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Potential Carbon Thickness on Ammonia Content in Nile Tilapia (*Oreochromis niloticus*) Aquaponics System with Water Spinach (*Ipomoea aquatica*)

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ABSTRACT

The application of aquaponics aquaculture is needed to improve water quality, especially the addition of filtration materials which act as absorbent planting mediums for ammonia nitrogen content in toxic water. The purpose of the current study was to determine the effect of the carbon thickness on ammonia levels in Nile tilapia in aquaponics systems with Water spinach. The study used an experimental method with a completely randomized design consisting of four treatments and five replications. The main parameter was ammonia level. Supporting parameters in this study included initial and final growth of Water spinach, fish survival rate, specific growth rate, and water quality which included temperature measurement, dissolved oxygen, and pH. Each treatment consisted of variations of carbon thicknesses at P0 (control), P1 (5 cm), P2 (7 cm), and P3 (9 cm). The results revealed that ammonia levels were significantly different at P1, P2, and P3 in the third and fourth weeks. Ammonia levels during the study decreased from 0.3969 ppm to 0.1741 ppm. The reported value of 7.2 was for acidity degree, 29.3°C for the temperature, 5.94 ppm for dissolved oxygen, 8.42 cm for the growth of Water spinach, 0.44% for the specific growth rate, and 90% for the survival rate. Carbon thickness caused a decrease in ammonia levels in Nile tilapia with aquaponics systems through the medium of Water spinach.

Keywords: Ammonia, Carbon thickness, *Ipomoea aquatica, Oreochromis niloticus*

INTRODUCTION

Nile tilapia (*Oreochromis Niloticus*) has become one of the leading commodities in aquaculture. Optimizing cultivation is deemed to be necessary to improve the quality of producing Nile tilapia including optimization of water quality. Aquaponics is a significant alternative system in improving water quality, especially waste management. In terms of utilization, it is necessary to have suitable planting and filter media for aquaponics to reduce ammonia levels in fish farming.

Nile tilapia is often used as fish in cultivation because of its superiority. It is easy to breed, has fast growth, eats all food ingredients, has extensive adaptive power, and has a high tolerance to various environmental conditions (Adhim et al., 2017; Soegianto et al., 2017). Accordingly, Nile tilapia has a very strategic position for cultivation development. Based on data from the Food and Agriculture Organization (FAO) in 2018, fish demands for the world market up to 2015 have had a supply shortfall of 2 million tons per year. Fulfillment of fish supply shortages could be fulfilled by optimizing Nile tilapia cultivation (FAO, 2021).

Optimizing cultivation could not be separated from waste management. Accumulation of waste would affect the growth, physiology, behavior, and mortality of fish. Fish in aquaculture contains a lot of ammonia because of the food leftovers and feces from excretion (Yin et al., 2018). High levels of ammonia in aquaculture ponds would reduce water quality and have a negative impact on cultivated fish. The application of aquaculture technology is needed to improve water quality, one that can be applied is integrated cultivation technology between fish and aquatic plants in principled aquaponics systems recirculation.

Aquaponics technology can produce fish optimally on narrow land with limited water sources and can be applied in urban areas (Diver, 2006). The choice of commodities for aquaponics systems played an important role in planning and obtaining results in accordance with what was desired (FAO, 2014). Aquaponics was a biological integration system through recirculation of aquaculture and hydroponics of vegetables, flowers, or medicinal plants that needed water continuously during the maintenance process (Diver, 2006). Aquatic plants could utilize nutrients so they could act as effective filters to improve water quality. Some types of plants commonly used in aquaponics systems were Water spinach, bok choi, spinach, chili, and tomato (Akter, 2018). Water spinach plants in aquaponics systems reduce ammonia by absorbing wastewater (Effendi et al., 2015) through roots so they could absorb ammonia undergoing an oxidation process with the help of oxygen and bacteria. Ammonia is then converted to nitrate which can be later used as source

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nutrition (Marschner, 1995; FAO, 2014). However, ammonia concentration should not be excessive. If it was excessive, the plant would be susceptible to disease attacks and it would slow down plant growth (Helali et al., 2010; Silva et al., 2016).

