Original Article

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

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How to Cite This Article Ebrahimzadeh Mousavi, H., Taheri Mirghaed, A., Hoseini, S. M., Ghelichpour, M., Aghaei Moghaddam, A., & Gharavi, B., et al. (2024). Effects of Myrcene Addition to Water on Plasma Biochemical Characteristics of Common Carp, *Cyprinus carpio*, During Transportation. *Iranian Journal of Veterinary Medicine, 18*(1), 131-140. http://dx.doi.org/10.32598/ijvm.18.1.1005360

http://dx.doi.org/10.32598/ijvm.18.1.1005360

ABSTRACT

Background: Sedative agents are utilized to mitigate stress and improve fish welfare during and after transportation.

Objectives: We aimed to assess the effects of myrcene addition to the transportation water on the plasma biochemical profile of common carp, *Cyprinus carpio*.

Methods: For this purpose, common carp (mean weight of 45.3±1.65 g) were transported in plastic bags containing myrcene at concentrations of 0 (CTL), 10 (10 M), 20 (20 M), 30 (30 M), and 50 (50 M) µL/L for 6 h. Then, their plasma parameters were compared to those values before transportation (BT).

Results: Transportation significantly ($P<0.05$) decreased plasma total protein, albumin, alternative complement, lysozyme, sodium, and chloride, while increasing potassium, calcium, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in the CTL treatment, as compared to BT. Adding 50 µL/L myrcene to water prevented total plasma protein and albumin loss. Supplementing 20 µL/L myrcene to water prevented or reduced the changes in plasma ions, alanine aminotransferase, and alkaline phosphatase. Addition of 10-50 µL/L myrcene to water decreased the changes in plasma aspartate aminotransferase. Adding 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 ($P>0.05$). However, a significant increase was observed in plasma globulin in the 20 M treatment ($P<0.05$).

Conclusion: In summary, adding 20 µL/L myrcene to the transport water of common carp reduced hepatic enzyme levels and improved immune parameters, and therefore, the use of myrcene improves fish welfare during transportation.

Keywords: Electrolytes, Fish welfare, Fish transport, Immune parameters, Transport stress

Article info:
Received: 16 Apr 2023
Accepted: 15 Feb 2023
Publish: 01 Jan 2024

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Introduction

With the rapid increase in global population, aquaculture stands out as an important agricultural and food production sector to ensure the worldwide demand for fish consumption and food security. For this reason, aquaculture production and income from this sector are rising every year. Undoubtedly, the new technologies and beneficial outcomes of scientific studies have contributed to promoting the aquaculture sector.

It is equally beneficial for fish and fish producers that fish experience a minimum stress level during aquaculture activities. Fish are susceptible to mechanical injury and physiological stress during routine procedures in aquaculture, which may result in undesirable conditions, such as reduced growth rate and increased fish morbidity. For example, the transportation process frequently used in fish farming is stressful for fish and may cause various injuries, leading to the loss of their scales and mucus and increasing the outbreak of bacterial and fungal diseases (Zeppenfeld et al., 2014; Adah Sylvanus et al., 2022).

One solution to this problem is using sedatives in transportation water. These chemicals may lower oxidative stress and fish metabolism and improve the welfare of aquatic species (Toni et al., 2015; Aydın & Barbas, 2020; Ventura et al., 2020). Accordingly, using sedative agents in the aquaculture sector is essential for producers.

Various studies have been performed in recent years on using sedatives in aquaculture (Aydın & Barbas 2020). Some studies approved using sedatives in fish transportation (Boaventura et al., 2020; Ventura et al., 2020). However, it was reported that anesthetic or sedative drugs used in farming procedures can cause stress at various levels and have undesirable effects on the physiology of fish (Zeppenfeld et al., 2014; Aydın & Barbas 2020; Rahman et al., 2020). Aydın and Barbas (2020) stated that while some drugs positively affect the blood biochemistry of fish, some medications, 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. Hence, it is crucial to investigate the detailed analysis of the side effects of sedative substances on fish.

For this purpose, some studies have been 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 for its rapid growth, good adaptation to farming conditions, and 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 & Ghelichpour, 2019; Hoseini et al., 2022; Mirzargar et al., 2022).

