ASSESSMENT OF DIFFERENT TYPES OF ANESTHETIC AGENTS IN *OREOCHROMIS NILOTICUS*

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CERTIFICATION

This is to certify that we have read this research paper entitled **'ASSESSMENT OF DIFFERENT TYPES OF ANESTHETIC AGENTS IN OREOCHROMIS NILOTICUS'** by Lim Wei Li, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course DVT 5436 – Research Project.

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DEDICATIONS

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parent, Lim Poh Thiam and Leow Poh Kiang, whose words of encouragement and push for tenacity ring in my ears. My brother, Lim Wei Xian who have never left my side and is very special.

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ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 5436 – Research Project.

Fish is also one of the living organisms that display neuroendocrine and physiologic stress responses toward environment. There are many anesthetics, sedatives and also analgesic drugs that are available and used in other vertebrates to reduce stress in fish, reduce handling trauma, restrict movement and physiologic changes in response to nociceptive stimuli and also euthanasia. This experiment aims to determine the minimum effective dose of 2-Phenoxyethanol, clove oil, Nika Transmore and MS-222 in adult Nile tilapia. Fishes were immersed in three different doses of each anesthetic and the minimal dose that produce stage 3 anestheisa within 10 minutes, maintain stage 3 anesthesia for 3 minutes was considered the optimal dose. As a result, the optimal dose for 2-Phenoxyethanol, clove oil, Nika Transmore and MS-222 in average 250g Nile Tilapia housed at 24°C were 300, 20, 75 and 90 ppm, respectively.

Keywords: Anesthetics; 2-Phenoxyethanol; Clove oil; Nika Transmore; MS-222

ABSTRACT

Abstrak kertas penyelidikan yang dibentangkan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, sebagai keperluan sebahagian daripada kursus DVT 5436 – Projek Penyelidikan.

Ikan juga merupakan salah satu organisma hidup yang memaparkan tindak balas tekanan neuroendokrin dan fisiologi terhadap alam sekitar. Terdapat banyak ubat anestetik, sedatif dan juga analgesik yang boleh didapati dan digunakan dalam vertebrata lain untuk mengurangkan tekanan pada ikan, mengurangkan trauma pengendalian, menyekat pergerakan dan perubahan fisiologi sebagai tindak balas kepada rangsangan nociceptive dan juga euthanasia. Eksperimen ini bertujuan untuk menentukan dos efektif minimum 2-Phenoxyethanol, Clove Oil, Nika Transmore & MS-222 dalam Nile Tilapia yang mempunyai berat purata 250g. Ikan direndam dalam tiga dos berbeza bagi setiap bius dan dos minimum yang menghasilkan bius peringkat 3 dalam masa 10 minit, mengekalkan bius peringkat 3, selama 3 minit dianggap sebagai dos optimum. Dos minimum untuk mendorong anestesia peringkat 3 dalam masa 10 minit, mengekalkan anestesia selama 3 minit ditentukan daripada eksperimen untuk 2-Phenoxyethanol (300ppm), Minyak Cengkih (20ppm), Nika Transmore (75ppm), MS-222 (90ppm) dalam purata 250g Nil Tilapia ditempatkan pada suhu 24°C.

Kata kunci: Anestetik; 2-Phenoxyethanol; Clove Oil; Nika Transmore; MS-222

1.0 Introduction

Fish are easily stressed during handling and transporting. Stressed fish will be immunosuppressed and leading to further complication. Anesthetic are used to stabilize the fish so that the fish can be handled easily during sampling, spawning procedures. Other than that, anesthetic are also used during transportation to reduce metabolism rate and prevent physical injury. (Coyole et al., 2004). A good anesthetic can reduce the plasma glucose and cortisol level and immobilize the fish during handling. (Park, Lee & Lim, 2018). Apart from that, it should have criterias like rapid induction with minimum adverse effects, easy administration and maintenance, recovery period should be rapid and it should be also effective at low doses and the toxic dose should greatly exceed the effective dose so that it is safe with wide safety margin. (Coyole et al., 2004). Inappropriate application of anesthetic can also lead to adverse effects for example, more physiological stress and death. Therefore, before administration of anesthetic it is crucial to determine the concentration and the dose to be given (Park et al, 2018). Anesthesia involves in 3 phases which are induction, maintenance, and recovery. While the depth of anesthesia has 4 stages which include sedation, anesthesia, surgical anesthesia and death.

