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Short Communication

Effect of *Aloe vera* extract on the improvement of the respiratory activity of leukocytes of matrinxã during the transport stress

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ABSTRACT - This study evaluated the effect of extract of *Aloe vera* in the transport water of matrinxã (*Brycon amazonicus*) fish on stress response and leukocyte respiratory activity. Fish was transported for 4 h in water containing Aloe at levels 0; 0.02; 0.2 and 2 mg/L, and sampled before transport 2, 4, 24 and 96 h after for determination of plasma glucose and respiratory activity of leukocytes. An additional *in vitro* assay was conducted with another fish species, pacu (*Piaractus mesopotamicus*), to test the respiratory burst of leukocytes exposed to Aloe extract (0.0, phosphate-buffered saline (PBS) only) at 0.1, 0.2, 0.5 and 1 mg/L). Plasma glucose increased after 2 and 4 h of transport and returned to control levels within 24 h, but the addition of Aloe in the transport water did not affect the level of blood glucose. However, at 2 h of transport, Aloe enhanced the respiratory activity of leukocytes in a dose-dependent way. The highest value of respiratory burst activity of leukocytes was observed in the fish transported in water containing Aloe at 2 mg/L. The enhancing effect of the plant extract on the production of oxygen radicals was confirmed *in vitro* in leukocytes of pacu incubated in Aloe at concentrations 0.1 and 0.2 mg/L. The results suggest that *Aloe vera* is a modulator of the immune system in fish improving the innate immune response tested.

Key Words: *Brycon amazonicus,* innate immune system, oxidative burst, white blood cells

Introduction

Transportation is among the farming stressors that compromise the ability of fish to perform essential life functions after their release in the destiny (Schreck et al., 1995; Wendelaar Bonga, 1997). Stress alters the immune function and increases susceptibility to diseases in fish (Maule et al., 1989; Jeney et al., 1997; Ramsay et al., 2009). Many stressors (including acute or chronic handling, crowding, transport, confinement, or a subordinate social position) in general cause a rapid increase in circulating neutrophils and reduction in the quantity of circulating lymphocyte (Wendelaar Bonga, 1997). Attempts to improve survival of transported fish have focused either on the physical systems *per se*, such as water quality (Pavilidis et al., 2003; Moran et al., 2008) or on variables associated with the fish themselves, such as density (Pavilidis et al., 2003; Urbinati et al., 2004) or through the use of water additives that can enhance metabolic and osmoregulatory processes such as salt (Carneiro & Urbinati, 2001), calcium (Mazik et al., 1991) and anesthetics (Ross & Ross, 2008). Studies have been conducted to investigate the use of immunostimulant substances to reduce the physiological stress in farmed fish, such as feeding high levels of vitamin C (Jaffa, 1989; Li et al., 1998) or using levamisole (Siwicki, 1989). In addition, feeding glucan at low doses several weeks before transportation of rainbow trout helped to prevent negative effects of stress (Jeney et al., 1997). High doses of vitamin C elevated the non-specific defense mechanisms of channel catfish (Li & Lovell, 1985; Hardie et al., 1991). Modulation of the immune response using medicinal plant products as a therapeutic measure has become the focus of extensive scientific investigation (Galina et al., 2009). A common theme running through recent research is the immunomodulatory properties of the gel polysaccharides, especially the acetylated mannans from *Aloe vera* (*Aloe barbadensis*), a perennial succulent plant belonging to the Liliaceal family (Choi & Chung, 2003). However, there is little information on the use of *Aloe vera* in aquaculture (Kim et al., 1999) and no information focusing on its effects on the stress in farmed fish. In the present study, *Aloe vera* powder was added to the transport water to investigate whether the plant extract could minimize the transport stress and affect the respiratory activity of leukocytes of

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matrinxã (*Brycon amazonicus*). To strengthen the influence of *Aloe vera* on the respiratory burst of fish leukocytes, an *in vitro* assay was performed using blood of pacu (*Piaractus mesopotamicus*).

Material and Methods

The *Aloe vera* used was a commercial powder (Aloe vera Powder 200:1 spray-dried Jung consult do Brasil Produtos Naturais Ltd.) produced from the gel obtained from the Aloe leaves spray-dried. Aloe powder was dissolved in the transport water to constitute the treatments (Aloe at 0 mg/L – control; 0.02 mg/L; 0.2 mg/L; and 2 mg/L), which had six replicates each one. For the matrinxã transportation, a total of 144 fishes (body weight 84.7±16.3 g) were stocked in four 1000 L tanks with constant water flow and aeration for adaptation. Fish were then transferred to 20 L plastic bags with 5.0 L water and 15.0 L of pure oxygen at a density of 200 g/L. Fish were sampled before transportation (initial; n=8), 2 hours after starting transportation (n=10), 4 hours (arrival; n=8), 24 h and 96 h after (recovery; n=8). No sampled fish returned to the experiment. At arrival, after fish sampling, remaining animals were placed back into the 1000 L tanks without *Aloe vera* for the subsequent monitoring.