The addition of biofiltration to the aquaponics system could reduce ammonia (Miller and Libey, 1985). This problem could be overcome by applying a recirculation system with the addition of filters to filter water to improve water quality so that it could be reused (Michaud, 2007; Interdonato, 2012). One of the materials used for planting media that could function as a good filter to reduce ammonia in aquaponics systems is carbon because it has good absorbent properties (Brennan et al., 2002).

Ammonia in the water would become ammonium because it reacts with H2O. Activated charcoal can absorb ammonia in the waters, where it has an active chemical group on the entire surface of the solid. There are free radical compounds in the active group, especially in Carbon (C) atoms that have free electrons so that C atoms that have a negative charge. Moreover, they have the ability to attract positively charged ammonium (Amin et al., 2016). The binding of ammonium ions to C results in reduced ammonia molecules (Amin et al., 2016). The ability to absorb C depends on its thickness so that the thicker the C used as an absorbent the better the results of its absorption (Mifbakhuddin, 2010). Therefore, the current study aimed to determine the effect of the carbon thickness on ammonia levels in Nile tilapia (*Oreochromis niloticus*) aquaponics systems with Water spinach (*Ipomoea aquatica*).

MATERIALS AND METHODS

Retrieval and acclimatization

All experimental protocols and procedures were approved by the Institutional Animal Care of Indonesia. All pieces of equipment, including aquariums, aeration hoses, aeration rock, water pipes, and gutters, were sterilized first. The used Carbon (C) was C from the coconut shell. The Nile tilapia seeds were in the size of 6-8 gr/each fish. The used water was previously grown for 2 weeks until it had a length of 5-8 cm (Setijaningsih and Suryaningrum, 2015).

The current study used aquaponics systems. The planting medium that was used in the present experiment was in the form of C, and the plants used were Water spinach. There were three treatments of variation in thickness of planting media in the form of C, namely P1 (5 cm), P2 (7 cm), P3 (9 cm) according to the method of Maharani and Pinjung (2016). Water spinach that had been shown to a length of 5-8 cm were planted with a distance of 10 cm per plant so that one tray of 35 × 20 cm required 11 Water spinach plants (Sumiarsih, 2021). Aquariums used as a maintenance container were 30 cm × 30 cm × 17 cm in size. Inlet and outlet holes were made of the same size so that the volume of water remained stagnant between the water entering and leaving the inside of the plant maintenance so that the maintenance of the plant remained inundated by water.

Preparation of Nile tilapia (*Oreochromis niloticus*) and rearing conditions

The study was conducted at the Faculty of Fisheries and Marine of Air Langa University (Indonesia) in 2019-2020.

Nile tilapia seeds used in the current study came from UPT PBAT Umbulan, Pasuruan, Indonesia. A total number of 500 tilapia seeds were purchased with a weight of 6-8 gr/each fish and an average length of 7-8 cm then maintained in an aquarium with a density of 10 fish/aquarium size 30 cm × 30 cm × 17 cm with a volume of 15 liters of water while Nile tilapia's stocking density was 5 g/L (Sace and Fitzsimmons, 2013). Nile tilapia fish which were maintained were given commercial feed three times a day (morning, afternoon, evening). Feeding administration was calculated as 3% of the average weight of fish.

Experimental design

The study began in January 2019 and was completed in July 2020. The present study was performed based on the experimental method of completely randomized design. In the current study four treatments were used, each treatment received five repetitions (group = 125, n = 25 per group). The treatments in the current study were based on the variation of C thickness as P1 (5 cm), P2 (7 cm), P3 (9 cm), and P0 for the control. The time interval between repetitions was one week. The main parameter observed was the ammonia (NH3) level. Ammonia measurement was carried out using spectrophotometry with the Fenat method (NSAI, 2005). Supporting parameters were initial and final Water spinach growth, survival rate (SR), specific growth rate (SGR), and water quality. Water quality analysis was carried out using procedures and (APHA, 2012). The measured parameters for water quality were temperature, pH, and dissolved oxygen (DO). These tests were performed every week.

