



Research on the Application of Fishery Anesthetic MS-222 and Eugenol in China

Xiao-jiang Chen*

Jiangsu Agri-animal Husbandry Vocational College

*Corresponding Author: Xiao-jiang Chen, Jiangsu Agri-animal Husbandry Vocational College.

Abstract: In order to clarify the research and application status of narcotic MS-222 and eugenol on aquatic animals, this article introduces the effects of anesthetic MS-222 and eugenol on oxygen consumption and ammonia excretion rates of different aquatic animals, the changes in resuscitation time and biochemical parameters, the safety of anesthetics and tissue residues after use of anesthetics, and points out that safety should be considered in the selection of anesthetics and the effect of anesthesia is second, and the development prospect of high-efficiency and environmental-friendly anesthetics is broad.

Keywords: Fishery anesthetic; MS-222; Eugenol; Oxygen Consumption Rate; Ammonia Excretion Rate

1. INTRODUCTION

As a large aquaculture industry country, China has been producing the world's largest number of aquaculture production for many years. In the operation to aquatic animals such as fishing, transportation, artificial propagation, individual marking, internal anatomy and external morphological measurement, etc., aquatic animals will produce strong stress reactions, such as mucus secretion, jumping and struggling, which seriously hamper the smooth development of related work and cause some harm to fish. The scientific and rational use of anesthetics can significantly reduce the stress response of fish, reduce the damage to fish, and facilitate manual operation.

At present, there are many kinds of anesthetics for aquatic animals. There are more than 30 kinds, such as eugenol, MS-222, benzocaine, 2-phenoxyethanol, etc. Among them, MS-222 and eugenol are the most widely used in breeding and production. MS-222 has been approved by the Food and Drug Administration (FDA) of the United States for its advantages of safe and convenient use, fast anaesthesia and recovery. It is the most widely used, safe and effective aquatic anaesthetic at present. MS-222 has been reported as an anesthetic for many kinds of fish [1-4]. Eugenol is a plant extract. It is cheap and easy to get, and the metabolites can be quickly discharged. Eugenol will not induce mutations in the body. It does not cause harm to human beings and the environment. It can be added directly in food. Research of eugenol, as a fish anesthetic of aquaculture animals such as *IctalurusPunetaus*, has been reported [5-6]. The writer summarizes the anesthetic MS222 and eugenol from the principles of anesthesia and its influencing factors and the current application in production.

2. ANESTHETIC PRINCIPLES AND INFLUENCING FACTORS OF FISHERY ANESTHETICS

2.1. Anesthetic Principles

The action principle of anesthetics: anesthetics first inhibit the cerebral cortex (tactile loss period), then act on the basal ganglia and cerebellum (excitatory period), and finally act on the spinal cord (anaesthesia period). Too much or too long exposure can make the anesthetics deep into the medulla, causing respiration and blood vessel shrink, and central paralysis, and eventually lead to death [7].

2.2. Influence Factor

2.2.1. Fish Species, Specifications and Ages

Different breeds react differently to different anesthetics and concentrations, while MS-222 has no effect on the crayfish (*Procambarusclarkii*). Generally, for the same variety with different specifications, the

higher the quality is, the more the anesthetic dose is need. As for the same kind and different ages of fish, because of metabolic reasons, respiratory organs are different, the effective anesthetic dose also has obvious changes. In the juvenile stage, skin breathing is the main method, while in the adult stage, gill breathing is the main method. As the age increases, the tolerance to anesthetics will lead to different effective anesthetic doses [8].

2.2.2. Temperature

In the aquatic environment where fish grow, when the water temperature rises but the concentration of fishery anesthetics is the same, the time for the receptor to reach anesthesia stage becomes shorter. When the water temperature rises to a certain temperature, the anesthetic effect of some anesthetics will decrease. In most cases, the survival rate of recipients after anesthesia is greatly reduced at higher temperatures.

2.2.3. pH Value and Salinity

The change of pH value has different effects on fish body because of different doses. Generally, it has little effect on freshwater fish and great influence on seawater fish. When high-dose MS-222 is used, a certain amount of NaHCO₃ should be added to buffer it so that the pH value is between 7.0 and 7.5 and reduce the stimulation to the fish [9].

At high salinity, in order to regulate the balance of osmotic pressure, fish need to consume more energy, and their respiratory rate is accelerated, and their oxygen consumption is increased. Therefore, at high salinity, fish consume more anesthetics, which shorten the time of entering anesthesia, prolong the recovery time and increase the mortality rate.

