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Faculty of Fisheries and Protection of Waters

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Effects of herbal-derived products on growth, digestibility, body composition, immune and stress response in fish

Doctoral thesis

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Doctoral thesis by Mahyar Zare

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. Introduction

World fisheries and aquaculture are vital industries worldwide, producing 177.8 million tons of aquatic food, including finfish and crustaceans (90.3 million tons total capture and 87.5 million tons total aquaculture) through both inland and marine aquaculture in 2020. Moreover, it is expected to increase globally by 1.2% to reach 184.1 million tonnes in 2022 (FAO, 2020). As one of the primary producers of animal protein worldwide, aquaculture holds a critical position in addressing the global food demand (Anderson et al., 2017). Aquatic foods, particularly fish, are a low-cost and highly nutritive source of indispensable vitamins, proteins, micronutrients, and minerals that are fundamental to the maintenance of human health (Khalili Tilami and Sampels, 2018). The consumption of fish and other aquatic foods has been linked to a reduced risk of various chronic diseases, including cardiovascular disease, diabetes, and cognitive impairment (Goel et al., 2018). Furthermore, fish consumption can help combat undernutrition, particularly in low-income communities where other sources of protein may be scarce (Toppe et al., 2012). As such, the production and consumption of aquatic foods are of paramount importance to ensure global food security and to promote public health (Jennings et al., 2016). In recent years, the aquaculture industry has made significant strides in promoting food security, bolstering economies, and enhancing human welfare (Pradeepkiran, 2019). While the production of affordable food is undoubtedly essential, ensuring the safety and quality of the food supply is equally critical. In response to this challenge, the industry has made concerted efforts over the past few decades to minimize the use of antibiotics and other chemicals, prioritizing the promotion of food safety, quality, and the welfare of both fish and humans (Jennings et al., 2016).

The aquaculture industry is faced with several serious challenges, including environmental issues such as water pollution, the global water crisis, and the need to ensure future food security. Climate change also poses a considerable threat to the industry's sustainability and resilience (Gupta et al., 2021). To address the issue of water scarcity, Recirculating Aquaculture Systems (RAS) have emerged as a viable alternative to conventional aquaculture systems. RAS is an intensive fish culture system that requires less water and space. Despite the high energy demand and production costs associated with RAS, it has gained popularity due to the limited availability of fresh water, limited space for new sites, and water pollution issues (Ahmed and Turchini, 2021). The development and implementation of sustainable and efficient aquaculture practices, such as RAS, are crucial to ensuring the future availability of safe and healthy aquatic foods. The aquaculture industry must continue to adopt and improve such practices to meet the growing demand for food while minimizing the impact on the environment and human health (Aguilar-Manjarrez, 2022). As a result, RAS systems have been developed and implemented by major European aquaculture producers, including Denmark, Norway, and the Netherlands, as a suitable option to promote sustainable aquaculture production (Badiola et al., 2012).

Moreover, the aquaculture industry is also confronted with a critical challenge in managing and controlling the spread of infectious diseases among fish (Assefa and Abunna, 2018), and shrimp farms (Macusi et al., 2022). Antibiotic usage is one of the common practices employed to control the spread of diseases and reduce mortalities in aquaculture (Zhao et al., 2021). However, the overuse and misuse of antibiotics can lead to the development of antibiotic-resistant bacteria, which poses a significant threat to human health (Lulijwa et al., 2020). Moreover, antibiotics that enter water environments from industrial sources can alter the microbial community and ecosystem, leading to further ecological problems (Grenni et al., 2018). To mitigate bacterial resistance to antibiotics in water environments, several strategies can be employed, including optimizing disinfection techniques and improving wastewater and fertilization management. Separating human and animal-oriented bacteria from environmental bacteria is also recommended (Manaia et al., 2016).

In recent decades, feed additives including a wide variety of candidates such as pre and probiotics (Yilmaz et al., 2022), feed enzymes such as digestive enzymes (amylases, lipases, proteases, cellulases, and hemicellulases), and non-digestive enzymes (phytases, glucose oxidase, and lysozyme) (Liang et al., 2022), trace minerals including iron, copper, manganese, zinc, iodine, selenium, and cobalt (Chanda et al., 2015; Dawood et al., 2021), and popular medicinal plant-based additive including garlic (*Allium sativum*), onion (*Allium cepa*), nettle (*Urtica dioica*), ginger (*Zingiber officinale*), black seed (*Nigella sativa*), thyme (*Thymus vulgaris*), dill (*Anethum graveolens*), horse mint (*Mentha longifolia*), turmeric (*Curcuma longa*), cinnamon (*Cinnamonum zeylanicum*) and etc. (Tadese et al., 2022). Amongst all, medicinal plants derived products (raw material powders of different parts of the plant, extracts, and essential oils have been widely used in the aquaculture industry to achieve cost-effective, healthy, and readily available alternatives to antibiotics (Reverter et al., 2021). These additives have demonstrated the ability to promote growth, improve immunity, and reduce stress in aquatic animals (Chapter 2). Additionally, plant-derived products have the potential to act as alternatives to antibiotics, thus reducing the risk of antibiotic resistance (Reverter et al., 2021).

The utilization of medicinal plants for therapeutic purposes dates back over 5000 years to ancient Sumerian civilization, as evidenced by the discovery of twelve medicinal drug protocols consisting of 250 plant species containing alkaloids such as henbane (*Hyoscyamus albus* L.), poppy (*Papaver somniferum*), and mandrake (*Bryonia alba*) (Kelly, 2009). Medicinal plants have since been widely used to treat various illnesses, with different plant parts such as barks, seeds, fruit bodies, leaves, and other parts being utilized (Jima and Megersa, 2018). Recent scientific research has revealed that medicinal plants have several beneficial effects on fish, such as enhancing growth, improving immune response, influencing hematological and biochemical parameters, boosting antioxidant enzymes, increasing disease resistance, altering body proximate composition, and elevating digestive enzyme activity (Nya and Austin, 2011; Ngugi et al., 2015; Saleh et al., 2015; Adel et al., 2017; Adineh et al., 2020; Mbokane and Moyo, 2020; Mehrabi et al., 2020; Gholamhosseini et al., 2021). The potential benefits of medicinal plants in aquaculture have been investigated in various fish species, and the results have been promising (Reverter et al., 2021).

A total of approximately 50 medicinal plant species are globally recognized as being significant and utilized worldwide (Van Wyk and Wink, 2018). Medicinal plants are classified based on various factors, including parts used, climates, and therapeutic properties, such as antioxidant, antibacterial, antifungal, anti-inflammatory, antiviral, anticancer, and immunostimulatory effects (Babich et al., 2020). The therapeutic value of medicinal plants can be attributed to bioactive compounds such as phenolics, flavonoids, thymols, and saponins, which they contain (Yusoff et al., 2022). In aquaculture, medicinal plant additives have been administered through bathing or oral feeding, and the development of plant-based vaccines for aquaculture is a promising future goal in the industry (Su et al., 2021). Recent studies have shown positive effects of nettle (De Vico et al., 2018), tarragon (*Artemisia dracunculus*) (Mbokane and Moyo, 2020, Gholamhosseini et al., 2021), and garlic (Valenzuela-Gutiérrez et al., 2021) in different fish species.

Among all species of importance in aquaculture rainbow trout (*Oncorhynchus mykiss*) and European perch (*Perca fluviatilis*) were chosen as main target species in this study. Rainbow trout, a member of the Salmonidae family, is a cold-water fish species that is found on the Pacific coast of North America. It is one of the seventy salmonid species that inhabit cold-water environments, and the family also includes Atlantic salmon (*Salmo salar*), Arctic char

(*Salvelinus alpinus*), and Arctic grayling (*Thymallus arcticus*), (Hardy, 2002). Rainbow trout is widely cultured in inland aquaculture due to its high tolerance for a wide range of water parameters, such as temperature, salinity, pH, dissolved oxygen, and ammonia (Molony and Molony, 2001). The growth performance of aquaculture species is influenced by various factors, such as water temperature and food quality. Its optimal temperature range for growth is between 13 and 18 °C, with a range of approximately 1 to 20 °C (Hardy, 2002). Turkey is the major producer of rainbow trout globally; Norway and Chile are in the next rank. However, Italy, Denmark, Spain, and France are other main rainbow trout producers in the world (D'Agaro et al., 2022; Fao, 2022).

The European perch is a carnivorous fish species belonging to the percid family, primarily inhabiting the northern regions of Eurasia. Due to its high tolerance for different habitats, including brackish water, estuaries, and rivers, it has been identified as a suitable candidate for the aquaculture industry (Policar et al., 2019). Policar et al. (2015) recommended three main farming systems for perch, including the traditional extensive polyculture system, semi-intensive, and intensive culture under the RAS platform. In recent decades, the European perch has been demonstrated to be a suitable species for RAS and intensive fish culture (Fontaine and Teletchea, 2019). Water quality is crucial for physiology, feed intake, and growth rate of perch. Despite this, the European perch has a fast growth rate from larvae to market size (130–150 g) in 14 months at 22–24 °C (Policar et al., 2015).

Aquaculture production of rainbow trout and European perch is expanding in response to changing consumer preferences and decreasing fishing sources in the seas and oceans (Fao, 2022). However, to meet the growing demand for fish products, it is necessary to increase aquaculture production and explore new cultural methods, fish species, and feed additives to improve production performance (Toner, 2015). In this regard, the use of plant-based additives is a promising alternative to growth hormones, chemical drugs, and antibiotics, as they can enhance growth performance, improve immune function, and increase resistance against stress and pathogens (Reverter et al., 2021).

Intensive culture of perch requires careful management of environmental conditions, particularly with regard to oxygen concentration. The recommended optimum oxygen range for perch intensive culture is between 5 mg $O_2 \cdot L^{-1}$ (60% oxygen saturation) (Policar et al., 2015) and 6.8 mg $O_2 \cdot L^{-1}$ (68–72% oxygen saturation) (Stejskal et al., 2009). In Switzerland, the main country involved in the Eurasian perch market, the local popularity and price of the species are contributing factors to its success (Toner, 2015).

In order to ensure a sustainable and secure food supply for the future, it is important to prioritize the development of environmentally responsible aquaculture practices that do not rely on growth hormones, chemical drugs, and antibiotics (Chakraborty et al., 2014). This can be achieved through the continued exploration and utilization of plant-based additives, as well as the promotion of responsible management practices that prioritize animal welfare and environmental sustainability. However, it requires further research into the long-term effects of plant additives on fish growth and health, as well as on the environment (Jennings et al., 2016). The use of plant additives in aquafeed should not compromise the nutritional value and quality of the final product, nor should it have adverse effects on the ecosystem (Reverter et al., 2021). Thus, it is crucial to evaluate the environmental impact of plant-based additives on water quality, metabolism, and microbial communities of water (Napier and Betancor, 2023). Furthermore, there is a need to investigate the potential synergistic effects of combining plant additives with other feed additives or management practices to optimize fish growth and health. For example, the use of probiotics, prebiotics, or synbiotics may enhance the effectiveness of plant additives by promoting a healthy gut microbiome in fish (Rohani et al., 2022; Wei et al., 2022). Similarly, the use of feed enzymes may improve the digestibility and availability of nutrients in plant-based feeds, thereby increasing their efficacy (Liang et al., 2022).

In summary, while the use of plant-based additives in aquafeed is promising, there are still significant gaps in our understanding of long-term effects, and potential synergies with different plant additives (Rakholiya et al., 2013). Further research in these areas is necessary to ensure the sustainable and efficient use of plant-derived additives in aquaculture, and to meet the growing demand for high-quality and environmentally friendly fish products.

1.2. Plants in human lifeline

Plants have been utilized by humans for a variety of purposes, including clothing, flavorings, food, shelter, and medicine (Beyene et al., 2016). The use of medicinal plants for healing purposes has been a part of human history for centuries, and the search for natural compounds for therapeutic use continues to this day (Thomford et al., 2018). Knowledge of medicinal plants has been acquired through trial and error, utilizing different plant parts such as leaves, stems, seeds, fruits, and flowers for treatment (Roy et al., 2018). Medicinal plants are popular worldwide, and research indicates that approximately 50% of drugs are directly derived from plants, while one-fourth of prescribed medicines have their origin in tropical plants. In addition, the World Health Organization (WHO) has reported that 80% of the world's population uses plant-based medicines for their healthcare needs (Khan and Ahmad, 2019).

The use of medicinal plant for human is approved and they are known as antimicrobial (Manandhar et al., 2019), hyperlipidemia treatment (Samakar et al., 2022), antiproliferative effect on prostate cancer cells (Chrastina et al., 2018), controlling type 2 diabetes (Franco et al., 2018), liver diseases treatments (Bagherniya et al., 2018), treatment of cardiovascular disease (Adegbola et al., 2017), and boosting immunity against COVID-19 (Khanal et al., 2022). Moreover, medicinal plants have been used in animal nutrition for various purposes including improvement of growth performance, and immune system function in broilers (Aroche et al., 2018), mice (Kamel and El-Shinnawy, 2015), birds (Pliego et al., 2022), pigs (Lan et al., 2017), and fish (Tadese et al., 2022).

The use of medicinal plants as safe, cost-effective, and renewable resources has gained attention in various fields due to their potential benefits. For example, Tungmunnithum et al. (2018) reported that medicinal plants have a positive impact on human health and can serve as a substitute for synthetic drugs. Similarly, Hailemeskel and Fullas (2015) and Ziaei et al. (2020) found that medicinal plants have been used traditionally in different cultures to treat various diseases and improve health. The use of stinging nettle as an antidiabetic medicinal plant for humans has been approved (Hailemeskel and Fullas, 2015). Nettle is known for its hyperlipidemia treatment properties as it stabilizes lipid peroxidation and increases the oxidation of fatty acids in the liver. Additionally, it is used as a hypotensive treatment due to its pathways such as vasorelaxation (Samakar et al., 2022). The stinging nettle root extract has shown an antiproliferative effect on human prostate cancer cells (Tungmunnithum et al., 2018), and nettle supplements have demonstrated their ability to control blood sugar levels in type 2 diabetes mellitus patients (Ziaei et al., 2020). Furthermore, nettle extract has been shown to increase blood serum high-density lipoprotein (HDL) and decrease low-density lipoprotein (LDL) in male mice, although no significant changes in serum lipids in female mice were observed (Domjanić Drozdek et al., 2022).

1.3. Applications of medicinal plants in aquaculture

In this study we chose the following medicinal plants due to their bioactive compounds including nettle (Jan et al., 2017; Bhusal et al., 2022), tarragon (Aglarova et al., 2008), and garlic (Ozma et al., 2022) powder that showed acceptable outcome on growth performance, haematological parameters, biochemical parameters, immune response, and enhanced resistance to pathogens in fish (De Vico et al., 2018; Mbokane and Moyo, 2020; Gholamhosseini et al., 2021; Valenzuela-Gutiérrez et al., 2021; Chapter 2, 3 and 4).

1.4. Nettle (*Urtica dioica*)

Nettle has been found to have several beneficial effects on animal health and performance. Juma et al. (2015) reported that nettle leaves water extract improved the haematological and biochemical parameters in mice, and also protected the liver against acetaminophen toxicity. Furthermore, Safamehr et al. (2013) observed that nettle powder improved growth performance and blood biochemical parameters in broiler chickens. Ahmadipour and Khajali (2019) found that feeding broiler chickens with nettle powder significantly improved growth performance, body proximate composition, and liver antioxidant enzymes-related gene expression. Similarly, Behboodi et al. (2021) reported that nettle hydroalcoholic extract elevated nutritional factors, body organ characteristics, blood serum biochemical parameters, and antioxidant enzyme activity in broiler chickens. Nettle extracts have been reported to exhibit antidiabetic, antihypertensive, hypolipidemic, and antiproliferative effects in humans (Hailemeskel and Fullas, 2015; Ziaei et al., 2020; Samakar et al., 2022). Additionally, studies have shown that nettle extracts can improve growth performance, immune system function, and resistance to pathogens in various animals, including mice, birds (Pliego et al., 2022), pigs (Lan et al., 2017), and fish (Ngugi et al., 2015; Adel et al., 2017; Chapter 2 and 3). The diverse range of beneficial effects associated with nettle extracts highlights their potential as a safe, cost-effective, and available source for improving animal health and performance in aquaculture (De Vico et al., 2018).

1.5. Tarragon (*Artemisia dracunculus*)

Tarragon is a perennial plant with stems ranging from 40 to 150 cm in height. It belongs to the *Asteraceae* family and has a rich background in both culinary and remedial properties (Obolskiy et al., 2011). Tarragon is found in various regions, including Asia, the Baltic States, the Mediterranean countries, central Europe, northern Africa, and North America (Aglarova et al., 2008). Tarragon has been found to exhibit a wide range of pharmacological activities, including antibacterial, antifungal, antiplatelet, anti-inflammatory, hepatoprotective, antioxidant, antihyperglycemic, and hypolipidemic actions (Adewumi et al., 2020). These actions are attributed to the presence of various active compounds, including flavonoids, phenylpropanoids, chromones/coumarins, alkamides, and benzodiazepines (Obolskiy et al., 2011). Tarragon supplements have been used as an antidepressant in humans (Aydin et al., 2020). On the other hand, Asteraceae family contains different species of tarragon including white wormwood (*Artemisia absinthium*) (Yousefi et al., 2021), sweet wormwood (*Artemisia annua*) (Mirghaed et al., 2020) and African wormwood (*Artemisia afra*) (Mbokane and Moyo, 2020) due to their bioactive compounds have been popular in aquaculture last years (Obolskiy et al., 2011). Gholamhosseini et al. (2021) reported that methanol extract of tarragon improved growth performance, haemato-biochemical parameters, immunological parameters, and resistance against *Yersinia ruckeri*. Moreover, tarragon powder can improve haematological and immunological factors in rainbow trout (Chapter 3). Some studies suggest that combining plant powder and/or extract can have a synergistic effect on fish by increasing the range of bioactive compounds (Jahanjoo et al., 2018; Rashidian et al., 2022; Chapter 3). According to Ji et al. (2007a), the combination of (*Massa medicata fermentata*), (*Crataegi fructus*), (*Artemisia capillaries*), and (*Cnidium officinale*) led to increased growth performance and saline water stress resistance in Japanese flounder (*Paralichthys olivaceus*) juveniles, and improved immune system and resistance to the pathogen in red sea bream (*Pagrus major*) juvenile (Ji et al., 2007b). Jahanjoo et al. (2018) found that the mixture of garlic, ginger, and thyme improved the growth performance, immune system, as well as haematological and biochemical parameters of sobaity seabream (*Sparidentex hasta*) fry. In different studies, the mixture of medicinal plants has been found to promote growth performance, immune system, haematological and biochemical parameters, digestive enzyme activity, and resistance to bacterial challenges in common carp (Ghafarifarsani et al., 2021; Rashidian et al., 2022; Rudiansyah et al., 2022; Chapter 3).

1.6. Garlic (*Allium sativum*) powder

Garlic is a bulb and perennial plant that belongs to the Liliaceae family. It can grow up to 100 cm in height and produces hermaphrodite blooms that are pollinated by butterflies, moths, bees, and other insects (Tahsin, 2019). It is native to South Asia, Central Asia, and northeastern Iran (Saif et al., 2020) but has since spread to China, the Mediterranean area, and East Asia before spreading to Central and Southern Europe, Northern Africa, and Mexico (Singh and Nigam, 2017).

Garlic has been used as a valuable spice and medicinal plant in different cultures as a traditional medicine (Saif et al., 2020). Garlic is one such plant that has been found to have multiple health benefits, including being a growth promoter, natural antibacterial, antioxidant, antiviral, and antiparasitic agent (Valenzuela-Gutiérrez et al., 2021) which originated from its bioactive compounds such as flavonoids and sulphur-containing compounds which have medicinal possessions (Tahsin, 2019). It contains a wide range of bioactive components, including alliin, allicin, ajoene, S-allyl-cysteine, S-trityl-L-cysteine, diallyl sulfide, and S-allylmercaptocysteine, which have been extracted from the garlic bulb (Ozma et al., 2022).

Garlic has been used for the prevention and treatment of many diseases *in vitro* and has shown pharmacological properties, including cancer prevention, prevention of some solid tumors (Mondal et al., 2022), reduced cholesterol, and lipase (Xie et al., 2022).

Various types of garlic additives, including essential oil, fresh mash, extract, and powder, have been used in aquaculture (Metwally, 2009; Adineh et al., 2020). Among these, garlic powder is the most widely used type, administered orally, and included in fish diets at concentrations ranging from 5 to 40 g/kg of feed in aquaculture (Valenzuela-Gutiérrez et al., 2021). Garlic has been used as a feed additive to enhance growth performance, body proximate composition, digestive enzyme activity, antioxidant enzyme activity, and immune response, as well as to improve biochemical and hematological parameters and boost resistance against pathogens in various fish species, including European perch (Chapter 4), rainbow trout (Nya and Austin, 2011), brown trout (*Salmo caspicus*) (Zaefarian et al., 2017), sterlet (*Acipenser ruthenus*) (Lee et al., 2014; Lee et al., 2012), grass carp (*Ctenopharyngodon idella*) (Adineh et al., 2021), European seabass (*Dicentrarchus labrax*) (Saleh et al., 2015), and Asian seabass (*Lates calcarifer*) (Talpur and Ikhwanuddin, 2012).

Handling stress is common in aquaculture and can harm fish health and growth. Understanding its impact is important for reducing negative effects and improving welfare (Martos-Sitcha et al., 2020). Handling can cause physiological responses like changes in heart and respiration rates and blood chemistry, leading to immune suppression, increased disease susceptibility, and reduced growth rates (Abdel-Tawwab et al., 2019). Effects can vary by species, age, size, and reproductive status. Studying these effects is crucial for developing species-specific protocols that minimize stress and improve welfare. Ultimately, this can contribute to the sustainability of the industry (Ciji and Akhtar, 2021).

Garlic powder has been found to increase rainbow trout resistance to acute ammonia stress (Esmaeili et al., 2017), and enhance resistance to stocking density stress in grass carp (Adineh et al., 2021) as well. Moreover, European perch have been under different stressors including the domestication process (Gebauer et al., 2021), transport, and handling stress (Acerete et al., 2004) but there was not found report about the effect of plant additives on European perch resistance to stress. Regarding the positive and promising outcomes from the use of garlic supplements in aquaculture mentioned above and European perch importance in the aquaculture industry (Policar et al., 2015) evaluation of garlic powder in European perch can be useful and improve resistance against high-density and net handling stress (Chapter 4). Thus, in this study, garlic powder was evaluated for its effects on growth performance, apparent digestibility, body proximate composition, hematological indices, immunological, and biochemical parameters, as well as resistance against high-density and net handling stress challenges which is routine in hatcheries and RAS culture in European perch.

1.7. Objectives of the thesis

The present investigation aimed to examine the impact of incorporating medicinal plant powders as a dietary supplement in fish feed, with the following objectives in focus:

1. Investigating the effect of medicinal plant supplementation on the growth performance of fish.
2. Determining the impact of medicinal plant supplementation on the body proximate composition of fish.
3. Evaluating the hematological and biochemical blood parameters of fish-fed diets supplemented with medicinal plants.
4. Assessing the immunomodulatory effects of medicinal plant supplementation in fish.
5. Investigating the potential of diets supplemented with medicinal plants to mitigate cortisol and glucose responses induced by high-density and net handling stress.

References

Abdel-Tawwab, M., Monier M.N., Hoseinifar, S.H., Faggio, C., 2019. Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. Fish Physiology and Biochemistry 45, 997–1013.

Acerete, L., Balasch, J., Espinosa, E., Josa, A., Tort, L., 2004. Physiological responses in Eurasian perch (*Perca fluviatilis*, L.) subjected to stress by transport and handling. Aquaculture 237, 167–178.

Adegbola, P., Aderibigbe I., Hammed W., Omotayo T., 2017. Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: a review. American Journal of Cardiovascular Disease 7, 19.

Adel, M., Caipang, C.M.A., Dawood, M.A., 2017. Immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles following dietary administration of stinging nettle (*Urtica dioica*). Fish and Shellfish Immunology 71, 230–238.

Adewumi, O.A., Singh, V., Singh, G., 2020. Chemical composition, traditional uses and biological activities of artemisia species. Journal of Pharmacognosy and Phytochemistry 9, 1124–1140.

Adineh, H., Harsij M., Jafaryan H., Asadi M., 2020. The effects of microencapsulated garlic (*Allium sativum*) extract on growth performance, body composition, immune response and antioxidant status of rainbow trout (*Oncorhynchus mykiss*) juveniles. Journal of Applied Animal Research 48, 372–378.

Adineh, H., Naderi M., Nazer A., Yousefi M., Ahmadifar E., 2021. Interactive effects of stocking density and dietary supplementation with Nano selenium and garlic extract on growth, feed utilization, digestive enzymes, stress responses, and antioxidant capacity of grass carp, *Ctenopharyngodon idella*. Journal of the World Aquaculture Society 52, 789–804.

Aglarova, A., Zilfikarov, I., Severtseva, O., 2008. Biological characteristics and useful properties of tarragon (Artemisia dracunculus L.). Pharmaceutical Chemistry Journal 42, 81–86.

Aguilar-Manjarrez, J., 2022. FAO Presence at AquaSur 2022 in Chile. FAO Aquaculture Newsletter, 15–16.

Ahmadi, S., Awliaei, H., Haidarizadeh, M., Rostamzadeh, J., 2015. The effect of ethanolic extract of *Urtica dioica* leaves on high levels of blood glucose and gene expression of glucose transporter 2 (Glut2) in liver of alloxan-induced diabetic mice. Gene Cell Tissue 2, e30355.

Ahmadipour, B., Khajali F., 2019. Expression of antioxidant genes in broiler chickens fed nettle (*Urtica dioica*) and its link with pulmonary hypertension. Animal Nutrition 5, 264–269.

Ahmed, N., Turchini, G.M., 2021. Recirculating aquaculture systems (RAS): Environmental solution and climate change adaptation. Journal of Cleaner Production 297, 126604.

Alagawany, M., Farag M.R., Abdelnour S.A., Dawood M.A., Elnesr S.S., Dhama K., 2021. Curcumin and its different forms: A review on fish nutrition. Aquaculture 532, 736030.

Anderson, J.L., Asche F., Garlock T., Chu J., 2017. Aquaculture: Its role in the future of food, world agricultural resources and food security: International Food Security. Emerald Publishing Limited, pp. 159–173.

Aroche, R., Martinez, Y., Ruan, Z., Guan, G., Waititu, S., Nyachoti, C.M., Más, D., Lan, S., 2018. Dietary inclusion of a mixed powder of medicinal plant leaves enhances the feed efficiency and immune function in broiler chickens. Journal of Chemistry 2018.

Assefa, A., Abunna F., 2018. Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. Veterinary Medicine International 2018.

Aydin, T., Akincioglu, H., Gumustas, M., Gulcin, I., Kazaz, C., Cakir, A., 2020. Human monoamine oxidase (hMAO) A and hMAO B inhibitors from *Artemisia dracunculus* L. herniarin and skimmin: human mononamine oxidase A and B inhibitors from *A. dracunculus* L. Zeitschrift für Naturforschung C. 75, 459–466.

Babich, O., Sukhikh, S., Prosekov, A., Asyakina, L., Ivanova, S., 2020. Medicinal plants to strengthen immunity during a pandemic. Pharmaceuticals 13, 313.

Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquacultural Engineering 51, 26–35.

Bagherniya, M., Nobili, V., Blesso, C.N., Sahebkar, A., 2018. Medicinal plants and bioactive natural compounds in the treatment of non-alcoholic fatty liver disease: A clinical review. Pharmacological Research 130, 213–240.

Behboodi, H., Alemi, M., Baradaran, A., 2021. *Urtica dioica* extract – suitable dietary supplement influencing the growth body characteristics, antioxidant status, and serum biochemical parameters of broiler chickens. Comparative Clinical Pathology 30, 913–920.

Beyene, B., Beyene, B., Deribe, H., 2016. Review on application and management of medicinal plants for the livelihood of the local community. Journal of Resources Development and Management 22, 33–39.

Bhusal, K.K., Magar, S.K., Thapa, R., Lamsal, A., Bhandari, S., Maharjan, R., Shrestha, S., Shrestha, J., 2022. Nutritional and pharmacological importance of stinging nettle (*Urtica dioica* L.): A review. Heliyon. 8, e09717.

Chakraborty, S.B., Horn, P., Hancz, C., 2014. Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Reviews in Aquaculture 6, 1–19.

Chanda, S., Paul, B., Ghosh, K., Giri, S., 2015. Dietary essentiality of trace minerals in aquaculture – A review. Agricultural Reviews 36, 100–112.

Chrastina, A., Baron, V.T., Abedinpour, P., Rondeau, G., Welsh, J., Borgström, P., 2018. Plumbagin-loaded nanoemulsion drug delivery formulation and evaluation of antiproliferative effect on prostate cancer cells. BioMed Research International 2018.

Ciji, A., Akhtar, M.S., 2021. Stress management in aquaculture: A review of dietary interventions. Reviews in Aquaculture 13, 2190–2247.

D'Agaro, E., Gibertoni, P., Esposito, S., 2022. Recent trends and economic aspects in the rainbow trout (*Oncorhynchus mykiss*) sector. Applied Sciences 12, 8773.

Dawood, M.A., Alagawany, M., Sewilam, H., 2021. The role of zinc microelement in aquaculture: A review. Biological Trace Element Research 1–13.

De Vico, G., Guida, V., Carella, F., 2018. *Urtica dioica* (Stinging Nettle): a neglected plant with emerging growth promoter/immunostimulant properties for farmed fish. Frontiers in Physiology 9, 285.

Domjanić Drozdek, S., Odeh, D., Đikić, D., Gračan, R., Oršolić, N., Dragović-Uzelac, V., Feher-Turković, L., Dragičević, P., Landeka Jurčević, I., 2022. The effects of nettle extract consumption on liver PPARs, SIRT1, ACOX1 and blood lipid levels in male and female C57Bl6 Mice. Nutrients. 14, 4469.

Esmaeili, M., Kenari, A.A., Rombenso, A., 2017. Immunohematological status under acute ammonia stress of juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) fed garlic (*Allium sativum*) powder-supplemented meat and bone meal-based feeds. Comparative Clinical Pathology 26, 853–866.

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.

FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO.

Fontaine, P., Teletchea, F., 2019. Domestication of the Eurasian perch (*Perca fluviatilis*), Animal domestication. IntechOpen. 1838811338.

Franco, R.R., da Silva Carvalho, D., de Moura, F.B.R., Justino, A.B., Silva, H.C.G., Peixoto, L.G., Espindola, F.S., 2018. Antioxidant and anti-glycation capacities of some medicinal plants and their potential inhibitory against digestive enzymes related to type 2 diabetes mellitus. Journal of Ethnopharmacology 215, 140–146.

Gebauer, T., Gebauer, R., Palińska-Żarska, K., Císař, P., Movchan, O., Tomášek, O., Prokešová, M., Matoušek, J., Hliwa, P., Król, J., 2021. Assessment of behavioural and physiological traits as indicators of suitability for European perch aquaculture. Aquaculture 544, 737048.

Ghafarifarsani, H., Hoseinifar, S.H., Adorian, T.J., Ferrigolo, F.R.G., Raissy, M., Van Doan, H., 2021. The effects of combined inclusion of *Malvae sylvestris*, *Origanum vulgare*, and *Allium hirtifolium boiss* for common carp (*Cyprinus carpio*) diet: Growth performance, antioxidant defense, and immunological parameters. Fish & Shellfish Immunology 119, 670–677.

Gholamhosseini, A., Hosseinzadeh, S., Soltanian, S., Banaee, M., Sureda, A., Rakhshaninejad, M., Ali Heidari, A., Anbazpour, H., 2021. Effect of dietary supplements of *Artemisia dracunculus* extract on the haemato-immunological and biochemical response, and growth performance of the rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research 52, 2097–2109.

Goel, A., Pothineni, N.V., Singhal, M., Paydak, H., Saldeen, T., Mehta, J.L., 2018. Fish, fish oils and cardioprotection: promise or fish tale? International journal of molecular sciences 19, 3703.

Grenni, P., Ancona, V., Caracciolo, A.B., 2018. Ecological effects of antibiotics on natural ecosystems: A review. Microchemical Journal 136, 25–39.

Gupta, P., Singh, J., Verma, S., Chandel, A.S., Bhatla, R., 2021. Impact of climate change and water quality degradation on food security and agriculture, Water Conservation in the Era of Global Climate Change. Elsevier, pp. 1–22.

Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27, 1–93.

Hailemeskel, B., Fullas F., 2015. The use of *Urtica dioica* (stinging nettle) as a blood sugar lowering herb: a case re-port and a review of the literature. Diabetes Research Open Journal. 1, 123–127.

Hardy, R.W., 2002. Rainbow trout, *Oncorhynchus mykiss*. Nutrient requirements and feeding of finfish for aquaculture, 184–202.

Jahanjoo, V., Yahyavi M., Akrami R., Bahri A.H., 2018. Influence of adding garlic (*Allium sativum*), Ginger (*Zingiber officinale*), thyme (*Thymus vulgaris*) and their combination on the growth performance, haematoimmunological parameters and disease resistance to *Photobacterium damselae* in sobaity sea bream (*Sparidentex hasta*) Fry. Turkish Journal of Fisheries and Aquatic Sciences 18, 633–645.

Jan, K.N., Zarafshan K., Singh S., 2017. Stinging nettle (*Urtica dioica* L.): a reservoir of nutrition and bioactive components with great functional potential. Journal of Food Measurement and Characterization 11, 423–433.

Jennings, S., Stentiford G.D., Leocadio A.M., Jeffery K.R., Metcalfe J.D., Katsiadaki I., Auchterlonie N.A., Mangi S.C., Pinnegar J.K., Ellis T., 2016. Aquatic food security: insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment. Fish and Fisheries 17, 893–938.

Ji, S.-C., Jeong G.-S., Gwang-Soon, I., Lee, S.-W., Yoo, J.-H., Takii, K., 2007a. Dietary medicinal herbs improve growth performance, fatty acid utilization, and stress recovery of Japanese flounder. Fisheries Science 73, 70–76.

Ji, S.-C., Takaoka, O., Jeong, G.-S., Lee, S.-W., Ishimaru, K., Seoka, M., Takii, K., 2007b. Dietary medicinal herbs improve growth and some non-specific immunity of red sea bream Pagrus major. Fisheries Science 73, 63–69.

Jima, T.T., Megersa, M., 2018. Ethnobotanical study of medicinal plants used to treat human diseases in Berbere District, Bale Zone of Oromia Regional State, South East Ethiopia. Evidence-Based Complementary and Alternative Medicine 2018.

Juma, K., Maina, S., Muriithi, J., Mwangi, B., Mworia, K., Mwonjoria, M., Ngeranwa, J., Mburu, D., 2015. Protective effects of *Urtica dioica* and cimetidine on liver function following acetaminophen induced hepatotoxicity in mice. Journal of Developing Drugs 4, 1–7.

Kamel, R.O., El-Shinnawy, N.A., 2015. Immunomodulatory effect of garlic oil extract on *Schistosoma mansoni* infected mice. Asian Pacific Journal of Tropical Medicine 8, 999–1005.

Kelly, K., 2009. The history of medicine. New York: Facts On File. Inc.

Khalili Tilami, S., Sampels S., 2018. Nutritional value of fish: lipids, proteins, vitamins, and minerals. Reviews in Fisheries Science & Aquaculture 26, 243–253.

Khan, M.S.A., Ahmad, I., 2019. Herbal medicine: current trends and future prospects, New look to phytomedicine. Elsevier, pp. 3–13.

Khanal, P., Duyu, T., Patil, B., Dey, Y.N., Pasha, I., Wanjari, M., Gurav, S.S., Maity, A., 2022. Network pharmacology of AYUSH recommended immune-boosting medicinal plants against COVID-19. Journal of Ayurveda and Integrative Medicine 13, 100374.

Lan, R., Park, J., Lee, D., Kim, I., 2017. Effects of *Astragalus membranaceus, Codonopsis pilosula* and allicin mixture on growth performance, nutrient digestibility, faecal microbial shedding, immune response and meat quality in finishing pigs. Journal of Animal Physiology and Animal Nutrition 101, 1122–1129.

Lee, D.-H., Lim, S.-R., Han, J.-J., Lee, S.-W., Ra, C.-S., Kim, J.-D., 2014. Effects of dietary garlic powder on growth, feed utilization and whole body composition changes in fingerling sterlet sturgeon, *Acipenser ruthenus*. Asian-Australasian Journal of Animal Sciences 27, 1303.

Lee, D.-H., Ra C.-S., Song Y.-H., Sung K.-I., Kim J.-D., 2012. Effects of dietary garlic extract on growth, feed utilization and whole body composition of juvenile sterlet sturgeon (*Acipenser ruthenus*). Asian-Australasian Journal of Animal Sciences 25, 577.

Liang, Q., Yuan M., Xu L., Lio E., Zhang F., Mou H., Secundo F., 2022. Application of enzymes as a feed additive in aquaculture. Marine Life Science & Technology 4, 208–221.

Lulijwa, R., Rupia E.J., Alfaro A.C., 2020. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. Reviews in Aquaculture 12, 640–663.

Macusi, E.D., Estor, D.E.P., Borazon, E.Q., Clapano, M.B., Santos, M.D., 2022. Environmental and socioeconomic impacts of shrimp farming in the philippines: A critical analysis using PRISMA. ustainability 14, 2977.

Manaia, C.M., Macedo, G., Fatta-Kassinos, D., Nunes, O.C., 2016. Antibiotic resistance in urban aquatic environments: can it be controlled? Applied microbiology and biotechnology 100, 1543-1557.

Manandhar, S., Luitel, S., Dahal, R.K., 2019. *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. Journal of Tropical Medicine 2019.

Martos-Sitcha, J.A., Mancera, J.M., Prunet, P., Magnoni, L.J., 2020. Welfare and stressors in fish: Challenges facing aquaculture. Frontiers Media SA, pp. 162.

Mondal, A., Banerjee, S., Bose S., Mazumder, S., Haber, R.A., Farzaei, M.H., Bishayee, A., 2022. Garlic constituents for cancer prevention and therapy: From phytochemistry to novel formulations. Pharmacological Research 175, 105837.

Mbokane, E.M., Moyo, N.A.G., 2020. Effect of dietary *Artemisia afra* on growth, some innate immunological parameters in *Clarias gariepinus* challenged with *Aeromonas hydrophila.* Aquaculture International. 28, 539–553.

Mehrabi, Z., Firouzbakhsh, F., Rahimi-Mianji, G., Paknejad, H., 2020. Immunity and growth improvement of rainbow trout (*Oncorhynchus mykiss*) fed dietary nettle (*Urtica dioica*) against experimental challenge with *Saprolegnia parasitica*. Fish & Shellfish Immunology 104, 74–82.

Metwally, M., 2009. Effects of garlic (*Allium sativum*) on some antioxidant activities in tilapia nilotica (*Oreochromis niloticus*). World Journal of Fish and Marine Sciences. 1, 56–64.

Mirghaed, A.T., Paknejad, H., Mirzargar, S.S., 2020. Hepatoprotective effects of dietary Artemisia (*Artemisia annua*) leaf extract on common carp (*Cyprinus carpio*) exposed to ambient ammonia. Aquaculture 527, 735443.

Molony, B., Molony, B., 2001. Environmental requirements and tolerances of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) with special reference to Western Australia: a review. Department of Fisheries, Government of Western Australia, pp.

Napier, J.A., Betancor, M.B., 2023. Engineering plant-based feedstocks for sustainable aquaculture. Current Opinion in Plant Biology 71, 102323.

Ngugi, C.C., Oyoo-Okoth, E., Mugo-Bundi, J., Orina, P.S., Chemoiwa, E.J., Aloo, P.A., 2015. Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*. Fish & Shellfish Immunology 44, 533–541.

Nya, E.J., Austin, B., 2011. Development of immunity in rainbow trout (*Oncorhynchus mykiss,* Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. Fish & Shellfish Immunology 30, 845–850.

Obolskiy, D., Pischel I., Feistel B., Glotov N., Heinrich M., 2011. *Artemisia dracunculus* L. (tarragon): a critical review of its traditional use, chemical composition, pharmacology, and safety. Journal of Agricultural and Food Chemistry 59, 11367–11384.

Ozma, M.A., Abbasi, A., Ahangarzadeh Rezaee, M., Hosseini, H., Hosseinzadeh, N., Sabahi, S., Noori, S.M.A., Sepordeh, S., Khodadadi, E., Lahouty, M., 2022. A critical review on the nutritional and medicinal profiles of garlic's (*Allium sativum* L.) Bioactive Compounds. Food Reviews International 1–38.

Pliego, A.B., Tavakoli, M., Khusro, A., Seidavi, A., Elghandour, M.M., Salem, A.Z., Márquez-Molina, O., Rene Rivas-Caceres R., 2022. Beneficial and adverse effects of medicinal plants as feed supplements in poultry nutrition: A review. Animal Biotechnology 33, 369–391.

Policar, T., Samarin, A.M., Mélard, C., 2015. Culture methods of Eurasian perch during ongrowing, Biology and Culture of Percid Fishes. Springer, pp. 417–435.

Policar, T., Schaefer, F.J., Panana, E., Meyer, S., Teerlinck, S., Toner, D., Żarski, D., 2019. Recent progress in European percid fish culture production technology – tackling bottlenecks. Aquaculture International 27, 1151–1174.

Pradeepkiran, J.A., 2019. Aquaculture role in global food security with nutritional value: a review. Translational Animal Science 3, 903–910.

Rakholiya, K.D., Kaneria, M.J., Chanda, S.V., 2013. Medicinal plants as alternative sources of therapeutics against multidrug-resistant pathogenic microorganisms based on their antimicrobial potential and synergistic properties. Fighting multidrug resistance with herbal extracts, essential oils and their components, 165–179.

Rashidian, G., Shahin, K., Elshopakey, G.E., Mahboub, H.H., Fahim, A., Elabd, H., Prokić, M.D., Faggio, C., 2022. The dietary effects of nutmeg (*Myristica fragrans*) extract on growth, hematological parameters, immunity, antioxidant status, and disease resistance of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila*. Journal of Marine Science and Engineering. 10, 325.

Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P., Caruso, D., 2021. Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. Reviews in Aquaculture. 13, 537–555.

Rohani, M.F., Islam, S.M., Hossain, M.K., Ferdous, Z., Siddik, M.A., Nuruzzaman, M., Padeniya, U., Brown, C., Shahjahan, M., 2022. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. Fish & Shellfish Immunology 120, 569–589.

Roy, A., Jauhari, N., Bharadvaja, N., 2018. 6 Medicinal plants as. Anticancer Plants 2, 109.

Rudiansyah, M., Abdelbasset, W.K., Jasim S.A., Mohammadi, G., Dharmarajlu, S.M., Nasirin, C., Jalil, A.T., Opulencia, M.J.C., Abid, M.K., Naserabad, S.S., 2022. Beneficial alterations in growth performance, blood biochemicals, immune responses, and antioxidant capacity of common carp (*Cyprinus carpio*) fed a blend of *Thymus vulgaris, Origanum majorana,* and *Satureja hortensis* extracts. Aquaculture 555, 738254.

Safamehr, A., Fallah, F., Nobakht, A., 2013. Growth performance and biochemical parameters of broiler chickens on diets consist of chicory (*Cichorium intybus*) and nettle (*Urtica dioica*) with or without multi-enzyme. Iranian Journal of Applied Animal Science 3, 131–137.

Saif, S., Hanif, M.A., Rehman, R., Riaz, M., 2020. Garlic, Medicinal plants of south asia. Elsevier, pp. 301–315.

Saleh, N.E., Michael, F.R., Toutou, M.M., 2015. Evaluation of garlic and onion powder as phyto-additives in the diet of sea bass (*Dicentrarcus labrax*). Egyptian Journal of Aquatic Research 41, 211–217.

Samakar, B., Mehri S., Hosseinzadeh H., 2022. A review of the effects of *Urtica dioica* (nettle) in metabolic syndrome. Iranian Journal of Basic Medical Sciences. 25, 543–553.

Singh, A.K., Nigam, S., 2017. Ancient alien crop introductions integral to Indian agriculture: An overview, Proc. Indian National Science Academy, pp. 549–568.

Stejskal, V., Kouřil, J., Valentová, O., Hamáčková, J., Policar, T., 2009. Size-related oxygen consumption and ammonia excretion of Eurasian perch (*Perca fluviatilis* L.) reared in a recirculating system. Aquaculture Research 41, 135–142.

Su, H., Yakovlev, I.A., Van Eerde, A., Su, J., Clarke, J.L., 2021. Plant-produced vaccines: future applications in aquaculture. Frontiers in Plant Science 12, 718775.

Tadese, D.A., Song, C., Sun, C., Liu, B., Liu, B., Zhou, Q., Xu, P., Ge, X., Liu, M., Xu, X., 2022. The role of currently used medicinal plants in aquaculture and their action mechanisms: A review. Reviews in Aquaculture 14, 816–847.

Tahsin, M.H., 2019. Survey on medicinal plants used by traditional healer for skin diseases at Pirganji upazila, Rangpur, Bangladesh. Hamdard University.

Talpur, A.D., Ikhwanuddin, M., 2012. Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). Aquaculture 364, 6–12.

Thomford, N.E., Senthebane, D.A., Rowe, A., Munro, D., Seele, P., Maroyi, A., Dzobo, K., 2018. Natural products for drug discovery in the 21st century: innovations for novel drug discovery. International Journal of Molecular Sciences 19, 1578.

