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Efficacy of Quinaldine Sulphate as an Anaesthetic for the Ornamental Carp (*Cyprinus carpio*) in Simulated Packaging for Long Distance Transport

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Abstract

High cost incurred in transporting fish in large volume of water is a major problem in airlifting ornamental fish to foreign markets. The present study was carried out to investigate the efficacy of the anaesthetic, quinaldine sulphate buffered with sodium bicarbonate on ornamental carps (Koi carps; *Cyprinus carpio*) in simulated packaging for air transport. Quinaldine sulphate significantly reduced the rate of oxygen consumption and the accumulation of ammonia in water. The most efficient concentration of buffered quinaldine sulphate which was responsible for the greatest reduction in accumulation of ammonia and the rate of oxygen consumption was 50 ppm. Young koi carps of 7.5 - 9.0 cm in total length anaesthetised with 50 ppm quinaldine sulphate at the density of 40% of fish body weight to weight of water ratio did not show any mortality at room temperature of 28 ^{0}C during the 40 hours of exposure time while unanaesthetized fish at the same density suffered 100% mortality. The recovery time during the post packaging period was found to be less than 5 minutes. The present study indicates that young koi carps could be transported at higher packing densities, using the suitable dosage of quinaldine sulphate which will maximize the effective utilization of space and weight during transportation.

Introduction

The export of live tropical ornamental fish from Sri Lanka has increased in an unprecedented manner during the past decade earning a substantial amount of foreign exchange to the country. According to the trade statistics, Sri Lanka has earned 30 million rupees in 1986 and it was envisaged to earn 250 million rupees by 1994 (Shariff & Subasinghe 1992; Anon. 1989). Efficient packaging of fish causing minimum mortality during transport plays an important role in the success of this trade. This is particularly important when these fish are transported over long distances to foreign markets.

The primary problems pertaining to the current closed system packaging are the low oxygen capacity of water and the accumulation of toxic excretory wastes. Another problem is the handling stress that results from initial capture, loading into transport containers, the actual transport, unloading and stocking. High transport density and poor water quality may be additional stresses (Robertson *et al.* 1988). Transportation often results in mortalities of fish which may occur immediately following the transport or secondarily due to osmoregulatory malfunctions or infectious diseases (Wedemeyer 1970, cited by Robertson *et al.* 1988).

Even though the low oxygen capacity can be solved by the use of pure oxygen, the accumulation of both ammonia and carbon dioxide is toxic to fish directly and also

indirectly by decreasing the ability of fish to extract oxygen from water (Teo & Chen 1989). This can be aggravated by an increase in temperature (Brookway 1950, cited by Teo & Chen 1993) which also increases the oxygen consumption rates thus making the situation even more severe for the fish. In order to avert these adverse effects the ornamental fish exporters use variety of methods such as pre-transport starvation, use of chilled shipping water, addition of salts to the fresh water and use of anaesthetics. Currently, the use of anaesthetics to tranquillize the fish to reduce the metabolic activities and stress responses during the transportation is becoming increasingly common. The present study was carried out to investigate the effects and efficacy of the anaesthetic, quinaldine sulphate (buffered with $NaHCO_3$) on ornamental carps (*Cyprinus carpio*) in simulated packaging for air transport.

Materials & Methods

Quinaldine sulphate solutions with different concentrations ranging from 25 ppm to 70 ppm were prepared and buffered with $NaHCO_3$ using 0.45 g of $NaHCO_3$ for every 1 g of Quinaldine sulphate. Acclimated and pre-starved koi carps for 48 hours ranging from 7.5 cm to 9.0 cm in total length were immersed in each concentration separately at a weight of fish to weight of water ratio of 1:10. The approximate concentration of buffered quinaldine sulphate to anaesthetize carps was selected using behavioural response in relation to the stage of anaesthesia (Table 1).

Double polyethylene bags were used for the packaging experiment designed to find the best concentration of quinaldine sulphate required to keep young koi carps under anaesthesia for a period of 40 hours. For a selected concentration, six such bags were employed as in Fig. 1 and experiment was carried out with three replicates.

After adding the chemicals and fish, the bags were flattened to expel the air and ¾ of the volume of each bag was inflated with industrial oxygen. These bags were then sealed with rubber bands and placed in a styrofoam box for 40 hours at room temperature (28.05 ± 0.28^0 C). At the end of the test period (40 hours), water in each bag was analysed for pH, temperature, dissolved oxygen (DO) and total ammonia. The amount of Excess Dissolved Oxygen (EDO) in the bags with the anaesthetic after 40 hours of exposure was obtained as below.

