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Effects of Myrcene Addition to Water on Plasma Biochemical Characteristics of Common Carp, *Cyprinus carpio*, During Transportation

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Abstract

Background: Sedative agents are used to mitigate stress and improve fish welfare during and after the transportation process. **Objectives:** We aimed to test the effects of myrcene addition to the transportation water on plasma biochemical characteristics of common carp, *Cyprinus carpio*. **Methods:** For this purpose, fish (average weight of 45.3 ± 1.65 g) were transported in the plastic bags 0 (CTL), 10 (10M), 20 (20M), 30 (30M), and 50 (50M) µL/L myrcene for 6 h and plasma parameters were compared to the before transportation (BT). **Results:** Transportation significantly ($P < 0.05$) decreased plasma total protein, albumin, alternative complement, lysozyme, sodium, and chloride, and increased potassium, calcium, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in the CTL treatment, compared to BT. Addition of 50 µL/L myrcene to water prevented the decreases in plasma total protein and albumin. Addition of 20 µL/L myrcene to water prevented/mitigated the changes in plasma ions, alanine aminotransferase, and alkaline phosphatase. Addition of 10-50 µL/L myrcene to water mitigated the changes in plasma aspartate aminotransferase. Addition of 20 and 30 µL/L myrcene to water mitigated the changes in plasma alternative complement, as 30 µL/L myrcene increased plasma lysozyme activity. Transportation and myrcene addition had no significant effects on plasma immunoglobulin ($P > 0.05$), but significant increase was observed in plasma globulin in the 20M treatment ($P < 0.05$). **Conclusions:** In conclusion, the addition of 20 µL/L myrcene to the transport water of common carp reduced hepatic enzyme levels and improved immune parameters, and therefore use of myrcene improves fish welfare during transportation.

Keywords Electrolytes, fish welfare, fish transport, immune parameters, transport stress

Introduction

While the global population growth is increasing rapidly, aquaculture stands out as an important agricultural and food production sector in order to ensure the worldwide demand for fish consumption and food security. For this reason, aquaculture production and income from this sector are increasing every year. There is no doubt that the development of technology and transferring positive results in scientific studies to the aquaculture sector are effective in the realization of this increase. It is of great importance both for the fish and for the producer that the fish are exposed to a minimum level of stress during aquaculture activities. Fish are susceptible to mechanical injury and physiological stress during routine procedures and experiments in aquaculture, and undesirable conditions such as reduced growth performance and increased fish morbidity may occur. Because, the transportation process which is frequently used in fish farming is stressful for fish and may cause various injuries that lead to loss of scales and mucus, increasing the outbreak of bacterial and fungal diseases (Zeppenfeld *et al.,* 2014; Adah Sylvanus *et al*., 2022). Sedatives in transportation water may be an essential tool to get lower oxidative stress, reduce fish metabolism and improve welfare of aquatic species (Toni *et al.,* 2015; Aydın and Barbas 2020; Ventura *et al.,* 2020). For this reason, the use of sedative agents in the aquaculture sector is of great importance to producers.

Various studies have been performed in recent years on the use of sedatives for fish in aquaculture (Aydın and Barbas 2020). In part of these studies, sedatives in the transportation processes are advisable for fish transport (Boaventura *et al.,* 2020; Ventura *et al.,* 2020). However, it was reported anesthetic/sedative drugs being used in farming procedures, the drugs themselves can cause stress at various levels and cause undesirable effect on the physiology of fish (Zeppenfeld *et al.,* 2014; Aydın and Barbas 2020; Rahman *et al.,* 2020). Aydın and Barbas

(2020) stated that while some drugs have a positive effect on the blood biochemistry of fish, some drugs, especially those of synthetics, have a negative effect. Furthermore, fish transport is a complex physiological process and more information is needed on the physiological responses of fish during and after transport using sedative agents. For this reason, it is crucial to investigate the detailed analysis of the side effects of sedative substances on fish. For this purpose, some studies are carried out on different types of sedatives synthetic drugs such as 2-phenoxyethanol (Shaluei *et al.,* 2012), benzocaine (Boaventura *et al.,* 2022), natural drugs, such as *Lippia alba* essential oil (EO) (Becker *et al.,* 2012), *Aloysia triphylla* EO (Becker *et al.,* 2012), and *Ocimum basilicum* EO (Ventura *et al.,* 2020), and active compounds such as menthol (da Silva *et al.,* 2016), citral (de Freitas Souza *et al.,* 2018), and citronellal (Yousefi *et al.,* 2019).

