
| Zn-0.50 | 153.88±3.83b | 107.9±3.8b | 2.47±0.08a | 85.83±0.83b |
| Zn-1.00 | 120.71±4.02c | 76.7±4.0c | 2.38±0.21b | 62.92±2.20c |
| Zn-1.50 | 78.33±6.17d | 32.3±6.2d | 2.25±0.40b | 44.17±3.97d |
| Zn-2.00 | 66.01±1.73d | 20.0±1.7e | 1.64±0.04c | 36.88±1.88d |

These results suggest that even though Zn is essential nutrient involved in several enzymatic and metabolic processes, its higher concentration in culture water may cause toxicity during early life stages of fish (Watanabe *et al.*, 1997). Spry and Wiener (1991) also highlighted that increase in Zn content in freshwater culture system can damage osmoregulatory function, gill health, and metabolic efficiency. Reduces survival and growth in Arabian killifish (*Aphanius dispar*) at higher Zn levels due to cellular damage and physiological stress was reported by Alkahem *et al.* (2011). Prolonged exposure to higher Zn led to decline in weight gain and survival as it may negatively affect nutrient assimilation and induce oxidative stress in developing fry of yellow catfish, *Pelteobagrus fulvidraco* (Wang *et al.*, 2012) and rainbow trout, *Oncorhynchus mykiss* (Hosseini *et al.*, 2016). Based on these results, it was noticed that high Zn negatively affected the survival and growth of rohu fry under tank system which prompted the need for further study with reduced sub-lethal Zn dosage, which can support the growth without causing toxicity.

**Lower concentrations**

Subsequent investigation wherein lower dosage of Zn was used, the average survival percentage under control, 0.10, 0.20, 0.30 and 0.50 Zn mg/l of water were recorded as 85.3, 84.6, 84.3, 83.9 and 84.6, respectively. Since there was no significant change of survival in any of the Zn treatments compared to control, it can be concluded that Zn application at low level did not cause any toxic effect on rohu fry under the experimental conditions. Further, as shown in Fig. 3, rohu fry exposed to 0.10 mg/l Zn exhibited significantly higher growth (7.57±0.05 cm; 3.27±0.15 g), compared to the control (6.02±0.11 cm; 2.53±0.04 g). However, further increase in Zn concentration failed to induce increment in length and weight rather it decreased with increase in Zn concentration beyond 0.10 to 0.50 mg/l.

**Fig. 3. Effect of different concentrations of Zn on length and weight of rohu at harvest**

Such results suggested threshold limit of Zn application up to 0.10 mg Zn/ L is beneficial for the growth of fish in microcosm rearing system however, further increase in Zn concentration becomes detrimental for fish growth. Several research reports suggest the above findings which demonstrates low Zn concentrations promote fish growth at optimal level, attributed to improvement in metabolism which primarily include protein synthesis, enzyme activity, blood cell formation which contributes to enhanced growth performance (Lall, 2002, Ogino and Yang, 1980, and NRC, 2011). In species like carp and catfish also moderate levels of Zn resulted in enhanced growth and feed efficiency while elevated Zn levels may cause toxicity, oxidative stress, and metabolic imbalance, leading to growth suppression (Spry and Wiener, 1991; Alkahem *et al.*, 2011; and Wang *et al.*, 2012). Reduced growth performance in channel catfish and common carp at higher Zn concentrations and optimal growth and moderate to low Zn levels have also been reported (Gatlin and Wilson, 1984 and Ebrahimi *et al.* 2012). Therefore, 0.10 mg/l Zn appears to be the optimal concentration for growth promotion in rohu fingerlings reared in tank system.

3.3 Effect of Zn Application on Rohu Fingerlings

**Survival and Growth performance**

The findings clearly indicate that Zn application at 0.10 mg/l resulted in statistically significant increase in length and weight gain in rohu fingerlings at harvest. The treatment receiving Zn (114.2 g; 22.84 cm) performed better than control (101.83 g; 20.10 cm) (Fig. 4). The survival (%) of fingerlings was recorded at around 88.5 and 90.3% in control and Zn treated groups, respectively which was statistically non-significant. The results evidently suggest that very small supplementation of Zn (0.10 mg/l) influenced both length and weight gain in rohu fingerlings, highlighting its beneficial role in growth during culture.