Toner, D., 2015. The market for Eurasian perch, Biology and culture of percid fishes. Springer, pp. 865–879.

Toppe, J., Bondad-Reantaso, M.G., Hasan, M.R., Josupeit, H., Subasinghe, R.P., Halwart, M., James, D., 2012. Aquatic biodiversity for sustainable diets: The role of aquatic foods in food and nutrition security. Sustainable Diets and Biodiversity, 94–101.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., Yangsabai, A., 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. Medicines 5, 93.

Valenzuela-Gutiérrez, R., Lago-Lestón, A., Vargas-Albores, F., Cicala, F., Martínez-Porchas, M., 2021. Exploring the garlic (*Allium sativum*) properties for fish aquaculture. Fish Physiology and Biochemistry 47, 1179–1198.

Van Wyk, B.-E., Wink M., 2018. Medicinal plants of the world. CABI, pp. 1786393255.

Wei, L.S., Goh, K.W., Abdul Hamid, N.K., Abdul Kari, Z., Wee, W., Van Doan, H., 2022. A mini-review on co-supplementation of probiotics and medicinal herbs: Application in aquaculture. Frontiers in Veterinary Science 9, 869564.

Xie, C., Gao, W., Li, X., Luo, S., Chye, F.Y., 2022. Study on the hypolipidemic properties of garlic polysaccharide in vitro and in normal mice as well as its dyslipidemia amelioration in type2 diabetes mice. Food Bioscience 47, 101683.

Yilmaz, S., Yilmaz, E., Dawood, M.A., Ringø, E., Ahmadifar, E., Abdel-Latif, H.M., 2022. Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. Aquaculture 547, 737514.

Yousefi, M., Zahedi, S., Reverter, M., Adineh, H., Hoseini, S.M., Van Doan, H., El-Haroun, E.R., Hoseinifar, S.H., 2021. Enhanced growth performance, oxidative capacity and immune responses of common carp, *Cyprinus carpio* fed with *Artemisia absinthium* extract-supplemented diet. Aquaculture 545, 737167.

Yusoff, I.M., Taher, Z.M., Rahmat, Z., Chua, L.S., 2022. A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. Food Research International 157, 111268.

Zaefarian, A., Yeganeh, S., Adhami, B., 2017. Dietary effects of garlic powder (*Allium sativum*) on growth, blood indices, carcass composition, and lysozyme activity in brown trout (*Salmo caspius*) and resistance against *Yersinia ruckeri* infection. Aquaculture International 25, 1987–1996.

Ziaei, R., Foshati, S., Hadi, A., Kermani, M.A.H., Ghavami, A., Clark, C.C., Tarrahi, M.J., 2020. The effect of nettle (*Urtica dioica*) supplementation on the glycemic control of patients with type 2 diabetes mellitus: A systematic review and meta-analysis. Phytotherapy Research 34, 282–294.

Zhao, H., Wei, L., Li, H., Zhang, M., Cao, B., Bian, J., Zhan, S., 2021. Appropriateness of antibiotic prescriptions in ambulatory care in China: a nationwide descriptive database study. The Lancet Infectious Diseases 21, 847–857.

CHAPTER 2

NETTLE (*URTICA DIOICA*) IN THE FISH DIET AS A PLANT ADDITIVE

Zare, M., Esmaeili N., Paolacci S., Stejskal V., 2023. Nettle (*Urtica dioica*) additive as a growth promoter and immune stimulator in fish. Aquaculture Nutrition 2023, 8261473.

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Review Article

Nettle (*Urtica dioica*) Additive as a Growth Promoter and Immune Stimulator in Fish

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Aquaculture will become an important food production sector for humans in the coming decades. However, disease outbreaks can be considered a significant obstacle to continually developing aquaculture. Plant powders and extracts are natural feed additives that, due to their bioactive compounds, including phenolic compounds, proteins, vitamins, and minerals, have antistress, antiviral, antibacterial, and antifungal effects on fish. One of these herbs is nettle (*Urtica dioica*), which has a long history of being used in traditional medicine. While it has been widely investigated in mammalian medicine, few studies have been done on aquaculture species. The positive effect of this herb on the growth performance, hematology, blood biochemistry, and immune system of fish species has been observed. When fish were exposed to pathogens, nettle-fed fish showed a higher survival rate and less stress than controls. Therefore, this literature review is aimed at reviewing the use of this herb in fish diets and its impacts on growth performance, hematology, blood biochemistry, liver enzymes, immune system stimulation, and challenges with pathogens.

1. Introduction

Aquaculture plays a vital role in supplying protein-rich food to humans [1]. Finfish and crustacean production occurs in different environments, from inland aquaculture to marine aquaculture, with annual production of more than 82 million tons in 2018. The aquaculture production demands hormones, antibiotics, and growth promoters to secure high and aquatic sustainable production [2]. Antibiotics are consumed widely in aquaculture, and in a recent report, at least 67 different kinds of antibiotics were used in 2008 and 2018 [3]. The use of antibiotics and other chemical substances in aquaculture raises concerns about food safety and quality, public health, the environment, and fish and human welfare [4]. These substrates cause pathogen resistance to antibiotics, and their accumulation in aquatic species is harmful when humans consume them [5]. Therefore, researchers and industry are seeking sustainable alternative options. Among the different options, herbal additives are sustainable and green alternatives to hormones and antibiotics used in fish feeds [6] to improve growth and health. Bioactive compounds extracted from herbs such as alkaloids, tannins, flavonoids, terpenoids, glycosides, and phenols [7] are responsible for positive outcomes, such as improved growth and health in fish [6]. Thus, in the last decades, several kinds of literature have reviewed the effects of different plant additives on aquatic species feeds [6, 8, 9] as well as livestock animals [10, 11].

Nettle (*Urtica dioica*) has been used for centuries among different medicinal herbs. Nettle is one of the nine plants invoked in the pagan Anglo-Saxon Nine Herbs Charm, recorded in 10th-century traditional medicine. This herb has a long list of features and benefits for humans [12]. For example, it has been known as a natural antihistamine diuretic, arthritis, anemia, hay fever blood purifier, astringent, antioxidant, antimicrobial, and anti-inflammatory agent [13]. Nettle is frequently used for allergies, anemia, a sluggish thyroid, restoring adrenal tonics, arthritis, and rheumatism [14]. The first reports about the use of nettle in the livestock diet are related to poultry in 1936 [15] and pig in 1974 [16]. Nettle supplements can result in a positive effect on growth, feed utilisation, blood lipids, antioxidants, and the immune system of poultry nutrition [17]. Administration of 1% nettle powder into broiler feed improved weight gain (WG), FCR, and liver antioxidant enzyme, including superoxide dismutase (SOD) and catalase (CAT), in the liver after 42 days of feeding trial [18]. In contrast, nettle powder and extract at levels 0.5 and 1% in broiler feed did not significantly improve WG, FCR, feed intake, blood serum cholesterol, triglyceride, glucose, alanine aminotransferase (ALT), and alkaline phosphatase (ALP) [19]. Studies related to supplementing nettle in the diets of pigs are limited. In the only available research, the use of 0.05 and 0.1% of nettle water extract in diets of pigs significantly improved fat and protein deposition and n-3 omega 3, decreased blood serum high-density lipoproteins, and had no change in cholesterol compared to the control [20]. But fishes live in different environments, temperatures, and salinity and are carnivorous, omnivorous, and herbivorous with a different physiology of digestion. Due to the long list of benefits, researchers in aquaculture have tested this herb in fish diets. Based on the literature, Awad and Austin [21] first tried 1% nettle in rainbow trout (*Oncorhynchus mykiss*). The results showed significant improvement in the immune system, including respiratory burst, lysozyme enzyme activity, bactericidal activity, and a high survival rate (96%), compared to the control (32%) after a 10-day challenge with *Aeromonas hydrophila*. In addition, 1% nettle increased hematocrit (Ht%) compared to the control. Nettle, as a medicinal plant additive, has been evaluated in fish feeds and has improved growth performance, proximate body composition [22], immune system, and resistance against pathogens [23, 24] and preserved fish fillet quality [25]. The literature has also briefly reviewed the effect of nettle on fish growth performance and the immune system [26]. There is a gap in knowledge regarding the effect of nettle on other parameters, such as proximate body composition, hematology, serum biochemistry, liver enzymes, immune stimulant, and resistance against pathogens. Moreover, we included the latest nettle articles in the present review. Therefore, this review paper is aimed at providing a comprehensive and updated view of the effect of nettle on aquaculture species and a short view of animals' monogastric farms (poultry and pig) to be compared with fish.

2. Botanical Characteristics and Bioactive Compounds of Nettle

Nettle, common nettle, or stinging nettle, which are all synonyms, is a perennial plant that belongs to the family of *Urticaceae*. It is found in moderate regions of Europe, Asia, North Africa, and North America up to 1,800 m altitude [27]. It is 2 to 4 meters high and creates sharp leaves [13], and both sides of the leaves are covered with tiny trichomes [27]. Moreover, it has white to yellowish flowers [13] and reddish or yellowish stems. A wide range of compounds was isolated from aerial parts, leaves, stems, flowers, seeds, and roots of nettle [27]. Essential amino acids alanine (6.4 μg/g), 4-aminobutyrate (GABA, 3.4 μg/g), glutamic acid (6.0 μg/g), isoleucine (1.9 μg/g), leucine (7.6 μg/g), phenylalanine (2.4 μg/g), proline (5.7 μg/g), tyrosine (1.2 μg/g), and valine (3.0 μg/g) are available in this herb. Organic acids, including malic acid (12.8 μg/g), acetic acid (29.3 μg/g), citric acid (3.6 μg/g), succinic acid (2.6 μg/g), and formic acid (6.9 μg/g), were found in the leaves. Some lipids, including steroids and triterpenoids 3-b-sitosterol (40 mg/kg), sitosterol-b-D-glucoside (30 mg/kg), (60-O-palmitoyl)-sitosterol-3-O-b-D-glucoside (5.6 mg/kg), 24R-ethyl-5a-cholestane3b,6a-diol (3.3 mg/kg), 7b-hydroxy-sitosterol (2.4 mg/kg), 7a-hydroxy sitosterol (2.9 mg/kg), 7b-hydroxy-sitosterol-b-D-glucoside (2.7 mg/kg), and 7a-hydroxy-sitosterol-b-D-glucoside (2.0 mg/kg), were detected in roots. Fatty acids, terpenoids, phenolic compounds, and volatile compounds were discovered in leaves, roots, seeds, and stems [27]. Furthermore, the nettle supplement (powder/extract) contains a wide range of vitamins and minerals, including both fat-soluble vitamins A, D, E, and K and water-soluble vitamins, such as vitamin C and the B vitamins (B1, B2, B3, and B9) (Supplementary table 1) [28].

In contrast, some antinutritional compounds, such as phytates (phytic acid), oxalates, and saponins, were found in nettle, limiting their supplementation level in diets and negatively impacting fish growth and health. These compound levels depend on the nettle's harvesting location and growth stage [29]. Therefore, the optimum dosage is required to positively affect growth and other physiological parameters; however, it has a certain level of antinutritional effects. These antinutritional effects cause many problems in fish and other animals at excessive levels. For example, phytic acid inhibits the absorption of minerals, such as iron, calcium, manganese, and zinc, in the digestive system of animals [30]. Oxalates can bind with calcium or magnesium in feed to shape insoluble calcium or magnesium oxalate. Then, these salts sediment in the kidneys of animals [31]. Saponins are steroid or triterpenoid glycosides and have shown positive effects, including membrane permeabilizing, immunostimulant, and anticarcinogenic effects in animals. By contrast, excessive levels of saponins demonstrated adverse effects, such as hypocholesterolemia, hypoglycemia, damaged digestion of protein, reduced uptake of vitamins and minerals in the gut, and negative impact on growth, feed intake, and reproduction in humans and animals [32]. Measuring these antinutritional parameters in diets supplemented with nettle can help formulate an optimum diet for fish. These compounds in any herb may react differently, and more research on the antinutritional parameters of nettle is required.

3. Preparation of Nettle Powder and Extract

Nettle, like other herbs, has been used in fish diets in two forms: powder and extract. Nettle powder is prepared from aerial parts [33, 34], leaves [22], and stems [35] similar to other herbs. Collected nettle samples were washed with distilled water [24]. Then, the samples were dried in the shade at room temperature [22, 36] and/or in the shade under ambient conditions under a flow of dry air [37]. The benefits of using powdered herbs like nettle include the fact that it is acceptable for fish, is widely available and cheap, and can easily be added to diets. However, sometimes, adding nettle to diets in its powdered form can be difficult to digest for carnivorous species. The antinutritional parameters are sometimes related to fiber, and using powders can cause them to be present in diets more than in the extract. On the other hand, with extracting, we exclude some antinutritional parameters and fiber, making them easier to digest for all fishes. Another benefit of extraction is that only a small amount of nettle is required for diets (usually less than 500 mg/kg). Based on our previous experience, to provide 1 g of dried extract, 200–250 g of powder is required. Therefore, more bioactive compounds can reach fish bodies by formulating diets with the extract. However, the extraction process requires laboratory equipment and is costly. These issues can be generalized to all herbs. Nettle extract, like other herbs, is sourced from aerial parts [38] and roots [39]. In the ethanol method, nettle samples are well-dried in a dark room and ground into a fine powder using a grinder. The prepared powder is then mixed in a 1 L volumetric flask at a ratio of 1 : 5 (w/v) with 80% ethanol for 48 h using a shaker. Then, the mixture is filtered using a Büchner funnel and filter paper. The primary extract is distilled via rotary distillation at 80°C for 4 h [33]. In the methanol method, after drying under the shade and grinding into good powder, a 50 g sample is percolated with 1 L methanol (40%) for 3 days and then filtered. The solvent is evaporated, and finally, the concentrate is dissolved in 50 mL of deionized water at 50°C [40]. In the acetone method, the nettle sample is dried for 7 days in the shade. The dried sample is ground into a fine powder. The sample is maintained in flasks with acetone (1 : 10 w/v). Subsequently, the extract is filtered through Whatman No. 1 filter paper, and the extract is concentrated under low pressure at 45°C with the pressure of a vacuum evaporator [38]. Depending on the extraction method and drying temperature, the final nutritional composition of herbs can differ slightly. A comprehensive investigation is required to determine how other extraction methods can affect the nutritional compositions of nettle species. Furthermore, it is interesting to see how fish growth and health can be affected by extraction methods. According to the documented results, both nettle powder (ranging from 0.5% to 12%) [21, 22, 35, 37] and extract with different methods (ranging from 0.01% to 3%) [23, 33, 40] in fish diets demonstrated positive effects on fish growth performance and health status [26]. This shows that both powder and extract of herbs could be effective and that extraction did not add any positive effects. However, their level in diet also matters. Herb powder or extract of nettle, like other herbal medicines, can be dried at room temperature and can be stored in the fridge, preferably at +4°C, until used.

4. Effects of Nettle on Growth Performance and Proximate Composition of Fish

Growth performance is the most crucial trait in aquaculture. In normal situations and supposing the same FCR, a system that can grow fish more effectively can be more sustainable and profitable. Farming fish with improved feed efficiency is necessary to reduce production costs and achieve sustainability in the aquaculture industry [41]. Therefore, considering that both phenotypes (growth and phenotype) are crucial for aquaculture, a balance between growth and feed efficiency should be considered. This makes all the more sense when we study the effect of supplements on fish diets, and only increased growth or feed efficiency is insufficient. Nettle is one of these supplements, and in the related literature, both growth and feed efficiency have been discussed. There can be a considerable difference between fish being fed with apparent satiation or limited feeding. The preferable approach is apparent satiation to test palatability when herbs such as nettle are added to diets. The experimental period and size of fish are also other important parameters that can directly affect growth and feed efficiency and should be considered in nettle and other herb studies.

Rainbow trout is the most common cold-water species that has been studied in supplementing herbal medicines to fish diets. This is because rainbow trout is one of the most commonly farmed aquaculture species in Iran and Turkey, where studies on the effects of herbs such as nettle on aquaculture are prevalent [16, 18, 33]. Administration of 3% nettle leaf powder to fish diet (7 g) significantly increased WG and specific growth rate (SGR) after 8 weeks of feeding based on apparent satiation with no change in FCR [22]. This herb significantly improved whole-body proximate protein and ash in this study and decreased fat content. Supplementation of 0.5% nettle powder showed the same result, as FCR was improved as well, in rainbow trout (12 g) after 4 [37] and 8 [24] weeks when fish were fed 3% of body weight. In another study, Awad et al. [42] administered 1% and 2% nettle leaf powder in rainbow trout diets (18 g) and saw an increase in WG and SGR and no change in FCR after an 8-week feeding trial; however, no significant effect on proximate body composition was observed. Studies on extracted nettle revealed the same trend and recorded improved WG, SGR, and FCR. For example, supplementing 3% nettle sourced from aerial parts with ethanol extract in fish (42 g) after 8 weeks of feeding based on 3% of body weight [33] and nettle methanol extract at 0.01% and 0.05% for 4 weeks based on apparent satiation [40] resulted in this output.

Studies that have investigated the effect of herbal supplements on warm-water species are usually found in freshwater. Iran, India, Egypt, and China are the top countries that focus on the effect of herbs, such as nettle, in warm-water species. As most of the studied fish species in this category are omnivorous, the inclusion of high levels of plant-based ingredients, such as nettle, did not impair fish growth. Nettle powder (5% of diet) improved WG, SGR, and FCR in Victoria Labeo (*Labeo victorianus*: 25 g) after 16 weeks of feeding based on 3-4% body weight [36]. In contrast, when beluga (*Huso huso*: 30 g) was fed with a diet including 1% nettle powder, it did not show a significant effect on growth performance after a 60-day feeding trial based on apparent satiation [34]. Similarly, administration of nettle acetone extract (0.015% of diet) after 3 months of feeding based on apparent satiation did not increase growth performance in electric yellow cichlid (*Labidochromis caeruleus*: 0.6 g) [38]. Use of 0.01% and 0.05% methanolic extract of nettle in female convict cichlid (1.3 g) (*Amatitlania nigrofasciata*) diet for 56 days significantly improved WG, SGR, feed intake, and FCR compared to the control [43].

It should be noted that the duration of administration is an essential factor. For example, a 4-week experiment is not enough to draw a solid conclusion. The fish's response to diet can be changed with adaptation (feeding more after 4 weeks or even less feeding due to a lack of palatability). The size of the fish was quite similar in these studies. The reason for these improvements in the abovementioned studies can be biocomponents such as flavonoids (kaempferol, quercetin, and quercitrin) [14], which can increase feed palatability [33, 42] and decrease FCR significantly [24, 37]. Moreover, Awad et al. [42] showed that the administration of 2% nettle powder in a fish diet after a two-month feeding trial could stimulate the stomach enzyme pepsin and other protease activities. Pepsin is an acidic protease found in gastric juice and is a main digestive enzyme that helps feed protein digestion by breaking down proteins into smaller peptides [44]. There is a long list of herbs that improve digestive enzymes and, eventually, growth [45, 46], which is not within the scope of this article. Nettle contains essential amino acids [47], and phenolic compounds, such as vanillic acid (which is used as a flavoring agent), could increase feed palatability [27]. The improved feed intake and digestibility in both cold-water and warm-water species can be explained by these reasons. Interestingly, an increased hemoglobin (Hb) level in experimental groups fed with a nettle supplement could improve growth performance in rainbow trout [22, 24, 37]. Hb, which has a vital function in transporting oxygen [48], affects the metabolism and growth performance of fish [49]. This protein plays a key role in cold-water species, which require more oxygen. The mechanism by which nettle improves Hb is an interesting topic for future studies. In short, different mechanisms can improve growth and feed efficiency by adding nettle to fish diets. For example, its bioactive compounds [27]; ability to modify colonization of various bacteria species in fish gut [50] and improve immunity [22, 40]; antioxidant activities [35]; hematological parameters [22, 33, 37]; blood metabolites [36, 43]; improved lipid, carbohydrate, and protein metabolism [22]; and stimulating digestive enzymes [42] are among them. In most cases, herbs such as nettle cannot change body composition, as the protein and lipid contents of experimental diets are similar. The effects of nettle supplementation on growth performance and proximate body composition of fish species are depicted in Table 1.

5. Effects of Nettle on Hematological and Blood Biochemical Parameters in Fish

Hematological parameters, including red blood cells (RBCs), white blood cells (WBCs) count, Hb, and Ht indices, have been known as bioindicators of fish health. Measuring hematological parameters is cheap and assessable and, therefore, commonly measured. The RBC of an organism is responsible for the carrying capacity of dissolved oxygen [51]. WBC is the first line of defense against pathogens [52]. The percentage of Ht shows the volume percentage of RBCs in the blood [53]. Hb is included in RBCs, and it is a globular protein with a quaternary structure constituted of four globin chains (two alpha and two betas) and a prosthetic group called heme bound to each one [54]. Given the diverse critical roles of blood, measuring blood parameters may provide a reliable picture of fish metabolism and health status [55]. As nettle can affect the growth and immunity of fish, it can directly impact hematological parameters. However, this question is raised whether the improved hematological parameter is the reason or/and result of improved growth and immunity.

Collecting blood from rainbow trout is easy, even in fish with a size less than 50 g. Therefore, we can see that hematological parameters have commonly been measured in rainbow trout studies. Nettle has positively affected hematological parameters in this fish species. Administration of 1% and 1.5% of nettle leaf powder in rainbow trout (10-11 g) diet increased WBCs, RBCs, Ht, and Hb after 4- [37] and 8-week [24] feeding trials. Mehrabi et al. [24] reported increased lymphocytes, decreased monocytes, and no significant effects on neutrophil percent by adding nettle to diets. By contrast, supplementing 3% nettle leaf powder in rainbow trout (7 g) diet for 8 weeks increased WBC, neutrophil, and monocyte counts but reduced RBC, Ht, and Hb levels [22]. In another report, Awad and Austin [21] mentioned that 1% nettle leaf powder in rainbow trout diet with an initial average body weight of 15 g after 14 days of feeding did not improve RBC, WBC, Hb, monocyte, and neutrophil counts but increased Ht significantly. It can be hypothesized that 14 days was not enough to change these parameters in rainbow trout. Administration of nettle sourced from aerial parts with ethanol extract in the diet of this species (42 g) at levels of 1%, 2%, and 3% after 4 weeks of feeding did not increase RBCs, WBC count, and monocyte percent but, at a dietary level of 3%, improved Ht, Hb, lymphocyte, and neutrophil percent [33]. However, after an 8-week feeding trial, nettle in this study improved RBC, WBC, Ht, Hb, and neutrophil percent [33]. This is further evidence that the experimental period plays a key role in making changes to blood parameters.

Warm-water species that were experimented with nettle supplementation usually have less blood, and collecting blood from them is a bit harder than from cold-water ones. For example, carps, acipenser, Victoria Labeo, and cichlids belong to this category. Supplementation of 1%, 2%, and 5% nettle leaf powder in Victoria Labeo (25 g) after 4 and 16 weeks in a feeding trial showed significant improvement of RBC, WBC, Ht, and Hb compared to controls [36]. In another study [35], nettle leaf powder (12% of diet) in beluga (204 g) after 4 weeks improved RBC, Ht, and Hb, but WBC, lymphocyte, and eosinophil levels did not show a significant difference. By contrast, in this research, after 8 weeks of the feeding trial, WBC, RBC, Ht, and Hb increased significantly in the nettle group, but not lymphocyte and eosinophil. These results indicate that, again, the time of administration plays a key role. Nobahar et al. [34] studied the effect of 1% nettle powder on beluga, with an initial average body weight

Table 1: Effects of nettle (*Urtica dioica*) supplement on growth performance and body proximate composition of fish.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Beneficial effects on growth performance and body proximate composition in fish significantly	References
Rainbow trout (*Oncorhynchus mykiss*)	Leaves	Dried in shade at room temperature	Powder	1% and 3%	8 weeks	Phenolic compounds and amino acids	3%	FW, WG, WG%, SGR%, body proximate protein, fat, and ash	[16]
Rainbow trout	Leaves	Dried in shade	Powder	0.5, 1, 1.5%	8 weeks	Capsaicin, carvacrol, cinnamaldehyde	0.5%, 1%, and 1.5%	FW, WG, SGR%, FCR	[18]
Rainbow trout	Leaves	Dried in the shade under ambient conditions by a flow of dry air	Powder	0.5, 1, 1.5%	4 weeks	Acetylcholine, histamine, serotonin, salicylic acid, lecithin, carotenoid, flavonoids, sterols, and thymol	0.5, 1%, and 1.5%	FW, WG, SGR%, FCR, and PER	[31]
Rainbow trout	Leaves	Dried leaves from a healthy food shop in Edinburgh	Powder	1% and 2%	2 months	Amino acids	1% and 2%	FW, WG, and SGR%	[47]
Rainbow trout	Leaves	Dried leaves obtained from Good n' Natural, Nuneaton, UK	Powder	1%	14 days	Not reported	—	Growth performance and body proximate composition were not evaluated	[15]
Victoria Labeo (*Labeo victorianus*)	Leaves	Air-dried	Powder	1%, 2%, and 5%	16 weeks	Vitamin A, vitamin B, vitamin B12, acetylcholine, serotonin, formic acid, salicylic acid, lecithin, carotenoids, flavonoids, sterols, and thymol	1%, 2%, and 5%	FW, WG%, SGR%, and FCR	[30]
Beluga (*Huso huso*)	Leaves and stem	Not reported	Powder	3%, 6%, and 12%	8 weeks	Flavonoids, polysaccharides, carotenoids, lignans, lectins, amino acids, minerals (especially iron) and vitamins (vitamin C), tannins, formic acid, salicylic acid, carvacrol, and thymol	12%	Growth performance and body proximate composition were not evaluated	[29]
Beluga	Not reported		Powder	1%	60 days		1%		[94]

Table 1: Continued.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Beneficial effects on growth performance and body proximate composition in fish significantly	References
		Oven-dried was obtained from the supermarket				Acetylcholine, serotonin, formic acid, minerals, amino acid, lecithin, carotenoid, flavonoids, sterols, tannins, and vitamins		Growth performance was not significant	
Electric yellow cichlid (*Labidochromis caeruleus*)	Not reported	Dried under shadow	Extract	0.015%	3 months	Carotenoids	0.015%	Growth performance was not significant	[32]
Convict cichlid (*Cichlasoma nigrofasciatum*)	Root	Ethanol extract	Extract	0.02% and 0.03%	8 weeks	Flavonoids	—	Reduced FW, WG%, and SGR%	[33]
Rainbow trout	Aerial parts	Ethanol extract	Extract	1%, 2%, and 3%	8 weeks	Carvacrol, flavonoids, carotenoids proteins, iron, and vitamins	3%	WG, SGR%, FCR, and SR%	[28]
Rainbow trout	Not reported	Methanol extract	Extract	0.01% and 0.05%	30 days	Quercetin	0.01% and 0.5%	FW, WG, SGR%, and FCR	[34]
Rainbow trout	Not reported	Nettle extract (quercetin) obtained from the laboratory of professor Amani Awaad	Extract	0.1%, 0.5%, and 1%	14 days	Quercetin	1%	Growth performance and body proximate composition were not evaluated	[17]
Convict cichlid (*Amatitlania nigrofasciata*)	Leaves	Methanol extract	Extract	0.01% and 0.05%	56 days	Capsaicin and carvacrol	0.01%	WG, SGR, FI, FCR, and PER	[48]

TABLE 2: Effects of nettle supplement on hematological parameters of fish.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Beneficial effects on hematological parameters of fish significantly	References
Rainbow trout	Leaves	Dried in shade at room temperature	Powder	1% and 3%	8 weeks	Phenolic compounds and amino acids	1% and 3%	WBC, RBC, Hct, Hb, monocyte, and lymphocyte	[16]
Rainbow trout	Leaves	Dried in shade	Powder	0.5, 1, 1.5%	8 weeks	Capsaicin, carvacrol, cinnamaldehyde	0.5%, 1%, and 1.5%	WBC, RBC, Hct, Hb, and lymphocyte	[18]
Rainbow trout	Leaves	Dried in the shade under ambient condition by a flow of dry air	Powder	0.5%, 1%, 1.5%	4 weeks	Acetylcholine, histamine, serotonin, salicylic acid, lecithin, carotenoid, flavonoids, sterols, and thymol	0.5%, 1%, and 1.5%	WBC, RBC, Hct, and Hb	[31]
Rainbow trout	Leaves	Dried leaves from a health food shop in Edinburgh	Powder	1% and 2%	2 months	Amino acids	1% and 2%	Hematology was not evaluated	[47]
Rainbow trout	Leaves	Dried leaves obtained from Good n' Natural, Nuneaton, UK	Powder	1%	14 days	Not reported	1%	Hb	[15]
Victoria Labeo	Leaves	Air-dried	Powder	1%, 2%, and 5%	16 weeks	Vitamin A, vitamin B, vitamin B12, acetylcholine, serotonin, formic acid, salicylic acid, lecithin, carotenoids, flavonoids, sterols, and thymol	1%, 2%, and 5%	WBC, RBC, Hct, Hb, and neutrophil	[30]
Beluga	Leaves and stem	Not reported	Powder	3%, 6%, and 12%	8 weeks	Flavonoids, polysaccharides, carotenoids, lignans, lectins, amino acids, minerals (especially iron) and vitamins (vitamin C), tannins, formic acid, salicylic acid, carvacrol, and thymol	12%	WBC, RBC, Hct, Hb, and neutrophil	[29]
Beluga	Not reported	Oven-dried was obtained from supermarket	Powder	1%	60 days	Acetylcholine, serotonin, formic acid, minerals, amino acid, lecithin,	1%	Hct	[94]

Table 2: Continued.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Beneficial effects on hematological parameters of fish significantly	References
						carotenoid, flavonoids, sterols, tannins, and vitamins			
Electric yellow cichlid	Not reported	Dried under shadow	Extract	0.015%	3 months	Carotenoids	0.015%	Hematology was not evaluated	[32]
Convict cichlid	Root	Ethanol extract	Extract	0.02% and 0.03%	8 weeks	Flavonoids	—	Hematology was not evaluated	[33]
Rainbow trout	Aerial parts	Ethanol extract	Extract	1%, 2%, and 3%	8 weeks	Carvacrol, flavonoids, carotenoids proteins, iron, and vitamins	3%	WBC, RBC, Ht, Hb, lymphocyte, and neutrophil	[28]
Rainbow trout	Not reported	Methanol extract	Extract	0.01% and 0.05%	30 days	Quercetin	0.01% and 0.5%	Hematology was not evaluated	[34]
Rainbow trout	Not reported	Nettle extract (quercetin) obtained from the laboratory of professor Amani Awaad	Extract	0.1%, 0.5%, and 1%	14 days	Quercetin	1%	Hematology was not evaluated	[17]
Convict cichlid	Leaves	Methanol extract	Extract	0.01% and 0.05%	56 days	—	—	Hematology was not evaluated	[48]

Table 3: Effects of nettle supplement on blood biochemical parameters of fish.

Fish species	Nettle (*U. dioica*) part used	Preparation of nettle (*U. dioica*) supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Beneficial effects on blood biochemical parameters on fish significantly	References
Rainbow trout	Leaves	Dried in shade at room temperature	Powder	1% and 3%	8 weeks	Phenolic compounds and amino acids	1% and 3%	Albumin and total protein	[16]
Rainbow trout	Leaves	Dried in shade	Powder	0.5, 1, 1.5%	8 weeks	Capsaicin, carvacrol, cinnamaldehyde	0.5%, 1%, and 1.5%	Albumin, total protein, and globulin	[18]
Rainbow trout	Leaves	Dried in the shade under ambient condition by a flow of dry air	Powder	0.5, 1, 1.5%	4 weeks	Acetylcholine, histamine, serotonin, salicylic acid, lecithin, carotenoid, flavonoids, sterols, and thymol	0.5%, 1%, and 1.5%	Albumin, total protein, and globulin	[31]
Rainbow trout	Leaves	Dried leaves from a health food shop in Edinburgh	Powder	1% and 2%	2 months	Amino acids	—	Blood biochemistry was not evaluated	[47]
Rainbow trout	Leaves	Dried leaves obtained from Good n' Natural, Nuneaton, UK	Powder	1%	14 days	Not reported	1%	No significant effect	[15]
Victoria Labeo	Leaves	Air-dried	Powder	1%, 2%, and 5%	16 weeks	Vitamin A, vitamin B, vitamin B12, acetylcholine, serotonin, formic acid, salicylic acid, lecithin, carotenoid, flavonoids, sterols, and thymol	1%, 2%, and 5%	Albumin, total protein, triglyceride, cholesterol, cortisol, and glucose	[30]
Beluga	Leaves and stem	Not reported	Powder	3%, 6%, and 12%	8 weeks	Flavonoids, polysaccharides, carotenoids, lignans, lectins, amino acids, minerals (especially iron) and vitamins (vitamin C), tannins, formic acid, salicylic acid, carvacrol, and thymol	12%	Total protein, triglyceride, and cholesterol	[29]
Beluga	Not reported	Oven-dried was obtained from supermarket	Powder	1%	60 days	Acetylcholine, serotonin, formic acid, minerals, amino acid, lecithin,	—	Blood biochemistry was not evaluated	[94]

Table 3: Continued.

Fish species	Nettle (*U. dioica*) part used	Preparation of nettle (*U. dioica*) supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Beneficial effects on blood biochemical parameters on fish significantly	References
						carotenoid, flavonoids, sterols, tannins, and vitamins			
Electric yellow cichlid	Not reported	Dried under shadow	Extract	0.015%	3 months	Carotenoids	—	Blood biochemistry was not evaluated	[32]
Convict cichlid	Root	Ethanol extract	Extract	0.02% and 0.03%	8 weeks	Flavonoids	—	Blood biochemistry was not evaluated	[33]
Rainbow trout	Aerial parts	Ethanol extract	Extract	1%, 2%, and 3%	8 weeks	Carvacrol, flavonoids, carotenoids proteins, iron, and vitamins	3%	Total protein and triglyceride	[28]
Rainbow trout	Not reported	Methanol extract	Extract	0.01% and 0.05%	30 days	Quercetin	—	Blood biochemistry was not evaluated	[34]
Rainbow trout	Not reported	Nettle extract (quercetin) obtained from the laboratory of professor Amani Awaad	Extract	0.1%, 0.5%, and 1%	14 days	Quercetin	1%	Total protein	[17]
Convict cichlid	Leaves	Methanol extract	Extract	0.01% and 0.05%	56 days	Carvacrol	0.01%	Glucose, triglyceride, cholesterol, total protein, and albumin	[48]

Table 4: Effects of nettle supplement in fish liver enzyme activity.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Measured liver enzymes in blood serum	Significant effect	References
Beluga	Leaves and stem	Not reported	Powder	3%, 6%, and 12%	8 weeks	Flavonoids, polysaccharides, carotenoids, lignans, lectins, amino acids, minerals (especially iron) and vitamins (vitamin C), tannins, formic acid, salicylic acid, carvacrol, and thymol	—	ALT, AST, and ALP	No significant	[29]
Rainbow trout	Aerial parts	Ethanol extract	Extract	1%, 2%, and 3%	8 weeks	Carvacrol, flavonoids, carotenoids proteins, iron, and vitamins	—	ALT, AST, and LDH	No significant	[28]

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase.

Table 5: Effects of nettle supplement on immunological parameters of fish.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Beneficial effects of immunological parameters of fish significantly	References
Rainbow trout	Leaves	Dried in shade at room temperature	Powder	1% and 3%	8 weeks	Phenolic compounds and amino acids	1% and 3%	Lysozyme activity and immunoglobulin	[16]
Rainbow trout	Leaves	Dried in shade	Powder	0.5%, 1%, and 1.5%	8 weeks	Capsaicin, carvacrol, cinnamaldehyde	0.5%, 1%, and 1.5%	Respiratory burst activity, lysozyme activity, and ACH50	[18]
Rainbow trout	Leaves	Dried in the shade under ambient condition by a flow of dry air	Powder	0.5%, 1%, and 1.5%	4 weeks	Acetylcholine, histamine, serotonin, salicylic acid, lecithin, carotenoid, flavonoids, sterols, and thymol	0.5%	Lysozyme activity	[31]
Rainbow trout	Leaves	Dried leaves from a health food shop in Edinburgh	Powder	1% and 2%	2 months	Amino acids	—	Immunology was not evaluated	[47]
Rainbow trout	Leaves	Dried leaves obtained from Good n' Natural, Nuneaton, UK	Powder	1%	14 days	Not reported	1%	Lysozyme, respiratory burst, and bactericidal activity	[15]
Victoria Labeo	Leaves	Air-dried	Powder	1%, 2%, and 5%	16 weeks	Vitamin A, vitamin B, vitamin B12, acetylcholine, serotonin, formic acid, salicylic acid, lecithin, carotenoid, flavonoids, sterols, and thymol	1%, 2%, and 5%	Total immunoglobulin (Ig), respiratory burst, and lysozyme activity	[30]
Beluga	Leaves and stem	Not reported	Powder	3%, 6%, and 12%	8 weeks	Flavonoids, polysaccharides, carotenoids, lignans, lectins, amino acids, minerals (especially iron) and vitamins (vitamin C), tannins, formic acid, salicylic acid, carvacrol, and thymol	12%	Total immunoglobulin (Ig) and respiratory burst activity	[29]
Beluga	Not reported		Powder	1%	60 days	Acetylcholine, serotonin, formic acid, minerals,	1%	Immunology was not evaluated	[94]

Table 5: Continued.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Beneficial effects of immunological parameters of fish significantly	References
		Oven-dried was obtained from supermarket				amino acid, lecithin, carotenoid, flavonoids, sterols, tannins, and vitamins			
Electric yellow cichlid	Not reported	Dried under shadow	Extract	0.015%	3 months	Carotenoids	0.015%	Immunology was not evaluated	[32]
Convict cichlid	Root	Ethanol extract	Extract	0.02% and 0.03%	8 weeks	Flavonoids	—	Immunology was not evaluated	[33]
Rainbow trout	Aerial parts	Ethanol extract	Extract	1, 2, and 3%	8 weeks	Carvacrol, flavonoids, carotenoids proteins, iron, and vitamins	3%	Immunoglobulin (IgM), complement 3, complement 4, lysozyme, and respiratory burst activity	[28]
Rainbow trout	Not reported	Methanol extract	Extract	0.01% and 0.05%	30 days	Quercetin	0.01% and 0.05%	Phagocytic, lysozyme, and myeloperoxidase activity	[34]
Rainbow trout	Not reported	Nettle extract (quercetin) obtained from the laboratory of professor Amani Awaad	Extract	0.1%, 0.5%, and 1%	14 days	Quercetin	0.5% and 1%	Lysozyme, myeloperoxidase, bactericidal, antiprotease activity, and immunoglobulin (IgM)	[17]
Convict cichlid	Leaves	Methanol extract	Extract	0.01% and 0.05%	56 days	Quercetin	0.01%	C3, C4, and IgM	[48]

Table 6: Effects of nettle supplement on pathogen challenge and survival rate in fish.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Pathogen species	Pathogen challenge period	Survival rate % in nettle groups after challenge significantly	Survival rate % in control after challenge	References
Rainbow trout	Leaves	Dried in shade at room temperature	Powder	1% and 3%	8 weeks	Phenolic compounds and amino acids	—	—	—	—		[16]
Rainbow trout	Leaves	Dried in shade	Powder	0.5, 1, 1.5%	8 weeks	Capsaicin, carvacrol, cinnamaldehyde	0.5%	*Saprolegnia parasitica*	3 weeks	86%	56%	[18]
Rainbow trout	Leaves	Dried in the shade under ambient condition by a flow of dry air	Powder	0.5%, 1%, and 1.5%	4 weeks	Acetylcholine, histamine, serotonin, salicylic acid, lecithin, carotenoid, flavonoids, sterols, and thymol	—	—	—	—	—	[31]
Rainbow trout	Leaves	Dried leaves from a healthy food Shop in Edinburgh	Powder	1% and 2%	2 months	Amino acids	—	—	—	—	—	[47]
Rainbow trout	Leaves	Dried leaves obtained from Good n' Natural, Nuneaton, UK	Powder	1%	14 days	Not reported	1%	*Aeromonas hydrophila*	10 days	96%	32%	[15]
Victoria Labeo	Leaves	Air-dried	Powder	1%, 2%, and 5%	16 weeks	Vitamin A, vitamin B, vitamin B12, acetylcholine, serotonin, formic acid, salicylic acid, lecithin, carotenoid, flavonoids, sterols, and thymol	1, 2, and 5%	*Aeromonas hydrophila*	21 days	50, 88, and 96%, respectively	0%	[30]
Beluga	Leaves and stem	Not reported	Powder	3%, 6%, and 12%	8 weeks	Flavonoids, polysaccharides, carotenoids, lignans, lectins, amino acids, minerals (especially iron) and vitamins (vitamin C), tannins, formic acid, salicylic acid, carvacrol, and thymol	—	—	—	—	—	[29]

Table 6: Continued.

Fish species	Nettle part used	Preparation of nettle supplement	Type of plant additive	Doses (%)	Period of feeding trial	Active compounds	Optimum dose	Pathogen species	Pathogen challenge period	Survival rate % in nettle groups after challenge significantly	Survival rate % in control after challenge	References
Beluga	Not reported	Oven-dried was obtained from the supermarket	Powder	1%	60 days	Acetylcholine, serotonin, formic acid, minerals, amino acid, lecithin, carotenoid, flavonoids, sterols, tannins, and vitamins	—	—	—	—	—	[94]
Electric yellow cichlid	Not reported	Dried under shadow	Extract	0.015%	3 months	Carotenoids	—	—	—	—	—	[32]
Convict cichlid	Root	Ethanol extract	Extract	0.02% and 0.03%	8 weeks	Flavonoids	—	—	—	—	—	[33]
Rainbow trout	Aerial parts	Ethanol extract	Extract	1%, 2%, and 3%	8 weeks	Carvacrol, flavonoids, carotenoids proteins, iron, and vitamins	3%	*Yersinia ruckeri*	14 days	78%	41%	[28]
Rainbow trout	Not reported	Methanol extract	Extract	0.01% and 0.05%	30 days	Quercetin	0.01% and 0.05%	*Aeromonas hydrophila*	14 days	58%	0%	[34]
Rainbow trout	Not reported	Nettle extract (quercetin) obtained from the laboratory of professor Amani Awaad	Extract	0.1%, 0.5%, and 1%	14 days	Quercetin	—	—	—	—	—	[17]

of 30 g. In that study, nettle did not increase WBC, RBC, Ht, or Hb after the 60-day feeding trial. While no significant difference was observed in lymphocytes, neutrophils, and monocytes, eosinophils decreased [34]. Blood is responsible for transferring different components, including water, gases, hormones, minerals, immune constituents, nutrients, microorganisms, toxins, and waste products in fish [56]. Thus, the normal range of hematologic parameters shows the fish health status [8]. Furthermore, increased hematological parameters in most cases are a good sign (not in the case of severe infections). Esmaeili [55] reviewed changes in hematological parameters in growth studies (based on more than 400 monitored cases). In most cases, fish with elevated hematologic parameters were healthier and had a higher growth rate. Furthermore, hematological parameters do not show the same trend across treatments and studies. Therefore, a new introduced formula (blood performance) can be a better indicator [55]. To test "blood performance" in nettle studies, we calculated this formula. While many variations in the trend of these parameters were observed, "blood performance," interestingly, in most of the nettle groups was significantly higher than controls [21, 24, 33, 35, 37]. It can be concluded that the higher hematological parameters in the studied supplemented nettle can be a good sign, as they had higher growth than the controls as well. Higher survival rates and fish resistance to pathogens [24, 33] are more evidence of this claim. Dügenci et al. [57] reported that vitamin C increased iron uptake in fish intestines. Since nettle is rich in vitamin C and iron [47], an increase in RBCs, Ht, and Hb can be attributed to these vitamins' synergistic effect via stimulation of the head kidney [22, 33]. The results of the administration of nettle in the fish diet on hematological parameters are presented in Table 2.

Blood biochemistry reveals the metabolic and health status of fish. They have become powerful tools and increasingly common measures in aquaculture studies. Using plant powder and/or extracts like nettle in the fish diet can improve blood biochemical parameters and enhance fish physiology and health conditions [22, 36, 37]. Nettle leaf powder at levels 3% [22] and 0.5, 1, and 1.5% [24] in rainbow trout (7-10 g) diet improved blood serum total protein and albumin after an 8-week feeding trial [37]. Furthermore, the use of 1% nettle extract (quercetin) in a rainbow trout diet (18 g) increased blood serum total protein after 14 days of feeding [23]. Conversely, 1% nettle leaf powder in a rainbow trout diet (15 g) after 2 weeks of feeding did not significantly affect blood serum total protein [21]. Adel et al. [33] reported that the ethanol extract of nettle aerial parts in rainbow trout (42 g) increased blood serum total protein and decreased blood serum glucose and triglyceride levels after 8 weeks of feeding. Furthermore, using 0.01% and 0.05% methanolic extract of nettle in female convict cichlid diet decreased glucose, triglyceride, and cholesterol. It significantly increased the total protein and albumin levels compared to the control [43]. Supplementation of nettle leaf powder at levels 1, 2, and 5% in Victoria Labeo (25 g) after a 4- and 16-week feeding trial, increased blood serum total protein, albumin, triglyceride, cholesterol, and decreased level of blood glucose [37]. An increase in serum total protein decreased blood serum triglycerides and cholesterol, and no change in glucose levels in beluga (204 g) after 8 weeks was observed [35].