In recent years, myrcene has been investigated for its sedative and anesthetic effects in fish (Taheri Mirghaed et al., 2016; Taheri Mirghaed et al., 2019). No studies have reported the common carp's biochemical profile transported under sedation with myrcene. Thus, this study aimed 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

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

Afterward, the plastic bags were divided into 5 groups, and 0 (CTL), 10 (10 M), 20 (20 M), 30 (30 M), and 50 (50 M) µL/L myrcene were added to either of the bags. The plastic lids were fastened and transported for 6 hours. After transportation, 4 fish were caught from each plastic bag, and their blood samples were taken. Fish were anesthetized with eugenol (100 mg/L), and their 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 a flame photometer. The photometric method was used to measure calcium with a commercial kit (Pars Azmoun). Chloride was measured by Zischem kit according to the manufacturer's instructions. 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 kinetic methods (Tulaby Dezuly et al., 2019).

Plasma lysozyme activity was measured at 530 nm using *Micrococcus luteus* as the target. Every 0.001 decrease in sample absorbance per minute was considered one unit of lysozyme (Mohseni et al., 2021a). Plasma alternative complement (ACH50) activity was measured according to hemolytic activity against sheep erythrocytes (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 treatment groups' means were compared by Duncan's test. SPSS software, version 22 was used to analyze the data. Significance was determined at the level of $P<0.05$. Data were presented as Mean±SD.

Results

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

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

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

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

Discussion

The positive or negative effects of sedatives in the transport water are not fully explored in aquaculture. It has been indicated that anesthetic or 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, this study examined the plasma biochemical profile of the common carp transported with different myrcene concentrations for 6-h medium-distance transportation. Blood protein reflects fish health, mainly liver and non-specific immunity, and our results suggest that myrcene in transportation water positively impacts the common carp's health condition. According to the current findings, eugenol and *O. basilicum* EO did not affect blood protein, albumin, and globulin levels of Nile

