UNIVERSITI PUTRA MALAYSIA

ANAESTHETIC EFFECTS OF SODIUM BICARBONATE ON RED TILAPIA JUVENILE FISH, *Oreochromis* spp.

MUHAMMAD HANIF BIN ABU HAMID

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FACULTY OF VETERINARY MEDICINE

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CERTIFICATIONS

It is hereby certified that we have read this project paper entitled "Anaesthetic Effects Of Sodium Bicarbonate On Red Tilapia Juvenile Fish, *Oreochromis spp.*" by Muhammad Hanif bin Abu Hamid and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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DR. CHEN HUI CHENG
DVM (UPM), MVM (UPM), DVSc (Guelph)
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

____

DR NUR DIYANA MOHAMAD TAHIR
DVM (UPM), PhD (UPM)
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

DEDICATIONS

This project paper is dedicated to my beloved family, lecturers, staffs, seniors and juniors of Faculty of Veterinary Medicine, UPM and to whom it might be beneficial.

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ABBREVIATIONS

CO_2	Carbon Dioxide
MS-222	Tricaine Methanesulfonate
ppm	Parts Per Million

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterianr untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

KESAN ANESTETIK NATRIUM BIKARBONAT PADA IKAN TILAPIA MERAH JUVENIL (*Oreochromis spp.*)

Oleh

Muhammad Hanif Abu Hamid

2023

Penyelia: Professor Madya Dr Chen Hui Cheng

Penyelia Bersama: Dr Nur Diyana Mohd Tahir

Ikan tilapia merah (*Oreochromis spp*.) merupakan salah satu spesies utama dalam industri akuakultur di Malaysia. Demi kebajikan ikan, penggunaan ejen anestetik yang sesuai semasa pelbagai prosedur adalah sangat penting untuk meminimumkan tekanan. Kajian ini menyelidiki potensi anestetik natrium bikarbonat, menilai keberkesanan dan keselamatannya pada ikan tilapia merah juvenil. Sejumlah 40 ekor ikan diagih secara rawak kepada tiga kepekatan natrium bikarbonat (25 g/L, 50 g/L, dan 7 5g/L) dan tricaine methanesulfonate (MS-222, pada 150 ppm), dengan n = 10 untuk setiap kumpulan rawatan. Dua bekas lutsinar bersiaz 1 litre masing-masing digunakan sebagai bekas aruhan dan bekas pemulihan Ikan dipindahkan ke dalam bekas pemulihan setelah mencapai anestesia pembedahan (peringkat 4), atau 15 minit perendaman, yang mana lebih awal ikan yang terdedah kepada natrium bikarbonat pada kepekatan 25 g/L dan 50 g/L hanya mencapai anestesia ringan (peringkat 3) dalam tempoh 15 minit perendaman. Sebaliknya, ikan pada 75 g/L dan MS-222 mencapai anestesia pembedahan (peringkat 4) pada 195 ± 88 dan 418 ± 74 saat. Selain itu, kesemua 40 ekor ikan berenang dengan normal dalam masa 10 minit pemulihan. Walau bagaimanapun, semua ikan yang terdedah kepada natrium bikarbonat mati dalam tempoh 2 hari selepas anestesia manakala semua ikan dalam kumpulan MS-222 hidup. Kajian ini menunjukkan bahawa natrium bikarbonat boleh mengaruh anestesia ringan hingga pembedahan. Walau bagaimanapun, kematian 100% pasca anestesia menimbulkan kebimbangan mengenai keselamatan dalam penggunaannya pada ikan tilapia merah juvenil.

Kata kunci: *Oreochromis spp*; anestesia; natrium bikarbonat; ikan juvenil

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999- Project.

ANAESTHETIC EFFECTS OF SODIUM BICARBONATE ON RED TILAPIA JUVENILE FISH (*Oreochromis spp.*)