Common carp, *Cyprinus carpio,* is an important freshwater fish widely cultured around the world with a production amount of about 4.24 million tons (FAO 2019). It is known to rapid growth, well adapted to farming conditions and fed low cost feed. To date, only a few studies have been conducted to evaluate the impacts of transport with sedative drugs on this species (Taheri Mirghaed and Ghelichpour 2019; Hoseini *et al.,* 2022; Mirzargar *et al.,* 2022).

In recent years, myrcene was investigated for fish showing that it is capable of sedative and anesthesia in fish (Taheri Mirghaed *et al.,* 2016; Taheri Mirghaed *et al.,* 2018). There are no studies reporting the biochemical characteristics of the common carp transported under sedation with myrcene. Thus, the objective of this study was to evaluate the effects of different concentrations of myrcene in transportation water on plasma biochemical characteristics, electrolytes, and immune parameters of common carp.

Material and methods

Experimental protocol

In this research, 126 pieces of carp (45.3 ± 1.65 g) were used. The fish with a density of 7 pieces were placed in eighteen 55-L aquaria. The fish were kept in these aquaria for 14 days to acclimatize and were fed twice a day with commercial carp food at a rate of 3% of biomass. Then, one fish was selected from each tank and anesthetized with eugenol at the rate of 100 mg/L, and blood was taken from the caudal vein with a heparinized syringe. Finally, 12 samples were considered as before transportation (BT) samples. Then the remaining fish were placed in plastic bags containing 2 L of water and 4 L of pure oxygen (6 individuals in each plastic).

Then the plastic bags were divided into five groups and 0 (CTL), 10 (10M), 20 (20M), 30 (30M), and 50 (50M) µL/L myrcene were added to either of bags. The plastic lids were fastened and transported for 6 hours. Right after transportation, 4 fish were caught from each plastic bag and the blood was sampled. In order to collect blood from the fish, anesthesia was performed with eugenol at the rate of 100 mg/L. Blood was collected from the caudal vein by heparin syringes. About 1 ml of blood was taken from each fish and centrifuged for plasma separation.

Plasma analysis

Sodium and potassium were measured with Flame Photometer. Calcium was measured with Pars Azmoun commercial kit by photometric method. Chloride was measured by Zischem kit accoding to the kit procedure. Plasma total protein and albumin were determined by Pars Azmoun commercial kits, based on the Biuret and bromocresol green methods, respectively (Alishahi *et al*., 2014). Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured using Pars Azmoun commercial kits, based on kinetically methods (Tulaby Dezuly *et al*., 2019).

Plasma lysozyme activity was measured at 530 nm using *Micrococcus luteus* as target. Every 0.001 decrease in sample absorbance per min was considered as one unit of lysozyme (Mohseni *et al.,* 2021a). Plasma alternative complement (ACH50) activity was measured according to hemolytic activity against sheep erythrocyte (Jami *et al.,* 2019). Plasma total immunoglobulin (Ig) was determined after precipitation with polyethylene glycol (Mohseni *et al.*, 2021b).

Statistical analysis

The normal distribution of the data was checked by the Shapiro-Wilk test. Then, the data were analyzed by one-way ANOVA test. The treatments' means were compared by Duncan's test. SPSS v.22 was used to analyze the data. Significance was determined at the level of $P>0.05$. Data were presented as mean ± standard error.

Results

Plasma total protein and albumin decreased significantly ($P < 0.001$) after transportation in the CTL, 10M, 20M and 30M treatments (Fig. 1). The greatest decrease in the plasma total protein and albumin levels was observed in the CTL, 10M, and 20M treatments. The plasma globulin after transportation remained unchanged in the CTL, 10M, 30M and 50M treatments, but increased significantly ($P < 0.001$) in the 20M treatment (Fig. 1).

Transportation caused a significant decrease ($P < 0.001$) of plasma sodium in the CTL, 10M, 20M and 30M treatments, and the greatest decrease was observed in the CTL treatment (Fig. 2). Plasma potassium increased significantly ($P = 0.006$) in the CTL and 10M treatments, but there was no significant change among the other treatments (Fig. 2). Transportation caused a significant decrease ($P < 0.001$) in the plasma chloride in all treatments, and the greatest decrease was observed in the CTL, 10M, and 30M treatments (Fig. 2). The plasma calcium increased significantly ($P < 0.001$) in the CTL, 10M, 30M and 50M treatments (Fig. 2).

The activity of ALT increased significantly ($P < 0.001$) in the treatments of the CTL, 10M, 30M and 50M treatments and the highest activity was observed in the treatments of the CTL, 30M and 50M treatments (Fig. 3). Plasma AST activity increased significantly ($P < 0.001$) in all treatments after transportation, but the highest increase was related to the CTL treatment (Fig. 3). Plasma ALP activity increased significantly ($P < 0.001$) in all treatments after transportation. The lowest increase was observed in the 20M treatment and the highest increase in the 50 M treatment (Fig. 3).

