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Influence of Tricaine Methanesulfonate on *Streptococcus agalactiae* Vaccination of Nile tilapia (*Oreochromis niloticus*)

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Abstract: Experiments were conducted to study the influence of tricaine Methanesulfonate (MS-222) on blood glucose levels and percent cumulative survival of Nile tilapia (*Oreochromis niloticus*) challenged with *Streptococcus agalactiae* 30 days post-vaccination with *S. agalactiae* vaccine or sham-vaccination with Tryptic Soy Broth (TSB). Anesthesia-treated fish were placed in an MS-222 (50 mg L^{-1}) solution to achieve stage 3 of anesthesia, while control fish were placed in a sham anesthetic solution containing water but no MS-222 for an equal amount of time. All fish were then injected intraperitoneally with *S. agalactiae* vaccine or TSB and later injected intraperitoneally with 750 cfu *S. agalactiae*/fish. Blood glucose levels among the groups immediately after anesthesia (92.8±5.6 and 73.2±4.2 mg dL^{-1}) or sham anesthesia (56.7±2.6 and 58.9±3.5 mg dL^{-1}) were significantly higher in the MS-222-treated groups ($p<0.0001$). No other significant differences in blood glucose levels between treatment groups were observed at subsequent sampling points. Percent cumulative survival among challenged vaccinated fish was 50% when treated with MS-222 versus 100% when not treated with MS-222 ($p<0.0001$). However, percent cumulative survival among challenged non-vaccinated fish was 80% when treated with MS-222 versus 29% when not treated with MS-222 ($p<0.0007$). Increased blood glucose levels prior to vaccination were significantly correlated to decreased percent cumulative survival after challenge ($r^2 = 0.5364$; $p = 0.0022$). Given increased blood glucose and decreased survival among MS-222-treated, vaccinated fish, consideration should be given when anesthetizing Nile tilapia with MS-222 prior to vaccination.

Key words: Fish, *Oreochromis niloticus*, tricaine methanesulfonate, MS-222, vaccination

INTRODUCTION

Tricaine Methanesulfonate (MS-222) is currently the only anesthetic approved by the United States Food and Drug Administration for use in foodfish. This anesthetic has been used to facilitate several manipulative processes, including handling, sorting, transport, diagnostic evaluation and vaccination (Burka *et al*., 1997; Wagner *et al*., 2003). Foodfish vaccination commonly involves the use of anesthetics to immobilize fish and limit stress responses, thus minimizing injury to the fish and hindering immunosuppressive stress responses that may affect vaccine efficacy (Summerfelt and Smith, 1990; Wedemeyer, 1997). However, other authors have reported that anesthetic treatment itself can be a stressor (Larsen, 1976; Bouck and Johnson, 1979; Barton and Peter, 1982; Iwama *et al*., 1989; Small, 2003) and thus would have a negative impact on vaccination. This study was conducted to assess the effects of MS-222 treatment on blood glucose levels and percent cumulative survival of Nile tilapia (*Oreochromis niloticus*) challenged with *Streptococcus agalactiae* 30 days post-vaccination with *S. agalactiae* vaccine or sham-vaccination with Tryptic Soy Broth (TSB).

MATERIALS AND METHODS

Fish: Fingerling Nile tilapia, (*Oreochromis niloticus*) with a mean weight of 15.34±0.34 g (mean±SEM) were housed at the USDA-ARS Aquatic Animal Health Research Laboratory in Chestertown, Maryland. The fish were kept in 9 L flow-through aquaria supplied by dechlorinated tap water and maintained on a 12 h: 12 h light: Dark period. The fish were fed daily to satiation with Aquamax Grower 400 fish feed (Brentwood, MO). Fish were maintained and handled according to Institutional Animal Care and Use Committee (IACUC)-approved guidelines. Water quality parameters (temperature, dissolved oxygen, Total

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Table 1: Blood glucose values (mean±SEM; mg dL^{-1}) of tricaine Methanesulfonate (MS-222)-treated and sham-treated control Nile tilapia (*Oreochromis niloticus*)[1]