2.0 Research Problem

In aquaculture fishes are always exposed to several stress factor such as handling for procedures like treatments, vaccination, blood withdrawal, spawnings and transportation. Due to the continue growth of the aquaculture industry, there are more and more research about the welfare of cultured fish despite the arguments about can fish experience pain and fear. There is lack of studies on fish anesthetic in Malaysia and it could be due to lack of resource. Certain anesthesia can be very costly and hard to sourcebut others can be the opposite. What is the minimum effective dose for all the 4 anesthetic agents. Because certain anesthetic agents are expensive, so it should be used efficiently in orderto save cost.

MS-222 is an anesthetic agent that is widely use in aquaculture industry. It is available in powder form which is water soluble. When dissolved it is very acidic, therefore a buffering agent like sodium hydroxide or sodium bicarbonate are needed. MS-222 can potentially induce neuromuscular blockage rather than just loss of consciousness which can in return causing stress and also affecting the vital parameters like reducing heart rates can increase the risk of death, especially under a prolonged anesthesia condition. There are several problems that associated with the usage of MS-222, it is very important to establish an anesthetic protocol using other agentstoo. (Obirikorang, Asante-Tuoh, Agbo, Amponsah, & Skov, 2020)

3.0 Research Question

What is the optimal dose and minimum effective concentration to anesthetize Nile tilapia (*Oreochromis niloticus*).

4.0 Research Hypothesis

Only the manufacturer recommended dose of the anesthetic can anesthetize the fish.

5.0 Research Objectives

To determine the optimal doses and minimum effective concentration of selected anesthetic agent in Nile tilapia (*Oreochromis niloticus*).

6.0 Literature Review

6.1 Anaesthethic Agents Used in Aquaculture

According to Coyle, Durborow and Tidwell (2004), fish anesthesia can be categorized into 4 stages which is the sedation, anesthesia, surgical anesthesia and death. Fish can be anesthetized by immersing them in an anesthetic bath containing the anesthetic agent. Then the anesthetic drug will be absorbed through the gills and rapidly enters the blood stream. While larger fish has another method by applying anesthetic solution to the gills with a spray bottle. At the same time the water temperature needs to be controlled. There are also a few factor that affect the efficacy of anesthetic in fish. The factors are divided into environmental and biological factors. The example of biological factors are cold water fish species have responded towards lower concentration of anesthetic than warm water fish species. Usually, larger fish requires high concentration of anesthetic than smaller fish. Active swimmer fish are more anesthetized faster than inactive fish. The environmental factors are fish are ectotherm which means the body temperature follows according to the environmental temperature therefore the physicochemical passage of the anesthetic agent will be affected by the temperature. For example, lower temperature tends to require higher concentration of anesthetic. Different stages can be reached based on the time of exposure and the dose given. After dosing, induction phase will occur. During the induction phase it is normal when an anesthetic is first administered to the fish become hyperactive for a few seconds. Once the desired depth of anesthesia is reached, the depth of anesthesia should be maintained for some time. Usually, the depth of anesthesia can be maintained by reducing the dose. During this phase, the condition of the animal must be monitored especially the respiratory rate. If the depth of anesthesia is too deep, the animal must be removed from the system and flushed with anesthetic-free water immediately. For the last phase, the recovery phase. The fish will be removed from the anesthetic and return to the normal state. Recovery period may vary from few seconds to minutes.