Water was collected in all samplings to monitor the temperature (27.7±2.02 °C), pH (7.25±0.54) and dissolved oxygen concentration (6.9±1.03 mg/L). Un-ionized ammonia was calculated (Emerson et al., 1975) and varied from 0.07 to 0.26 mg/L during transport. The levels were at around 0.03 mg/L during the recovery period.

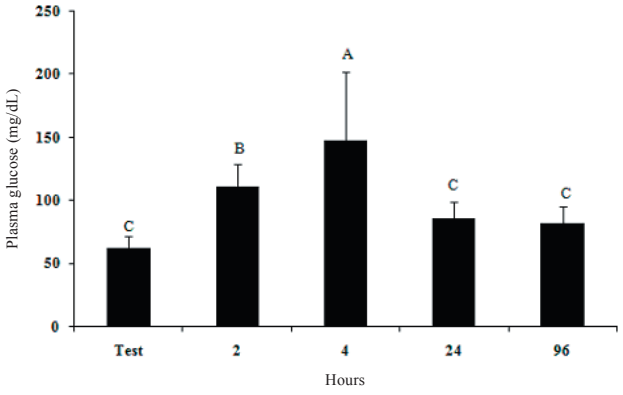
Blood was collected from anesthetized fish (benzocaine, 0.1 g/L) via puncture of the caudal vein for determination of the plasma glucose level (kit Labtest, Sao Paulo, Brazil, code 84) and the respiratory burst of leukocytes by the production of intracellular superoxide anion (O_2^-) by reduction of NBT (nitroblue tetrazolium) following assay protocol of Anderson & Siwicki (1995).

To strengthen the influence of *Aloe vera* on the respiratory burst of fish leukocytes, an *in vitro* assay was performed using blood of pacu (*Piaractus mesopotamicus*) (84.13±5.4 g). Five samples of 100 µL of total blood added to 100 µL of Aloe solutions prepared with phosphate-buffered saline (PBS, Aloe at 0.1, 0.2, 0.5 and 1 mg/L) were incubated for 1 h, at room temperature. The control solution was only PSB. The respiratory burst of leukocytes was determined following the protocol above.

The experiment of transport was conducted in a completely randomized design in a 4 × 4 factorial arrangement + initial condition to test the influence of the main treatments (four Aloe solutions and four sampling times) and the interaction between them. The results were submitted to ANOVA and means were compared using Tukey test ($P<0.05$). The data obtained from the *in vitro* assay were submitted to one-way ANOVA. Means were compared using Duncan's multiple range test ($P<0.05$). Results are presented as means ± standard deviation. All data were analyzed by SAS (Statistical Analysis System, version 9.2).

Results and Discussion

No mortality was observed in any group of fish throughout the experimental period. In the experiment herein reported, transported fish showed an increase in plasma glucose concentrations indicating the stress condition of fish; however, *Aloe vera* had no effect on such response. Plasma glucose concentrations increased in fish of all treatments within 2 hours of transport (111.7 mg/dL) peaking at 4 h (147.9 mg/dL) ($P<0.05$; Figure 1). Those levels returned to levels indistinguishable from those of the control fish within 24 and 96 h at the end of transport. The increase of glucose in fish blood helps to satisfy the increased energy demand during stress, allowing the fish to react to stressors (Wendelaar Bonga, 1997). In matrinxã, hyperglycemia has been associated with stressful conditions such as capture, transport and crowding (Rocha et al., 2004; Urbinati et al., 2004; Hoshiba et al., 2009). To the knowledge of the authors, there are no studies available on the effect of *Aloe vera* on the reduction of stress in fish that can be compared to the present research.

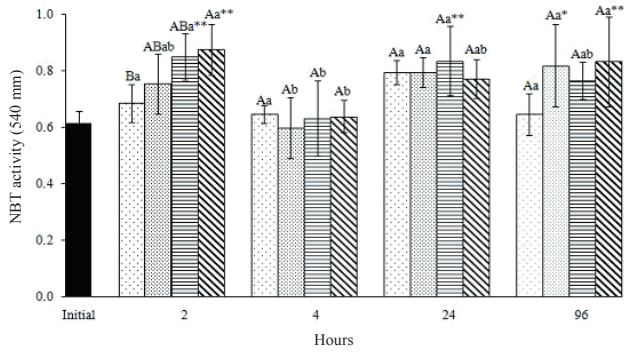


Different letters indicate differences between sampling times (all *Aloe vera* treatments are represented in the same bar).
Bars represent means of samplings.
Vertical bars represent the standard deviation.

