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WATER ADDITIVES IN MITIGATING TRANSPORTATION STRESS IN FISH: A MINI REVIEW

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ABSTRACT : Transportation of live fish is an inevitable process of aquaculture, whether it may be for the trading of ornamental fishes or for availing seeds in grow-out culture ponds. Netting, grading, handling, water movement and variation in water quality parameters cause severe stress to the fishes during transportation, which ultimately leads to physiological disturbance and mortality in the transported fishes. For maintaining optimum physiological balance during transportation of commercially important fish species, the use of differenttypes of water additives is being promoted and being available in the market. Therefore, a holistic review on the application of different types of water additives is required in order to explore their effect during fish transportation.

Key words : Fish transportation, stress, water additive, water quality.

INTRODUCTION

The aquaculture sector is a flourishing sector of the food production industry, which is capable enough to supplement the animal protein and nutritional demand of the ever-increasing population. With the static growth rate of capture fisheries, the aquaculture sector is predicted to fulfill the demand of fishes in the consumer market in the future (Bhujel, 2012). However, several stressful practices associated with aquaculture such as transportation, handling, confinement poor water quality are threatening the economic growth of this sector by causing huge mortality of fishes (Barton, 2002; Zahl *et al*, 2012). Depending on the species, duration, and mode of transportation, the degree of stress exposed to the fishes varies. In some cases, transportation stress can cause as high as 80% mortality in fishes (Rubec and Cruz, 2005). Further, it was observed that the mortality rate was increased considerably during the recovery period after transportation (Sadovy, 2002).

Stress is a phenomenon where stressors result in disturbance to the homeostasis of fishes (Portz *et al*, 2006). Release of the stress hormone cortisol, occurrence of hyperglycemia and decrease in body ions such as sodium, potassium, and chloride are the major physiological response which indicates the onset of stress by the teleost fishes (Barton, 2002). Keeping because of these in the present scenario, the focus of the researchers needs to be shifted to mitigate the transportation stress in aquaculture species. Various types of products are being used in transport water to decrease the stress response and to increase the survival of the transported fishes. This review has focused on providing a holistic view of the use of the various range of water additive chemicals in transport water for different fish species so that a clear depiction of availability and efficacy of different additive compounds can be provided to the readers.

Synthetic anesthetics

Synthetic anesthetics are one of the most conventionally applied water additives in the transportation of fish (Harmon, 2009). Anesthetics depress the nervous system of the transported fishes causing immobilization and loss of sensation to the fishes (Coyle *et al*, 2004; Mirzargar *et al*, 2011). Thus, these sedatives lower the metabolic rate of fishes and improving water quality conditions in transport water (Pattanasiri *et al*, 2017), ultimately leading to decreased stress response and higher survival (Mirzargar *et al*, 2011). Several different types of anesthetics are used for various purposes in the aquaculture sector. However, the vital criteria for selecting an ideal anesthetic for any kind of aquaculture activity generally depends on the efficacy (rapid induction and smooth recovery process), availability, ease for application, cost and toxicity to animals (Marking and

Meyer, 1985; Zahl *et al*, 2012). However, the cost, availability of any sedative will vary from region to region and the efficacy will vary from species to species. Several reports are available on the use of various synthetic anesthetic in the transportation of different species of fishes.

One of the most commonly recommended anesthetics used for transportation of fish is MS-222 (Ross and Ross, 2009). The active base compound of MS-222 which is responsible for causing sedation in fishes is ethyl 3-aminobenzoate (Husen and Sharma, 2014). MS-222 in a concentration of 10-15 mg per liter of water was reported to be efficient for mitigating stress during transportation of *Siganus rivulatus* (Ghanawi, 2013). This was also reported to potent enough to diminish the handling stress of channel catfish (*Ictalurus punctatus,* Rafinesque) at the concentration of 90 mg L^{-1} (Welker *et al*, 2007).

Quinaldine is another popularly used anesthetic, which is comparatively lower in price and shorter recovery periods (Sills and Allen, 1971; Lambert, 1982). Quinaldine was reported to induce sedation effect in tilapia (*Sarotherodon melanotheron*) in the concentration of 25 mg L^{-1} (Sado, 1985). In the case of sea bream fingerlings (*Sparus sarba*), quinaldine was found to induce quicker lethargy at the concentration of 9 ml L^{-1} in comparison to other sedatives such as quinate, 2-phenoxy ethanol and MS-222 (Hseu *et al*, 1998).