6.2 Tricaine methane-sulfonate (MS-222) application in fish anaesthesia

Referring to Topic Popovic et al. (2012), MS-222 is also known as Tricaine methane-sulfonate. It is one of the most widely used anesthetic agent in fish. Usage of MS-222 can potentially lower the water pH to 2.8. Low pH can cause the formation of methanesulfonic acid. Low pH condition can also cause disturbance in osmotic and ionic balance leading to hemoconcentration, increase in blood pressure, reduction in metabolic rate. Therefore, there are a few buffer that can be used along with MS-222 like imidazole, sodium hydrogen phosphate, sodium bicarbonate and sodium hydroxide. Like other anesthetic agent, MS-222's efficacy is also dependent on environment factor and biological factor. (Topic Popovic et al., 2012)

6.3 Effect of anaesthesia with clove oil in fish

According to Javahery et al., 2012, clove oil is a natural product extracted from Clove tree (*Syzygium aromaticum*). It has been used as a topical local anesthetic to help with toothache, headache and joint pains. It is cheaper compared to other fish anesthetic agent. It is also a very potent anesthetic agent to be used in fish. Clove oil can be used to anesthetize fish by immersion method. After being absorbed through gills, clove oil can be excreted in the urine within 24hours without any negative effect in fish. Thus, it is considered safe to use. (Javahery, Nekoubin, & Moradlu, 2012).

6.4 Efficiency of 2-phenoxyethanol

Referring to Barata, Soares, 2016, 2-Phenoxyethanol (2-PE) has rapid effect, quick recovery, easy preparation, and low cost. It is a colorless liquid, slightly aromatic and moderately soluble in water. (Barata et al., 2016).

6.5 Nika Transmore as fish anesthetic

Nika transmore contains the active ingredient of α-methylquinoline. According to Stoskopf & Posner (2008), α-methylquinoline has been used for fish anesthesia. During anesthesia it does not completely stop muscle movement and many anesthetist find it to be a sedative than anesthetic. Long term sedation or anesthesia has been associated with death. α-methylquinoline can be an irritant to gills and it can cause corneal damage when high concentration has been used for rapid induction after captures. It is generally administered at concentration of 15-60mg/L for induction. (Stoskopf & Posner, 2008).

According to Rairat et al. (2021) to maximize the anesthetic's efficacy and at the same time minimizing its toxicity, an optimal dose for a give fish species in a specified environment is important. Nile tilapia at 400-600gram size requires an induction time of less than 5 minutes and 5 minute recovery time at a dose of 300ppm. The optimal dose for tricaine methanesulfonate (MS-222) are 25-400ppm, for 2-phenoxyethanol (2-PE) is 0.25-1200ppm, and for clove oil is 20-200ppm. (Rairat et al., 2021)

7.0 Materials & Methods

7.1 Chemical

MS-222, Sodium Bicarbonate and Clove Oil were provided by Aquatic Laboratory, Universiti Malaysia Kelantan. The 2-Phenoxyethanol was from EvaChem. Lastly, the Nika Transmore was bought from local fish store.

7.2 Experimental Fish

A total of 15 clinically healthy Nile tilapia that weighs 200-300g were kept in a 350 litre indoor fiber tank at Aquatic Laboratory, Universiti Malaysia Kelantan. They were acclimatized for 7days before the experiment. Water parameters such as dissolved oxygen (DO), temperature, pH and total ammonia nitrogen were maintained at 4.12ppm, 24°C, 6.0, and <1.0ppm, respectively.

7.3 Preparation of Anesthetic Solution

All the anesthetic solution were prepared by dissolving in the water by using syringe to the accurate amount (Table 1.) and were mixed into the aquarium that contained 45 liter of water. All anesthetic agents are in liquid form except MS-222 is powder. MS-222 powder was weighed by using a weighing scale.and it was prepared with a buffer (Sodium bicarbonate) at a ratio of 1:2 (MS-222: $NaHCO_3$) to maintain the water pH at about 7. The doses use for all the anesthetic agents were shown in Table 1.

Table 1: Different doses use for different anesthetic agents

Anesthetic Agent	**Dose (ppm)**
2-Phenoxyethanol	100, 200, 300
Clove Oil	20, 40, 60
Nika Transmore	50, 75, 100
MS-222	30, 60, 90

7.4 Determination of the Minimum Effective Dose

The purpose of the study, the Minimum Effective Dose is defined as the minimum dose that could induce stage 3 anesthesia within 10minutes, maintain stage 3 anesthesia for 3minutes and has a recovery time of less than 5minutes. The criterias are chosen based on the anesthetic agent and scientific procedures and activities that requires sedation.