By

Muhammad Hanif Abu Hamid

2023

Supervisor: Associate Professor Dr Chen Hui Cheng

Co-Supervisor: Dr Nur Diyana Mohd Tahir

Red tilapia fish (*Oreochromis spp.*) is one of the major species in the aquaculture industry in Malaysia. The welfare of these fish during various procedures is paramount, necessitating the use of suitable anaesthetic agents to minimise stress. This study delves into the anaesthetic potential of sodium bicarbonate, evaluating its effectiveness and safety in red tilapia juvenile fish. A total of 40 fish were randomly assigned to the three concentrations of 25 g/L, 50 g/L, and 75 g/L sodium bicarbonate and 150 ppm tricaine methanesulfonate (MS-222), with n = 10 per treatment group. Two transparent 1-litre tanks were used as the anaesthetic induction and recovery chamber respectively. Fish were transferred into recovery chamber once attaining surgical (Stage IV) anaesthesia, or 15 minutes of immersion, whichever earlier. Fish subjected to sodium bicarbonate at concentrations of 25 g/L and 50 g/L achieved only light anaesthesia (Stage III) within the 15 minutes of immersion. In contrast, fish in 75 g/L and MS-222 reached surgical anaesthesia (Stage IV) at 195 ± 88 and 418 ± 74 seconds, respectively. All 40 fish regained normal swimming patterns within 10 minutes of recovery. However, all fish exposed to sodium bicarbonate died within 2 days post-anaesthesia while all fish in MS-222 survived. In summary, this study shows that sodium bicarbonate can induce light to surgical anaesthesia. However, the 100% mortality post-anaesthesia raises safety concern on its use in red tilapia juvenile fish.

Keywords: *Oreochromis spp*; anaesthesia; sodium bicarbonate; juvenile fish

1. INTRODUCTION

1.1 Background

Various anesthetics have been long employed in fishery operations for their research studies as well as routine processes to mitigate stress in fishes (Dong et al., 2017; Hoseini et al., 2019; Nordgreen et al., 2014). It is crucial to mitigate stress during fish handling, sampling and surgery by anesthesia as diseases caused by pathogens are commonly found in the aquatic ecosystem (Tuttle et al., 2023; Xu et al., 2023; Yu et al., 2023; Zhu et al., 2023). Stress encountered by the fish may cause lowered immunity and render the fish to become more susceptible to opportunistic diseases. Sodium bicarbonate, also known as bicarbonate of soda and baking soda, is inexpensive and commonly available in various countries as a safe chemical anesthetics on fish (Anesthetic Options for Fish | IVIS, 2001). The efficiency of sodium bicarbonate as an anesthetic agent is said to depend on its ability to give off carbon dioxide gas (CO_2). It is reported to be effective in sedating fish while not having effects on humans and fish (Altun et al., 2009; Hasimuna et al., 2021). Sodium bicarbonate has been studied on common carp juveniles (*Cyprinus carpio*) in Turkey, african catfish juveniles (*Clarias gariepinus*) in Kenya and mozambique tilapia (*O. mossambicus*) in Namibia (Altun et al., 2009; Githukia, 2016; Gabriel et al., 2020). In Malaysia, there is no published report on the use of sodium bicarbonate on fish. This study aims to evaluate the effectiveness and safety of sodium bicarbonate to anaesthetise red tilapia juvenile fish.

1.2 Hypothesis

The hypotheses for this study are:

$1H_0$: There is no significant difference in the induction and recovery time at different concentrations of sodium bicarbonate.

$1H_a$: There is significant difference in the induction and recovery time at different concentrations of sodium bicarbonate.

1.3 Objectives

The objective of this study is:

1. To determine anaesthetic effects of sodium bicarbonate in red tilapia juvenile fish.

2 LITERATURE REVIEW

Tricaine methanesulfonate, or also known as MS-222, is commonly used in aquaculture and it is the only fish sedative that has been approved by United States Food and Drug Administration (FDA). Anaesthetic effects of MS-222 has been studied on yellow catfish (Pelteobagrus fulvidraco), a freshwater species in China (Liu et al., 2022). Results from this study indicates that usage of MS-222 in aquaculture is very affective as an authorised anaesthetic agent which could reduce the physiological stress of the fish during transportation. However, fish treated with MS-222 require 21-days of withdrawal period (Bowker et al., 2018). Therefore, there is a need to look for alternative agents with less residue or safety concerns for human consumption. One such potential agent is the sodium bicarbonate.

The anaesthetic effects of sodium bicarbonate has been studied on Mozambique tilapia (*O. Mossambicus*), a freshwater species in Namibia (Gabriel et al., 2020). In this study, the authors have identified that if the aquatic temperature is increased along with sodium bicarbonate concentration, the anesthetic induction time will increase. Additionally, the induction time increases with the body size of the fish. Furthermore, it is also found that the recovery time of the fish is positively correlated to the fish size and sodium bicarbonate concentration. With the evidence of this study, it indicates that sodium bicarbonate is effective as anesthesia in Mozambique tilapia at all examined fish sizes and aquatic temperature.