After transportation, plasma lysozyme decreased significantly ($P < 0.001$) in the CTL, 10M, 20M, and 30M treatments and increased in the 50M treatment (Fig. 4). Plasma ACH50 decreased significantly ($P < 0.001$) in all treatments after transportation, and the lowest values were observed in the CTL and 50M treatments, while the 30M treatment had the highest activity (Fig. 4). Transportation did not have a significant effect on the plasma total Ig levels (Fig. 4).

Discussion

The positive or negative effects of sedatives used in the transport water are not fully known in fish and aquaculture. It has been indicated that anesthetic/sedative type, concentration and exposure time affect immune and stress-related responses in fish (Cao *et al.*, 2019), characterized by changes in plasma biochemical (Ventura *et al.*, 2020; Ventura *et al.*, 2021), and gene expression (Cao *et al.*, 2019; Zapata-Guerra *et al.*, 2020) findings. Therefore, in this study, we examined the plasma biochemical characteristics of the common carp transported with different myrcene concentrations for 6-h medium-distance transportation. Blood protein reflects fish health, mainly liver, and the non-specific immunity, and our results suggest that myrcene in transportation water has positive impacts on health condition of the common carp. In accordance with the current findings, eugenol and *Ocimum basilicum* EO did not affect blood protein, albumin and globulin levels of Nile tilapia transported for 2h (Ventura *et al.*, 2020). Similarly, no significant changes were observed in total protein levels of fish transferred with 5-10 mg/L eugenol and benzocaine (Boaventura *et al.*, 2022). It should be noted that research findings between studies can vary due to the fish, sedative concentration, water temperature, and transport time (Bowker *et al.,* 2015). For example, long-duration transportation process has been indicated to suppress the immune system, and increases fish morbidity or mortality (Gomes *et al.,* 2003).

Plasma electrolytes are important for the normal physiological function of fish. During stress, freshwater fish exhibit chloride loss due to increased ventilation from the gills (Barton *et al.,* 2003; Mirzargar *et al.,* 2022). In this study, chloride levels in the transport treatments were decreased significantly compared to BT treatment. This finding has also been reported in different fish such as *Stizostedion vitreum* (Barton *et al.,* 2003), *Labeo rohita* (Biswal *et al.,* 2021). The 20M treatment chloride level were found less influenced by transportation, compared to the other treatments in the present study. Myrcene prevented hyperkalemia in the 20M-50M treatments, which was similar to Ventura *et al.,* (2020), who reported there was no change in Nile tilapia plasma potassium values after transportation with eugenol, compared to not-transported fish. It is seen in the results that the myrcene concentration is of great importance and affects electrolyte concentrations. In the present study, the concentration of 20 µL/L myrcene was the most efficient to mitigate osmotic disturbance after transportation of common carp. Similar results were observed in tambaqui sedated with *Ocimum basilicum* EO at 800 µL/L (Ventura *et al.,* 2021). Differently, chloride levels exhibited significant elevation after 3-h transportation using thymol as a sedative (Mirzargar *et al.,* 2022). This difference might be due to the hyperventilation and different physiological and pharmacodynamics effects of the sedative drugs on the fish.

ALT, AST, and ALP enzymes as markers of hepatic function have been frequently used for investigation of tissue damage and health status of fish (Yousefi *et al.,* 2022), and in this study, the 6-h transportation stress altered these parameters upwards. The present results are consistent with earlier studies showing the benefits of the use of 10 mg/L *Ocimum gratissimum* EO (Boaventura *et al.,* 2020) and 30 µg/L 1,8-cineole (Liu *et al.,* 2022) in transportation water on fish plasma enzymes. Highest level of AST in transported treatments in the current study may be related to the AST participation for the production of glucose. In 20M treatment, AST and ALP values were found to be closest to BT, whereas ALT values were similar. These results indicated that myrcene improved hepatic function and lowered the effect of liver damage in common carp exposed to the transportation process.

