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THE EFFECT OF AERATION METHOD ON NILE TILAPIA BIOLOGICAL INDICATORS

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Abstract

This study examined the impact of applying the optimum operating condition for fine bubbles aeration of 0.554 $m^3.h^{-1}$ air flow rate, tube depths from the water surface of 0.7 m, tube inner diameter of 11 mm, and circular design shape at aquaculture greenhouse for rearing Nile Tilapia and compared with conventional water change system in three replicates for every treatment. For the classic water change method and fine bubbles, the corresponding water change ratios were 10 and 30, respectively. Before reaching commercial size, the experiment lasted for 8 weeks. Biological indicators estimated for blood, blood bio-chemical, digestive enzymes, anti-oxidant enzymes and serum bio-chemical parameters. Aeration method has a significant effect on red blood cells (Rbcs) where P value was 0.033. Also, a significant effect obtained on hemoglobin content (HB) where P value is 0.04. fine bubbles aeration has highest value of total protein by 4.93 g/dl compared with 4.46 g/dl for water change treatment. Amylas mean values were 21.047 and 12.307 U/L for fine bubbles aeration and water change treatment, respectively. mean values for SOD were 8.87 and 8.187 U/gm, CAT were 10.617 and 10.367 U/gm and MDA mean values were 12.93 and 18.367 nmol/g for fine bubbles aeration and water change treatment, respectively.

Key words**:** *aquaculture, biological indicators, dissolved oxygen, aeration diffusion by fine bubbles*

INTRODUCTION

The quantity of fish produced increased by 5.4% to 2.0 million tons in 2019 from 1.90 million tons in 2018. Lakes came in second with a production percentage of 7.97%, followed by marine waters with 4.9%, fresh water with 3.8% and rice fields with 0.8% of the total amount of fish produced. The revenue of fish production increased by 26.6% in 2019 to 61.1 billion LE from 48.3 billion LE in 2018. Also, the area of aquaculture farms decreased by 3.9% from 307.2 thousand feddan in 2018 to 295.2 thousand feddan in 2019 [6].

Between 1970 and 2000, the average annual cumulative growth rate of aquaculture production was 9%, compared to only 1.3% for catch fisheries. Global aquaculture increased steadily by 6.3% between 2000 and 2017, reaching 111.95 million metric tons (mmt). Finfish accounted for 53.40 mmt, or 47.7%, of the total fish production produced globally in 2017 and 83% of them originated from freshwater sources [11].

The most crucial period to introduce more aeration is shortly before dawn, when DO concentrations are often lowest because this is when they frequently drop below tolerable levels. Early morning DO for warmwater fish should stay above 3–4 mg/L and above 5–6 mg/L for cold-water fish. Warmwater and cold-water fish can survive with concentrations as low as 1.0-1.5 mg/L and 2.5-3.5 mg/L, respectively. However, these concentrations can raise stress, reduce appetite or aggression to eat and if low enough for a long length of time they can be deadly [5].

As well as serving as a sign of healthy liver function, variations in the activity of liver enzymes like lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) can also be utilized as biomarkers for tissue injury [2].

The health of fish is undoubtedly impacted by the quality of the water because they are the largest and most diverse group of aquatic species that are closely tied to the aquatic

environment. They are extremely sensitive to both immediate and subtle changes in the aquatic environment [4].

Hematological investigations are crucial for environmental monitoring because they shed light on how blood properties relate to habitat and how well a species can adapt to its surroundings [13].

Enzymes are sensitive indicators that can be used to identify stress in fish that have been exposed to different types of water contaminants [26].

It is crucial to completely take into account the habitat when doing research on the effect of habitat on fish health since physical and chemical changes in the aquatic environment frequently cause blood changes that lead to physiological abnormalities in fish.

An excellent approach for evaluating the health of aquatic habitat is the use of biochemical and enzymatic blood serum indicators in fish. Blood's biochemical and enzymatic properties have been shown to be a reliable way to assess an animal's state of health [17].