Benzocaine is another group of synthetic sedative, which is structurally and functionally similar to MS-222. However, its solubility in water is very less, and it's preferably dissolved in organic solvents like ethanol and acetone primarily before application. This nontoxic form of sedative is also cost-effective than other synthetic sedatives (Coyle *et al*, 2004; Ross and Ross, 2009). It has been reported that benzocaine at the concentration of 5-10 mg.L^{-1} effectively reduced the handling and transportation stress of marbled spinefoot (*S. rivulatus*) (Ghanawi *et al*, 2013). In the case of *M. estor,* the potential benzocaine dose for induction of sedation was 15 mg L^{-1} and 18 mg L^{-1} (Ross *et al*, 2007). Also, the use of benzocaine at the rate of 30 mg L^{-1} led to a decrease in serum glucose, cortisol concentration, and mortality of transported *Labeo rohita* fingerlings (Hasan and Bart, 2007).

Metomidate hydrochloride (Aquacalm) is another potential sedative, which has been already added to the Food and Drug administration's index of legally marketed unapproved New Animal Drugs for Minor Species (FDA, 2009). FDA approved drug MS-222 is reported to cause elevation of cortisol level due to its antecedent excitatory response (Davis *et al*, 1982). However, this result can be avoided by the addition of metomidate hydrochloride as a water additive. It has been reported that with the addition of 3.0 gm/L^{-1} metomidate hydrochloride, the inhibition in upregulation of plasma cortisol level was achieved along with no adverse effect was in the behavior of transported koi carps (Crosby *et al*, 2010). In Hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) metomidate observed to suppress the cortisol response and hyperglycemia during transportation (Davis and Griffin, 2004).

It has been demonstrated that although these synthetic anesthetics decrease the stress response, they initially induce the stress response leading to a delayed stress reduction effect (Ims, 2011). Therefore, as an alternative to this several natural anesthetic compounds are being used.

Natural anesthetics

Natural anesthetics are generally the plant extracts and essential oils that are effective in the sedation of fishes during transportation. There are many kinds of natural anesthetic compounds extracted from various diversified species of plants. These substances are reported to have antioxidant properties and stress alleviating effect (Hoseini and Ghelichpour, 2013). Because of the cost-effectiveness and health benefits, herbal anesthetic could be considered as the pertinent alternatives of synthetic drugs (Hoseini *et al*, 2019).

Clove oil can be considered as one of the most sought-after plant oils to be used as a sedative during transportation. Clove oil is extracted from the species of *Eugenia aromaticum* or *Eugenia caryophyllata* (Ross and Ross, 2009). Being natural it doesn't possess any kind of ecological risk and is effective at very low concentrations. At the concentration of 15 mg L^{-1} clove oil found to reduce the transportation stress of freshwater prawn (*Macrobrachium rosenbergii*) (Vartak and Singh, 2006). Further, within the concentration of 5 to 9 mg L^{-1} clove oil was potential to ameliorate the stress of sub-adult largemouth bass (Cooke *et al*, 2004). However, during transportation of *lophiosilurus alexandri*, clove oil was found to increase the plasma glucose level and decrease hematocrit count, thus clove oil was considered to be inefficient in reducing the physiological stress of this species (Favero *et al*, 2019).

The addition of essential oil of *Lippia alba* in the transportation of juvenile silver catfish (*Rhamdia quelen*) has shown to improve the redox state. By increasing the activity of SOD (the enzyme responsible for breaking down the first reactive oxygen species), it acclimatizes the fish to adapt to oxidative stress. The addition of *Lippia alba* at the concentration of 10 ìL L^{-1} has proved to be beneficial during hypoxia and hyperoxia conditions of transportation (Azambuza *et al*, 2011).

Another natural sedative is the essential oil of *Aloysia triphylla*, which found to reduce the ionic imbalance in the grey strain of silver catfish (*Rhamdia quelen*) during transportation. Within the concentration of 100-800 µ/L of water, this anesthetic was the potential to reduce the physiological stress of both albino and grey silver catfish (Parodi *et al*, 2014). Besides the use of anesthetics, several other water additives are being used during the transportation of fishes for several purposes such as zeolite for water quality control, sodium chloride for osmoregulation maintenance, probiotics for immunity modulation, etc.