Analytical procedure

To make a calibration curve with the Fenat method the following steps were performed. At first, 10 mg/L ammonia standard solution was piped as much as 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL, 3.5 mL, 4 mL, 5 mL, and 6 mL, then put each into a 100 mL volumetric flask. After that distilled water was added to the anchovy until the ammonia level were

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0.1 mg/L, 0.15 mg/L, 0.2 mg/L, 0.25 mL, 0.3 mg/L, 0.35 mg/L, 0.4 mg/L, 0.5 mg/L, 0.6 mg/L, and homogenized. Testing the samples was done by moving 25 mL of the test sample into a 50 mL volume tube, then adding 1 mL of phenol solution, and homogenizing it, followed by adding 1 mL of sodium nitroprusside, and homogenizing it was done once more. 2.5 mL oxidizing solution was added and homogenized. The volume tube was closed and biased in a dark space for 1 hour for color formation. The solution was inserted into the cuvette on a spectrophotometer, read, and recorded for absorption at a wavelength of 640 nm.

Here is how to calculate ammonia levels using the following formula (NSAI, 2005):

Ammonia level (mg N/L) = C x df

Where, C is content obtained from the measurement results (mg/L), df signifies Dilution factor (SNI 06-6989.30-2005).

Measuring water temperature and DO was done by using a DO meter, and pH was measured using a pH pen. Initial and final Water spinach growth (cm) were carried out by manual measurement using a ruler as a measuring instrument and measuring from the root to the tip of the leaf.

Specific Growth Rate was calculated as follows (Steffens, 1989):

$$SGR = \frac{InWt - InWo}{t} \text{x}100\%$$

Where, InWt denotes fish weight at the end of the study (gr), InWo is fish weight at the beginning of the study (gr), and T refers to maintenance time (minutes).

The SR was calculated as follows (Effendie, 2002):

$$SR = \frac{Nt}{No} \text{ x } 100\%$$

Where, Nt is the number of fish at the beginning of the study and No signifies the number of fish at the end of the study

Water samples for the ammonia test were taken by inserting water into the bottle using a hose. Water samples for the ammonia test were needed as much as 25 mL. Sample water quality can be maintained using a temperature of 4^oC.

Statistical analysis

The data obtained were analyzed using the ANOVA (Analysis of Variance) test since the data obtained were homogeneous. The significantly different treatment means were investigated using Duncan's new multiple range test. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

The analysis of ammonia content during the study revealed a decrease in ammonia concentration. The decrease in ammonia concentration can be seen in Table 1 and Figure 1.

Ammonia content values based on the results of the study ranged from 0.1741 to 0.4875 ppm. Duncan's Multiple Range Test indicated that treatments in the first week did not present significant differences ($p > 0.05$), but in the second week, each treatment presented significant differences. There were significant differences in ammonia levels in the third and fourth weeks in P1, P2, and P3 ($p < 0.05$), but were no significant differences between P0 and P1 ($p > 0.05$). The statistical test results also presented the highest results of ammonia values found in the fourth week of P0 as much as 0.4875 ppm and the lowest value was in the fourth week of P3 as much as 0.1741 ppm.

The analysis results of the average pH, temperature and DO during the study period can be seen in Table 2**.** The pH value of aquaculture water based on the results of the current study was within the range of 7.2-8. The pH value at the first measurement averaged 7.675 and experienced gradual fluctuations until it reached 8 on the fourth week in the P0 measurement. In addition to P0, the pH value decreased in the measurement of the second week in each treatment. The pH value in P2 and P3 in the third week decreased while in P1 it increased and P0 tended to be stable. The increase in the measurement of the fourth week was observed at P0, P1, and P2 while in the fourth week of P3 it continued to experience a decrease in succession. However, the temperature in the study revealed a high value of fluctuation with the lowest value of 29.3 at P3 in the fourth week. The highest temperature value during the study was found in the first week for P1, P2, and the fourth week for P0. Dissolved oxygen values of the water ranged from 4.56 to5.94 ppm. Judging from the effect of the treatments given, P0 decreased from the first week of 5.39 ppm to the fourth week of 4.56 ppm. On the other hand, the increase occurred in P3 from the first week by 5.32 ppm until the fourth week at 5.94 ppm.

The results of the initial and final Water spinach measurements during the study have been demonstrated in Figure 2 which performed a significant increase with the best performance on P3 treatment (10.45 to 18.87 cm, $p < 0.05$). The results from the analysis of the average specific growth rate and survival rate of Nile tilapia could be seen in Table 3.