Natural water systems are susceptible to contamination from a variety of sources, including bacterial contamination, oil pollution, organic contamination, and inorganic contamination. All types of species are negatively impacted by water pollution. Additionally, fish health is impacted by the physicochemical characteristics of water, including temperature, pH, dissolved oxygen, nitrites, nitrates, and phosphates [22].

The main aims of the research were evaluating effect of aeration method on biological indicators.

MATERIALS AND METHODS

The aquatic fish of Nile tilapia *(Oreochromis niloticus*) was obtained from private farm at Damro village, Kafr El-Sheikh Governorate, Egypt. The experimental fish weight is of 140 grams with density of 5 fish/m^3 (90 fish/pond) for every treatment replicate of the experiment.

Fish feed were from extruded floating 25% CP, 3 mm diameter. The ingredients and the chemical composition of the used fish feed as shown in Table 1.

Aquaculture water used at aquaculture is mixture from lake Burullus water and agricultural drainage water. Secchi disk mean value before conducting experiments was 41 cm. The chemical analysis of aquaculture water at the begin of the experiment at kafr el-Sheikh university laboratories is shown at Table 2.

Table 1. The chemical composition of experimental fish feed

Composition	Value
Crude protein (%)	25
Crude fat (%)	4.5
NFE (%)	53.1
Ash (%)	5.1
Fibre (%)	4.3
P (%)	0.7
Gross energy (MJ)	17.5
Digestible energy (MJ)	7.5

Source: Feed datasheet.

Table 2. The chemical analysis of aquaculture water.

Test item	**Value**	**Unit**
pH	7.36	—
EC	3.22	ds/m
TDS	1.61	g/l
Na	35.63	meq/l
K	0.5	meq/l
Ca	10.0	meq/l
Mg	18.6	meq/l
CO_3	0.00	meq/l
HCO_3	10.0	meq/l
CL	50.0	meq/l
SO_4	4.73	meq/l
Fe	Nd	meq/l
Mn	Nd	meq/l

Source: Faculty of Agriculture laboratory.

The application of aeration by fine bubbles tubes and compare with traditional aquaculture system of water change occurred at green house in three replicates.

The greenhouse has design of quonset double span and covered with polyethylene (PE) plastic sheet.

It has 18 concrete ponds as shown at figure. Every pond has dimensions of 3 m width and 6 m length. Also, every pond filled with 18 m^3 of water.

An electric single phase compressor model APT (SGBM9037, China) of 1.5 hp, 25 L capacity, maximum pressure of 8 bar and

maximum air delivery up to 130 $L.min^{-1}$ used as a source of air injection with regulator valve to control airflow rate.

Methods

The optimum operational conditions for oxygen productivity were applicated at aquaculture greenhouse and compared with traditional water change system in three replicates for every treatment. Water change ratios were 10 and 30% for fine bubbles and traditional water change method, respectively. The experiment period were 8 weeks until reach commercial size.

Operational conditions were: air flow rates of 0.554 $m^3.h^{-1}$, tube depth of 0.7 m from water surface for aeration tube and holder, tube wall inner diameter of 11 mm and circular design shape according to [16].

A statistical analysis of *t in pairs* attempted by using SPSS 25 program to conduct significant effect of treatments on biological characteristics in the study. The experiment divided into two treatments of water change (W.C.T.) and fine bubbles tube (F.B.T.) treatments in three replicates for every treatment.

Blood parameters

Blood sampling:

The blood samples were collected from the caudal vertebral vein [14].

Erythrocytic and leukocytic counts determination:

The erythrocytes and leukocytes were counted according to the method described [24]. using hemocytometer and Natt- Herrik solution.

Hemoglobin concentration determination:

Hemoglobin concentration was determined using the cyanomet hemoglobin method Drabkin's solution [24]. The cyanomet hemoglobin method converts all hemoglobin derivatives to methemoglobin using ferricyanide and cyanide ion. Methemoglobin is a stable red compound and can be measured color metrically.

Packed cell volume determination:

The micro hematocrit method was used for estimation of the PCV% [8].