The present study found no difference in total Ig among treatments after 6-h transportation process. But, transportation process led to a significant decrease in plasma ACH50 activities and the highest decrease was found in the control and 50M treatments compared to other treatments. In this study, it was determined that the use of 20-30 µL/L myrcene concentrations in the transport water was effective in significantly improving lysozyme and ACH50 activities of common carp. Complement proteins are important in fish non-specific immune responses, which involve in opsonization, inflammation, and resistance to various stress conditions (Ghafarifarsani *et al.,* 2021). Therefore, stimulating immune functions may increase fish resistance against transport stress and diseases. In the current study findings, addition of myrcene (20-30 µL/L) to the transport water has a positive contribution to the immune responses of common carp. In line with our results, in a recent study, eugenol (20 mg/L) and MS-22 (100 mg/L) was found play role activation of immune gene expression in transported *Carassius auratus* (Cao *et al.,* 2019). This improvement may be related to the sedative effect of sedative drugs during the transport period and its direct effect on the immune system. These results are inconsistent with another study that there was no significant difference in lysozyme levels of *Pelteobagrus fulvidraco* between MS-222 treatment and the control (Liu *et al.,* 2022).

In conclusion, compared with CTL, 20 µL/L myrcene concentrations significantly improved the biochemical (globulin), hepatic enzyme activity (ALT, AST, and ALP), plasma electrolytes (sodium, calcium, potassium and chloride), and immune (lysozyme and ACH50) parameters. As a result of this study, 20 mg/L myrcene was shown to be most applicable and provides greater protection against transport stress for common carp.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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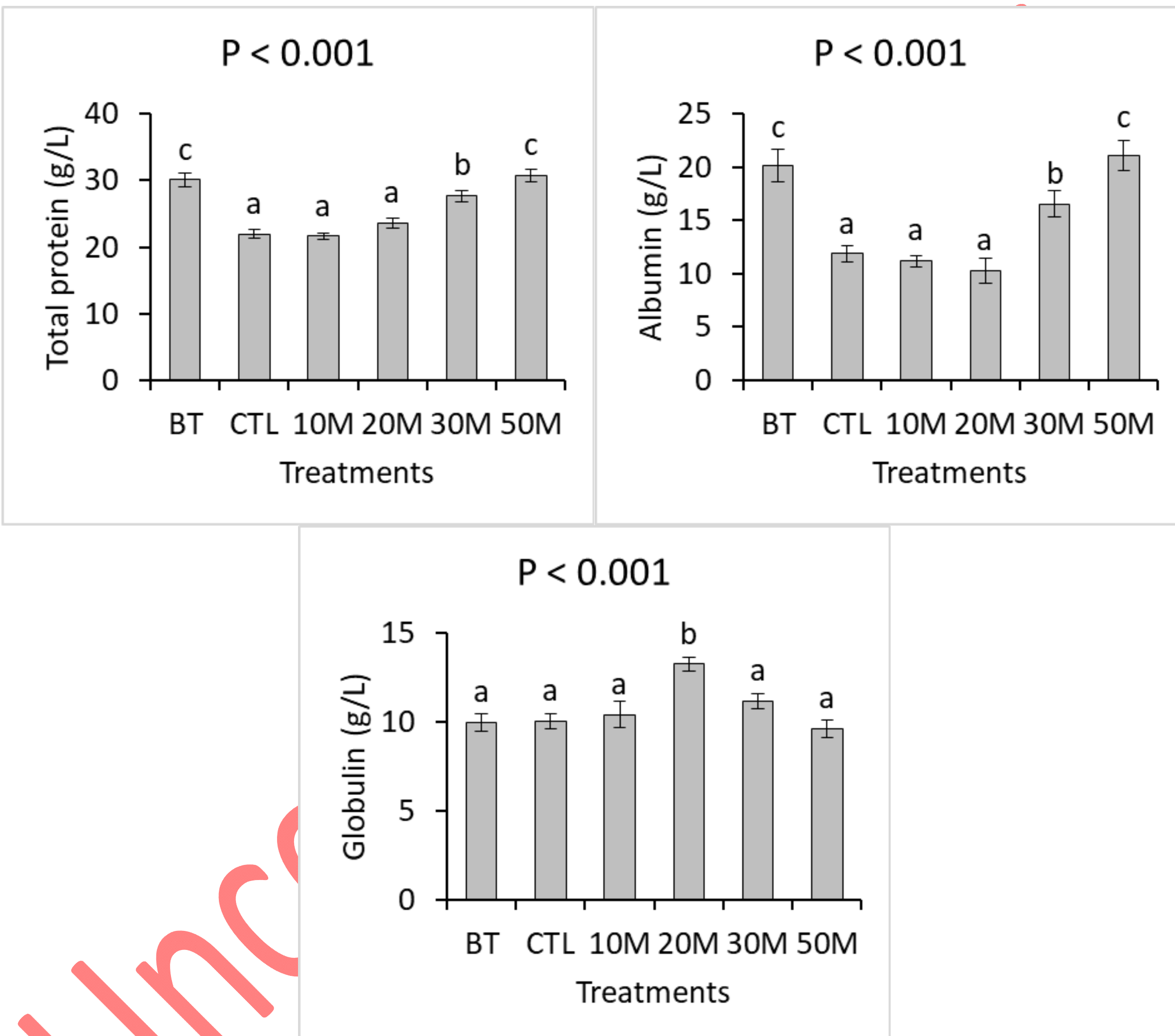
Figure captions

Fig. 1: Plasma total protein, albumin, and globulin levels of common carp after transportation with different concentrations of myrcene. Different letters above the bars show significant differences (n = 6). BT: before transportation; CTL: transported in myrcene-free water; 10M-50M: transported with 10-50 µL/L myrcene.

