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# EFFECTS OF TRICAINE ON BLUE TILAPIA AT DIFFERENT SALINITIES AND CONCENTRATIONS

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***Abstract***

*This experiment was devised to evaluate the effects of tricaine methanesulfonate (MS-222) on blue tilapia, Oreochromis aureus at five different salinities (0, 8, 16, 20, 24 ppt) and four different tricaine concentrations (200, 300, 400, 500 mg $l^{-1}$). Even though, a body of literature exist about the tricaine usage on fish, not much information is present on tricaine with salt. The requirement time to anaesthetize fish depends on intensity of tricaine concentration and salinity. Induction time of fish decreased as tricaine concentrations increased. When exposed to any of tricaine concentrations, fish entered a deep state of anaesthesia (induction time ranged between 0.19 and 2.54 min). Recovery time was highest at 400-500 mg $l^{-1}$ of tricaine as salinity increased. Tricaine + salt combination is strongly recommended to use in blue tilapia culture. Ideal tricaine concentration was 200 mg $l^{-1}$ of tricaine at 8 ppt of salinity to reduce stress in blue tilapia.*

***Key words:*** *anaesthetic, blue tilapia, salinity, tricaine.*

## INTRODUCTION

Anaesthetics are generally used for sedating or immoving fish in aquacultural practices such as scientific researches and fish farming. They are crucial for decreasing stress caused by handling procesures (Hseu et al., 1998). Several chemicals have established to anaesthetize fish such as tricaine methanesulfonate, quinaldine, phenoxyethanol, clove oil, benzocaine, metomidate, sedanol (Mylonas et al., 2005; Küçük et al., 2016). Each one has its own benefits and balks (Anderson et al., 1997; Munday and Wilson, 1997; Hseu et al., 1998; Wagner et al., 2003; King et al., 2005; Mylonas et al., 2005; Weber et al., 2009; Küçük, 2010; Pramod et al., 2010; Mercy et al., 2013; Mazik and Simco, 2014).

Tricaine methanesulfonate is one of the most widely used anaesthetics in aquaculture (Ross and Ross, 1999). It is structured a white crystalline powder and is solved easily in water. It is purchased as Tricine-S or Finquel and registered only chemical by the Food and Drug Administration (FDA) to use for market fish in the USA and United Kingdom (Hseu et al., 1998; Coyle et al., 2004).

The aim of the study was to expose tilapia to four different MS-222 concentrations (200, 300, 400 and 500 mg $l^{-1}$) at 0, 8, 16, 20, 24 ppt of salinities and to evaluate induction time, recovery time and survival for each concentration.

## MATERIALS AND METHODS

The study was done on blue tilapia, *Oreochromis aureus*, which was commercially attained from Adana, Turkey. Some of water quality parameters of water source were given in Table 1.

Fish average length (147.03±8.53 mm) and weight (47.53±6.54 g) were measured at the beginning of experiment. Fish had been starved for 24 h prior to the trial.

Tricaine methansulfonate (Sigma Aldrich catalog no E10521) stock solution (0.4%, 100 ml) buffered with 1 M Tris-Cl (pH 9.0) and working solution (0.2%, 100 ml) were prepared. Anesthetization practise was done in an aerated 500 ml beaker. Fish was exposed 200, 300, 400, 500 mg $l^{-1}$ concentrations of MS-222 at 0, 8, 16, 20, 24 ppt of salinities at 23.7°C and pH 8.13 untill anaesthesia stage of 3 for induction and recovery times were reported for each concentration. After recovery, fish were taken care in the maintenance aquariums for 48 h in order to see any adverse event for fish situation. Experiment was undertaken on ten fish for each concentration.

Table 1. Some water quality parameters of water source

| Parameter | Value |
|---|---|
| EC (µs $cm^{-1}$) | 856.2 |
| Total hardness (mg $l^{-1}$ $CaCO_3$ ) | 712.6 |
| Alkalinity (mg $l^{-1}$ $CaCO_3$) | 588.0 |
| Bicarbonate (mg $l^{-1}$) | 360.4 |
| Calcium (mg $l^{-1}$) | 90.2 |
| Magnesium (mg $l^{-1}$) | 12.8 |
| Ammonia (mg $l^{-1}$) | 0.33 |
| Nitrite (mg $l^{-1}$) | 0.0016 |

The induction time was put down for each fish when fish suffered total equilibrium, its operculum rate stopped and fish did not reponse to presure on its body (SIII) (Table 2). Anaesthetized each fish was weighed and measured its length. After that, recovery time was recorded when fish began swimming as usual (RIII) (Table 2).