Determination of differential leukocytic count (DLC):

A thin blood films were obtained, air dried, fixed with methanol for 3-5 min. and stained with Gimsa stain for 8-10 min., then rinsed with distilled water and left to dry. The white blood cells were counted among one hundred of blood smear [24].

The absolute DLC was calculated [25] according to the following formula:

Absolute DLC = no. of each white cell x no. of total leukocytic count/100.

Blood bio-chemical

Lysozyme concentrations assays:

The lysozyme activity of sera was assayed according to the method [9] based on the ability of lysozyme to lyses Gram positive lysozyme sensitive bacterium; Micrococcus lysodeikticus.

The lysozyme substrate was 75 mg/ml Micrococcus lysodeikticus lyophilized cells, suspended in 0.1M sodium phosphate/ citric acid buffer; pH 5.8.

A 25µl of the undiluted serum samples were placed into the 96- well micro plate, in triplicates.

A 175 µl of the substrate solution was then added to each micro titer plate well and kept at 25ºC; thereafter, rapidly mixed, the changes in turbidity was measured every 30 sec. for 5 min. at the wave length 450nm using the micro plate ELISA reader.

The unit of lysozyme present in serum (µg/ml) was obtained by matching with the standard curve made with lyophilized hen egg white lysozyme.

Serum total proteins (REF:310 001 Spectrum. Egyptian company for Biotechnology. Egypt) were determined colorimetrically at the wave length 546 nm [10].

Albumins (CAT. No. AB 10 10 Biodiagnostic co. Egypt.) were determined colorimetrically at the wave length 630 nm [10].

Globulins content was calculated mathematically.

Serum bio-chemical

Activities of aspartate aminotransferase (AST), CAT. No. AS 10 61 (45) Biodiagnostic co. Egypt. were determined colorimetrically at the wave length 505 nm [20].

Alanine aminotransferase (ALT) CAT. No. AL 10 31 (45) Biodiagnostic co. Egypt. were

determined colorimetrically at the wave length 505 nm [20].

Creatinine (CAT. No. CR 12 51 Biodiagnostic co. Egypt.) were determined colorimetrically at the wave length 495 nm [3].

Urea (CAT. No. UR 21 10 Biodiagnostic co. Egypt.) were determined colorimetrically at the wave length 550 nm [12].

Glucose (GOD-PAP) (REF. 1180 VitroScient co. Egypt.) were determined colorimetrically at the wave length 500 nm [7].

Triglycerides (CAT. No. TR 20 30 Biodiagnostic co. Egypt.) were determined colorimetrically at the wave length 505 nm [15].

Cholesterol (CAT. No. CH 12 20 Biodiagnostic co. Egypt.) were determined colorimetrically at the wave length 500 nm [21].

Antioxidants enzymes

Superoxide dismutase (SOD) (CAT. No. SD 25 21 Biodiagnostic co. Egypt) were determined colorimetrically at the wave length 560 nm [19].

Catalase (CAT. No. CA 25 17 Biodiagnostic co. Egypt.) were determined colorimetrically at the wave length 510 nm [1].

Lipid peroxide (***Malondialdehyde***) (MDA) (CAT. No. MD 25 29 Biodiagnostic co. Egypt.) were determined colorimetrically at the wave length 534 nm [23].

Digestive enzymes activity

Lipase (REF:281 001 Spectrum. Egyptian company for Biotechnology. Egypt) were determined colorimetrically at the wave length 580 nm [18].

Amylase (CAT. NO. AY 10 50 Biodiagnostic co. Egypt.) were determined colorimetrically at the wave length 660 nm [7].

A statistical analysis attempted by using SPSS 25 program to show significant effect of treatments on biological characteristics in the study.

The experiment divided into two treatments of water change (A) and fine bubbles tube (B) treatments in three replicates for every treatment.