		Treatment			
Hours post-vaccination	Hours post-challenge	MS-222 Vaccinated	MS-222 Non-vaccinated	No MS-222 Vaccinated	No MS-222 Non-vaccinated
0		92.8±5.6ab**	73.2±4.2bc*	56.7±2.6b	58.9±3.5bc
2		144.6±18.4a	111.6±12.5a	150.9±14.3a	110.1±13.0ab
24		71.8±16.6ab	59.3±7.2cd	68.5±12.3b	60.6±5.4bc
720	0	32.0±3.9b	36.6±4.7cd	30.8±0.6b	30.1±2.3c
722	2	49.0±23.1ab	37.1±5.3d	32.0±6.0b	72.9±30.4bc
724	4	71.8±15.3ab	79.7±21.1bc	60.7±16. 6b	138.6±36.5a
744	24	99.7±21.3ab	98.2±14.9ab	97.0±20.4ab	114.3±23.7ab
1056	336	51.5±5.5ab	77.7±11.2bc	79.7±28.7ab	52.0±5.0bc

[1]Nile tilapia were either treated with MS-222 (as treated groups) or no MS-222 (as control groups), bled and then intraperitoneally injected with either *Streptococcus agalactiae* vaccine (vaccinated) or TSB (non-vaccinated). All fish were subsequently injected intraperitoneally with 750 cfu *Streptococcus agalactiae*/fish without MS-222 treatment at 30 days (720 h; underlined) post-vaccination. Blood glucose levels were monitored through 1056 h post-vaccination. Data from treated and control fish are represented as mean blood glucose levels±SE. Different letters indicate significant differences ($p<0.05$) between time intervals within each treatment group (column) and asterisks denote significant differences between treatment groups within a time period (row)

Ammonia Nitrogen (TAN) and pH) were measured daily or weekly using a YSI 85 m (Yellow Spring Meter, Yellow Springs, Ohio) and a Fresh Water Aquaculture Kit Model AG-2 (LaMotte, Chestertown, MD) or HACH test kit (Hach Company, Loveland, CO). The mean water quality conditions throughout the duration of the experiment were as follows: Temperature, 29.67±0.63°C; DO, 4.10±0.79 mg L^{-1}; TAN, 0.09±0.02 mg L^{-1}; pH, 7.26±0.43.

MS-222 treatment and *Streptococcus agalactiae* vaccination: Two groups of 10 Nile tilapia each in 6 aquaria were treated with either MS-222 (treated) or not treated with MS-222 (control) for an equal amount of time. The MS-222 solution was prepared by dissolving MS-222 powder (50 mg L^{-1}; pH, 6.2; Argent Chemical Laboratories, Redmond, WA) in water inside a 40 L bucket with supplemental aeration from an airstone. The fish were placed in the MS-222 solution without bicarbonate buffering long enough to achieve stage 3 of anesthesia (equilibrium loss and movement termination) as in previous anesthesia studies (Schoettger and Julin, 1969; Small, 2003; Welker *et al*., 2007). Most fish exhibited these signs after approximately 4-6 min. Control fish were placed in a sham anesthetic solution containing water but no MS-222 for an equal amount of time as the MS-222-treated fish. After the time required to achieve stage 3 of anesthesia in the treated fish, one group of 30 MS-222-treated fish and another group of 30 non-treated MS-222 control fish were removed from water, weighed, intraperitoneally injected with 0.1 mL *S. agalactiae* vaccine (Evans *et al*., 2004a) and segregated equally into three replicate aquaria. Another group of 30 MS-222-treated fish and another group of 30 non-treated MS-222 control fish were removed from water, weighed, intraperitoneally injected with TSB (non-vaccinated) and segregated equally into 3 replicate aquaria. All fish were subsequently challenged by intraperitoneal injection with 750 cfu/*S. agalactiae* fish as described by Evans *et al*. (2006) without MS-222 treatment prior to challenge injection at 30 days (720 h) post-vaccination. Mortalities were monitored for 14 days post-challenge.

Measurement of blood glucose levels: Blood glucose levels were measured at 0 h (following MS-222 treatment but prior to vaccination), 2 and 24 h post-MS-222 treatment and post-vaccination and 720 h post-MS-222 treatment and post-vaccination (but prior to challenge). Blood glucose levels were also measured at 722, 724, 744 and 1056 h post-vaccination or 2, 4, 24 and 336 h post-challenge (Table 1). Neither fish treated with MS-222 prior to vaccination nor control fish were treated with MS-222 prior to challenge. At each sampling time, five fish from each tank were bled; however, some tanks did not have 5 fish after challenge and all fish in such tanks were bled in these cases.