Blood serum total protein and albumin are important factors for vertebrate health [58] and can boost the immune response [59]. It can be concluded that the use of nettle supplements in the fish diet can increase blood serum total protein due to bioactive nettle compounds, such as quercetin [23]. Compared to other herbs, nettle is reasonably high in quercetin (2 mg/100 g), and it is a rich source of antioxidants and vitamins A and C. Increasing blood serum albumin due to nettle supplementation can improve transportation of substances, including bilirubin, metals, ions, enzymes, amino acids, hormones, free fatty acids, drugs, and phospholipids, by providing many surface-charged groups and many particular binding places [22, 24, 37, 60]. There is no study related to the effect of nettle on glucose, cholesterol, and triglyceride in rainbow trout to check the glucose- and lipid-lowering effects of this herb. The glucose-lowering effect of nettle was reviewed in mammal studies [61]. Furthermore, many studies have reported the lipid-lowering effect of this herb in a mammal, which reduces cholesterol, low-density lipoproteins, and triglyceride in blood and improves high-density lipoproteins [62]. Parham et al. [63] reported that nettle stimulated the secretion of insulin and affected the peroxisome proliferator-activated receptor- (PPAR-) gamma agonist and the repressing effect on alpha-glucosidase, which has an inhibitory role in reducing blood glucose levels [64]. Alpha-glucosidase is a carbohydrate-hydrolyzing enzyme such as α-amylase and α-glucosidase, which is located in the brush border of the small intestine and breaks down oligosaccharides and disaccharides into monosaccharides that are suitable for absorption [65]. Moreover, hydroalcoholic extract of nettle showed a protective and hypoglycemic effect on pancreatic beta cells in hyperglycemic rats, which can be related to antioxidant possessions of nettle [66] such as phenolic compounds including quercetin and kaempferol [27, 67]. Moreover, the efficiency of nettle on fish glucose levels can be related to fish species, parts of nettle used as a supplement, and the type of nettle supplement (powder and/or extract) [35, 36, 67]. Decreasing the level of cholesterol and triglycerides in the blood serum can be related to nettle sterols, including stigmasterol, campesterol, β-sitosterol [68], and phenolic compounds [27], which affect cholesterol biosynthesis [69]. In conclusion, the positive effect of nettle on protein, glucose, and lipid metabolism was also observed, like many other herbs. The effects of supplementation nettle in the fish diet on blood biochemical parameters are displayed in Table 3. In the studies where the same output was not observed, there were short-term (2 weeks) studies. This can indicate the importance of the administration period, and at least 4 weeks are required. By matching these data with growth, most of the treatments with higher growth had higher total protein (which can indicate better health). Fish growth is closely related to its health status. A fish with a higher growth rate is more likely to be healthy [55]. Furthermore, improved blood biochemistry due to nettle supplementation coincided with a higher survival rate resistance against stress [33, 36].

6. Effects of Nettle Additive on Liver Enzyme Activity in Fish

Any change in the physiological status of the fish, from pollution to nutritional stress, can cause changes in liver enzymes. The fish liver is a key organ of numerous vital metabolic reactions related to carbohydrates, lipids, and protein [70]. Evaluation of liver indicators, including ALT, AST, lactate dehydrogenase (LDH), and ALP, provides a suitable index for the health condition of fish [71]. There is a long list of herbs that decrease these enzymes and eventually provide a healthier status (with a higher immunity and antioxidant response) for fish [72, 73]. Interestingly, in some of these investigations, fish with a lower level of liver enzymes also had a higher growth rate [33, 72, 74–80]. Studies regarding the effect of nettle on these enzymes are limited. Researchers found that nettle sourced from aerial parts with ethanol extract at levels 2% and 3% in rainbow trout and beluga did not significantly demonstrate an effect on blood serum ALT, AST, and LDH [33, 35]. It can be hypothesized that the levels used in these studies were not high enough (antinutritional factors are high as well) to affect fish negatively. Usually, if the herbs are high in fish diets, liver enzymes tend to be higher to reduce or eliminate antinutritional factors from the fish body. However, more studies are required to test this hypothesis. The effects of supplementation nettle on liver enzyme activity in fish are displayed in Table 4.

7. Effects of Nettle on the Immune System of Fish

An immunostimulant is a substance, medicine, or compound that improves the defensive system and immune response of fish [81]. Herbal medicine has been considered a strong immunostimulant, and numerous herbs have indicated this effect in aquaculture species [6, 82]. Generally, the most studied positive effects of herbal medicine in fish are related to its immunostimulatory effect. Several studies have indicated the positive impact of nettle in fish. This effect is due to bioactive compounds, including phenolic compounds and flavonoids [27].

We observed that 8 weeks of feeding rainbow trout (7 g) with a diet content of 3% nettle leaf powder improved blood serum lysozyme enzyme activity [22]. Moreover, others found that supplementation of this herb in rainbow trout at levels 0.5, 1, and 1.5% after 4 weeks of feeding did not improve respiratory burst activity and blood serum alternative complement activity (ACH50), but in the 0.5% group, the serum lysozyme was higher than in the controls [37]. In another study in this fish species (10 g), after 8 weeks of feeding nettle leaf powder at dietary levels of 0.5%, 1%, and 1.5%, increased respiratory burst activity and blood serum lysozyme enzyme ACH50 were observed [24]. Moreover, Awad and Austin [21] reported that supplementing 1% nettle leaf powder to the diet of rainbow trout (15 g) improved respiratory burst, blood serum lysozyme, and bactericidal activity, and no change in phagocytic activity, blood serum α2-macroglobulin, myeloperoxidase content, complement, or antiprotease activity after 14 days feeding was observed. After 30 days of providing rainbow trout (10 g) with nettle methanol extract at dietary levels of 0.01% and 0.05%, increased phagocytic activity, blood serum lysozyme, and myeloperoxidase activity were reported [40].

Use of 0.01% and 0.05% methanolic extracts of nettle in female convict cichlid diet significantly increased complement component 3, complement component 4, and IgM levels at a level of 0.01% of the diet compared to the control [43]. In this context, Ngugi et al. [36] reported that nettle leaf powder in Victoria Labeo juvenile and adult diets with an initial average body weight of 25 g at levels 1%, 2%, and 5% stimulated respiratory burst and blood serum lysozyme activity significantly after 4- and 16-week feeding trials. Another study showed that nettle leaf powder at dietary levels of 3%, 6%, and 12% in beluga (11 g) did not stimulate the immune system, including blood serum total immunoglobulin level, lysozyme, and respiratory burst activity after a 4-week feeding trial [35]. By contrast, after 8 weeks of feeding, total blood serum immunoglobulin levels and respiratory burst activity significantly increased in beluga (204 g) in the 12% nettle group [35]. Improvement of nonspecific immune parameters can be interpreted as the effect of the nettle supplement in the diet of fish due to its different essential amino acids [47] and phenolic compounds, including quercetin, kaempferol, myricetin, and rutin [67], especially quercetin [23], which is known as a phenolic compound, and stimulated immune parameters in fish [21, 24, 33, 36, 37]. Nettle can boost serum total protein level and immune response of fish, as previously mentioned. The effects of supplementing nettle in the fish diet on the immune system are shown in Table 5.

8. Nettle Helps Fish Fight Pathogens

A long list of herbs has positively affected fishes' ability to fight against pathogens [80, 83]. Challenging fish with pathogens has been considered one of the most common and best ways to measure fish and larvae quality. Numerous parameters, from stress to nutritional change, can alter the stress response and eventually the survival rate of fish. Herbal medicine has been one of the greenest ways to improve fish resistance to pathogens. In the past few decades, nettle powder and/or extract have been used as feed additives in fish diets to increase immune resistance and combat pathogens [21, 24, 33, 40].

Mehrabi et al. [24] reported that supplementing 0.5% nettle leaf powder in rainbow trout for 8 weeks showed the highest survival rate (86%) after 3 weeks of challenge with *Saprolegnia parasitica* compared to the control with a 56% survival rate. Moreover, others reported that 1% nettle leaf powder in rainbow trout (15 g) after 14 days of feeding showed the highest survival rate (96%) compared to the control (32%) after 10 days of an *A. hydrophila* challenge [21]. Similarly, a higher survival rate in nettle-supplemented groups (50%-80%) compared with the 35% survival rate in the control in rainbow trout (42 g) after 8 weeks of feeding, followed by 14 days of a *Yersinia ruckeri* challenge, was observed [33]. Bilen et al. [40] used 0.01% and 0.05% nettle methanol extracts in a rainbow trout diet with an initial average body weight of 10 g after a 30-day feeding trial, and their results showed that nettle groups achieved the highest survival rate compared to the controls after a 14-day *A. hydrophila* challenge. In warm-water species, the same improvement in survival rate after a challenge by nettle was observed. Therefore, the use of 1%, 2%, and 5% nettle leaf powder in Victoria Labeo after 4 and 16 weeks of feeding could increase the survival rate (more than 80%) in nettle's groups (2% and 5%) compared to the control group (0%) after a 21-day *A. hydrophila* challenge [36].

There are many possible explanations behind this improvement by nettle. The antimicrobial activity of phenolic components of nettle, such as carvacrol, thymol, and quercetin, has been well documented [7, 84]. Moreover, quercetin has a wide range of biological functions that modulate oxidative stress and inflammatory reactions in mammals [85]. Administration of nettle powder and or extract in fish diet could improve WBC and stimulate nonspecific immune parameters, including immunoglobulin, lysozyme, respiratory burst, ACH50, phagocytic, complements, bactericidal, and antiprotease activity in fish, and increased resistance against pathogens [21, 24, 36, 40]. All these parameters can improve stress responsiveness and survival rates separately or with each other. Furthermore, in terms of gene expression levels, some evidence has indicated that nettle could have positive effects. Cytokines are small protein intermediaries created by immune cells that regulate the immune system, inflammation, and hematopoiesis [86]. Some relative cytokine genes, including interleukin 6 (IL-6) and interleukin 8 (IL-8), showed high expression in nettle treatments [24]. IL-6 is a key multifunctional cytokine that is known as a B-cell separation factor involved in the maturation of antibody-producing cells in mammals [87], and IL-6 is expressed at high levels in inflammatory diseases in mammals [88]. IL-8 is strongly associated with cytokines. It is created by phagocytes and mesenchymal cells exposed to inflammatory stimulants and activates neutrophils and respiratory bursts in fishes and mammals [21, 24, 33, 89]. Moreover, other genes, such as tumor necrosis factor α (TNF-α) and interleukin 1 beta (IL-1β), showed high expression in nettle treatments in fish [24]. TNF-α is one of the cytokines involved in developing inflammation and regulating immune cells in different animals and humans [90]. It removes pathogens during the induction of different cellular responses [91]. Furthermore, IL-1β has a central role in response to pathogens and tissue damage in various taxa [92], and it controls immune responses by increasing cytokines [93]. The effects of the application of nettle in fish diets in terms of ability to fight against pathogens are presented in Table 6.

9. Conclusion

Literature on the applications of nettle has been reviewed on various aquaculture species, with a focus on important and common aquaculture species such as rainbow trout. Nettle has useful features, including growth promotion, immune-boosting, antiviral, antibacterial, and antiparasitic activities. It also improved hematological and blood biochemistry and lowered stress. Nettle improved most of these features in different sizes or species of fish. Natural additives such as nettle could represent a green and sustainable replacement for hormones, antibiotics, and chemical drugs to enhance growth performance, the immune system, and resistance against pathogens due to active compounds, including alkaloids, flavonoids, phenolics, terpenoids, pigments, minerals, and essential oils. Nettle can potentially help to produce healthy food, increase aquaculture production quality and quantity, and reduce environmental pollution through the reduction of chemical usage in aquafeed and fish farm effluent. The combination of molecular studies on gene levels, proteins, and omics (i.e., transcriptomics, proteomics, metabolomics, and microbiomics) in addition to classic analysis can provide a better understanding of nettle's mechanism of action, as well as promote the use of this herb in aquaculture. Finally, reaching a plateau in growth and finding the optimum dosage is necessary, as various fish species have different requirements to achieve maximum growth.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

The supplementary file 2 presents different isolated compounds, type of compounds, and level of compounds from aerial parts, leaves, stems, flowers, seeds, and roots of nettle. *(Supplementary Materials)*

References

[1] The State of World Fisheries and Aquaculture, *Meeting the Sustainable Development Goals*, FAO, Rome, 2018.

[2] Food and Agriculture Organization, *Sustainability in action*, vol. 200, State of World Fisheries and Aquaculture, Rome, 2020.

[3] R. Lulijwa, E. J. Rupia, and A. C. Alfaro, "Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers," *Reviews in Aquaculture*, vol. 12, no. 2, pp. 640–663, 2020.

[4] S. Jennings, G. D. Stentiford, A. M. Leocadio et al., "Aquatic food security: insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment," *Fish and Fisheries*, vol. 17, no. 4, pp. 893–938, 2016.

[5] M. Dorucu, U. Ispir, S. Colak, B. Altinterim, and Y. Celayir, "The effect of black cumin seeds, *Nigella sativa*, on the immune response of rainbow trout, *Oncorhynchus mykiss*," *Mediterranean Aquaculture Journal*, vol. 2, no. 1, pp. 27–33, 2009.

[6] M. Reverter, N. Tapissier-Bontemps, S. Sarter, P. Sasal, and D. Caruso, "Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance," *Reviews in Aquaculture*, vol. 13, no. 1, pp. 537–555, 2021.

[7] K. K. Ghaima, N. M. Hashim, and S. A. Ali, "Antibacterial and antioxidant activities of ethyl acetate extract of nettle (*Urtica dioica*) and dandelion (*Taraxacum officinale*)," *Journal of Applied Pharmaceutical Science*, vol. 3, no. 5, pp. 96–99, 2013.

[8] T. Citarasu, "Herbal biomedicines: a new opportunity for aquaculture industry," *Aquaculture International*, vol. 18, no. 3, pp. 403–414, 2010.

[9] D. A. Tadese, C. Song, C. Sun et al., "The role of currently used medicinal plants in aquaculture and their action mechanisms: a review," *Reviews in Aquaculture*, vol. 14, no. 2, pp. 816–847, 2022.

[10] J. P. Dzoyem, R. T. Tchuenteu, K. Mbarawa et al., "Ethnoveterinary medicine and medicinal plants used in the treatment of livestock diseases in Cameroon," in *Ethnoveterinary medicine*, pp. 175–209, Springer, 2020.

[11] K. C. Chinsembu, J. Negumbo, M. Likando, and A. Mbangu, "An ethnobotanical study of medicinal plants used to treat livestock diseases in Onayena and Katima Mulilo, Namibia," *South African Journal of Botany*, vol. 94, pp. 101–107, 2014.

[12] M. Rubin, *Medieval Christianity in Practice*, Princeton University Press, 2021.

[13] K. K. M. Ahmed and S. Parsuraman, "*Urtica dioica* L., (*Urticaceae*): a stinging nettle," *Systematic Reviews in Pharmacy*, vol. 5, no. 1, pp. 6–8, 2014.

[14] K. N. Jan, K. Zarafshan, and S. Singh, "Stinging nettle (*Urtica dioica* L.): a reservoir of nutrition and bioactive components with great functional potential," *Journal of Food Measurement and Characterization*, vol. 11, no. 2, pp. 423–433, 2017.

[15] R. Marracino, "Nettles in poultry feeding," *Quaderni della Nutrizione*, vol. 3, pp. 480–494, 1936.

[16] W. K. Wilson, "Feeding dried nettles to livestock," *Nature*, vol. 147, no. 3739, pp. 796–798, 1941.

[17] B. Milosevic, I. Omerovic, Z. Savic, L. Andjusic, V. Milanovic, and S. Ciric, "Stinging nettle (*Urtica dioica*) in broiler nutrition," *World's Poultry Science Journal*, vol. 77, no. 4, pp. 901–912, 2021.

[18] B. Ahmadipour and F. Khajali, "Expression of antioxidant genes in broiler chickens fed nettle (*Urtica dioica*) and its link with pulmonary hypertension," *Animal Nutrition*, vol. 5, no. 3, pp. 264–269, 2019.

[19] M. Keshavarz, V. Rezaeipour, and S. Asadzadeh, "Growth performance, blood metabolites, antioxidant stability and carcass characteristics of broiler chickens fed diets containing nettle (*Urtica dioica*. L) powder or essential oil," *International Journal of Advanced Biological and Biomedical Research*, vol. 2, no. 9, pp. 2553–2561, 2014.

[20] A. Szewczyk, E. Hanczakowska, and M. Świątkiewicz, "The effect of nettle (*Urtica* dioica) extract on fattening performance and fatty acid profile in the meat and serum lipids of pigs," *Journal of Animal and Feed Sciences*, vol. 15, Supplement 1, pp. 81–84, 2006.

[21] E. Awad and B. Austin, "Use of lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, as feed additives to prevent *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum)," *Journal of Fish Diseases*, vol. 33, no. 5, pp. 413–420, 2010.

[22] M. Zare, M. K. Mirzakhani, and V. Stejskal, "Growth, body proximate composition and selected blood parameters of rainbow trout *Onchorhynchus mykiss* fingerlings fed on a diet supplemented with nettle *Urtica dioica* and tarragon *Artemisia dracunculus*," *Aquaculture Research*, vol. 52, no. 11, pp. 5691–5702, 2021.

[23] E. Awad, D. Austin, and A. R. Lyndon, "Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum)," *Aquaculture*, vol. 388-391, pp. 193–197, 2013.

[24] Z. Mehrabi, F. Firouzbakhsh, G. Rahimi-Mianji, and H. Paknejad, "Immunity and growth improvement of rainbow trout (*Oncorhynchus mykiss*) fed dietary nettle (*Urtica dioica*) against experimental challenge with *Saprolegnia parasitica*," *Fish & Shellfish Immunology*, vol. 104, pp. 74–82, 2020.

[25] K. Alirezalu, M. Yaghoubi, Z. Nemati, B. Farmani, and A. Mousavi Khaneghah, "Efficacy of stinging nettle extract in combination with ε-polylysine on the quality, safety, and shelf life of rainbow trout fillets," *Food Science & Nutrition*, vol. 9, no. 3, pp. 1542–1550, 2021.

[26] G. De Vico, V. Guida, and F. Carella, "*Urtica dioica* (stinging nettle): a neglected plant with emerging growth promoter/immunostimulant properties for farmed fish," *Frontiers in Physiology*, vol. 9, p. 285, 2018.

[27] L. Grauso, B. de Falco, V. Lanzotti, and R. Motti, "Stinging nettle, (*Urtica dioica* L.): botanical, phytochemical and pharmacological overview," *Phytochemistry Reviews*, vol. 19, no. 6, pp. 1341–1377, 2020.

[28] A. A. H. Said, I. S. El Otmani, S. Derfoufi, and A. Benmoussa, "Highlights on nutritional and therapeutic value of stinging nettle (*Urtica dioica*)," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 7, no. 10, pp. 8–14, 2015.

[29] P. Bagade, V. Pant, and S. T. Pandey, "Nutritional and antinutritional profiling of stinging nettle (*Urtica dioica* L.)," *International Journal of Food Science and Nutrition*, vol. 6, no. 4, pp. 111–116, 2021.

[30] J. Nissar, T. Ahad, H. R. Naik, and S. Z. Hussain, "A review phytic acid: as antinutrient or nutraceutical," *Journal of Pharmacognosy and Phytochemistry*, vol. 6, no. 6, pp. 1554–1560, 2017.

[31] M. M. Rahman, R. B. Abdullah, and W. E. W. Khadijah, "A review of oxalate poisoning in domestic animals: tolerance and performance aspects," *Journal of Animal Physiology and Animal Nutrition*, vol. 97, no. 4, pp. 605–614, 2013.

[32] G. Francis, Z. Kerem, H. P. S. Makkar, and K. Becker, "The biological action of saponins in animal systems: a review," *British Journal of Nutrition*, vol. 88, no. 6, pp. 587–605, 2002.

[33] M. Adel, C. M. A. Caipang, and M. A. Dawood, "Immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles following dietary administration of stinging nettle (*Urtica dioica*)," *Fish & Shellfish Immunology*, vol. 71, pp. 230–238, 2017.

[34] Z. Nobahar, H. Gholipour-Kanani, S. Kakoolaki, and H. Jafaryan, "Effects of garlic (*Allium sativum*) and nettle (*Urtic adioica*) on growth performance and on hematological parameters of beluga (*Huso huso*)," *Iranian Journal of Aquatic Animal Health*, vol. 1, no. 1, pp. 63–69, 2015.

[35] M. Binaii, M. Ghiasi, S. M. V. Farabi et al., "Biochemical and hemato-immunological parameters in juvenile beluga (*Huso huso*) following the diet supplemented with nettle (*Urtica dioica*)," *Fish & Shellfish Immunology*, vol. 36, no. 1, pp. 46–51, 2014.

[36] C. C. Ngugi, E. Oyoo-Okoth, J. Mugo-Bundi, P. S. Orina, E. J. Chemoiwa, and P. A. Aloo, "Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*," *Fish & Shellfish Immunology*, vol. 44, no. 2, pp. 533–541, 2015.

[37] Z. Mehrabi and F. Firouzbakhsh, "Short-term effects of feeding powdered Aloe vera (*Aloe barbadensis*) and nettle (*Urtica dioica*) on growth performance and stimulation of innate immune responses in rainbow trout (*Oncorhynchus mykiss*)," *Comparative Clinical Pathology*, vol. 29, no. 2, pp. 441–449, 2020.

[38] N. Yeşilayer, G. Mutlu, and A. Yıldırım, "Effect of nettle (*Urtica spp.*), marigold (*Tagetes erecta*), alfalfa (*Medicago sativa*) extracts and synthetic xanthophyll (zeaxanthin) carotenoid supplementations into diets on skin pigmentation and growth parameters of electric yellow cichlid (*Labidochromis caeruleus*)," *Aquaculture*, vol. 520, article 734964, 2020.

[39] P. Babahajiani, B. Shokrollahi, and M. Gharibkhani, "The effect of gokshura (*Tribulus terrestris*) and nettle root (*Urtica dioica*) extracts on growth rate and sex reversal in convict cichlid (*Cichlasoma nigrofasciatum*)," *Iranian Journal of Fisheries Sciences*, vol. 17, no. 3, pp. 620–628, 2018.

[40] S. Bilen, S. Ünal, and H. Güvensoy, "Effects of oyster mushroom (*Pleurotus ostreatus*) and nettle (*Urtica dioica*) methanolic extracts on immune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*)," *Aquaculture*, vol. 454, pp. 90–94, 2016.

[41] M. Esmaeili, C. G. Carter, R. Wilson et al., "Proteomic investigation of liver and white muscle in efficient and inefficient Chinook salmon (*Oncorhynchus tshawytscha*): fatty acid metabolism and protein turnover drive feed efficiency," *Aquaculture*, vol. 542, article 736855, 2021.

[42] E. Awad, B. Austin, and A. Lyndon, "Effect of dietary supplements on digestive enzymes and growth performance of rainbow trout (*Oncorhynchus mykiss*, Walbaum)," *Journal of American Science*, vol. 8, no. 12, pp. 858–864, 2012.

[43] N. Jafari, H. Abdollahpour, M. Karimzadeh, and B. Falahatkar, "A potential role of nettle (*Urtica dioica*) extract on growth, biochemical changes and reproductive performance of convict cichlid (*Amatitlania nigrofasciata*)," *Fish Physiology and Biochemistry*, vol. 48, no. 1, pp. 133–144, 2022.

[44] L. Zhao, S. M. Budge, A. E. Ghaly, and M. S. Brooks, "Extraction, purification and characterization of fish pepsin: a critical review," *Journal of Food Processing & Technology*, vol. 2, no. 6, p. 126, 2011.

[45] Z. Tülek, H. Alaşalvar, B. Başyiğit et al., "Extraction optimization and microencapsulation of phenolic antioxidant compounds from lemon balm (*Melissa officinalis* L.): instant soluble tea production," *Journal of Food Processing and Preservation*, vol. 45, no. 1, article e14995, 2021.

[46] S. Ramezanzadeh, A. Abedian Kenari, M. Esmaeili, and A. Rombenso, "Effects of different forms of barberry root (*Berberis vulgaris*) on growth performance, muscle fatty acids profile, whole-body composition, and digestive enzymes of rainbow trout (*Oncorhynchus mykiss*)," *Journal of the World Aquaculture Society*, vol. 52, no. 2, pp. 284–302, 2021.

[47] L. K. Rutto, Y. Xu, E. Ramirez, and M. Brandt, "Mineral properties and dietary value of raw and processed stinging nettle (*Urtica dioica* L.)," *International Journal of Food Science*, vol. 2013, Article ID 857120, 9 pages, 2013.

[48] C. Thomas and A. B. Lumb, "Physiology of haemoglobin," *Continuing Education in Anaesthesia Critical Care & Pain*, vol. 12, no. 5, pp. 251–256, 2012.

[49] A. K. Imsland, A. Foss, G. Nævdal et al., "Variations in growth in haemoglobin genotypes of Atlantic cod," *Fish Physiology and Biochemistry*, vol. 30, no. 1, pp. 47–55, 2004.

[50] N. Van Hai, "The use of medicinal plants as immunostimulants in aquaculture: a review," *Aquaculture*, vol. 446, pp. 88–96, 2015.

[51] A. L. Val, "Organic phosphates in the red blood cells of fish," *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, vol. 125, no. 4, pp. 417–435, 2000.

[52] G. J. Lieschke and N. S. Trede, "Fish immunology," *Current Biology*, vol. 19, no. 16, pp. R678–R682, 2009.

[53] F. Moreno, D. Sanz-Guajardo, J. M. López-Gómez, R. Jofre, and F. Valderrábano, "Increasing the hematocrit has a beneficial effect on quality of life and is safe in selected hemodialysis patients," *Journal of the American Society of Nephrology*, vol. 11, no. 2, pp. 335–342, 2000.

[54] P. C. de Souza and G. O. Bonilla-Rodriguez, "Fish hemoglobins," *Brazilian Journal of Medical and Biological Research*, vol. 40, no. 6, pp. 769–778, 2007.

[55] M. Esmaeili, "Blood performance: a new formula for fish growth and health," *Biology*, vol. 10, no. 12, p. 1236, 2021.

[56] B. Ciesla, *Hematology in Practice*, F. A. Davis company Publishing, third edition, 2018.

[57] S. K. Dügenci, N. Arda, and A. Candan, "Some medicinal plants as immunostimulant for fish," *Journal of Ethnopharmacology*, vol. 88, no. 1, pp. 99–106, 2003.

[58] M. Baker, "Albumin, steroid hormones and the origin of vertebrates," *The Journal of Endocrinology*, vol. 175, no. 1, pp. 121–127, 2002.

[59] G. F. Wiegertjes, R. M. Stet, H. K. Parmentier, and W. B. van Muiswinkel, "Immunogenetics of disease resistance in fish: a comparative approach," *Developmental & Comparative Immunology*, vol. 20, no. 6, pp. 365–381, 1996.

[60] J. Hankins, "The role of albumin in fluid and electrolyte balance," *Journal of Infusion Nursing*, vol. 29, no. 5, pp. 260–265, 2006.

[61] A. Chehri, R. Yarani, Z. Yousefi et al., "Anti-diabetic potential of (*Urtica dioica*): current knowledge and future direction," *Journal of Diabetes & Metabolic Disorders*, vol. 21, no. 1, pp. 931–940, 2022.

[62] W. Long, H. Tang, X. Zhong, H. Wang, and H. Wang, "Hypoglycemic and lipid-lowering effects of extracted from the aerial part of *Urtica cannabina* L. on alloxan-induced hyperglycemic mice," *IOP Conference Series: Earth and Environmental Science*, vol. 512, no. 1, article 012097, 2020.

[63] M. Parham, M. Bagherzadeh, M. Asghari et al., "Evaluating the effect of a herb on the control of blood glucose and insulin-resistance in patients with advanced type 2 diabetes (a double-blind clinical trial)," *Caspian Journal of Internal Medicine*, vol. 11, no. 1, pp. 12–20, 2020.

[64] F. A. Van de Laar, P. L. Lucassen, R. P. Akkermans, E. H. Van de Lisdonk, and W. J. De Grauw, "Alpha-glucosidase inhibitors for people with impaired glucose tolerance or impaired fasting blood glucose," *Cochrane Database of Systematic Reviews*, vol. 4, pp. 1–69, 2006.

[65] D. Sinha, T. Satapathy, M. K. Dewangan, A. Kumar, and A. Roy, "Recent status on carbohydrate metabolizing enzyme inhibitors in regulation of diabetes: a mechanism based review," *Journal of Applied Pharmaceutical Research*, vol. 3, no. 2, pp. 1–7, 2015.

[66] M. J. Golalipour and V. Khori, "The protective activity of (*Urtica dioica*) leaves on blood glucose concentration and beta-cells in streptozotocin-diabetic rats," *Pakistan Journal of Biological Sciences*, vol. 10, no. 8, pp. 1200–1204, 2007.

[67] S. Otles and B. Yalcin, "Phenolic compounds analysis of root, stalk, and leaves of nettle," *The Scientific World Journal*, vol. 2012, Article ID 564367, 12 pages, 2012.

[68] Z. Y. Petkova, G. A. Antova, and M. Y. Angelova-Romova, "Biologically active components and health benefits of nettle seed oil," *Grasas y Aceites*, vol. 71, no. 1, pp. 347–347, 2020.

[69] K.-W. Lee, H. Everts, H. J. Kapperst, K.-H. Yeom, and A. C. Beynen, "Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens," *Journal of Applied Poultry Research*, vol. 12, no. 4, pp. 394–399, 2003.

[70] V. Mitra and J. Metcalf, "Metabolic functions of the liver," *Anaesthesia & Intensive Care Medicine*, vol. 10, no. 7, pp. 334-335, 2009.

[71] R. A. Ardeshir, A.-A. Movahedinia, and S. Rastgar, "Fish liver biomarkers for heavy metal pollution: a review article," *American Journal of Toxicology*, vol. 2, no. 1, pp. 1–8, 2017.

[72] R. Akrami, A. Gharaei, M. R. Mansour, and A. Galeshi, "Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato-biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile," *Fish & Shellfish Immunology*, vol. 45, no. 2, pp. 828–834, 2015.

[73] M. Yousefi, S. V. Shabunin, Y. A. Vatnikov et al., "Effects of lavender (*Lavandula angustifolia*) extract inclusion in diet on growth performance, innate immunity, immune-related gene expression, and stress response of common carp, *Cyprinus carpio*," *Aquaculture*, vol. 515, article 734588, 2020.

[74] M. Ravardshiri, S. Bahram, S. R. Javadian, and M. Bahrekazemi, "Cinnamon promotes growth performance, digestive enzyme, blood parameters, and antioxidant activity of rainbow trout (*Oncorhynchus mykiss*) in low-carbohydrate diets," *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 21, no. 7, pp. 309–322, 2021.

[75] H. Ghafarifarsani, S. H. Hoseinifar, T. J. Adorian, F. R. G. Ferrigolo, M. Raissy, and H. Van Doan, "The effects of combined inclusion of *Malvae sylvestris*, *Origanum vulgare*, and *Allium hirtifolium* boiss for common carp (*Cyprinus carpio*) diet: growth performance, antioxidant defense, and immunological parameters," *Fish & Shellfish Immunology*, vol. 119, pp. 670–677, 2021.

[76] H. Ghafarifarsani, M. Yousefi, S. H. Hoseinifar et al., "Beneficial effects of Persian shallot (*Allium hirtifolium*) extract on growth performance, biochemical, immunological and antioxidant responses of rainbow trout *Oncorhynchus mykiss* fingerlings," *Aquaculture*, vol. 555, article 738162, 2022.

[77] A. Gholamhosseini, S. Hosseinzadeh, S. Soltanian et al., "Effect of dietary supplements of *Artemisia dracunculus* extract on the haemato-immunological and biochemical response, and growth performance of the rainbow trout (*Oncorhynchus mykiss*)," *Aquaculture Research*, vol. 52, no. 5, pp. 2097–2109, 2021.

[78] G. Rashidian, H. H. Mahboub, A. Fahim et al., "Mooseer (*Allium hirtifolium*) boosts growth, general health status, and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Streptococcus iniae* infection," *Fish & Shellfish Immunology*, vol. 120, pp. 360–368, 2022.

[79] H. Adineh, M. Harsij, H. Jafaryan, and M. Asadi, "The effects of microencapsulated garlic (*Allium sativum*) extract on growth performance, body composition, immune response and antioxidant status of rainbow trout (*Oncorhynchus mykiss*) juveniles," *Journal of Applied Animal Research*, vol. 48, no. 1, pp. 372–378, 2020.

[80] H. Hosseini, M. Pooyanmehr, A. Foroughi, M. Esmaeili, F. Ghiasi, and R. Lorestany, "Remarkable positive effects of figwort (*Scrophularia striata*) on improving growth performance, and immunohematological parameters of fish," *Fish & Shellfish Immunology*, vol. 120, pp. 111–121, 2022.

[81] R. Harikrishnan, C. Balasundaram, and M.-S. Heo, "Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish," *Aquaculture*, vol. 317, no. 1-4, pp. 1–15, 2011.

[82] D. Stratev, G. Zhelyazkov, X. S. Noundou, and R. W. M. Krause, "Beneficial effects of medicinal plants in fish diseases," *Aquaculture International*, vol. 26, no. 1, pp. 289–308, 2018.

[83] M. Li, D. Wei, S. Huang et al., "Medicinal herbs and phytochemicals to combat pathogens in aquaculture," *Aquaculture International*, vol. 30, no. 3, pp. 1239–1259, 2022.

[84] İ. Gülçin, Ö. İ. Küfrevioğlu, M. Oktay, and M. E. Büyükokuroğlu, "Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.)," *Journal of Ethnopharmacology*, vol. 90, no. 2-3, pp. 205–215, 2004.

[85] A. W. Boots, G. R. M. M. Haenen, and A. Bast, "Health effects of quercetin: from antioxidant to nutraceutical," *European Journal of Pharmacology*, vol. 585, no. 2-3, pp. 325–337, 2008.

[86] R. Savan and M. Sakai, "Genomics of fish cytokines," *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, vol. 1, no. 1, pp. 89–101, 2006.

[87] T. Kishimoto, S. Akira, M. Narazaki, and T. Taga, "Interleukin-6 family of cytokines and gp130," *Blood*, vol. 86, no. 4, pp. 1243–1254, 1995.

[88] C. Gabay, "Interleukin-6 and chronic inflammation," *Arthritis Research & Therapy*, vol. 8, Supplement 2, pp. S3–S6, 2006.

[89] M. Baggiolini and I. Clark-Lewis, "Interleukin-8, a chemotactic and inflammatory cytokine," *FEBS Letters*, vol. 307, no. 1, pp. 97–101, 1992.

[90] T. Krakauer, J. Vilcek, and J. Oppenheim, "Proinflammatory cytokines: TNF and IL-1 families, chemokines, TGFβ and others," in *Fundamental Immunology*, pp. 775–811, Lippincott Williams & Wilkins, 1999.

[91] S. Subramanian, S. L. MacKinnon, and N. W. Ross, "A comparative study on innate immune parameters in the epidermal mucus of various fish species," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 148, no. 3, pp. 256–263, 2007.

[92] S. Bird, J. Zou, T. Wang, B. Munday, C. Cunningham, and C. J. Secombes, "Evolution of interleukin-1β," *Cytokine & Growth Factor Reviews*, vol. 13, no. 6, pp. 483–502, 2002.

[93] C. Low, S. Wadsworth, C. Burrells, and C. J. Secombes, "Expression of immune genes in turbot (*Scophthalmus maximus*) fed a nucleotide-supplemented diet," *Aquaculture*, vol. 221, no. 1-4, pp. 23–40, 2003.

[94] M. Seliya and P. Kothiyal, "*Urtica dioica* (stinging nettle): a review of its chemical, pharmacological, toxicological and ethnomedical properties," *International Journal of Pharmacy*, vol. 4, no. 1, pp. 270–277, 2014.

CHAPTER 3

NETTLE *URTICA DIOICA* AND TARRAGON *ARTEMISIA DRACUNCULUS* IN RAINBOW TROUT *ONCORHYNCHUS MYKISS* FINGERLINGS DIET

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Growth, body proximate composition and selected blood parameters of rainbow trout *Onchorhynchus mykiss* fingerlings fed on a diet supplemented with nettle *Urtica dioica* and tarragon *Artemisia dracunculus*

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Abstract

Diet supplemented with nettle *Urtica dioica* and tarragon *Artemisia dracunculus* leaf powder on fingerling rainbow trout *Oncorhynchus mykiss* was assessed. Fish of initial body weight 7.41 ± 0.16 g, were distributed in six groups, three replications, and were fed 8 weeks. 0 (control), 10 nettle (N1), 30 nettle (N3), 10 tarragon (T1), 30 tarragon (T3) and 10 nettle +10 tarragon g/kg (N1+T1) diet. The N3 improved FBW, WG, WG% and SGR significantly ($p \leq 0.05$). N3 increased body protein, ash and decreased body fat significantly ($p \leq 0.05$). The highest haematocrit was observed in T3 significantly ($p \leq 0.05$). The highest WBC in T3, and RBC and Hb in N1+T1 were observed ($p \leq 0.05$). N1 and N1+T1 showed the highest MCV ($p \leq 0.05$). N1, T1 and N1+T1 showed the highest MCH ($p \leq 0.05$). The highest MCHC was observed in T1 significantly ($p \leq 0.05$). N3, T3 and N1+T1 demonstrated the highest albumin levels ($p \leq 0.05$). The T1 increased serum lysozyme activity compared to control ($p \leq 0.05$). The highest serum total immunoglobulin and total protein in N3 were observed ($p \leq 0.05$). The results showed beneficial effects of N3, T3 and N1+T1 on growth performance, body proximate composition and blood parameters.

KEYWORDS
bioactive compounds, feed conversion ratio, leaf powder, lysozyme activity, survival rate, weight gain

1 | INTRODUCTION

Aquaculture plays an important role in world food production, and particularly in supplying nutritious, high-quality protein to an expanding world population (Kobayashi et al., 2015). As demand for food rises, fish farmers have increasingly replaced extensive systems with semi-intensive and intensive systems. These productive systems do, however, carry higher risks of outbreaks of infectious disease (Yan et al., 2017). As a result, there is now a strong interest in how diet quality can not only improve growth, but also the health and immunity of fish (Ji, Jeong, et al., 2007; Ji, Takaoka, et al., 2007).

In recent years, an important theme in aquaculture research has been fish growth and immunity (Bilen et al., 2016; Bilen et al., 2016; Binaii et al., 2014; Ji, Jeong, et al., 2007; Mehrabi et al., 2019; Ngugi et al., 2015; Yan et al., 2017). Specifically, organic, inorganic and synthetic feed additives have been investigated in order to increase growth performance and immune responses (Li & Gatlin, 2005). The use of antibiotics and other types of medication to combat various diseases has disadvantages such as negative effects on the environment and human health (Jennings et al., 2016), and may increase pathogen resistance against medicines (Seyfried et al., 2010). Thus, there has been a marked increase in studies of exploring the potential effects of plant products in order to prevent and control aquatic diseases (Mehrabi et al., 2019; Zaefarian et al., 2017). The use of plant products has begun to reduce the applications of some synthetic drugs (Banaee et al., 2011). Plants are natural, and cost-effective sources of various active compounds, some of which can be used to combat fish stress, promote growth and stimulate food intake and immune system function (Citarasu, 2010). Plant compounds can also control pathogenic and non-pathogenic bacteria in the gut, and further increase growth performance (Bedford, 2000). Hence, plant extracts have been used to increase growth rate (Ji, Jeong, et al., 2007; Lee et al., 2014; Adel et al., 2017; Talpur & Ikhwanuddin, 2012), improve body proximate composition (Hwang et al., 2013), and as an alternative to antibiotics and chemical therapeutics (Bilen, Ünal, et al., 2016; Immanuel et al., 2009; Ngugi et al., 2015; Nya & Austin, 2011; Stratev et al., 2018; Vaseeharan & Thaya, 2014). One of the plant species exploited in aquaculture is the stinging nettle *Urtica dioica*, which belongs to the *Urticaceae* family. This is a native plant species in Asia, Europe, North Africa and North America (Ahmed & Parsuraman, 2014; Borchers et al., 2000). A large number of active compounds including amino acids, proteins (Dalev et al., 1996), carotenoids, vitamins (Akbay et al., 2003), agglutinin (Does et al., 1999), minerals, amino acids, lignans, terpenoids (Ahmed & Parsuraman, 2014) and phenolic components including syringic acid, myricetin, quercetin, kaempferol, rutin, ellagic acid, isorhamnetin, ferulic acid and naringin (Otles & Yalcin, 2012) are detected in nettle, but it is not known which compounds affect fish. A limited number of papers describe the effects of nettle extract applied as part of fish nutrition. For example alcoholic extract of nettle increased growth performance and stimulated the immune capacity of rainbow trout (Bilen, Ünal, et al., 2016; Adel et al., 2017). Nettle leaf powder improved growth performance, biochemical, haematological and immunological parameters in Victoria labeo (*Labeo victorianus*) (Ngugi et al., 2015). Similarly, nettle leaf powder improved blood biochemical parameters and immune factors in juvenile beluga *Huso huso* (Binaii et al., 2014).

Tarragon, *Artemisia dracunculus*, belongs to *Asteraceae* family. The species has a long history of use as a culinary herb and medical remedy (Obolskiy et al., 2011) due to medical properties including antibacterial and antifungal effects (Eisenman et al., 2013). Different kind of active compounds including vitamins, sesquiterpenoids (Greger, 1979; Logendra et al., 2006), coumarins/chromones, flavonoids, phenylpropanoids, alkamides, estragole, methyl eugenol (Obolskiy et al., 2011), polyacetylene (Lanciotti et al., 2004), tannins, alkaloids, saponin, terpene and anthraquinones (Behbahani et al., 2017) are found in tarragon, but it is not known which compound affect fish specifically. In the last decade, investigations have documented how tarragon powder or extract can improve growth performance and immune system function in fish (Ekanem & Brisibe, 2010; Mbokane & Moyo, 2018, 2020). There are also some reports about the effects of tarragon (*A. dracunculus*) powder on broiler growth performance and body composition (Tunç et al., 2019; Yildirim & Tunç, 2018). An exciting new development is the use of combinations of extracts from different plant species as a feed additive. Combinations that have been trialled on fish include *Astragalus radix and Lonicera japonica* (Zakęś et al., 2008), and *Massa medicata fermentata*, *Crataegi fructus*, *Artemisia capillaries* and *Cnidium officinale* (Ji, Jeong, et al., 2007). The observations of improved growth performance, body proximate composition and a stimulated immune system (Ji, Jeong, et al., 2007; Zakęś et al., 2008) demonstrate the potential benefits of using combinations of extracts from different plants as a feed additive. The combination of nettle and tarragon powder warrants investigation because of the distinct pharmacological properties of nettle (Ahmed & Parsuraman, 2014; Otles & Yalcin, 2012) and tarragon (Gregeret al., 1979; Logendra et al., 2006; Obolskiy et al., 2011), and the effects each individual powder or extract has on haematological parameters (Binaii et al., 2014; Ekanem & Brisibe, 2010; Mbokane & Moyo, 2018, 2020; Ngugi et al., 2015; Soares et al., 2020). Here, rainbow trout (*Onchorhynchus mykiss*) was chosen as an experimental species, given that it is extensively cultured across the world (Pauly & Froese, 2012). Therefore, the aims of this study were to determine the effects of nettle and tarragon powder individually and in combination on growth performance, body proximate composition and selected blood parameters of rainbow trout.

2 | MATERIALS AND METHODS

2.1 | Fish and rearing condition

Rainbow trout (*O. mykiss*) fingerlings were purchased from a private hatchery, Rezvanshahr, Guilan, Iran. Rainbow trout fingerlings were fed a basal diet formulation (Table 1) without added plant biomass for a 14-day acclimation period prior to the start of the trial. After the acclimation period, all fish initial weight was taken individually. Twenty rainbow trout fingerlings, with a mean body weight of 7.41 ± 0.08 g (mean ± pooled SE), were randomly stocked into each of eighteen 35 L flow-through tanks filled with well water with water flow-through rate 3 L/min. The eighteen tanks comprise three replications of six diets (Mehrabi et al., 2012). The following diets (Table 1) were compared:

- Control, standard diet formulation without plant supplement
- N1 - 10 g nettle leaf powder per 1,000 g diet
- N3 - 30 g nettle leaf powder per 1,000 g diet
- T1 - 10 g tarragon leaf powder per 1,000 g diet
- T3 - 30 g tarragon leaf powder per 1,000 g diet
- N1+T1 - 10 g nettle leaf powder per 1,000 g diet +10 g tarragon leaf powder per 1,000 g diet

Fish was fed till apparent satiation (Bilen, Altunoglu, et al., 2016; Bilen, Ünal, et al., 2016), three times per day, manually at 08:00, 12:00 and 16:00 h for 8 weeks (Mehrabi et al., 2019; Adel et al., 2017). Uneaten food was collected after each meal (Bilen, Ünal,

TABLE 1 Ingredients and proximate composition of experimental diets with and without nettle and tarragon leaf powder

Test ingredients (g kg^{-1})	Control	N1	N3	T1	T3	N1+T1
Fish meal[a]	460	460	460	460	460	460
Soybean meal[a]	215	210	205	210	205	205
Wheat flour[a]	180	175	160	175	160	170
Nettle leaf powder	0	10	30	0	0	10
Tarragon leaf powder	0	0	0	10	30	10
Soy bean oil[a]	50	50	50	50	50	50
Fish oil[b]	50	50	50	50	50	50
Dicalcium phosphate[a]	5	5	5	5	5	5
Mineral mix[c]	15	15	15	15	15	15
Vitamin mix[d]	15	15	15	15	15	15
BHT[e]	6	6	6	6	6	6
Choline chloride[a]	4	4	4	4	4	4
Proximate composition						
Moisture (%)	8.9	8.4	8.7	9.1	9.4	8.9
Crude protein (%)	45.7	45.5	45.1	44.9	45.2	45.4
Lipid (%)	16.8	16.2	16.4	16.3	16.5	16.3
Ash (%)	8.3	9.1	8.6	9.2	9.1	8.9
Gross energy (Kj g^{-1})[f]	20.9	20.6	20.5	20.7	20.3	20.5

Note: Control: no plant supplement; N1: 10 g nettle leaf powder per 1,000 g diet; N3: 30 g nettle leaf powder per 1,000 g diet; T1: 10 g tarragon leaf powder per 1,000 g diet; T3: 30 g tarragon leaf powder per 1,000 g diet; N1+T1: 10 g nettle leaf powder per 1,000 g diet +10 g tarragon leaf powder per 1,000 g diet.