In addition, the application of sodium bicarbonate as anesthesia in euryhaline fish as a model species, specifically juvenile red tilapia hybrids, was studied by

Avillanosa & Caipang (2019). The study of interest was focused on the effectiveness of using sodium bicarbonate as a sedating agent in both freshwater and brackishwater environments. The study showed that sodium bicarbonate has proven its effectiveness as a safe anesthesia for red tilapia hybrid juveniles. Further to this, they found that brackish water is slower than freshwater for the tilapia fish to fully sedate the fish regardless of the dose. The authors have concluded that at higher temperature, higher concentration of sodium bicarbonate is required to sedate the tilapia fish, however, the recovery time showed insignificant difference in both environment and anesthetic dose.

Based on the available published studies, the use of sodium bicarbonate seems to be inexpensive, and safe as a chemical anaesthetic in tilapia fish. As there were no published report on the use of sodium bicarbonate on juvenile red tilapia fish in Malaysia, further studies in this area is needed.

3 MATERIALS AND METHODS

3.1 Experimental Fish

A total of 40 red tilapia juvenile fish (*Oreochromis spp.*) were bought from Aquamas Resources, Puchong. The fish were placed in 2 water tanks, each.each tank divided into two using a plastic divider. The fish were randomly assorted into the 4 groups with 10 fish for each group. Mesh net was used for the transfer of the fish into the partitions. Fish were acclimatised for 2 days and fasted 24 hours prior to the experiment day to prevent from regurgitation of feed which will increase the ammonia level in the water (Hasimuna et al., 2019).

3.2 Experimental Design

In this study, the experiments were conducted using two transparent 1-litre tanks, which would become the anaesthetic chamber and recovery chamber, and a 10-litre tank for the monitoring chamber. The sodium bicarbonate with the brand of Arm&Hammer in powder form was bought from local mart. The company claimed that this product contains 100% sodium bicarbonate. Tricaine-S (MS-222) was used as the controlled anaesthetic agent for the control group. A concentration of 150 ppm of MS-222 and three different concentrations of sodium bicarbonate (25, 50, and 75g/L) were used by following tested concentrations from previous successful experiments on juvenile hybrid red tilapia fish and greenhead tilapia (Avillanosa et al., 2019; Hasimuna et al., 2019). Each group of A,B,C, and D will be exposed to different concentrations of 25, 50, 75g/L of sodium bicarbonate and 150 ppm of MS-222 respectively. The solutions were stirred until all the solvents are fully dissolved.

The experiment was performed by anaesthetising and recovering one fish at a time. One fish was transferred from the main tank to the anaesthetic chamber for the treatment. A stop watch was started, and the time taken to reach the various stages of anaesthesia within a time limit of 15 minutes were recorded. Descriptions of the various stages of anaesthesia and recovery are summarised in Table 1.

Once fish reached Stage IV of anaesthesia, or 15 minutes of exposure, which ever earlier, fish were removed to be weighed on a digital weighing scale and photo taken for the measurement of the fish. Fish then was transferred into the recovery chamber and time taken to reach the various stages of recovery was recorded. Following full recovery (Stage III recovery), the fish was transferred into a monitoring chamber to be observed for 12 hours before transferred to the main tank.

Table 1: Stages of anaesthesia and recovery in fish (Abbas et al., 2006).

Condition	Stages	Description
Anaesthesia	I	Sedation, partial loss of external stimuli
	II	Partial loss of equilibrium, uncoordinated movement followed by active, erratic swimming
	III	Total loss of equilibrium
	IV	Anaesthesia, loss activity, fish fails to respond to external stimuli
Recovery	I	Opercular movement without body

		movement
	II	Regular opercular movements and gross body movements beginning
	III	Equilibrium regained and swim normally

3.3 Measurement of Fish

Photo of each fish was taken and the measurement was taken for each fish by using Image Processing and Analysis in Java (Image J).

3.4 Water Parameter Analysis

Water parameter analysis was done only once before and after the addition of the anaesthetic agents into the anaesthetic chamber for each different concentration, water parameters such as ammonia, temperature, pH, nitrite and nitrate were measured by using a water test kit (API Freshwater Master Test Kit). The results are stated in table below (Table 2).