Blood glucose levels are a recognized and reliable indicator of fish stress responses (Andersen *et al*., 1991; Chen *et al*., 1995; Cech *et al*., 1996; Reubush and Heath, 1996; Ortuño *et al*., 2001) and they were determined using the methods of Evans *et al*. (2003, 2004b, 2006). Briefly, blood glucose was measured with a One Touch Ultra Brand Meter and test strips (Lifescan, Milpitas, CA). The blood sample was taken from the caudal vein using a tuberculin syringe and 27-gauge needle, a 5-10 μL blood drop was placed onto a clean glass slide, the test strip was dipped in the blood drop and the confirmation window was allowed to completely fill with the blood drop (Diouf *et al*., 2000). The blood glucose level was displayed in mg dL^{-1} in about 5 sec. The one touch accuracy in determining Nile tilapia blood glucose levels has been established by Evans *et al*. (2003, 2004b, 2006). The authors used ten replicates of blood samples from healthy Nile tilapia to determine blood glucose protocol sensitivity at 20 mg dL^{-1} and observed a 3.25% intra-assay variance (mean = 20.4±0.66 mg dL^{-1}). The blood glucose meter results were also correlated with a colorimetric commercial laboratory method and the resulting correlation coefficient was 0.928 at a $p<0.001$.

Statistical analysis: All data analysis was performed using SAS System version 8 software (SAS Institute, 1999; Cary, NC). Blood glucose data were analyzed by ANOVA followed by means comparisons using Duncan's multiple-range test. Percent cumulative survival patterns post-challenge were analyzed by SAS System proc lifetest (Lifetest) with Wilcoxon comparison and correlation between blood glucose levels and percent cumulative survival patterns was determined by SAS System proc corr (correlation) analysis. For all data analysis, differences were considered significant at $p<0.05$.

RESULTS

Anesthesia: MS-222-treated fish achieved stage 3 anesthesia most often within approximately 4-6 min and recovered after vaccination and placement into fresh water. Control fish were kept in the sham control solution water for a corresponding period of time. None of the fish exhibited an observable physical reaction or struggle.

Responses to MS-222 treatment prior to and following vaccination: Blood was drawn from MS-222-treated fish and control fish and blood glucose levels were analyzed (Table 1). Mean blood glucose levels among the groups after anesthesia or sham anesthesia were as follows: MS-222-treated (92.8±5.6 and 73.2±4.2 mg dL^{-1}) and control (56.7±2.6 and 58.9±3.5 mg dL^{-1}). The mean blood glucose levels in MS-222-treated fish were significantly higher ($p<0.0001$) than the control (non-treated) mean blood glucose levels prior to vaccination, but blood glucose levels were not significantly different between treated and control fish at any other time post-vaccination. When comparing blood glucose levels within each group, considerable and sometimes significant increases in both treated and control blood glucose levels were observed at 2 h post-vaccination. At 24 h post-vaccination, blood glucose levels had decreased to levels statistically similar to blood glucose levels before vaccination.

Responses to MS-222 treatment following vaccination and experimental *S. agalactiae* challenge: Fish were challenged 30 days (720 h) post-vaccination with *S. agalactiae* and blood samples were obtained to measure blood glucose. Mean blood glucose levels among the groups prior to injection challenge were as follows: MS-222-treated (32.0±3.9 and 36.6±4.7 mg dL^{-1}) and control (30.8±0.6 and 30.1±2.3 mg dL^{-1}). The mean blood glucose levels in the treated fish were not significantly different than the control levels at any time post-challenge. When comparing blood glucose levels within each group post-challenge, considerable and sometimes significant increases in both the treated and control fish blood glucose levels were observed at 4 and 24 h post-challenge, but blood glucose levels at 336 h post-challenge (1056 h post vaccination) decreased to levels statistically similar to those immediately prior to challenge (Table 1).

Mortalities began within 24 h post-challenge and were observed in all groups except the vaccinated, challenged fish not treated with MS-222. In the absence of MS-222 treatment, percent cumulative survival among the vaccinated and the non-vaccinated fish was 100 and 29%, respectively ($p<0.0001$; Table 2). Percent cumulative survival among the vaccinated and the non-vaccinated fish treated with MS-222 was 50 and 80%, respectively ($p<0.0908$). Percent cumulative survival among challenged vaccinated fish was 50% when treated with MS-222 versus 100% when not treated with MS-222 ($p<0.0001$). Percent cumulative survival among challenged non-vaccinated fish was 80% when treated with MS-222 versus 29% when not treated with MS-222 ($p<0.0007$). Increased blood glucose levels prior to vaccination were significantly correlated to decreased percent cumulative survival among vaccinated fish after challenge ($r^2 = 0.5364$; $p = 0.0022$).