[a]Clopeonella meal (Mazandaran Animal and Aquatic feed (Manaqua) Co. Iran).

[b]Kilka oil (Manaqua Co. Iran).

[c]Mineral mix provided (mg Kg-1): Fe: 6,000, Cu: 600, Mn: 5,000, Zn: 10,000, I: 600, Se: 20, Co: 100, choline chloride: 6,000, Career up to 1 kg (Kemin, MasterMIN™ Fish, USA).

[d]Vitamin mix provided (Unit Kg-1): A: 1,200,000 IU, D3: 400,000 IU, E: 50,000 mg, K3: 800 mg, B9: 1,000 mg, C: 30,000 mg, B1: 2,500 mg, B2: 4,000 mg, B6: 25,000 mg, B12: 8 mg, Biotin: 150 mg, Niacin: 35,000 mg, Inositol: 50,000 mg, Career up to 1 kg.

[e]Antioxidant (Gluba Tiox, French).

[f] Estimated energy was calculated based on 1 g crude protein at 23.6 Kj, 1 g crude fat being 39.5 Kj and 1 g carbohydrate being 17.2 Kj (Jobling (NRC), 2011).

TABLE 2 Proximate composition of nettle and tarragon leaf powder

Plants test ingredients	Moisture (%)	Crude protein (%)	Lipid (%)	Ash (%)
Nettle leaf powder	12.66	21.86	5.50	20.33
Tarragon leaf powder	10.00	23.56	7.86	12.16

et al., 2016). Water parameters including temperature, pH and dissolved oxygen (DO) were measured daily by HQ40D portable multimeter and were found to be on average 16.2 ± 0.1°C, pH 7.5 ± 0.1, 7.71 ± 0.82 mg/L respectively. The photoperiod was 16 L: 8 D (Adel et al., 2017).

2.2 | Diet preparation

All nettle (*U. dioica*) was collected from rural Rezvanshahr, Guilan, in the north of Iran. Tarragon (*A. dracunculus*) was bought once from a market in Rezvanshahr, Guilan, in the north of Iran. Nettle and tarragon leaves were separated from stems, cleaned and washed with distilled water and dried for 3 weeks in the shade at room temperature. At that stage, the moisture content of nettle and tarragon were 12.6 and 10.0% respectively (Table 2). Dried leaves were powdered using a grinder and the resulting powder was packed and stored at −4°C till used for feed preparation (Ngugi et al., 2015). The leaf powder was mixed into the basal diet using a further grinder and was subsequently pelleted through a 1.0 mm diameter pore. Pellets were dried at room temperature (25°C) for 24 h, packed into plastic bags and stored at +4°C until used (Ahmadifar et al., 2011). Soybean meal and wheat flour were used as carbohydrate sources (Cho & Lee, 2012) and they were replaced with nettle and tarragon powder (Cho & Lee, 2012; Immanuel et al., 2009; Xu et al., 2020) as detailed in Table 1.

2.3 | Growth performance

At the end of week eight, all remaining fish were counted and weighed separately to assess growth performance and determine the survival rate. Initial body weight (IBW), final body weight (FBW), feed intake (FI), weight gain (WG), weight gain percent (WG %), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and survival rate (SR%) were calculated as follows. (n = remaining number of fish per each group).

IBW (g) = Initial body weight.
FBW (g) = Final body weight.
FI (g/fish) = dry feed consumed/fish number.
WG (g) = [Final body weight (g) − Initial body weight (g)].
WG% = [Final body weight (g) − Initial body weight (g)]/Initial body weight (g) ×100.
PER =Weight gain (g)/Total protein intake (g).
FCR =dry feed intake (g)/WG (g).
SGR (% day^{-1}) =100 × [ln final body weight (g) − ln initial body weight (g)]/(time) days.
Survival rate (%SR) = (number of survived fish after feeding trial/initial number of stocked fish) ×100.

2.4 | Whole-body proximate composition

At the end of the feeding trial, three intact fish were taken randomly from each tank, packed in plastic bags and stored at −20°C for whole-body proximate composition analysis (Khosravi et al., 2015). Feed and whole-body proximate composition analysis was performed according to the method detailed in Latimer. (2016). Whole-body crude fat was estimated by the Soxhlet extractor (Soxtec System HT6; Tecator, Hoganas, Sweden). Protein was determined by the Kjeldahl method (BUCHI Labortechnik AG, type K-360, Switzerland). Ash content was determined by incineration in a muffle furnace at 550°C for 4 h (LAC, type Ht40AL, Czech Republic), and moisture at 105°C for 24 h until constant weight (Nüve, type FN 400 (P), Turkey) (n = 9 per group).

2.5 | Blood sampling

At the end of week eight, 24 h after last feeding, three fish from each tank were randomly collected and anaesthetized using tricaine methane sulphonate (MS-222) at a concentration of 0.1 mg/L water (Mehrabi et al., 2012). Blood samples were collected from the caudal vein using heparinized (sodium heparin 5,000 U/ml) and non-heparinized syringes. Heparinized blood samples were transferred to 1.5 ml heparinized Eppendorf tubes kept on ice for haematological analysis, and non-heparinised blood samples were transferred to non-heparinized 1.5 ml Eppendorf tubes and left at room temperature to be clotted (Ngugi et al., 2015) Following clotting, serum was separated by centrifugation (Heraeus Megafuge 16 R Centrifuge), 5000 $_{\times}$g, 10 min, +4°C and stored at −80°C to be assessed later (Khosravi et al., 2015). (n = 9 per group).

2.6 | Haematology and biochemistry analysis

Red blood cells (RBC) and white blood cells (WBC) were counted with a Neubaur haemocytometer, and different white blood cells were counted following Giemsa staining (Adel et al., 2017). Haemoglobin (Hb) in grams per decilitre was assessed according to the cyanmethaemoglobin procedure (Mehrabi et al., 2019). Haematocrit (Hct) was measured after centrifugation (Heraeus Megafuge 16 R Centrifuge) at 14,000 rpm for 15 min at +4°C (Dawood et al., 2017). Mean corpuscular volume (MCV fL) = [Hct/RBC (×106/mm^3)] × 10, mean corpuscular haemoglobin (MCH Pg) = [Hb/RBC (×106/mm^3)] × 10 and mean erythrocyte haemoglobin concentration (MCHC g dL) = (Hb/Hct) × 100 were calculated according to Firouzbakhsh et al.,. (2011). Biochemical blood factors such as serum albumin and serum total protein were measured according to Mehrabi et al., (2019), using a commercial kit (Zist-shimi Iran) (n = 9 per group).

2.7 | Immunological factors

Immunological blood factors including serum lysozyme activity were assessed with the turbidimetric method explained by Mehrabi et al., (2019) with slight modifications. Briefly, 175 µl of a bacterial suspension (*Micrococcus lysodeikticus*, Sigma M3770) was added to 96-well ELISA plates, then 25 µl of serum was mixed and the optical density was measured at 450 nm. Lyophilized egg white lysozyme (HEWL; Sigma, USA) was used to obtain a standard curve.

Serum total immunoglobulin was measured according to Khosravi et al., (2015). Briefly, serum total immunoglobulin was measured by the microprotein method (C-69; Sigma) and by applying a 12% solution of polyethylene glycol (Sigma-Aldrich) before and after precipitating the total immunoglobulin molecules. The difference in protein content showed the total immunoglobulin content (n = 9 per group).

2.8 | Statistical analysis

Data normality and homogeneity were checked by Kolmogorov–Smirnov test. Data were analysed by one-way ANOVA using (SPSS statistics software version 24, IBM, USA). Statistical differences among mean values were performed using the Duncan post hoc test ($p \leq 0.05$).

TABLE 3 Growth parameters of rainbow trout provided feed supplemented with nettle and/or tarragon leaf powder

Treatments (*n* = 60)	IBW (g)	FBW (g)	FI (g fish^{-1})	WG (g fish^{-1})	WG% (%)	FCR	SGR (% day^{-1})	PER	Survival rate (%)
Control	7.43^a	29.15^a	25.80^a	21.72^a	292.59^a	1.23^a	2.43^a	1.77^a	90.00^a
N1	7.40^a	29.48^a	27.67^a	22.08^a	296.67^a	1.27^a	2.45^a	1.72^a	93.33^a
N3	7.38^a	33.59^b	28.77^a	26.21^b	354.99^b	1.13^a	2.70^b	1.90^a	90.00^a
T1	7.38^a	29.89^a	27.50^a	22.50^a	304.57^a	1.24^a	2.49^a	1.81^a	93.33^a
T3	7.52^a	29.57^a	27.75^a	22.05^a	293.76^a	1.29^a	2.44^a	1.70^a	91.66^a
N1+T1	7.35^a	28.20^a	26.26^a	20.87^a	283.88^a	1.30^a	2.39^a	1.69^a	91.66^a
Pooled SE	0.08	0.58	1.60	0.58	9.12	0.06	0.04	0.09	2.04

Note: Values are presented as mean and pooled SE. Mean values with different superscripts within a column vary significantly according to one-way ANOVA ($p \le 0.05$).

Control: without supplement; N1: 10 g nettle leaf powder per 1,000 g diet; N3: 30 g nettle leaf powder per 1,000 g diet; T1: 10 g tarragon leaf powder per 1,000 g diet; T3: 30 g tarragon leaf powder per 1,000 g diet; N1+T1: 10 g nettle leaf powder per 1,000 g diet +10 g tarragon leaf powder per 1,000 g diet.

Abbreviations: FBW, final weight; FCR, feed conversion ratio; IBW, initial weight; PER, protein efficiency ratio; SGR, specific growth rate; WG%, weight gain percent; WG, weight gain.

2.9 | Ethic statement

All applicable national and institutional guidelines for the care and use of rainbow trout were according to the Iranian Veterinary Organization and Tarbiat Modares University (The guidelines adopted from the Declaration of Helsinki [1975] and the Society for Neuroscience Animal Care and Use guidelines [1998]). These guidelines were approved for implementation by the Medical Ethics Committee, School of Medical Sciences of the Tarbiat Modares University on 17th April 2006.

3 | RESULTS

The results showed that N3 group was significantly ($p \le 0.05$) associated with higher FBW, and WG compared to other groups. Fish fed the N3 diet showed higher WG% and SGR% compared to other groups significantly ($p \le 0.05$). There was no significant effect of any diet on FI, FCR, PER and SR% ($p \ge 0.05$). (Table 3).

Analysis of whole-body proximate composition showed a significantly ($p \le 0.05$) higher moisture in fish fed a N1+T1 diet and the lowest moisture in N3 group compared to all other groups ($p \ge 0.05$). There was no significant difference between control and N1+T1 ($p \ge 0.05$). There were no significant differences in moisture between control, N1, T1 and T3 fed fish ($p \ge 0.05$). Fish consuming the N3 diet exhibited significantly higher protein content compared to all groups ($p \le 0.05$). Fat content in fish fed the N1, N3, T3 and N1+T1 diet was significantly less than in other groups ($p \le 0.05$), but there were no significant differences amongst fish on N1, N3, T3, N1+T1 and N1 diets ($p \ge 0.05$). Moreover, no significant differences were seen between control and T1 ($p \ge 0.05$). The highest fat content was observed in control and T1 groups compared to N1, N3, T3 and N1+T1 significantly ($p \le 0.05$), but no significant differences between control, and T1 were observed ($p \ge 0.05$). The highest ash content in the N3 group was significantly higher than in other groups ($p \le 0.05$). (Table 4).

TABLE 4 Body proximate composition of rainbow trout provided feed supplemented with nettle and/or tarragon leaf powder

Treatments (*n* = 9)	Moisture %	Protein %	Fat %	Ash %
Control	72.16^{cd}	17.31^a	9.10^b	1.11^a
N1	71.64^{bc}	17.51^a	8.12^a	2.44^c
N3	70.11^a	18.86^b	7.86^a	2.98^e
T1	71.38^b	17.37^a	8.91^b	2.10^b
T3	71.83^{bc}	17.34^a	7.81^a	2.67^d
N1+T1	72.51^d	17.42^a	7.73^a	2.11^b
Pooled SE	0.20	0.21	0.18	0.02

Note: Values are presented as mean and pooled SE. Mean values with different superscripts within a column vary significantly according to one-way ANOVA ($p \le 0.05$).

Control: without supplement; N1: 10 g nettle leaf powder per 1,000 g diet; N3: 30 g nettle leaf powder per 1,000 g diet; T1: 10 g tarragon leaf powder per 1,000 g diet; T3: 30 g tarragon leaf powder per 1,000 g diet; N1+T1: 10 g nettle leaf powder per 1,000 g diet +10 g tarragon leaf powder per 1,000 g diet.

The T3 group was associated with a significantly higher WBC count compared to other groups significantly ($p \le 0.05$). The highest RBC count and Hb were observed in fish fed the N1+T1 diet compared to all groups significantly ($p \le 0.05$). The highest Hct was detected in T3 diet compared to other groups significantly ($p \le 0.05$). The highest MCV was observed in fish fed the N1 and N1+T1 groups compared to other groups significantly ($p \le 0.05$). The highest MCH was observed in fish fed the N1, T1 and N1+T1 compared to other groups significantly ($p \le 0.05$). Fish fed T1 diets showed higher MCHC than other groups ($p \le 0.05$) (Table 5). The highest lymphocytes count was observed in control compared to other groups significantly ($p \le 0.05$). The highest monocytes count was observed in

TABLE 5 Blood parameters of rainbow trout provided feed supplemented with nettle and/or tarragon leaf powder

Treatments (n = 9)	WBC (×10^{3}/mm^{3})	RBC (×10^{6}/mm^{3})	Hb (g dl^{-1})	Hct (%)	MCV (Fl)	MCH (Pg)	MCHC (g dl^{-1})
Control	5,933^{a}	1,150,000^{b}	6.73^{b}	46.33^{b}	402.33^{b}	58.33^{a}	14.77^{ab}
N1	6,333^{a}	1,240,000^{c}	7.53^{c}	52.00^{c}	419.00^{c}	60.66^{b}	14.44^{a}
N3	7,433^{bc}	1,050,000^{a}	6.13^{a}	41.44^{a}	393.77^{a}	57.88^{a}	15.66^{bcd}
T1	7,600^{c}	1,039,888^{a}	6.16^{a}	41.66^{a}	400.77^{b}	59.66^{b}	16.11^{d}
T3	9,233^{d}	1,138,333^{b}	6.67^{b}	54.33^{d}	397.77^{ab}	58.33^{a}	14.88^{abc}
N1+T1	7,000^{b}	1,356,666^{d}	8.23^{d}	47.33^{b}	419.77^{c}	60.33^{b}	15.77 cd
Pooled SE	153	11,883	0.06	0.54	1.42	0.33	0.28

Note: Values are presented as mean and pooled SE. Mean values with different superscripts within a column vary significantly according to one-way ANOVA ($p \le 0.05$).

Control: without supplement; N1: 10 g nettle leaf powder per 1,000 g diet; N3: 30 g nettle leaf powder per 1,000 g diet; T1: 10 g tarragon leaf powder per 1,000 g diet; T3: 30 g tarragon leaf powder per 1,000 g diet; N1+T1: 10 g nettle leaf powder per 1,000 g diet +10 g tarragon leaf powder per 1,000 g diet.

Abbreviations: HB, Haemoglobin; HCT, Haematocrit; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; MCV, Mean corpuscular volume; RBC, Red blood cells; WBC, White blood cells.

TABLE 6 Lymphocyte, monocyte and neutrophil count in blood of rainbow trout provided feed supplemented with nettle and/or tarragon leaf powder

Treatments (n = 9)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)
Control	77.00^{d}	2.66^{a}	20.55^{a}
N1	70.72^{c}	2.44^{a}	26.33^{b}
N3	68.00^{b}	3.55^{b}	28.00^{c}
T1	70.33^{c}	3.66^{bc}	25.77^{b}
T3	65.00^{a}	4.44^{c}	30.00^{d}
N1+T1	67.77^{b}	3.55^{b}	28.33^{c}
Pooled SE	0.36	0.78	0.35

Note: Values are presented as mean and pooled SE. Mean values with different letters within a column vary significantly according to one-way ANOVA ($p \le 0.05$).

Control: without supplement; N1: 10 g nettle leaf powder per 1,000 g diet; N3: 30 g nettle leaf powder per 1,000 g diet; T1: 10 g tarragon leaf powder per 1,000 g diet; T3: 30 g tarragon leaf powder per 1,000 g diet; N1+T1: 10 g nettle leaf powder per 1,000 g diet +10 g tarragon leaf powder per 1,000 g diet.

the T3 compared to other groups significantly ($p \le 0.05$), but there were no significant differences between T1 and T3 ($p \ge 0.05$). The T3 group resulted in significantly higher neutrophils counts compared to all groups ($p \le 0.05$) (Table 6).

The N3, T3, N1+T1 diet resulted in significantly higher albumin levels compared to other groups significantly ($p \le 0.05$). The highest lysozyme activity was detected in fish on the T3 diet compared to other groups significantly ($p \le 0.05$), but there was no significant difference between T1 and T3 ($p \ge 0.05$). The total immunoglobulin (Ig) in fish fed N3 group was significantly higher compared to other experimental groups ($p \le 0.05$). The highest serum total protein was measured in N3 compared to all groups significantly ($p \le 0.05$), but there was no significant difference between N3 and T1 (Table 7).

4 | DISCUSSION

In recent decades, the effects of hormones, antibiotics and chemical compounds on the growth parameters and immune system of aquatic animals have been investigated (Sakai, 1999). Plant compounds have been used in aquaculture to stimulate growth performance, body proximate composition, biochemical and immunological parameters (Bilen, Altunoglu, et al., 2016; Bilen, Ünal, et al., 2016; Citarasu, 2010; Mehrabi & Firouzbakhsh, 2020; Mehrabi et al., 2019; Adel et al., 2017). Moreover, plants have been suggested as appropriate alternatives to antibiotics and chemical therapeutics (Immanuel et al., 2009; Mehrabi et al., 2019; Ngugi et al., 2015; Adel et al., 2017; Stratev et al., 2018; Zaefarian et al., 2017). In the present research, we investigated the effects of nettle and tarragon powder individually and one level combination on rainbow trout (*O. mykiss*) fingerlings.

We found that nettle powder supplementation of rainbow trout diet enhances growth performance. The N3 diet showed beneficial effects on FBW, WG, WG% and SGR. In a recent study, ethanolic extract of stinging nettle (*U. dioica*) improved WG, SGR, FCR and survival rate percent compare to control in rainbow trout (Adel et al., 2017). Furthermore, in another study, Ngugi et al., (2015) reported that nettle powder added to a *Labeo victorianus* diet, improved FW, WG%, FCR and SGR compared to the control. Another study showed that nettle methanolic extract increased WG and SGR in rainbow trout (Bilen, Ünal, et al., 2016). Administration of nettle powder increased feed palatability, feed intake and growth performance in fish (Adel et al., 2017). It seems that bioactive compounds in nettle (*U. dioica*) leaf including capsaicin, cinnamaldehyde and carvacrol improved growth performance (Jamroz & Kamel, 2002; Ngugi et al., 2015) due to increase food utilization efficiency (Bedford, 2000), digestive enzyme secretion, digestion and nutrient absorption (Lee & Gao, 2012). Furthermore, maybe phenolic compounds stimulated growth performance due to inhibition of the colonization of various

TABLE 7 Albumin, lysozyme activity, total immunoglobulin and total protein in plasma of rainbow trout provided feed supplemented with nettle and/or tarragon leaf powder

Treatments (*n* = 9)	Albumin (g dl^{-1})	Lysozyme activity (U ml^{-1})	Total immunoglobulin (mg ml^{-1})	Total protein (g dl^{-1})
Control	1.62^{a}	57.33^{a}	18.66^{a}	3.73^{a}
N1	1.55^{a}	58.33^{ab}	19.88^{b}	4.00^{b}
N3	1.94^{c}	62.33^{b}	22.50^{d}	4.50^{d}
T1	1.73^{b}	81.33^{d}	21.50^{c}	4.30 cd
T3	1.89^{c}	80.00 cd	21.50^{c}	4.10^{bc}
N1+T1	1.89^{c}	75.66^{c}	21.44^{c}	4.17^{bc}
Pooled SE	0.02	1.43	0.29	0.13

Note: Values are presented as mean and pooled SE. Mean values with different superscripts within a column vary significantly according to one-way ANOVA ($p \le 0.05$).

Control: without supplement; N1: 10 g nettle leaf powder per 1,000 g diet; N3: 30 g nettle leaf powder per 1,000 g diet; T1: 10 g tarragon leaf powder per 1,000 g diet; T3: 30 g tarragon leaf powder per 1,000 g diet; N1+T1: 10 g nettle leaf powder per 1,000 g diet +10 g tarragon leaf powder per 1,000 g diet.

pathogenic and non-pathogenic species of bacteria in fish intestine (Van Hai, 2015).

In the present investigation, we did not find significant growth performance improvements following the dietary inclusion of tarragon. These data are similar to those from previous studies where the inclusion of tarragon powder did not stimulate growth in African catfish (*Clarias gariepinus*) (Mbokane & Moyo, 2020), and Mozambique tilapia (*Oreochromis mossambicus*) (Mbokane & Moyo, 2018). These results are in agreement with our study where tarragon powder did not increase WG% and SGR to a similar extent as 30 g/kg nettle powder. The combination of dried Astragalus root (*Radix astragalin*) and Chinese angelica root (*R. angelicae sinensis*) powder at a ratio of 5:1 (w/w) in Jian carp (*Cyprinus carpio var. Jian*) diet in two levels 10 and 15 g/kg improved body weight (Jain et al., 2008). Besides, in previous research, the addition of garlic powder improved growth performance in Asian sea bass (*Lates calcarifer*) (Talpur & Ikhwanuddin, 2012). Ji, Jeong, et al., (2007) reported that use of a plant powder combination, including *Massa medicata fermentata*, *Crataegi fructus*, *Artemisia capillaries* and *Cnidium officinale*, in the ratio of 2:2:1:1, improved weight gain in juvenile Japanese flounder (*Paralichthys olivaceus*). In our study, dietary inclusion of a combination of nettle and tarragon did not improve growth performance. Previous studies reported that tarragon (*A. dracunculus*) compounds like coumarin reduced diet palatability (Favaretto et al., 2015), and feed intake (Mbokane & Moyo, 2018, 2020). Moreover, partial differences between present and previous studies can be attributed to fish species, size, physiology, plant additive doses and culture condition (Akrami et al., 2015). Ngugi et al., (2015) confirmed that an increase in nettle powder in the Victoria labeo (*L. victorianus*) diet, increased growth performance. In this study, we observed FCR and PER improvement at the highest rate of nettle powder (N3) addition, but the effects were not significantly. One recent study demonstrated that garlic (*Allium sativium*) powder increased FBW, WG and SGR in brown trout (*Salmo caspius*), but there were no significant differences in survival rate between groups (Zaefarian et al., 2017). Moreover, the use of green tea ethanol extract in black rockfish (*Sebastes schlegeli*) (Hwang et al., 2013), and nettle acetone extract in electric yellow cichlid (*Labidochromis caeruleus*) (Yeşilayer et al., 2020) showed the same result in survival rate. But Adel et al., (2017) reported that 20, and 30 g/kg diet nettle ethanolic extract showed a higher survival rate compared to control and 10 g/kg nettle in rainbow trout. It can be interpreted because of fish size, stocking density and ambient culturing situation. Another study reported that different levels of nettle powder enhanced growth performance including, WG, SGR and FCR in rainbow trout, but there were no significant differences in survival rate between groups (Mehrabi & Firouzbakhsh, 2020; Mehrabi et al., 2020). In our study, improvements in FBW, WG, WG% and SGR in rainbow trout diet could be the effects of nettle compounds (Ahmed & Parsuraman, 2014; Akbay et al., 2003; Dalev et al., 1996) which stimulate appetite, increase nutrients intake and eventually improve growth performance (Adel et al., 2017). Although Jain et al., (2008) suggested that certain plant additives may have the appetite and digestion-stimulating effects like to nettle (*U. dioica*) (Mehrabi et al., 2020; Ngugi et al., 2015; Adel et al., 2017), but in our study, tarragon supplementation did not stimulate growth performance because of coumarin (Obolskiy et al., 2011), and its appetite-suppressing properties, which can hinder animals from consuming tarragon (*A. dracunculus*) (Favaretto et al., 2015). Thus, in our study dietary inclusion of tarragon could not increase diet palatability, feed intake and growth performance in fingerling rainbow trout (Mbokane & Moyo, 2018, 2020).

Investigation of body proximate composition of rainbow trout which were fed with different levels of nettle and tarragon powder, showed the highest and the least percent of moisture in fish fed on N1+T1 and N3 respectively. In previous studies, garlic powder and black cumin oil (*Nigella sativa*) reduced body proximate moisture in rainbow trout (Öz et al., 2018), brown trout (*S. caspius*) (Zaefarian et al., 2017) and demonstrated high moisture in control (Öz et al., 2018; Zaefarian et al., 2017) similar to our result. Another study showed the highest level of moisture in control and the least level of moisture in sterlet sturgeon which was fed with garlic powder (Lee et al., 2014). While, at the same time use of garlic ethanol extract showed no differences in moister between garlic group and control in sterlet sturgeon (Lee et al., 2012).

In our study, the highest body proximate protein was detected in fish fed on N3. In this line, the inclusion of garlic powder (Zaefarian et al., 2017), green tea ethanol extract (Hwang et al., 2013) and black cumin oil (*Nigella sativa*) (Öz et al., 2018) increased body protein in brown trout (*S. caspius*), black rockfish (*S. schlegeli*) and rainbow trout respectively. Furthermore, garlic powder and oil increased body proximate protein and reduced fat in Nile tilapia (*Oreochromis niloticus*) (Metwally, 2009). Hwang et al., (2013) reported the inclusion of green tea ethanol extract decreased fat content in black rockfish (*S. schlegeli*), and garlic powder showed the same result in starlet (*Acipenser ruthenus*) fingerling (Lee et al., 2014) and brown trout (*S. caspius*) (Zaefarian et al., 2017). These reports agree with our results which showed higher fat in control and higher protein in fish fed on the N3 diet. In this study, nettle components including amino acids (Ahmed & Parsuraman, 2014), and bioactive compounds could increase feed utilization and intake nutrients (Jain et al., 2008). Besides, nettle phenolic compounds (Otles & Yalcin, 2012) reduced fat, and increased protein accumulation in the body composition (Lee et al., 2014; Metwally, 2009; Zaefarian et al., 2017). In our study, the highest body proximate ash was observed in fish fed on N3. In this line, garlic powder increased body ash in *Acipenser ruthenus* fingerling (Lee et al., 2014), and brown trout (*S. caspius*) (Zaefarian et al., 2017). Moreover, green tea ethanol extract improved black rockfish body proximate composition ash as well (Hwang et al., 2013). As it was mentioned, in previous investigations, a high level of plant supplement showed higher crude ash (Hwang et al., 2013; Lee et al., 2014; Zaefarian et al., 2017) as well as our result. While, onion (*Allium cepa*) powder did not show any effect on proximate composition of olive flounder (*Paralichthys olivaceus*) (Cho & Lee, 2012). It can be attributed to different type of additives (powder/extract), fish and plant species (Citarasu, 2010; Hashemi & Davoodi, 2011).

Supplementation of fish diets with plants powder or extract has been reported to enhance haematological, immunological and biochemical parameters (Bilen, Altunoglu, et al., 2016; Bilen, Ünal, et al., 2016; Binaii et al., 2014; Mehrabi & Firouzbakhsh, 2020; Ngugi et al., 2015; Adel et al., 2017). We found that nettle and tarragon powder individually and in combination in rainbow trout diet to be associated with higher RBC, WBC, Hct, Hb, MCV, MCH, MCHC, some immune and biochemical blood factors. In the previous studies *Artemisia annua* extract (Soares et al., 2020), and *Artemisia arfa* powder (Mbokane & Moyo, 2020) improved WBC, neutrophils, monocytes, RBC counts and Hct in Nile tilapia (*O. niloticus*), and African catfish (*Clarias gariepinus*) respectively. Moreover, *Artemisia arfa* powder showed WBC counts improvement in Mozambique tilapia, (*O. mossambicus*) (Mbokane & Moyo, 2018). These reports agree with our result which we observed higher WBC counts, monocytes, neutrophils and Hct at least in T3. In a previous investigation, nettle powder caused to higher Hct, Hb and MCHC, RBC, WBC count in *Huso* (Binaii et al., 2014). Another study showed the highest Hb, Hct, MCV, MCH, MCHC WBC and RBC count in *L. victorianus* which fed with nettle powder (Ngugiet al., 2015). Another investigation showed WBC, RBC, Hct and Hb increase in dietary inclusion of nettle powder in rainbow trout (Mehrabi & Firouzbakhsh, 2020; Mehrabi et al., 2020). Moreover, Adel et al., (2017) reported that nettle ethanolic extract improved RBC, WBC, Hct, Hb and neutrophil in rainbow trout. Moreover, ginger powder showed the same results in rainbow trout (Nya & Austin, 2009). Binaii et al., (2014) reported that nettle powder had no effects on lymphocytes in beluga (*H. huso*). This result is in line with our study where nettle and tarragon powder did not increase lymphocytes, and higher lymphocytes were observed in control. Furthermore, garlic (*A. sativium*) powder had no effects on haematological parameters including RBC, WBC, Hct, Hb, MCV, MCH and MCHC in brown trout (*Salmo caspius*) which could be related to fish size and species (Zaefarian et al., 2017). Previous studies demonstrated the effects of nettle and tarragon separately on haematological parameters (Binaii et al., 2014; Mbokane & Moyo, 2018; Mehrabi et al., 2020; Ngugi et al., 2015; Adel et al., 2017; Soares et al., 2020). In our investigation, we observed a higher RBC count, and Hb in N1+T1. It can be related to synergistic effects of nettle and tarragon in combination like to combination of garlic, ginger and thyme powder in sea bream (*Sparidentex hasta*) (Jahanjoo et al., 2018), and black seeds and turmeric powder in sea bass (Saad et al., 2013) which showed the same result in RBC and haemoglobin. It could be attributed to iron, vitamin A, B, C and B12 which are important for RBC production. Moreover, it was mentioned that vitamin C promotes iron absorption in fish intestine (Dügenci et al., 2003). Vitamins and minerals support haematopoiesis (Binaii et al., 2014), and plants due to a variety of vitamins and minerals contents could improve haematological parameters in fish (Mbokane & Moyo, 2018; Ngugi et al., 2015; Adel et al., 2017; Soares et al., 2020).

The innate immune system in fish, is a vital defensive line to combat pathogens (Saurabh & Sahoo, 2008), and lysozyme is known to be an opsonin, and activates the complement system and phagocytes in fish (Grinde, 1989). Based on our results, immune parameters investigation showed the highest lysozyme activity in T1, and the highest level of total immunoglobulin in N3 was detected. In last studies, were mentioned that nettle powder increased serum total immunoglobulin and lysozyme activity in Victoria labeo (*L. victorianus*) (Ngugi et al., 2015), and rainbow trout (Mehrabi & Firouzbakhsh, 2020). Another research confirmed that ethanol extract of nettle in rainbow trout improved IgM and lysozyme activity (Adel et al., 2017). Total immunoglobulin improvement can be related to nettle and tarragon effects on B lymphocytes, and increased immunoglobulin production (Ngugi et al., 2015; Adel et al., 2017). In previous studies, tarragon powder (Mbokane & Moyo, 2018, 2020), nettle (Bilen, Ünal, et al., 2016) and tarragon (Soares et al., 2020) extract separately rose lysozyme activity in African catfish, Mozambique tilapia, rainbow trout and Nile tilapia respectively. Besides, caper (*Capparis spinosa*) methanol extract (Bilen, Altunoglu, et al., 2016), aloe vera (*A. barbadensis*) (Mehrabi et al., 2019), garlic (*A. sativum*) (Zaefarian et al., 2017) and onion powder (Cho & Lee, 2012) improved lysozyme activity in rainbow trout and brown trout, and olive flounder (*Paralichthys olivaceus*). Jian and Wu. (2004) documented that administration of Astragalus root (*R. astragalin*) and Chinese angelica root (*R. angelicae sinensis*) powder combination (5:1 w/w) increased lysozyme activity in Jian carp (*C. carpio var. Jian*), and black seeds and turmeric powder combination in sea bass showed the same results in lysozyme activity (Saad et al., 2013). Besides, the combination of garlic, ginger and thyme in sea bream (*Sparidentex hasta*) showed high lysozyme activity (Jahanjoo et al., 2018). In this study, although we observed numerically improvement in lysozyme activity improvement of nettle treatments, the highest level of lysozyme was observed in T1, T3 and N1+T1. Lysozyme enzyme is found among alive organisms. It is important as an innate immune parameter of fish. It is documented that fish lysozyme has lytic activity in contradiction of both Gram-positive bacteria and Gram-negative bacteria, and lysozyme is recognized to be related to nature and activates the complement system and phagocytes (Saurabh & Sahoo, 2008).

In our study, blood biochemical analysis showed that, N3, T3 and N1+T1 increased albumin, and the highest level of total protein in N3 was observed. Serum total protein is a key factor for fish health (Yang & Chen, 2003), and albumin as an important serum protein, which has a key role to transport nutrients, metabolites and xenobiotics (Peters Jr, 1995). In previous studies, nettle powder increased serum total protein and albumin in Victoria labeo (*L. victorianus*) (Ngugi et al., 2015) and, Adel et al., (2017) reported that ethanol extract of nettle increased total protein in rainbow trout. These results are in line with our study where nettle powder increased serum total protein in rainbow trout fingerling. Another study mentioned that nettle powder rose total protein in beluga (*H. huso*), but had no effects on albumin (Binaii et al., 2014), and the same result found due to the administration of garlic (*A. sativum*) powder in brown trout (*S. caspius*) (Zaefarian et al., 2017). Mehrabi et al., (2019) reported that aloe vera (*A. barbadensis*) powder increased total protein and albumin in rainbow trout. Nonetheless, the use of onion (*A. cepa*) powder was not effective on serum total protein content in olive flounder (*P. olivaceus*) (Cho & Lee, 2012). In this study, we observed serum total protein improvement in T1 and T3 treatments mathematically as like as Mbokane and Moyo. (2020) and Soares et al., (2020) who reported serum total protein improvement in African catfish and Nile tilapia respectively. In our study, we detected the highest amount of serum total protein in N3. Serum total proteins include albumin and globulin were mentioned as the important innate immune system in previous reports (Binaii et al., 2014; Mehrabi & Firouzbakhsh, 2020; Mehrabi et al., 2020; Adel et al., 2017; Zaefarian et al., 2017). Moreover, we detected a high level of albumin in N1+T1 as a combination which the same result was reported by Saad et al., (2013) where black seeds and turmeric powder combination increased serum albumin in sea bass. Besides, in this study the highest level of serum albumin was measured in N3 (Mehrabi & Firouzbakhsh, 2020; Ngugi et al., 2015; Adel et al., 2017) and T3 (Mbokane & Moyo, 2020) the same as previous reports. Albumin, total protein and lysozyme inhibit adherence and colonization of bacteria as first line of non-specific defensive system (Kaleeswaran et al., 2011). Regarding plants as safe and beneficial natural additives (Hashemi & Davoodi, 2011), also nettle (Gülcin et al., 2004), and tarragon compounds (Logendra et al., 2006; Obolskiy et al., 2011), investigation of apparent digestibility, digestive enzymes and antioxidant enzyme activity, intestine microflora and challenge with pathogens are suggested in the future research.

Our results showed that 30 g/kg nettle powder improved final weight, weight gain, weight gain percent and specific growth rate. Supplementation of 30 g/kg nettle powder increased body proximate composition including protein and ash. The 30 g/kg tarragon powder and 10 g/kg nettle +10 g/kg tarragon powder increased improve immune system, biochemical and haematology in rainbow trout fingerlings. Based on our results, 30 g/kg nettle, 30 g/kg tarragon and 10 g/kg nettle +10 g/kg tarragon powder according to cultivate aims are suggested as suitable levels to exert beneficial effects on growth performance, body composition and selected blood parameters.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Mahyar Zare; Experiment design, feeding trial, sampling, samples analysis and writing manuscript. Mohammad Kazem Mirzakhani; Feed formulation, samples analysis, statistical analysis and improve manuscript. Vlastimil Stejskal; Improve manuscript and financial support.

DATA AVAILABILITY STATEMENT

All data retrieved from this research have been included in this article.

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REFERENCES

Ahmadifar, E., Falahatkar, B., & Akrami, R. (2011). Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, *Oncorhynchus mykiss. Journal of Applied Ichthyology*, *27*(4), 1057–1060.

Ahmed, K. M., & Parsuraman, S. (2014). *Urtica dioica* L., (*Urticaceae*): A stinging nettle. *Systematic Reviews in Pharmacy*, *5*(1), 6.

Akbay, P., Basaran, A. A., Undeger, U., & Basaran, N. (2003). In vitro immunomodulatory activity of flavonoid glycosides from *Urtica dioica* L. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *17*(1), 34–37.

Akrami, R., Gharaei, A., Mansour, M. R., & Galeshi, A. (2015). Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato–biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. *Fish & Shellfish Immunology*, *45*(2), 828–834.

Banaee, M., Sureda, A., Mirvaghefi, A. R., & Rafei, G. R. (2011). Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, *37*(4), 885–896.

Bedford, M. (2000). Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimise subsequent problems. *World's Poultry Science Journal*, *56*(4), 347–365.

Behbahani, B. A., Shahidi, F., Yazdi, F. T., Mortazavi, S. A., & Mohebbi, M. (2017). Antioxidant activity and antimicrobial effect of tarragon (*Artemisia dracunculus*) extract and chemical composition of its essential oil. *Journal of Food Measurement and Characterization*, *11*(2), 847–863.

Bilen, S., Altunoglu, Y. C., Ulu, F., & Biswas, G. (2016). Innate immune and growth promoting responses to caper (*Capparis spinosa*) extract in rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology*, *57*, 206–212.

Bilen, S., Ünal, S., & Güvensoy, H. (2016). Effects of oyster mushroom (*Pleurotus ostreatus*) and nettle (*Urtica dioica*) methanolic extracts on immune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *454*, 90–94.

Binaii, M., Ghiasi, M., Farabi, S. M. V., Pourgholam, R., Fazli, H., Safari, R., Alavi, S. E., Taghavi, M. J., & Bankehsaz, Z. (2014). Biochemical and hemato-immunological parameters in juvenile beluga (*Huso huso*) following the diet supplemented with nettle (*Urtica dioica*). *Fish & Shellfish Immunology*, *36*(1), 46–51.

Borchers, A. T., Keen, C. L., Stern, J. S., & Gershwin, M. E. (2000). Inflammation and Native American medicine: The role of botanicals. *The American Journal of Clinical Nutrition*, *72*(2), 339–347.

Cho, S. H., & Lee, S. M. (2012). Onion powder in the diet of the olive flounder, *Paralichthys olivaceus*: Effects on the growth, body composition, and lysozyme activity. *Journal of the World Aquaculture Society*, *43*(1), 30–38.

Citarasu, T. (2010). Herbal biomedicines: A new opportunity for aquaculture industry. *Aquaculture International*, *18*(3), 403–414.

Dalev, P., Ljubomirova, A., & Ivanov, I. (1996). Enzyme extraction, functional properties and application of leaf protein fractions of nettle. *Biotechnology Letters*, *18*(1), 107–110.

Dawood, M. A., Koshio, S., El-Sabagh, M., Billah, M. M., Zaineldin, A. I., Zayed, M. M., & Omar, A.-A.-E.-D. (2017). Changes in the growth, humoral and mucosal immune responses following β-glucan and vitamin C administration in red sea bream, Pagrus major. *Aquaculture*, *470*, 214–222.

Does, M. P., Ng, D. K., Dekker, H. L., Peumans, W. J., Houterman, P. M., Van Damme, E. J., & Cornelissen, B. J. (1999). Characterization of *Urtica dioica* agglutinin isolectins and the encoding gene family. *Plant Molecular Biology*, *39*(2), 335–347.

Dügenci, S. K., Arda, N., & Candan, A. (2003). Some medicinal plants as immunostimulant for fish. *Journal of Ethnopharmacology*, *88*(1), 99–106.

Eisenman, S. W., Juliani, H. R., Struwe, L., & Simon, J. E. (2013). Essential oil diversity in North American wild tarragon (*Artemisia dracunculus* L.) with comparisons to French and Kyrgyz tarragon. *Industrial Crops and Products*, *49*, 220–232.

Ekanem, A. P., & Brisibe, E. A. (2010). Effects of ethanol extract of *Artemisia annua* L. against monogenean parasites of Heterobranchus longifilis. *Parasitology Research*, *106*(5), 1135–1139.

Favaretto, A., Ortiz Chini, S., Scheffer-Basso, S. M., Sobottka, A. M., Bertol, C. D., & Perez, N. B. (2015). Pattern of allelochemical distribution in leaves and roots of tough lovegrass ('*Eragrostis plana*'Nees.). *Australian Journal of Crop Science*, *9*(11), 1119–1125.

Firouzbakhsh, F., Noori, F., Khalesi, M. K., & Jani-Khalili, K. (2011). Effects of a probiotic, protexin, on the growth performance and hematological parameters in the Oscar (*Astronotus ocellatus*) fingerlings. *Fish Physiology and Biochemistry*, *37*(4), 833–842.

Greger, H. (1979). Aromatic acetylenes and dehydrofalcarinone derivatives within the *Artemisia dracunculus* group. *Phytochemistry*, *18*, 1319–1322.

Grinde, B. (1989). Lysozyme from rainbow trout, *Salmo gairdneri* Richardson, as an antibacterial agent against fish pathogens. *Journal of Fish Diseases*, *12*(2), 95–104.

Gülcin, I., Küfrevioglu, O., Oktay, M., & Büyükokuroglu, M. (2004). Study of some antioxidant parameters in mice livers affected with *Urtica Pilulifer* extracts. *Journal of Ethnopharmacology*, *90*, 205–215.

Hashemi, S. R., & Davoodi, H. (2011). Herbal plants and their derivatives as growth and health promoters in animal nutrition. *Veterinary Research Communications*, *35*(3), 169–180.

Hwang, J.-H., Lee, S.-W., Rha, S.-J., Yoon, H.-S., Park, E.-S., Han, K.-H., & Kim, S.-J. (2013). Dietary green tea extract improves growth performance, body composition, and stress recovery in the juvenile black rockfish, Sebastes schlegeli. *Aquaculture International*, *21*(3), 525–538.

Immanuel, G., Uma, R., Iyapparaj, P., Citarasu, T., Punitha Peter, S., Michael Babu, M., & Palavesam, A. (2009). Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*. *Journal of Fish Biology*, *74*(7), 1462–1475.

Jahanjoo, V., Yahyavi, M., Akrami, R., & Bahri, A. H. (2018). Influence of adding garlic (*Allium sativum*), ginger (*Zingiber officinale*), thyme (*Thymus vulgaris*) and their combination on the growth performance, haematoimmunological parameters and disease resistance to *Photobacterium damselae* in sobaity sea bream (*Sparidentex hasta*) fry. *Turkish Journal of Fisheries and Aquatic Sciences*, *18*(4), 633–645.

Jain, M., Ganju, L., Katiyal, A., Padwad, Y., Mishra, K. P., Chanda, S., Karan, D., Yogendra, K. M. S., & Sawhney, R. C. (2008). Effect of *Hippophae rhamnoides* leaf extract against Dengue virus infection in human blood-derived macrophages. *Phytomedicine*, *15*(10), 793–799.

Jamroz, D., & Kamel, C. (2002). Plant extracts enhance broiler performance. In non-ruminant nutrition: Antimicrobial agents and plant extracts on immunity, health and performance. *Journal of Animal Sience*, *80*(1), 41–46.

Jennings, S., Stentiford, G. D., Leocadio, A. M., Jeffery, K. R., Metcalfe, J. D., Katsiadaki, I., Auchterlonie, N. A., Mangi, S. C., Pinnegar, J. K., Ellis, T., Peeler, E. J., Luisetti, T., Baker-Austin, C., Brown, M., Catchpole, T. L., Clyne, F. J., Dye, S. R., Edmonds, N. J., Hyder, K., … Verner-Jeffreys, D. W. (2016). Aquatic food security: Insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment. *Fish and Fisheries*, *17*(4), 893–938.