3.5 Statistical Analysis

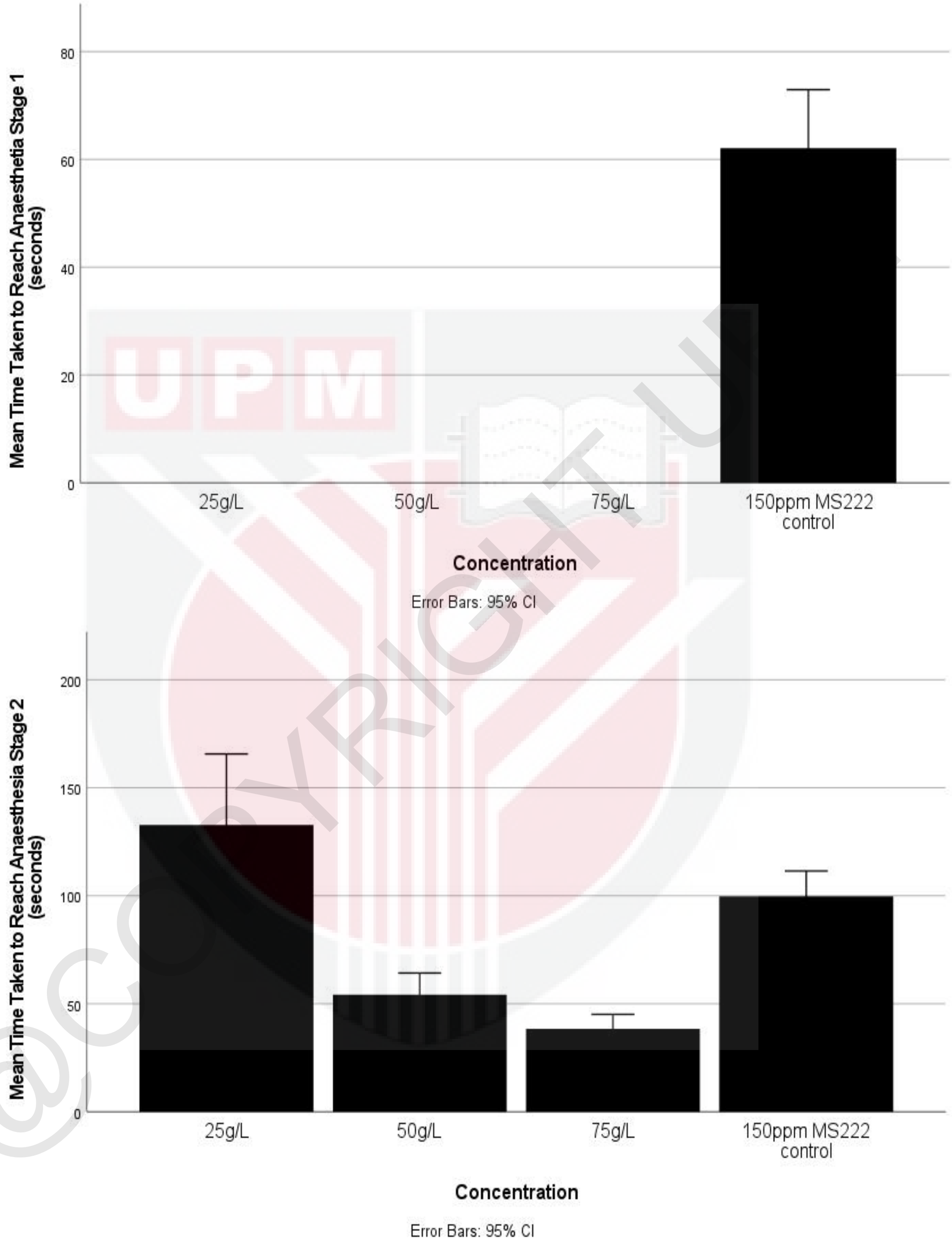
Statistical analysis was performed using Statistical Package for Social Sciences (SPSS version 27.0, IBM Corp). After checking for normality and homogeneity of variance, one-way analysis of variance (ANOVA), followed by post-hoc Tukey test, was performed to check on treatment effects on the induction and recovery times.

