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Growth and health parameters of Nile tilapia (*Oreochromis niloticus*) grown in four different aquaculture systems in Kerala, India

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Abstract
Nile tilapia is successfully farmed in a wide range of environmental conditions using different culture systems and is an important aquaculture fish species in many parts of the world. The objective of this study was to compare the growth, hematological, serological and antioxidant status of Nile tilapia grown in different culture systems such as earthen pond (control), biofloc (BF), aquaponics (AP), and polythene lined (PL) ponds. Fish samples were collected from ten sites for each culture system. No significant difference between the growth in different culture systems, although Feed consumption ratio was higher in PL ponds. Among the haematological parameters studied, the fish cultured in BF and PL ponds showed significantly high ($p<0.05$) White Blood Cell (WBC) values. Red Blood Cell (RBC) and hematocrit were high in fish reared in PL ponds, and mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were significantly higher in fish reared in biofloc. Serum parameters showed significantly less glucose level in AP compared all the other groups. Antioxidant enzymes were significantly higher ($p<0.05$) in fish reared in BF and AP compared to control. The study revealed that most of the health indices used in the study gave better values for fishes grown in biofloc and aquaponics. Biofloc and aquaponics system with stocking density and production per unit area almost 20 and 15 times higher than the earthen ponds respectively, provides better health status to the fish, thus reducing chances of disease outbreaks in these systems, if well managed.

Keywords: Haematology, serology, antioxidant enzymes, biofloc, aquaponics

Introduction
Finfish production from aquaculture has seen rapid growth in quantity, providing nearly 50 percent of all fish production and the industry has great global economic impact. Nile tilapia (*Oreochromis niloticus*) [6, 8, 21, 24, 25, 34] is one of the most widely used aquaculture species and represents approximately 80% of cultured tilapia worldwide (FAO, 2018) [14]. Various traditional and non-traditional tilapia farming methods are adapted in different countries in accordance with the socioeconomic and ecological condition (Lèveque, 2002) [20]. In India, Nile tilapia is cultured in several systems like biofloc, aquaponics, cages, earthen ponds, polythene lined ponds and recirculatory aquaculture systems (RAS). Among these, pond culture is the most traditional system where semi-intensive culture is practiced. In areas where there is problem of seepage, plastic lined ponds are used which do not allow soil-water interaction, and therefore provide fewer natural fish food organisms. Intensive culture of Nile tilapia in tanks has been globally expanding (El-Sayed, 2006) [13] due to less requirement of land and water. Biofloc technology is an approach in aquaculture that has gained much interest in the last decade in Kerala. In this system, a nitrogen cycle is created in zero water exchange aquaculture system by encouraging heterotrophic microbial growth to assimilate nitrogenous waste, which in turn becomes feed source for the cultured species (Avnimelech, 1999) [4]. Aquaponics system exhibits a composite and flexible balance of interactions between aquaculture and hydroponic systems, where, wastes in one sub-system (aquaculture) serve as nutrients in another sub-system (hydroponic) and along with fish, a crop of vegetable also, can be obtained. All these systems are used for the farming of Nile Tilapia in Kerala, India. Intensive farming practices is always associated with infectious diseases, causing heavy loss to farmers. Therefore, it is essential to give more importance to the health of the fish when it is cultured in high capital invested intensive culture systems. Blood parameters are useful for detecting physiological changes in fishes and can provide valuable information related

to health. Total erythrocyte count (RBC), hematocrit (HCT), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), and thrombocyte count, are all routinely studied in fish as tools to assess the health status. Evaluation of these parameters along with routine analysis of serum biomarkers such as glucose, triglyceride, total protein, etc. helps to diagnose and assess stress and/or diseases that have a direct impact on productivity (Öner, Atli and Canli, 2008; Fazio, 2019) [25, 15]. Stress in aquatic environments can increase the intracellular formation of reactive oxygen species (ROS), which can trigger or cause oxidative damage to biological systems (Di Giulio *et al*., 1989a). The amount of antioxidant enzymes, thus, gives an indication of the health status of any organism including fish. The aim of the present study was to compare and evaluate variations in hematological and serum parameters and antioxidant enzyme status in Nile tilapia reared in four different culture systems such as earthen ponds, aquaponics, biofloc and polythene lined ponds, along with growth parameters.

