Journal of Animal and Veterinary Advances 9 (3): 547-550, 2010
ISSN: 1680-5593

The Anaesthetic Effects of Quinaldine Sulphate, Muscle Relaxant Diazepam and Their Combination on Convict Cichlid, *Cichlasoma nigrofasciatum* (Günther, 1867) Juveniles

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Abstract: The goal of this study was to determine the safety dosage of anaesthetic Quinaldine Sulphate (QS) alone and together with a muscle relaxant, Diazepam (D) on Convict cichlid, *Cichlasoma nigrofasciatum* (0.54±0.01 g) juveniles for ornamental fish sector. The trial showed that the QS with D administration significantly increased the anaesthesia level in the Convict cichlid. The fish entered light anaesthesia at 9.75 ppm QS+0.5 ppm D (0.83±0.02 min). Moreover, a deep anaesthesia level was reached at 9.75 ppm QS+1 ppm D (1.20±0.09 min) as compared to 13 ppm of QS (6.40±0.28 min). When used together with QS and D eliminated the excitement and hyperactivity of the fish compared to QS alone. In addition, no mortality occurred in any anaesthesia levels except high concentrations (40-54 ppm QS). This study indicate that the advisable light and deep phases of anaesthesia for experimental treatments, handling, immobilization and transportation of the convict cichlid juveniles were achieved with dosages of 9.75 ppm QS+0.5 ppm D and 9.75 ppm QS+1 ppm D, respectively.

Key words: Anaesthesia, quinaldine sulphate, diazepam, convict cichlid, *cichlasoma nigrofasciatum*

INTRODUCTION

Anaesthetics are widely used in aquaculture to minimize fish stress response and to aid in handling fish and to minimize stress (Ortuno *et al*., 2002). In many cases their effectiveness depends on the procedure used because severe anaesthesia may itself induce a stress response in fish (Iwama *et al*., 1989; Thomas and Robertson, 1991). Several anaesthetic agents, e.g., Quinaldine (Small, 2003), Quinaldine sulphate (Massee *et al*., 1995; Small, 2003), MS-222 (Wagner *et al*., 2002), Propanidid (Jeney *et al*., 1986), Benzocaine-hydrochloride (Ferreira *et al*., 1984), Metomidate, Chlorobutanol and Phenoxyethanol (Summerfelt and Lynwood, 1990; Molinero and Gonzales, 1995), Clove oil (Griffiths, 2000) are used in aquaculture. Quinaldine is economical and effective at very low concentrations and has a low toxicity and short fish recovery time (Bell, 1964). However, poor solubility in water long induction time and strong odour are the drawbacks of this anaesthetic. Quinaldine sulphate eliminates the water solubility and odour problems and reduces the induction time (Summerfelt and Lynwood, 1990). At present, quinaldine sulphate is one of the most commonly used anaesthetics in aquaculture. It is demonstrated to be a convenient and safe anaesthetic for use with fish. Both onset of anaesthesia and recovery are significantly more rapid than with the parent compound quinaldine. Quinaldine sulphate was first reported to act as an anaesthetic in fish by Jodlbauer and Salvendi (Schoettger and Steucke, 1972; Yanar and Kumlu, 2001). It has proved to be an excellent anaesthetic because it is rapidly absorbed and appears to be excreted largely unaltered. As a consequence, anaesthesia is rapidly induced and fish recover very quickly (Tort *et al*., 2002).

Although, quinaldine sulphate produces a total loss of equilibrium at deep anaesthesia level, the fish do not completely lose reflex responses (Tytler and Hawkins, 1981). This is undesirable during handling and particularly in the case of surgical procedures of fish (Schram and Black, 1984). It is suggested that the use of quinaldine sulphate together with triacin overcomes the reflex twitching problem (Schoettger and Steucke, 1972; Piper *et al*., 1982). It is known that muscle relaxants