Zeolite

Transportation leads to a change in water quality such as ammonia accumulation, a decrease in pH, high CO_2 build-up and a lower concentration of DO. All these altered water quality parameters lead to the disturbance of fish physiology and leading to the mortality of transported fishes. Ammonia builds up in transport water decreases the blood oxygen-carrying capacity and thus leads to stress and exhaustion of transported fishes.

Zeolite is the crystalline form of sodium or calcium aluminum silicate. The mechanism of action of zeolite is to exchange the sodium ion for ammonium ions and thereby it completes the selective absorption of ammonia (Singh *et al*, 2004). Thus, there will be less effective on the physiology of the stressed fishes due to the decreased ammonia concentration in transport water. It has been demonstrated that the application of zeolite in the range of 7 g L^{-1} to 28 g L^{-1} decreased the ammonia concentration in water during the 48-hour transportation of catla, rohu, and mrigal. Further, 7g L^{-1} of zeolite was found to be the best dose as water additive resulting in 100% survivability of all the experimental fish (Singh *et al*, 2004). The addition of 7g L^{-1} of clinoptilolite reported decreasing the ammonia concentration during rainbow trout fingerling transportation (Oz *et al*, 2010). During the live transportation of goldfish, the addition of clinoptilolite at the concentration of 10 g L^{-1}, 20 g L^{-1}, and 40 g L^{-1} found to decrease the ammonia load by 73, 87 and 93% respectively (Bower and Turner, 1982). On the contrary, the addition of zeolite has not been proved to be beneficial during the transportation of a few fishes. For example, during the transportation of *A. triradiatus,* the addition of zeolite did not seem to reduce the stress and mortality rate (Ramirez-Duarte *et al*, 2013).

Sodium chloride

During the transportation of fishes, the accumulation of ammonia in the aquatic environment leads to the passive ion loss and water influx into the body, ultimately leading to the occurrence of hydromineral imbalance (Bonga, 1997). Thus, to maintain the hydromineral balance, fish increases its body metabolic rate during transportation leading to ultimate exhaustion and death. It has been suggested that to reduce the energy cost for maintaining osmotic regulation during stress, the osmotic gradient difference between the body fluid and external medium should be reduced by keeping the fishes in the isosmotic environment (Redding and schreck, 1983). The addition of sodium chloride in the ambient water increases the ionic strength of water thereby, decreases the osmotic gradient difference among fish blood and ambient water (Harmon, 2009). This leads to lower osmoregulatory disturbance and a reduction in stress during transportation (McDonald and Milligan, 1997). During six-hour transportation of *L. victorianus,* use of 1 to 8 psu saline water found to decrease the loss of sodium chloride ion from the fish body and among them 4psu salinity was found to be the best mode for transportation due elicitation of least stress response (Oyoo-okoth *et al*, 2011). Similarly, 0.6% sodium chloride led to a reduction in the blood glucose, cortisol hormone, and plasma ammonia concentration along with decreased ionic loss during the transportation of Gunther, *Brycon cephalus* (Carneiro and Urbinati, 2001). Thus, sodium chloride can be used as a potential water additive to decrease the transportation stress yet, the efficacy of this compound in various other species needs to be verified. Biswal *et al* (2020a) reported that the combinatorial dose of 1g.L^{-1} glucose and 4 g.L^{-1} NaCl could curtail the stress response and highest cumulative survival in transported rohu fingerlings. They also reported that a significantly ($p<0.05$) lower stress response and water quality values were observed, indicating the potency of water additives in ameliorating the transportation stress, and in turn increases the survival rate of the IMC, *Labeo rohita* fingerlings after the transportation (Biswal *et al*, 2020b).