The average value of specific growth rates of Nile tilapia indicated that the highest average value of the specific growth rate was found in treatment P3 (9cm), which was equal to 0.44%. However, the lowest specific growth rate was

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found in treatment P0 (control) which was equal to 0.40%. The graphical mean value of the specific growth rate of Nile tilapia indicated that the highest average specific growth rate was found in treatment P3 (9 cm) which was equal to 0.44 %. Whereas, the lowest specific growth rate was found in treatment P0 (control) which was equal to 0.40%.

Table 1. Average results of ammonia levels during the study

Treatment	Ammonia Value (ppm) ± SD First week	Ammonia Value (ppm) ± SD Second week	Ammonia Value (ppm) ± SD Third week	Ammonia Value (ppm) ± SD Fourth week
P0	$0.3985^{a} \pm 0.0127$	$0.4009^{d} \pm 0.0127$	$0.4426^{c} \pm 0.0151$	$0.4875^{c} \pm 0.0419$
P1	$0.3993^{a} \pm 0.0133$	$0.3737^{c} \pm 0.0054$	$0.4229^{c} \pm 0.0183$	$0.4609^{c} \pm 0.0269$
P2	$0.3965^{a} \pm 0.0184$	$0.3340^{b} \pm 0.0340$	$0.3111^{b} \pm 0.0251$	$0.3108^{b} \pm 0.0169$
P3	$0.3969^{a} \pm 0.0169$	$0.2594^{a} \pm 0.0099$	$0.2290^{a} \pm 0.0287$	$0.1741^{a} \pm 0.0414$

Different superscripts show significant differences ($p < 0.05$). P0: No charcoal, P1: 5 cm of charcoal thickness, P2: 7 cm of charcoal thickness, P3: 9 cm of charcoal thickness

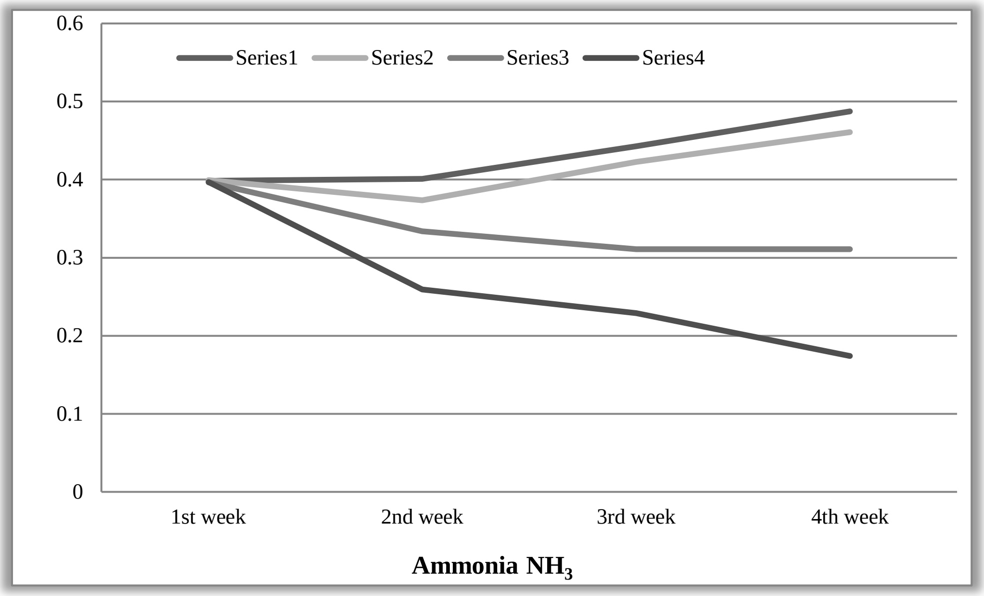

Figure 1. Ammonia level (mg/l). Note: P1: 5 cm of charcoal thickness, P2: 7 cm of charcoal thickness, P3: 9 cm of charcoal thickness