Ji, S. –C., Jeong, G. –S., Im, G. –S., Lee, S. –W., Yoo, J. –H., & Takii, K. (2007). Dietary medicinal herbs improve growth performance, fatty acid utilization, and stress recovery of Japanese flounder. *Fisheries Science*, *73*(1), 70–76.

Ji, S.-C., Takaoka, O., Jeong, G.-S., Lee, S.-W., Ishimaru, K., Seoka, M., & Takii, K. (2007). Dietary medicinal herbs improve growth and some non-specific immunity of red sea bream *Pagrus major*. *Fisheries Science*, *73*(1), 63–69.

Jian, J., & Wu, Z. (2004). Influences of traditional Chinese medicine on non-specific immunity of Jian carp (*Cyprinus carpio var. Jian*). *Fish & Shellfish Immunology*, *16*(2), 185–191.

Jobling, M. (2012). National Research Council (NRC): Nutrient requirements of fish and shrimp. *Aquaculture International*, *20*, 601–602. https://doi.org/10.1007/s10499-011-9480-6

Kaleeswaran, B., Ilavenil, S., & Ravikumar, S. (2011). Dietary supplementation with *Cynodon dactylon* (L.) enhances innate immunity and disease resistance of Indian major carp, *Catla catla* (Ham.). *Fish & Shellfish Immunology*, *31*(6), 953–962.

Khosravi, S., Rahimnejad, S., Herault, M., Fournier, V., Lee, C. –R., Dio Bui, H. T., Jeong, J. –B., & Lee, K. –J. (2015). Effects of protein hydrolysates supplementation in low fish meal diets on growth performance, innate immunity and disease resistance of red sea bream *Pagrus major*. *Fish & Shellfish Immunology*, *45*(2), 858–868.

Kobayashi, M., Msangi, S., Batka, M., Vannuccini, S., Dey, M. M., & Anderson, J. L. (2015). Fish to 2030: The role and opportunity for aquaculture. *Aquaculture Economics & Management*, *19*(3), 282–300.

Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M., & Gardini, F. (2004). Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. *Trends in Food Science & Technology*, *15*(3–4), 201–208.

Latimer, G. W. (2016). *Official Methods of analysis of AOAC international*.

Lee, D.-H., Lim, S.-R., Han, J.-J., Lee, S.-W., Ra, C.-S., & Kim, J.-D. (2014). Effects of dietary garlic powder on growth, feed utilization and whole body composition changes in fingerling sterlet sturgeon, *Acipenser ruthenus*. *Asian-Australasian Journal of Animal Sciences*, *27*(9), 1303–1310.

Lee, D.-H., Ra, C.-S., Song, Y.-H., Sung, K.-I., & Kim, J.-D. (2012). Effects of dietary garlic extract on growth, feed utilization and whole body composition of juvenile sterlet sturgeon (*Acipenser ruthenus*). *Asian-Australasian Journal of Animal Sciences*, *25*(4), 577–583.

Lee, J. Y., & Gao, Y. (2012). Review of the application of garlic, *Allium sativum*, in aquaculture. *Journal of the World Aquaculture Society*, *43*(4), 447–458.

Li, P., & Gatlin, D. M. III (2005). Evaluation of the prebiotic GroBiotic®-A and brewers yeast as dietary supplements for sub-adult hybrid striped bass (*Morone chrysops× M. saxatilis*) challenged in situ with *Mycobacterium marinum*. *Aquaculture*, *248*(1–4), 197–205.

Logendra, S., Ribnicky, D. M., Yang, H., Poulev, A., Ma, J., Kennelly, E. J., & Raskin, I. (2006). Bioassay-guided isolation of aldose reductase inhibitors from *Artemisia dracunculus*. *Phytochemistry*, *67*(14), 1539–1546.

Mbokane, E., & Moyo, N. (2018). A preliminary investigation into the potential effect of *Artemisia afra* on growth and disease resistance in sub-adults of *Oreochromis mossambicus*. *Aquaculture*, *482*, 197–202.

Mbokane, E. M., & Moyo, N. A. G. (2020). Effect of dietary *Artemisia afra* on growth, some innate immunological parameters in *Clarias gariepinus* challenged with *Aeromonas hydrophila*. *Aquaculture International*, *28*(2), 539–553.

Mehrabi, Z., & Firouzbakhsh, F. (2020). Short-term effects of feeding powdered Aloe vera (*Aloe barbadensis*) and nettle (*Urtica dioica*) on growth performance and stimulation of innate immune responses in rainbow trout (*Oncorhynchus mykiss*). *Comparative Clinical Pathology*, *29*(2), 441–449.

Mehrabi, Z., Firouzbakhsh, F., & Jafarpour, A. (2012). Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Journal of Animal Physiology and Animal Nutrition*, *96*(3), 474–481.

Mehrabi, Z., Firouzbakhsh, F., Rahimi-Mianji, G., & Paknejad, H. (2019). Immunostimulatory effect of Aloe vera (*Aloe barbadensis*) on non-specific immune response, immune gene expression, and experimental challenge with *Saprolegnia parasitica* in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *503*, 330–338.

Mehrabi, Z., Firouzbakhsh, F., Rahimi-Mianji, G., & Paknejad, H. (2020). Immunity and growth improvement of rainbow trout (*Oncorhynchus mykiss*) fed dietary nettle (*Urtica dioica*) against experimental challenge with *Saprolegnia parasitica*. *Fish & Shellfish Immunology*, *104*, 74–82.

Metwally, M. (2009). Effects of garlic (*Allium sativum*) on some antioxidant activities in tilapia nilotica (*Oreochromis niloticus*). *World Journal of Fish and Marine Sciences*, *1*(1), 56–64.

Ngugi, C. C., Oyoo-Okoth, E., Mugo-Bundi, J., Orina, P. S., Chemoiwa, E. J., & Aloo, P. A. (2015). Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, *44*(2), 533–541.

Nya, E. J., & Austin, B. (2009). Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, *32*(11), 971–977.

Nya, E. J., & Austin, B. (2011). Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. *Fish & Shellfish Immunology*, *30*(3), 845–850.

Obolskiy, D., Pischel, I., Feistel, B., Glotov, N., & Heinrich, M. (2011). *Artemisia dracunculus* L. (tarragon): A critical review of its traditional use, chemical composition, pharmacology, and safety. *Journal of Agricultural and Food Chemistry*, *59*(21), 11367–11384.

Otles, S., & Yalcin, B. (2012). Phenolic compounds analysis of root, stalk, and leaves of nettle. *The Scientific World Journal*, *2012*, 1–12.

Öz, M., Dikel, S., & Durmus, M. (2018). Effect of black cumin oil (*Nigella sativa*) on the growth performance, body composition and fatty acid profile of rainbow trout (*Oncorhynchus mykiss*). *Irainian Journal of Fisheries and Sciences*, *17*(4), 713–724.

Pauly, D., & Froese, R. (2012). Comments on FAO's state of fisheries and aquaculture, or 'SOFIA 2010'. *Marine Policy*, *36*(3), 746–752.

Peters, T. Jr (1995). *All about albumin: Biochemistry, genetics, and medical applications*. Academic press.

Saad, T., Abou El-Geit, E., El-Hammady, A., & Mona, S. (2013). Effect of black cumin seeds (*Nigella sativa*) and/or turmeric (curcumin) on hematological, biochemical and immunological parameters of sea bass vaccinated with *Pseudomonas fluorescence* bacterin. *Life Science Journal*, *10*(2), 1292–1303.

Saeidi asl, M. R., Adel, M., Caipang, C. M. A., & Dawood, M. A. O. (2017). Immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles following dietary administration of stinging nettle (*Urtica dioica*). *Fish & Shellfish Immunology*, *71*, 230–238.

Sakai, M. (1999). Current research status of fish immunostimulants. *Aquaculture*, *172*(1–2), 63–92.

Saurabh, S., & Sahoo, P. (2008). Lysozyme: An important defence molecule of fish innate immune system. *Aquaculture Research*, *39*(3), 223–239.

Seyfried, E. E., Newton, R. J., Rubert, K. F., Pedersen, J. A., & McMahon, K. D. (2010). Occurrence of tetracycline resistance genes in aquaculture facilities with varying use of oxytetracycline. *Microbial Ecology*, *59*(4), 799–807.

Soares, M. P., Cardoso, I. L., Ishikawa, M. M., de Oliveira, A. D. S. S., Sartoratto, A., Jonsson, C. M., de Queiroz, S. C. D. N., Duarte, M. C. T., Rantin, F. T., & Sampaio, F. G. (2020). Effects of *Artemisia annua* alcohol extract on physiological and innate immunity of Nile tilapia (*Oreochromis niloticus*) to improve health status. *Fish & Shellfish Immunology*, *105*, 369–377.

Stratev, D., Zhelyazkov, G., Noundou, X. S., & Krause, R. W. (2018). Beneficial effects of medicinal plants in fish diseases. *Aquaculture International*, *26*(1), 289–308.

Talpur, A. D., & Ikhwanuddin, M. (2012). Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). *Aquaculture*, *364*, 6–12.

Tunç, M. A., Yildirim, S., & Yorük, M. A. (2019). Effects of tarragon (*Artemisia dracunculus*) powder on broiler performance parameters and histopathology of internal organs. *Austral Journal of Veterinary Sciences*, *51*(3), 113–118.

Van Hai, N. (2015). The use of medicinal plants as immunostimulants in aquaculture: A review. *Aquaculture*, *446*, 88–96.

Vaseeharan, B., & Thaya, R. (2014). Medicinal plant derivatives as immunostimulants: An alternative to chemotherapeutics and antibiotics in aquaculture. *Aquaculture International*, *22*(3), 1079–1091.

Xu, A., Shang-Guan, J., Li, Z., Gao, Z., Huang, Y., & Chen, Q. (2020). Effects of garlic powder on feeding attraction activity, growth and digestive enzyme activities of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture Nutrition*, *26*(2), 390–399.

Yan, J., Guo, C., Dawood, M., & Gao, J. (2017). Effects of dietary chitosan on growth, lipid metabolism, immune response and antioxidant-related gene expression in *Misgurnus anguillicaudatus*. *Beneficial Microbes*, *8*(3), 439–449.

Yang, J.-L., & Chen, H.-C. (2003). Effects of gallium on common carp (*Cyprinus carpio*): Acute test, serum biochemistry, and erythrocyte morphology. *Chemosphere*, *53*(8), 877–882.

Yeşilayer, N., Mutlu, G., & Yıldırım, A. (2020). Effect of nettle (*Urtica* spp.), marigold (*Tagetes erecta*), alfalfa (*Medicago sativa*) extracts and synthetic xanthophyll (*zeaxanthin*) carotenoid supplementations into diets on skin pigmentation and growth parameters of electric yellow cichlid (*Labidochromis caeruleus*). *Aquaculture*, *520*, 734964.

Yildirim, F., & Tunç, M. (2018). The effect of dietary tarragon (*Artemisia dracunculus*) powders in different levels on carcass characteristics and some internal organ's weight of broiler chickens. *Brazilian Journal of Poultry Science*, *20*(1), 179–182.

Zaefarian, A., Yeganeh, S., & Adhami, B. (2017). Dietary effects of garlic powder (*Allium sativum*) on growth, blood indices, carcass composition, and lysozyme activity in brown trout (*Salmo caspius*) and resistance against *Yersinia ruckeri* infection. *Aquaculture International*, *25*(6), 1987–1996.

Zakęś, Z., Kowalska, A., Demska-Zakęś, K., Jeney, G., & Jeney, Z. (2008). Effect of two medicinal herbs (*Astragalus radix* and *Lonicera japonica*) on the growth performance and body composition of juvenile pikeperch [*Sander lucioperca* (L.)]. *Aquaculture Research*, *39*(11), 1149–1160.

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CHAPTER 4

GARLIC *ALLIUM SATIVUM* POWDER IN EURASIAN PERCH *PERCA FLUVIATILIS* JUVENILES DIET

Zare, M., Tran, H.Q., Prokešová, M., Stejskal, V., 2021. Effects of garlic *Allium sativum* powder on nutrient digestibility, haematology, and immune and stress responses in Eurasian perch *Perca fluviatilis* juveniles. Animals 11, 2735.

My share of this work was about 70%.

animals

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Article

Effects of Garlic *Allium sativum* Powder on Nutrient Digestibility, Haematology, and Immune and Stress Responses in Eurasian Perch *Perca fluviatilis* Juveniles

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Simple Summary: Herbal medicine feed supplements are used as growth promoters, immune system stimulants, and to combat stress. We evaluated the effects of garlic powder in the diet of European perch. The inclusion of garlic powder was shown to improve whole body composition, feed digestibility, and biochemical and immunohematological effects, and increased resistance against overcrowding stress.

Abstract: The supplementation of fish diets with phytogenics can increase growth performance and can modulate immune system response. European perch *Perca fluviatilis* (initial weight 25.0 ± 0.4 g) were fed a diet including 0 (Control), 10 (G10), 20 (G20), and 30 (G30) g kg^{-1} garlic powder. No significant difference in the growth parameters and somatic indices were observed. Significantly higher fat digestibility was observed in G10 and G30 diets compared to in the control and G20 diets($p < 0.05$). Significantly greater red blood cell and white blood cell counts were observed with the G10 diet ($p < 0.05$). Garlic significantly decreased serum cholesterol in all of the experimental groups. Serum albumin was significantly higher in the G10 and G20 diets ($p < 0.05$). Immediately after the overcrowding stress challenge, the garlic groups showed significantly higher cortisol levels than the control group, while no significant difference was observed in the glucose concentration among groups. At 1 h post-stress, all of the groups that had been fed a garlic-supplemented diet showed lower cortisol levels than the control group, and this trend was maintained at 6 and 24 h post stress ($p < 0.05$), and glucose level in all garlic groups was significantly lower than control ($p < 0.05$). Garlic at 10 g kg^{-1} in feed can improve apparent fat digestibility and selected blood parameters and can enhance resistance against high-density and net handling stress in Eurasian perch.

Keywords: aquaculture; cortisol; fish; haematology; immunology; myeloid cells; stress

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1. Introduction

Commercial production of fish, shellfish, and seafood is projected to increase by approximately 62% by 2030 [1]. Thus, the aquaculture rearing system is changing from an extensive system to intensive and semi-intensive systems [2], which might increase the chance of infectious disease outbreaks occurring [3]. Botanical derivatives and extracts, also known as phytogenics, have been used in fish diets as natural growth promoters and as immune stimulants. Currently, many plant extracts are considered as safe and cost-effective additives to aquafeed [4]. Antibiotics that can control pathogens on fish farms present concerns with respect to consumer health, animal welfare, and environmental pollution [5].

Garlic *Allium sativum* belongs to the *Liliaceae* family [6]. It has long been used as a herbal medicine and may be relevant to aquaculture because of its immunostimulant properties [7]. Garlic contains alliin, which has sulfur compounds including gammaglutamyl-s-allyl-cysteine and S-allyl-L-cysteins sulfoxides. Moreover, garlic contains allicin (diallyl thiosulfate), which is responsible for garlic's typical pungent smell and its medicinal properties [6], and other bioactive compounds, including vitamins (ascorbic acid, thiamine and riboflavin), minerals (potassium, phosphorus, calcium, magnesium, sodium, iron, selenium, and germanium), flavonoids (phenolic acids) [8], and amino acids [9]. Garlic powder has been reported to promote growth in Japanese seabass (*Lateolabrax japonicus*) [10] and rainbow trout *Oncorhynchus mykiss* [11] and to improve body composition in brown trout (*Salmo caspius*) [12]. However, garlic powder was not shown to improve growth performance in Asian sea bass (*Lates calarifer*) [13] or rohu (*Labeo rohita*) [14]. In addition, it has been reported to increase apparent nutrient digestion in rainbow trout [15] and Nile tilapia (*Oreochromis niloticus*) [16]. Dietary garlic powder has shown a favourable effect on blood total protein, albumin, and phagocytic activity in rainbow trout [17,18]; this plant increased fish resistance to ammonia stress [19]. Garlic powder increased immunoglobulins in European seabass (*Dicentrarchus labrax*) [20]. Garlic microencapsulated extract improved growth performance, body proximate composition, and biochemical and immunohematological parameters in rainbow trout (*Oncorhynchus mykiss*) [21]. These studies, regardless of the form of garlic presentation within diets, suggest that garlic may be used as an alternative to antibiotics [22].

Eurasian perch (*Perca fluviatilis*) is a carnivorous percid fish inhabiting northern Eurasia [23]. It is a domesticated species with a wide habitat range, and can be found in brackish water, estuaries, and rivers in recent decades [24], showing potential for European inland culture [23]. Eurasian perch can be a suitable species for recirculation aquaculture system (RAS) production and intensive culture [25], but handling through counting, sorting, and tank cleaning as well as high density stocking, may potentially increase energy consumption and decrease feed intake and growth [26].

Regarding to use of Eurasian perch in RAS culture in recent decades [25], the aim of the present study was to investigate the effects of garlic powder in feed on growth performance, body proximate composition, apparent nutrient digestibility, selected blood and immune parameters, and resistance to high-density and net handling stress in Eurasian perch juveniles.

2. Materials and Methods

2.1. Ethics Approval

The experimental procedures were performed under guidelines of the European Communities Directive (No. 2010/63/EU) on the protection of animals used for scientific purposes and have been approved by the Czech Ministry of Health (MSMT–6744/2018–2).

2.2. Preparation of Garlic Powder and Feed

Garlic powder was purchased from EQUISERVIS, Prague, in Czech Republic. Garlic powder was produced by Pommier Nutrition, Thymerais—France. (Accessed: 4 June 2019) (www.pommier-nutrition.com). Experimental feeds (Table 1) were extruded at Exot Hobby s.r.o. (Černá v Pošumaví, Czech Republic), packed in plastic vacuum bags, and stored at 4 °C until use. In the present study, corn meal was replaced by 10, 20, and 30 g garlic powder per kilogram of diet feed. The proximate composition of the basal diet, including dry matter, crude protein, crude fat, and ash, was 93.48%, 47.20%, 16.33%, and 8.91%, respectively (Table 1).

2.3. Experimental Design

Eurasian perch juveniles with an initial weight of 25.0 ± 0.4 g were obtained from Anapartners s.r.o fish farm (Prague, Czech Republic). Fish were transferred to the aquaria at the Institute of Aquaculture and Protection of Waters (České Budějovice, Czech Republic) and were fed a basal diet formulation (Table 1) without garlic powder for a 14-day acclimation period before the feeding trial [12]. After adaptation, 1320 fish were randomly distributed into twelve 150 L tanks (110 fish per tank) with a water flow rate 10 L min^{-1} in RAS [25].

Each diet was tested with three replicates. Fish were fed manually to apparent satiation at 08:00, 12:00, and 16:00 for 87 days, and uneaten feed was collected at maximum of 30 min after each meal. Water temperature, pH, and dissolved oxygen (DO) were measured daily by an HQ40D portable multi-meter (Hach Lange GmbH, Düsseldorf, Germany) and were maintained at 22.1 ± 0.5 °C, pH 7.14 ± 1.61, and DO 8.16 ± 0.42 mg L^{-1}, respectively. The photoperiod was 12L:12D [27].

Table 1. Proximate composition of experimental diets with and without garlic powder.

Ingredients (g kg^{-1})	Control	G10	G20	G30
Fish meal	271	271	271	271
Soybean concentrate	290	290	290	290
Corn meal	97	87	77	67
Soybean meal	128.5	128.5	128.5	128.5
Garlic powder [a]	0	10	20	30
Fish oil	77	77	77	77
Rapeseed oil	58	58	58	58
Methionine [b]	8	8	8	8
Lysine [c]	5	5	5	5
Valine [d]	2	2	2	2
L-Threonine [e]	0.5	0.5	0.5	0.5
Vitamins & minerals [f]	8	8	8	8
Binder [g]	50	50	50	50
Yttrium oxide (Y_2O_3) [h]	5	5	5	5
Proximate composition analysis				
Dry matter%	93.48	93.54	93.71	93.17
Crude protein%	47.20	46.84	46.59	46.33
Fat%	16.33	16.51	15.98	16.13
Ash%	8.91	8.78	8.78	8.82
Fiber%	1.82	3.87	3.98	2.42
Nitrogen-free extract (NFE) [f,i]	19.22	17.54	18.38	19.47
Gross energy (Kj g^{-1}) [g,j]	17.90	18.24	17.99	17.72

[a] Garlic powder was purchased from EQUISERVIS, Prague, in Czech Republic. [b] Adisseo, Shanghai, China; [c] Inner Mongolia Eppen Biotech Co., Ltd., Ningxia, China.; [d] Ajinomoto Animal Nutrition Europe; [e] Ningxia Eppen Biotech, China; [f] Aminovitan Sak, Trouw Nutrition Biofaktory s.r.o, Prague, Czech Republic; [g] binder (NutriBind, Adisseo, Shanghai, China) (3.0%); [h] yttrium oxide (Y_2O_3), Sigma, Ronkonkoma, NY, USA; [i] nitrogen-free extracts (NFE) = dry matter—(crude protein + crude lipid + ash + fiber) [15]; [j] gross energy was calculated according to following formula: gross energy (MJ/kg) = (protein × 23.6 kJ g^{-1}) + (fat × 39.5 kJ g^{-1}) + (carbohydrates × 17.2 kJ g^{-1}) [15]; control: without garlic supplement; G10: 10 g garlic powder per 1000 g diet; G20: 20 g garlic powder per 1000 g diet; G30: 30 g garlic powder per 1000 g diet.

2.4. Growth Performance

At the end of the feeding trial, feed was withheld for 24 h. The fish were anesthetized by tricaine methane sulphonate (MS-222) at 200 mg L^{-1} water [21] and counted, and individual length and weight were measured. Growth performance and survival rate were calculated [21]. Final body weight (FBW), feed intake (FI), weight gain (WG), weight gain percentage (WG%), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), survival rate (SR%), condition factor (CF%), hepatosomatic index (HSI), and viscerosomatic index (VSI) were calculated as follows:

FBW (g) = Final body weight
WG (g) = [Final body weight (g) − Initial body weight (g)]
WG% = [Final body weight (g) − Initial body weight (g)]/initial body weight (g) × 100
FI (g/fish) = dry feed consumed/number of fish
PER = Weight gain (g)/total protein intake (g)
FCR = dry feed intake (g)/WG (g)
SGR (% day^{-1}) = 100 × [ln final body weight (g) − ln initial body weight (g)]/time (days)
SR% = (number of fish after test/initial number of stocked fish) × 100
CF% = [fish body weight (g)/(fish length)^3(cm)] × 100
HSI% = [liver weight (g)/body weight (g)] × 100

VSI% = [viscera weight (g)/body weight (g)] × 100

2.5. Whole Body Proximate Composition

At the end of experiment, two fish per each tank (n = 6 per group) were randomly selected. Fish were anesthetized with MS-222 at 200 mg L^{-1} water [21] and were killed by a sharp blow to the head. The entire fish body was ground, packed individually into plastic bags, and stored at −20 °C for whole body proximate composition analysis. Both body and feed proximate composition analyses were conducted according to the methods of the Association of Official Analytical Chemists (AOAC) [28]. Crude fat was analyzed by the extraction method using hexane–isopropanol (3:2) according to Hara and Radin, [29] with slight modifications [30]. Crude protein was measured using the Kjeldahl method (BUCHI Labortechnik AG, type K-360, Königswinter, Germany) [12]. Dry matter was analyzed by drying in a NÜVE type FN 400P oven (NÜVE, Ankara, Turkey) at 105 °C to a stable weight [21]. Ash was analyzed in a muffle furnace L 40/11 BO (Nabertherm GmbH, Lilienthal, Germany)) at 550 °C for 4 h [12].

2.6. Apparent Digestibility Coefficients

Diets contained 5 g kg^{-1} yttrium oxide (Y_2O_3) as an inert marker (Table 1). Two hours after the final daily meal, the tanks were brushed and cleaned, and the remaining faeces were discarded. After cleaning, the faeces were collected overnight by sieving every two hours from outlets until the first daily feeding in the morning. The faeces from each tank were stored separately at −20 °C after centrifugation at 3000 rpm for 15 min for analysis [31]. The Y_2O_3 in the diet and faeces was measured using inductively coupled plasma emission spectrometry following digestion with nitric acid at 180 °C for 48 h. The apparent digestibility coefficients (ADC) of the dry matter, protein, and fat were calculated with the following formula [15]:

% digestibility = 100 × 100 [(yttrium in feed/yttrium in faeces) × (nutrient in faeces/nutrient in feed)].

2.7. Haematology and Biochemistry

After 24 h starvation to induce the post-absorptive condition, two fish from each tank (n = 6 per group) were randomly netted and anesthetized by tricaine methane sulphonate (MS-222) at 200 mg L^{-1} water [21]. Duplicate blood samples were drawn from the caudal vein into heparinized and non-heparinized sterile syringes. The heparinized blood samples were transferred to heparinized Eppendorf tubes and were placed on ice for haematological analysis. The non-heparinized blood samples were transferred to non-heparinized Eppendorf tubes, and samples were left on ice for 2 h to clot [32]. The serum was subsequently separated by centrifugation (Heraeus Megafuge 16 R Centrifuge) at 4200 rpm for 15 min at 4 °C and was stored at −80 °C until analysis [33]. Red blood cells (RBC), white blood cells (WBC), and subpopulations were counted according to a modified method of Korytář et al. [34]. The heparinized blood (10 µL) was diluted in 200 µL RPMI medium on ice for cell composition evaluation by a FACS Canto flow cytometer (Heidelberg, Germany). The biochemical parameters of the blood were assessed using an Abbott Architect c8000 clinical chemistry analyzer (Abbott, Chicago, IL, USA) and assay kits [19] according to manufacturer's instructions, as follows: serum total cholesterol, B7D6C7 G3-5321/R02 (Abbott, Chicago, IL, USA); triglycerides B7D7E7 G3-9334/R03 (Abbott, USA); alanine aminotransferase (ALT), B8L9x7 G5-4432/R05 (Abbott, USA); aspartate aminotransferase, G8-1502/R06 B8LY7 (Abbott, USA); albumin, 7D53-2030-3927/R6 (Abbott, USA); total protein, G6-6667/R04 B7D7D7 (Abbott, USA); and glucose, B3L8X7 G3-5375/R02) (Abbott, USA). Cortisol levels were analyzed with a cortisol assay kit (L2KCO2) using an immunochemistry analyzer Immulite 2000Xpi Siemens (Siemens Healthcare GmbH, Erlangen, Germany) at the Stafila laboratory, České Budějovice, Czech Republic.

2.8. Respiratory Burst and Phagocytic Activity

Two fish per tank (*n* = 6 per group) were anesthetized with MS-222 (200 mg L^{-1} water) [21]. The head kidney was removed, and the leukocytes were separated by pushing them through a nylon sieve with RPMI-1640 medium, according to the method by Biswas et al. [35]. A respiratory burst activity assay was conducted using nitro blue tetrazolium with minor modifications according to Zaineldin et al. [36]. Briefly, the leukocyte suspension was transferred into 96-well plates, and an equivalent volume of 0.2% nitro blue tetrazolium solution (Sigma, Ronkonkoma, NY, USA) was added to each well and was incubated for 30 min at room temperature. After incubation, N-dimethylformamide (Sigma, USA) was added and centrifuged for 5 min at 3000 rpm. The respiratory burst activity was reported as the mean fluorescence intensity.

Phagocytic activity was assessed using a modified method from Morimoto et al. [37]. The leukocytes of head kidney were separated by washing with PBS (5×10^5 cells mL^{-1}) and were incubated with latex beads at 25 °C for 2 h, after which cell-related fluorescence was evaluated, and the samples were transferred into 96-well plates and assessed with a FACS Canto flow cytometer (Heidelberg, Germany) to detect the fluorescence of the beads engulfed by the phagocytic cells.

2.9. High-Density and Net Handling Stress Challenge

At the end of the feeding period, working with one tank at a time, the volume of water was decreased to leave the fish in a high-density condition (0.67 kg/L) for one minute with adequate aeration to avoid additional stress [38]. The fish were netted and removed from the water for 30 s [27] and were then returned to the tank, where the water level of the tank was increased back to the original volume, and the density was reduced [38]. Immediately after, two fish per tank were randomly selected (*n* = 6 per group), and the tank was refilled. The fish were anaesthetized with MS-222, and 1 mL of blood was drawn from the caudal vein with non-heparinized sterile syringes. All of these fish were killed after sampling. Sampling was conducted prior to the stress challenge, immediately after stress, and 1, 6, and 24 h post-stress [27]. Blood sampling in each tank was completed within 5 min.

2.10. Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics v. 22 (Armonk, NY, USA). Data normality and homogeneity were checked using the Kolmogorov–Smirnov test. Data were analyzed by one-way ANOVA. Significant differences among the mean values was set at $p < 0.05$ using the Duncan test. In addition, to determine if the effect was linear and/or quadratic, a follow-up trend analysis using orthogonal polynomial contrasts was performed. The results are presented as mean ± SD (standard deviation of the mean).

3. Results

3.1. Growth Performance and Feed Utilization

No significant differences in growth performance and feed utilization, including final weight, weight gain, weight gain percent, specific growth rate, feed intake, feed conversion ratio, protein efficiency ratio, or survival rate, were observed among the groups ($p > 0.05$). The condition factor was significantly lower in the G30 diet group compared to the other groups ($p < 0.05$). In particular, the condition factor significantly linearly decreased with the increasing dietary garlic powder levels ($p = 0.04$). In addition, no significant differences in the viscerosomatic or hepatosomatic index were found ($p > 0.05$). (Table 2).

Table 2. Growth performance and feed utilization of juvenile Eurasian perch consuming feed supplemented with garlic powder.

							Linear Trend		Quadratic Trend	
Parameters	**Control**	**G10**	**G20**	**G30**	**ANOVA**	η_p^2	***P*-Value**	R^2	***P*-Value**	R^2
Initial weight (g)	24.75 ± 0.47	25.37 ± 0.41	24.77 ± 0.13	25.18 ± 0.18	0.13					
Final weight (g)	66.29 ± 1.99	67.39 ± 1.89	64.89 ± 1.53	65.52 ± 2.93	0.55					
Feed intake (g fish^{-1})	68.49 ± 1.82	68.41 ± 1.06	68.00 ± 0.80	67.83 ± 1.76	0.92					
Weight gain (g)	41.53 ± 2.02	42.02 ± 1.51	40.12 ± 1.40	40.34 ± 3.09	0.65					
Weight gain%	167.82 ± 9.25	165.55 ± 3.67	161.97 ± 4.83	160.29 ± 13.38	0.71					
Feed conversion ratio	1.68 ± 0.15	1.64 ± 0.07	1.73 ± 0.08	1.71 ± 0.16	0.85					
Specific growth rate (% day^{-1})	1.12 ± 0.04	1.11 ± 0.01	1.10 ± 0.02	1.09 ± 0.05	0.76					
Protein efficiency ratio	1.25 ± 0.09	1.29 ± 0.03	1.24 ± 0.07	1.26 ± 0.13	0.90					
Survival rate (%)	96.36 ± 2.40	98.18 ± 0.91	96.96 ± 1.89	97.57 ± 2.78	0.75					
Condition factor%	1.25 ± 0.05 ^{b}	1.24 ± 0.02 ^{b}	1.18 ± 0.03 ^{b}	1.10 ± 0.03 ^{a}	0.00	0.78	0.04	0.33	0.05	
Hepatosomatic index%	1.59 ± 0.36	1.56 ± 0.28	1.53 ± 0.37	1.38 ± 0.30	0.44					
Viscerosomatic index%	12.06 ± 2.28	13.13 ± 2.17	12.88 ± 1.56	13.19 ± 1.85	0.49					

Values are presented as (mean ± SD; *n* = 110). Mean values with different superscripts within a row vary significantly according to one-way ANOVA ($p < 0.05$). R^2 = R squared. η_p^2 = partial eta squared. Control: without garlic supplement; G10: 10 g garlic powder per 1000 g diet; G20: 20 g garlic powder per 1000 g diet; G30: 30 g garlic powder per 1000 g diet.

3.2. Body Proximate Composition

No significant differences were observed the among groups in terms of whole body dry matter, fat, or ash ($p > 0.05$). The level of protein in fish consuming the G30 diet was significantly higher than the G1 group ($p < 0.05$), but there were no significant differences among the controls, G10, and G20 groups ($p > 0.05$) or among the controls, G20, and G30 groups ($p > 0.05$). There was a significant linear ($p = 0.01$) and quadratic ($p = 0.04$) trend regarding the dietary garlic powder level for body protein content, where body protein content decreased with the inclusion of garlic powder at G10 and then increased with the inclusion of garlic powder at G20 and G30. (Table 3).

Table 3. Body proximate composition of Eurasian perch consuming feed supplemented with garlic powder.

							Linear Trend		Quadratic Trend	
Parameters	**Control**	**G10**	**G20**	**G30**	**ANOVA**	η_p^2	***P*-Value**	R^2	***P*-Value**	R^2
Dry matter%	32.08 ± 1.15	31.75 ± 1.33	32.10 ± 1.71	32.26 ± 1.09	0.92					
Fat%	11.16 ± 1.50	10.96 ± 1.44	10.78 ± 1.13	9.76 ± 1.24	0.29					
Protein%	17.42 ± 0.60 ^{ab}	16.98 ± 0.69 ^{a}	17.39 ± 0.98 ^{ab}	18.31 ± 0.67 ^{b}	0.04	0.33	0.01	0.23	0.04	0.25
Ash%	3.38 ± 0.43	3.01 ± 0.60	3.16 ± 0.19	3.53 ± 0.29	0.16					

Values are presented as (mean ± SD; *n* = 6). Mean values with different superscripts within a row vary significantly according to one-way ANOVA ($p < 0.05$). R^2 = R square. η_p^2 = partial eta squared. Control: without garlic supplement; G10: 10 g garlic powder per 1000 g diet; G20: 20 g garlic powder per 1000 g diet; G30: 30 g garlic powder per 1000 g diet.

3.3. Apparent Digestibility Coefficient (ADC%)

Significantly higher dry matter digestibility was observed in all of the garlic-supplemented groups compared to the controls ($p < 0.05$). Moreover, significantly higher fat digestibility was found in the G10 and G30 groups compared to the control and G20 groups ($p < 0.05$). No differences in protein digestibility were observed among the groups ($p > 0.05$). A positive linear ($p = 0.00$) and quadratic ($p = 0.00$) trend was found for dietary garlic powder levels and protein digestibility, where protein digestibility increased with the inclusion of garlic powder at G10 and then decreased with the inclusion of garlic powder at G20 and G30. (Table 4).

Table 4. Apparent digestibility coefficient for dry matter, fat, and protein in Eurasian perch provided feed supplemented with garlic powder.

							Linear Trend		Quadratic Trend	
Parameters	Control	G10	G20	G30	ANOVA	$\eta_p{}^2$	*P*-Value	R^2	*P*-Value	R^2
ADCd%	77.53 ± 0.59 [a]	80.78 ± 0.50 [b]	79.60 ± 0.87 [b]	81.12 ± 1.83 [b]	0.01	0.71	0.69	-	0.27	-
ADCf%	78.29 ± 0.46 [a]	79.89 ± 0.68 [b]	78.35 ± 0.72 [a]	80.16 ± 1.08 [b]	0.03	0.65	0.94	-	0.09	-
ADCp%	92.41 ± 0.30	93.33 ± 0.28	92.66 ± 0.32	92.49 ± 0.55	0.06					

Values are presented as (mean ± SD; n = 6). Mean values with different superscripts within a row vary significantly according to one-way ANOVA ($p < 0.05$). R^2 = R squared. $\eta_p{}^2$ = partial eta squared. Control: without garlic supplement; G10: 10 g garlic powder per 1000 g diet; G20: 20 g garlic powder per 1000 g diet; G30: 30 g garlic powder per 1000 g diet; ADCd: Apparent digestibility coefficient of dry matter. ADCf: Apparent digestibility coefficient of fat. ADCp: Apparent digestibility coefficient of protein.

3.4. Haematology and Serum Biochemistry

The number of RBCs and WBCs in G10 were significantly higher than those observed in the other groups ($p < 0.05$). The WBCs had positive quadratic trend ($p = 0.01$) to the dietary garlic powder and reached a peak in the G10 group. The RBCs had positive quadratic trend to the dietary G10 group ($p = 0.01$). (Table 5).

Table 5. Haematological parameters of Eurasian perch fed with feeds supplemented with garlic powder.

							Linear Trend		Quadratic Trend	
Parameters	Control	G10	G20	G30	ANOVA	$\eta_p{}^2$	*P*-Value	R^2	*P*-Value	R^2
Red blood cells ($n \times 10^6$ µL^{-1})	283,896 ± 77,236 [ab]	464,543 ± 78,157 [c]	256,285 ± 16,266 [a]	352,395 ± 46,442 [b]	0.00	0.68	0.13	-	0.01	0.34
White blood cells ($n \times 10^6$ µL^{-1})	19,711 ± 5397	30,589 ± 7884 [b]	18,520 ± 4312 [a]	21,245 ± 5152 [a]	0.00	0.44	0.03	0.19	0.01	0.33
Lymphocytes (%)	91.84 ± 3.51	89.77 ± 4.56	93.91 ± 1.84	94.11 ± 2.87	0.11					
Myeloid cells (%)	8.15 ± 3.51	10.22 ± 4.56	6.08 ± 1.84	5.88 ± 2.87	0.11					

Values are presented as (mean ± SD; n = 6). Mean values with different superscripts within a row vary significantly according to one-way ANOVA ($p < 0.05$). R^2 = R square. $\eta_p{}^2$ = partial eta squared. Control: without garlic supplement; G10: 10 g garlic powder per 1000 g diet; G20: 20 g garlic powder per 1000 g diet; G30: 30 g garlic powder per 1000 g diet.

No significant differences in blood serum ALT and AST activity, triglycerides, or total protein were observed among the groups ($p > 0.05$). At all levels, garlic powder was associated with significantly lower levels of cholesterol ($p < 0.05$). Significantly higher levels of albumin were detected in the G10 and G20 groups compared to in the other groups ($p < 0.05$). A significant linear trend ($p = 0.00$) was observed regarding the dietary garlic powder level for albumin, where albumin increased with the inclusion of garlic powder at G10 and then decreased with the inclusion of garlic powder at G20 and G30. (Table 6).

Table 6. Serum biochemistry of Eurasian perch provided feed supplemented with garlic powder.

							Linear Trend		Quadratic Trend	
Parameters	Control	G10	G20	G30	ANOVA	$\eta_p{}^2$	*P*-Value	R^2	*P*-Value	R^2
Alanine aminotransferase (ukat L^{-1})	0.28 ± 0.10	0.27 ± 0.07	0.28 ± 0.08	0.19 ± 0.09	0.27					
Aspartate aminotransferase (ukat L^{-1})	1.39 ± 0.89	1.92 ± 1.26	1.83 ± 1.42	0.75 ± 0.40	0.24					
Cholesterol (mmol L^{-1})	8.28 ± 1.43 [b]	6.02 ± 1.13 [a]	7.01 ± 0.61 [a]	6.27 ± 0.44 [a]	0.00					
Triglycerides (mmol L^{-1})	9.28 ± 1.94	9.79 ± 6.20	14.59 ± 4.41	11.30 ± 3.63	0.17					
Albumin (g L^{-1})	11.16 ± 0.77 [b]	13.10 ± 1.28 [c]	12.83 ± 1.47 [c]	9.53 ± 1.28 [a]	0.00	0.61	0.00	0.55	0.00	0.55
Total protein (g L^{-1})	43.18 ± 3.52	42.86 ± 2.26	43.46 ± 1.94	41.51 ± 1.35	0.52					

Values are presented as (mean ± SD; n = 6). Mean values with different superscripts within a row vary significantly according to one-way ANOVA ($p < 0.05$). R^2 = R square. $\eta_p{}^2$ = partial eta squared. Control: without garlic supplement; G10: 10 g garlic powder per 1000 g diet; G20: 20 g garlic powder per 1000 g diet; G30: 30 g garlic powder per 1000 g diet.

3.5. Respiratory Burst and Phagocyte Activity

Garlic powder inclusion did not affect respiratory burst activity ($p > 0.05$) or lymphocyte and myeloid cell phagocytic activity and index ($p > 0.05$) (Table 7).

Table 7. Immunological parameters of Eurasian perch provided feed supplemented with garlic powder.

							Linear Trend		Quadratic Trend	
Parameters	**Control**	**G10**	**G20**	**G30**	**ANOVA**	η_p^2	***P*-Value**	R^2	***P*-Value**	R^2
Respiratory burst activity (MFI)	9290.33 ± 1185.85	7873.66 ± 1267.97	7688.00 ± 1675.28	10,838.00 ± 4639.88	0.60					
Lymphocytes phagocytic activity%	42.10 ± 8.51	46.81 ± 7.86	40.25 ± 10.62	41.87 ± 6.24	0.57					
Lymphocytes phagocytic index	14.45 ± 2.70	14.43 ± 4.86	14.14 ± 5.16	14.47 ± 5.25	0.99					
Myeloid phagocytic activity%	52.95 ± 7.69	48.03 ± 8.25	54.08 ± 10.61	52.20 ± 6.78	0.63					
Myeloid phagocytic index	16.15 ± 2.50	19.41 ± 7.48	17.37 ± 7.43	17.91 ± 6.97	0.85					

Values are presented as (mean ± SD; $n = 6$). R^2 = R square. η_p^2 = partial eta squared. Control: without garlic supplement; G10: 10 g garlic powder per 1000 g diet; G20: 20 g garlic powder per 1000 g diet; G30: 30 g garlic powder per 1000 g diet. MFI: Mean fluorescence intensity.

3.6. High-Density and Net Handling Stress Challenge

No significant differences in the level of serum cortisol and glucose were observed among the groups before stress ($p > 0.05$). Immediately after stress, all garlic diet groups showed significantly higher levels of cortisol compared to the control group ($p < 0.05$). No significant differences in glucose levels were observed among the groups ($p > 0.05$). At 1 h, significantly higher cortisol was observed in the controls, and there was a significantly lower level in the G30 group compared to in the other groups ($p < 0.05$). At 1 h, a positive quadratic trend was found between the increasing levels of garlic powder and serum cortisol ($p = 0.00$), where serum cortisol decreased with the inclusion of garlic powder at G10, increased with the inclusion of garlic powder at G20, and then decreased with the inclusion of garlic powder at G30. At 1 h, the control and G10 groups showed significantly higher glucose compared to in the G20 and G30 groups, while the glucose level of the G20 group was significantly lower than that of the other groups ($p < 0.05$). At 6 h, significantly higher and lower levels of cortisol were observed in the control and G20 groups, respectively, compared to in the other groups ($p < 0.05$), where the highest level of glucose was found in the control and G10 groups, and the lowest level of glucose was found in the G30 group at significant levels ($p < 0.05$). At 6 h, a significant linear trend ($p = 0.00$) regarding the dietary garlic powder level was observed for serum glucose. With increasing levels of garlic powder, the serum glucose decreased linearly. A positive quadratic trend was observed between the increasing levels of garlic powder and the serum glucose, where the serum glucose increased with the inclusion of garlic powder at G10 and then decreased with the inclusion of garlic powder at G20 and G30. At 24 h, significantly higher and lower cortisol levels were detected in the control and G30 groups, respectively ($p < 0.05$), while glucose was significantly higher in the control group than in the garlic-fed groups ($p < 0.05$) (Figures 1 and 2).

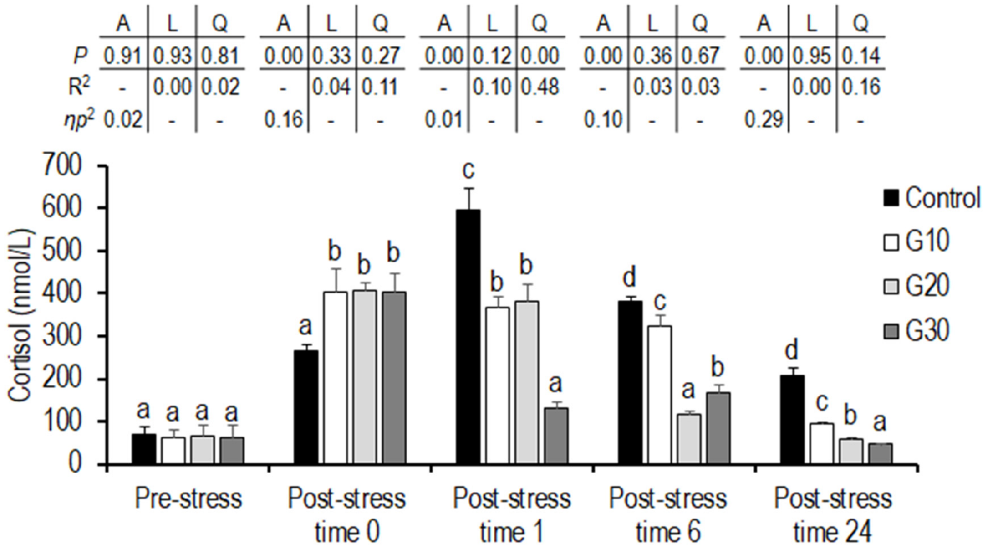

Figure 1. Serum cortisol of Eurasian perch provided feed supplemented with garlic powder under high-density and net handling stress. Values are presented as (mean ± SD; *n* = *6*). Mean values with different superscripts within each time vary significantly according to one-way ANOVA ($p < 0.05$). A = the variance analysed by one-way ANOVA; L = the linear trend analysed by orthogonal polynomial contrasts; Q = the quadratic trend analysed by orthogonal polynomial contrasts. R^2 = R square. $\eta_p{}^2$ = partial eta squared. Control: without garlic supplement; G1: 10 g garlic powder per 1000 g diet; G2: 20 g garlic powder per 1000 g diet; G3: 30 g garlic powder per 1000 g diet. Pre-stress: before stress; Post-stress time 0: immediately after stress; Post-stress time 1: one hour after stress; Post-stress time 6: 6 h after stress; Post-stress time 24: 24 h after stress.