Table 2. Stages of induction and recovery in fish (Küçük and Çoban, 2016)

| Stages of Induction | Description | Behavior/Response |
|---|---|---|
| | Sedation | Slight loss of reactivity to external stimuli; operculum rate slightly decreased; equilibrium normal |
| I | Anesthesia | Partial loss of muscle tone; swimming erratic; increased operculum rate; reactivity only to strong tactile and vibration stimuli |
| II | | |
| III | Deep anesthesia | Total loss of muscle tone and equilibrium; slow but regular operculum rate; loss of spinal reflexes |
| IV | Death | Breathing and heart beat stop; eventual death |
| **Stages of Recovery** | **Desciption** | **Behavior/Response** |
| I | Deep anesthesia | No body movements but opercular movements start |
| II | Anesthesia | Regular opercular movements and body movements start |
| III | Sedation | Equilibrium regained with preanesthetic appearance |

Differences between tricaine and salinity concentrations were analyzied by SSPS. Induction time, recovery times and survival value were set up by comparing each salinity and tricaine concentration. The data are given as mean±SD. Analysis of variance and Duncan's multipe range tests were followed out for signicant differences ($P \leq 0.05$).

## RESULTS AND DISCUSSIONS

In the present study, induction and recovery times at each salinity and concentration were demonstrated in the Table 3. The induction time of *Oreochromis aureus* decreased as tricaine concentration increased ($P<0.05$). It ranged between 0.19 and 2.54 min at 200-500 mg $l^{-1}$ of tricaine.

Increasing salinity did not affect induction time of blue tilapia. But, at > 8 ppt, recovery time was signifantly different.

Recovery time became shorter than that of higher salinities and tricaine concentrations. Survival was not significantly affected during trial. Even no fish died within 24 h after trial.

Table 3. Induction time, recovery time, induction rage and survival of blue tilapia in six salinities and four tricaine concentrations

| Salinity (ppt) | Tricaine conc. (mg $l^{-1}$) | Induction time (min) | Recovery time (min) | Induction rage (min) | Survival (%) |
|---|---|---|---|---|---|
| 0 | 200 | $1.34\pm0.34^{A, a}$ | $0.64\pm0.35^{A, a}$ | 1.05-2.21 | 100 |
| | 300 | $0.77\pm0.25^{A, b}$ | $0.67\pm0.37^{A, b}$ | 0.43-1.01 | 100 |
| | 400 | $0.59\pm0.23^{A, c}$ | $1.39\pm0.30^{A, c}$ | 0.36-1.03 | 100 |
| | 500 | $0.47\pm0.22^{A, d}$ | $1.82\pm0.42^{A, c}$ | 0.27-1.04 | 100 |
| 8 | 200 | $1.23\pm0.29^{A, a}$ | $0.49\pm0.19^{C, a}$ | 0.51-1.54 | 100 |
| | 300 | $0.72\pm0.34^{A, b}$ | $0.83\pm0.46^{C, b}$ | 0.35-1.29 | 100 |
| | 400 | $0.41\pm0.06^{A, c}$ | $1.13\pm0.46^{C, c}$ | 0.35-0.51 | 100 |
| | 500 | $0.34\pm0.06^{A, d}$ | $1.21\pm0.11^{C, c}$ | 0.22-0.45 | 100 |
| 16 | 200 | $1.31\pm0.44^{A, a}$ | $0.52\pm0.26^{BC, a}$ | 0.42-2.19 | 100 |
| | 300 | $0.90\pm0.32^{A, b}$ | $0.83\pm0.26^{BC, b}$ | 0.51-1.25 | 100 |
| | 400 | $0.58\pm0.23^{A,c}$ | $1.24\pm0.51^{BC, c}$ | 0.39-1.03 | 100 |
| | 500 | $0.39\pm0.05^{A, d}$ | $1.31\pm0.31^{BC, c}$ | 0.28-0.45 | 100 |
| 20 | 200 | $1.39\pm0.29^{A, a}$ | $0.59\pm0.17^{ABC, a}$ | 1.00-2.01 | 100 |
| | 300 | $0.73\pm0.32^{A, b}$ | $0.90\pm0.21^{ABC, b}$ | 0.40-1.19 | 100 |
| | 400 | $0.51\pm0.21^{A, c}$ | $1.22\pm0.10^{ABC, c}$ | 0.31-1.07 | 100 |
| | 500 | $0.33\pm0.08^{A, d}$ | $1.25\pm0.32^{ABC, c}$ | 0.21-0.45 | 100 |
| 24 | 200 | $1.40\pm0.48^{A, a}$ | $0.86\pm0.25^{AB, a}$ | 0.90-2.54 | 100 |
| | 300 | $0.69\pm0.29^{A, b}$ | $1.08\pm0.30^{AB, b}$ | 0.40-1.06 | 100 |
| | 400 | $0.41\pm0.11^{A, c}$ | $1.20\pm0.17^{AB, c}$ | 0.29-0.58 | 100 |
| | 500 | $0.31\pm0.09^{A, d}$ | $1.18\pm0.11^{AB, c}$ | 0.19-0.48 | 100 |

$^{a-d, A-C}$ Mean values within a row having different superscripts are significantly different by least significant difference test; upper case for salinity; lower case for tricane concentration.

