



USES OF EUGENOL IN AQUACULTURE: BENEFITS AND APPLICATIONS – A REVIEW

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Abstract

Aquaculture is a pivotal industry for global food production, facing challenges such as environmental conditions, and disease. Stress, a significant factor compromising fish health, suppresses immune functions and increases disease susceptibility. Anesthetic agents like eugenol (EU), derived from clove oil (*Eugenia caryophyllata*), offer promising stress mitigation in aquaculture. EU, known for its anesthetic and analgesic properties in human medicine, shows potential as a safe anesthetic in aquaculture. This review examines EU's effects on cortisol levels, hematological, and biochemical parameters across various fish species, highlighting its role in stress reduction and improving production efficiency.

Key words: herbal, hematological and biochemical parameters, stress, cortisol, fish species

Global fish stocks are declining at an accelerating rate (Yue and Shen, 2022). The aquaculture industry has a longstanding tradition of supplying high-quality proteins to the global population (Gui et al., 2018; Nash, 2010). However, aquaculture faces significant challenges, including diseases (Gholamhosseini et al., 2020, 2021), environmental pollution, nutritional issues, and inadequate management (Bagheri et al., 2023). Scaling up production in aquaculture necessitates the adoption of advanced technologies (Yue and Shen, 2022). One of the primary challenges contributing to disease outbreaks and reduced production in aquaculture is stress. Various factors can induce stress in fish, and suppress their immune systems (Jafari et al., 2023; Yaghoobpour et al., 2022), ultimately leading to illness. Anesthetics are employed to reduce stress during activities such as transportation (Bosworth et al., 2007), handling (Small, 2003), artificial insemination (McGovern-Hopkins et al., 2003), tagging, sampling (Ackerman and Bellwood, 2002; Baker et al., 2005), and surgical preparations (Adel et al., 2020) for research purposes within the aquaculture industry (Warren et al., 2004).

In a 2020 study, the sedative effects of propofol and clove oil were compared in the Levantine scraper (*Capoeta damascina*), with propofol being preferred for this species due to advantages such as rapid induction and longer duration (Adel et al., 2020). Anesthesia is a biological state induced by chemical or non-chemical

means, resulting in partial or complete loss of sensation or voluntary neuromotor control (Raisi et al., 2020). Various anesthetics are utilized in the aquaculture industry (Alishahi et al., 2019). The stages of anesthesia include: sedation (first stage), characterized by a partial reduction in reaction to external stimuli; partial loss of balance and involuntary movements while swimming (second stage); total loss of balance and irregular operculum movements (third stage); anesthesia (fourth stage), marked by loss of reflexes and swimming ability, and lack of response of operculum to external stimuli, with recovery possible without anesthesia (optimal phase); and medullary collapse (fifth stage), characterized by relaxation of the limbs and neck, is a hazardous stage that should be avoided (Adel et al., 2017).

A good anesthetic possesses several characteristics, including effective muscle relaxation and inactivity, short recovery time without side effects, no adverse effects on fish safety and physiology, easy excretion from the body, harmlessness to humans, water solubility, and ready availability (Akbulut et al., 2012; Beheshti et al., 2018). The choice of anesthetic compound depends on availability, effectiveness, cost-effectiveness, study requirements, ease of use, and safety for both users and the environment (Iversen et al., 2003; Mylonas et al., 2005).

Fish respire through their gills, necessitating the addition of an anesthetic to the reservoir water for absorp-

tion through these gills (Schaeffer, 1997). Factors such as the surface area of the gill epithelium, body volume, and epithelial thickness influence the effectiveness of anesthetics. Individual parameters such as age, species, size, weight, body condition, lipid content, and disease status can also influence the pharmacokinetics of the anesthetic agent and its metabolism rate (Iversen et al., 2003). Water conditions such as pH and temperature are additional factors that impact the efficacy of an anesthetic solution (Batt et al., 2005; Guo et al., 1995).