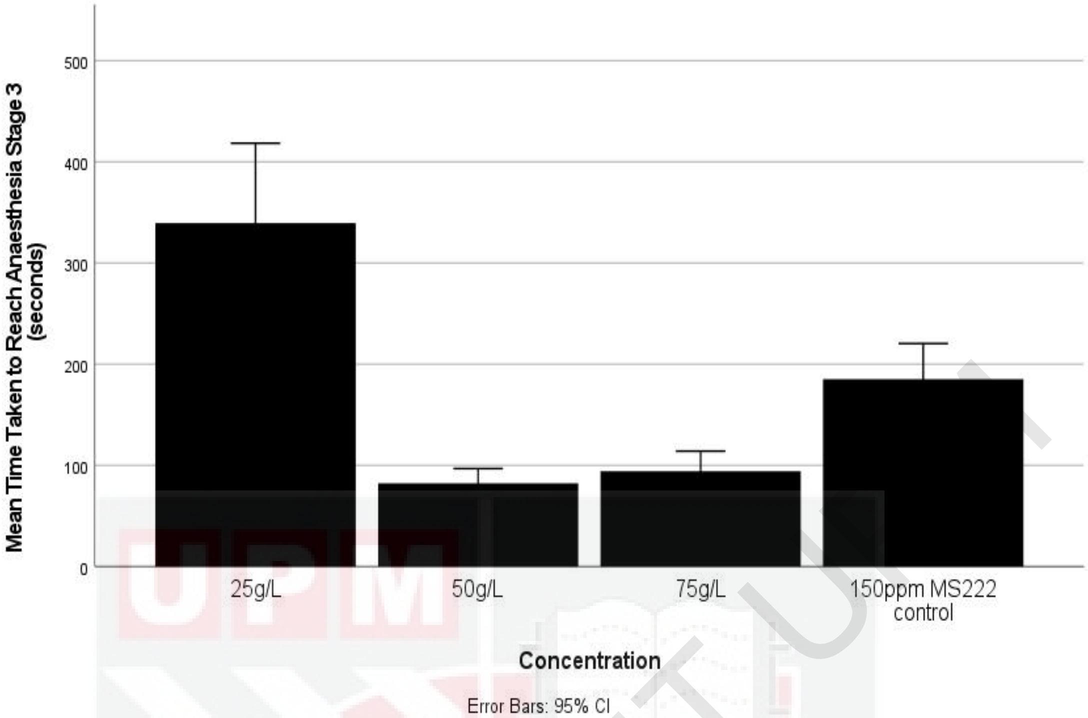
Significance was set at *p*<0.05 for all tests.