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reduce excitation, hyperactivity, respiration rate and rigidity of the muscles. Intramuscular injection of muscle relaxants (e.g., gallamine triethiodide, tubocurarine chloride and pancuronium bromide) has been used to eliminate the reflex problem encountered in anaesthetised fish by quinaldine sulphate (Summerfelt and Lynwood, 1990). Diazepam anaesthesia is simple to administer, effective allows rapid delivery and is comfortable and safe for man. Also, it is is frequently used to decrease muscle rigidity and excitation in combination with appropriate anaesthetics in man (Kayaalp, 1992). The use of quinaldine sulphate, diazepam and their combination as anaesthetic agents have been extensively studied in some finfish species such as Sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) juveniles and tilapia (*Oreochromis niloticus*) (Kumlu and Yanar, 1999; Yanar and Kumlu, 2001; Yanar and Genç, 2004). The combination of quinaldine sulphate and diazepam significantly decreased the excitement and hyperactivity of these fish in confined space without leading to mortality. Due to the small amount of information available on suitable concentrations in ornamental fish culture, the effects of anaesthetic quinaldine sulphate alone and in combination with a muscle relaxant (diazepam) on convict cichlid, *Cichlasoma nigrofasciatum* juveniles were studied in the study.

MATERIALS AND METHODS

Convict cichlid, Cichlasoma nigrofasciatum juveniles were from the Mustafa Kemal University Aquaculture Research Unit. Prior to starting the experiment, four hundred eighty juvenile fish with an avarage body weight of 0.54±0.01 g were acclimatized to experimental conditions for 2 weeks in four 100 L Aquaria. The experiment was performed in 4-L flat bottom glass flasks in two replicates. The tests were conducted at 26±1°C. The pH and dissolved oxygen were maintained at 7.5±0.17 and 7.35±0.23 mg l-1, respectively. All fish were fed with 4% body weight ornamental fish feed daily. Quinaldine Sulphate (QS) was distributed into the flasks at concentrations of 3.25, 6.5, 9.75, 13, 27, 40 and 54 ppm. In addition to QS, diazepam (Deva Company, Istanbul, Turkey) obtained from a local pharmacy in 10 mg ampoules was also added into some of the flasks at concentrations of 0.5 and 1.0 ppm . After stirring the water of the flask with a glass rod for better dispersal of the anaesthetic, 10 fish which had been starved for 48 h were stocked into each of the flasks. Continuous aeration was supplied through airstones attached to a plastic tube. The response of each individual fish in each test media was immediately recorded from the stocking until the end of the experiment. The exact time taken for the fish to partially or completely lose equilibrium was noted. The fish were observed for 1, 5, 10, 24 and 48 h after the stocking. These periods, any fish that lost its equilibrium was transferred to a 4-L glass flask filled with anaesthetic free water to record recovery time. The fish that recovered from the anaesthesia were also observed for another 48 h to observe the post-exposure effect of the treatments. The fish were not fed either during or after the experimental period. The onset of different phases of anesthetic and recovery was measured in sec and min with digital stopwatch, according to Hamackova *et al*. (2001) and presented in Table 1.

In the experiment, all data were subjected to a one-way analysis of variance to determine if there is a difference in treatments. Duncan test was used to compare the means of the treatments when differences occurred (Norusis, 1993).

RESULTS AND DISCUSSION

The time for the fish to enter the desired anaesthesia level (induction time) ranged between 0.29±0.06 and 6.40±0.28 min depending on concentration of the anaesthetic used (Table 2). Increase in the concentration of QS decreased the time of entrance to anaesthesia. Induction time was 0.66±0.03 and 3.73±0.75 min for light sedation and 1.20±0.09 and 6.40±0.28 min for deep sedation. Minimum and the maximum recovery time were ranged between 2.29±0.12 and 17.50±0.71 min, respectively (Table 2).

The cichlid juveniles show only tranquility period in 3.25 ppm QS+0.5 ppm D and 6.5 ppm QS+0.5 ppm D dosage groups. QS alone was not suitable for the light and/or deep anaesthesia of convict cichlid juveniles. Even, 10% of the fish died at 40 ppm QS. No mortality occurred in any anaesthesia levels except high concentrations (40-54 ppm QS) and 0.5 D and 1 D alone (for control dosages) did not produce anaesthesia in fish.