EDO in test water (with anaesthetic) = Amount of DO in test water - Amount of DO in the control

The water in the bags was then replaced gradually with aged tap water and mortality of fish was recorded. The fish were kept for five subsequent days in aquaria and the mortality was recorded. The concentration of anaesthetic that resulted in the highest EDO after 40 hours of exposure was used to determine the maximum loading capacity of young koi carps under anaesthesia.

Results

Table 1 shows the stages of anaesthesia observed in young koi carps for the different concentrations of buffered quinaldine sulphate solutions. At 25 ppm quinaldine sulphate, fish began to show the signs of slight sedation and at 45 ppm, fish were under light anaesthesia and the speed of opercular movements became lower than that of the un-

anaesthetized (control) fish. At 60 ppm, fish began to show some signs of stress and increased mucous secretion was observed. Fish stopped responding to any stimuli at 70 ppm quinaldine sulphate and were at the stage of surgical anaesthesia. At 90 ppm, the opercular movements of fish stopped within ten minutes and fish were in the stage of medullary collapse.

Table 2 shows the effects of different concentrations of quinaldine sulphate on the packaging water and the survival of young koi carps. A slight drop in the pH was recorded in the bags with quinaldine sulphate. The amount of total ammonia was lower in the water with quinaldine sulphate than in the control except at the concentration of 70 ppm. The lowest value (9.9 mg/l) was recorded at 50 ppm quinaldine sulphate which is about 23% lower than that of the control. This value was significantly different from the amounts of ammonia at other concentrations of quinaldine sulphate ($p<0.0001$).

Table 2 also shows the amount of excess oxygen in the packaging water at different concentrations of quinaldine sulphate. The amount of excess oxygen in the water increased up to the concentration of 50 ppm of quinaldine sulphate and then decreased. The recorded values of dissolved oxygen in packaging water at 70 ppm was less than that of the control. Oxygen consumption rate (OCR) of koi carps decreased with increasing concentration of quinaldine sulphate from 25 ppm to 50 ppm in the packaging water and then increased. The OCR of koi carps at the concentration of 50 ppm of quinaldine sulphate was smaller than that of the control. A negative second order relationship between the anaesthetic concentration and the oxygen consumption rate was evident (Fig. 2).

At the end of the exposure period of 40 hours, there was no significant difference between the amounts of dissolved oxygen in the bags with quinaldine sulphate only (Bag No 4) and the blank (Bag No 6 - without anaesthetic). This indicates that the chemical did not have a significant effect on the dissolved oxygen content of packaging water.

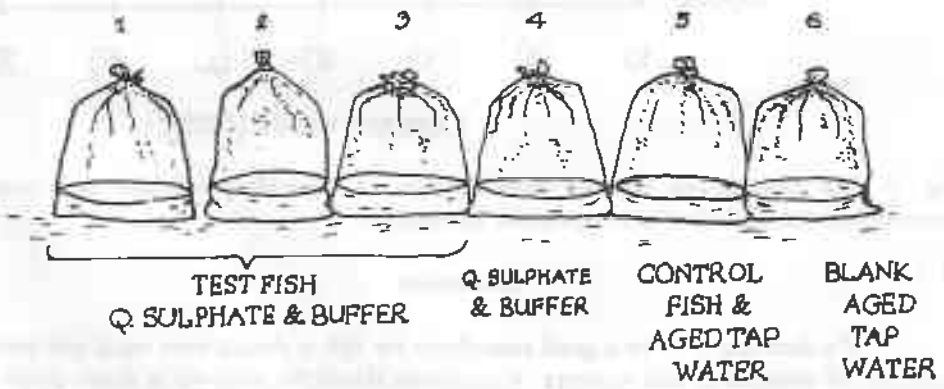

Fig. 1. Arrangement of polythene bags.

Table 3 shows the effects of packing density on the survival of young koi carps anaesthetised with 50 ppm quinaldine sulphate. Up to the packing density of 30% of body weight to weight of water ratio, there was no mortality in the controls and test fish.

Mortalities of 66.6% and 100% were recorded in the controls at the densities of 35% and 40% respectively, while no mortality in test fish treated with 50 ppm quinaldine sulphate were recorded at these densities.