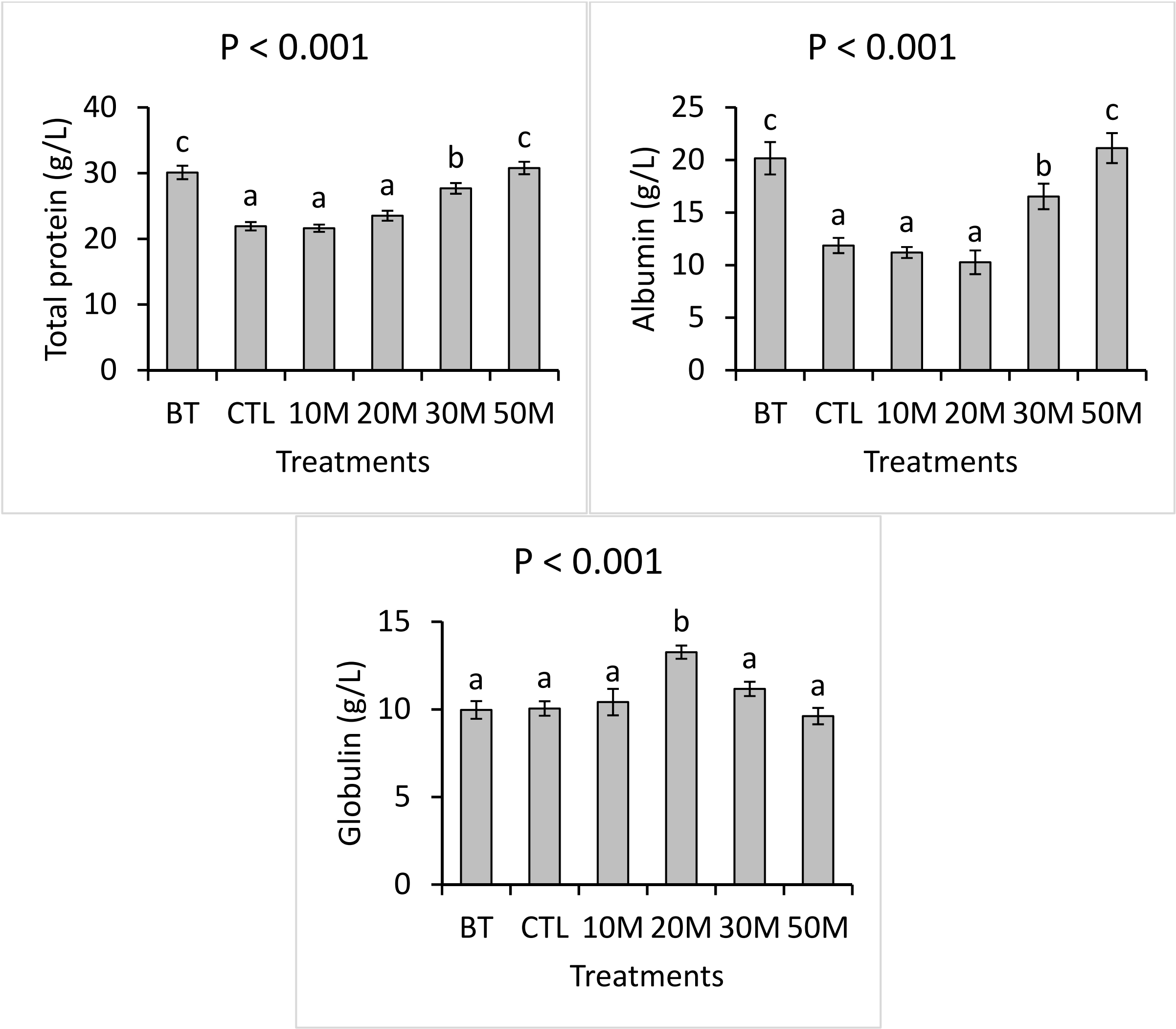

Figure 1. Plasma total protein, albumin, and globulin levels of common carp after transportation with different concentrations of myrcene

Different letters above the bars show significant differences (n=6).

Note: CTL, transported in myrcene-free water; 10M-50M, transported with 10-50 µL/L myrcene.

BT: Before transportation.

tilapia transported for 2 h (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 of studies can vary due to the fish species, sedative concentration, water temperature, and transport time (Bowker et al., 2015). For example, a long-duration transportation process suppresses the immune system and increases fish morbidity or mortality (Gomes et al., 2003).

Plasma electrolytes are essential for the normal physiological function of fish. Freshwater fish exhibit chloride loss during stress due to increased ventilation from the gills (Barton et al., 2003; Mirzargar et al., 2022). In this study, chloride levels in the transport treatments decreased significantly compared to BT. This finding has also been reported in different fish species, such as *Stizostedion vitreum* (Barton et al., 2003) and *Labeo rohita* (Biswal et al., 2021). The 20 M treatment chloride level was less influenced by transportation than the other treatments in the present study. Myrcene prevented hyperkalemia in the 20 M-50 M treatments, similar to Ventura et al. (2020), who reported no change in Nile tilapia plasma potassium values after transportation with eugenol, compared to not-transported fish. The results indicate that the myrcene concentration affects electrolyte concentrations. In the present study, myrcene (20 µL/L) was the