Probiotics

In addition to the aforementioned products, several probiotics are also being used during the transportation of fishes. Efinol® L (Manufacture by Bentoli, Inc., USA) is a commercial probiotic which is a combination of beneficial microorganisms and selected nutrients generally applied in reptile, fish, and shrimp hatcheries. Addition of commercial probiotic Efinol® L at a rate of 10 ppm in *Catla calta* fry tank before two hours of catching and transportation has proven to be beneficial for increasing the survival. Both additions of probiotic before and after transportation have led to increased survival and especially the addition of probiotic after transportation causes increased growth rate of fry during the post transformational period (Raj *et al*, 2008).

In another experiment, the treatment of *Saccharomyces cerevisiae* (5.6 × 108 cfu.g^{-1}) and Bacillus spp. (1 × 106 cfu.g^{-1}) as probiotics were applied in culture water for 120 days before the transportation of Nile tilapia. The transportation was then carried out for four hours and it was observed that the addition of probiotics showed improved survival, biochemical profile, hematological profile and expression of immune-related genes in comparison to the non-additive added control group. However, the addition of Bacillus as a probiotic was found to be the most beneficial between the aforementioned two treatments (Sutthi and Van Doan, 2020).

In addition to different probiotics, the use of several plants extracts having immunomodulatory properties are being used for transporting fishes. Aloe vera (*Aloe barbadensis*) is the plant possessing certain gel polysaccharides, oligosaccharides especially mannans. The extract of *Aloe vera* has been applied in transport water of matrixana and it was concluded that the respiratory burst activity was increasing in a dose-dependent manner and the highest activity was observed in fish transported in water containing aloe vera extract at 2 mg.L^{-1} (Zanuzzo *et al*, 2012). However, the presence of research in the area of herbal supplements is quite scarce and it needs to be addressed as a future research prospect.

CONCLUSION

Transportation of fishes is a common aquaculture practice which is causes severe physiological disturbance and stress due to the altered water quality parameter of transport water. To maintain the optimum physiological balance during transportation, there is a growing market establishing for the production of compounds causing stress mitigation. Several anesthetic drugs such as MS-222, benzocaine, quinaldine has found to be efficient in maintaining proper water quality during transportation so that decreased metabolic stress and the lower mortality rate has been achieved in transported fishes. The addition of zeolite has led to the achievement of proper water quality and salt has led to a decrease in the ionic imbalance in fish body. Certain probiotics have shown their potency for generating immunomodulatory response whereas, studies on the proper use of the herbal supplement in the transportation of fish are still scarce. Therefore, to improve the welfare of traded and transported fishes, many more studies on these emerging fields need to be conducted in the future.

REFERENCES

Azambuja C R, Mattiazzi J, Riffel A P K, Finamor I A, de Oliveira Garcia L, Heldwein C G and Llesuy S F (2011) Effect of the essential oil of *Lippia alba* on oxidative stress parameters in silver catfish (*Rhamdia quelen*) subjected to transport. *Aquaculture* **319**, 156-161.

Barton B A (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr. Comp. Biol*. **42**, 517-525.

Bhujel R C (2012) Increasing role and potential of Small-Scale Aquaculture: National, regional and global perspectives. In: *Proceedings of the National Symposium on Small-scale Aquaculture for Increasing Resilience of Rural Livelihoods in Nepal (Eds. MK Shrestha and J. Pant). Institute of Agriculture and Animal Science*, Tribhuvan University, Rampur, Chitwan, Nepal, and The World Fish Center, Penang, Malaysia, 191.

Biswal A, Srivastava P P, Jayant M, Varghese T and Gupta S (2020a) Optimization of transportation condition of *Labeo rohita* fingerling using potential water additives: Based on primary and secondary stress responses and survival. *J. Exp. Zool. India* **23**, 1219-1226.

Biswal A, Srivastava P P, Gupta S, Jayant M and Varghese T (2020b) Study on alteration of critical water quality parameters and selected metabolic response of Labeo rohita fingerling subjected to transportation stress. *J. Anim. Res.* (In Press).

Bower C E and Turner D T (1982) Ammonia removal by clinoptilolite in the transport of ornamental freshwater fishes. *Progress Fish Cult*. **44**, 19-23.

Carneiro P C F and Urbinati E C (2001) Salt as a stress response mitigator of matrinxã, *Brycon cephalus* (Günther), during transport. *Aquac. Res.* **32**, 297-304.