There was no significant difference in pH, water temperature and dissolved oxygen levels in the treated bags and controls at all the densities of fish. However, there was a significant difference ($p<0.05$) in the amount of total ammonia in treated (9.9 mg/l) and control (20 mg/l) bags at 40% density of fish.

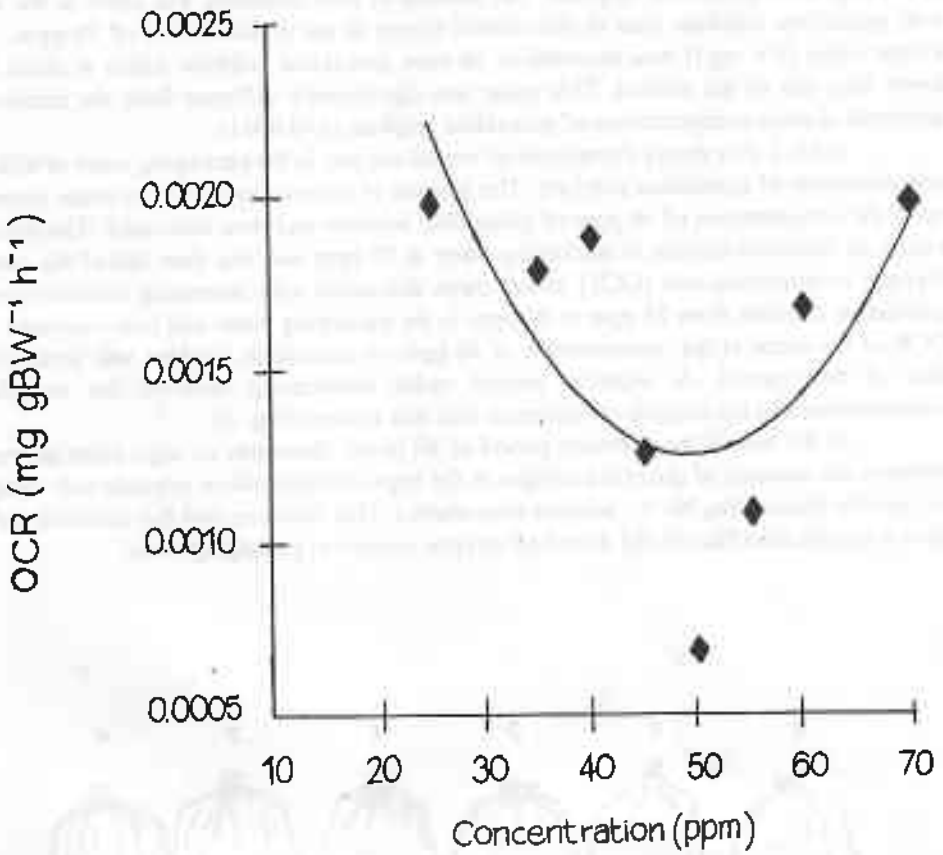

Fig. 2. The relationship between concentration of quinaldine sulphate and oxygen consumption rate (OCR) of koi carps under anaesthesia.

Discussion

If a chemical is to be a good anaesthetic for fish it should have rapid and smooth induction of anaesthesia and recovery. Anaesthesia should be achieved at doses which are not toxic either to the fish or to the user. Anaesthetic should also be soluble in water (Brown 1993). Results of the present study show that quinaldine sulphate when buffered with sodium bicarbonate possesses almost all the above characters to be used as an anaesthetic for young koi carps. If the fish are to be kept under anaesthesia for a long duration of about 40 hours, the best concentration appears to be 50 ppm. Higher dosage than this would result in a higher degree of anaesthesia (Table 1) which would be suitable for medical purposes.

Table 1. Stages of anaesthesia in young koi carps anaesthetised with quinaldine sulphate.