Fig. 2: Plasma sodium, chloride, potassium, and calcium levels of common carp after transportation with different concentrations of myrcene. Different letters above the bars show significant differences (n = 6). BT: before transportation; CTL: transported in myrcene-free water; 10M-50M: transported with 10-50 µL/L myrcene.

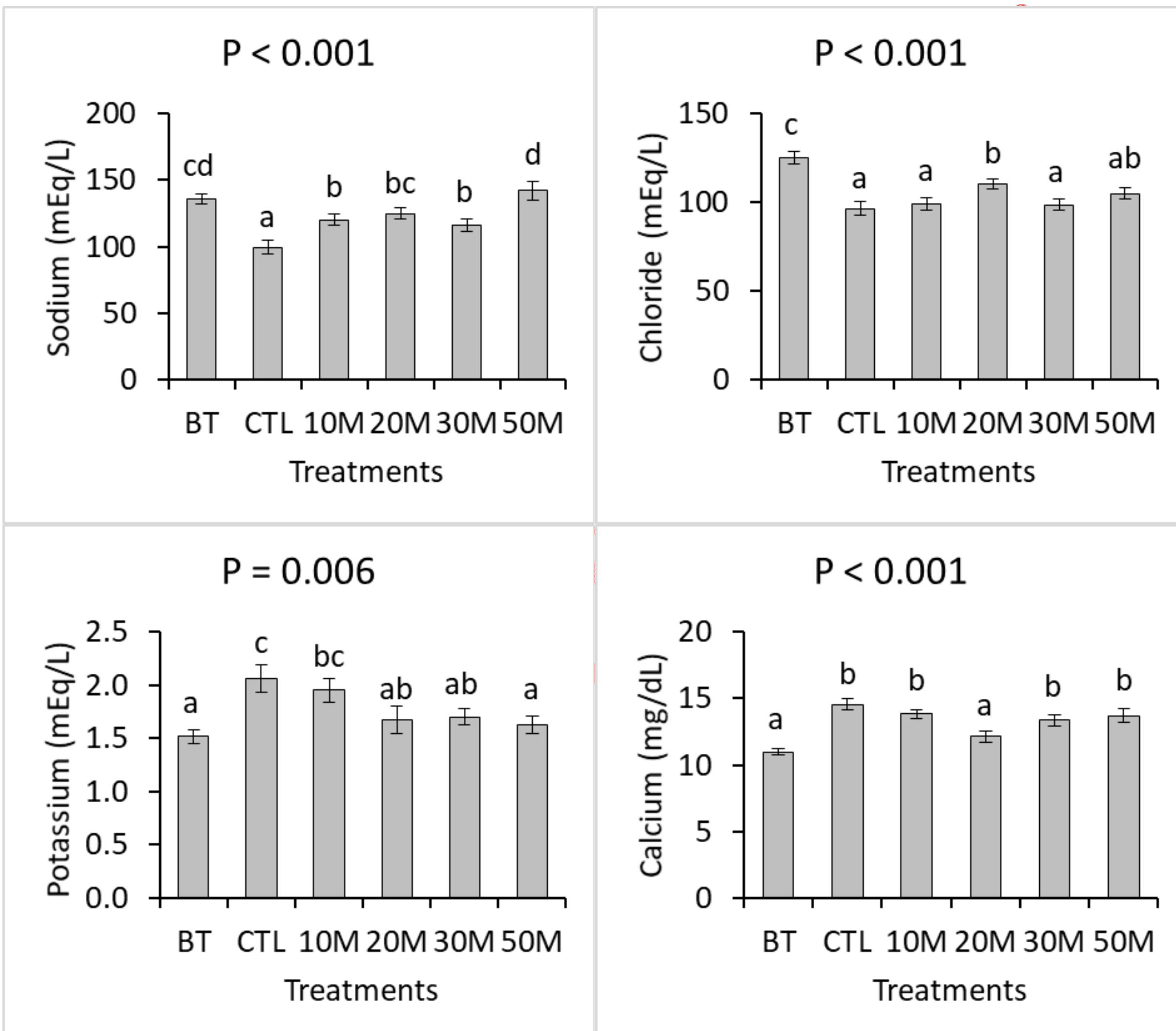
Fig. 3: Plasma ALT, AST, and ALP of common carp after transportation with different concentrations of myrcene. Different letters above the bars show significant differences (n = 6). BT: before transportation; CTL: transported in myrcene-free water; 10M-50M: transported with 10-50 µL/L myrcene.