Table 2: Percent cumulative survival of Nile tilapia (*Oreochromis niloticus*) treated and not treated with MS-222 and challenged with *Streptococcus agalactiae* at 30 days post-vaccination with *S. agalactiae* vaccine or sham-vaccinated with TSB[1]

Treatment	Percent cumulative survival
MS-222 (Vaccinated)	50xy
MS-222 (Non-vaccinated)	80y
No MS-222 (Vaccinated)	100z
No MS-222 (Non-vaccinated)	29x

[1]Nile tilapia were either treated with MS-222 (as treated groups) or no MS-222 (as control groups), bled and then intraperitoneally injected with either *Streptococcus agalactiae* vaccine (vaccinated) or TSB (non-vaccinated). All fish were subsequently injected intraperitoneally with 750 cfu *Streptococcus agalactiae*/fish without MS-222 treatment at 30 days (720 h) post-vaccination. Mortalities were monitored for 14 days post-challenge and different letters indicate significant differences ($p<0.05$) in percent cumulative survival between treatments (column)

DISCUSSION

Blood glucose levels: The results in this study indicate that anesthetizing Nile tilapia, *O. niloticus*, with MS-222 immediately prior to injection vaccination can cause a transient stress response. A significant increase in mean blood glucose levels among MS-222-treated fish above controls was observed following immersion in MS-222 solution, but there were no other significant differences between MS-222-treated and control blood glucose levels. These results suggest that immersion in MS-222 anesthetic or sham anesthetic solution and vaccination can cause transient stress responses within 2-24 h and that MS-222 treatment does not cause chronic stress responses. After the apparent blood glucose level peak at 2 h post-vaccination or 4 and 24 h post-challenge, the blood glucose levels at 24 h post-vaccination and 366 h post-challenge in both groups decreased to statistically similar levels as at 0 h. Several authors have observed this recovery of blood glucose levels to basal levels 24 h after stressor removal (Evans *et al*., 2003; Gomes *et al*. 2003; Cnaani *et al*., 2004; Evans *et al*., 2004b, 2006, 2003).

The baseline control blood glucose levels just prior to vaccination and challenge were similar to blood glucose levels observed in Nile tilapia held at 31°C by Evans *et al*. (2004b) (41.0 -53.0 mg dL^{-1}) and at 30°C by Evans *et al*. (2006) (30-45 mg dL^{-1}). Slightly, lower blood glucose levels were reported in Nile tilapia held at 25-26°C by Evans *et al*. (2003) (26.0 -32.0 mg dL^{-1}) and by Benli and Yildiz (2004) (21.8 mg dL^{-1}). After immersion in sham anesthetic solution and vaccination, blood glucose levels in controls were slightly higher than previously reported Nile tilapia basal levels; the higher levels may be attributed to the injection procedure and these blood glucose levels were still lower than those for the MS-222-treated fish. This suggests that the MS-222 treatment elicited the significantly higher blood glucose levels above controls.

Though, this is the first study regarding MS-222 treatment and its effects on blood glucose responses in vaccinated Nile tilapia, other researchers have reported that MS-222 anesthesia can reduce stress responses in several fish species, including chinook salmon, *Oncorhynchus tshawytscha* (Strange and Schreck, 1978), hybrid striped bass, *Morone chrysops* × *M. saxatilis* (Tomasso *et al*., 1980), red drum, *Sciaenops ocellatus* (Robertson *et al*., 1988) and rainbow trout, *O. mykiss* (Morales *et al*., 1990). Nonetheless, several researchers indicated that MS-222 treatment alone may elicit significant stress responses. Robertson *et al*. (1988) and Thomas and Robertson (1991) noted MS-222 dose-related stress responses with as little as 15 min treatment. Davis and Griffin (2004) assessed the adequacy of MS-222 by exposing hybrid striped bass to MS-222 for 15 min and then to a low-water, crowding stress for 15 min. The authors observed that MS-222 treatment induced significant increases in blood glucose and cortisol and failed to decrease stress responses in fish exposed to the low water crowding stress. Schreck (1981) suggested that anesthetics can reduce stress responses by affecting stressor perception. However, Davis and Griffin (2004) found that fish in their experiment thrashed and may have perceived the low-water situation, suggesting that the MS-222 dose (25 mg L^{-1}) was too low to suppress awareness.