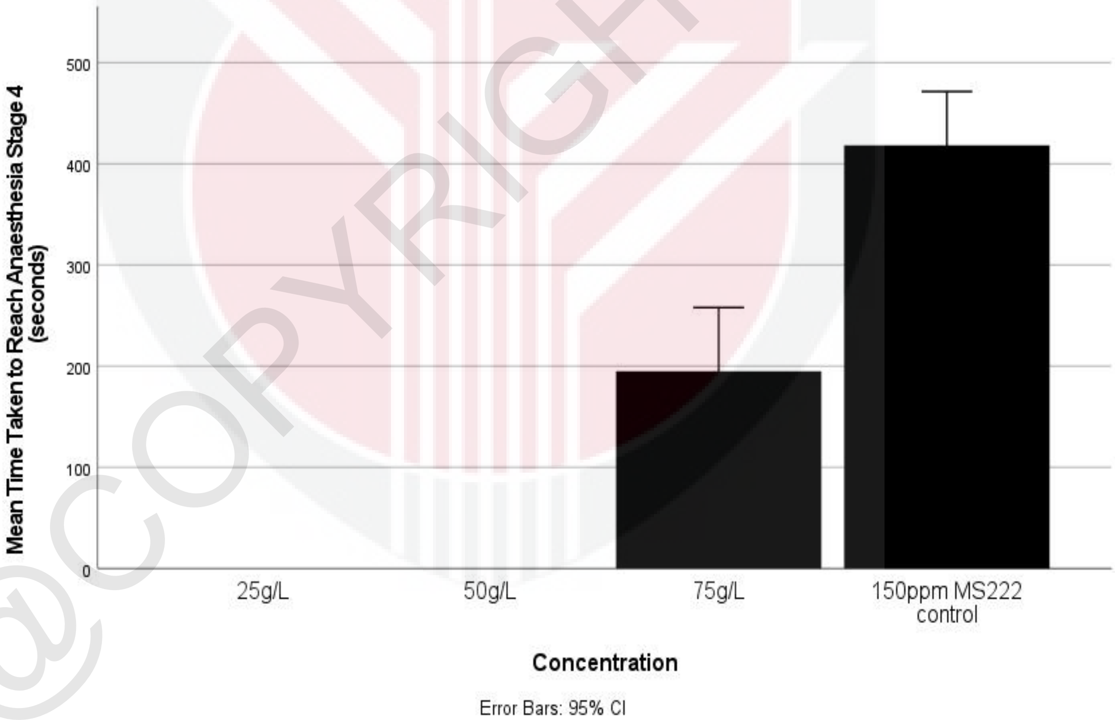
4 RESULTS

The induction time taken of anaesthesia of red tilapia juvenile fish for this study is reported in Figure 1 below. Different concentrations of sodium bicarbonate had significant effects on the induction times ($p<0.05$). In general, the time taken to reach anaesthesia was in the order of 75<50<25g/L (Figure 1). However, groups that were treated with sodium bicarbonate did not show signs of Stage I anaesthesia when compared to the control group. The fish exposed to 75 g/L of sodium bicarbonate showed the shortest time to reach anaesthesia Stage II, with the induction time of (mean ± SD) 38 ± 9 seconds, compared to 133 ± 46 seconds in 25 g/L sodium bicarbonate. Similar patterns are observed for anaesthesia Stage III, with 94 ± 28 seconds in 75 g/L compared to 339 ± 110 seconds in 25 g/L sodium bicarbonate. However, fish treated with 25 and 50 g/L sodium bicarbonate could not reach anaesthesia Stage IV. Fish exposed to 75 g/L sodium bicarbonate could reach anaesthesia Stage IV with 195 ± 88 seconds, compared to the control (MS222) at 418 ± 74 seconds.

Figure 1: Anaesthesia induction times of red tilapia juvenile fish exposed to 25, 50, 75 g/L sodium bicarbonate.