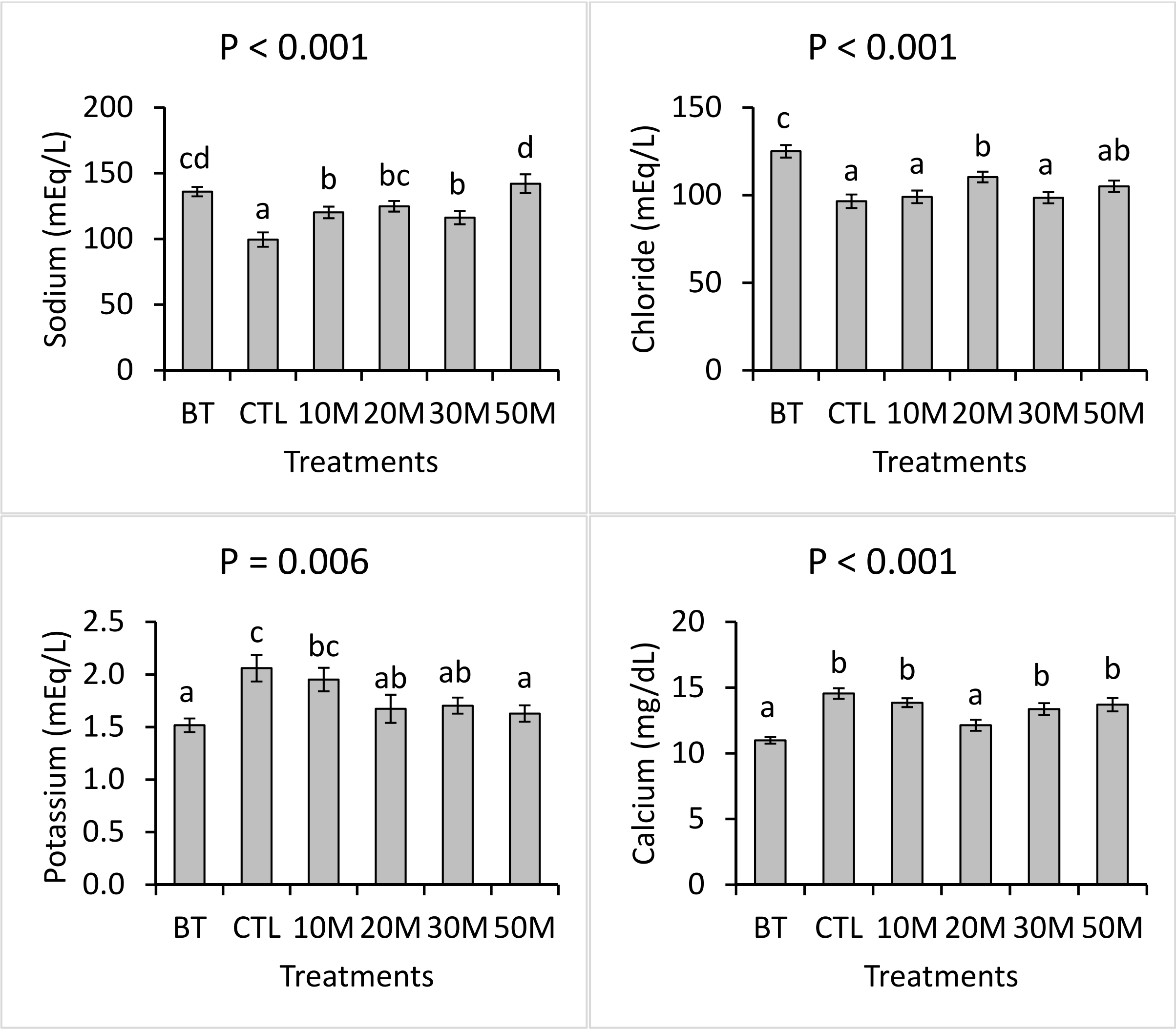

Figure 2. Plasma sodium, chloride, potassium, and calcium levels of common carp after transportation with different concentrations of myrcene

Different letters above the bars show significant differences (n=6).

Note: CTL, transported in myrcene-free water; 10M-50M, transported with 10-50 µL/L myrcene.

BT: Before transportation.

most efficient concentration for mitigating osmotic disturbance after transporting common carp. Similar results were observed in tambaqui sedated with *O. basilicum* EO at 800 µL/L (Ventura et al., 2021). However, 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 pharmacodynamic effects of the sedative drugs on the fish.

ALT, AST, and ALP enzymes as markers of hepatic function have been frequently used to investigate tissue damage and the health status of fish (Yousefi et al., 2022). In this study, the 6-h transportation stress elevated these parameters. The present results are consistent with earlier studies showing the benefits of using *Ocimum gratissimum* EO (10 mg/L) (Boaventura et al., 2020) and 1,8-cineole (30 µg/L) (Liu et al., 2022) in transportation water on fish plasma enzymes. The highest level of AST in transported treatments in the current study may be related to AST participation in glucose production. In the 20 M treatment, AST and ALP values were 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.