Materials and Methods

Experimental site and design

Fish samples were collected from 40 sites, with 10 sites for each culture system. All these cultures were carried out during a period from November to April 2021. All the sites were located within 9°37'10.70"N-10°24'18.5"N and 76°11'28.5"E-76°32'9.40"E distributed in the three district of Kerala such as Thrissur, Ernakulam and Alappuzha where there were no differences in the climatic conditions during the study period.

Being a conventional system, earthen pond system was taken as control. In earthen ponds, the stocking density was 4-5 fish/m^3, water exchange was not done, but loss of water due to seepage and evaporation was compensated and water depth was maintained at 1 m. Aeration was not provided in these ponds. Biofloc tanks were of uniform size, having 5 m diameter and 1 m depth. In all the 10 sites covered in the study, same procedures were followed in the preparation and maintenance of biofloc. The stocking density was 100 fish/m^3 and the floc volume was maintained at 10-30 cm/L with continuous aeration. Aquaponics systems were stocked with 75-80 fish/m^3. Vegetables grown were same in all the aquaponics systems covered in the present study, i.e., Tomato, Chilli and Spinach. Aeration was provided throughout the culture period. Filters were provided to filter and recirculate the water and the speed of filtration was 1.6-2 L/min. Polythene lined ponds were stocked with 12-13 fish/m^3 and aeration was given. Water level was maintained at 1 m, compensating for evaporation loss, when needed. Feeding was done as per commercial feeding chart.

Sampling

From each site of sampling, 10 fish were selected randomly at the start of culture and at the end of culture, for growth performance studies. Mortality, if any, was recorded daily. Blood was collected at the site itself for hematological studies, to avoid the error that may happen due to stress while transportation. Fish samples were collected from various places and brought to wet lab of the Department of Aquatic Animal Health Management, Kerala University of Fisheries and Ocean Studies for various assays and analysis. Water samples were collected and analyzed on a weekly basis.

Water quality parameters

Water quality parameters like temperature, dissolved oxygen (DO) [12, 37], pH, alkalinity, hardness, total ammonia nitrogen and nitrite-nitrogen were analyzed at 7 days intervals. Dissolved oxygen, alkalinity and hardness were analyzed by standard method (APHA, 1998) [3]. Total ammonia nitrogen (TAN) and nitrite-nitrogen were estimated by Strickland and Parsons (1972) method.

Growth parameters

In order to assess the growth performances of Nile Tilapia in different culture systems, the following indices were used:

$$\text{Specific growth rate (SGR)} = 100 \times [\ln(\text{final weight (g)}) - \ln \text{initial weight(g))}] \times \text{days of culture}^{-1}$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake (g)} \times \text{wet biomass gain (g)}^{-1}$$

$$\text{Production per unit area (Kg/m}^2) = \text{Final wt (g)} \times \text{survival rate x stocking density} \times \text{area under culture} - 1\ (\text{m}^2) \times 10^{-3}$$

$$\text{Survival rate (\%)} = \text{Number of fish stocked / Number of fish caught at time of harvesting} \times 100$$

Blood sampling

Randomly selected fish from different culture systems were anesthetized with clove oil prior to blood sampling. Blood was collected from caudal vein using a sterile 1 ml disposable syringe containing 0.5 M EDTA as an anticoagulant. It was transferred to EDTA vial and closed air tight to prevent hemolysis. Serum was separated from whole blood collected without anti- coagulant by centrifugation at 3500 rpm for 15 min at 4 °C and thereafter stored at -20 °C for further analysis. The whole blood was analyzed for RBC, WBC, HGB, HCT, MCV, MCH, MCHC, PLT, and PWD using Mindray Auto-Veterinary hematology analyzer (BC2800, China). Serum parameters like total glucose, total protein and albumin were estimated using commercially available kits (Agappe Diagnostics Ltd, Kerala, India). The parameters were measured according to the manufacturer’s instructions and the absorbance was read at specific wavelengths using spectrophotometer.