Concentration of q sulphate (ppm)	Stage of anaesthesia	Behavioural response in fish
15	0 Normal	Fish were active and behaved normally.
20	0 Normal	-do-
25	I - 1 Light sedation	Voluntary swimming continued. No change in the speed of opercular movements. Equilibrium normal. Slight loss of reactivity to visual and tactile stimuli.
30	I - 2 Deep sedation	Voluntary swimming stopped. Slight reduction in the speed of opercular movements. Equilibrium normal. Total loss of reactivity to visual and tactile stimuli, but responded to positional changes.
35	II - 1 Light narcosis	Loss of equilibrium, but efforts were made to right itself. Fish became excited and the speed of opercular movements increased; weakly responded to positional changes.
40	II - 2 Deep narcosis	Speed of opercular movements decreased approximately to normal; total loss of equilibrium and no efforts to right itself. Weakly responded to strong tactile and vibrational stimuli; No response to positional changes.
45	III - 1 Light anaesthesia	Speed of opercular movements became lower than normal. Other behaviours as in deep narcosis.
50	III - 2 Light anaesthesia	Further decrease in the speed of opercular movements. Other behaviours as in deep narcosis.
60	III - 3 Stage between Light anaesthesia & surgical anaesthesia	A slight increase in the speed of opercular movements. Fish showed signs of stress. Increased mucus secretion on the gills.
70	III - 4 Surgical anaesthesia	Total loss of reactivity to any stimuli. Speed of opercular movements very law.
90	IV Medullary collapse	Total loss of opercular movements.

Modified from M. K. Stoskopf, cited by Brown (1993).

Table 2. Effect of different concentrations of quinaldine sulphate on the water quality and survival of young koi carps after 40 hours of exposure (weight of fish/water ratio is 15%; Results are averages of 3 replicates). Tem = Temperature; Ex. Oxy = Excess Oxygen,; OCR = Oxygen Consumption Rate; Indc time = Induction time; Rec time = Recovery time; Mort = Mortality; * Control

No. of Fish	Total Weight of fish (g)	Concentration of Quinaldine sulphate (ppm)	After Exposure to 40 hours							
			pH	Tem (^{0}C)	NH_3	Ex. Oxy (mg l^{-1})	OCR (O_2 mg gBW^{-1} h^{-1})	Indc time (min)	Rec time (min)	% Mort
11	91.0	25	6.66	27.9	12.2	0.00	0.0020	3.50	0.75	0.0
10	90.3	35	6.65	28.7	12.0	1.10	0.0018	3.00	0.75	0.0
12	90.6	40	6.70	28.1	11.0	1.83	0.0019	2.50	1.00	0.0
12	90.8	45	6.55	28.0	11.4	2.10	0.0013	1.50	1.20	0.0
12	92.3	50	6.37	28.2	9.9	4.90	0.0007	1.00	2.00	0.0
11	90.5	55	6.39	28.1	11.3	3.90	0.0011	0.75	2.00	0.0
12	91.8	60	6.69	27.6	11.8	1.00	0.0017	0.50	3.50	0.0
10	92.0	70	6.30	27.9	15.5	-0.74	0.0020	0.50	4.00	37.4
10	91.6	00*	7.02	28.0	12.9	-	0.0020	-	-	0.0

Table 3. Effect of packing density on the survival of young koi carps after exposing for 50 ppm Quinaldine sulphate for 40 hours. (Results are averages of 2 replicates). T- Treated containers; C - Control.

No. of fish	Total weight of fish (g)	Packing density %	Temperature (^{0}C)		Dissolved Oxygen (mg l^{-1})		pH		NH_3 (mg l^{-1})		% Mortality	
			T	C	T	C	T	C	T	C	T	C
08	34.0	20	28.6	28.9	13.8	12.2	6.60	6.80	9.9	14.0	0.00	0.0
10	42.1	25	27.8	27.6	13.0	11.8	6.60	6.79	10.1	14.0	0.00	0.0
13	50.0	30	27.3	27.5	13.0	10.2	6.70	6.65	10.0	15.0	0.00	0.0
15	58.1	35	27.9	27.8	12.0	9.9	6.90	7.00	10.4	17.0	0.00	66.6
17	68.0	40	28.0	28.1	10.0	8.5	6.60	7.40	9.9	20.0	0.00	100.0

Blasiola (1976, cited by Brown 1993) stated that quinaldine is considered to be a good anaesthetic for fish by some researchers and he recommended a concentration of 200 ppm for tropical marine fish. Generally, for warm water fish species a concentration of 15 - 70 ppm is used. The dose required to reach stage III anaesthesia in many species is 16 ppm (Brown 1993). However, Sado (1985, cited by Brown 1993) reported that concentrations ranging from 50 ppm to 1000 ppm are used for tilapia. Brown (1993) stated that quinaldine is less effective at pH values less than 5 and becomes more potent at high pH values. During the present study, the pH of the treatment water ranged between 6.3 and 6.7 and increase in pH using higher doses of sodium bicarbonate may reduce the effective dose of quinaldine sulphate for young koi carps.