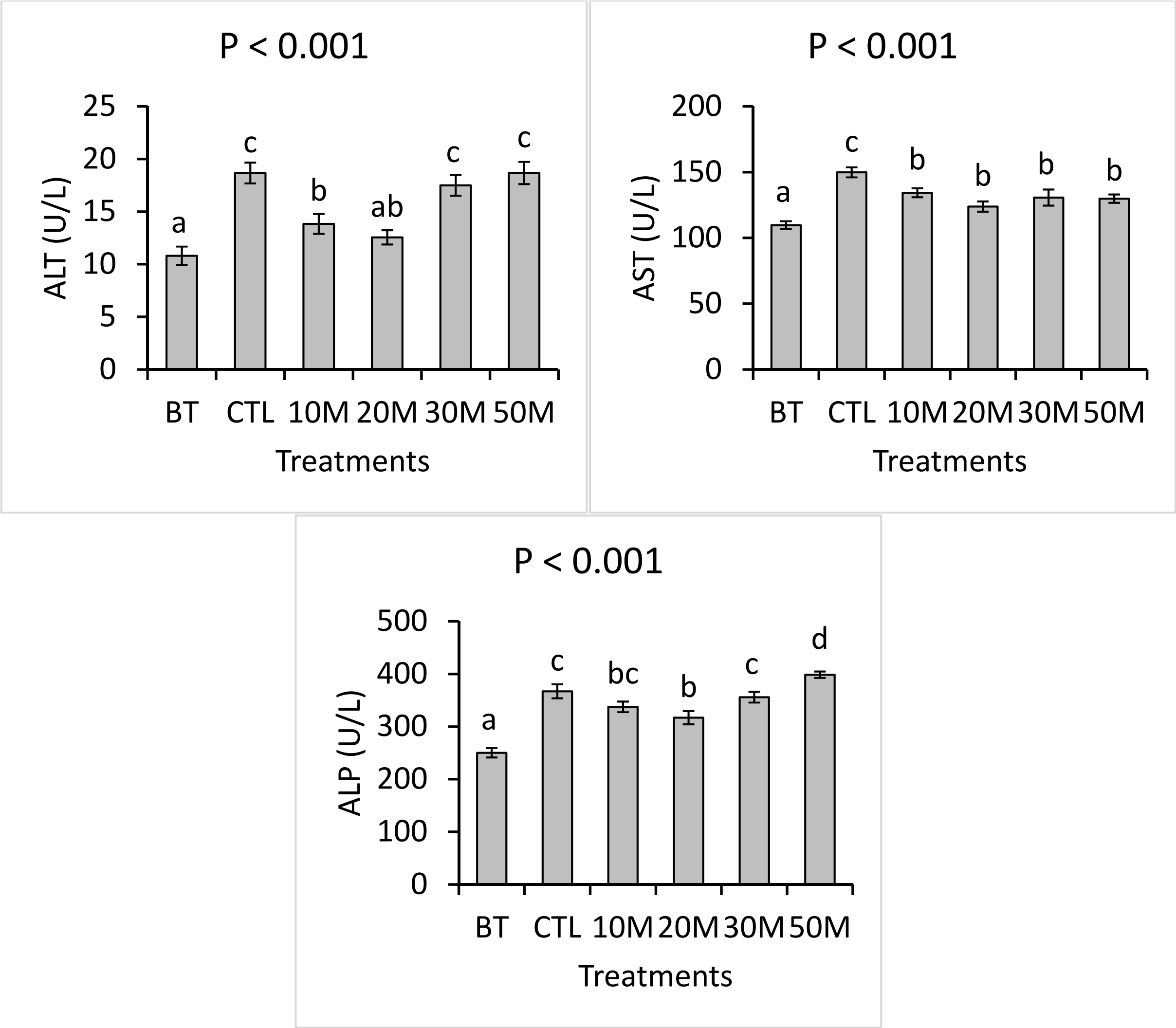

Figure 3. Plasma ALT, AST, and ALP of common carp after transportation with different concentrations of myrcene

Different letters above the bars show significant differences (n=6).

Note: CTL, transported in myrcene-free water; 10M-50M, transported with 10-50 µL/L myrcene.

Abbreviations: BT: Before transportation; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

The present study found no difference in total Ig among treatments after the 6-h transportation process. Nevertheless, the transportation process led to a significant decrease in plasma ACH50 activities, and the highest reduction was found in the control and 50 M treatments compared to other treatments. This study determined that using myrcene at 20-30 µL/L concentrations in the transport water was effective in significantly improving lysozyme and ACH50 activities of common carp. Protein supplements are essential in fish non-specific immune responses involving 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, adding myrcene (20-30 µL/L) to the transport water positively contributed 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) were found to activate immune gene expression in transported *Carassius auratus* (Cao et al., 2019). This improvement may be attributed 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 in that there was no significant difference in lysozyme levels of *Pelteobagrus fulvidraco* between the MS-222 treatment and the control (Liu et al., 2022).