Innate antioxidant enzyme assays

The innate antioxidant enzyme activity was assayed with liver tissue homogenates. The fish samples were dissected immediately after harvest for taking the liver samples in order to estimate antioxidant activity. Each sample was homogenised in a mortar and pestle with appropriate buffers (Phosphate buffer/Tris-HCl). The assay for Superoxide Dismutase (SOD) activity was done according to the method of Das *et al*. (2000) [9]. SOD was defined as unit / mg protein (One unit of SOD activity was calculated using the amount of superoxide dismutase required to inhibit the auto-oxidation reaction by 50%). Catalase (CAT) activity was assayed according to the method of Sinha (1972). CAT activity was defined as the amount of CAT required to transform 1µmol of H_2O_2 per min/mg protein. Glutathione peroxidase (GPx) activity was determined by the method described by Rotruck *et al.* (1973) [32]. GPx was expressed as GSH consumed/min/mg protein. One unit of GPx activity was defined as the amount of GPx required to oxidize 1 μmol of substrate per min.

Statistical analysis

All the data except water quality parameters are expressed in mean±standard error (Mean±SE). The data were compared using a one-way analysis of variance (ANOVA), and the difference between means were tested for significance using Tukey test. The significance level was set at $p<0.05$. Statistical analyses were performed with the software package SPSS 22.

Results

Water quality of the experimental system

The range of variations in the water quality parameters in the experimental tanks during the experimental period are given in the table 1.

Table 1: Physico-chemical parameters of water in the experimental system

Water parameter	Earthen	Biofloc	Aquaponics	Polythene
DO (mg/L)	4.1-4.7	5.2-6.0	4.0-5.0	3.4-4.9
Temperature (°C)	27.6-31.2	28.4-29.8	28.6-30.4	26.8-31.5
pH	6.0-7.5	5.5-7.5	6.5-7.5	6.5-7.5
Alkalinity (mg/L)	5.0-150.0	9.75-141.52	120-230	24-175
Hardness (mg/L)	25.0-275.0	62.0-428.0	150.-300.0	25.0-125.0
Ammonia (mg/L)	0.03-1.17	0.31-7.08	0.24-4.17	0.02-1.95
Nitrite (mg/L)	0.008-0.07	0.002-0.622	0.126-2.600	0.011-0.060
Salinity (ppt)	0-5.0	0-2.0	0-1.0	0-1.0

Growth performances

The growth parameters of fish in different culture system in terms of SGR, FCR, survival (%) and production per unit area were calculated and shown in table 2. Among the four culture systems no significant difference ($p>0.05$) was observed in SGR although the biofloc system (4.5±0.3) showed highest SGR than other culture systems and lowest was obtained from polythene lined ponds (3.7±0.37). FCR values were not significantly different ($p>0.05$) between fishes reared in earthen ponds (1.30±0.13), biofloc (1.12±0.09) and aquaponics (1.36±0.07), but those reared in polythene lined ponds showed a significantly high ($p<0.05$) FCR. The survival rate was not significantly different ($p>0.05$) in the four culture systems. The production per unit area showed significant difference ($p<0.05$) with biofloc (24.38±1.05 Kg/m^2) being the highest followed by aquaponics (14.47±0.44 Kg/m^2). The values obtained for earthen ponds and polythene lined ponds were not different significantly ($p>0.05$), but these values were significantly ($p<0.05$) lower than that of biofloc system and aquaponics.

Hematological analysis

The results of hematological analysis are given in table 2. The results showed significant difference in the different parameters such as WBC, RBC, Hb, HCT, MCV, MCH and MCHC ($p<0.05$). WBC, RBC and thrombocyte count were highest in polythene lined ponds, but MCV, MCH and MCHC were lowest here. Fishes reared in biofloc tanks also showed significantly higher ($p<0.05$) values for WBC, MCH and MCHC compared to control. In the case of fish reared in aquaponics, the haematological parameters were not significantly different ($p>0.05$) from control.

Serum analysis

The mean values of serum parameters such as glucose, total protein, albumin and globulin of Nile Tilapia reared in culture systems such as earthen pond, biofloc, aquaponics and polythene lined ponds are given in table 2. Among the serum parameters, only glucose showed significant difference and the value was significantly higher in fish from earthen and polythene lined ponds whereas in the case of fish reared in aquaponics, the lowest value of 97.25±3.05 mg/dl was obtained.