Cooke S J, Suski C D, Ostrand K G, Tufts B L and Wahl D H (2004) Behavioral and physiological assessment of low concentrations of clove oil anaesthetic for handling and transporting large mouth bass (*Micropterus salmoides*). *Aquaculture* **239**, 509-529.

Coyle S D, Durborow R M and Tidwell J H (2004) *Anesthetics in aquaculture* (No. 3900). Texas: Southern Regional Aquaculture Center.

Crosby T C, Petty B D, Hamlin H J, Guillette Jr L J, Hill J E, Hartman K H and Yanong R P (2010) Plasma cortisol, blood glucose, and marketability of koi transported with metomidate hydrochloride. *N Am J Aquacult*. **72**, 141-149.

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

Davis K B, Parker N C and Suttle M A (1982) Plasma corticosteroids and chlorides in striped bass exposed to tricaine methanesulfonate, quinaldine, etomidate and salt. *Progress Fish Cult*. **44**, 205-207.

Favero G C, Silva W D S, Boaventura T P, Leme F D O P and Luz R K (2019) Eugenol or salt to mitigate stress during the transport of juvenile *Lophiosilurus alexandri*, a Neotropical carnivorous freshwater catfish. *Aquaculture* **512**, 734321.

FDA (2009) The index of legally marketed unapproved new animal drugs for minor species. FDA, Rockville, Maryland. http://www.fda.gov/

Ghanawi J, Monzer S and Saoud I P (2013) Anaesthetic efficacy of clove oil, benzocaine, 2 phenoxyethanol and tricaine methanesulfonate in juvenile marbled spinefoot (*Siganus rivulatus*). *Aquac. Res.* **44**, 359-366.

Harmon T S (2009) Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Rev. Aquacult*. **1**, 58-66.

Hasan M and Bart A N (2007) Improved survival of rohu, *Labeo rohita* (Hamilton : Buchanan) and silver carp, *Hypophthalmichthys molitrix* (Valenciennes) fingerlings using low dose quinaldine and benzocaine during transport. *Aquac. Res.* **38**, 50-58.

Hoseini S M and Ghelichpour M (2013) The effects of different eugenol concentration and anesthesia time on blood biochemical parameters in goldfish (*Carassius auratus*). *J. Appl. Ichthyol. Res*. **1**, 29-40.

Hoseini S M, TaheriMirghaed A and Yousefi M (2019). Application of herbal anaesthetics in aquaculture. *Rev. Aquacult*. **11**(3), 550-564.

Hseu J R, Yeh S L, Chu Y T and Ting Y Y (1998) Comparison of efficacy of five anesthetics in goldlined sea bream, *Sparus sarba*. *Acat Zool. Taiwan* **9**, 35-41.

Husen A and Sharma S (2014) Efficacy of anaesthetics for reducing stress in fish during aquaculture practices-a review. *Kathmandu Univ. J. Sci. Eng. Tech*. **10**, 104-123.

Ims S (2011) The Efficacy and Stress-Reducing Capacity of MS-222, Benzoak and Aqui-S for the Ornamental Cichlid Fish, Metriaclimaestherae (*Master's thesis*, Institute for Biology).

Lambert T C (1982) Techniques for the capture and handling of Atlantic mackerel with special reference to the use of quinaldine. *Progress Fish Cult*. **44**, 145-147.

Marking L L and Meyer F P (1985) Are better anaesthetics needed in fisheries? *Fisheries* **10**, 2-5.

McDonald G and Milligan L (1997) Ionic, osmotic and acid-base regulation in stress. *Fish stress and Health in Aquaculture* **62**, 119-145.

Mirzargar S S, Soltani M, Ahmadi M, Abrishamifar A, Bahonar A and Yousefi P (2011) Anesthetic effect of tricaine methanesulfonate, clove oil and electroanesthesia on lysozyme activity of *Oncorhynchus mykiss*. *Iran. J. Fish. Sci.* **10**, 393-402.

Oyoo-Okoth E, Cherop L, Ngugi C C, Chepkirui-Boit V, Manguya-Lusega D, Ani-Sabwa J and Charo-Karisa H (2011) Survival and physiological response of *Labeo victorianus* (Pisces: Cyprinidae, Boulenger 1901) juveniles to transport stress under a salinity gradient. *Aquaculture* **319**, 226-231.