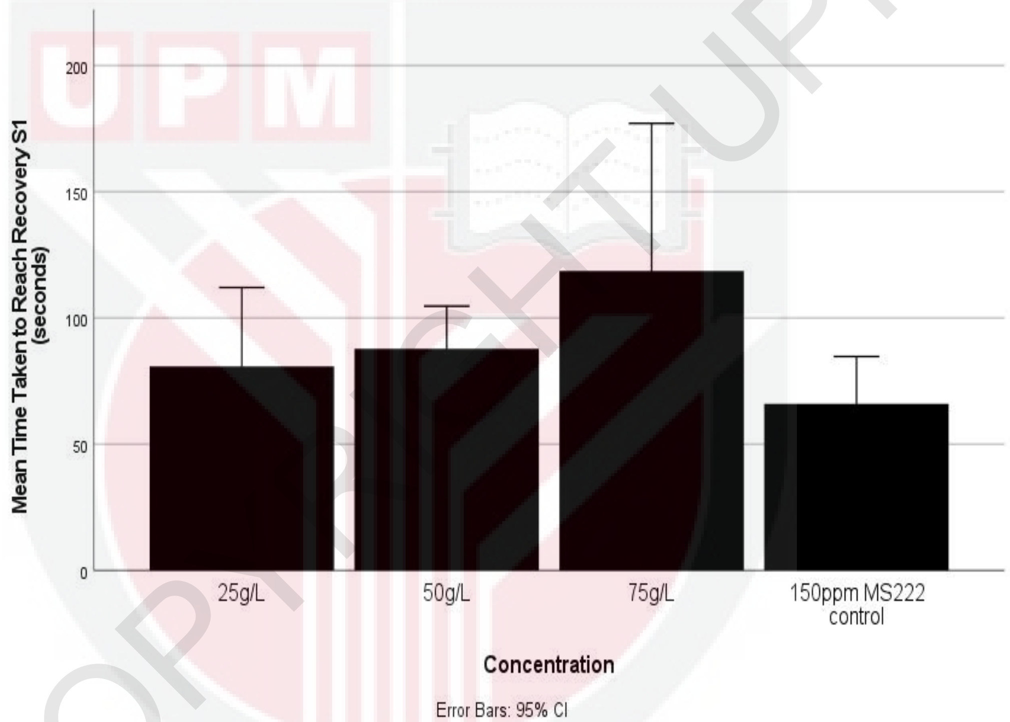


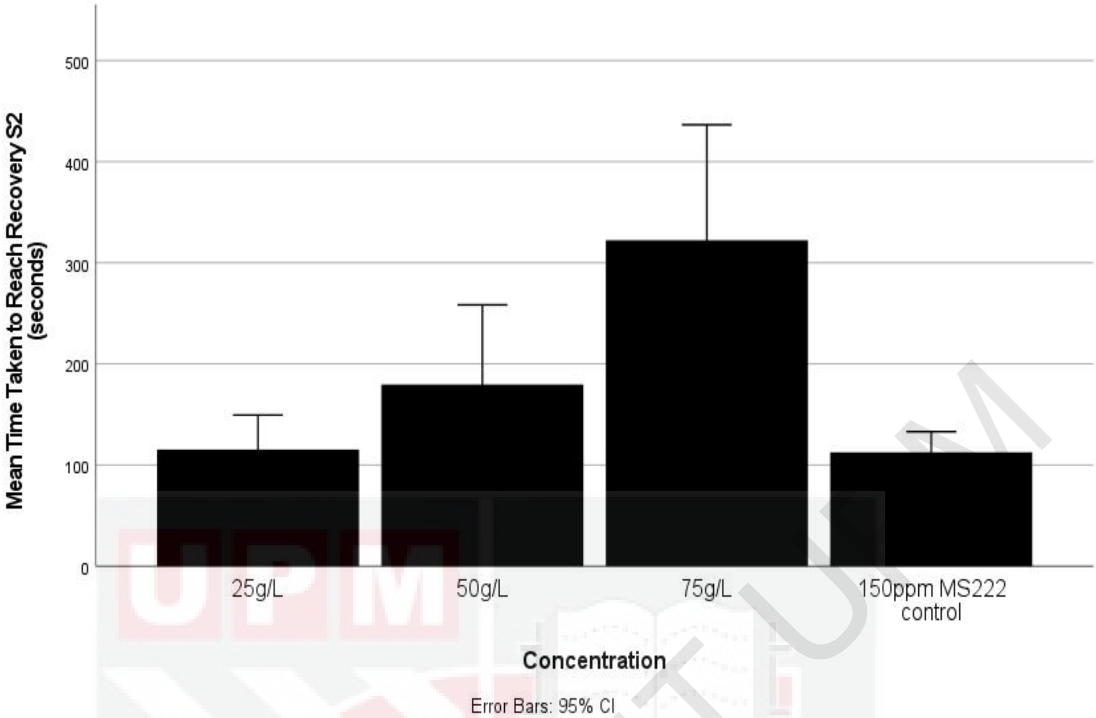


The time taken to reach for red tilapia fish to reach full recovery for this study is reported in Figure 2 below. The time taken was in the order of 25<50<75g/L respectively. Fish exposed to 75g/L of sodium bicarbonate showed the longest time to reach full recovery with mean of time of 402 ± 162 seconds, while fish exposed to

25g/L of sodium bicarbonate showed the shortest time (179 ± 61 seconds) to reach full recovery. However, all fish that were treated with sodium bicarbonate died within 2 days of the experiment. Fish treated with MS-222 survived the 7 days of observation post anaesthesia.

Figure 2: Recovery times of red tilapia juvenile fish exposed to 25, 50, 75 g/L sodium bicarbonate.




Mean Time Taken to Reach Recovery S2 (seconds)
0
100
200
300
400
500
25g/L
50g/L
75g/L
150ppm MS222 control
Concentration
Error Bars: 95% CI