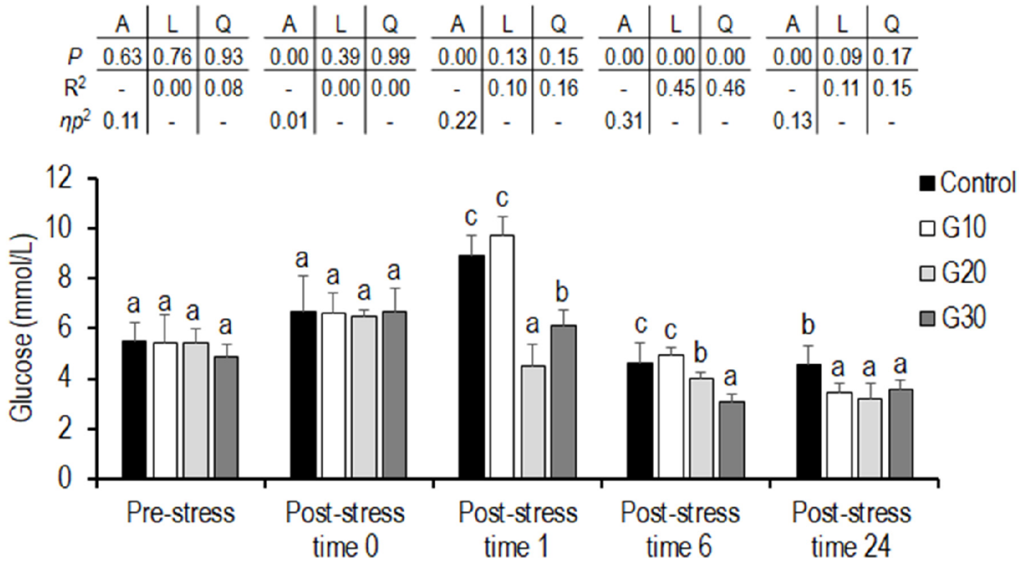

Figure 2. Serum glucose of Eurasian perch provided feed supplemented with garlic powder under high-density and net handling stress. Values are presented as (mean ± SD; *n* = 6). Mean values with different superscripts within each time vary significantly according to one-way ANOVA ($p < 0.05$). A = the variance analysed by one-way ANOVA; L = the linear trend analysed by orthogonal polynomial contrasts; Q = the quadratic trend analysed by orthogonal polynomial contrasts; R^2 = R square. $\eta_p{}^2$ = partial eta squared. Control: without garlic supplement; G1: 10 g garlic powder per 1000 g diet; G2: 20 g garlic powder per 1000 g diet; G3: 30 g garlic powder per 1000 g diet. Pre-stress: before stress; Post-stress time 0: immediately after stress; Post-stress time 1: one hour after stress; Post-stress time 6: 6 h after stress; Post-stress time 24: 24 h after stress.

4. Discussion

In the present study, the inclusion of garlic powder in compound diets for European perch did not show significant effects on growth performance. This finding agrees with Sahu et al. [14], who reported that garlic powder in the diet of rohu at 1, 5, and 10 g kg^{-1} feed did not significantly improve SGR or FCR. Another report documented that the use of garlic powder at the level of 40 g kg^{-1} in European sea bass did not have a significant effect on final weight, while 60 g kg^{-1} significantly decreased final weight, specific growth rate, and feed intake [39]. In contrast, garlic powder improved growth performance in Japanese sea bass at 25 g kg^{-1} [10], in brown trout at 20 and 30 g kg^{-1} [12], and in European sea bass at 10 g kg^{-1} [40]. Enhanced growth performance can be attributed to garlic bioactive compounds, including alliin, allicin, and organosulfur compounds, especially thiosulfinates [8], which increase digestion, nutrient uptake, and growth [16]. Differences among the results can be related to differences in the experimental design, fish species [10,12,40], fish size [39,40], garlic supplement type (powder or extract), and its purity [41,42] and garlic supplement level in the diet [18,39].

The liver is active in fish metabolisms, and HSI can be a marker of the harmful effects from the environment or diet [43]. In our research, the HSI and VSI indices did not differ among groups. This agrees with Shalaby et al. [16], who reported no effect of garlic powder at 10, 20, 30, and 40 g kg^{-1} feed on HSI in Nile tilapia. In contrast, 30 g kg^{1} garlic powder in the diet of brown trout [12] and 32 g kg^{-1} in the diet of Nile tilapia [42] were associated with significantly decreased HSI. In contrast, the inclusion of 10 g kg^{-1} garlic powder in the diet of brown trout also significantly increased HSI and VSI [12]. Furthermore, Lee et al. [44] confirmed that 5 g kg^{-1} of garlic extract did not have an effect on HSI in sterlet (*Acipenser ruthenus*) after 5 weeks, but 5 g kg^{-1} of garlic extract increased the somatic index (HSI) in sterlet after 10 weeks. Moreover, the use of garlic powder at levels 5, 10, 15, 20, and 30 g kg^{-1} in the sterlet diet significantly decreased HSI after a 12-week feeding trial in all garlic groups [45]. These reports showed that feeding trial duration has a strong effect on the hepatosomotic index. In contrast, our results confirm that no significant difference in HSI and VSI among groups can be significantly related to non-accumulation fat in the whole body and liver [40,46] or reduced fat accumulation in the whole body and liver in the garlic groups [21,42].

The biological characteristics of fish along with environmental parameters, feeding protocols, and parasitic infections, affect the fish condition factor [47]. In recent studies, the addition of garlic powder to brown trout feed [12] did not increase the condition factor. In the present study, the condition factor in the G30 group was significantly reduced. Lower levels—10 g kg^{-1} garlic powder in Japanese sea bass [10] and 20 g kg^{-1} in sterlet [45] feed—significantly increased the condition factor, suggesting increased diet palatability [10,45]. In contrast, garlic powder at levels of 10, 20, and 30 g kg^{-1} significantly decreased the condition factor in Indian major carp, which is in line with our results [48]. In our study, the decrease in the condition factor can be attributed to the pungent odour of garlic in G30, which may have reduced feed palatability [49] and feed intake [39]. Moreover, previous reports proved that use of garlic powder in levels of 25 g kg^{-1} in the diet of Japanese seabass [10] and 60 g kg^{-1} [39] and 20 g kg^{-1} of garlic powder in European sea bass feed [40] decreased feed intake. In the present study, feed intake decreased in the G30 groups and subsequently decreased the condition factor [48] for Eurasian perch.

The whole-body proximate composition of perch fed garlic powder did not show significant differences in dry matter, fat, or ash, while the G30 diet significantly increased body proximate protein. These results are comparable to those with 30 g kg^{-1} garlic powder in brown trout [12] and 30 g kg^{-1} in monosex redbelly tilapia (*Tilapia zilli*) [50], which improved body proximate protein composition. The inclusion of garlic powder in the diet of European seabass [40] and Nile tilapia [16,42] improved body proximate composition. Studies have shown that garlic supplementation can increase body proximate protein. Increasing protein and decreasing fat can be attributed to the organosulfur compounds found in garlic such as allicin, S-allyl cysteine, and diallyl-di-sulfide, which reduce fat aggregation in the body [42] due to the increasing bile acids in the garlic treatments [51]. Bile acids are considered to be regulatory molecules, and they have been considered to stimulate specific nuclear receptors in cells in the liver and gastrointestinal tract [52]. Increased protein can be interpreted as a result of the essential amino acids contained in garlic [9], increasing free amino acids in the muscle and resulting in protein synthesis [40].

Plant ingredients in fish diets can balance some micronutrients or bioactive compounds [53]. The evaluation of the digestibility coefficients of feed ingredients specify the nutrient utilization for different fish species [54]. At our lowest test level, garlic powder significantly improved dry matter and fat digestibility. Esmaeili et al. [15] observed higher dry matter, fat, and protein digestibility in rainbow trout fed with 30 g kg^{-1} of garlic powder in feed. Shalaby et al. [16] demonstrated that 30 g kg^{-1} of garlic powder increased protein and fat digestibility in Nile tilapia, similar to our results in perch. Other studies have confirmed that garlic powder improved the digestibility of nutrients and SGR and decreased FCR in European seabass at 20 and 30 g kg^{-1} [40], in Nile tilapia at 32 g kg^{-1} [42], and in rainbow trout at 0.5, 1, 5, and 10 g kg^{-1} [18]. Moreover, we found some studies showing that the use of 10 g kg^{-1} of garlic powder in the diet of sobaity sea bream (*Sparidentex hasta*) [55] and 5, 10, 15, and 20 g kg^{-1} of garlic powder in the diet of Asian sea bass significantly improved nutrient digestibility, SGR, and FCR [13]. Furthermore, the administration of microencapsulated garlic extract in rainbow trout at a level of 10 g kg^{-1} improved nutrient digestibility, SGR, and FCR as well [21]. These reports reveal that the administration of garlic as either a powder or an extract in different fish species increases growth performance [21,55] and nutrient digestibility due to the bioactive compounds found in garlic, such as allicin, which improved growth performance and nutrient digestibility in Nile tilapia [16,42] and European sea bass [40].

Red blood cell and withe blood cell counts are good key indices for evaluating fish physiology and pathology [56]. In our research, the administration of garlic at 10 g kg^{-1} increased RBC and WBC numbers compared to the other groups. Garlic powder has shown similar results in rainbow trout at 0.5, 1, 5, and 10 g kg^{-1} [18] and in rohu at 10 g kg^{-1} [14]. Nya and Austin [18] reported that 10 g kg^{-1} of garlic powder increased the WBCs in rainbow trout but did not affect RBC numbers. In contrast, the administration of 10 g kg^{-1} of garlic extract (allicin) in the diet of rainbow trout increased RBC numbers, but significantly decreased WBCs [41]. The use of garlic powder did not alter RBC and WBC numbers in brown trout at 10, 20, or 30 g kg^{-1} [12] or in beluga (*Huso huso*) at 10 g kg^{-1} [57], and it had no effect on RBC numbers in European sea bass at 10, 20, or 30 g kg^{-1}, while 30 g kg^{-1} of garlic powder increased the WBCs in sea bass [40]. The higher number of WBCs found in perch in our study may be related to the immunostimulatory effect of garlic compounds on the kidney, spleen, and thymus [58], as reported in previous studies [13,18]. RBCs play important roles in oxygen transfer, decreasing hypoxia stress, and contributing to fish health [59]. Our findings of higher RBC counts can be attributed to the effect of garlic compounds such as allicin [41] on the head kidney as the main erythropoietic site in teleost fish [60]. In our study, diets containing garlic powder did not increase concentrations of blood lymphocytes or myeloid cells. This result is similar to the inclusion of 5, 10, 15, and 20 g kg^{-1} in the diet of Asian sea bass [13]. Nya et al. [41] reported that 10 g kg^{-1} of allicin in the diet of rainbow trout increased neutrophil concentration but showed no effect on lymphocyte and monocyte percentage. WBCs, including lymphocytes [61] and myeloid cells [62], have key functions against pathogens as a first line of defence [63]. Myeloid cells include neutrophils and eosinophils (granulocytes) along with monocytes (macrophages) in fish [62].

Fish health can be evaluated by blood serum biochemical parameters [33], specifically the levels of ALT and AST [21,55], which are affected by diet, environment, and stress [64]. The level of ALT and AST activity is considered an indicator of liver health [33]. The levels of blood serum ALT and AST can be affected by stocking density [65]; water parameters [66]; and fish species [55,57], age, and sex [67]. In the present study, garlic powder did not show significant effects on serum ALT and AST activity. In agreement with our results, garlic powder in the 40 g kg^{-1} diet did not show significant effect on ALT and AST activity in Asian sea bass (*Lates calcarifer*) [68]. Furthermore, a mixture of cumin seeds (*Nigella sativa*) and turmeric (*Curcuma longa* Linn.) powder at the levels of 5 and 10 g kg^{-1} feed (1:1 w/w) did not show significant difference in the levels of ALT and AST in the Asian sea bass (*L. Calcarifer*), which is the same as in our study [69]. Other studies showed no effect on ALT activity in sobaity sea bream [55] or beluga at 10 g kg^{-1} feed [57]. Serum AST activity significantly increased in sobaity sea bream with 10 g kg^{-1} of garlic [55] and decreased in beluga [57]. Garlic powder at 32 g kg^{-1} [42] and 30 and 40 g kg^{-1} significantly decreased blood serum ALT and AST activity in Nile tilapia [16]. Moreover, garlic powder at the levels 5, 10, and 15 g kg^{-1} in feed decreased the level of blood serum ALT and AST significantly in common carp (*Cyprinus carpio*) [70]. In contrast, the inclusion of 40 and 50 g kg^{-1} of garlic powder significantly increased blood serum ALT and AST activity in rainbow trout [33]. The present study showed that the levels of ALT and AST can at least be related to fish species and to herbal medicine level and species [68,70] in the diet, similar to previous studies [55,69]. Moreover, no significant difference in the level of blood serum ALT and AST in our experimental fish, indicating that 10, 20, and 30 g kg^{-1} of garlic powder in perch diet were safe doses, as they did not disturb liver finction, as confirmed in the previous studies [68,69]. The reduction of ALT and AST activity in the blood serum can be attributed to the antioxidant compounds found in garlic, including S-allyl cysteine and diallyl-di-sulfide [71] and the flavonoids rutin, tangeretin, and nobiletin [72]. These antioxidant compounds hinder fat peroxidation in the cell membrane and prevent ALT and AST secretion into the blood [55].

Triglyceride and cholesterol were measured as blood serum biochemical parameters [55]. We observed no significant differences in the triglyceride levels among groups, while cholesterol was significantly lower in the garlic-fed groups. Garlic powder at 5, 10, 15, and 20 g kg^{-1} feed reduced cholesterol and triglycerides in Asian sea bass [13] as well as in rainbow trout at 20, 30, and 50 g kg^{-1} [33]. In contrast, 10 g kg^{-1} garlic powder in feed increased cholesterol and triglyceride levels in sobaity sea bream [5]. Apparently, garlic sulphur compounds reduce triglyceride levels in the blood serum [42]. Allicin is a main bioactive compound found in garlic that is responsible for hypolipidemia and hypocholesterolemia [73] and inhibits cholesterol biosynthesis [74]. In this line, our result showed that garlic powder at the higher level of G30 (30 g garlic powder per kg feed) significantly decreased blood serum cholesterol levels in our experimental species. In line with our study, Shalaby et al. [16] confirmed that garlic powder improved nutrient digestibility, SGR%, and FCR and increased fat digestibilty. Moreover, garlic powder decreased whole body fat and blood plasma lipids in Nile tilapia (*O. niloticus*). In another research study that was of a similar design to ours, garlic powder improved SGR, FCR, and nutrient digestibility and decreased total blood serum lipids, triglycerides, and cholesterol in Asian sea bass [13]. Moreover, Adineh et al. [21] reported the use of microencapsulated garlic extract at the level of 10 g kg^{-1} feed in rainbow trout improved SGR%, FCR, and nutrient digestibility and decreased whole body fat, which is the same as our results. Another study showed that garlic oil (0.15 g kg^{-1} feed) and powder (32 g kg^{-1} feed) increased nutrient digestibility by improving SGR% and FCR and decreased fat accumulation in the whole body and in the blood serum triglycerides and cholesterol [42] like our study. Previous studies [13,16,21,42] confirm our results and have demonstrated that whole body fat accumulation, apparent fat digestibility, and levels of blood serum triglycerides and cholesterol are related. In fact, those studies confirmed that increasing fat digestibility decreases fat accumulation in the whole body and reduces blood serum triglycerides and cholesterol [16,42].

In the present study, blood serum albumin was significantly higher in the G10 and G20 groups. Albumin has a protein structure. Albumin is primarily produced in the liver and prevents blood from leaking out of blood vessels. Albumin also transfers medicines and other substances across the blood for tissue growth and is used for tissue growth and healing [75]. Garlic powder increased blood serum albumin in amur carp [76] and rainbow trout [18]. The inclusion of garlic powder at levels of 10, 20, and 30 g kg^{-1} in brown trout feed did not significantly increase blood serum albumin [12], but an increase was seen in Asian sea bass at the levels of 5, 15, and 20 g kg^{-1} feed [13]. These differences in results can be related to the garlic dose and fish species as well as feed ingredient composition.

Blood serum protein parameters specifically show the status of fish as they react to internal and external factors [42]. Blood serum protein provides energy, creates new cells, reconstructs muscles, transports other nutrients such as messengers in the body, and supports the immune system [70]. We did not find blood serum total protein to differ among groups. This was also reported by Talpur and Ikhwanuddin [13], who administered garlic powder to Asian sea bass at the levels of 5, 10, 15, 20 g kg^{-1} feed, and by Nya and Austin [17], who used 5 and 10 g kg^{-1} in the feed of rainbow trout. In contrast, garlic powder at 10 g kg^{-1} in the diet of sobaity sea bream [55] and at 20 g kg^{-1} in brown trout [12] increased blood serum total protein. Total protein indicates immune system status [77]. Increased blood serum protein in the garlic groups can be interpreted as a higher amount of amino acids in the garlic groups as well as higher amounts of sulfur compounds including S-allyl cysteine sulfoxide [9] and and stimulate liver to synthesize blood serum proteins [42].

Phytogenics enhance the immune system of fish [78], but in our study, garlic in the diet of perch did not improve respiratory burst activity. This finding is in agreement with Mahfouz et al. [79], who reported that 20 g kg^{-1} of garlic powder in Nile tilapia feed did not increase respiratory burst activity, which may be related to fish species, culture, and feeding conditions. Respiratory burst is a latent metabolic route in the cells and is activated upon pathogen exposure. It destroys pathogens through the synthesis of powerful oxidizing compounds [80]. The use of 5 and 10 g kg^{-1} of garlic powder in rainbow trout increased respiratory burst reactive oxygen species [17] and 15 g kg^{-1} in Amur carp (*Cyprinus carpio haematopterus*) diets [76] was shown to increase respiratory burst activity. Increasing superoxide anion production elevates reactive oxygen species [14]. The administration of 10 g kg^{-1} garlic powder to Asian sea bass [13] and 0.5 and 1 g kg^{-1} to rainbow trout [18] increased superoxide anion production ($p < 0.05$).

Phagocytic activity is considered to be an indicator of fish immune system activity [81]. We did not find the inclusion of garlic powder in the diet of Eurasian perch to be associated with the phagocytic activity of lymphocytes or myeloid cells, unlike another reports that indicate that garlic powder increased phagocytic activity and the phagocytic index in Nile tilapia at 10 and 20 g kg^{-1} [82], Asian sea bass at 20 g kg^{-1} [13], and rainbow trout at 10 g kg^{-1} [18]. Garlic extract (allicin) increased phagocytic activity in rainbow trout at 5 and 10 g kg^{-1} feed [41]. Fish species and the level of garlic can determine its effect on the immune system. The phagocytic boost of garlic powder or garlic extract [18,41] can be attributed to the immunostimulatory effect of compounds such as allicin [41], germanium, and lectin [83]. However, the present study showed that garlic powder cannot boost phagocytic activity, at least in perch. Although we did not find a significant immune response in our experimental fish in our study, immune response may happen during a longer feeding trial, at higher levels of garlic powder [14], or with the use of garlic extract in the diet [41]. In light of this, Sahu et al. [14] mentioned that superoxide anion production, which elevates reactive oxygen species was significantly higher in garlic groups compared to in control groups after 20-, 40-, 60- and 70-day feeding trials. However, the level of superoxide anion production after 60 days was higher than it was at 20, 40, and 70 days. This result shows that immune response can at the very least be related to feeding trial duration.

A mixture of 200 ppm garlic and labiatae essential oils (Delacon, Austria) (PHYTO diet) did not reduce blood plasma cortisol or glucose in European sea bass [84]. Garlic powder at 10, 20, and 30 g kg^{-1} feed in brown trout [12] and at 1, 5, and 10 g kg^{-1} in rohu [14] showed no significant effect on serum glucose, while it decreased levels of blood serum glucose at 5, 10, 15, and 20 g kg^{-1} in the feed of Asian sea bass [13] and 40 g kg^{-1} in Nile tilapia feed [16]. Zaefarian et al. [12] suggested that the efficacy of garlic supplementation intake can be related to culture conditions and fish species. The reduction of glucose in blood serum can be attributed to the effect of garlic organosulfur compounds such as alliin (S-allyl cysteine sulfoxide) [85] and diallyl trisulfide [86], which have been shown to stimulate insulin secretion in diabetic mice [85] and rats [86], respectively. Although increasing levels of amino acids elevate insulin secretion, especially in carnivorous fish [87], increasing blood glucose levels in fish also elevate insulin levels [88]. Garlic organosulfur compounds increase glycemic control through enhanced insulin secretion and increase insulin sensitivity [85].

Blood cortisol and glucose are considered primary and secondary stress indicators in fish [89]. Cortisol is the key circulating glucocorticoid in fish, and its level is indicated by its cytosolic receptor, which regulates the expression of genes involved in growth, metabolism, and immune function [90]. Cortisol, a common stress indicator increased blood glucose in response to stress [91].

In the present study, post-challenge, the observed blood serum cortisol was significantly higher in all of the garlic groups compared to the control group, while there was no difference in the serum levels ($p > 0.05$) among groups. Elevated blood serum glucose indicates a higher stress level, requiring fish to increase energy expenditure [92]. Along with serum cortisol, glucose increases in response to energy demands [93]. Under stress, catecholamines and cortisol exert an effect on hepatocytes and induce glycolysis and gluconeogenesis, leading to an increase serum glucose [94].

At 24 h post-stress, the G30 group showed lower blood serum cortisol and glucose compared to the other groups ($p > 0.05$). At 1, 6, and 24 h post-stress, blood serum cortisol was lower in all of the garlic groups compared to the levels in the control grpi. High-density stocking [95], handling [27], heat stress [96], and low water pH [66] have been reported to increase levels of cortisol and glucose in fish. The inclusion of 2 mg nano selenium and 2 ppm garlic extract reduced blood plasma cortisol and glucose in grass carp (*Ctenopharyngodon idella*) under stocking density stress [97], while 200 ppm of a mixture of garlic and labiatae essential oil (Delacon, Austria) (PHYTO diet) reduced blood serum cortisol after 2 h overcrowding stress but did not show any effect on blood glucose ($p > 0.05$) in European sea bass [84]. In the present study, lower cortisol and glucose may be attributed to the bioactive compounds found in garlic, including alliin and diallyl trisulfide [98], which were higher in the G30 diet compared to in the other diets [13,21,42].

5. Conclusions

Garlic powder at 10 g kg^{-1} diet shows beneficial effects on haematology, blood biochemical parameters, and the apparent digestibility of nutrients including fat. The inclusion of garlic at 30 g kg^{-1} improved whole-body protein composition and increased resistance against high-density and net handling stress in European perch.

Further research should include garlic *A. sativum* powder in the diets of European perch of different sizes and developmental stages to evaluate growth performance and haematological and immunological parameters, including digestive enzymes and liver antioxidant activity. We suggest further study to identify bioactive compounds in garlic that are effective in immune-related gene expression.

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Data Availability Statement: The data presented in this research is available on request from the corresponding author.

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References

1. Griffin, W.; Wang, W.; de Souza, M.C. The sustainable development goals and the economic contribution of fisheries and aquaculture. *FAO Aquac. Newsl.* **2019**, *60*, 51–52.
2. Dawood, M.A.; Koshio, S. Recent advances in the role of probiotics and prebiotics in carp aquaculture: A review. *Aquaculture* **2016**, *454*, 243–251. [CrossRef]
3. Yan, J.; Guo, C.; Dawood, M.A.O.; Gao, J. Effects of dietary chitosan on growth, lipid metabolism, immune response and antioxidant-related gene expression in *Misgurnus anguillicaudatus*. *Benef. Microbes.* **2017**, *8*, 439–449. [CrossRef]
4. Citarasu, T. Herbal biomedicines: A new opportunity for aquaculture industry. *Aquac. Int.* **2010**, *18*, 403–414. [CrossRef]
5. Jennings, S.; Stentiford, G.D.; Leocadio, A.M.; Jeffery, K.R.; Metcalfe, J.D.; Katsiadaki, J.D.I.; Auchterlonie, N.A.; Mangi, S.C.; Pinnegar, J.K.; Ellis, T.; et al. Aquatic food security: Insights into challenges and solutions from an analysis of interactions between fisheries, aquaculture, food safety, human health, fish and human welfare, economy and environment. *Fish Fish.* **2016**, *17*, 893–938. [CrossRef]
6. Santhosha, S.; Jamuna, P.; Prabhavathi, S.N. Bioactive components of garlic and their physiological role in health maintenance: A review. *Food Biosci.* **2013**, *3*, 59–74. [CrossRef]
7. Firmino, J.P.; Galindo-Villegas, J.; Reyes-Lo'pez, F.E.; Gisbert, E. Phytogenic bioactive compounds shape fish mucosal immunity. *Front. Immunol.* **2021**, *12*, 695973. [CrossRef]
8. Lawson, L.D. Garlic: A review of its medicinal effects and indicated active compounds. In *Phytomedicines of Europe: Chemistry and Biological Activity*; Lawson, L.S., Bauer, R., Eds.; American Chemical Society: Washington, DC, USA, 1998; pp. 176–209.
9. Sasaki, J.; Lu, C.; Machiya, E.; Tanahashi, M.; Hamada, K. Processed black garlic (*Allium sativum*) extracts enhance anti-tumor potency against mouse tumors. *Med. Aromat. Plant Sci. Biotechnol.* **2007**, *2*, 278–281.
10. Xu, A.; Shang-Guan, J.; Li, Z.; Gao, Z.; Huang, Y.; Chen, Q. Effects of garlic powder on feeding attraction activity, growth and digestive enzyme activities of Japanese seabass, *Lateolabrax japonicus*. *Aquac. Nutr.* **2020**, *26*, 390–399. [CrossRef]
11. Büyükdeveci, M.E.; Balcázar, J.L.; Demirkale, I.; Suat Dikel, S. Effects of garlic-supplemented diet on growth performance and intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **2018**, *486*, 170–174. [CrossRef]
12. Zaefarian, A.; Yeganeh, S.; Adhami, B. Dietary effects of garlic powder (*Allium sativum*) on growth, blood indices, carcass composition, and lysozyme activity in brown trout (*Salmo caspius*) and resistance against *Yersinia ruckeri* infection. *Aquac. Int.* **2017**, *25*, 1987–1996. [CrossRef]
13. Talpur, A.D.; Ikhwanuddin, M. Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). *Aquaculture* **2012**, *364*, 6–12. [CrossRef]
14. Sahu, S.; Das, B.K.; Mishra, B.K.; Pradhan, J.; Sarangi, N. Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *J. Appl. Ichthyol.* **2007**, *23*, 80–86. [CrossRef]
15. Esmaeili, M.; Abedian Kenari, A.; Rombenso, A.N. Effects of fish meal replacement with meat and bone meal using garlic (*Allium sativum*) powder on growth, feeding, digestive enzymes and apparent digestibility of nutrients and fatty acids in juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Aquac. Nutr.* **2017**, *23*, 1225–1234. [CrossRef]
16. Shalaby, A.M.; Khattab, Y.A.; Abdel Rahman, A.M. Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). *J. Venom. Anim. Toxins Incl. Trop. Dis.* **2006**, *12*, 172–201. [CrossRef]
17. Nya, E.J.; Austin, B. Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. *Fish Shellfish Immunol.* **2011**, *30*, 845–850. [CrossRef]
18. Nya, E.J.; Austin, B. Use of garlic, *Allium sativum*, to control control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.* **2009**, *32*, 963–970. [CrossRef] [PubMed]
19. Esmaeili, M.; Abedian Kenari, A.; Rombenso, A. Immunohematological status under acute ammonia stress of juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) fed garlic (*Allium sativum*) powder-supplemented meat and bone meal-based feeds. *Comp. Clin. Path.* **2017**, *26*, 853–866. [CrossRef]
20. Abdel-Tawwab, M.; Khalil, R.H.; Diab, A.M.; Khallaf, M.A.; Abdel-Razek, N.; Abdel-Latif, H.M.; Khalifa, E. Dietary garlic and chitosan enhance the antioxidant capacity, immunity, and modulate the transcription of HSP70 and Cytokine genes in Zearalenone-intoxicated European seabass. *Fish Shellfish Immunol.* **2021**, *113*, 35–41. [CrossRef]
21. Adineh, H.; Harsij, M.; Jafaryan, H.; Asadi, M. The effects of microencapsulated garlic (*Allium sativum*) extract on growth performance, body composition, immune response and antioxidant status of rainbow trout (*Oncorhynchus mykiss*) juveniles. *J. Appl. Anim. Res.* **2020**, *48*, 372–378. [CrossRef]

22. Lee, J.Y.; Gao, Y. Review of the Application of Garlic, *Allium sativum*, in Aquaculture. *J. World Aquac. Soc.* **2012**, *43*, 447–458. [CrossRef]
23. Policar, T.; Samarin, A.M.; Mélard, C. Culture methods of Eurasian perch during on growing. In *Biology and Culture of Percid Fishes: Principles and Practices*; Kestemont, P., Dabrowski, K., Summerfelt, R.C., Eds.; Springer: Dordrecht, The Netherlands, 2015; pp. 417–435.
24. Ozerov, M.Y.; Ahmad, F.; Gross, R.; Pukk, L.; Kahar, S.; Kisand, V.; Vasemägi, A. Highly continuous genome assembly of Eurasian perch (*Perca fluviatilis*) using linked-read sequencing. *G3* **2018**, *8*, 3737–3743. [CrossRef]
25. Stejskal, V.; Kouril, J.; Valentova, O.; Hamackova, J.; Policar, T. Size-related oxygen consumption and ammonia excretion of Eurasian perch (*Perca fluviatilis* L.) reared in a recirculating system. *Aquac. Res.* **2009**, *41*, 135–142. [CrossRef]
26. Strand, Å.; Magnhagen, C.; Alanärä, A. Effects of repeated disturbances on feed intake, growth rates and energy expenditures of juvenile perch, *Perca fluviatilis*. *Aquaculture* **2007**, *265*, 163–168. [CrossRef]
27. Acerete, L.; Balasch, J.C.; Espinosa, E.; Josa, A.; Tort, L. Physiological responses in Eurasian perch (*Perca fluviatilis*, L.) subjected to stress by transport and handling. *Aquaculture* **2004**, *237*, 167–178. [CrossRef]
28. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 2005.
29. Hara, A.; Radin, N.S. Lipid extraction of tissues with a low toxicity solvent. *Anal. Biochem.* **1987**, *90*, 420–426. [CrossRef]
30. Zajic, T.; Mraz, J.; Samples, S.; Pickova, J. Fillet quality changes as a result of purging of common carp (*Cyprinus carpio* L.) with special regard to weight loss and lipid profile. *Aquaculture* **2013**, *400*, 111–119. [CrossRef]
31. Vandenberg, G.W.; De La Noüe, J. Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. *Aquac. Nutr.* **2001**, *7*, 237–245. [CrossRef]
32. Awad, E.; Austin, D.; Lyndon, A.R. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture* **2013**, *388*, 193–197. [CrossRef]
33. Mohebbi, A.; Nematollahi, A.; Dorcheh, E.E.; Asad, F.G. Influence of dietary garlic (*Allium sativum*) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). *Aquac. Res.* **2012**, *43*, 1184–1193. [CrossRef]
34. Korytář, T.; Thi, H.D.; Takizawa, F.; Köllner, B. A multicolour flow cytometry identifying defined leukocyte subsets of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol.* **2013**, *35*, 2017–2019. [CrossRef]
35. Biswas, G.; Korenaga, H.; Nagamine, R.; Takayama, H.; Kawahara, S.; Takeda, S.; Kikuchi, Y.; Dashnyam, B.; Kono, T.; Sakai, M. Cytokine responses in the Japanese pufferfish (*Takifugu rubripes*) head kidney cells induced with heat-killed probiotics isolated from the Mongolian dairy products. *Fish Shellfish Immunol.* **2013**, *34*, 1170–1177. [CrossRef] [PubMed]
36. Zaineldin, A.I.; Hegazi, S.; Koshio, S.; Ishikawa, M.; Bakr, A.; El-Keredy, A.M.S.; Dawood, M.A.O.; Dossou, S.; Wang, W.; Yukuna, Z. *Bacillus subtilis* as probiotic candidate for red sea bream: Growth performance, oxidative status, and immune response traits. *Fish Shellfish Immunol.* **2018**, *79*, 303–312. [CrossRef] [PubMed]
37. Morimoto, T.; Biswas, G.; Kono, T.; Sakai, M.; Hikima, J. Immune responses in the Japanese pufferfish (*Takifugu rubripes*) head kidney cells stimulated with particulate silica. *Fish Shellfish Immunol.* **2016**, *49*, 84–90. [CrossRef] [PubMed]
38. Di Marco, P.; Priori, A.; Finoia, M.G.; Massari, A.; Mandich, A.; Marino, G. Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. *Aquaculture* **2008**, *275*, 319–328. [CrossRef]
39. İrkin, L.C.; Yiğit, M. The use of garlic (*Allium sativum*) meal as a natural feed supplement in diets for European seabass (*Dicentrarchus labrax*) juveniles. *J. Aquac. Eng. Fish. Res.* **2015**, *2*, 128–141. [CrossRef]
40. Saleh, N.E.; Michael, F.R.; Toutou, M.M. Evaluation of garlic and onion powder as phyto-additives in the diet of sea bass (*Dicentrarcus labrax*). *Egypt. J. Aquat. Res.* **2015**, *41*, 211–217. [CrossRef]
41. Nya, E.J.; Dawood, Z.; Austin, B. The garlic component, allicin, prevents disease caused by *Aeromonas hydrophila* in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.* **2010**, *33*, 293–300. [CrossRef]
42. Metwally, M.A.A. Effects of Garlic (*Allium sativum*) on Some Antioxidant Activities in Tilapia Nilotica (*Oreochromis niloticus*). *World J. Fish Mar. Sci.* **2009**, *1*, 56–64.
43. Pait, A.S.; Nelson, J.O. Vitellogenesis in male *Fundulus heteroclitus* (Killifish) induced by selected estrogenic compounds. *Aquat. Toxicol.* **2003**, *64*, 331–342. [CrossRef]
44. Lee, D.-H.; Ra, C.-S.; Song, Y.-H.; Sung, K.-I.; Kim, J.-D. Effects of dietary garlic extract on growth, feed utilization and whole body composition of juvenile sterlet sturgeon (*Acipenser ruthenus*). *Asian-australas. J. Anim. Sci.* **2012**, *25*, 577–583. [CrossRef] [PubMed]
45. Lee, D.H.; Lim, S.R.; Han, J.J.; Lee, S.W.; Ra, C.S.; Kim, J.D. Effects of dietary garlic powder on growth, feed utilization and whole-body composition changes in fingerling Sterlet Sturgeon, *Acipenser ruthenus*. *Asian-australas. J. Anim. Sci.* **2014**, *25*, 577–583. [CrossRef]
46. Hwang, J.-H.; Lee, S.-W.; Rha, S.-J.; Yoon, H.-S.; Park, E.-S.; Han, K.-H.; Kim, S.-J. Dietary green tea extract improves growth performance, body composition, and stress recovery in the juvenile black rockfish, *Sebastes schlegeli*. *Aquac. Int.* **2013**, *21*, 525–538. [CrossRef]
47. Froese, R.; Tsikliras, A.C.; Stergiou, K.I. Editorial note on weight–length relations of fishes. *Acta Ichthyol. Piscat.* **2011**, *41*, 261–263. [CrossRef]
48. Kaur, H.; Ansal, M.D. Efficacy of garlic powder as a growth promoting feed additive for higher growth and improved flesh quality in an Indian major carp, *Labeo rohita* (Ham.) fingerlings. *J. Entomol.* **2020**, *8*, 25–29.
49. Platel, K.; Srinivasan, K. Digestive stimulant action of spices: A myth or reality? *Indian J. Med. Res.* **2004**, *119*, 167–179.

50. Ajiboye, O.O.; Yakubu, A.F.; Simpa, J.O.; Balogun, S.A. Effect of garlic-supplemented diets on growth response, survival, nutrient utilization and body composition of monosex *Tilapia zillii*. *World J. Fish Mar. Sci.* **2016**, *8*, 115–122.
51. Platel, K.; Srinivasan, K. Stimulatory influence of select spices on bile secretion in rats. *Nutr. Res.* **2000**, *20*, 1493–1503. [CrossRef]
52. Hylemon, P.B.; Zhou, H.; Pandak, W.M.; Ren, S.; Gil, G.; Dent, P. Bile acids as regulatory molecules. *J. Lipid Res.* **2009**, *50*, 1509–1520. [CrossRef]
53. Barrows, F.T.; Gaylord, T.G.; Sealey, W.M.; Smith, C.E.; Porter, L. Supplementation of plant-based diets for rainbow trout (*Oncorhynchus mykiss*) with macro-minerals and inositol. *Aquac. Nutr.* **2010**, *16*, 654–661. [CrossRef]
54. Lee, S.-M. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). *Aquaculture* **2002**, *207*, 79–95. [CrossRef]
55. Jahanjoo, V.; Yahyavi, M.; Akrami, R.; Bahri, A.H. Influence of adding garlic (*Allium sativum*), ginger (*Zingiber officinale*), thyme (*Thymus vulgaris*) and their combination on the growth performance, haemato-immunological parameters and disease resistance to *Photobacterium damselae* in sobaity sea bream (*Sparidentex hasta*) fry. *Turkish J. Fish. Aquat. Sci.* **2018**, *18*, 633–645.
56. Fazio, F. Fish haematology analysis as an important tool of aquaculture: A review. *Aquaculture* **2019**, *500*, 237–242. [CrossRef]
57. Kanani, H.G.; Nobahar, Z.; Kakoolaki, S.; Jafarian, H. Effect of ginger- and garlic-supplemented diet on growth performance, some hematological parameters and immune responses in juvenile *Huso huso*. *Fish Physiol. Biochem.* **2014**, *40*, 481–490. [CrossRef] [PubMed]
58. Press, C.M.; Evensen, Ø. The morphology of the immune system in teleost fishes. *Fish Shellfish Immunol.* **1999**, *9*, 309–318. [CrossRef]
59. Mbokane, E.M.; Moyo, N.A.G. Effect of dietary *Artemisia afra* on growth, some innateimmunological parameters in *Clarias gariepinus* challenged with *Aeromonas hydrophila*. *Aquac. Int.* **2020**, *28*, 539–553. [CrossRef]
60. Witeska, M. Erythrocytes in teleost fishes: A review. *Zool Ecol.* **2013**, *23*, 275–281. [CrossRef]
61. Scapigliati, G. Functional aspects of fish lymphocytes. *Dev. Comp. Immunol.* **2013**, *41*, 200–208. [CrossRef] [PubMed]
62. Crowhurst, M.O.; Layton, J.E.; Lieschke, G.J. Developmental biology of zebrafish myeloid cells. *Int. J. Dev. Biol.* **2002**, *46*, 483–492.
63. Lieschke, G.; Trede, N. Fish immunology. *Curr. Biol.* **2009**, *19*, R678–R682. [CrossRef]
64. Barcellos, L.J.G.; Kreutz, L.C.; Souza, C.; Rodriguez, L.B.; Fioreze, I.; Quevedo, R.M.; Cericato, L.; Soso, A.B.; Fagundes, M.; Conrad, J.; et al. Haematological changes in jundia (*Rhamdia quelen*) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. *Aquaculture* **2004**, *237*, 229–236. [CrossRef]
65. Battisti, E.K.; Rabaioli, A.; Uczay, J.; Sutili, F.J.; Lazzari, R. Effect of stocking density on growth, hematological and biochemical parameters and antioxidant status of silver catfish (*Rhamdia quelen*) cultured in a biofloc system. *Aquaculture* **2020**, *524*, 735213. [CrossRef]
66. Chan, T.L.; Roy, S.D.; Sharma, A.; Biswas, P.; Siddhya, G.M.; Das, A. Antistress potential of acetone extract of *Zingiber officinale* Roscoe on biochemical and oxidative stress parameters in *Labeo calbasu* (Hamilton, 1822) fingerlings subjected to acid stress. *Indian J. Fish.* **2014**, *61*, 69–77.
67. Akrami, R.; Gharaei, A.; Karami, R. Age and sex specific variation in hematological and serum biochemical parameters of Beluga (*Huso huso* Linnaeus, 1758). *Int. J. Aquat. Biol.* **2013**, *1*, 132–137.
68. Abdelwahab, A.M.; El-Bahr, S.M.; Al-Khamees, S. Influence of dietary garlic (*Allium sativum*) and/or ascorbic acid on performance, feed utilization, body composition and hemato-biochemical parameters of juvenile Asian Sea Bass (*Lates calcarifer*). *Animals* **2020**, *10*, 2396. [CrossRef]
69. Abdelwahab, A.; El-Bahr, S. Influence of black cumin seeds (*Nigella sativa*) and turmeric (*Curcuma longa* Linn.) mixture on performance and serum biochemistry of Asian sea bass, Lates calcarifer. *World J. Fish Mar. Sci.* **2012**, *4*, 496–503.
70. Yousefi, M.; Vatnikov, Y.A.; Kulikov, E.V.; Plushikov, V.G.; Drukovsky, S.G.; Hoseinifar, S.H.; Van Doan, H. The protective effects of dietary garlic on common carp (*Cyprinus carpio*) exposed to ambient ammonia toxicity. *Aquaculture* **2020**, *526*, 735400. [CrossRef]
71. Amagase, H.; Petesch, B.L.; Matsuura, H.; Kasuga, S.; Itakura, Y. Recent advances on the nutritional effects associated with the use of garlic as a supplement intake of garlic and its bioactive components. *J. Nutr.* **2001**, *131*, 955S–962S. [CrossRef] [PubMed]
72. Berginc, K.; Milisav, I.; Kristl, A. Garlic flavonoids and organosulfur compounds: The impact on saqunavir's and darunavir's hepatic pharmacokinetics. *Drug Metab.* **2010**, *25*, 521–530. [CrossRef] [PubMed]
73. Liu, L.; Yeh, Y.-Y. S-alk (en) yl cysteines of garlic inhibit cholesterol synthesis by deactivating HMG-CoA reductase in cultured rat hepatocytes. *J. Nutr.* **2002**, *132*, 1129–1134. [CrossRef] [PubMed]
74. Yamamoto, Y.; Oue, E. Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 933–939. [CrossRef]
75. Dhinaa, A.N.; Palanisamy, P.K. Z-Scan technique: To measure the total protein and albumin in blood. *J. Biomed. Eng.* **2010**, *3*, 285–290. [CrossRef]
76. Chesti, A.; Chauhan, R.S.; Khati, A. Evaluation of immunostimulatory effect of dietary garlic (*Allium sativum*) in fingerlings of Amur carp, (*Cyprinus carpio haematopterus*). *J. Pharm. Innov.* **2018**, *7*, 375–379.
77. Jha, A.K.; Pal, A.K.; Sahu, N.P.; Kumar, S.; Mukherjee, S.C. Haemato-immunological responses to dietary yeast RNA, w-3fatty acid and beta-carotene in *Catla catla* juveniles. *Fish Shellfish Immunol.* **2007**, *23*, 917–927. [CrossRef] [PubMed]
78. Van Hai, N. The use of medicinal plants as immunostimulants in aquaculture: A review. *Aquaculture* **2015**, *446*, 88–96. [CrossRef]
79. Mahfouz, N.B.; Fathi, M.; Zead, M.Y.A.; Mesalhy, S. Effect of garlic and yeast in the culture of Nile tilapia (*Oreochromis niloticus*). *Abbassa Int. J. Aquac.* **2009**, *4*, 453–465.