For each group of experiment, 3 fishes were randomly selected from the main tank. Each fish was immersed with the selected anesthetic agent and dose of 2-Phenoxyethanol (100, 200, 300ppm) in a 45litre tank until stage 3 anesthesia was achieved. Time was recorded for stage 1-3 of anesthesia. Then the fish were monitored for another 3minutes for the anesthesia status (maintenance phase). After that, they were put in to a 60litre recovery tank for recovery. Water temperature during the entire experiment was controlled at 24°C. Similarly the doses of Clove Oil (20, 40, 60ppm), Nika Transmore (50, 75, 100ppm) and MS-222 (30, 60, 90ppm) were tested. Induction and recovery duration of all groups were recorded.

8.0 Results

In table 2, for 2-Phenoxyethanol 100 ppm and 200 ppm failed to induce stage 3 anesthesia. Only 300 ppm successfully induced stage 3 anesthesia in Nile tilapia at 3.17 minutes, it successfully passed the 3minutes maintenance, with 3.48 minutes of recovery time. Therefore, the minimum effective dose for 2-Phenoxyethanol is 300ppm. For clove oil, the minimum effective dose is at 20 ppm. It successfully induced stage 3 anesthesia at 6.47 minutes, successfully maintain for 3minutes and with 5.52 minutes of recovery duration. For Nika Transmore, 50 ppm failed to induce stage 3 anesthesia. But at 75 ppm it successfully induced stage 3 anesthesia at 5.34 minutes, successfully maintained for 3 minutes with 4.51 minutes of recovery time. Therefore, the minimum effective dose for Nika Transmore is 75ppm. Lastly, MS-222 failed to induce stage 3 anesthesia at 30 ppm and 60 ppm. But, at 90 ppm it successfully induced stage 3 anesthesia at 7.14 minutes, successfully passed the 3 minutes maintenance with 5.32 minutes of recovery time. Therefore, the minimum effective dose for MS-222 is 90 ppm.

Table 2: Result of the anesthesia duration based on different doses of anesthetic agents

Anesthetic Agents	**Route**	**Dose (ppm)**	**Stages of Anesthesia**	**Average Duration of anesthesia (min)**	**Quality control for 3 minutes maintenance**	**Average Recovery Duration of 3 Fishes**
2-Phenoxyethanol	Immersion	100	1	5.24	Failed	-
			2	-		
			3	-		
		200	1	2.39	Failed	-

			2	5.57		
			3	-		
		300	1	1.08	Passed	3.48
			2	2.11		
			3	3.17		
Clove Oil		20	1	1.48	Passed	5.52
			2	4.56		
			3	6.47		
		40	1	0.56	Passed	5.08
			2	1.22		
			3	2.52		
		60	1	0.24	Passed	6.29
			2	0.40		
			3	0.48		
Nika Transmore		50	1	0.35	Failed	-
			2	1.53		
			3	-		
		75	1	0.37	Passed	4.51
			2	1.26		
			3	5.34		
		100	1	0.22	Paased	6.1
			2	1.06		
			3	3.16		
MS-222		30	1	6.24		
			2	-		
			3	-		
		60	1	3.21		
			2	-		
			3	-		
		90	1	1.18	Passed	5.32
			2	3.37		
			3	7.14		

9.0 Discussion

There are many ongoing debates among the researches about can fishes feel pain. Due to the animal welfare, it is wiser to use anesthetic in fish experiments, especially for those invasive procedures. Effectiveness of anesthetic agents for fishes depends on several factors which are fish species, size of the fish, duration of anesthetic exposure, water temperature. To maximize the anesthetic agent efficacy and at the same time minimizing its toxicity, the optimal dose for different environment should be determined. In this experiment the mean weight of the fish is 250 g. Three different dose is determined for every anesthetic agent to find out which one is the minimum effective dose to induce stage 3 anesthesia. 3 minutes maintenance is included in this experiment is because certain procedures require longer time to perform, therefore the minimum effective dose should be able to provide maintenance of at least 3 minutes.

The minimum effective dose for 2-Phenoxyethanol in this experiment is 300ppm, it managed to anesthetize the fish to stage 3 of anesthesia at 3.17minute and maintained for 3minutes. While at 100ppm & 200ppm, it was unable to anesthetize the fish to stage 3.