Antioxidant enzyme analyses

The mean values of SOD, CAT and GPx from the liver tissue of the Nile tilapia grown in different culture systems are given in table 2. The results reveal that the antioxidant enzyme status of the fish reared in biofloc and aquaponics are significantly higher than those reared in earthen and polythene lined ponds. All values on the same row with the different superscripts are significantly difference ($p<0.05$)

Table 2: Various parameter of Nile tilapia cultured in different culture systems

Parameter	Earthen	Biofloc	Aquaponics	Polythene
SGR	4.1±0.18	4.5±0.31	3.9±0.20	3.7±0.37
FCR	1.30±0.13^a	1.12±0.09^a	1.36±0.07^a	2.20±0.25^b
Survival (%)	94±1.18	94.4±2.08	94.5±1.10	89.2±1.95
Production per unit area (Kg/m^2)	0.83±0.03^a	24.38±1.05^b	14.47±0.44^c	1.80±0.09^a
WBC × 10^3/μl	157.82±17.69^a	181.21±16.23^b	149.60±4.06^a	189.15±3.05^b
RBC × 10^6/μl	0.82±0.02^a	0.82±0.06^a	0.90±0.07^{ab}	1.10±0.05^b
Hb (g/dl)	9.12±0.089	10.66±0.45	9.46±0.18	10.21±0.30
HCT (%)	14.97±0.39^a	12.16±0.14^a	16.95±0.08^a	21.06±0.52^b
MCV	180.04±3.35	166.83±5.21	175.44±11.50	179.61±3.85
MCH (pg)	119.55±3.80^a	129.68±8.69^b	109.64±11.39^a	97.78±4.04^a
MCHC (g/dl)	61.29±1.92^a	93.19±4.04^b	58.04±1.44^a	51.52±1.08^a
Platelets × 10^3	45.67±3.52	57.16±5.42	46.20±2.45	58.90±2.97
PDW	19.76±0.23	20.56±0.22	20.06±0.15	19.81±0.21

Glucose (mg/dl)	130.09±10.72^{a}	116.80±9.49^{a}	98.77±7.19^{b}	125.37±4.34^{a}
Total protein (g/dl)	2.04±0.09	2.21±0.12	2.01±0.05	1.87±0.14
Albumin (g/dl)	1.07±0.05	1.28±0.04	1.22±0.08	1.12±0.04
Globulin (g/dl)	1.14±0.10	0.73±0.06	0.65±0.13	0.82±0.09
A/G Ratio	0.94±0.03	1.75±0.04	1.88±0.01	1.37±0.08
SOD (U/mg)	2.87±0.27^{a}	4.74±0.37^{b}	4.0±0.48^{b}	3.21±0.70^{a}
CAT (µ mole of H_2O_2 consumed/min/mg protein)	43.82±2.42^{a}	60.11±3.08^{b}	59.68±1.59^{b}	55.72±5.15^{a}
GPx (µmole oxidised/min/mg protein)	2.35±0.18^{a}	4.51±0.24^{b}	4.38±0.23^{b}	3.47±0.49^{a}

* All values on the same row with the different superscripts are significantly difference (p<0.05)