2.2.4. Dissolved Oxygen

The level of dissolved oxygen has a profound effect on the respiration of fish. When dissolved oxygen is low, fish anesthetics play a faster role, but at the same time, recovery time is slower. When dissolved oxygen is high, the receptor is more sensitive to fishery anesthetics, and the dissolved oxygen the receptor needs is low, and the coming of anaesthesia stage of the receptor is slower, but the recovery time is shorter and the survival rate is higher.

3. STUDY ON THE APPLICATION OF MS-222 IN AQUATIC ANIMALS

After MS-222 anesthetize aquatic animals, it mainly concentrates in organs such as spleen and liver, and its content in muscle tissue is very small. In clean water environment, it is easy to transfer from aquatic animals to water. Therefore, the anesthetic effect of MS-222 is fast, the recovery time of the receptor is short, the safety is high, and it is harmless to the recipient and the human body contacted. But MS-222 also has its shortcomings. When MS-222 is used during transportation, the CO₂ emission cannot be reduced, and the water solution is weak acidic, which lead to the increase of cortisol in plasma after deep anaesthesia. In addition, when we use MS-222 solution, direct sunlight should be avoided, otherwise it will produce strong toxicity to fish in sea water.

3.1. Anesthesia Effect of MS-222

The most suitable concentration of MS-222 for bass is 20~50mg/L under the condition of 25°C. After 24 hours of anesthesia, the recovery rate could reach 100% in clear water environment [10]. The effective concentration of MS-222 for *Acipenser schrenckii* was 80-100 mg/L under the condition of 16~18°C, which was the safest, and the fish could recover within 3-5 minutes when held in hand [11]. MS-222 had a good anesthetic effect on *Pelteobagrus fulvidraco* at 40-80 mg/L, and the most suitable concentration for short-time anesthesia was 60 mg/L [12]. Yan found that, when the concentration of MS-222 was lower than 40 mg/L, it had no anesthetic effect on the *Coiliamacrogathos Bleeker*, but had a certain sedative effect [13]; Sun showed that, the safest concentration of MS-222 was 60 mg/L in the transport of *cynoglossus semilaevis* [14]; Ding found that, the reasonable range of MS-222 anesthesia for bream was between 60 and 80 mg/L [2]; Xu found that MS-222 had no anesthetic effect on crayfish (*Procambarus clarkii*). The anesthetic effect of the same anesthetic for different aquatic products is different. Therefore, it is the research direction to continue to explore more anaesthesia mechanisms and

metabolic pathways of aquatic animals.

3.2. Effects of MS-222 on Oxygen Consumption Rate and Ammonia Excretion Rate of Aquatic Animals

Zhang studied the anesthesia of juvenile *Oreochromis niloticus*. When the concentration of MS-222 was 10mg/L, 20mg/L and 30mg/L, the average oxygen consumption rate decreased by 10%, 31.4%, 40.5%, and the average ammonia excretion rate decreased by 12.4%, 37.8%, 42.8% [15]. When the concentration of MS-222 was 20-100 mg/L, the oxygen consumption rate of *Procambarus clarkia* could not be reduced, but decreased when the concentration exceeded 100 mg/L. The general trend was that with the increase of the concentration, the oxygen consumption rate and ammonia excretion rate increased first and then decreased.

3.3. Changes of Biochemical Parameters

Wang found that the serum cortisol content of bass was significantly increased at 25°C after bass was anesthetized and transported, and the activity of serum AST increased slightly. After 10h, serum UREA level increased significantly, serum GLU increased significantly, and the activity of serum LDH increased significantly during anesthesia [10].

3.4. Tissue Residues and Safety Of MS-222

Xue determined the MS-222 residues in anaesthetized fish. It was found that the half-life period of elimination in muscle and liver were 5.54-5.27h (30 mg/L) and 8.72-7.15h (60 mg/L) respectively [16]. When the concentration was 30 mg/L, the withdrawal period was at least 4.5d; if the concentration was 60 mg/L, the withdrawal period was at least 7.5day. With the deepening of research and the emphasis on food safety, many developed countries have strictly stipulated MS-222 withdrawal period. For example, the United States stipulated that MS-222 withdrawal period is 21day, Canada is 5day, New Zealand is 10day [17]. Sun suggested that the withdrawal period of *cynoglossussemilaevis* after being anaesthetized by MS-222 should not be less than 16day [14].

4. RESEARCH AND EXPERIMENT OF EUGENOL IN AQUATIC ANIMALS

Eugenol, its molecular formula $C_{10}H_{12}O_2$, is a colorless to yellowish slightly-thickened liquid. If exposed to air for a long time, it will turn into a black-brown liquid. Eugenol and its metabolites can be quickly discharged from blood and tissues, and it will not induce mutant substances in the body. Eugenol has the advantages of high efficiency and low dose. Compared with MS-222, its recovery time is longer. Owing to its volatility, its effect will gradually decrease during anesthesia.