RESULTS AND DISSCUSIONS

Blood parameters

Aeration method has a significant effect on red blood cells (Rbcs) where P value is 0.033, while fine bubbles aeration has highest value of 3.49X106/mm^3 compared with 2.96 X106/mm3 for water change treatment as shown in Tables 3 and 4. Also, a significant effect obtained on hemoglobin content (HB) where P value is 0.04, HB content mean value was 9.017 and 10.57 g/100ml in treatment of water change and fine bubbles tube, respectively as shown in Tables 3 and 4. In addition to that, Packed cell volume (PCV) has a significant different in favor of fine bubble tube aeration treatment with mean value of 33.33 and 28.33 % for fine bubble tube and water change aeration treatments, respectively at P value of 0.046 as shown in Tables 3 and 4. On the other hand, there is no significance between replicates mean values of fine bubbles aeration treatment and water change treatment for mean corpuscular volume (MCV), Mean corpuscular Hemoglobin (MCH) and mean corpuscular hemoglobin, concentration (MCHC) indicators as shown in Tables 3 and 4.

Blood bio-chemical parameters

The results showed that fine bubbles tube treatment and water change treatment has a significant effect on total protein and globulin with p value of 0.035 and 0.032, respectively. While fine bubbles tube treatment and water change treatment have no significant effect on albumin and lysozyme as shown in Tables 3 and 5.

While fine bubbles aeration has highest value of total protein by 4.93 g/dl compared with 4.46 g/dl for water change treatment as shown in Tables 3 and 5.

Also, fine bubbles aeration has highest value of globulin by 3.41 g/dl compared with 2.93 g/dl for water change treatment as shown in Tables 3 and 5.

Table 3. Analysis of variance for blood test

Independent Samples Test					
		Trt	Mean	Std. Error Mean	Sig.
1	RBCS	A	2.96	0.05508	0.033
		B	3.49	0.30534	
2	HB	A	9.0167	0.19359	0.04
		B	10.5733	0.88939	
3	PCV	A	28.3333	0.66667	0.046
		B	33.3333	2.848	
4	MCV	A	95.71	0.98419	0.221
		B	95.5433	0.51596	
5	MCH	A	30.4567	0.11893	0.629
		B	30.31	0.09504	
6	MCHC	A	31.8333	0.40251	0.099
		B	31.7267	0.16128	
7	WBcs	A	9.0567	0.78065	0.084
		B	10.0467	0.17947	
8	basophil	A	0.0667	0.03383	0.024
		B	0.1	0	
9	esinophil	A	0.1267	0.04177	0.774
		B	0.1	0.05774	
10	monocyte	A	0.7267	0.08511	0.886
		B	0.7733	0.08667	
11	lymphocyt	A	6.7467	0.53261	0.112
		B	7.67	0.22898	
12	heterophil	A	1.39	0.18009	0.468
		B	1.41	0.1365	
13	Glucose	A	11.2133	0.59468	0.086
		B	11.5167	1.56263	
14	Protein	A	4.4633	0.40134	0.035
		B	4.93	0.06245	
15	Globulin	A	2.9333	0.42912	0.032
		B	3.41	0.07095	
16	Albumin	A	1.53	0.03786	0.284
		B	1.52	0.07371	
17	lysozyme	A	5.56	0.94495	0.713
		B	6.7633	0.78154	
18	Ast	A	30.3067	1.90398	0.669
		B	29.97	2.64195	
19	Alt	A	21.9267	1.71928	0.146
		B	19.9867	3.88684	
20	Urea	A	4.09	0.09866	0.175
		B	3.9133	0.19411	
21	creatinine	A	0.4467	0.0318	0.688
		B	0.4167	0.02333	
22	cholestrol	A	89.9433	5.01694	0.811
		B	90.3	6.23695	
23	triglyceride	A	99.0767	6.52827	0.467
		B	95.7333	3.52254	
24	Lipase	A	29.39	0.89034	0.049
		B	34.2633	3.49955	
25	Amylas	A	12.3067	0.92113	0.726
		B	21.0467	0.70516	
26	MDA	A	18.3667	0.84881	0.576
		B	12.9333	1.03577	
27	CAT	A	10.3667	0.33138	0.141
		B	10.6167	0.89841	

Source: Own results.