Several researchers have indicated that anesthetic treatment generates decreased or increased stress responses based on the administration conditions, including water quality, location, time, anesthetic dose and water quality (Strange and Schreck, 1978; Iwama *et al*., 1989; Morales *et al*., 1990; Thomas and Robertson, 1991; Welker *et al*., 2007). Morales *et al*. (1990) indicated that anesthesia may not subsequently eliminate the handling stress when fish are caught and then transferred into a container with anesthetic solution. Some authors have suggested that anesthetizing fish in their original tank before handling may reduce the stress response (Robertson *et al*., 1988; Morales *et al*., 1990; Wagner *et al*., 2003), but still other researchers have conversely observed little to no benefit from anesthetization prior to stressor exposure (Barton and Peter, 1982). Given the apparent contradictory outcomes of MS-222 treatment on stress responses, several factors should be considered when working with fish and anesthetics.

Since, the water pH changed from 7.2-6.2 with administration of the MS-222, the water acidification may be a stressor of MS-222-exposed fish. Indeed, low pH around 4.0 can be a stressor to *Oreochromis* sp. under certain conditions (Van Dijk *et al*., 1993; Lamers *et al*., 1994). Van der Salm *et al*. (2005) indicated that a pH decrease from 7.8-4.5 over 2 days was stressful to tilapia, though no significant change in blood glucose levels were noted. Other authors indicated that pH can be lowered to pH 4.0 (Van Ginneken *et al*., 1997) without eliciting stress responses in *Oreochromis* sp. The effect of the pH change in this study was not fully assessed but may have influenced the stress response of the tilapia. The MS-222 solution was not buffered because this mimics the protocols used by culture facilities and this allows comparison with other studies that did not buffer the MS-222 solution (Schoettger and Julin, 1969; Small, 2003; Welker *et al*., 2007). Furthermore, while MS-222 has been approved for use by the United States Food and Drug Administration for use in foodfish, the buffering agent sodium bicarbonate has not been approved. However, it has been designated as drugs of low regulatory priority and could possibly be used under certain conditions (United States Food and Drug Administration, 2008).

Percent cumulative survival: As observed in previous studies, percent cumulative survival data indicates that the vaccine helped fish survive after *S. agalactiae* challenge (Evans *et al*., 2004b; Pasnik *et al*., 2005).

However, MS-222 treatment helped increase post-challenge percent cumulative survival among non-vaccinated fish, but decreased percent cumulative survival in vaccinated fish. Percent cumulative survival among challenged non-vaccinated fish was higher when treated with MS-222 versus when not treated with MS-222, but percent cumulative survival among challenged vaccinated fish was significantly lower when treated with MS-222 versus when not treated with MS-222. While, MS-222 treatment historically has some beneficial effects when performing procedures that risk physical injury (i.e. handling, bleeding, transporting, etc.) (Burka *et al.*, 1997; Wedemeyer, 1997; Wagner *et al.*, 2003), MS-222 treatment appears to have a detrimental effect when vaccinating Nile tilapia. Anesthesia may trigger stress responses that modulate immuno-suppression, thereby hindering vaccine-generated immune responses and increasing disease susceptibility (Iwama *et al.*, 1989; Thomas and Robertson, 1991). Because increased blood glucose levels following MS-222 treatment but prior to vaccination were correlated with decreased percent cumulative survival after challenge, such anesthetic effects may have played a role in this study. Ortuño *et al.* (2002) indicated that the anesthetic agent itself also influences the immune system through direct interaction with the immune system or through interaction with the nervous system. Most studies indicate changes among the innate immune response activities following anesthetic treatment (Ortuño *et al.*, 2002; Bressler and Ron, 2004) and this study indicates that MS-222 treatment may be detrimental in vaccination protocols, particularly during vaccination of Nile tilapia. Though these findings contradict rationale for current commonly-used animal welfare protocols, the consequences of anesthesia in fish vaccination protocols may outweigh the utility of MS-222 in some cases.

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REFERENCES

Andersen, D.E., S.D. Reid, T.W. Moon and S.F. Perry, 1991. Metabolic effects associated with chronically elevated cortisol in rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish Aquat. Sci., 48: 1811-1817.