Clove oil, derived from the clove plant and containing eugenol (4-allyl-2-methoxyphenol) as its active component, has been widely used as a safe anesthetic for human consumption (Anderson et al., 1997; Iversen et al., 2003; Keene et al., 1998). It possesses several properties that make it an ideal anesthetic, including its analgesic effects (Keene et al., 1998). Clove oil-based anesthetics are cost-effective, readily available in low doses, and capable of reducing stress, without having a harmful effect on the consumer or the environment (Iversen et al., 2003; McGovern-Hopkins et al., 2003). Therefore, clove oil represents a safe option for use in aquaculture, prompting numerous studies to assess its efficacy across various species (Anderson et al., 1997; Cooke et al., 2004; Davis and Griffin, 2004; Iversen et al., 2003; Keene et al., 1998; Munday and Wilson, 1997; Mylonas et al., 2005; Ribas et al., 2007; Sladky et al., 2001; Small, 2003). In Iran, clove oil is predominantly employed as an effective anesthetic in fish farms to alleviate stress during transportation, grading, and artificial spawning. This effective anesthetic also influences other parameters such as growth, digestive physiology, and gene expression. Given these factors, this study aims to investigate the anesthetic effects of eugenol on serum cortisol levels, biochemical

responses, and hematological parameters across different fish species, to determine its suitability for farm use and stress reduction in fish.

Eugenol

Eugenol (EU) is a volatile phenolic compound extracted from clove essential oil. Essential oils are natural, concentrated extracts of plants, fruits, and vegetables obtained through various methods. Clove buds or leaves (*Eugenia caryophyllata*) contain clove oil, with EU being a functional component in limited concentrations in the food and cosmetic industries. It is also used in medicine as an antiseptic and local anesthetic. Eugenol exhibits various effects including analgesic, antimicrobial, cardiovascular, antioxidant, and anti-inflammatory properties. The aquaculture industry uses the EU for stress reduction, anesthesia, and sedation.

In a study (Mosavi et al., 2013), a concentration of 1 mg L⁻¹ was found optimal for sedation, while 40 mg L⁻¹ was optimal for inducing anesthesia in *Barbus sharpeyi*. However, a concentration of 200 mg L⁻¹ was determined to be highly toxic. Based on statistical analysis, the 48-hour LC50 of eugenol was determined to be 61.22±76.1 mg L⁻¹, indicating its average toxicity in *Barbus sharpeyi*. While generally considered safe, concerns about eugenol's toxicity have arisen due to its broad range of applications (Nejad et al., 2017). Eugenol has also been extensively used in dentistry for its anesthetic and analgesic effects, with studies investigating its pharmacokinetics and metabolism in aquatic animals (Tang et al., 2022; Xu et al., 2023). It may be used as a trigger for induction in specific situations (Pramod et al., 2010). In aquaculture, the EU has diverse applications and effectively reduces fish stress.