**Fig. 4. Effect of different concentrations of Zn on length and weight of rohu at harvest**

The findings bring into line with several other studies which reported helpful role of Zn in fish growth and metabolism. Being an essential micronutrient, Zn is vital for numerous physiological activities like protein assimilation, numerous enzyme activities, and cell division etc. (Watanabe *et al.*, 1997). Enzymes like carbonic anhydrase and alkaline phosphatase which are involved in osmoregulation and digestion require Zn as co-factor which means external Zn fertilization has a positive role in influencing growth performance in fish (Hardy *et al.*, 1996). Under intensive culture system, wherein free Zn availability in water may be limited, Zn supplied through diet resulted in increase in growth performance of rainbow trout (Hughes and Johnston, 1990). Zinc supplementation has been reported to enhance weight gain in channel catfish Gatlin and Wilson (1984) and Indian major carps (Reddy and Venugopal, 1990) as well. Zn also promotes absorption and utilization of other nutrients thereby improving gain in body weight (Prasad, 1995). The above reports advocate that Zn has a vital role to play in metabolism and tissue development in fish species which are in consonance with the results observed in the current study on rohu fingerlings.

**Haematopoietic activity and innate immune responsiveness**

With the application 0.10 mg/l Zn, a significant increase in haemoglobin (%), packed cell volume (PCV%), total RBC & WBC counts, and neutrophil (%) percentage was observed which indicated greater haematopoietic activity and immune responsiveness in fingerlings (Table 3). However, eosinophils (%) remained statistically at par, while small reduction in lymphocyte (%) was observed with Zn application. Thus the results clearly suggest that Zn application at very low concentration (0.10 mg/l) results in promoting blood health and cellular immunity in rohu fingerlings. In addition to haematological improvements, Zn supplementation positively influenced innate immune responses in rohu fingerlings. In Zn treated groups, significant increase in important non-specific immunity markers like respiratory burst activity and myeloperoxidase activity was observed compared to control. These results highlight the effect of Zn fertilisation in enhancing innate immune parameters, indicator of healthy defense mechanisms in *Labeo rohita* fingerlings. Zn supplementation in common carp (*Cyprinus carpio*) increased RBC and WBC counts as well as proportion of neutrophils (Ebrahimi *et al.* (2012). Similar results have been reported by Hosseini *et al.* (2016) also which revealed that dietary Zn supplementation improved haematopoietic activities in significantly rainbow trout. Increased NBT activity in Nile tilapia following Zn application has also been reported by Tawfik (2009). Role of Zn in increasing enzymatic responses of phagocytic cells was demonstrated by Deng *et al.* (2010) wherein increased level of myeloperoxidase activity was seen in hybrid tilapia.

**Table 3: Changes in blood and innate immunity parameters of rohu fingerlings as influenced by Zn supplementation**

|  |  |  |
| --- | --- | --- |
| **Parameters** | **Control** | **Zn @ 0.10 mg/l** |
| **Blood Parameters** | | |
| Haemoglobin (%) | 6.28±0.05a | 6.50±0.05b |
| PCV (Packed Cell Volume) (%) | 18.5±0.5a | 21.0±0.5b |
| Total RBC Count (mil/cumm) | 1.44±0.03a | 1.56±0.02b |
| Total WBC Count (per cmm) | 1,81,000±2,500a | 1,92,333±1,500b |
| Neutrophils (%) | 8.85±0.0a | 11.67±0.05b |
| Eosinophils (%) | 1.07±0.005a | 1.00±0.003a |
| Lymphocytes (%) | 91.0±0.5b | 88.0±1.0a |
| **Innate immunity Parameters** | | |
| Respiratory Burst/ NBT assay *(absorbance)* | 0.069a | 0.073a |
| Myeloperoxidase activity *(absorbance)* | 1.075a | 1.177b |

4. Conclusion

The current experiment showed that Zn has an important role to play in promoting plankton productivity and enhancing the survival, growth, and health of fry and fingerlings of *Labeo rohita* at the moderate concentrations. Zinc supplementation in water up to 1.00 mg/l considerably enhanced plankton biomass and chlorophyll-a content under photo-bioreactor conditions. However, higher concentrations of Zn (>1.00 mg/l) had the potential to be harmful as it reduced plankton production, slowed down fry survival and growth. Nonetheless, lower Zn level (0.10 mg/l) was found to be the optimum dose for fry and fingerlings, supporting better growth and significantly improving haematological and some important immune markers such as respiratory burst and myeloperoxidase activity. These observations emphasize the need for cautious application of Zn in aquaculture systems to maximize productivity without triggering toxicity. Therefore, regulated Zn supplementation at 0.10 mg/l is suggested for sustainable and immune-enhancing aquaculture practices in *Labeo rohita* under tank system.

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