The minimum effective dose for Clove oil in this experiment is 20ppm. Clove oil at 20ppm, it can induce stage 3 anesthesia at 6.47minutes, managed to maintain for 3minutes. Besides that, as the dose increase 40ppm & 60ppm the induction duration becomes faster.

The minimum effective dose for Nika Transmore in this experiment is 75ppm. At 75ppm, it can induce stage 3 anesthesia as fast as 5.34minutes, and managed to maintain for 3minutesas well. As the dose increase, the induction time reduced at 100ppm. At 50ppm, it only can induce to stage 2 anesthesia.

The minimum effective dose for MS-222 is 90ppm. It induced stage 3 anesthesia at 7.14minutes, and managed to maintain for 3minutes. At 30ppm and 60ppm, both dose did not manage to induce stage 3 anestheisa this could be due to the concentration is too low.

From the result obtained, it is concluded that higher dose can achieve stage 3 anesthesia in a shorter time. This is because that higher dose of anesthetic, the drugs can reach the threshold of concentration much ealier than lower dose. Therefore, it shortens the induction. Based on the minimum effective concentration of the 4 anesthetic agents 2-Phenoxyethanol (300ppm), Clove oil (20ppm), Nika Transmore (75ppm), MS-222 (90ppm), suggesting that the higher potency of the clove oil and lesser potency of the 2-Phenoxyethanol in stage 3 anesthesia induction in Nile tilapia. From the result, it is also shown that increasing dose of the anesthetic agent will prolong the recovery time (Rairat et al., 2021). But certain studies show that increasing dose of anesthetic agent can shorten the recovery time. This could be due to higher dose will lead to shorter exposure time and therefore faster removal of the anesthetic agent (Rairat et al., 2021). In short, the effect of dose on the recovery duration is based on the anesthetic agent properties.

The choice of the sanesthetic agents in aquaculture is depend on the cost, availability, ease of use, user safety, and the duration. MS-222 is the most common standard for fish anesthetic. It is widely used in aquaculture, but the concern for MS-222 are aversion and stress induction. MS-222 is only available in powder form which can be dissolved in water. When dissolved it is very acidic, therefore a buffering agent like sodium bicarbonate or sodium hydroxide or Tris buffer is needed. MS-222 function by blocking the neuromuscular system rather than loss of consciousness which can potentially cause stress in addition to affecting the vital organs like reduction in heart rates and increase the risk death, especially under a prolonged sedation condition. There are several problems associated with the use of MS-222 in fishes, therefore it is very important to establish an anesthetic protocol of different anesthetic agents. MS-222's efficacy and safety in fish are still rare and poorly explained. An alternative to MS-222 is Propofol. Propofol has been used in fishes. It is done intravenously or by immersion baths in a few species of fishes like Sturgeons, Nile tilapia, Bamboo shark, Goldfish, Silver Catfish and Zebra fish. Propofol induces quick anesthesia with rapid recovery. Propofol immersion method shows smoothers anesthetization than MS-222. Because of the side effects of using anesthetics in fishes, it is very crucial to establish an anesthetic regimen encompassing doses and combination that suits research or production procedures and also minimize collateral effects. Factors like species, age, gender and developmental stage should be taken into consideration of anesthetic protocol for fish to ensure the efficacy and safety of the anesthetic agents. (Obirikorang, Asante-Tuoh, Agbo, Amponsah, & Skov, 2020)

10. Conclusion

As the conclusion, the minimum doses to induce stage 3 anesthesia within 10minutes, maintain anesthesia for 3minutes were determine from the experiment for 2-Phenoxyethanol (300ppm), Clove Oil (20ppm), Nika Transmore (75ppm), MS-222 (90ppm) in average 250 g Nile tilapia housed at 24°C. This experiment shows that the benefits of obtaining information specific to the anesthetic agent and its minimum effective dose. Manufacturer recommended dose is higher than the experiment proven dose, but we have to take into consideration that several factors can affect the results like temperature and size of the fish.

11. Recommendation

There are a few setbacks throughout this experiment. More species can be done for example climbing perch and carps because the dose to anesthetize other species of fish could be different. The experiment can also be conducted at different temperature.

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