4.1. Anesthetic Effect of Eugenol

The anesthetic effect of clove oil on loach was the most suitable when it was at 60-100 mg/L, and it would not cause harm to loach in breeding production and scientific research [18]. Kuang showed that when the concentration of eugenol anesthetics was not higher than 27 mg/L, the anesthetic effect on *ictalurus punctatus* was the safest [19]; when the concentration of eugenol anesthetics was 5 mg/L and 8 mg/L, it would not produce anesthetic effect on *Coiliaetenes* within 12 hours [13]. 40 mg/L eugenol can produce anesthetic effect on *procambarusclarkii*.

4.2. Effects of Eugenol on Oxygen Consumption Rate and Ammonia Excretion Rate of Aquatic Animals

Generally, the oxygen consumption rate of aquatic animals anesthetized by eugenol anesthetic will rise first and then decrease. When the concentration increased further, the oxygen consumption rate of juvenile *acipenserbaeri* decreased gradually, and there were obvious differences occurring between different concentration [20]. When the concentration of eugenol was 10 mg/L, the lowest oxygen consumption rate of *trachinotusblochii* was 0.33 mg/(g h) [21], and when the optimum concentration of eugenol for the carp anesthesia was 20 mg/L, the oxygen consumption rate and ammonia excretion rate decreased significantly [22].

4.3. Changes of biochemical parameters

After juveniles of *acipenserbaeri* were anesthetized with eugenol 60~180 mg/L for 10 min., the concentrations of high-density lipoprotein, low density lipoprotein and triglyceride in the young group

were significantly higher than those in the control group ($P < 0.05$), while the total bilirubin concentration was lower than that in the control group ($P < 0.05$); in the 90~180 mg/L concentration group, the magnesium concentration in the blood of the young fish was higher than that in the control group; in the 120~180 mg/L concentration group, the blood concentration of the young fish was higher than that of the control group. The concentration of glutamic oxaloacetic transaminase in the test group was higher than that in the control group. The concentration of albumin and lactic dehydrogenase in each test group fluctuated greatly. 8~30 mg/L eugenol could be applied to short-distance transportation of juvenile *acipenserbaeri*, and the influence of 60~90 mg/L eugenol on the blood biochemistry of juvenile *acipenserbaeri* was relatively small.

4.4. Tissue residues and safety of eugenol

No carcinogenic or toxic effects of eugenol have been found in studies in various countries. The Standard for the Use of Food Additives GB2760-2014 specifies that natural plant extracts containing eugenol can be used as food additives [23]. However, there is no clear prescription for the use of eugenol as a fishery anesthetic. Moreover, the maximum residue limit of eugenol in food is not specified in Standard for the Use of Food Additives GB2760-2014 and Contaminant Limit in Food GB2762-2012[24]. Preliminary evaluation shows that eugenol used as a fish anesthetic has no effect on food safety.

Many countries have not approved the use of clove oil because it may contain methyl eugenol. In Japan and other countries, eugenol has been allowed to circulate in the market as a fish anesthetic. At present, there are no relevant regulations on the administration of eugenol used as fishery anesthetics in China. Some researchers suggest Chinese government that, for eugenol use management, we can refer to Japanese regulations on eugenol, and set temporary use and management limits. The specific dosage of medicine bath is 50~200 $\mu\text{g/mL}$, and the residue limit is 0.05 $\mu\text{g/mL}$. For the period of drug withdrawal, the dosage of fish is 7day and that of crustaceans is 10day [25].

5. SUMMARY

With the development of the concept of “blue granary” in China, the development of fishery will reach an unprecedented speed and height. But if we want to develop modern fisheries, the standardization and use of anesthetics is an important step. China’s research on fishery anesthetics started late, and there are many problems in the management and use of fishery anesthetics. ①The relevant laws and regulations concerning the use of anesthetics in fisheries in China need to be improved and perfected, and there is a lack of allowed-to-use product catalog and using standards for the use of anesthetics for fisheries. ②The pharmacology and metabolic pathways of anesthetic drugs in fish are relatively little studied. For many problems, such as intramuscular drug residues, safety range, toxicity and harm to human body, there is no credible basis for evaluation. ③At present; there is a phenomenon of non-standard use of fishery anesthetics. To sum up, under the strategy of maritime power, it is imperative to solve the problems of fishery anesthetics if we want to develop the marine economy.

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