Fig. 4: Plasma lysozyme, ACH50, and total Ig of common carp after transportation with different concentrations of myrcene. Different letters above the bars show significant differences (n = 6). BT: before transportation; CTL: transported in myrcene-free water; 10M-50M: transported with 10-50 µL/L myrcene.



P < 0.001
Total protein (g/L)
40
30
20
10
0
c
a
a
a
b
c
BT CTL 10M 20M 30M 50M
Treatments
P < 0.001
Albumin (g/L)
25
20
15
10
5
0
c
a
a
a
b
c
BT CTL 10M 20M 30M 50M
Treatments
P < 0.001
Globulin (g/L)
15
10
5
0
a
a
a
b
a
a
BT CTL 10M 20M 30M 50M
Treatments

390 Fig. 1


P < 0.001
Sodium (mEq/L)
200
150
100
50
0
cd
a
b
bc
b
d
BT
CTL
10M
20M
30M
50M
Treatments
P < 0.001
Chloride (mEq/L)
150
100
50
0
c
a
a
b
a
ab
BT
CTL
10M
20M
30M
50M
Treatments
P = 0.006
Potassium (mEq/L)
2.5
2.0
1.5
1.0
0.5
0.0
a
c
bc
ab
ab
a
BT
CTL
10M
20M
30M
50M
Treatments
P < 0.001
Calcium (mg/dL)
20
15
10
5
0
a
b
b
a
b
b
BT
CTL
10M
20M
30M
50M
Treatments
Fig. 2