Table 4. Blood parameters values

Treatment	Rep.	RBCS ($x10^6$ /mm^3)	HB (g/100 ml)	PCV (%)	MCV	MCH	MCHC
Water change	1	2.86	8.69	27	94.41	30.38	**32.19**
	2	3.05	9.36	29	95.08	30.69	**32.28**
	3	2.97	9	29	97.64	30.3	**31.03**
	Mean	**2.96**	**9.02**	**28.33**	**95.71**	**30.46**	**31.83**
Fine bubble	1	3.21	9.76	31	96.57	30.4	**31.48**
	2	4.1	12.35	39	95.12	30.12	**31.67**
	3	3.16	9.61	30	94.94	30.41	**32.03**
	Mean	**3.49**	**10.57**	**33.33**	**95.54**	**30.31**	**31.73**

Source: Own results.

Digestive enzymes parameters

Aeration method has a significant effect on Lipase at P value of 0.049. While fine bubbles aeration has value of 34.26 U/L compared to 29.39 U/L for water change treatment as shown Tables 3 and 6. While fine bubbles tube treatment and water change treatment have no significant effect on Amylas. However, Amylas mean values were 21.047 and 12.307 U/L for fine bubbles aeration and water change treatment, respectively as shown in Tables 3 and 6.

Anti-oxidant enzymes parameters

The results showed that fine bubbles tube treatment and water change treatment haven't a significant effect on superoxide dismutase (SOD), catalase (CAT) and Malondialdehyde (MDA) as shown in Tables 3 and 7. Also, results showed that mean values for SOD were 8.87 and 8.187 U/gm for fine bubbles aeration and water change treatment, respectively as shown in Tables 3 and 7.

In addition to that mean values for CAT were 10.617 and 10.367 U/gm for fine bubbles aeration and water change treatment, respectively as shown in Tables 3 and 7.

While, MDA mean values were 18.367 and 12.93 nmol/g for fine bubbles aeration and water change treatment, respectively as shown in Tables 3 and 7.

Serum bio-chemical parameters

The results showed that fine bubbles tube treatment and water change treatment haven't a significant effect on white blood cells (WBcs), eosinophil, monocyte, lymphocyte, heterophil, glucose, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), urea, creatinine, cholesterol and triglyceride.

Table 5. Blood bio-chemical parameters values

Treatment	Replicates	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Water change	1	3.98	1.52	**2.46**
	2	5.26	1.47	**3.79**
	3	4.15	1.6	**2.55**
	Mean	**4.46**	**1.53**	**2.93**
Fine bubble	1	4.96	1.41	**3.55**
	2	5.02	1.66	**3.36**
	3	4.81	1.49	**3.32**
	Mean	**4.93**	**1.52**	**3.41**

Source: Own results.

Table 6. Digestive enzymes parameters values

Treatment	Replicates	Amylase	Lipase
		(U/L)	(U/L)
Water change	1	12.35	29.98
	2	10.69	27.64
	3	13.88	30.55
	Mean	12.31	29.39
Fine bubble	1	20.98	41.24
	2	19.86	31.26
	3	22.3	30.29
	Mean	21.05	34.26

Source: Own results.

Table 7. Anti-oxidant enzymes parameters values

Treatment	Replicates	SOD	CAT	MDA
		U/gm	U/gm	nmol/g
Water change	1	8.67	10.99	19.85
	2	6.94	9.86	16.91
	3	8.95	10.25	18.34
	Mean	8.17	10.37	18.37
Fine bubble	1	8.01	9.34	14.25
	2	10.65	10.16	13.66
	3	7.96	12.35	10.89
	Mean	8.87	10.62	12.93

Source: Own results.

Table 8. Serum bio-chemical parameters values, part (1).