Barton, B.A. and R.E. Peter, 1982. Plasma cortisol stress response in fingerling rainbow trout, *Salmo gairdneri* Richardson, to various transport conditions, anaesthesia and cold shock. J. Fish Biol., 20: 39-51.

Benli, A.C.K. and H.Y. Yildiz, 2004. Blood parameters in Nile tilapia (*Oreochromis niloticus* L.) spontaneously infected with *Edwardsiella tarda*. Aquac. Res., 35: 1388-1390.

Bouck, G. and D. Johnson, 1979. Medication inhibits tolerance to sea water of coho salmon smolts. Trans. Am. Fish Soc., 108: 63-66.

Bressler, K. and B. Ron, 2004. Effect of anesthetics on stress and the innate immune system of gilthead seabream (*sparus aurata*). The Israeli J. Aquac. Bamidgeh, 56: 5-13.

Burka, J.F., K.L. Hammell, T.E. Horsberg and G.R. Johnson, 1997. Drugs in salmonid aquaculture-a review. J. Vet. Pharmacol. Ther., 20: 333-349.

Cech, J.J., S.D. Bartholow, P.S. Young and T.E. Hopkins, 1996. Striped bass exercise and handling stress in freshwater: Physiological responses to recovery environment. Trans. Am. Fish Soc., 125: 308-320.

Chen, G.R., L.T. Sun, Y.H. Lee and C.F. Chang, 1995. Characteristics of blood in common carp, *Cyprinus carpio*, exposed to low temperatures. J. Applied Aquac., 5: 21-31.

Cnaani, A., S. Tinman, Y. Avidar, M. Ron and G. Hulata, 2004. Comparative study of biochemical parameters in response to stress in *Oreochromis aureus*, *O. mossambicus* and two strains of *O. niloticus*. Aquac. Res., 35: 1434-1440.

Davis, K.B. and B.R. Griffin, 2004. Physiological responses of hybrid striped bass under sedation by several anesthetics. Aquaculture, 233: 531-548.

Diouf, B., P. Rioux, P. Blier and D. Rajotte, 2000. Use of brook char (*Salvelinus fontinalis*) physiological responses to stress as teaching exercise. Adv. Physiol. Edu., 23: 18-23.

Evans, J.J., P.H. Klesius and C.A. Shoemaker, 2004a. Efficacy of *Streptococcus agalactiae* (Group B) vaccine in tilapia (*Oreochromis niloticus*) by intraperitoneal and bath immersion administration. Vaccine, 22: 3769-3773.

Evans, J.J., P.H. Klesius, C.A. Shoemaker and B.T. Fitzpatrick, 2004b. *Streptococcus agalactiae* vaccination and infection stress in Nile tilapia, *Oreochromis niloticus*. J. Applied Aquac., 16: 105-115.

Evans, J.J., D.J. Pasnik, G.C. Brill and P.H. Klesius, 2006. Un-ionized ammonia exposure in Nile tilapia: Toxicity, stress response and susceptibility to *Streptococcus agalactiae*. N. Am. J. Aquac., 68: 23-33.

Evans, J.J., C.A. Shoemaker and P.H. Klesius, 2003. Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to *Streptococcus agalactiae* in Nile tilapia, *Oreochromis niloticus*. J. Aquat. Anim. Health, 15: 202-208.

Gomes, L.C., C.A.R.M. Araujo-Lima, R. Roubach, A.R. Chippari-Gomes, N.P. Lopes and E.C. Urbinati, 2003. Effect of fish density during transportation on stress and mortality of juvenile tambaqui *Colossoma macropomum*. J. World Aquac. Soc., 34: 76-84.

Iwama, G.K., J.C. McGeer and M.P. Pawluk, 1989. The effects of five fish anesthetics on acid-base balance, hematocrit, blood gases, cortisol and adrenaline in rainbow trout. Can. J. Zool., 67: 2065-2073.

Lamers, A.E., G. Flik and S.E. Wendelaar Bonga, 1994. A specific role for TRH in release of diacetyl alpha-MSH in *tilapia* stressed by acid water. Am. J. Physiol., 267: R1302-R1307.

Larsen, L.O., 1976. Blood glucose levels in intact and hypophysectomized river lampreys (*Lampetra fluviatilis* L.) treated with insulin, stress, or glucose, before and during the period of sexual maturation. Gen. Comp. Endocrinol., 29: 1-13.