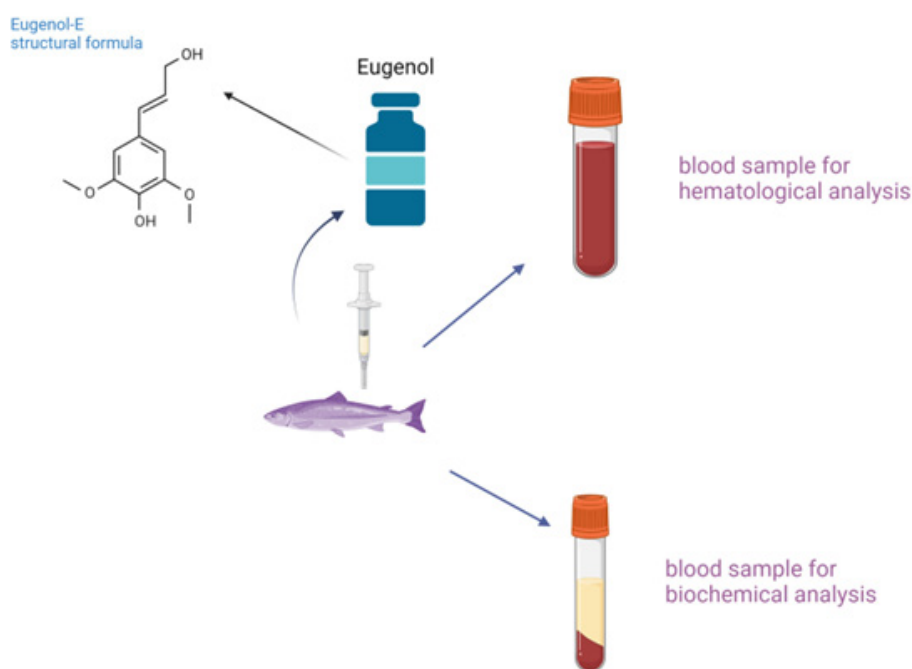


Figure 1. Appearance and structure of eugenol

Cortisol and its functions in fish

Plasma cortisol concentrations are a crucial indicator of stress in various fish species (Bortoletti et al., 2021). Cortisol levels in the blood increase significantly in response to stressful conditions and low temperatures (Speers-Roesch et al., 2010). The elevation of cortisol levels is a result of activation of the hypothalamic-pituitary-interrenal (HPI) axis in response to environmental stressors (Baker et al., 2005). For example, studies have shown that transportation induces stress and raises cortisol levels in live juvenile walleyes (Barton et al., 2003), which can subsequently increase the risk of losses due to bacterial infections.

Effects of eugenol on cortisol levels

EU does not elevate cortisol levels, unlike other anesthetics, such as metomidate, which are known to suppress cortisol production and increase cortisol levels (Hansen et al., 2003). Research has shown that cortisol concentrations can increase significantly (up to five times the control levels) 30 minutes after the onset of stress in various fish species (Barton, 2002). While EU does not induce a significant change in serum cortisol levels, it prevents a decline in degranulation (Palić et al., 2006).

EU also inhibits the rise in cortisol levels in *Rhamdia quelen* exposed to acute stress (Cunha et al., 2010). Elevated plasma cortisol levels are associated with immunosuppression (Barcellos et al., 2004). A 2021 study (Cao et al., 2021) on the transport and recovery of turbot (*Scophthalmus maximus*) sedated with MS-222 and EU showed that cortisol levels in EU and MS-222-treated turbot were significantly lower compared to the control group. Similar findings were reported by Jerez-Cepa et al. (2019), indicating anesthetics can mitigate the effects of transport stress. These results suggest that anesthetics like EU can lower cortisol levels and improve survival rates. For

instance, in a study involving kelp grouper (*Epinephelus bruneus*), cortisol levels remained unchanged 1–6 hours after sedation with clove oil but significantly increased at 12–48 hours (Park et al., 2008). Conversely, in rock bream, a significant increase in cortisol was observed 0–12 hours after anesthesia with clove oil, with levels returning to normal after 24–72 hours (Park et al., 2009). A 2011 study (Bagheri and Imanpour, 2011) evaluated the efficacy, physiological, and hematological responses of Persian sturgeon (*Acipenser persicus*) to clove oil. The study found that clove oil effectively inhibited increases in cortisol levels (Olsen et al., 1995).

Eugenol as an anesthetic agent has been demonstrated to reduce cortisol levels in several fish species, including crucian carps (*Carassius auratus*) (Le et al., 2019), South American catfish (*Rhamdia quelen*) (Corso et al., 2019), and the European eel (*Anguilla anguilla* L.) (Renault et al., 2011). In one study, fathead minnows (*Pimephales promelas*) exhibited decreased cortisol concentrations in the EU treatment group after 12 hours of transport. This reduction is likely due to the inhibition of adrenocorticotrophic hormone (ACTH) actions and interference with cortisol synthesis (Palić et al., 2006).

A 2021 study investigated the anesthetic effects of EU on the preservation and transport of yellow catfish (*Pelteobagrus fulvidraco*). The results showed a significant decrease in blood cortisol levels in the anesthetized transportation group compared to the control group ($P < 0.05$). Conversely, other studies have reported increased cortisol levels during anesthesia and recovery in common carp (Hassan et al., 2016). In a 2021 study (Zahran et al., 2021), the efficacy and health effects of propofol (PR) and EU were compared in Nile tilapia. The results indicated no significant change in cortisol levels in the PR group regarding monitoring and recovery, unlike the EU group (Table 1).