80. Dale, D.C.; Boxer, L.; Liles, W.C. The phagocytes: Neutrophils and monocytes. *Blood* **2008**, *112*, 935–945. [CrossRef] [PubMed]
81. Abarike, E.D.; Kuebutornye, F.K.; Jian, J.; Tang, J.; Lu, Y.; Cai, J. Influences of immunostimulants on phagocytes in cultured fish: A mini review. *Rev. Aquac.* **2019**, *11*, 1219–1227. [CrossRef]
82. Diab, A.M.; Saker, O.; Eldakroury, M.; Elseify, M. Effects of garlic (*Allium sativum*) and curcumin (Turmeric, *Curcuma longa* Linn) on Nile tilapia immunity. *Vet. Med. J.-Giza* **2014**, *60*, 1–19.
83. Magnadóttir, B. Innate immunity of fish (overview). *Fish Shellfish Immunol.* **2006**, *20*, 137–151. [CrossRef]
84. Serradell, A.; Torrecillas, S.; Makol, A.; Valdenegro, V.; Fernández-Montero, A.; Acosta, F.; Izquierdo, M.S.; Montero, D. Prebiotics and phytogenics functional additives in low fish meal and fish oil based diets for European sea bass (*Dicentrarchus labrax*): Effects on stress and immune responses. *Fish Shellfish Immunol.* **2020**, *100*, 219–229. [CrossRef] [PubMed]
85. Zhai, B.; Zhang, C.; Yao, S.; Zhao, C.; He, X.; Xu, W.; Huang, K.; Luo, Y. Hypoglycemic and hypolipidemic effect of S-allyl-cysteine sulfoxide (alliin) in DIO mice. *Sci. Rep.* **2018**, *8*, 3527. [CrossRef] [PubMed]
86. Liu, C.T.; Hse, H.; Lii, C.K.; Chen, P.S.; Sheen, L.Y. Effects of garlic oil and diallyl trisulfide on glycemic control in diabetic rats. *Eur. J. Pharmacol.* **2005**, *516*, 165–173. [CrossRef]
87. Navarro, I.; Rojas, P.; Capilla, E.; Albalat, A.; Castillo, J.; Montserrat, N.; Codina, M.; Gutiérrez, J. Insights into insulin and glucagon responses in fish. *Fish Physiol. Biochem.* **2002**, *27*, 205–216. [CrossRef]
88. Moon, T.W. Glucose intolerance in teleost fish: Fact or fiction? *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2001**, *129*, 243–249. [CrossRef]
89. Santos, G.; Schrama, J.; Mamauag, R.; Rombout, J.; Verreth, J. Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (*Dicentrarchus labrax*): The combined effects of fish crowding and water quality deterioration. *Aquaculture* **2010**, *299*, 73–80. [CrossRef]
90. Fought, E.; Vijayan, M.M. Mechanisms of cortisol action in fish hepatocytes. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2016**, *199*, 136–145. [CrossRef]
91. Barton, B.C. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integr. Comp. Biol.* **2002**, *42*, 517–525. [CrossRef] [PubMed]
92. Kavitha, C.; Malarvizhi, A.; Senthil Kumaran, S.; Ramesh, M. Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp. *Catla catla. Food Chem. Toxicol.* **2010**, *48*, 2848–2854. [CrossRef]
93. Yousefi, M.; Paktinat, M.; Mahmoudi, N.; Pérez-Jiménez, A.; Hoseini, S.M. Serum biochemical and non-specific immune responses of rainbow trout (*Oncorhynchus mykiss*) to dietary nucleotide and chronic stress. *Fish Physiol. Biochem.* **2016**, *42*, 1417–1425. [CrossRef] [PubMed]
94. Makvandi, H.; Khodadadi, M.; Keyvanshokoh, S.; Makvandi, Z.M. Effect of salinity stress on cortisol hormone and glucose in Grass carp fingerlings (*Ctenopharyngodon idella*). *Int. J. Aquac. Fish Sci.* **2012**, *2*, 77–84.
95. Odhiambo, E.; Angienda, P.O.; Okoth, P.; Onyango, D. Stocking density induced stress on plasma cortisol and whole blood glucose concentration in Nile tilapia fish (*Oreochromis niloticus*) of lake Victoria, Kenya. *Int. J. Zool.* **2020**, *2020*, 9395268. [CrossRef]
96. Dawood, M.A.; Eweedah, N.M.; El-Sharawy, M.E.; Awad, S.S.; Van Doan, H.; Paray, B.A. Dietary white button mushroom improved the growth, immunity, antioxidative status and resistance against heat stress in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* **2020**, *530*, 735229. [CrossRef]
97. Adineh, H.; Naderi, M.; Afieh Nazer, A.; Yousefi, M.; Ahmadifar, E. Interactive effects of stocking density and dietary supplementation with Nano selenium and garlic extract on growth, feed utilization, digestive enzymes, stress responses, and antioxidant capacity of grass carp, *Ctenopharyngodon Idella*. *J. World Aquac. Soc.* **2020**, *48*, 372–378. [CrossRef]
98. Han, J.; Lawson, L.; Han, G.; Han, P. Spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates. *Anal. Biochem.* **1995**, *225*, 157–160. [CrossRef] [PubMed]

CHAPTER 5

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLEDGEMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING THE STUDY
CURRICULUM VITAE

General discussion

Growth performance

The optimization of aquaculture practices aims to improve the growth performance and reduce the associated production costs through the minimization of the feed conversion ratio (FCR), which quantifies the amount of feed required to produce a unit of fish biomass. A reduction in the FCR leads to an increase in production efficiency and profitability (Esmaeili et al., 2021). The present study investigated the efficacy of supplementing rainbow trout feed with 30 g/kg of nettle leaf powder for a duration of eight weeks, with a focus on determining the effects on final body weight (FBW), weight gain (WG), weight gain percent (WG%), and specific growth rate percent (SGR%). While significant improvements in FBW, WG, WG%, and SGR% were observed, no significant changes were observed in the feed conversion ratio FCR (Zare et al., 2021a).

In previous studies, nettle leaf powder has been investigated as a potential feed additive to enhance the growth performance of rainbow trout. Awad et al. (2012) reported that the inclusion of 10 or 20 g/kg of nettle leaf powder in rainbow trout feed for eight weeks resulted in improved WG and SGR%, but no significant changes were observed in FCR. Similarly, our study found that adding 30 g/kg of nettle leaf powder to rainbow trout feed for eight weeks resulted in increased FBW, WG, WG%, and SGR%, while FCR remained unchanged (Zare et al., 2021a).

Furthermore, Mehrabi and Firouzbakhsh (2020) and Mehrabi et al. (2020) investigated the effects of administering 5 g/kg of nettle leaf powder in rainbow trout feed for four and eight weeks, respectively, based on 3% of the biomass. Their results showed improved WG, SGR%, and FCR. Similarly, Bilen et al. (2016) studied the effects of administering methanol extract of nettle at levels of 0.1 and 0.5 g/kg diet in rainbow trout for four weeks based on *ad libitum* feeding which improved FBW, WG%, FCR, and SGR%. Adel et al. (2017) also investigated the effects of adding 30 g/kg ethanol extract of nettle aerial part to rainbow trout feed for eight weeks based on 3% of biomass and found improved WG, FCR, and SGR%.

Rainbow trout fed with feed containing 10 or 20 g/kg of nettle leaf powder for eight weeks showed improved WG and SGR%, but no significant effect was observed on FCR (Awad et al., 2012) the same as our result on rainbow trout (Zare et al., 2021a). Besides, administration of 5 g/kg feed nettle powder improved WG, SGR%, and FCR in rainbow trout after four weeks (Mehrabi and Firouzbakhsh, 2020) and eight weeks (Mehrabi et al., 2020) feeding trial based on 3% of biomass (Mehrabi and Firouzbakhsh, 2020; Mehrabi et al., 2020). Additionally, methanol extract of nettle at levels 0.1 and 0.5 g/kg diet in rainbow trout diet based on *ad libitum* for four weeks (Bilen et al., 2016), and 30 g/kg feed ethanol extract of nettle aerial part for eight weeks feeding trial based on 3% of biomass (Adel et al., 2017) improved growth performance as well as our results (Zare et al., 2021a) (Chapter 2 and 3).

The literature indicates that several factors can influence growth performance outcomes in fish, including feed palatability, adaptation, feeding trial duration, fish size (Ngugi et al., 2015; Nobahar et al., 2015), medicinal plants' bioactive and antinutritional compounds (Rutto et al., 2013; Grauso et al., 2020). Nettle bioactive compounds, such as flavonoids, have been identified, including kaempferol, quercitrin, and quercetin (Jan et al., 2017), which can enhance feed palatability (Awad et al., 2012) and significantly reduce FCR (Mehrabi et al., 2020). Additionally, the use of nettle powder at a level of 20 g/kg feed has been shown to enhance the activity of the main digestive enzyme, pepsin, in the stomach. Pepsin is an acidic protease that regulates feed protein digestion by breaking down proteins into smaller peptides (Zhao et al., 2011). Furthermore, phenolic compounds such as vanillic acid (Grauso et al., 2020) and amino acids (Rutto et al., 2013) have been found to improve feed palatability and increase feed intake and digestibility in both warm-water (Ngugi et al., 2015) and cold-water fish species (Adel et al., 2017). Nettle bioactive compounds have also been found to modulate the colonization of different bacterial species in the gut of fish (Van Hai, 2015), enhance digestive enzymes activity (Awad et al., 2012), and increase carbohydrate, lipid, and protein metabolism (Zare et al., 2021a) (Chapter 3).

The effects of plant-based additives on the growth performance of fish can vary depending on several factors, such as the type of plant material (Gholamhosseini et al., 2021), concentration, and duration of the feeding trial, as well as the species and size of the fish Mbokane and Moyo, 2018; Mbokane and Moyo, 2020a; Yousefi et al., 2021). In the present study, we investigated the effects of tarragon and nettle leaf powder, alone and in combination, on the growth performance of rainbow trout. However, our results showed no significant effect on growth parameters, indicating that the use of tarragon leaf powder additives may not be as effective for rainbow trout (Zare et al., 2021a).

Interestingly, the use of tarragon extract in rainbow trout has been shown to significantly improve growth performance (Gholamhosseini et al., 2021), which is contrary to our findings with tarragon leaf powder (Zare et al., 2021a). The disparity in results could be due to differences in the chemical composition of the extract and the leaf powder, as well as the concentration used. Similarly, the use of African wormwood (*Artemisia afra*) powder did not show any significant effect on growth performance in both African catfish and Mozambique tilapia (Mbokane and Moyo 2018, 2020a), which is consistent with our findings on rainbow trout (Zare et al., 2021a). However, the use of white wormwood (*Viscum album*) extract in common carp has been found to significantly improve growth parameters, which highlights the potential benefits of plant-based additives for fish growth (Yousefi et al., 2021). Further research is needed to identify the most effective plant additives and concentrations for different fish species to optimize growth performance and reduce production costs.

The lack of significant effect observed in our study regarding the use of tarragon and nettle-tarragon combination on growth performance and nutritional factors of rainbow trout suggests that the type and dosage of the supplements may be crucial for their efficacy. The findings of our study are consistent with those of Mbokane and Moyo (2018, 2020a), who observed no significant effect on growth performance and nutritional factors of African catfish and Mozambique tilapia when fed with African wormwood at various levels. In contrast, a study by Gholamhosseini et al. (2021) reported an improvement in growth performance and feed utilization in rainbow trout fed with tarragon extract. This discrepancy could be attributed to the different supplement types used in these studies (powder vs. extract) (Mbokane and Moyo 2020a; Gholamhosseini et al., 2021), as well as the potential presence of antinutritional compounds like coumarin in tarragon, which could negatively affect feed palatability and consumption (Obolskiy et al., 2011).

Interestingly, other studies have shown that the combination of medicinal plants can improve growth performance and nutrient utilization in fish. For example, Chowdhury et al. (2021) reported that a mixture of turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*) powder at a level of 10 g/kg feed improved growth performance and nutrient utilization in *Labeo rohita*. These findings suggest that exploring the potential synergistic effects of different medicinal plants could be a promising avenue for improving fish growth performance and nutrition.

Overall, our study highlights the importance of carefully selecting the type and dosage of supplements when formulating fish diets, as well as considering the potential presence of antinutritional compounds that may affect feed palatability and consumption. Further research is needed to investigate the potential synergistic effects of different medicinal plant combinations on fish growth performance and nutrition.

In Chapter 4 of our research, we investigated the effects of administering garlic powder at levels of 10, 20, and 30 g/kg feed in the diet of European perch on growth performance and feed utilization. Our results, as reported by Zare et al. (2021b), indicated that the use of garlic powder at the aforementioned levels did not result in significant improvements in growth performance and feed utilization. These findings are consistent with those reported in previous studies involving fingerlings Nile tilapia (Diab et al., 2008), as well as those reported in our study and in other studies on various fish species (Zare et al., 2021b).

Indeed, previous research has shown that the use of garlic powder at levels of 10 g/kg in the diet of beluga (Nobahar et al., 2015), and 10, 40, and 80 g/kg in the diet of grouper (*Epinephelus coioides*) (Huang et al., 2022) did not result in improvements in growth performance. These findings are consistent with our results regarding the use of garlic powder in the diet of European perch (Zare et al., 2021b).

In contrast, other studies have reported positive effects of garlic powder supplementation on the growth performance of fish. For instance, the use of garlic powder at a level of 30 g/kg in the diet of Nile tilapia (Shalaby et al., 2006) and 20 g/kg in the diet of brown trout (Zaefarian et al., 2017) resulted in significant improvements in FBW, WG%, and SGR%. These findings contrast with our results with the use of garlic powder in the diet of European perch (Zare et al., 2021b).

Moreover, it has been reported that the type of garlic supplement used, including raw garlic, garlic powder, and essential oil, can significantly impact the growth performance of fish (Metwally, 2009). Garlic supplements have been found to positively impact fish growth and nutritional factors, which is thought to be due to the bioactive compounds present in garlic, including alliin, allicin, and organosulfur compounds, especially thiosulfinates (Zaefarian et al., 2017; Tavares et al., 2021). These compounds have been shown to enhance feed digestion and nutrient intake, thus improving the growth performance of fish (Patel et al., 2022).

It is important to note that the effectiveness of garlic supplements on fish growth performance and nutritional factors can be influenced by a number of factors, including the type of supplement used, experimental facility conditions, fish size, fish species, and supplement level in the diet (Nya and Austin, 2009; Lee et al., 2012; İrkin and Yiğit, 2015; Chesti et al., 2018; Jahanjoo et al., 2018; Huang et al., 2022). Therefore, further research is needed to fully understand the potential benefits and limitations of using garlic supplements in the diets of fish, particularly in relation to specific fish species and environmental conditions.

The liver plays a vital role in fish metabolism, and the hepatosomatic index (HSI) is widely used as a reliable indicator of the impact of various environmental stressors or dietary factors on fish health (Asaoka et al., 2013). Our recent investigation (Chapter 4) on European perch found that the addition of garlic powder to the diet did not result in any significant changes in HSI or viscerosomatic index (VSI) (Zare et al., 2021b). This finding is consistent with previous studies that have reported no significant effects of garlic supplementation on HSI and VSI in rainbow trout (Adineh et al., 2020) and African catfish juveniles (Gabriel et al., 2019). In contrast, some studies have reported increases in HSI and VSI in rainbow trout (Xu et al., 2020) and brown trout (Zaefarian et al., 2017) following garlic supplementation. However, our results on European perch agree with studies reporting decrease of fat aggregation in the liver and whole-body composition may explain significant changes in HSI and VSI (Shalaby et al., 2006; Metwally, 2009). Hence, further research is required to determine the factors that may contribute to the inconsistent findings of previous studies on the effects of garlic supplementation on HSI and VSI in different fish species.

In the context of fish nutrition, the condition factor is an important index that reflects the overall health status and well-being of fish. Our study on the effect of garlic powder on European perch revealed a significant reduction in the condition factor in the 30 g/kg garlic group (Zare et al., 2021b). This finding agrees with a study on Indian major carp (*Labeo rohita*) where the use of garlic powder at levels of 10, 20, and 30 g/kg in the diet resulted in a significant reduction of the condition factor (Kaur and Ansal, 2020). The observed reduction in the condition factor in the 30 g/kg garlic group (Zare et al., 2021b) may be attributed to the pungent aroma of garlic, which at a higher level, decreased the palatability and food intake (Kaur and Ansal, 2020). Similar observations have been made in European seabass (İrkin and Yiğit, 2015) and Japanese seabass (Xu et al., 2020). In contrast, the supplementation of 10 g/kg garlic powder significantly increased the condition factor in Japanese sea bass (Xu et al., 2020) and sterlet (Lee et al., 2014). This improvement in the condition factor can be attributed to the enhanced feed palatability achieved with lower levels of garlic powder supplementation for Japanese sea bass (Xu et al., 2020) and sterlet (Lee et al., 2014). However, in our study on European perch, the use of garlic powder at levels of 10 and 20 g/kg feed did not show a significant effect on the condition factor (Zare et al., 2021b). This difference in response may be attributed to several factors such as the type of garlic supplement, fish species (Gholipour Kanani et al., 2014; Zaefarian et al., 2017; Adineh et al., 2020), and the level of garlic supplementation (İrkin and Yiğit, 2015; Xu et al., 2020).

Whole-body proximate composition

Herbal supplements, owing to their bioactive compounds, have been shown to modulate the whole-body proximate composition in various fish species (Obolskiy et al., 2011; Lee and Gao, 2012; Behbahani et al., 2017; Zaefarian et al., 2017; Van Wyk and Wink, 2018; Gabriel et al., 2019; Grauso et al., 2020; Zare et al., 2021a; Öz and Dikel, 2022; Patel et al., 2022; Zare et al., 2023).

In our recent studies, the effect of nettle, tarragon, and garlic powder on the whole-body proximate composition of rainbow trout (Zare et al., 2021a) and European perch (Zare et al., 2021b) were investigated. The results showed that supplementation of the feed with 30 g/kg nettle powder significantly reduced the whole-body moisture and fat content while increasing the protein and ash content in rainbow trout (Zare et al., 2021a). However, in contrast to the findings for European perch, the supplementation of the feed with garlic powder at levels of 10 and 20 g/kg did not have a significant effect on the whole-body proximate composition (Zare et al., 2021b). This difference in results may be due to variations in fish size and supplement levels in the feed (Awad et al., 2012).

In the same study, tarragon leaf powder at levels of 10 and 30 g/kg feed, as well as the combination of nettle and tarragon (N10+T10), did not have a significant effect on the whole-body proximate composition of rainbow trout (Zare et al., 2021a). Similarly, a study on largemouth bass (*Micropterus salmoides*) showed that fennel (*Foeniculum vulgare*) and sweet wormwood (*Artemisia annua*) extract, both separately and as a combination (0.5 fennel + 0.5 sweet wormwood) at a level of 0.5 g/kg feed, did not have a significant effect on the whole-body proximate composition (He et al., 2022).

The observed effects of nettle compounds, including vitamins, minerals, and phenolics (Kk and Parsuraman, 2014), as well as a variety of available essential amino acids (Rutto et al., 2013), on increased digestibility and nutrient intake (Ngugi et al., 2015; Adel et al., 2017; Jafari et al., 2022) may contribute to decreased fat and increased protein accumulation in fish body proximate composition (Hwang et al., 2013; Al-Shakarchi and Mohammad, 2021). However, further research is needed to fully understand the mechanisms underlying these effects, as well as the optimal levels and combinations of herbal supplements to achieve desired outcomes.

In our investigation (Chapter 4), we conducted a comprehensive evaluation of the impact of garlic powder supplementation on the whole-body proximate composition of fish. Our results indicate that the inclusion of 30 g/kg of garlic powder in the diet of fish led to a significant improvement in the protein content of the whole-body proximate composition (Zare et al., 2021b). Our findings are consistent with previous studies that have reported similar improvements in fish protein levels with the supplementation of garlic powder (Xu et al., 2020; Öz and Dikel, 2022). Additionally, other studies have demonstrated that the use of higher levels of garlic powder (40–60 g/kg) in the diet of fish can result in a significant reduction in body fat while simultaneously increasing protein levels, although it had no significant effect on moisture content (İrkin and Yiğit, 2015).

The enhancements in the proximate composition of fish following the addition of garlic powder to their diet may be attributed to the bioactive compounds in garlic, including alliin, allicin, and other sulfur-containing compounds. These compounds are well-known for their antimicrobial, antioxidant, and immunomodulatory properties, and likely contribute to the observed improvements in fish nutrition (Shang et al., 2019). These compounds have been shown to improve fish growth, FCR, and nutrient utilization (Adineh et al., 2020; Feng et al., 2021). Furthermore, garlic is rich in essential amino acids, vitamins, and minerals that can enhance fish growth and development, leading to improved body composition (Huang et al., 2018; Abdelwahab et al., 2020).

Our study (Zare et al., 2021b) found that garlic powder has an impact on the whole-body proximate composition of European perch, and this effect can be attributed to the presence of bioactive compounds, such as S-allyl cysteine, allicin, and diallyl-di-sulfide, in garlic (Song et al., 2021). These compounds have been shown to reduce fat accumulation in the fish body (Shalaby et al., 2006) through their effects on bile acid levels (Pande and Srinivasan, 2012). The increase in protein availability in the garlic treatment group may be due to the presence of essential amino acids in garlic (Valenzuela-Gutiérrez et al., 2021), which have been demonstrated to increase the availability of free amino acids in muscle and promote protein synthesis (Zaefarian et al., 2017). These findings are in line with previous studies reporting an increase in protein levels in the whole-body proximate composition of rainbow trout fed diets supplemented with garlic powder (Öz and Dikel, 2022; Xu et al., 2020).

Apparent digestibility coefficient

Medicinal plants are increasingly being utilized in aquafeeds as they contain bioactive compounds that could modify the intestinal microbiota and enhance nutrient uptake in fish (Foysal et al., 2019). One of the crucial indicators of the efficiency of digestion and nutrient utilization in fish is the digestibility coefficient of feed (Abang Zamhari and Yong, 2021).

In our study (Chapter 4), we observed a significant increase in dry matter and fat digestibility with the inclusion of 10 g/kg garlic powder in the diet of European perch (Zare et al., 2021b). These findings are consistent with those of previous studies, such as Esmaeili et al. (2017) in rainbow trout and Shalaby et al. (2006) in Nile tilapia, who also reported improved apparent digestibility of protein, dry matter, and fat with garlic supplementation. Garlic's sulfur compounds, such as allicin, aliin, and isoaliin, are known to improve feed digestion, nutrient uptake, and utilization in fish (Valenzuela-Gutiérrez et al., 2021). Moreover, garlic powder has been found to enhance the growth performance of various fish species (Lee and Gao, 2012).

However, there are reports indicating that garlic supplements in fish diets may not always lead to significant improvements in feed utilization and FCR. Studies conducted on beluga (Gholipour Kanani et al., 2014; Nobahar et al., 2015), European seabass (İrkin and Yiğit, 2015), brown trout (Zaefarian et al., 2017), and rainbow trout (Büyükdeveci et al., 2018) have reported inconsistent results. These discrepancies in the findings may be attributed to various factors, such as fish species (Nobahar et al., 2015; Zaefarian et al., 2017) or size (İrkin and Yiğit, 2015; Saleh et al., 2015), and highlight the need for further investigation.

Haematological parameters

Evaluation of haematological parameters is a crucial aspect of assessing the health status and metabolic processes in fish (Esmaeili, 2021). Our recent review highlights the positive impact of nettle supplementation on haematological parameters in both cold-water and warm-water fish species (Zare et al., 2023) (Chapter 2).

In particular, the inclusion of nettle powder in fish diets has been shown to significantly improve various haematological parameters in rainbow trout. For instance, the supplementation of 10 and 15 g/kg feed with nettle powder has been found to enhance leukocyte count, erythrocyte count, haemoglobin (Hb), and haematocrit% (Hct%) in rainbow trout after four and eight weeks of feeding trials (Mehrabi and Firouzbakhsh, 2020; Mehrabi et al., 2020). Furthermore, nettle supplementation led to a decrease in monocytes% (Mon%), an increase in lymphocytes% (Lym%), and no significant impact on the percentage of neutrophils% (Neu%) (Mehrabi et al., 2020).

In contrast, our recent study found that the addition of 30 g/kg nettle powder to rainbow trout diet decreased erythrocyte count, Hb, and Hct%, while increasing leukocyte counts, Mon%, and Neu% compared to the control group (Zare et al., 2021a). It should be noted, however, that other studies have reported different findings. For example, Awad and Austin (2010) found that a 10 g/kg feed of nettle leaf powder in rainbow trout improved Hct% significantly but did not have a significant effect on erythrocyte count, leukocyte count, Mon%, Neu%, and Hb significantly after a 14-day feeding trial. This discrepancy may be attributed to differences in the duration of the feeding trial, as our study utilized an 8-week feeding trial.

Moreover, the use of 30 g/kg nettle extract in a longer feeding trial of 4 weeks was found to improve erythrocyte count, leukocyte count, Hb, Hct%, Neu%, and Mon% in rainbow trout (Adel et al., 2017). Our evaluation (Chapter 3) revealed that nettle leaf powder at a level of 30 g/kg improved leukocyte count, neutrophils%, and monocytes%, while at a level of 10 g/kg feed, it increased erythrocyte count, Hb, and Hct% in rainbow trout compared to the control group after an 8-week feeding trial (Zare et al., 2021a). Overall, the positive impact of nettle supplementation on haematological parameters in fish highlights its potential as a functional feed ingredient in aquaculture (Zare et al., 2023).

In warm-water fish species, Binaii et al. (2014) reported that nettle leaf powder at 120 g/kg feed increased erythrocyte count, Hct%, and Hb in beluga after four weeks of feeding. However, leukocyte count and Lym% were not affected. After eight weeks, nettle supplementation increased leukocyte count, erythrocytes count, Hb, and Hct%, but had no significant impact on lymphocytes%. Similarly, Ngugi et al. (2015) observed an increase in erythrocyte count, leukocyte count, Hb, and Hct% in Victoria Labeo fed with 10, 20, and 50 g/kg nettle leaf powder after 4 and 16 weeks of feeding trial. While several studies have reported the positive effect of nettle supplementation on haematological parameters in fish (Awad and Austin, 2010; Binaii et al., 2014; Adel et al., 2017; Mehrabi and Firouzbakhsh, 2020; Mehrabi et al., 2020), our review of the effect of nettle on haematological parameters in cold and warm water fish species (Zare et al., 2023) and our study on rainbow trout (Zare et al., 2021a) confirmed the beneficial effect of nettle on haematological parameters (Chapter 2 & 3).

Regarding tarragon supplementation, our study (Chapter 3) showed that tarragon powder at a level of 30 g/kg feed significantly increased leukocyte count, Hct%, Neu% and Mon% in rainbow trout (Zare et al., 2021a). Gholamhosseini et al. (2021) also reported an increase in leukocyte count, Neu%, and Hct% in rainbow trout fed with tarragon extract at a level of 20 g/kg feed but observed no significant effect on Mon%. Conversely, African wormwood supplementation did not have any significant effect on Hb, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) in rainbow trout (Zare et al., 2021b).

In our study (Chapter 3), the (N10+T10) group exhibited significantly higher levels of MCV, mean corpuscular hemoglobin (MCH), and MCHC in comparison to the control group, thus indicating a positive impact of nettle and tarragon supplementation on red blood cell indices in rainbow trout (Zare et al., 2021a). Our investigation also revealed that the (N10+T10) group displayed elevated erythrocyte count and Hb levels in comparison to the other experimental groups, thus supporting the beneficial impact of this combination on hematological parameters (Zare et al., 2021a). In a study involving African catfish juveniles, the addition of garlic and *Aloe vera* crude extract at 10 g/kg feed, in a weight1: weight1 ratio, resulted in improved erythrocyte count, Hct%, and Hb levels, with MCH being enhanced at levels of 20 and 40 g/kg feed. Nevertheless, this supplement did not significantly impact leukocyte count, Lym%, Mon%, granulocytes count, MCV, and MCHC in the African catfish diet (Gabriel et al., 2021).

Furthermore, a combination of turmeric and ginger and another combination of ginger and garlic powder, both in a ratio of weight1: weight1 at a level of 10 g/kg feed, enhanced Hb levels in rohu (Chowdhury et al., 2021), which is in line with our findings in rainbow trout (Zare et al., 2021a). Additionally, combinations of turmeric and ginger, turmeric and garlic, and ginger and garlic in a weight1: weight1 ratio at a level of 10 g/kg feed increased erythrocyte count, monocyte, and neutrophil counts in rohu (Chowdhury et al., 2021), which further corroborates our findings in rainbow trout (Zare et al., 2021a). Our study highlights the beneficial effects of combining nettle and tarragon at the (N10+T10) level in the feed, which may be attributed to the minerals, such as iron and calcium, present in nettle, and vitamins such as A, B, B12, and C found in nettle, as well as the L-ascorbic acid present in tarragon. It has been confirmed that vitamin C enhances iron uptake in the intestine, and the role of vitamins and minerals in hematopoiesis is well-established (Binaii et al., 2014). Nettle and tarragon are rich in vitamins and minerals, and their combined use has shown synergistic effects, believed to stimulate the head kidney and enhance hematopoiesis in fish (Adel et al., 2017; Chowdhury et al., 2021).

Our investigation has revealed that the addition of garlic powder to the diet of fish can have a significant effect on erythrocyte and leukocyte counts. Specifically, the incorporation of 10 g/kg of garlic powder into the diet of European perch resulted in increased erythrocyte and leukocyte counts compared to other groups (Zare et al., 2021b) (Chapter 4). This finding is consistent with previous studies that have examined the effects of garlic powder on erythrocyte and leukocyte counts in fish. For example, Talpur and Ikhwanuddin (2012) reported that the inclusion of 5, 10, 15, and 20 g/kg of garlic powder in the diet of Asian seabass resulted in an increase in erythrocyte and leukocyte counts compared to the control group. Similarly, Sahu et al. (2007) found that the inclusion of 1, 5, and 10 g/kg of garlic powder in the diet of Indian major carp resulted in an increase in erythrocyte and leukocyte counts compared to the control group, which is consistent with our results (Zare et al., 2021b).

In contrast, studies on beluga and brown trout have shown that the addition of garlic powder to the diet at levels of 10, 20, and 30 g/kg had no significant effect on erythrocyte and leukocyte counts (Gholipour Kanani et al., 2014; Nobahar et al., 2015; aNazerian et al., 2016; Zaefarian et al., 2017).

The observed improvement in leukocyte count in European perch fed with garlic powder may be due to the immunostimulatory effects of garlic on the spleen, thymus, and kidney (Fazio, 2019). The increase in erythrocyte count observed in our study may be due to the presence of bioactive compounds, including allicin, in garlic. Previous research has shown that allicin has a similar effect on the head kidney, which is the primary erythropoietic organ in teleost fish such as rainbow trout (Nya et al., 2010; Witeska, 2013). While our study did not show a significant effect of garlic inclusion on lymphocytes or myeloid cells, this result is consistent with previous studies, including those conducted on rainbow trout (Nya et al., 2010; Talpur and Ikhwanuddin, 2012). Similarly, garlic powder at levels of 5 and 10 g/kg in rainbow trout did not have a significant effect on the number of lymphocytes, as observed in our study (Nya and Austin, 2011; Zare et al., 2021b) (Chapter 4).

Blood biochemical parameters

Blood biochemical factors play a crucial role in the assessment of fish health status, and their evaluation is a common practice in aquaculture (Binaii et al., 2014). Recent studies have highlighted the potential benefits of nettle supplements, including nettle powder or extract, in improving fish health status and biochemical factors (Ngugi et al., 2015; Mehrabi and Firouzbakhsh, 2020).

In rainbow trout, feeding trials with 10 g/kg nettle extract (quercetin) for 14 days resulted in increased blood serum total protein levels (Awad et al., 2013). Similarly, in 8-week feeding trials, administration of nettle powder at levels of 5, 10, 15, and 30 g/kg feed improved blood serum total protein and albumin levels (Mehrabi and Firouzbakhsh, 2020; Mehrabi et al., 2020; Zare et al., 2021a). These findings suggest that nettle supplements can enhance fish health status by improving blood serum biochemical factors.

However, it is worth noting that not all nettle supplements have the same effects on fish blood serum biochemical factors. For instance, nettle leaf powder at a level of 10 g/kg feed had no significant effect on blood serum total protein in rainbow trout after a two-week feeding trial (Awad and Austin, 2010). In contrast, aerial parts of nettle ethanol extract improved blood serum total protein in rainbow trout at a level of 30 g/kg feed after eight weeks of feeding trial, while reducing glucose and triglyceride levels (Adel et al., 2017). Additionally, in a study on female convict cichlid, the methanolic extract of nettle at levels of 0.4 and 0.5 g/kg feed improved the levels of blood serum total protein and albumin but decreased cholesterol, glucose, and triglyceride levels (Jafari et al., 2022), consistent with the findings of Zare et al. (2021a).

Moreover, in feeding trials with beluga and Victoria Labeo, nettle leaf and stem powder at levels of 120 g/kg feed and nettle leaf powder at levels of 10, 20, and 50 g/kg feed, respectively, increased blood serum albumin and total protein levels and reduced blood serum cholesterol and triglyceride levels (Binaii et al., 2014; Ngugi et al., 2015). In Victoria Labeo, nettle leaf powder also reduced blood serum glucose levels after 4 and 16 weeks of the feeding trial (Ngugi et al., 2015).

Blood serum total protein and albumin are important biomarkers of fish health and can play a significant role in enhancing their immune system (Abdel-Tawwab et al., 2018). Quercetin, a flavonoid compound found in nettle, has been shown to elevate blood serum total protein levels in fish (Awad et al., 2013). Nettle is also a rich source of vitamins A and C, and antioxidants, and contains quercetin at a concentration of 2 mg/100 g (Rutto et al., 2013). In addition, blood serum albumin has a crucial function in the transport of various materials such as hormones, ions, amino acids, enzymes, metals, phospholipids, bilirubin, fatty acids, and drugs (Belinskaia et al., 2020) by providing suitable binding sites (Mehrabi and Firouzbakhsh, 2020; Mehrabi et al., 2020).

While no studies have yet been conducted on the effects of nettle on cholesterol and triglyceride levels in rainbow trout, research has shown that nettle may have the potential to reduce these levels in mammals (Chehri et al., 2022). For instance, the hydroalcoholic extract of nettle has been found to have preservative and hypoglycemic effects on pancreatic beta cells in hyperglycemic rats, which may be attributed to the antioxidant properties of nettle (Saad et al., 2017). These properties include phenolic compounds such as kaempferol and quercetin (Otles and Yalcin, 2012; Grauso et al., 2020). Furthermore, the efficacy of nettle on fish glucose levels may be influenced by various factors, including fish species, the type of nettle supplement (powder and/or extract), and the part of the nettle used as a supplement (Otles and Yalcin, 2012; Binaii et al., 2014; Ngugi et al., 2015).

The reduction of cholesterol and triglyceride levels in the blood serum may be attributed to nettle sterols, including phenolic compounds (Grauso et al., 2020), stigmasterol, β-sitosterol, campesterol (Petkova et al., 2020), and phenolic compounds (Grauso et al., 2020), which can impact cholesterol biosynthesis (Lee et al., 2003).

In conclusion, nettle has shown potential benefits for fish health and immunity, particularly in terms of elevating blood serum total protein levels. It is also a rich source of vitamins A and C, as well as antioxidants such as kaempferol and quercetin (Rutto et al., 2013). Furthermore, nettle has the potential to lower cholesterol and triglyceride levels, as seen in studies conducted on mammals. The reduction in cholesterol and triglyceride levels may be attributed to the presence of sterols, such as stigmasterol, β-sitosterol, and campesterol, in addition to phenolic compounds (Grauso et al., 2020). However, further research is needed to determine the exact mechanisms through which nettle exerts its effects on fish health and to establish optimal dosages and application methods. The findings of this review suggest that nettle may be a promising natural supplement for promoting fish health and immunity, with potential applications in aquaculture and fisheries management.

The condition of fish in response to internal and external factors can be reflected through their blood serum protein levels (Metwally, 2009). Blood serum protein has various roles in the body, including generating energy, producing new cells, repairing muscles, transporting other essential nutrients, serving as messengers within the body, and boosting the immune system (Yousefi et al., 2020). Albumin plays a crucial role in transferring medicines and other substances necessary for growth and healing through the blood in fish (Khasani and Astuti, 2019).

Our study did not find any significant impact of garlic treatments on the level of blood serum total protein in European perch. Nonetheless, administering garlic powder at a rate of 10 and 20 g/kg feed increased the level of blood serum albumin in European perch (Zare et al., 2021b). Unlike our study on European perch (Zare et al., 2021b), administering 10 g/kg feed garlic powder to sobaity seabream fry resulted in an increase in blood serum total protein levels but did not affect the albumin levels (Jahanjoo et al., 2018). Furthermore, in brown trout, administering garlic powder at levels of 10, 20, and 30 g/kg feed did not lead to a significant increase in blood serum albumin, as observed in the study by Zaefarian et al. (2017). In contrast, administering garlic powder at levels of 5, 15, and 20 g/kg feed resulted in increased blood serum albumin levels in Asian seabass (Talpur and Ikhwanuddin 2012). However, some other studies have reported different findings. For instance, administering garlic powder at levels of 32 g/kg (Metwally 2009) and 10, 20, and 30 g/kg feed (Shalaby et al., 2006) resulted in an increase in blood serum total protein in Nile tilapia, as reported by Metwally (2009) and Shalaby et al. (2006). On the other hand, some reports suggest that garlic powder can increase blood serum albumin levels in rainbow trout (Nya and Austin 2009) and amur carp (Chesti et al., 2018), similar to our findings on European perch (Zare et al., 2021b).

The elevated blood serum protein levels observed in the garlic-treated groups can be ascribed to the higher concentration of amino acids in these groups, along with increased levels of sulfur compounds, such as S-allyl cysteine sulfoxide, which have been shown to stimulate the liver to synthesize blood serum proteins (Metwally 2009; Valenzuela-Gutiérrez et al., 2021).

To sum up, the variations in the results obtained from different studies could be attributed to the garlic dosage, fish species, and feed composition (Shalaby et al., 2006; Metwally 2009; Nya and Austin 2009; Jahanjoo et al., 2018). Further research is necessary to determine the optimal dose and type of garlic supplement for different fish species.

The effects of garlic powder on fish lipid profile have been investigated in several studies. In our study (Zare et al. 2021b), garlic powder did not have a significant effect on European perch triglyceride, but it did significantly decrease cholesterol levels in all groups. Conversely, a study by Jahanjoo et al. (2018) showed that garlic powder at level 10 g/kg diet increased blood serum triglyceride and cholesterol significantly in sobaity seabream. It should be noted that the effects of garlic supplementation on fish lipid profile can be influenced by several factors, including the fish species, the type and level of garlic supplement, and the mode of administration.

In rainbow trout, Mohebbi et al. (2012) found that garlic powder at levels of 20, 30, 40, and 50 g/kg diet significantly decreased blood serum triglyceride and cholesterol levels. Similarly, Metwally (2009) reported that supplementation of natural garlic, garlic powder, and garlic oil in Nile tilapia significantly decreased blood serum triglyceride and cholesterol levels. Additionally, garlic powder at 30 g/kg feed was found to reduce blood serum total lipid in Nile tilapia (Shalaby et al., 2006).

The sulfur compounds in garlic, including the main active compound allicin, are responsible for the beneficial effects on fish lipid profile. These compounds are known to reduce levels of triglycerides and cholesterol in the blood serum by decreasing lipid and cholesterol synthesis, which contributes to the observed improvements (Shalaby et al., 2006; Talpur and Ikhwanuddin, 2012) and inhibiting cholesterol biosynthesis (Metwally, 2009). It is important to note that the efficacy of garlic supplementation can vary depending on the type and level of garlic supplement, as well as the fish species being studied.

Garlic powder has been shown to reduce cholesterol levels in different fish species, including European perch (Zare et al., 2021b) and Nile tilapia (Metwally, 2009), while its effect on triglyceride levels varies among species. The variability of garlic's effects on lipid metabolism may be attributed to differences in the type of supplement (Metwally, 2009), the level of supplementation (Metwally, 2009; Jahanjoo et al., 2018), and the specific sulfur compounds present in garlic, such as allicin (Valenzuela-Gutiérrez et al., 2021).

Overall, garlic supplementation can be a potential dietary strategy for improving lipid metabolism in fish, but further research is needed to determine the optimal dosage and duration of supplementation, as well as its potential impact on fish growth performance and health.

Research has shown that plant-based supplements in fish diets can affect the levels of cortisol and glucose in fish after long-term feeding trial (Zaefarian et al., 2017; Yousefi et al., 2020). Our own study (Chapter 4) investigated the effect of adding garlic powder to the diet of European perch at levels of 10, 20, and 30 g/kg on the cortisol and glucose levels in the fish after long-term feeding trial (Zare et al., 2021b). Contrary to previous studies, our findings showed that garlic powder supplementation did not significantly affect the cortisol and glucose levels in European perch in long-term feeding trial. Similarly, a study on brown trout found that the supplementation of garlic powder at levels of 10, 20, and 30 g/kg did not have a significant effect on glucose levels after 6 weeks feeding trial (Zaefarian et al., 2017).

In contrast, the addition of garlic powder at levels of 0.5, 1, and 1.5 g/kg in the feed of common carp significantly reduced the levels of both cortisol and glucose (Yousefi et al., 2020) at the end of long-term feeding trial. This may be attributed to the presence of organosulfur compounds such as diallyl trisulfide and alliin (S-allyl cysteine sulfoxide) in garlic (Tavares et al., 2021). Furthermore, garlic contains amino acids that may increase insulin secretion in carnivorous fish, in addition to the organosulfur compounds present in garlic (Polakof and Panserat, 2016). This is due to the rising glycemic regulator function, which increases insulin sensitivity and secretion (Nasim and Dhir, 2012).

Liver enzymes activity

The physiological status of fish is known to be influenced by a variety of stressors, including nutrition and pollution, which can result in altered secretion of liver enzymes (Khalil et al., 2017). The liver is a vital organ for various metabolic processes involved in protein, carbohydrate, and lipid metabolism in fish (Mitra and Metcalf, 2012). The assessment of liver function by measuring the activity of enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine transaminase (ALT) is a useful approach to evaluate the health status of fish (Taheri Mirghaed et al., 2019).

Medicinal plants have been found to have beneficial effects on the immune and antioxidant responses of different fish species (Metwally, 2009; Akrami et al., 2015; Adineh et al., 2020). Consumption of medicinal plants by fish has been shown to lower the levels of liver enzymes while promoting higher growth rates (Akrami et al., 2015; Adel et al., 2017; Adineh et al., 2020; Gholamhosseini et al., 2021). However, there have been only a few reports investigating the effects of nettle on liver enzymes in fish. Previous studies have found that feeding rainbow trout and beluga with aerial parts of nettle ethanol extract at levels of 20 and 30 g/kg did not significantly affect blood serum AST, ALT, and LDH levels (Binaii et al., 2014; Adel et al., 2017).

The present study examined the effect of garlic powder on the activity of blood serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes in European perch, and found no significant impact (Zare et al., 2021b) (Chapter 4). These findings are consistent with a previous study that reported no significant effect on ALT and AST enzyme activity in Asian sea bass when fed a diet containing 40 g/kg garlic powder (Abdelwahab et al., 2020). Conversely, studies conducted on common carp and Amur carp (*Cyprinus carpio haematopterus*) found that supplementing the diet with garlic powder at levels of 5–15 g/kg led to reduced blood serum ALT and AST enzyme activity (Chesti et al., 2018; Yousefi et al., 2020). In rainbow trout, supplementing the diet with garlic powder at levels of 40 and 50 g/kg increased ALT and AST enzyme activity, while no significant effect was observed at lower levels (Mohebbi et al., 2012).

It is important to note that the activity levels of ALT and AST enzymes can vary among fish species (Shalaby et al., 2006; Mohebbi et al., 2012; Chitsaz et al., 2018), and that the type of supplement (powder/extract) (Metwally, 2009; Adineh et al., 2020) and level of supplementation are important factors to consider (Mohebbi et al., 2012). Antioxidant compounds in garlic may play a protective role against lipid peroxidation in cell membranes, which could prevent the release of ALT and AST enzymes into the bloodstream (Yousefi et al., 2020). Then consequently, the reduction in ALT and AST enzyme activity in fish fed with garlic powder may be attributed to the antioxidant properties of garlic, specifically its organosulfur compounds, including S-allyl cysteine and diallyl-di-sulfide (Shang et al., 2019).

In conclusion, the impact of garlic supplementation on lipid metabolism in fish is species-specific, with varying effects on blood serum ALT and AST enzymes. However, it requires more specific further evaluations according to feed supplement levels, fish size and species.

Immune system stimulation

Numerous reports have examined the impact of medicinal plants on the immune system of fish, as documented by Reverter et al. (2021). Among these plants, nettle supplements have been found to have a positive effect on the immune system of different fish species, including warm and cold-water species, due to their bioactive compounds such as phenolic compounds and flavonoids, as noted by Grauso et al. (2020) in Chapter 2.

For example, the addition of nettle leaf powder at a rate of 10 g/kg to the diet of rainbow trout for a period of 14 days has been found to enhance lysozyme and bactericidal activity in blood serum, as well as respiratory burst. However, there was no significant impact on α2-macroglobulin, complement, myeloperoxidase content, and antiprotease activity, according to Awad and Austin (2010). Additionally, Mehrabi and Firouzbakhsh (2020) reported that administering nettle powder at rates of 5, 10, and 15 g/kg feed in rainbow trout had no significant effect on blood serum alternative complement activity (ACH50) and respiratory burst activity, but the level of blood serum lysozyme activity in the 5 g/kg treatment was significantly higher than the control. These results are consistent with our findings that nettle powder increased blood serum lysozyme enzyme activity in rainbow trout compared to the control, as reported by Zare et al. (2021a) in Chapter 3.

Furthermore, our results are consistent with those of Mehrabi et al. (2020), who found that blood serum lysozyme enzyme activity in rainbow trout improved when fed with nettle leaf powder at rates of 5, 10, and 15 g/kg feed for 8 weeks. Bilen et al. (2016) reported that the use of nettle methanol extract at rates of 0.1 and 0.5 g/kg feed in rainbow trout improved blood serum lysozyme activity after a 30-day feeding trial. In our investigation, however, nettle powder at a rate of 30 g/kg feed significantly enhanced blood serum lysozyme enzyme activity and total immunoglobulin in rainbow trout after an 8-week feeding trial, according to Zare et al. (2021a). In the study by Adel et al. (2017), nettle extract at a level of 30 g/kg in the diet of rainbow trout significantly improved lysozyme enzyme activity and immunoglobulin M. Similarly, Jafari et al. (2022) observed a significant increase in the immunoglobulin M levels in female convict cichlid fed with nettle methanolic extract at a level of 0.1 g/kg in feed, which supports the findings of Zare et al. (2021a) in rainbow trout.