Discussion

The present study aimed to study the health parameters of Nile Tilapia grown in different culture systems along with the different indices for production performance, to understand whether the fish is in any form of stress, which may invite diseases in these culture systems. The growth and production performances were assessed using specific growth rate, feed conversion ratio, survival rate and production per unit area. The health status was evaluated based on hematology, serology and antioxidant enzyme studies. Among the different growth parameters studied, specific growth rate and survival showed no significant difference between the treatments. Thus, the present study revealed that although stocking density was almost 20 times higher in biofloc and 15 times in aquaponics system compared to earthen ponds, the growth rate and survival was not affected which proves the hardiness of the species for intensive culture. In Walleye also similar growth rate in polythene and earthen ponds have been reported (Ward & Blackwell, 2021) [37]. However, in the present study, the feed conversion ratio was found to be highest in polythene lined ponds whereas the other systems showed a better FCR and was not significantly different between each other. The high FCR obtained the polythene lined ponds may be because of the high stocking density in those ponds (12 /m^2). A stocking density above 5 /m^2 has been found to retard growth in *O. niloticus* grown in polythene lined ponds (Ray *et al*., 2020) [31], which may be because of the wide fluctuation in the water quality parameters due to the high stocking density in polythene lined ponds. A lower FCR (1.7-1.8) in biofloc and aquaponics compared to conventional clear water system has been reported by Azim and Little (2008) [6] and Rakocy *et al*. (2016) [30] in Nile Tilapia. Compared to these studies, the FCR obtained in the present experiment, was still lower. Moreover, the FCR values from biofloc system, aquaponics and earthen ponds showed no significant difference. The results indicate that even at a very high stocking density, fish in the biofloc and aquaponics showed similar growth rate, survival rate and FCR compared to earthen ponds which resulted in higher production per unit area compared to earthen and polyethene lined ponds. The production in the biofloc system was almost 25 times of that from the earthen ponds. The production in aquaponics was almost 15 times of that from the earthen ponds. The present study is the first of its kind comparing aquaponics with other culture systems. The study revealed that the health status of the fish grown in aquaponics were similar to that grown in biofloc and earthen system. Although the production in aquaponics was lower compared to biofloc, the vegetable production in the system helps in diversification of products compensating the farmers and also facilitate a balance of vegetarian and nonvegetarian diets. The diversification possible in the aquaponics for increased food production has been discussed in detail by Kotzen *et al*., (2019) [19]. Blood parameter analyses have proven to be valuable tools to analyze the health status of farmed and wild fishes, as these indices provide reliable information on possible exposure to mutagens, metabolic disorders, deficiencies and chronic stress status before clinical symptoms appear (Bahmani *et al*., 2001) [7]. Hematological profile is an important tool to assess the physiological and pathological conditions of fish (Adhikari *et al*., 2004) [1]. In the present study the hematological tools used to assess the health status of the fish were WBC, RBC, Hb, HCT, MCV, MCH, MCHC, Platelets and PDW. The WBC are important cells in immune system with a variety of functions including phagocytosis, antibody production and release of immune-related molecules (Shoemaker *et al*., 2015) [33]. In the present study, WBC count in polythene lined ponds and biofloc showed a significantly higher value than earthen pond. Hoseini *et al*., (2019) [17] showed that increased stocking density significantly increased WBC. In the biofloc tank, the increase in WBC may be a result of higher stocking density and increased amount of suspended solids in water. In the case of polythene lined tanks also, the increase in WBC may be an indication of stress. There was no significant difference in the RBC count of the fish from biofloc, aquaponics and earthen ponds. Long *et al*., (2015) [21, 25] compared RBC, hematocrit and hemoglobin between biofloc and clear water system and found there was no significant difference in these parameters. Rafatnezhad *et al*., (2008) [29] also reported that there was no significant difference observed in the Hb at different stocking densities. In the present study, RBC and HCT values were significantly high in polythene pond. The erythrocytes count is positively correlated with the hemoglobin and negatively correlated with MCV MHC and MCHC (Bittencourt *et al*., 2003) [8]. In the present study the MHC and MCHC were significantly lower in polythene ponds corresponding to the increase in RBC. This again reveal some stress for the fish in those ponds. Serum factors have also been used as an indicator for the overall wellbeing of different aquatic species (Fazio, 2019) [15]. The total serum protein concentration is also used as an indicator of health and its main function is maintaining blood pH and osmotic pressure (De Lisle, 1971) [10]. It is the protein component of the blood and it decreases with stress (Oluwalola *et al*., 2020) [24]. Apart from stress, reduced appetite of fish, dropping capacities for synthesis and absorption, or increasing protein loss through hemodilution also are reported to be reasons for less serum protein (Patriche *et al*., 2011) [27]. In the present study, there was no significant difference in the serum protein value of the cultured fish, between the different culture systems, revealing minimal stress on the fish. Even though stocking density was high in aquaponics and biofloc, compared to traditional systems, there was no stress for the fish in these systems, showing the antistress efficacy of the systems. The main function of albumin is the regulation of colloidal osmotic pressure of the blood and transport of some exogenous components such as drugs and endogenous chemicals (Peyghan *et al*., 2014) [28]. The low A/G ratio may be characterized by inflammation or a shift from albumin production to globulin in response to stress (Foott *et al*, 1996) [16]. In the current study, the values obtained for albumin and globulin and A/G ratio also showed no significant difference revealing a stress and disease free condition in all the culture system studied. The elevated glucose level in the serum is treated as a secondary stress response (Mommsen *et al*., 1999) [23]. In this study, the serum glucose levels from fish cultured in earthen ponds and polythene ponds were significantly higher compared to other systems, which indicate some level of stress of these fish compared to biofloc and aquaponic systems. Adineh *et al*., (2019) [2] have reported that the serum glucose levels in common carp cultured in biofloc was lower compared to a clear water system with water exchange, pointing the antistress effects of biofloc. In aquaponics, the glucose level was lowest revealing the fact that this environment provided the maximum stress-free environment among all the other culture systems. Induction of antioxidant enzymes is an important line of defense against oxidative stress in biological systems (Parihar & Dubey, 1995) [26]. Superoxide dismutase activity and catalase activity play important roles to protect cells against H_2O_2 production (Karadag *et al,* 2014) [18]. SOD interacts with superoxide anion to produce H_2O_2 and O_2, while CAT and GPx can rapidly decompose H_2O_2 into H_2O and O_2, keeping free radicals at a low level. Lower levels of SOD and CAT indicate cell damage due to the accumulation of high-level free radicals in cells affecting the quality and palatability of fish which impacts human consumption (Menaga *et al*., 2019) [22]. In the present study, SOD activity was significantly high in biofloc compared to all other systems. CAT showed significantly high values in biofloc and aquaponics compared to earthen ponds. Long *et al*. (2015) [21, 25] and Shourbela *et al.* (2021) [34] also reported similar results in O. niloticus [6, 8, 21, 24, 25, 34]. In the present study, the GPx activity in the liver tissue homogenate of tilapia cultured in the biofloc and aquaponics showed significantly high values compared to earthen and polythene lined ponds. According to Do *et al*., (2019) [12], GPx is the key enzyme that protects fish from oxidative stress. Similar results were also obtained by Long *et al*., (2015). [21, 25] Thus, in the present study the antioxidant enzyme status was higher in biofloc and aquaponics compared to other culture systems, thus revealing the better health status of the fish in these systems.