Öz M, ʻahÝn D and Aral O (2010) Using of the natural zeolite clinoptilolite in transportation of fingerling trout (*Oncorynchus mykiss*, W., 1792). *J. Fish Sci*. **4**, 264-268.

Parodi T V, Cunha M A, Becker A G, Zeppenfeld C C, Martins D I, Koakoski G and Baldisserotto B (2014) Anesthetic activity of the essential oil of *Aloysia triphylla* and effectiveness in reducing stress during transport of albino and gray strains of silver catfish, *Rhamdia quelen*. *Fish Physiol. Biochem*. **40**, 323-334.

Pattanasiri T, Taparhudee W and Suppakul P (2017) Anaesthetic efficacy of clove oil-coated LDPE bag on improving water quality and survival in the Siamese fighting fish, *Bettas plendens*, during transportation. *Aquac Int*. **25**, 197-209.

Portz D E, Woodley C M and Cech J J (2006). Stress-associated impacts of short-term holding on fishes. *Rev Fish Biol. Fisher*. **16**, 125-170.

Raj A J A (2008) Probiotic Performance on Fish Fry during Packing, Transportation Stress and Post-transportation Condition. *J. Fish Aquat. Sci.* **3**, 152-157.

Ramírez-Duarte W F, Pineda-Quiroga C, Martínez-Rueda N and Eslava-Mocha P R (2013) Evaluation of the use of sodium chloride, eugenol and zeolite in confinement of *Ancistrus triradiatus*. *Orinoquia* **17**(1), 84-95.

Redding J M and Schreck C B (1983) Influence of ambient salinity on osmoregulation and cortisol concentration in yearling coho salmon during stress. *Trans. Am. Fish. Soc.* **112**(6), 800-807.

Ross L G and Ross B (2009). *Anaesthetic and sedative techniques for aquatic animals*. John Wiley & Sons.

Ross L G, Sanchez Blanco J, Martínez Palacios C, Racotta I S and Toledo Cuevas M (2007) Anaesthesia, sedation and transportation of juvenile *Menidiaestor* (Jordan) using benzocaine and hypothermia. *Aquac. Res.* **38**, 909-917.

Rubec P J and Cruz F P (2005). Monitoring the chain of custody to reduce delayed mortality of net-caught fish in the aquarium trade. *SPC live reef fish Information Bulletin* **13**, 13-23.

Sado E K (1985) Influence of the anaesthetic quinaldine on some tilapias. *Aquaculture* **46**(1), 55-62.

Sadovy Y (2002) Death in the live reef fish trades. *SPC Live Reef Fish Information Bulletin* **10**, 3-5.

Sills J B and Allen J L (1971). The influence of pH on the efficacy and residues of quinaldine. *Trans. Am. Fish. Soc.* **100**(3), 544-545.

Singh R K, Vartak V R, Balange A K and Ghughuskar M M (2004) Water quality management during transportation of fry of Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton). *Aquaculture* **235**, 297-302.

Sutthi N and Van Doan H (2020). *Saccharomyces crevices* and *Bacillus* spp. effectively enhance health tolerance of Nile tilapia under transportation stress. *Aquaculture* 735527.

Vartak V and Singh R K (2006). Anaesthetic effects of clove oil during handling and transportation of the freshwater prawn, *Macrobrachium rosenbergii* (de man). *Isr. J. Aquacult-Bamid,* **58**, 46-54.

Welker T L, Lim C, Yildirim-Aksoy M and Klesius P H (2007) Effect of buffered and unbuffered tricaine methanesulfonate (MS-222) at different concentrations on the stress responses of channel catfish, *Ictalurus punctatus*. *J. Appl. Aquac.* **19**, 1-18.

Wendelaar Bonga S E (1997) The stress response in fish. *Physiol. Rev*. **77**, 591-625.

Zahl I H, Samuelsen O and Kiessling A (2012) Anaesthesia of farmed fish: implications for welfare. *Fish Physiol. Biochem.* **38**(1), 201-218.

Zanuzzo F S, Biller-Takahashi J D and Urbinati E C (2012) Effect of Aloe vera extract on the improvement of the respiratory activity of leukocytes of matrinxa during the transport stress. *RBZ* **41**, 2.