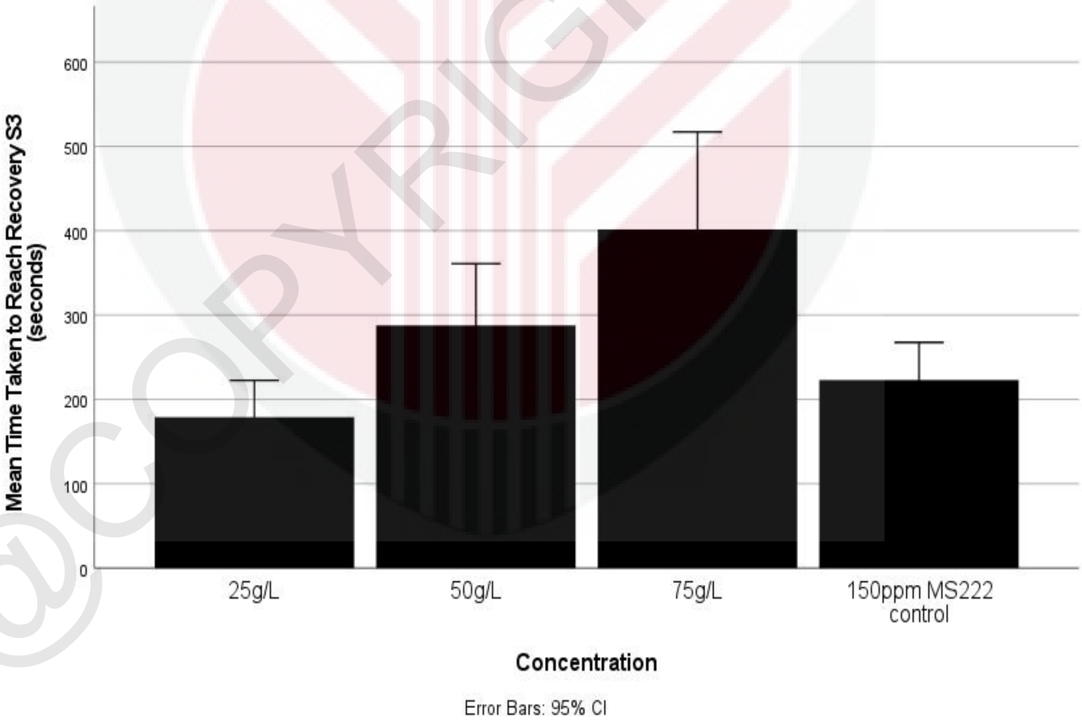

Mean Time Taken to Reach Recovery S3 (seconds)
0
100
200
300
400
500
600
25g/L
50g/L
75g/L
150ppm MS222 control
Concentration
Error Bars: 95% CI

Table 2: Water parameter analysis

Parameters	Value	
	Before added sodium bicarbonate	**After added sodium bicarbonate**
pH	7.2	8.2
Ammonia	0 ppm	0 ppm
Nitrite	0 ppm	0 ppm
Nitrate	0 ppm	0 ppm
Temperature	26°C	26°C

5 DISCUSSIONS

In this study, signs of sedation and gradual loss of equilibrium as described in Stage I of anaesthesia were not seen in fish tested in any concentrations of sodium bicarbonate. When fish showed loss of equilibrium they were all hyperactive and in excitement, indicating Stage II of anaesthesia. This finding is similar with the study on common carp (*Cyprinus caprio*) by Altun *et al.,* (2009).

Akinrotimi *et al.,* (2015) suggested in their study of effectiveness of clove seed extracts as anaesthetic agent on adult guinean tilapia, an induction time of less than 3-5 minutes and with recovery in 5 minutes is an ideal time for anaesthetic agent. In this study, the induction time to reach stage IV anaesthesia is 3 minutes 14 seconds, which were observed from fish exposed to concentrations of 75 g/L. However, the group of fish treated with 25 and 50 g/L of sodium bicarbonate did not reach stage IV of anaesthesia, which the fish still react to external stimuli within 15 minutes. This contradicted with the findings by Hasimuna *et al.,* (2021) where *Oreochromis macrochir* juveniles were reported to reach Stage III of anaesthesia with (complete ceased of opercular and body movement) within 3 minutes when immersed in 25 g/L. However, comparing directly may not be possible due to we were experimenting on two different species, and how we evaluate the anaesthesia stages whereby the reaction to stimuli of the fish was not tested for Hasimuna *et al.,* (2021).

In this study, the mortality rate for fish exposed to sodium bicarbonate is 100% after 2 days post-treatment. All these fish were observed to attain full recovery, with normal swimming behavior in the recovery chamber. However, they were less active and did not react to food when compared to the control group. The cause of death were not further researched in this study, however, a study on physiological response of of rapid hypothermia on Arctic char (*Salvelinus alpinus*) with high levels of CO_2, showed that high CO_2 is highly stressful even not mitigated by hypothermia (Seth et al., 2013).

In this study, red tilapia juvenile fish exposed to 75 g/L of sodium bicarbonate induced faster to reach Stage IV of anaesthesia but took a longer time to reach full recovery. This could be explained by higher level of CO_2 produced, resulting in longer time to eliminate the CO_2.This result is comparable to the findings by Altun *et al.,* (2009) on common carp, where the higher the amount of sodium bicarbonate, the longer the time taken to reach full recovery.

6 CONCLUSION

In conclusion, this present study has shown that there is significant difference in the induction and recovery times at different concentration of sodium bicarbonate on red tilapia juvenile fish (*Oreochromis spp.*). However, the 100% mortality rate raised safety concern on its use in red tilapia juvenile fish.

7 RECOMMENDATIONS

Recommendations that can be suggested for further study would be that the study should be conducted with laboratory-grade of sodium bicarbonate This will ensure the authenticity of the ingredients to have only sodium bicarbonate and no other additives are included. Following confirmation of safety, further study should be conducted on other local aquaculture species in Malaysia, such as riverine catfish, walking catfish, climbing perch, and common snakehead. Moreover, the research should include measurement of cortisol level to assess the physiological stress response on the fish.

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