Table 1. Effects of eugenol on cortisol levels of fish

Species	Anesthetic agent	Effects	References
Turbot (<i>Scophthalmus maximus</i>)	MS-222 and eugenol	Cortisol level ↓	(Cao et al., 2021)
Kelp grouper (<i>Epinephelus bruneus</i>)	Clove oil	Cortisol level ↑	(Park et al., 2008)
Rock bream	Clove oil	Cortisol level ↑	(Park et al., 2009)
Persian sturgeon (<i>Acipenser persicus</i>)	Clove oil	Prevention of an increase in cortisol level	(Olsen et al., 1995)
Crucian carp (<i>Carassus auratus</i>), South American catfish (<i>Rhamdia quelen</i>) and European eel (<i>Anguilla anguilla</i> L.)	Eugenol	Cortisol level ↓	(Le et al., 2019; Corso et al., 2019; Renault et al., 2011)
Yellow catfish (<i>Pelteobagrus fulvidraco</i>)	Eugenol	Cortisol level ↓	(Xu et al., 2021)
Common carp	Eugenol	Cortisol level ↑	(Hassan et al., 2016)
Nile tilapia	Propofol (PR) and Eugenol	No significant changes in the PR group compared with its control and recovery level, versus eugenol group	(Zahran et al., 2021)
Persian sturgeon (<i>Acipenser persicus</i>)	Eugenol	Suppressed the normal plasma cortisol and increased GLU level	(Imanpoor et al., 2010)

Abbreviations: EU: eugenol, MS-222: tricaine methanesulfonate, PR: propofol.

Further research on stress reduction in aquatic animals using various anesthetics has been conducted (Soltani et al., 2010; Soltanian et al., 2018; Souza et al., 2019, 2018, 2017 b). For instance, concentrations of 100 and 300 $\mu\text{L L}^{-1}$ of two separate chemotypes of *Lippia alba* essential oil (citral EO-C and linalool EO-L) used to anesthetize silver catfish (*Rhamdia quelen*) did not increase plasma cortisol levels, however alterations in energetic metabolism were observed (Souza et al., 2018). Treatment with bactericidal *Melaleuca alternifolia* essential oil (TTO) nanoparticles in silver catfish reduced or prevented hepatic damage caused by *Pseudomonas aeruginosa* infection. Exposure to both essential oils (citral and linalool chemotypes), except for 100 $\mu\text{L L}^{-1}$ EO-L in most cases, decreased plasma cortisol levels compared to control and ethanol treatments (Souza et al., 2017 a). However, citral essential oil was found to be stressful for silver catfish and is not recommended, in contrast linalool essential oil is advised as it does not cause significant changes in the HPI axis (Souza et al., 2019). Additionally, different concentrations of *Lippia alba* essential oil (EOLA) admin-

istered in silver catfish did not provide protective effects under stressful conditions (Souza et al., 2015).

Effects on hematological and immunological parameters

Increasing the dose of clove oil and eugenol has been associated with a decrease in white blood cell (WBC) level in hybrid Amazon catfish (*Pseudoplatystoma reticulatum* $\times$ *Leiarius marmoratus*). This decrease may result from WBC death or reduced production during anesthetic administration. In anesthetized fish, neutrophil counts were significantly higher five hours after handling than measurements taken at zero and one hour ($P < 0.05$). Additionally, five hours post-stress, neutrophil levels in anesthetized fish were significantly greater than in control fish ($P < 0.05$). Other hematological parameters, such as erythrocytes, monocytes, lymphocytes, and total leukocytes, did not vary significantly across treatments. The increase in neutrophils enhances the primary line of defense in the immune system, indicating that eugenol may have enhanced the non-specific immune response in young Amazon catfish.

Table 2. Effects of eugenol on hematological and biochemical measurements in fish