According to Teo & Chen (1989), the period between packing and unpacking of tropical ornamental fish varies from 12 to 40 hours depending on the destination, and ammonia and oxygen are the two most important factors in a packaging system of fish in polyethylene bags. Results of the present study show that the dissolved oxygen levels in the packaging water at the end of 40 hours were high even in the bags without the anaesthetic (Table 2). This shows that the current practice of supplying oxygen by charging pure oxygen into polyethylene bags to maintain 3 : 1 ratio of pure oxygen to water volume can ensure sufficient oxygen for the fish although it takes up a considerable portion of the space in the bags. Similar observations were made by Chow *et al.* (1994) for clown fish and Teo & Chen (1989) for guppy in simulated transport experiments. Teo & Chen (1989) suggested that it might not be necessary to charge such a large quantity of oxygen into the bags; the space saved from charging less oxygen could be used to add more water so that harmful metabolic wastes would be diluted. However, the increase of weight due to more water in the packing bags might not be accepted by exporters.

Results of the present study show that even though the dissolved oxygen supply is adequate, when the packing density is increased up to 35% of body weight to weight of water ratio without the anaesthetic, mortality of young koi carps was very high (66.6%). This could perhaps be largely attributed to the accumulation of metabolic wastes, especially ammonia. According to Winstone & Solomon (1976), high levels of oxygen reduce respiratory strain and promote survival of fish on long journeys. Some species such as rainbow trout, perch and roach have been found to be less susceptible to ammonia, under high oxygen tensions (Merkens & Downing 1957). However McFarland & Norris (1958, cited by Guo *et al.* 1995) have reported that metabolic waste level must be low enough to allow sufficient uptake of oxygen by fish even at adequate levels of dissolved oxygen. Chow *et al.* (1994) also pointed out that unacceptable levels of mortalities occur in the current system of packaging fish in polyethylene bags, although the dissolved oxygen supply is adequate.

During the present study, carps were starved for 48 hours before the experiment commenced, in order to reduce defecation as well as to stop regurgitation of food which would cause pollution of packaging water. Many studies have indicated that the problem of ammonia accumulation in closed systems such as packaging bags is partly solved by starving the fish for 2 - 3 days depending on their size (Amend *et al.* 1982; Frose 1986; Teo & Chen 1989; Guo *et al.* 1995). However, according to Teo & Chen (1989), starvation of fish prior to packaging did not have a considerable effect on the metabolic rates of guppies. Meanwhile, Phillips & Brookway (1954, cited by Teo & Chen 1993) found that starvation of brook trout for 63 hours, enhanced the effect of anaesthetic on the metabolic rate. Brown (1993) stated that food should be withheld for up to 24 hours and fish should not be disturbed prior to anaesthesia.

During the present study, it was observed that the amount of ammonia excreted by anaesthetised young koi carps at 15% fish body weight to weight of water ratio and at a concentration of 50 ppm of quinaldine sulphate was 23% lower than that of the control. Guo *et al.* (1995) reported similar results for platy fish (*Xipophorus maculatus*). Even at a density of 40% of fish weight to weight of water ratio, no mortality was observed during the present study in the bags with 50 ppm of quinaldine sulphate and the recorded total ammonia content in these bags were significantly lower than that in the control ($p<0.02$). Therefore, it was evident that use of quinaldine sulphate has significantly reduced the amount of ammonia released by young koi carps even at high densities.

Many authors have pointed out that accumulation of ammonia could be suppressed by addition of an ion-exchange resin such as clinoptilolite in to transport water (Nemato 1957; Amend *et al.* 1982; Bower & Turner 1982). Toxic carbon dioxide can be converted to bicarbonate by maintaining the pH of the water above 7; however, ammonia toxicity increases at pH values above 7 (Teo & Chen 1989). Therefore, use of a suitable buffer to maintain pH at or close to neutral levels will reduce the toxicity of both these metabolites in transport water. Meanwhile, some exporters try to reduce the activity of tropical fish by lowering the temperature. During the present study, the ceiling of loading capacity for young koi carps, anaesthetised with 50 ppm of quinaldine sulphate was not determined. Further research is suggested to determine the maximum loading capacity for young koi carps with quinaldine sulphate as the anaesthetic, in the presence of an ion-exchange resin, an anti-microbial agent and an efficient buffer at low temperature which would further increase the survival of fish during transportation while reducing the weight and space rendering higher profits.

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