Morales, A.E., L. García-Rejón and M. De la Higuera, 1990. Influence of handling and/or anaesthesia on stress response in rainbow trout. Effects on liver primary metabolism. Comp. Biochem. Physiol., 95A: 87-93.

Ortuño, J., M.A. Esteban and J. Meseguer, 2001. Effects of short-term crowding stress on gilthead seabream (*Sparus aurata* L.) innate immune response. Fish Shellfish Immunol., 11: 187-197.

Ortuño, J., M.A. Esteban and J. Meseguer, 2002. Effects of four anesthetics on the innate immune response of gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol., 12: 49-59.

Pasnik, D.J., J.J. Evans, V.S. Panangala, P.H. Klesius, R.A. Shelby and C.A. Shoemaker, 2005. Antigenicity of *Streptococcus agalactiae* extracellular products and vaccine efficacy. J. Fish Dis., 28: 205-212.

Reubush, K.J. and A.G. Heath, 1996. Metabolic responses to acute handling by fingerling inland and anadromous striped bass. J. Fish Biol., 49: 830-841.

Robertson, L., P. Thomas and C.R. Arnold, 1988. Plasma cortisol and secondary stress responses of cultured red drum (*Sciaenops ocellatus*) to several transportation procedures. Aquaculture, 68: 115-130.

Schoettger, R.A. and A.M. Julin, 1969. Efficacy of quinaldine as an anesthetic for seven species of fish. Investigations in Fish Control, U.S. Department of Interior, 22: 3-10.

Schreck, C.B., 1981. Stress and Compensation in Telesotean Fishes: Response to Social and Physical Factors. In: Pickering, A.D. (Ed.). Stress and Fish. Academic Press, London, pp: 295-321.

Small, B.C., 2003. Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish *Ictalurus punctatus*. Aquaculture, 218: 177-185.

Strange, R.J. and C.B. Schreck, 1978. Anesthetic and handling stress on survival and cortisol concentration in yearling chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Board of Canada, 35: 345-349.

Summerfelt, R.C. and L.S. Smith, 1990. Anaesthesia, Surgery and Related Techniques. In: Schreck, C.B. and P.B. Moyle (Eds.) Methods for Fish Biology. Am. Fish. Soc. Bethesda, MD., pp: 213-272.

Thomas, P. and L. Robertson, 1991. Plasma cortisol and glucose stress responses of red drum (*Sciaenops ocellatus*) in handling and shallow water stressors and anesthesia with MS 222, quinaldine sulfate and metomidate. Aquaculture, 96: 68-86.

Tomasso, J.R., K.B. Davis and N.C. Parker, 1980. Plasma corticosteroid and electrolyte dynamics of hybrid striped bass (white bass × striped bass) during netting and hauling. Proc. World Aquac. Soc., 11: 303-310.

United States Food and Drug Administration, 2008. www.fda.gov.

Van der Salm, A.L., F.A.T. Spanings, R. Gresnigt, S.E. Wendelaar Bonga and G. Flik, 2005. Background adaptation and water acidification affect pigmentation and stress physiology of tilapia, *Oreochromis mossambicus*. Gen. Comp. Endocrinol., 144: 51-59.

Van Dijk, P.L.M., G.E.E.J.M. Van den Thillart and S.E. Wendelaar Bonga, 1993. The influence of gradual water acidification on the oxygen consumption pattern of fish. Comp. Biochem. Physiol., 105C: 421-427.

Van Ginneken, V.J.T., R. Van Eersel, P. Balm, M. Nieveen and G. Van den Thillart, 1997. Tilapia are able to withstand long-term exposure to low environmental pH, judged by their energy status, ionic balance and plasma cortisol. J. Fish. Biol., 51: 795-806.

Wagner, G.N., T.D. Singer and R.S. McKinley, 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac. Res., 34: 1139-1146.

Wedemeyer, G.A., 1997. Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In: Iwama, G.K., A.D. Pickering, J.P. Sumpter and C.B. Schreck (Eds.). Fish Stress and Health in Aquaculture. Cambridge University Press, Cambridge, pp: 35-72.

Welker, T.L., C. Lim, M. Yildirim-Aksoy and P.H. Klesius, 2007. Effect of buffered and unbuffered tricaine methanesulfonate (MS-22) at different concentrations on the stress responses of channel catfish, *Ictalurus punctatus* rafinesque. J. Applied Aquac. (in Press).