Table 2. Average results of pH, temperature, and dissolved oxygen

pH				Temperature ºC				DO			
W1	W2	W3	W4	W1	W2	W3	W4	W1	W2	W3	W4
7.7	7.9	7.9	8	29.5	29.6	29.7	29.8	5.39	4.92	4.62	4.56
7.6	7.6	7.8	7.9	29.8	29.5	29.5	29.5	5.63	5.72	5.61	5.32
7.7	7.5	7.5	7.6	29.8	29.5	29.5	29.6	5.44	5.53	5.57	5.58
7.7	7.4	7.3	7.2	29.5	29.5	29.4	29.3	5.32	5.59	5.65	5.94

W: Week, DO: Dissolved oxygen

Table 3. Average specific growth rate and survival rate

Treatment	Specific Growth Rate	Survival Rate
P0	0.40%	72%
P1	0.42%	78%
P2	0.43%	82%
P3	0.44%	90%

P1: 5 cm of charcoal thickness, P2: 7 cm of charcoal thickness, P3: 9 cm of charcoal thickness

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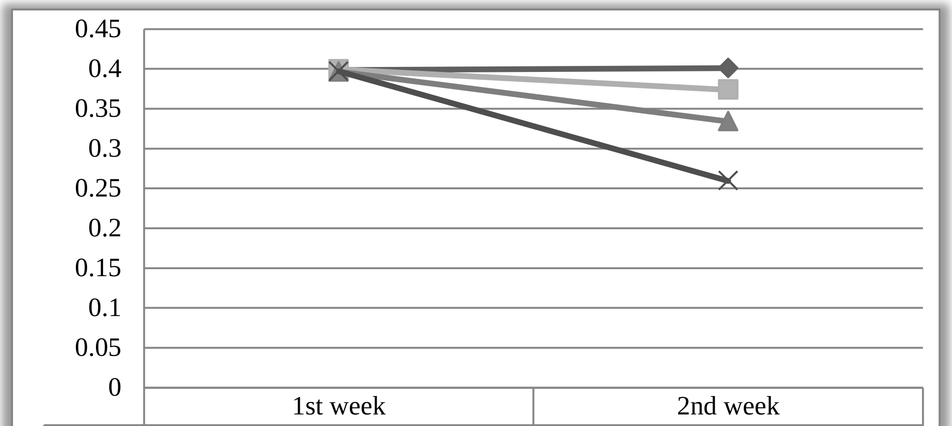

	1st week	2nd week
P0	0.3985	0.4009
P1	0.3993	0.3737
P2	0.3965	0.334
P3	0.3969	0.2594

Figure 2. Water spinach growth results. P1: 5 cm of charcoal thickness, P2: 7 cm of charcoal thickness, P3: 9 cm of charcoal thickness

DISCUSSION

The obtained results indicated that ammonia levels were significantly different at P1, P2, and P3 in the third and fourth weeks with the best treatment at P3 (9 cm thickness). However, in P0 and P1, there were no significant differences. There was a decrease in ammonia levels with different values due to the treatment given in administering different carbon with each thickness in each treatment. This was appropriate because the ability to absorb carbon was adjusted to its thickness, so that the thicker the media used, the better the results of its absorption (Mifbakhuddin, 2010). On the other hand, in the first week, ammonia measurements were not significantly different in each treatment. The average ammonia value in all four treatments at the first week was 0.39801 ppm since in the first week, there was no administration of carbon in all treatments so that the ammonia value obtained from the measurement results presented no significant difference.

Ammonia levels at P0 gradually increased until the measurement of the fourth week to 0.4875 ppm. As the absorption of ammonia was not optimal, so ammonia continues to increase every week. The longer the maintenance time, the higher the accumulation of ammonia concentration produced. Ammonia concentration during the maintenance period of fish has increased maintenance time (Handy et al., 1999). Ammonia concentrations that were increasingly high affected the ability of Water spinach plants to absorb accumulated ammonia.

Ammonia levels were significantly different in all treatments in the second week. Ammonia levels in P1, P2, and P3 decreased in the second week because of the administration of carbon so that ammonia was absorbed by the carbon. However, in P0 ammonia levels increased. NH_3 in water is NH_4OH because it reacts with H_2O. $NH_3 + H_2O \rightarrow NH_4OH$, NH_4OH is broken down into NH_4 + and OH- ions (Amin et al., 2016). Activated carbon can adsorb NH_4 + ions in water because it has an active group on all solid surfaces, where there are free radical compounds in the active group at C atoms which have free electrons so that negative C atoms have the ability to attract positively charged NH_4+ ion.