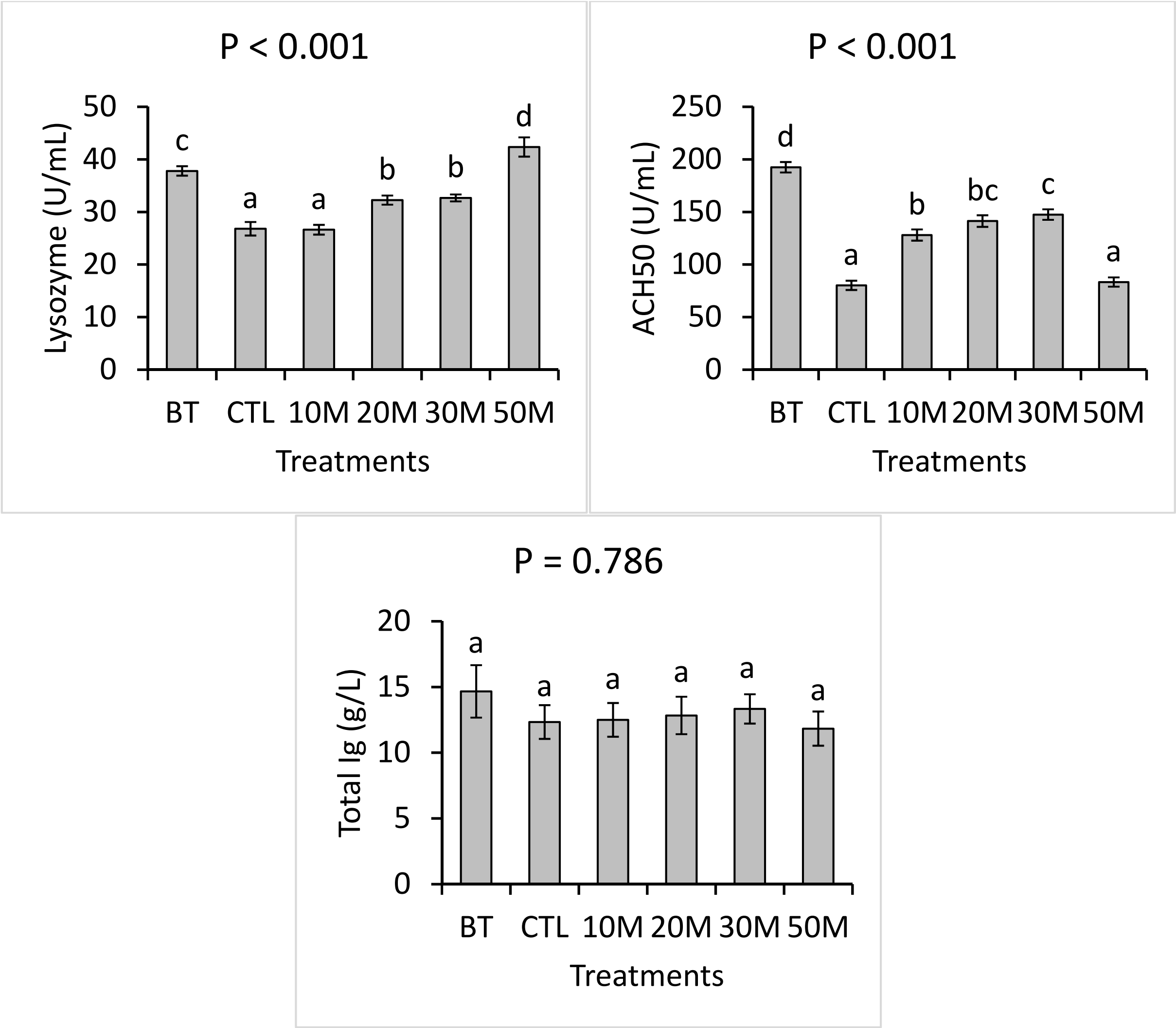

Figure 4. Plasma lysozyme, ACH50, and total Ig of common carp after transportation with different concentrations of myrcene

Different letters above the bars show significant differences (n=6).

Note: CTL, transported in myrcene-free water; 10-50 M, transported with 10-50 µL/L myrcene.

BT: Before transportation.

In conclusion, compared with CTL, myrcene at 20 µL/L concentration 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. Thus, myrcene (20 mg/L) can provide greater protection against transport stress for common carp.

Ethical Considerations

Compliance with ethical guidelines

All parts of the study were conducted under a protocol approved by the Committee of Ethics of the Faculty of Sciences of the University of Tehran (No.: 357; 8 November 2000).

Funding

This study has been supported by the Center for International Scientific Studies and Collaboration (CISSC), Ministry of Science Research and Technology.