When Diazepam (D) was used together with Quinaldine Sulphate (QS), the fish entered anaesthesia at lower concentrations than when only QS was used. The fish entered light anaesthesia at 9.75 ppm QS+0.5 ppm D (0.83±0.02 min). Moreover, a deep anaesthesia level was reached at 9.75 ppm QS+1 ppm D (1.20±0.09 min) as compared to 13 ppm of QS (6.40±0.28 min) (Table 2). When used together with QS and D eliminated the excitement and hyperactivity of the fish compared to QS alone. We observed best and safety recovery time 2.51±0.03 min at 9.75 ppm QS+1 ppm D. This study shows that the convict cichlid can be successfully anesthetized

Table 1: Description of the respective stages of anesthesia and recovery in fish (modified from Hamackova *et al.*, 2001)

Stage 0:	Tranquility period	Slow swimming, decreased reactivity to external stimuli
Stage 1:	Excitation period	Restlessness, voluntary swimming still possible, strong withdrawal reflex
Stage 2:	Light anaesthesia	Decreased activity, low turning to one side, still reaction to external stimuli particularly in the fish, loss of co-ordination, excrement discharge, respiratory motion regular, slower and deep
Stage 3:	Deep anaesthesia	Lying on one side without movement, increase in excrement discharge, loss of motility, none of the withdrawal reflexes but the acoustic one, respiratory motions regular deep

Table 2: The effects of Quinaldine Sulphate (QS) and Quinaldine Sulphate+Diazepam (QS+D) on convict cichlid, Cichlasoma nigrofasciatum juveniles

Anaesthetic concentrations (ppm)	Induction time (minute) Anaesthesia level				Recovery time (Minute)	Survival rate (%)				
	Tranquility	Excitation	Light	Deep		1 h	5 h	10 h	24 h	48 h
3.25 QS	0^{g}	0^{c}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
3.25 QS+0.5 D	1.62 ± 2.28^{bcd}	0^{c}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
3.25 QS+1 D	3.36 ± 12.73^{a}	0^{c}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
6.5 QS	2.14 ± 0.02^{b}	0^{c}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
6.5 QS+0.5 D	1.50 ± 0.01^{bcde}	0^{c}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
6.5 QS+1D	1.13 ± 0.18^{bcdefg}	2.46 ± 0.15^{a}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
9.75 QS	1.77 ± 0.33^{bc}	1.23 ± 1.74^{b}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
9.75 QS+0.5 D	1.45 ± 0.09^{bcdef}	1.05 ± 1.48^{bc}	0.83 ± 0.02^{e}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
9.75 QS+1 D	1.10 ± 0.14^{bcdefg}	1.41 ± 0.21^{b}	3.73 ± 0.75^{a}	1.20 ± 0.09^{h}	2.51 ± 0.03^{ef}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
13 QS	0.71 ± 0.06^{cdefg}	0.97 ± 0.19^{bc}	3.41 ± 0.13^{a}	6.40 ± 0.28^{a}	8.05 ± 0.07^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
13 QS+0.5 D	0.34 ± 0.01^{fg}	0.39 ± 0.02^{bc}	0.66 ± 0.03^{e}	2.51 ± 0.01^{e}	6.25 ± 2.48^{c}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
13 QS+1 D	0.34 ± 0.01^{fg}	0.70 ± 0.03^{bc}	1.10 ± 0.14^{d}	1.23 ± 0.18^{h}	3.25 ± 0.07^{def}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
27 QS	0.45 ± 0.03^{efg}	0.80 ± 0.02^{bc}	2.60 ± 0.09^{b}	5.39 ± 0.01^{b}	8.94 ± 0.09^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
27 QS+0.5 D	0.29 ± 0.06^{fg}	0.68 ± 0.01^{bc}	1.29 ± 0.01^{d}	2.40 ± 0.04^{e}	4.13 ± 0.04^{d}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
27 QS+1 D	0.33 ± 0.00^{fg}	0.61 ± 0.03^{bc}	0.99 ± 0.02^{e}	1.13 ± 0.03^{h}	2.52 ± 0.09^{ef}	100^{a}	100^{a}	100^{a}	100^{a}	90.0 ± 14.14^{a}
40 QS	0.37 ± 0.06^{efg}	0.49 ± 0.02^{bc}	2.35 ± 0.07^{bc}	5.12±0.02c	17.50 ± 0.71^{a}	100^{a}	100^{a}	100^{a}	100^{a}	90.0 ± 14.14^{a}
40 QS+0.5 D	0.46 ± 0.01^{efg}	0.62 ± 0.02^{bc}	1.24 ± 0.33^{d}	2.32 ± 0.03^{ef}	2.29 ± 0.12^{f}	100^{a}	100^{a}	100^{a}	100^{a}	90.0 ± 14.14^{a}
40 QS+1 D	0.49 ± 0.02^{efg}	0.82 ± 0.02^{bc}	1.00 ± 0.00^{de}	1.70 ± 0.42^{g}	3.36 ± 0.20^{def}	100^{a}	100^{a}	100^{a}	100^{a}	90.0 ± 14.14^{a}
54 QS	0.41 ± 0.04^{efg}	0.76 ± 0.01^{bc}	2.00 ± 0.00^{c}	3.10 ± 0.01^{d}	6.81 ± 0.69^{c}	100^{a}	100^{a}	100^{a}	100^{a}	60.0 ± 0.0^{b}
54 QS+0.5 D	0.34 ± 0.12^{fg}	0.95 ± 0.03^{bc}	1.36 ± 0.08^{d}	2.40 ± 0.14^{e}	4.23 ± 0.04^{d}	100^{a}	100^{a}	100^{a}	100^{a}	70.0 ± 14.14^{b}
54 QS+1 D	0.53 ± 0.04^{defg}	0.88 ± 0.06^{bc}	1.19 ± 0.13^{d}	2.13 ± 0.04^{f}	3.73 ± 0.38^{de}	100^{a}	100^{a}	100^{a}	30.0 ± 14.14^{b}	10.0 ± 14.14^{c}
0.5 D	0^{g}	0^{c}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
1 D	0^{g}	0^{c}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Control	0^{g}	0^{c}	0^{f}	0^{i}	0^{g}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Means followed by different letters in the same column are significantly different ($p<0.05$) (mean±SD)