Treatment	Rep.	WBcs	Heter-ophil%	Heter-ophil	Lymp-hocyt%	Lymp-hocyt
		(x10³/ mm³)		(x10³/ mm³)		(x10³/ mm³)
Water change	1	8.67	18	1.56	71	6.16
	2	7.94	13	1.03	79	6.27
	3	10.56	15	1.58	74	7.81
	Mean	9.06	15.3	1.39	74.67	6.74
Fine bubble	1	9.69	12	1.16	78	7.56
	2	10.26	14	1.44	79	8.11
	3	10.19	16	1.63	72	7.34
	Mean	10.05	14	1.41	76.3	7.67

Source: Own results.

Table 9. Serum bio-chemical parameters values, part (2)

Treatment	Rep.	Mon-ocyte%	Mon-ocyte (x10³/ mm³)	Esino-phil%	Esino-phil (x10³/ mm³)	Baso-phil%	Baso-phil (x10³/ mm³)
Water change	1	9	0.78	1	0.09	1	**0.09**
	2	7	0.56	1	0.08	0	**0**
	3	8	0.84	2	0.21	1	**0.11**
	Mean	**8**	**0.73**	**1.3**	**0.13**	**0.67**	**0.07**
Fine bubble	1	8	0.78	1	0.1	1	**0.1**
	2	6	0.62	0	0	1	**0.1**
	3	9	0.92	2	0.2	1	**0.1**
	Mean	**7.7**	**0.77**	**1**	**0.1**	**1**	**0.1**

Source: Own results.

Table 10. Serum bio-chemical parameters values, part (3)

Treatment	Rep.	Urea	Alt (U/L)	Ast (U/L)	Trigly-ceride (mg/dl)	Choles-terol (mg/dl)	Glu-cose (mg/dl)	Creat-inine (mg/dl)
Water change	1	4.25	25.34	30.15	99.34	80.64	10.32	**0.45**
	2	4.11	19.86	27.09	110.25	91.34	10.98	**0.39**
	3	3.91	20.58	33.68	87.64	97.85	12.34	**0.5**
	Mean	**4.09**	**21.93**	**30.3**	**99.01**	**89.9**	**11.21**	**0.45**
Fine bubble	1	4.3	27.64	29.86	94.66	101.24	9.86	**0.38**
	2	3.75	17.34	34.6	102.3	90.02	14.64	**0.41**
	3	3.69	14.98	25.45	90.24	79.64	10.05	**0.46**
	Mean	**3.91**	**19.99**	**29.97**	**95.7**	**90.3**	**11.52**	**0.42**

Source: Own results.

However, they have a significant effect on basophil at p of 0.024 as shown in Tables 3, 8, 9 and 10.

White blood cells (WBcs) mean values were 10.05 and 9.057 x10³/mm³ for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Eosinophil mean values were 0.1 and 0.127 x10³/mm³ for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Also, Monocyte mean values were 0.773 and 0.726 x10³/mm³ for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Eosinophil mean values were 0.1 and 0.127 x10³/mm³ for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Lymphocyte mean values were 7.67 and 6.647 x10³/mm³ for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Besides, Heterophil mean values were 1.41 and 1.39 x10³/mm³ for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Also, Glucose mean values were 11.5167 and 11.213 mg/dl for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Aspartate aminotransferase (AST) mean values were 29.97 and 30.30 U/l for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Alanine aminotransferase (ALT) mean values were 19.987 and 21.92 U/l for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10. Urea mean values were 3.91 and 4.09 mg/dl for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

Creatinine mean values were 0.4167 and 0.467 U/l for fine bubbles aeration and water change treatment, respectively as shown in tables VII.22. While, Cholesterol mean values were 0.4167 and 0.467 mg/dl for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

In addition to that, Triglyceride mean values were 95.73 and 99.07 mg/dl for fine bubbles aeration and water change treatment, respectively as shown in Tables 3, 8, 9 and 10.

CONCLUSIONS

There is no significance between replicates mean values of fine bubbles aeration treatment and water change treatment for mean corpuscular volume (MCV), Mean corpuscular Hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) indicators. Fine bubbles tube treatment and water change treatment have no significant effect on albumin and lysozyme. Aeration method has a significant effect on Lipase where P value is 0.049. While fine bubbles tube treatment and water change treatment have no significant effect on Amylas.

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