Induction time did not change significantly when salinity increased. But it decreased as tricaine concentration increased (Figure 1).

Recovery time was highest at 0 ppt and 400-500 mg $l^{-1}$ of tricaine. It was particially suppressed by salinity at 8 ppt of salinity.

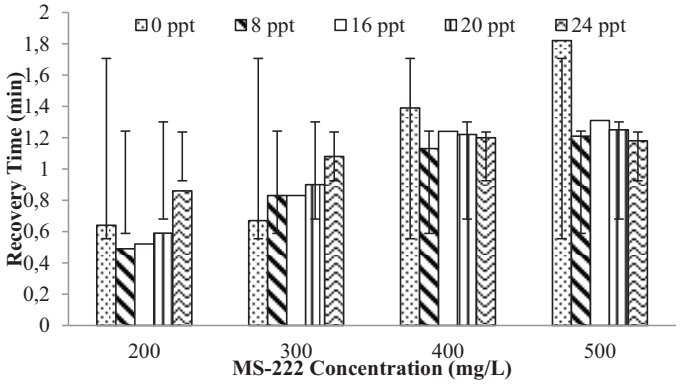


Figure 1. Mean±SD of induction time of blue tilapia immersed to anesthesia

There was negative relation between induction time and tricaine concentration. Induction times significantly decreased with increasing tricaine concentrations. It is agree with previous studies (Mylonas et al., 2005; Küçük, 2010; Pramod et al., 2010; Pawar et al., 2011 and Mercy et al., 2013). But, there was a positive relation between recovery time and tricaine concentration. Recovery time increased as tricaine concentration arised. The same relation was reported by Küçük (2010). Recovery time and mortality risk inceased when anaesthetic concentration rised up.

Ideal tricaine concentration was 200 mg $l^{-1}$ of tricaine at 8 ppt of salinity. Induction and recovery times were 1.23 min and 0.49 min respectively. Hseu et al., (1998) verified our results. They indicated that those were 1.31 min and 0.63 min at 100 mg $l^{-1}$ of tricaine, respectively for goldlined sea bream.

Tilapia were tolerant fish to high tricaine concentrations (200-500 mg $l^{-1}$) and high salinities (0-24 ppt) concentrations comparing with goldfish *Carassius auratus* exposed to 150-500 mg $l^{-1}$ of tricaine concentration and 0-16 ppt of salinity (Küçük and Çoban, 2016). Induction time and recovery time of tilapia were 0.31 and 1.18 min at highest degrees of salinity and tricaine (24 ppt and 500 mg $l^{-1}$ of MS-222, respectively). Those of goldfish were 0.27 and 1.27 min at 16 ppt and 500 mg $l^{-1}$ of tricaine. The values are almost close each other. The results showed that tilapia were more tolerant than goldfish when exposed to high salinity and tricaine concentration.

When an anaesthetic is chosen, a lot of considerations are thought such as efficiency, price, handiness to use, security to fish, user and environment, structure of experiment and fish species (Mylonas et al., 2005). Tricaine provided all these considerations, except for price and withdrawal time. Cost of tricaine was the higher than other anaesthetic (Hseu et al., 1998) and before marketing fish 21 days are needed to draw the residue from fish body (Coyle et al., 2004).

Tricaine + salt combination alleviated fish from handling stress and increased survival in stripped bass (Mazik et al., 1991; Mazik and Simco, 2014). Survival percentage were also excellent (100%) in this trial. On the other hand, in our study, tilapia tolerated to high tricaine concentration. Because alkalinity and total hardness of the water source were high (Table 1). Coyle et al. (2004) mentioned that tricaine has high potency to warm water fish with low hardness. High hardness of water protected tilapia from advers effect of high tricaine concentrations.

In this study, use of anaesthetics + salt combination was tested in tilapia to explaine why tricaine was used with salt to anaesthetize fish. This combination alleviated blue tilapia. Because salt is used to reduce stress in fish. Adding 8 g $l^{-1}$ of salt take off the difference between fish blood and environment (Wurst, 1995). For blue tilapia, 200 mg $l^{-1}$ of tricaine + 8 g $l^{-1}$ of salt combination is suggested to use. Even, identical combination (200 mg $l^{-1}$ of tricaine + 12 g $l^{-1}$ of salt) was used for goldfish by Küçük and Çoban (2016).

## CONCLUSIONS

Ideal concentration of tricaine was 200 mg $l^{-1}$ at 8 ppt of salinity for blue tilapia (1.23 min). As tricaine concentration increased, induction time decreased. Tilapia quite tolerant to tricaine and salt. Survival was perfect for all concentrations and salinities.

At high salinity tilapia recovered quickly, eventhough concentration and salinity were high. High salinity alleviated tricaine effect on recovery time of tilapia (1.18 min at 500 mg $l^{-1}$ and 24 ppt). Recovery time was . 1.82 min at 500 mg $l^{-1}$ and 0 ppt. As a result of that, tricaine + salt combination made induction and recovery times faster.

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