Species	Anesthetic	Hematological effects	Biochemical effects	References
Amazon fish	Eugenol	Thrombocytes number $\uparrow$ Neutrophil values $\uparrow$	Cortisol level $\downarrow$ AST level $\downarrow$	(de Oliveira et al., 2019)
European eel (<i>Anguilla anguilla</i>)	0.0, 0.025, 0.05 and 0.075 ml of clove oil or 0.0, 2.250, 3.375 and 4.500 ml of eugenol	WBC counts $\downarrow$, RBC counts, hematocrit value $\uparrow$	GLU level $\uparrow$	(Altun et al., 2006)
<i>Rhamdia quelen</i>	40 and 50 mg L^{-1} eugenol	Not detected	Cortisol level $\downarrow$	(Corso et al., 2019)
Spotted knifejaw (<i>Oplegnathus punctatus</i>)	Clove oil (10, 20, 30, 35, 40) mg L^{-1}	WBC number $\uparrow$ RBC number and Hb and Hct levels $\uparrow$	The serum AST and ALT $\uparrow$ glucose levels $\uparrow$	(Jia et al., 2022)
<i>Piaractus brachipomus</i>	0, 25, 50, 75, 100 and 125 mg L^{-1} eugenol	50 mg L^{-1} of eugenol having little influence on hema- tological and biochemical parameters after handling.	50 mg L^{-1} of eugenol prevent an increase in plasma GLU	(Ferreira et al., 2021)
<i>Pelteobagrus fulvidraco</i>	0, 15, 20, 25 and 30 mg L^{-1}	Not detected	Blood cortisol level $\downarrow$ the levels of GLU and lysozyme $\uparrow$	(Xu et al., 2021)
Common carp (<i>Cyprinus carpio</i>)		No change in hematocrit value	GLU level $\uparrow$	(Velisek et al., 2005)
Russian sturgeon	Propofol (PR) and EU	Expressed changes in WBC system values of PCV, Hb increased	GLU, triacylglycerols, inorganic phos- phates, calcium and ammonia $\uparrow$	(Gomulka et al., 2015)
Siberian sturgeon (<i>Acipenser baerii</i>)	MS-222 and EU	Leukocyte number $\downarrow$	Calcium, inorganic phosphate, ALP activity $\downarrow$, albumin (ALB) and glucose concentrations and the activity of lactate dehydrogenase, aminotransferase (AST) and creatine kinase (unchanged), total protein, total globulin, triacylglycerol concentration and ALT activity $\uparrow$	(Gomulka et al., 2008)

Abbreviations: Hb: hemoglobin, Htc: hematocrit, RBS: red blood cells, WBC: white blood cells, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

Thrombocytes may play a role in bridging the innate immune system and the fish's stress response. Research suggests that thrombocytes have a high affinity for phagocytosis (Tavares-Dias et al., 2007) and contribute to blood coagulation, a process known as hemostasis, and are often observed at inflammation sites (Matushima and Matiano, 1996). Increased levels of thrombocytes and neutrophils in anesthetized fish might reflect a compensatory defense mechanism against stress-induced disorders (de Oliveira et al., 2019). Anesthetized fish showed significantly higher thrombocyte counts than the control group one hour after handling stress ($P < 0.05$).

In another study, the effects of eugenol at lower concentrations and for longer durations than typically used for anesthesia were examined in silver catfish. After five days of exposure, no significant changes in hematological parameters were observed. The only significant difference was that fish treated with ethanol had higher hematocrit levels compared to controls. Furthermore, EU-exposed silver catfish did not show significantly different phagocytic activity in coelomic cells compared to controls. The formation of superoxide anions (O_2^-) and serum myeloperoxidase activity were also unaffected by eugenol baths. There were no substantial differences in serum hemolytic activity or natural bacterial agglutination against pathogenic, formalin-inactivated *Aeromonas hydrophila* (Suttili et al., 2014).

Eugenol's impact on immunological markers can be variable. It has been described as both a stimulant and a suppressant of specific immunological events, depending on the context. For instance, clove oil anesthesia in rainbow trout (*Oncorhynchus mykiss*) decreased respiratory burst activity for 24 hours following anesthesia but did not affect alternative complement hemolytic activity (Soltani and Mirzargar, 2013).

Twenty-four hours after receiving clove oil anesthesia, rainbow trout exhibited signs of lymphopenia, neutrophilia, and elevated lysozyme activity (Mirzargar et al., 2011). In a study by Halder et al. (2011), clove oil suppressed cell-mediated immune responses while increasing humoral immunity and decreasing lymphokine release in rats. Eugenol reduced macrophage production of IL-1 and IL-6 (Rodrigues et al., 2009) and exerted immunomodulatory/anti-inflammatory effects by inhibiting lipopolysaccharide (LPS) action, possibly through NF- κ B inhibition (Bachiega et al., 2012). EU also demonstrated anti-inflammatory and antioxidant effects in fish infected with *Aeromonas* (Leem et al., 2011; Yogalakshmi et al., 2010). Sub-inhibitory levels of EU significantly reduced red blood cell hemolysis in fish infected by *Aeromonas* and extended the lifespan of infected silver catfish.

These findings suggest that the immunological and hematological parameters studied remained stable, indicating that EU can be used not only as an anesthetic or tranquilizer but also as a therapeutic agent to treat or prevent disease in fish. The use of eugenol, either alone or in combination with other drugs, is a priority for aqua-

culture research due to its diverse pharmacological and biological properties (Suttili et al., 2014).