Authors' contributions

Conceptualization and supervision: Hoseinali Ebrahimzadeh Mousavi, Ali Taheri Mirghaed and Seyyed Morteza Hoseini; Methodology: Behrouz Gharavi; Visualization: Melika Ghelichpour; Investigation: Behrouz

Gharavi and Melika Ghelichpour; Data curation: Seyyed Morteza Hoseini; Formal analysis: Hoseinali Ebrahimzadeh Mousavi, Seyyed Morteza Hoseini, Melika Ghelichpour and Abbasali Aghaei Moghaddam; Writing - original draft: Seyyed Morteza Hoseini, Baki Aydın and Seyyed Morteza Hoseini; Review & editing: Seyyed Morteza Hoseini and Baki Aydın.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors thanks staffs of University of Tehran and Inland Waters Aquatics Resources Research Center (Gorgan for their supports.

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مقاله پژوهشی

اثر افزودن میرسن به آب بر شاخص‌های بیوشیمیایی پلاسمای ماهی کپور معمولی در حین حمل‌ونقل

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How to Cite This Article Ebrahimzadeh Mousavi, H., Taheri Mirghaed, A., Hoseini, S. M., Ghelichpour, M., Aghaei Moghaddam, A., & Gharavi, B., et al. (2024). Effects of Myrcene Addition to Water on Plasma Biochemical Characteristics of Common Carp, *Cyprinus carpio*, During Transportation. *Iranian Journal of Veterinary Medicine, 18*(1), 131-140. http://dx.doi.org/ 10.22059/IJVM.2022.351535.1005333

doi http://dx.doi.org/ 10.22059/IJVM.2022.351535.1005333

تاریخ دریافت: ۲۶ بهمن ۱۴۰۱
تاریخ پذیرش: ۲۷ فروردین ۱۴۰۲
تاریخ انتشار: ۱۱ دی ۱۴۰۲

چکیده

زمینه مطالعه: از عوامل آرام‌بخش برای کاهش استرس و بهبود سلامت ماهی در حین و پس از فرآیند حمل‌ونقل استفاده می‌شود.

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 ۱/۶۵±۴۵/۳ گرم) در کیسه‌های پلاستیکی حاوی صفر (CTL)، ۱۰، ۲۰، ۳۰ و ۵۰ میکرولیتر در لیتر میرسن به‌مدت ۶ ساعت حمل‌ونقل شدند و پارامترهای پلاسما با قبل از حمل‌ونقل مقایسه شد.

نتایج: حمل‌ونقل باعث کاهش معنادار 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<۰/۰۵). افزودن ۵۰ میکرولیتر در لیترمیرسن به آب از کاهش پروتئین کل پلاسما و آلبومین جلوگیری کرد. افزودن ۲۰ میکرولیتر در لیتر میرسن به آب تغییرات یون‌های پلاسما، آلانین آمینوترانسفراز و آلکالین فسفاتاز را کاهش داد یا مانع از این تغییرات شد. افزودن ۱۰-۵۰ میکرولیتر در لیتر میرسن به آب، تغییرات آسپارتات آمینوترانسفراز پلاسما را کاهش داد. افزودن ۲۰ و ۳۰ میکرولیتر در لیتر میرسن به آب، تغییرات در فعالیت کمپلمان فرعی پلاسما را کاهش داد، افزودن ۳۰ میکرولیتر در لیترمیرسن باعث افزایش فعالیت لیزوزیم پلاسما شد. حمل‌ونقل و افزودن میرسن تأثیر معناداری بر ایمونوگلوبولین پلاسما نداشتند (P>۰/۰۵)، اما افزایش معناداری در گلوبولین پلاسما در تیمار ۲۰ میکرولیتر مشاهده شد (P<۰/۰۵).

نتیجه‌گیری نهایی: درنتیجه افزودن ۲۰ میکرولیتر در لیترمیرسن به آب حمل‌ونقل ماهی کپور معمولی باعث کاهش سطح آنزیم کبدی و بهبود پارامترهای ایمنی شد و بنابراین استفاده از میرسن باعث بهبود سلامت ماهی در حین حمل‌ونقل می‌شود.

کلیدواژه‌ها: الکترولیت‌ها، سلامت ماهی، حمل‌ونقل ماهی، پارامترهای ایمنی، استرس حمل‌ونقل

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