Conclusion

The study revealed a better health status and better production of *O. niloticus* in biofloc and aquaponics compared to earthen ponds and polythene line earthen ponds. The overall results of the present study reveal that the health parameters of the fish from high stocking density systems such as biofloc and aquaponics are better compared to the traditional earthen pond and polythene lined pond system, with no significant different in growth and survival, indicating that these systems can be used extensively, provided all the conditions required are practiced as per guidelines given by the governmental agencies based on research findings.

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Declarations

Ethical Approval and consent to participate

All experiments were performed in strict accordance with the guidelines of the Committee for Purpose of Control and Supervision of Experiments on Animal (CPCSEA) registration number: 1174/ac/08/CPCSEA. The protocol was reviewed and approved by the institutional animal ethics committee of Kerala University of Fisheries and Ocean Studies, India.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent for publication

The authors consent to the publication of the work.

Authors' contributions

Sagar Gorakh Satkar: Conceptualization, Methodology, Data Curation, Investigation, Visualization, Writing - Original Draft, Writing**;** Omanakuttan Nibin: Data Curation, investigation, Methodology, Formal analysis**;** Sooraj Suresh Nediyirippil: Methodology, investigation, Data Curation, Formal analysis, Software; Gijo Ittoop: Project administration, Investigation Supervision, Resources, Writing - Review & Editing**;** Vadavanath Prabhakaran Vineetha: Methodology, investigation, Statistical analysis**;** Pillai Devika: Funding acquisition, Project administration, Investigation, Supervision, Resources, Conceptualization, Methodology, Data Curation, Writing - Original Draft, Writing - Review & Editing.

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Availability of data and materials

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