In one study, fish exposed to clove oil and eugenol showed lower red blood cell (RBC) counts and hematocrit levels during the induction phase compared to the application phase. This decline may be attributed to hypoxia, as the blood may need more oxygen when fish are under anesthesia for long times. Anesthetics can induce stress in fish (İetinkaya and Şahin, 2005), and white blood cell counts, and blood glucose concentrations are reliable markers of physiological stress (Wagner et al., 2002). These markers typically rise due to stress but may decrease when exposed to certain chemicals, such as copper (Svobodova et al., 1994).

In comparative studies of different EU concentrations, it was found that 50 g mL⁻¹ of EU significantly reduced erythrocyte lysis in fish (35%) compared to controls and 25 g mL⁻¹ EU, which did not affect erythrocyte hemolytic activity (10%) against *Aeromonas hydrophila* supernatant. While it is unlikely that the effects of eugenol in *in vivo* studies are due to its bactericidal properties, sub-inhibitory concentrations of eugenol may reduce the virulence of *A. hydrophila* (Suttili et al., 2014) (Table 2).

Effects on biochemical parameters

In a study, control fish exhibited higher plasma cortisol levels than anesthetized fish (0 hours) (de Oliveira et al., 2019). Control fish also had elevated plasma AST (aspartate aminotransferase) and albumin levels. In the control group, levels of glucose, cortisol, total protein, and albumin significantly decreased 5 hours after stress manipulation. Additionally, thrombocyte and neutrophil counts were lower in the control group compared to the anesthetized group at 1 and 5 hours, respectively.

EU at a concentration of 53.0 mg L⁻¹ effectively anesthetized Amazon catfish and reduced post-handling stress by lowering mortality rates and improving biochemical and immune responses (de Oliveira et al., 2019). A concentration of 10.6 mg L⁻¹ of eugenol or less was ineffective for anesthesia in juveniles. For males of *Rhamdia quelen* (Quoy and Gaimard, *Pimelodidae*), EU induced drowsiness and anesthesia, with the minimum effective dose for sedation being 21.2 mg L⁻¹. Plasma levels of cortisol and glucose generally increase following manipulation, reflecting both primary and secondary stress responses (Jose et al., 2003). EU was effective in reducing plasma cortisol levels and thereby mitigating stress responses.

The control group exhibited the highest plasma glucose values within one hour of eugenol anesthesia, suggesting an adaptation to the new physiological demands imposed by the environment (Vickers, 2017). Stressed fish release catecholamines and corticosteroids, which stimulate gluconeogenesis and glycogenolysis, increasing blood glucose levels (Wedemeyer, 1970). However, fish sedated with 25 mg L⁻¹ EU also showed signs of hyperglycemia one hour after induction, indicating possible

restlessness and hypoxia (Boaventura et al., 2020; Teixeira et al., 2011).

A concentration of 50 mg L⁻¹ of EU was used to minimize handling stress effects. Compared to menthol, glucose levels decreased 24 hours after recovery. This observation may result from glycogenolysis and increased plasma glucose during initial fish handling or could be attributed to fasting, which may have depleted glycogen reserves and caused hyperglycemia (Ribeiro et al., 2019). In another study, blood plasma glucose levels rose post-anesthesia, indicating stress due to the treatment. Significant increases in blood plasma glucose levels were observed in *Cyprinus carpio* and *Oncorhynchus mykiss* 10 minutes after clove oil anesthesia (Velisek et al., 2005). Glucose levels normalized 24 hours later (Altun et al., 2006).