using appropriate concentrations of QS and D for ornamental fish sector, experimental treatments, handling, immobilization and transportation. This is the first report to the knowledge regarding the use of Quinaldine sulphate and Diazepam combination in convict cichlid. This observation agrees with the finding of Yanar and Kumlu (2001), who defined that the combination of QS and diazepam significantly decreased the excitement and hyperactivity of the fish in confined space without leading to mortality. Similar statements have also been made by Yanar and Genc (2004) for Nile tilapia, *Oreochromis niloticus* by Kumlu and Yanar (1999) for sea bream, *Sparus aurata* and by Schram and Black (1984) for grass carp, *Ctenopharyngodon idella*. It is well known that some anaesthetics may be more suitable for one species than others (Jolly *et al.*, 1972; Sylvester and Holland, 1982; Josa *et al.*, 1992; Weyl *et al.*, 1996). Though, there is dispute in the literature, it is generally accepted that light anaesthesia is desirable during the transportation of fish (Summerfelt and Lynwood, 1990). Anaesthetised fish at deep sedation levels lose equilibrium and hence may sink to the bottom, pile up and finally suffocate (Dupree and Huner, 1984). It appears that a concentration of 9.75 ppm QS plus 0.5 ppm diazepam is suitable for light anaesthesia and may be used for the transportation of cichlid juveniles. The concentration of 9.75 ppm QS plus 1 ppm diazepam which provides deep anaesthesia may be suitable for marking, surgery and handling. The combination of QS and diazepam significantly decreased the excitement and hyperactivity of the fish in confined space without leading to mortality.

CONCLUSION

In diazepam, when administered with quinaldine sulphate at considerably low concentrations, enhances anaesthesia and eliminates the undesirable effects of quinaldine sulphate. The combination of quinaldine sulphate and diazepam is a potent anesthetic for convict cichlid, having both rapid induction and recovery times. We strongly advised that the desirable and the safety concentrations for light and deep anesthesia were determined to be 9.75 ppm quinaldine sulphate plus 0.5 ppm Diazepam and 9.75 ppm Quinaldine sulphate plus 1 ppm Diazepam respectively for the convict cichlid juveniles as an ornamental fish model.

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