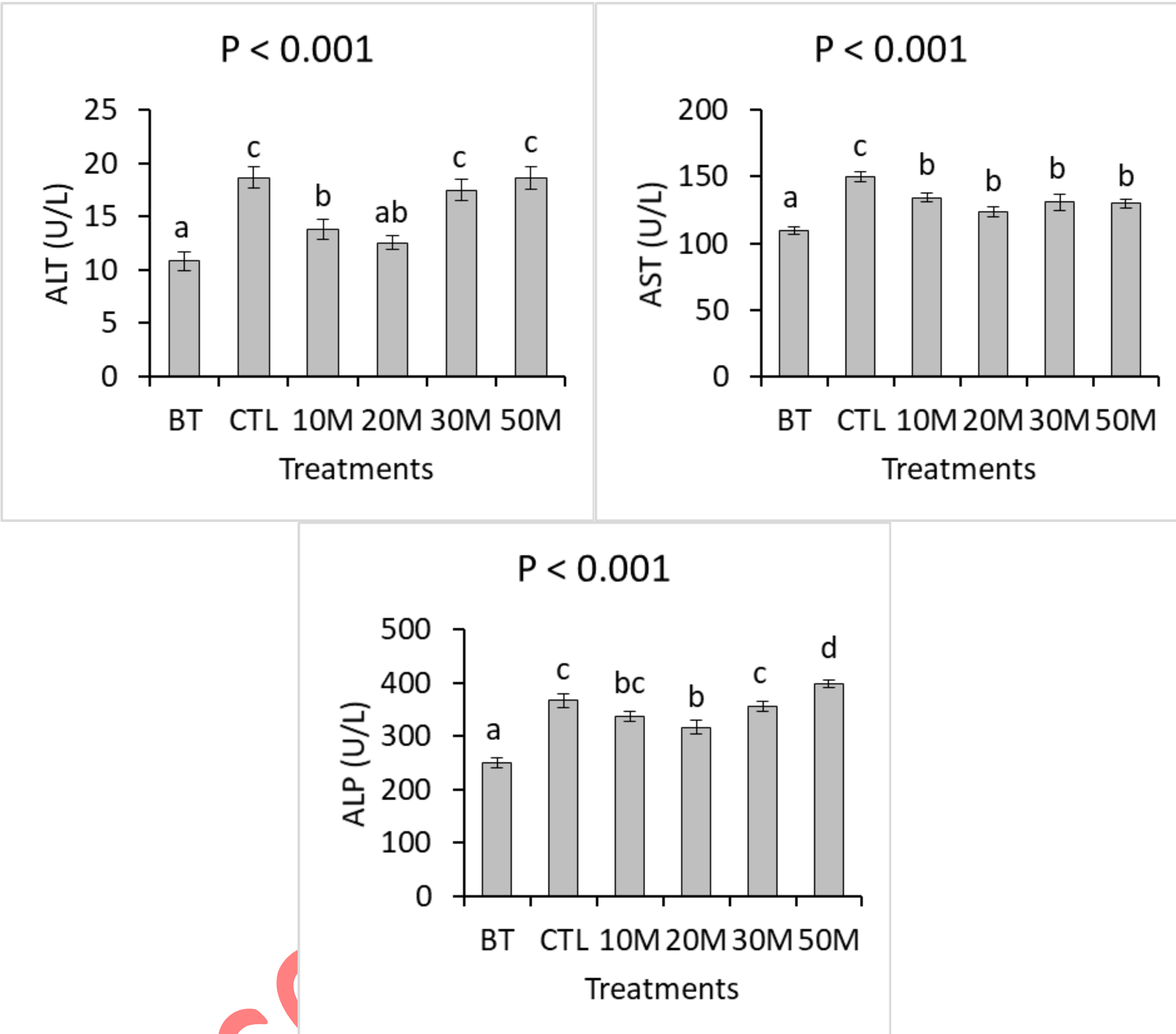

P < 0.001
ALT (U/L)
25
20
15
10
5
0
a
c
b
ab
c
c
BT CTL 10M 20M 30M 50M
Treatments
P < 0.001
AST (U/L)
200
150
100
50
0
a
c
b
b
b
b
BT CTL 10M 20M 30M 50M
Treatments
P < 0.001
ALP (U/L)
500
400
300
200
100
0
a
c
bc
b
c
d
BT CTL 10M 20M 30M 50M
Treatments