Besides measuring ammonia, there was also observation of several water quality parameters, such as pH, temperature, and DO. This is because the supporting parameters could affect ammonia concentration directly or indirectly. Based on the measurement results, it was found that the pH value during the study ranged from 7.2 to 8. The concentration of ammonia in toxic water could increase with increasing pH values and would decrease with pH decrease (Wurst, 2003). Increased ammonia levels from the measurement of the first week to the fourth-week measurement were followed by an increase in the pH value, as well as the subsequent measurements. On the other hand, pH and temperature also played an important role in ammonia levels. During the research, it was found that the temperature in the pond water ranged from 29.3 to 29.8^{o}C. When the temperature was compared to pH, the effect of temperature would be lower (Boyd, 1982). Therefore, it could be seen that the pattern of temperature fluctuations did not resemble the pattern of fluctuations in ammonia and pH. Differences in fluctuations in temperature values were not more than 1^{o}C in each treatment. Because, during cultivation media maintenance, fish were not exposed to direct sunlight exposure. Dissolved oxygen played an important role in determining ammonia levels in the water. The DO content in the water was around 4.56 -5.96 ppm. The DO value obtained was still relatively safe to support fish life in the cultivation process.

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Water spinach growth during the study experienced a significant increase. The best Water spinach growth value was found in the P3 treatment ($p < 0.05$). In treatment P3, Water spinach growth rose up to 8.42 cm with an initial size of 10.45 to 18.87 cm. The results of measurements of Water spinach at the beginning and end of the study indicated optimal Water spinach growth. This was due to Water spinach plants could absorb nitrate optimally, sufficiently dissolving oxygen, adequate lighting, and the appropriate spacing. The process of absorption of organic matter by plants occurs through the roots. Proper spacing based on the type of plant would make the plants grow well by giving inadequate growth space. The spacing which used for planting Water spinach is 10 cm. Besides, the ammonia content that has been converted into nitrate is also an important factor in the optimal growth of Water spinach in the aquaponics system.

The average value of the specific growth rate (SGR) of Nile tilapia revealed the highest was found in P3 treatment which was equal to 0.44% per day. However, the lowest specific growth rate was found in treatment P0 (control) which was equal to 0.40% per day. These results were obtained because the measurement of ammonia levels in P3 had the lowest value compared to the other treatments. On the other hand, P0 has the highest average ammonia level compared to the other treatments. The high level of ammonia could trigger the onset of disease, cause stress and reduce fish appetite so that growth became inhibited (Lin and Chen, 2003; Barbieri and Bondioli, 2015).

The highest value of survival rate was found in the P3 treatment which was equal to 90%, while the lowest survival rate was found in treatment P0 (control) which was 72%. In treatment P1, P2, and P3, fish mortality also occurred, but not as much as in P0 (control) treatment. The lowest survival rate in treatment P0 was caused by higher ammonia concentration compared to other treatments of P1, P2, and P3. In a zero water exchange system, such as in a calm water pool, the concentration of cultivated waste, such as ammonia (NH3), nitrite (NO2), and carbon dioxide CO2, would increase. High ammonia concentrations resulted in physiological disorders and triggered stress in fish (Lin and Chen, 2003; Barbieri and Bondioli, 2015). Stress in fish caused a decrease in endurance so that fish were susceptible to disease and decreased appetite which led to fish death.

CONCLUSION

Based on the results of the present study, it can be concluded that the thickness of carbon affects ammonia levels in Nile tilapia (*Oreochromis niloticus*) with aquaponics systems through the medium of Water spinach (*Ipomoea aquatica*). The optimum level to reduce ammonia levels was presented in P3 of the current study with 9 cm of carbon thickness which from in the first week was 0.3969 ppm and then became 0.1741 ppm in the fourth week.

DECLARATIONS

Competing interests

Authors declare no competing interests.

Authors' contribution

All authors participated equally in this study.

Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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