Additionally, the total protein and energy requirements of fish are correlated (Javed and Usmani, 2015). Notably, this study showed similar trends in plasma glucose concentration and total plasma protein levels. The increase in plasma total protein levels in non-anesthetized fish (Jose et al., 2003) likely indicates that proteins are mobilized for hepatic glycogenolysis, with cortisol playing a role in regulating hepatic metabolism. This response can be triggered by elevated cortisol concentrations in the control group during stress manipulation (0 hours) (Vijayan et al., 1994). Elevated albumin levels in non-anesthetized fish may also reflect increased hepatic metabolism (Javed and Usmani, 2015), as albumin can serve as an additional energy source to address the physiological stress caused by handling. Plasma AST (aspartate aminotransferase) is a critical marker for assessing fish welfare, as elevated levels may indicate hepatotoxicity and liver damage (El-Sayed et al., 2007). EU was therefore used in this study to prevent increases in plasma AST levels in anesthetized juveniles, thereby maintaining physiological stability and preventing liver damage. One hour after induction, fish anesthetized with 50 mg L⁻¹ of EU had the lowest triglyceride concentrations, likely due to changes in lipid movement and circulation between tissues and the liver, which are involved in cholesterol and phospholipid synthesis (Jun et al., 2015). The eugenol group also had lower overall ammonia nitrogen levels. Sedated yellow catfish exhibited a significant decrease in plasma cortisol levels compared to the control group, along with a substantial increase in lysozyme and glucose levels. After transportation, there was a notable increase in the expression of hsp70 and hsp90 mRNA in the liver of sedated yellow catfish. In conclusion, eugenol proved to be an effective anesthetic for yellow catfish. To reduce stress, it is recommended to use concentrations of 25–30 mg L⁻¹ for short-term anesthesia and 10 mg L⁻¹ for long-term transportation (Table 2).

Effects on growth parameters

Several studies have examined the impact of eugenol on growth parameters in various fish species. One study found that increasing EU levels significantly im-

proved fish growth and feed consumption (Adeshina et al., 2019). Another study reported that EU administration enhanced growth performance, feed utilization, antioxidant capacity, and innate immunity in African catfish (Abdel-Tawwab et al., 2018).

Effects on gene expression

The administration of EU as a stress-reducing agent affects the expression of several genes, as documented in various studies. For instance, research on crucian carp (*Carassius auratus*) showed that EU was associated with earlier activation of immune-related gene expression (Cao et al., 2019). Similarly, in studies on Chinese sea bass (*Lateolabrax maculatus*), EU administration was linked to increased expression of genes related to detoxification and xenobiotic biodegradation (Dong et al., 2020).

Effects on digestive physiology

EU also influences digestive physiology. In African catfish (*Clarias gariepinus*), dietary EU significantly increased intestinal villus length/width and absorption area in a dose-dependent manner (Adeshina et al., 2019). This improvement in intestinal structure led to enhanced ingestion, digestive activity, and nutrient absorption.

Negative impacts of eugenol on fish

While the EU is a widely used anesthetic agent due to its effectiveness and low cost, it may have negative impacts on fish. It is known to cause respiratory depression, which can lead to hypoxia and potentially death. Additionally, EU can alter enzyme activity and disrupt the ionic balance in fish, with potential long-term effects on health and survival, especially with repeated or prolonged exposure (Aydın and Barbas, 2020). EU can also induce seizures and be highly toxic to the fish brain. Therefore, its use for short-term anesthesia or euthanasia should be carefully monitored due to concerns regarding fish welfare and ethics (Barbas et al., 2021).

Another concern is the potential environmental impact of the EU. Although EU is a natural compound found in plants like cloves, its use as an anesthetic can lead to environmental accumulation through wastewater and runoff. This accumulation can adversely affect aquatic organisms, including fish and crustaceans, and impact water quality and ecosystem health. Thus, using eugenol as an anesthetic should be carefully regulated to minimize its environmental impact (Javahery et al., 2012).

Conclusion

Eugenol has emerged as a promising anesthetic in aquaculture due to its ability to minimize stress and its associated impacts on fish health and productivity. As a natural and effective agent, EU offers several benefits, including reducing cortisol levels and stabilizing hematological and biochemical parameters across various fish species. These items help to maintain immune function

and overall fish welfare, which is essential for sustainable aquaculture practices. Further research is necessary to optimize its application across different species and conditions, ensuring its role as a safe and efficient tool in fish farming.

Authors' contributions

Data curation: Bagheri and Yaghoobpour; investigation: Gholamhosseini and Adel; writing – review and editing: Bagheri.

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Declaration of competing interests

The authors declare that they have no conflicts of interest.

Data availability statement

The data supporting the findings of this study are available from Shiraz University. However, due to licensing restrictions, these data are not publicly available. Data can be accessed from the authors upon reasonable request and with permission from Shiraz University.

Ethical approval

This article does not contain any studies involving human participants. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

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