Fig. 3

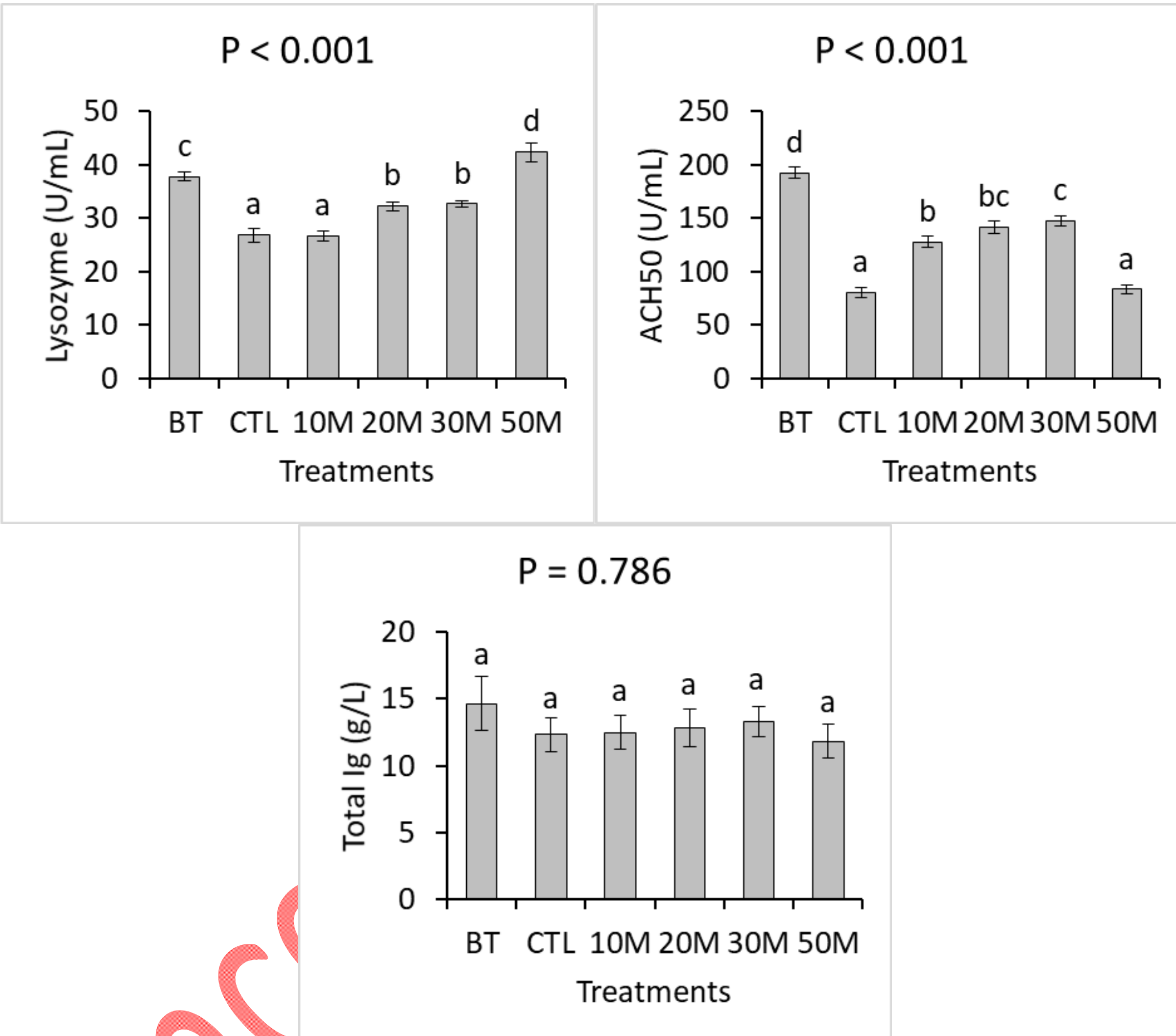

P < 0.001
Lysozyme (U/mL)
50
40
30
20
10
0
c
a
a
b
b
d
BT CTL 10M 20M 30M 50M
Treatments
P < 0.001
ACH50 (U/mL)
250
200
150
100
50
0
d
a
b
bc
c
a
BT CTL 10M 20M 30M 50M
Treatments
P = 0.786
Total Ig (g/L)
20
15
10
5
0
a
a
a
a
a
a
BT CTL 10M 20M 30M 50M
Treatments

Fig. 4

اثر افزودن میرسن به آب بر شاخصهای بیوشیمیایی پلاسمای ماهی کپور معمولی *Cyprinus carpio* در حین حمل و نقل

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زمینه مطالعه: از عوامل آرام بخش برای کاهش استرس و بهبود سلامت ماهی در حین و پس از فرآیند حمل و نقل استفاده می شود. **هدف**: این تحقیق با هدف بررسی اثرات افزودن میرسن به آب حمل‌ونقل بر شاخصهای بیوشیمیایی پلاسمای ماهی کپور معمولی (*Cyprinus carpio*) انجام شد. **روش کار**: برای این منظور، ماهیان (متوسط وزن 1/65 ± 45/3 گرم) در کیسه‌های پلاستیکی حاوی صفر (CTL)، 10 (10M)، 20 (20M)، 30 (30M) و 50 (50M) میکرولیتر در لیتر میرسن میرسن به مدت 6 ساعت حمل و نقل شدند و پارامترهای پلاسما با قبل از حمل و نقل (BT) مقایسه شد. **نتایج**: حمل و نقل باعث کاهش معنی‌دار پروتئین کل پلاسما، آلبومین، فعالیت کمپلمان فرعی، لیزوزیم، سدیم و کلراید، و افزایش پتاسیم، کلسیم، آلانین آمینوترانسفراز، آسپارتات آمینوترانسفراز و آلکالین فسفاتاز در تیمار CTL نسبت به BT شد ($P < 0/05$). افزودن 50 میکرولیتر

در لیتر میرسن به آب از کاهش پروتئین کل پلاسما و آلبومین جلوگیری کرد. افزودن 20 میکرولیتر در لیتر میرسن به آب تغییرات یون‌های پلاسما، آلانین آمینوترانسفراز و آلکالین فسفاتاز را کاهش داد یا از این تغییرات جلوگیری نمود. افزودن 10-50 میکرولیتر در لیتر میرسن به آب، تغییرات آسپارتات آمینوترانسفراز پلاسما را کاهش داد. افزودن 20 و 30 میکرولیتر در لیتر میرسنبه آب، تغییرات در فعالیت کمپلمان فرعی پلاسما را کاهش داد، و افزودن 30 میکرولیتر در لیترمیرسن باعث افزایش فعالیت لیزوزیم پلاسما شد. حمل و نقل و افزودن میرسن تأثیر معنی‌داری بر ایمونوگلوبولین پلاسما نداشتند ($P > 0/05$)، اما افزایش معنی‌داری در گلوبولین پلاسما در تیمار 20M مشاهده شد ($P < 0/05$). **نتیجه گیری نهایی**: در نتیجه افزودن 20 میکرولیتر در لیتر میرسن به آب حمل و نقل ماهی کپور معمولی باعث کاهش سطح آنزیم کبدی و بهبود پارامترهای ایمنی شد و بنابراین استفاده از میرسن باعث بهبود سلامت ماهی در حین حمل و نقل می شود.

واژکان کلیدی: الکترولیت ها، سلامت ماهی، حمل و نقل ماهی، پارامترهای ایمنی، استرس حمل و نقل