Figure 1 - Plasma glucose of matrinxã before (initial), during (2 and 4 hours) and after (24 and 96 hours) transport in water containing *Aloe vera*.

No changes were observed in the respiratory burst activity of matrinxã during the stress response considering the control fish. However, at 2 h of transport, *Aloe vera* significantly enhanced the respiratory activity of leukocytes of matrinxã. There was a dose-dependent increase in the respiratory burst of leukocytes when compared with the control fish (Figure 2). The highest values of respiratory burst of leukocytes, as measured by the production of oxygen radicals, were observed in the fish transported in water containing *Aloe vera at* 2 mg/L (0.876 OD (optical density), respectively). No changes were observed in the subsequent samplings.

Contrarily to the observations in this study, Jeney et al. (1997) verified that respiratory burst activity, phagocytosis, serum protein and lysozyme levels were significantly reduced in rainbow trout stressed by 2 hours of transport. Maule et al. (1989) examined the effects of acute stress on the immune system and disease resistance of juvenile chinook salmon (*Oncorhynchus tshawytscha*) and found that the ability of lymphocytes from the anterior kidney to generate specific antibody-producing cells (APC) *in vitro* and resistance to the fish pathogen, *Vibrio anguillarum*, were depressed 4 hours after stress regardless of the type of stressor applied: i.e., being held out of water in a dipnet for 30 s, manipulation during hatchery operations for 4 hours, or transportation for 9 hours. Recently, effect of stress and elevated cortisol was demonstrated on the morbidity and prevalence of clinical diseases associated with mycobacterial infections in zebrafish (Ramsay et al., 2009).

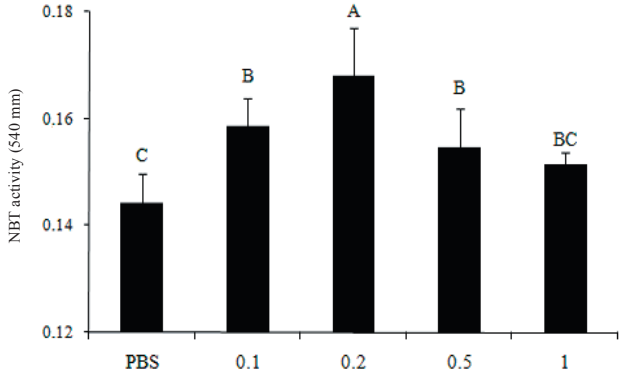


Different capital letters indicate differences between treatments within each sampling time and lowercase letters, in the different sampling times.
* and ** indicate differences (P<0.05 and P<0.01, respectively) between each treatment and initial condition.
Vertical bars represent the standard deviation.
Initial (■); control (□); 0.02 mg/L (▨); 0.2 mg/L; (▤) and 2 mg/L Aloe (▧).

Figure 2 - Respiratory activity of leukocytes of matrinxã before (initial), during (2 and 4 h) and after (24 and 96 h) transport in water containing *Aloe vera.*

The *in vitro* assay of respiratory burst of leukocytes in pacu (Figure 3) confirmed the enhancing effect of *Aloe vera* on the production of oxygen radicals verified during the transport of matrinxã. The respiratory burst increased when leukocytes were incubated in *Aloe vera* at 0.1 and 0.2 mg/L (0.158 and 0.168 OD, respectively) when compared with the control values (0.144 OD) and decreased in 0.5 and 1 mg/L (0.154 and 0.151 OD, respectively).

Except for a study showing that feeding *Aloe vera* in moderate doses can help to control *Vibrium alginolyticus* in juvenile rockfish (*Sebastes schlegeli*), no results are known about the use of *Aloe* in aquaculture. In contrast, many other immunostimulants have been investigated to reduce the physiological stress in farmed fish, such as vitamin C, levamisole, glucan, lactoferrin, chitin and lipopolysaccharide, among others (Li & Lovell, 1985; Jaffa, 1989; Siwicki, 1989; Hardie et al., 1991; Jeney et al., 1997; Li et al., 1998). The results found in this study suggest that *Aloe vera*, a medicinal plant extensively used for humans (Galina et al., 2009), is a promising natural product for aquaculture and should be more investigated as a modulator of the immune system in fish farming, since its use in matrinxã improved its non-specific immune response.



Different letters indicate differences between treatments.
Bars represent means of treatments.
Vertical bars represent the standard deviation.

Figure 3 - *In vitro* respiratory burst of leukocytes of pacu incubated in *Aloe vera* solutions (0.1; 0.2; 0.5 and 1 mg/L *Aloe vera*; phosphate-buffered saline (PBS) control).

Conclusions

The extract of *Aloe vera* improves the respiratory activity of leukocytes in transported matrinxã and seems to be a modulator of the immune system in the response of this species.

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