However, Binaii et al. (2014) reported that nettle leaf powder supplementation at levels of 30, 60, and 120 g/kg feed in beluga had no significant effect on blood serum total immunoglobulin and lysozyme activity after a 4-week feeding trial, which contradicts the results of the study conducted by Zare et al. (2021a) on the use of nettle leaf powder in the diet of rainbow trout. On the other hand, in Victoria Labeo juvenile and adult fish, supplementation of nettle leaf powder at levels of 10, 20, and 30 g/kg feed in their diet for 4 and 16 weeks significantly improved blood serum lysozyme activity compared to the control group (Ngugi et al., 2015) as well as in our study on rainbow trout (Zare et al. 2021a).

The improvement of the innate immune system in fish can be attributed to the bioactive content of nettle in fish diet, including myricetin, quercetin, rutin, and kaempferol (Otles and Yalcin, 2012), particularly quercetin (Awad et al., 2013), which are considered phenolic compounds that can enhance the immune system in fish (Awad and Austin, 2010; Binaii et al., 2014; Ngugi et al., 2015; Bilen et al., 2016; Adel et al., 2017; Mehrabi and Firouzbakhsh, 2020; Mehrabi et al., 2020). These findings suggest that nettle can be a potential dietary supplement for improving the immune system of fish (Zare et al., 2023).

The impact of garlic powder as a dietary supplement on respiratory burst activity and phagocytic activity in various fish species has been investigated in several studies. Our study on European perch (Chapter 4) examined the effects of different levels of garlic powder supplementation on respiratory burst and phagocytic activities in comparison to other studies conducted on Nile tilapia, rainbow trout, and Amur carp. The results indicated that adding garlic powder to the diet of European perch did not result in significant changes in respiratory burst or phagocytic activity. Similar findings were reported by Mahfouz et al. (2009) in Nile tilapia, where garlic powder at a level of 20 g/kg feed did not have a significant effect on respiratory burst activity. In contrast, supplementation of garlic powder at levels of 5 and 10 g/kg feed in rainbow trout (Nya and Austin, 2011) and 5, 10, and 15 g/kg feed in Amur carp (Chesti et al., 2018) resulted in improved respiratory burst activity. These contrasting results suggest that the effects of garlic powder supplementation on respiratory burst activity are species-dependent and may vary with the level of supplementation.

Respiratory burst activity is considered an important indicator of immunity in fish, as it is activated against pathogen exposure (Biller and Takahashi, 2018). Phagocytic activity, which is linked to innate and adaptive immunity, is another important indicator of immunity in fish. In our study on European perch, the garlic groups did not show a significant increase in phagocytic activity in lymphocytes or myeloid cells compared to the control group. This finding agrees with the study by Huang et al. (2022), where raw garlic and garlic powder at levels 10, 40, and 80 g/kg did not enhance phagocytic activity in grouper.

In contrast, Nya and Austin (2009) reported that garlic powder at levels of 5 and 10 g/kg feed and allicin extract at levels of 5 and 10 ml/kg feed stimulated phagocytic activity in rainbow trout. The presence of bioactive compounds, such as allicin in garlic, may account for the observed improvement in phagocytic activity in previous studies. However, our study on European perch did not demonstrate a significant difference in phagocytic activity among groups fed with garlic powder (Chapter 4).

Overall, our study on European perch (Chapter 4) and previous research on other fish species suggest that the effects of garlic powder supplementation on respiratory burst and phagocytic activities may be species-dependent and vary with the level of supplementation. Further research is necessary to understand the mechanisms underlying these variations and to optimize the use of garlic powder as a dietary supplement in fish farming.

Pathogens challenge

In addition to nettle, other medicinal plants have been found to have antimicrobial and immunostimulatory effects in fish. For instance, garlic has been reported to have strong antimicrobial properties due to its organosulfur compounds, such as allicin and alliin (Bhatwalkar et al., 2021). Feeding rainbow trout (weighing 15 g) with 10 g/kg feed nettle leaf powder for 14 days resulted in the highest survival rate of 96% compared to the control group's survival rate of 32% after a 10-day challenge with *Aeromonas hydrophila* (Nya and Austin, 2010). Moreover, Bilen et al. (2016) found that rainbow trout, with an average initial weight of 10 g, fed with nettle methanol extracts at levels of 0.1 and 0.5 g/kg feed for a 30-day feeding trial exhibited the highest survival rate among all groups after a 14-day challenge with *A. hydrophila.* Their study revealed that the nettle groups had the highest survival rate compared to the control group after a 14-day *A. hydrophila* challenge. The survival rate of warm-water species improved after a nettle challenge. Thus, feeding Victoria Labeo with 10, 20, and 50 g/kg feed nettle leaf powder for 4 and 16 weeks could increase the survival rate to over 80% in the 2% and 5% nettle groups compared to the control group, which received 0% nettle. This improvement was observed after a 21-day *A. hydrophila* challenge (Ngugi et al., 2015).

The inclusion of nettle supplements in fish feed has been reported to enhance the innate immune response and leukocyte count, as indicated by increased levels of various immune factors such as complements, ACH50, bactericidal, immunoglobulin, respiratory burst, phagocytic, antiprotease, and lysozyme activity (Awad and Austin, 2010; Ngugi et al., 2015; Bilen et al., 2016; Mehrabi et al., 2020; Zare et al., 2021a). Furthermore, studies have demonstrated that nettle supplementation can upregulate the expression of immune-related genes in fish (Mehrabi et al., 2020). For example, Mehrabi et al. (2020) reported an increase in cytokine-related gene expression, including interleukin 6 (IL-6) and interleukin 8 (IL-8) in fish fed with nettle supplements. Cytokines are small proteins produced by immune cells that play a vital role in regulating inflammation, the immune system, and hematopoiesis (Savan and Sakai, 2006). In addition, nettle supplementation has also been shown to upregulate the expression of other genes such as interleukin 1 beta (IL-1β) and tumor necrosis factor α (TNF-α) (Mehrabi et al., 2020). Overall, the use of medicinal plants and plant-based supplements as feed additives in fish diets has shown promising results in improving fish health and disease resistance. However, the effectiveness of these supplements may depend on various factors, such as fish species, dosage, duration of feeding, and the specific pathogen challenge.

High density and net handling stress challenge

Cortisol and glucose levels are commonly used as primary and secondary stress markers in fish, respectively (Bordin and Freire, 2021). Cortisol, a glucocorticoid hormone, circulates in fish and its levels are regulated by cytosolic receptors that control various physiological processes such as metabolism, growth, and the expression of immune-related genes (Faught and Vijayan, 2016). Under stress, cortisol raises blood glucose levels (Bordin and Freire, 2021). Our study investigated the effects of garlic powder supplementation on cortisol and glucose levels in European perch after high density and net handling stress challenge. We found that immediately after stress, although cortisol levels were higher in all garlic-fed groups compared to the control group, the difference in glucose levels was not statistically significant. However, at 1, 6, and 24 hours after stress, the garlic-fed groups showed significantly lower levels of both cortisol and glucose compared to the control group. These findings suggest that garlic supplementation may have a potential protective effect against high density and net handling stress-induced cortisol and glucose elevation in fish.

Previous studies have also examined the effects of garlic supplementation on cortisol and glucose levels in fish. For instance, Adineh et al. (2021) found that a combination of 2 mg nano selenium and 2 g/kg feed garlic extract decreased cortisol and glucose levels in grass carp under different stocking densities. Ahmed and Al-Hamdani (2022) reported that garlic extract at 5 g/kg feed in common carp lowered glucose levels and increased resistance under heating stress. In contrast, Gabriel et al. (2019) found that the inclusion of 5–40 g/kg feed garlic powder in the African catfish diet did not result in significant mortality under low pH stress. The sulfur bioactive properties of garlic, such as alliin and allicin, may be responsible for its effects on cortisol and glucose levels in fish (Tavares et al., 2021).

Altogether, our findings suggest that the use of medicinal plants (nettle leaf and garlic powders) can significantly improve fish growth, protein digestibility, immune system function, and fish endurance to handling stress. However, it is important to note that our results were influenced by various factors, including fish species, size, age, rearing conditions, plant additives source, and feed ingredients. In addition to the remarkable findings from the present studies, which have significant commercial implications for the aquaculture industry, we strongly recommend complementing the analysis with histological and gene expression studies. Doing so will provide a clearer and broader view of the results and help explain the potential mechanisms of action.

Conclusion

This dissertation encompasses three research publications that investigated the use of nettle and garlic powder as additives in fish diets. The main conclusions from these studies are as follows:

The use of nettle leaf powder as a feed additive in warm-water fish species and rainbow trout have been shown to enhance growth performance, nutritional factors, haemato-biochemical parameters, and immune system function. As such, it presents a promising alternative to chemical and medicinal treatments for controlling pathogens in fish.

The inclusion of nettle and tarragon leaf powders in rainbow trout fingerling diets has been found to promote growth performance, body composition, haemato-biochemical factors, and immune response. Specifically, a combination of nettle and tarragon leaf powders at a minimum of 10 g/kg each in the feed, or 30 g/kg of nettle leaf powder alone, was found to be optimal.

Garlic powder at a level of 10 g/kg feed has been demonstrated to improve haemato-biochemical factors and nutrient digestibility, particularly fat, in European perch. Furthermore, the use of 30 g/kg feed garlic powder increased whole-body protein composition and elevated resistance against high density and net handling stress in European perch.

References

Abang Zamhari, D.N.J.b., Yong, A.S.K., 2021. Dietary herbs supplementation improves growth, feed efficiency and apparent digestibility coefficient of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) juvenile. Aquaculture Research 52, 5239–5246.

Abdelwahab, A.M., El-Bahr, S.M., Al-Khamees, S., 2020. Influence of dietary garlic (*Allium sativum*) and/or ascorbic acid on performance, feed utilization, body composition and hemato-biochemical parameters of juvenile Asian Sea Bass (*Lates calcarifer*). Animals. 10, 2396.

Abdel-Tawwab, M., Adeshina, I., Jenyo-Oni, A., Ajani, E.K., Emikpe, B.O., 2018. Growth, physiological, antioxidants, and immune response of African catfish, *Clarias gariepinus* (B.), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to Listeria monocytogenes infection. Fish & Shellfish Immunology 78, 346–354.

Adel, M., Caipang, C.M.A., Dawood, M.A., 2017. Immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles following dietary administration of stinging nettle (*Urtica dioica*). Fish & Shellfish Immunology. 71, 230-238.

Adineh, H., Harsij, M., Jafaryan, H., Asadi, M., 2020. The effects of microencapsulated garlic (*Allium sativum*) extract on growth performance, body composition, immune response and antioxidant status of rainbow trout (*Oncorhynchus mykiss*) juveniles. Journal of Applied Animal Research. 48, 372–378.

Adineh, H., Naderi, M., Nazer, A., Yousefi, M., Ahmadifar, E., 2021. Interactive effects of stocking density and dietary supplementation with Nano selenium and garlic extract on growth, feed utilization, digestive enzymes, stress responses, and antioxidant capacity of grass carp, *Ctenopharyngodon idella*. Journal of the World Aquaculture Society. 52, 789–804.

Ahmed, I.B., Al-Hamdani, A., 2022. Effect of garlic on blood parameters in thermal stressed common carp fish (*Cyprinus Carpio* L). Egyptian Journal of Veterinary Sciences 53, 573–581.

Akrami, R., Gharaei, A., Mansour, M.R., Galeshi, A., 2015. Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato–biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. Fish & Shellfish Immunology. 45, 828–834.

Al-Shakarchi, H.H., Mohammad, M.A., 2021. Effect of different levels of cinnamon and coriander powder on growth performance, feed utilization, survival rate and body chemical composition of common carp *Cyprrinus carpio* L. Plant Archives. 21, 46–54.

Asaoka, Y., Terai, S., Sakaida, I., Nishina, H., 2013. The expanding role of fish models in understanding non-alcoholic fatty liver disease. Disease Models & Mechanisms 6, 905–914.

Awad, E., Austin, B., 2010. Use of lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, as feed additives to prevent *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 33, 413–420.

Awad, E., Austin, B., Lyndon, A., 2012. Effect of dietary supplements on digestive enzymes and growth performance of rainbow trout (*Oncorhynchus mykiss*, Walbaum). Journal of American Science 8, 858–864.

Awad, E., Austin, D., Lyndon, A.R., 2013. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture 388, 193–197.

Behbahani, B.A., Shahidi, F., Yazdi, F.T., Mortazavi, S.A., Mohebbi, M., 2017. Antioxidant activity and antimicrobial effect of tarragon (*Artemisia dracunculus*) extract and chemical composition of its essential oil. Journal of Food Measurement and Characterization. 11, 847–863.

Belinskaia, D.A., Voronina, P.A., Shmurak, V.I., Vovk, M.A., Batalova, A.A., Jenkins, R.O., Goncharov, N.V., 2020. The universal soldier: Enzymatic and non-enzymatic antioxidant functions of serum albumin. Antioxidants 9, 966.

Bhatwalkar, S.B., Mondal, R., Krishna, S.B.N., Adam, J.K., Govender, P., Anupam, R., 2021. Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). Frontiers in Microbiology 12, 1869.

Bilen, S., Ünal, S., Güvensoy, H., 2016. Effects of oyster mushroom (*Pleurotus ostreatus*) and nettle (*Urtica dioica*) methanolic extracts on immune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 454, 90-94.

Biller, J.D., Takahashi, L.S., 2018. Oxidative stress and fish immune system: phagocytosis and leukocyte respiratory burst activity. Anais da Academia Brasileira de Ciências 90, 3403-3414.

Binaii, M., Ghiasi M., Farabi, S.M.V., Pourgholam, R., Fazli, H., Safari, R., Alavi, S.E., Taghavi, M.J., Bankehsaz, Z., 2014. Biochemical and hemato-immunological parameters in juvenile beluga (*Huso huso*) following the diet supplemented with nettle (*Urtica dioica*). Fish and Shellfish Immunology. 36, 46–51.

Bordin, D., Freire, C.A., 2021. Remarkable variability in stress responses among subtropical coastal marine teleosts. Marine Biology. 168, 1–17.

Büyükdeveci, M.E., Balcázar, J.L., Demirkale, İ., Dikel, S., 2018. Effects of garlic-supplemented diet on growth performance and intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 486, 170–174.

Chehri, A., Yarani, R., Yousefi, Z., Novin Bahador, T., Shakouri, S.K., Ostadrahimi, A., Mobasseri, M., Pociot, F., Araj-Khodaei, M., 2022. Anti-diabetic potential of *Urtica dioica*: Current knowledge and future direction. Journal of Diabetes & Metabolic Disorders. 21, 931–940.

Chesti, A., Chauhan, R.S., Khati, A., 2018. Evaluation of immunostimulatory effect of dietary garlic (*Allium sativum*) in fingerlings of Amur carp, *Cyprinus carpio haematopterus*. The Pharma Innovation Journal. 7, 375–379.

Chitsaz, H., Oraji, H., Keramat Amirkolaei, A., Akrami, R., 2018. Effect of garlic peel on haematological, biochemical and digestive enzyme activity in beluga juvenile (*Huso huso*). Iranian Journal of Aquatic Animal Health. 4, 13-28.

Chowdhury, D.K., Sahu, N.P., Sardar, P., Deo, A.D., Bedekar, M.K., Singha, K.P., Maiti, M.K., 2021. Feeding turmeric in combination with ginger or garlic enhances the digestive enzyme activities, growth and immunity in *Labeo rohita* fingerlings. Animal Feed Science and Technology. 277, 114964.

Diab, A.S., Aly, S.M., John, G., Abde-Hadi, Y., Mohammed, M.F., 2008. Effect of garlic, black seed and Biogen as immunostimulants on the growth and survival of Nile tilapia, *Oreochromis niloticus* (Teleostei: Cichlidae), and their response to artificial infection with Pseudomonas fluorescens. African Journal of Aquatic Science 33, 63–68.

Esmaeili, M., 2021. Blood performance: a new formula for fish growth and health. Biology. 10, 1236.

Esmaeili, M., Carter, C.G., Wilson, R., Walker, S.P., Miller, M.R., Bridle, A., Symonds, J.E., 2021. Proteomic investigation of liver and white muscle in efficient and inefficient Chinook salmon (*Oncorhynchus tshawytscha*): Fatty acid metabolism and protein turnover drive feed efficiency. Aquaculture. 542, 736855.

Esmaeili, M., Kenari, A.A., Rombenso, A., 2017. Immunohematological status under acute ammonia stress of juvenile rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) fed garlic (*Allium sativum*) powder-supplemented meat and bone meal-based feeds. Comparative Clinical Pathology 26, 853–866.

Faught, E., Vijayan, M.M., 2016. Mechanisms of cortisol action in fish hepatocytes. Comp. Biochem. Physiol. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 199, 136–145.

Fazio, F., 2019. Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture. 500, 237–242.

Foysal, M.J., Alam, M., Momtaz, F., Chaklader, M.R., Siddik, M.A., Cole, A., Fotedar, R., Rahman, M.M., 2019. Dietary supplementation of garlic (*Allium sativum*) modulates gut microbiota and health status of tilapia (*Oreochromis niloticus*) against *Streptococcus iniae* infection. Aquaculture Research. 50, 2107–2116.

Gabriel, N.N., Wilhelm, M.R., Habte-Tsion, H.-M., Chimwamurombe, P., Omoregie, E., 2019. Dietary garlic (*Allium sativum*) crude polysaccharides supplementation on growth, haematological parameters, whole body composition and survival at low water pH challenge in African catfish (*Clarias gariepinus*) juveniles. Scientific African 5, e00128.

Gabriel, N.N., Wilhelm, M.R., Habte-Tsion, H.-M., Chimwamurombe, P., Omoregie, E., 2021. The effects of dietary garlic (*Allium sativum*) and *Aloe vera* crude extract mixtures supplementation on growth performance, feed utilization, hematological parameters, whole body composition, and survival at low pH in African catfish (*Clarias gariepinus*) juveniles. Scientific African 11, e00671.

Gholamhosseini, A., Hosseinzadeh, S., Soltanian, S., Banaee, M., Sureda, A., Rakhshaninejad, M., Ali Heidari, A., Anbazpour, H., 2021. Effect of dietary supplements of *Artemisia dracunculus* extract on the haemato-immunological and biochemical response, and growth performance of the rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research 52, 2097–2109.

Gholipour Kanani, H., Nobahar, Z., Kakoolaki, S., Jafarian, H., 2014. Effect of ginger-and garlic-supplemented diet on growth performance, some hematological parameters and immune responses in juvenile *Huso huso*. Fish Physiology and Biochemistry 40, 481–490.

Grauso, L., de Falco, B., Lanzotti, V., Motti, R., 2020. Stinging nettle, *Urtica dioica* L.: Botanical, phytochemical and pharmacological overview. Phytochemistry Reviews. 19, 1341–1377.

He, G., Sun, H., Liao, R., Wei, Y., Zhang, T., Chen, Y., Lin, S., 2022. Effects of herbal extracts (*Foeniculum vulgare* and *Artemisia annua*) on growth, liver antioxidant capacity, intestinal morphology and microorganism of juvenile largemouth bass, *Micropterus salmoides*. Aquaculture Reports 23, 101081.

Huang, H.-T., Lee, P.-T., Liao, Z.-H., Huang, C.-T., Chen, H.-Y., Huang, Z.-G., Nan, F.-H., Wu, Y.-S., 2022. Raw garlic (*Allium sativum*) improves nonspecific immune responses and resistance against Vibrio alginolyticus infection in grouper (*Epinephelus coioides*). Journal of Marine Science and Technology 29, 757–766.

Hwang, J.-H., Lee, S.-W., Rha, S.-J., Yoon, H.-S., Park, E.-S., Han, K.-H., Kim, S.-J., 2013. Dietary green tea extract improves growth performance, body composition, and stress recovery in the juvenile black rockfish, *Sebastes schlegeli*. Aquaculture International. 21, 525–538.

İrkin, L.C., Yiğit, M., 2015. The use of garlic (*Allium sativum*) meal as a natural feed supplement in diets for European seabass (*Dicentrarchus labrax*) juveniles. Journal of Aquaculture Engineering and Fisheries Research. 2, 128–141.

Jafari, N., Abdollahpour, H., Karimzadeh, M., Falahatkar, B., 2022. A potential role of nettle (*Urtica dioica*) extract on growth, biochemical changes and reproductive performance of convict cichlid (*Amatitlania nigrofasciata*). Fish Physiology and Biochemistry 48, 133–144.

Jahanjoo, V., Yahyavi M., Akrami R., Bahri A.H., 2018. Influence of adding garlic (*Allium sativum*), Ginger (*Zingiber officinale*), thyme (*Thymus vulgaris*) and their combination on the growth performance, haematoimmunological parameters and disease resistance to *Photobacterium damselae* in sobaity sea bream (*Sparidentex hasta*) fry. Turkish Journal of Fisheries and Aquatic Sciences. 18, 633–645.

Jan, K.N., Zarafshan, K., Singh, S., 2017. Stinging nettle (*Urtica dioica* L.): a reservoir of nutrition and bioactive components with great functional potential. Journal of Food Measurement and Characterization 11, 423-433.

Kaur, H., Ansal, M.D., 2020. Efficacy of garlic powder as a growth promoting feed additive for higher growth and improved flesh quality in an Indian major carp, *Labeo rohita* (Ham.) fingerlings. Journal of Entomology and Zoology Studies 8, 25–29.

Khalil, M.T., Gad, N.S., Ahmed, N.A., Mostafa, S.S.E.-D., 2017. Antioxidant defense system alternations in fish as a bio-indicator of environmental pollution. Egyptian Journal of Aquatic Biology and Fisheries 21, 11–28.

Khasani, I., Astuti, D.N., 2019. Albumin level, growth and survival rate of snakehead fish (*Channa striata*) from three islands of Indonesia. Aquaculture, Aquarium, Conservation & Legislation 12, 1688–1697.

Kk, M.A., Parsuraman, S., 2014. *Urtica dioica* L.,(Urticaceae): A stinging nettle. Systematic Reviews in Pharmacy. 5, 6–8.

Lee, D.-H., Lim, S.-R., Han, J.-J., Lee, S.-W., Ra, C.-S., Kim, J.-D., 2014. Effects of dietary garlic powder on growth, feed utilization and whole body composition changes in fingerling sterlet sturgeon, *Acipenser ruthenus*. Asian-Australasian Journal of Animal Sciences. 27, 1303.

Lee, D.-H., Ra, C.-S., Song, Y.-H., Sung, K.-I., Kim, J.-D., 2012. Effects of dietary garlic extract on growth, feed utilization and whole body composition of juvenile sterlet sturgeon (*Acipenser ruthenus*). Asian-Australasian Journal of Animal Sciences 25, 577.

Lee, J.Y., Gao, Y., 2012. Review of the application of garlic, *Allium sativum*, in aquaculture. Journal of the World Aquaculture Society. 43, 447–458.

Lee, K.-W., Everts, H., Kapperst, H., Yeom, K.-H., Beynen, A., 2003. Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. Journal of Applied Poultry Research 12, 394–399.

Mahfouz, N., Fathi, M., Abou, Zead, M., Mesalhy, S., 2009. Effect of garlic and yeast in the culture of Nile tilapia (*Oreochromis niloticus*). Abbassa International Journal for Aquaculture, Special Issue for Global Fisheries & Aquaculture Research Conference, Cairo International Convention Center, 24–26 October 2009, 453–465.

Mahmoud, H.K., Reda, F.M., Alagawany, M., Farag, M.R., El-Naggar, K., 2023. The role of dietary chia seed powder in modulating cold stress-related impacts in Nile tilapia, *Oreochromis niloticus*. Aquaculture 739246.

Mbokane, E., Moyo, N., 2018. A preliminary investigation into the potential effect of *Artemisia afra* on growth and disease resistance in sub-adults of *Oreochromis mossambicus*. Aquaculture. 482, 197–202.

Mbokane, E.M., Moyo, N.A.G., 2020a. Effect of dietary *Artemisia afra* on growth, some innate immunological parameters in *Clarias gariepinus* challenged with *Aeromonas hydrophila*. Aquaculture International 28, 539–553.

Mbokane, E.M., Moyo, N.A.G., 2020b. Effects of dietary levels of essential oil extracts from Moringa oleifera and *Artemisia afra* on kidney histology, haemato-immunological parameters and disease resistance in *Clarias gariepinus*. Aquaculture Research 51, 410–425.

Mehrabi, Z., Firouzbakhsh, F., 2020. Short-term effects of feeding powdered Aloe vera (*Aloe barbadensis*) and nettle (*Urtica dioica*) on growth performance and stimulation of innate immune responses in rainbow trout (*Oncorhynchus mykiss*). Comparative Clinical Pathology. 29, 441–449.

Mehrabi, Z., Firouzbakhsh, F., Rahimi-Mianji, G., Paknejad, H., 2020. Immunity and growth improvement of rainbow trout (*Oncorhynchus mykiss*) fed dietary nettle (*Urtica dioica*) against experimental challenge with *Saprolegnia parasitica*. Fish & Shellfish Immunology 104, 74–82.

Metwally, M., 2009. Effects of garlic (*Allium sativum*) on some antioxidant activities in tilapia nilotica (*Oreochromis niloticus*). World Journal of Fish and Marine Sciences. 1, 56–64.

Mitra, V., Metcalf, J., 2012. Metabolic functions of the liver. Anaesth. Anaesthesia & Intensive Care Medicine. 13, 54–55.

Mohebbi, A., Nematollahi, A., Dorcheh, E.E., Asad, F.G., 2012. Influence of dietary garlic (*Allium sativum*) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research 43, 1184–1193.

Nasim, S., Dhir, B., 2012. Reduction in serum glucose with garlic extracts. Bioactive Food as Dietary Interventions for Diabetes: Bioactive Foods in Chronic Disease States, 97.

Nazerian, S., Jafaryan, H.A., Soltani, M., Patimar, R., 2016. Effect of purple coneflower (*Echinacea purpora*) and garlic (*Allium satvium*) as a supplemented dietary intake on some non-specific immune status, hematological parameters and growth performance in grower (*Huso huso*). Iranian Journal of Veterinary Science and Technology. 8, 29–39.

Ndimele, P., Kumolu-Johnson, C., Aladetohun, N., Ayorinde, O., 2010. Length-weight relationship, condition factor and dietary composition of *Sarotherodon melanotheron*, Rüppell, 1852 (Pisces: cichlidae) in Ologe Lagoon, Lagos, Nigeria. Agriculture and Biology Journal of North America. 1, 584–590.

Ngugi, C.C., Oyoo-Okoth, E., Mugo-Bundi, J., Orina, P.S., Chemoiwa, E.J., Aloo, P.A., 2015. Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult Victoria Labeo (*Labeo victorianus*) challenged with *Aeromonas hydrophila*. Fish & Shellfish Immunology. 44, 533–541.

Nobahar, Z., Gholipour-Kanani H., Kakoolaki S., Jafaryan H., 2015. Effects of garlic (*Allium sativum*) and nettle (*Urtic adioica*) on growth performance and on hematological parameters of beluga (*Huso huso*). Iranian Journal of Aquatic Animal Health. 1, 63–69.

Nya, E.J., Austin, B., 2009. Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 32, 963–970.

Nya, E.J., Austin, B., 2011. Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. Fish & Shellfish Immunology. 30, 845–850.

Nya, E.J., Dawood, Z., Austin, B., 2010. The garlic component, allicin, prevents disease caused by *Aeromonas hydrophila* in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases 33, 293–300.

Obolskiy, D., Pischel, I., Feistel, B., Glotov, N., Heinrich, M., 2011. *Artemisia dracunculus* L. (tarragon): a critical review of its traditional use, chemical composition, pharmacology, and safety. Journal of Agricultural and Food Chemistry 59, 11367–11384.

Otles, S., Yalcin, B., 2012. Phenolic compounds analysis of root, stalk, and leaves of nettle. The Scientific World Journal. 2012, 12.

Öz, M., Dikel, S., 2022. Effect of garlic (*Allium sativum*)-supplemented diet on growth performance, body composition and fatty acid profile of rainbow trout (*Oncorhynchus mykiss*). Cellular and Molecular Biology. 68, 217–225.

Pande, S., Srinivasan, K., 2012. Potentiation of the hypolipidemic influence of dietary tender cluster bean (*Cyamopsis tetragonoloba*) by garlic in cholesterol fed rats. Food Chemistry. 133, 798–805.

Patel, P., Borichangar, R., Vanza, J., 2022. Effect of garlic (*Allium sativum*) supplementation on growth, survival and body composition of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878). The Pharma Innovation Journal 11, 642–652.

Petkova, Z.Y., Antova, G., Angelova-Romova, M., 2020. Biologically active components and health benefits of nettle seed oil. Grasas y Aceites. 71, e347–e347.

Polakof, S., Panserat, S., 2016. How Tom Moon's research highlighted the question of glucose tolerance in carnivorous fish. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 199, 43–49.

Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P., Caruso, D., 2021. Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. Review in Aquaculture. 13, 537–555.

Rutto, L.K., Xu, Y., Ramirez, E., Brandt, M., 2013. Mineral properties and dietary value of raw and processed stinging nettle (*Urtica dioica* L.). International Journal of Food Science 2013, 857120.

Saad, B., Zaid, H., Shanak, S., Kadan, S., Saad, B., Zaid, H., Shanak, S., Kadan, S., 2017. Antidiabetic medicinal plants. Anti-diabetes and Anti-obesity Medicinal Plants and Phytochemicals: Safety, Efficacy, and Action Mechanisms, 147–174.

Sadauskas-Henrique, H., Sakuragui, M.M., Paulino, M.G., Fernandes, M.N., 2011. Using condition factor and blood variable biomarkers in fish to assess water quality. Environmental Monitoring and Assessment. 181, 29–42.

Sahu, S., Das, B., Mishra, B., Pradhan, J., Sarangi, N., 2007. Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Journal of Applied Ichthyology. 23, 80–86.

Saleh, N.E., Michael, F.R., Toutou, M.M., 2015. Evaluation of garlic and onion powder as phyto-additives in the diet of sea bass (*Dicentrarcus labrax*). The Egyptian Journal of Aquatic Research. 41, 211–217.

Savan, R., Sakai, M., 2006. Genomics of fish cytokines. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics. 1, 89–101.

Shalaby, A., Khattab, Y., Abdel Rahman, A., 2006. Effects of Garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). Journal of Venomous Animals and Toxins including Tropical Diseases. 12, 172–201.

Shang, A., Cao, S.-Y., Xu, X.-Y., Gan, R.-Y., Tang, G.-Y., Corke, H., Mavumengwana, V., Li, H.-B., 2019. Bioactive compounds and biological functions of garlic (*Allium sativum* L.). Foods 8, 246.

Song, X., Yue, Z., Nie, L., Zhao, P., Zhu, K., Wang, Q., 2021. Biological functions of diallyl disulfide, a garlic-derived natural organic sulfur compound. Evidence-Based Complementary and Alternative Medicine 2021.

Taheri Mirghaed, A., Fayaz, S., Hoseini, S.M., 2019. Dietary 1, 8-cinoele affects serum enzymatic activities and immunological characteristics in common carp (*Cyprinus carpio*) exposed to ambient ammonia. Aquaculture Research 50, 146–153.

Talpur, A.D., Ikhwanuddin, M., 2012. Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against Vibrio harveyi infection in Asian sea bass, *Lates calcarifer* (Bloch). Aquaculture 364, 6–12.

Tavares, L., Santos, L., Noreña, C.P.Z., 2021. Bioactive compounds of garlic: A comprehensive review of encapsulation technologies, characterization of the encapsulated garlic compounds and their industrial applicability. Trends in Food Science & Technology. 114, 232–244.

Valenzuela-Gutiérrez, R., Lago-Lestón, A., Vargas-Albores, F., Cicala, F., Martínez-Porchas, M., 2021. Exploring the garlic (*Allium sativum*) properties for fish aquaculture. Fish Physiology and Biochemistry 47, 1179–1198.

Van Hai, N., 2015. The use of medicinal plants as immunostimulants in aquaculture: A review. Aquaculture. 446, 88–96.

Van Wyk, B.-E., Wink, M., 2018. Medicinal plants of the world. CABI, pp. 1786393255.

Witeska, M., 2013. Erythrocytes in teleost fishes: a review. Zoology and Ecology 23, 275–281.

Xu, A., Shang-Guan, J., Li, Z., Gao, Z., Huang, Y., Chen, Q., 2020. Effects of garlic powder on feeding attraction activity, growth and digestive enzyme activities of Japanese seabass, *Lateolabrax japonicus*. Aquaculture Nutrition. 26, 390–399.

Yousefi, M., Vatnikov, Y.A., Kulikov, E.V., Plushikov, V.G., Drukovsky, S.G., Hoseinifar, S.H., Van Doan, H., 2020. The protective effects of dietary garlic on common carp (*Cyprinus carpio*) exposed to ambient ammonia toxicity. Aquaculture. 526, 735400.

Yousefi, M., Zahedi, S., Reverter, M., Adineh, H., Hoseini, S.M., Van Doan H., El-Haroun E.R., Hoseinifar S.H., 2021. Enhanced growth performance, oxidative capacity and immune responses of common carp, *Cyprinus carpio* fed with *Artemisia absinthium* extract-supplemented diet. Aquaculture. 545, 737167.

Zaefarian, A., Yeganeh, S., Adhami, B., 2017. Dietary effects of garlic powder (*Allium sativum*) on growth, blood indices, carcass composition, and lysozyme activity in brown trout (*Salmo caspius*) and resistance against *Yersinia ruckeri* infection. Aquaculture International. 25, 1987–1996.

Zare, M., Esmaeili, N., Paolacci, S., Stejskal, V., 2023. Nettle (*Urtica dioica*) additive as a growth promoter and immune stimulator in fish. Aquaculture Nutrition. 2023, 21.

Zare, M., Mirzakhani, M.K., Stejskal, V., 2021a. Growth, body proximate composition and selected blood parameters of rainbow trout *Oncorhynchus mykiss* fingerlings fed on a diet supplemented with nettle *Urtica dioica* and tarragon *Artemisia dracunculus*. Aquaculture Research. 52, 5691–5702.

Zare, M., Tran, H.Q., Prokešová, M., Stejskal, V., 2021b. Effects of garlic *Allium sativum* powder on nutrient digestibility, haematology, and immune and stress responses in Eurasian perch *Perca fluviatilis* juveniles. Animals 11, 2735.

Zhao, L., Budge, S.M., Ghaly, A.E., Brooks, M.S., Dave, D., 2011. Extraction, purification and characterization of fish pepsin: a critical review. Journal of Food Processing & Technology 2, 126.

English summary

Effects of herbal derived products on growth, digestibility, body composition, immune and stress response in fish

In the upcoming decades, aquaculture is expected to play a vital role in food production for the growing human population. Nevertheless, disease outbreaks can hinder the continuous development of this industry. One promising solution to address this challenge is the use of natural feed additives, such as plant powders and extracts, which contain bioactive compounds, including phenolic compounds, proteins, vitamins, and minerals. These compounds have been shown to possess antistress, antiviral, antibacterial, and antifungal effects on fish.

Chapter 2 is the literature review focused on nettle, a medicinal herb with a long history of use in traditional medicine. While nettle has been extensively studied in mammalian medicine, its effects on aquaculture species have not been well documented. Recent studies have demonstrated the positive impact of nettle on the growth performance, hematology, blood biochemistry, and immune system of fish species. In particular, nettle-fed fish showed a higher survival rate and less stress than the control group when exposed to pathogens. This literature review aims to provide an overview of the use of nettle in fish diets and its effects on growth performance, hematology, blood biochemistry, liver enzymes, immune system stimulation, and resistance to pathogenic challenges.

Chapter 3 of the dissertation examines the effects of nettle and tarragon leaf powder, both alone and in combination, as dietary additives in the diet of rainbow trout fingerlings. Nettle leaf powder at a rate of 30 g/kg feed, resulted in significant improvements in the FBW, WG, WG%, and SGR% of rainbow trout fingerlings. Additionally, nettle leaf powder increased whole-body proximate protein and ash content while reducing whole-body proximate fat. Tarragon leaf powder, when added at a rate of 30 g/kg feed, significantly increased the Hct% and leukocyte count. The combination of 10 g/kg feed nettle leaf powder and 10 g/kg feed tarragon leaf powder resulted in the highest levels of erythrocyte count and Hb. Nettle leaf powder at 30 g/kg feed, tarragon leaf powder at 30 g/kg feed, and a combination of 10 g/kg feed nettle leaf powder and 10 g/kg feed tarragon leaf powder significantly increased albumin levels in blood serum. Tarragon leaf powder at 10 g/kg feed showed the highest blood serum lysozyme enzyme activity compared to the control. Blood serum total immunoglobulin and total protein levels were significantly associated with nettle leaf powder at 30 g/kg feed. The study confirms the positive effects of these additives on growth performance, whole body proximate composition, immunological, haematological, and selected blood serum biochemical factors.

In Chapter 4, the study found that different levels of garlic powder did not significantly affect growth performance and somatic indices in European perch. However, adding 30 g/kg feed garlic powder resulted in a significantly higher level of whole-body proximate protein. Both 10 g/kg feed and 30 g/kg feed garlic powder showed significantly higher fat digestibility than the control and 20 g/kg feed garlic powder groups. The 20 g/kg feed garlic powder diet resulted in significantly higher erythrocyte and leukocyte count. Furthermore, all garlic groups had significantly lower blood serum cholesterol levels compared to the control. Garlic powder improved European perch's resistance against high density and net handling stress, as well as their haematological and immunological parameters. Immediately after stress, the level of cortisol in all garlic-fed groups was significantly higher than in the control group, and there was no significant difference in glucose levels among the groups. However, after 24 hours, the levels of both cortisol and glucose were lower in all garlic-fed groups compared to the control group significantly. These results indicate that feeding European perch with garlic powder may

have a positive effect on managing stress response. Garlic powder at a level of 10 g/kg feed improved apparent fat digestibility, whole body proximate protein, hematological parameters, immunological parameters, and increased resistance in high density and net handling stress. Overall, garlic powder could be considered as a potential dietary supplement for managing stress in European perch, however, further research is needed to determine the optimal dosage and long-term effects.

Results of present thesis suggest that the use of natural feed phyto-additives could be a potential solution to address health issues in aquaculture, leading to more sustainable and efficient food production.

Czech summary

Účinky rostlinných produktů na růst, stravitelnost, složení těla, imunitní a stresovou reakci u ryb

Očekává se, že v nadcházejících desetiletích bude akvakultura hrát zásadní roli při produkci potravin pro rostoucí lidskou populaci. Nicméně propuknutí nemocí může bránit dalšímu rozvoji tohoto odvětví. Jedním ze slibných řešení pro řešení tohoto problému je použití přírodních krmných aditiv, jako jsou rostlinné moučky a extrakty, které obsahují bioaktivní sloučeniny, včetně fenolických sloučenin, proteinů, vitamínů a minerálů. Bylo prokázáno, že tyto sloučeniny mají na ryby antistresové, antivirové, antibakteriální a antifungální účinky.

Druhá kapitola má podobu přehledového článku a zaměřuje se na kopřivu dvoudomou jakožto léčivou bylinu s dlouhou historií použití v tradiční medicíně. Zatímco kopřiva byla rozsáhle studována v medicíně savců, její účinky na druhy využívané v akvakultuře nebyly dostatečně zdokumentovány. Nedávné studie však prokázaly pozitivní vliv kopřivy na růstovou výkonnost, hematologii, biochemii krve a imunitní systém ryb. Ryby krmené přídavkem kopřivy vykazovaly vyšší míru přežití a menší úroveň stresu než kontrolní skupiny, když byly chované ryby vystaveny patogenům. Tento přehled literatury si klade za cíl poskytnout souhrn současných poznatků o použití kopřivy v rybí stravě a jejích účincích na růstovou výkonnost, hematologii, biochemii krve, jaterní enzymy, stimulaci imunitního systému a odolnost vůči patogenům.

Třetí kapitola disertační práce zkoumá účinky moučky z kopřivy a moučky z listů estragonu, a to jak samotných, tak v kombinaci, jako krmých aditiv v krmivu pstruha duhového. Prášek z listů kopřivy v dávce 30 g/kg krmiva vedl k významnému zlepšení konečné hmotnosti ryb, hmotnostního přírůstku, procentuálního hmotnostního a specifické rychlosti růstu pstruha duhového. Přídavek moučky z listů kopřivy navíc zvýšil celkový obsah bílkovin a popelovin v těle ryb a zároveň snížil obsah tuku. Přídavek moučky z listů estragonu, v dávce 30 g/kg krmiva, významně zvýšil Hct% a počet leukocytů. Kombinace 10 g/kg moučky z listů kopřivy a 10 g/kg moučky z listů estragonu se projevila nejvyššími hladinami erytrocytů a hemoglobinu. Moučka z listů kopřivy v dávce 30 g/kg krmiva, moučka z listů estragonu v množství 30 g/kg krmiva a kombinace moučky z listů kopřivy v dávce 10 g/kg a 10 g/kg moučky z listů estragonu v krmivu významně zvýšily hladiny albuminu v krevním séru. Moučka z listů estragonu v dávce 10 g/kg krmiva vykazovala nejvyšší aktivitu enzymu lysozymu v krevním séru ve srovnání s kontrolou. Hladiny celkového imunoglobulinu v krevním séru a celkového proteinu byly významně spojeny s použitím moučky z listů kopřivy v dávce 30 g/kg krmiva. Studie potvrzuje pozitivní účinky těchto aditiv na růstovou výkonnost, složení celého těla, imunologické, hematologické a vybrané biochemické faktory krevního séra.

V kapitole 4 je prezentována studie, která zjistila, že různé úrovně česnekového prášku významně neovlivnily rychlost růstu a somatické indexy u okouna říčního. Přidání 30 g/kg česnekového prášku v krmivu však vedlo k výrazně vyšší hladině bílkovin v těle ryb. Použití jak dávky 10 g/kg krmiva, tak 30 g/kg krmného česnekového prášku se projevilo ve významně vyšší stravitelnosti tuku v porovnání s kontrolní skupinou a 20 g/kg krmivového česnekového prášku. Dieta obsahující 20 g/kg česnekového prášku vedla k významně vyššímu počtu erytrocytů a leukocytů. Kromě toho měly všechny skupiny s přídavkem česnekuvýznamně nižší hladiny cholesterolu v krevním séru ve srovnání s kontrolou.

Česnekový prášek zlepšil odolnost okouna říčního proti stresu z vysoké hustoty a manipulace, jakož to i jeho hematologické a imunologické parametry. Bezprostředně po stresu byla hladina kortizolu ve všech skupinách krmených česnekem významně vyšší než v kontrolní skupině a mezi skupinami nebyl žádný významný rozdíl v hladinách glukózy. Po 24 hodinách však byly hladiny kortizolu i glukózy významně nižší ve všech skupinách krmených česnekem ve srovnání s kontrolní skupinou. Tyto výsledky naznačují, že krmení okouna říčního dietou s česnekovým práškem může mít pozitivní vliv na zvládání stresové reakce. Česnekový prášek v množství 10 g/kg krmiva zlepšil stravitelnost tuků, obsah proteinu v těle ryb, hematologické parametry, imunologické parametry a odolnost při vysoké hustotě a manipulaci. Celkově lze česnekový prášek považovat za potenciální doplněk stravy pro zvládání stresu u okouna evropského, je však zapotřebí dalšího výzkumu, aby se určilo optimální dávkování a dlouhodobé účinky.

Výsledky disertační práce naznačují, že používání přírodních krmných fytoaditiv by mohlo být potenciálním řešením pro řešení zdravotních problémů v akvakultuře, což povede k udržitelnější a efektivnější produkci potravin.

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تقدیم به ایران

جلوه ها کردم و نشناخت مرا اهل دلی / منم آن سوسن وحشی که به ویرانه دمید

«رهی معیری»

List of publications

Peer-reviewed journals with IF

Bagheri, D., Moradi, R., **Zare, M.,** Sotoudeh, E., Hoseinifar, S.H., Oujifard, A., Esmaeili, N., 2023. Does dietary sodium alginate with low molecular weight affect growth, antioxidant system, and haemolymph parameters and alleviate cadmium stress in white leg shrimp (*Litopenaeus vannamei*)? Animals 13, 1805. (IF 2022 = 3.0; AIS 2022 = 0.498)

Fanizza, C., Trocino, A., Stejskal, V., Dvořáková Prokešová, M., **Zare, M.,** Tran, H.Q., Brambilla, F., Xiccato, G., Bordignon, F., 2023. Practical low-fishmeal diets for rainbow trout (*Oncorhynchus mykiss*) reared in RAS: effects of protein meals on fish growth, nutrient digestibility, feed physical quality, and faecal particle size. Aquaculture Reports 28, 101435 (IF 2022 = 3.7, AIS 2022 = 0.560)

Hosseini, H., Esmaeili, M., Sepehr, A., **Zare, M.,** Rombenso, A., Badierah, R., Redwan, M.E., 2023. Does supplement laying hen diets with a herb mixture mitigate the negative impacts of excessive inclusion of extruded flaxseed? Animal Bioscience 36, 629–641. (IF 2022 = 2.2, AIS 2022 = 0.360)

Rashidian, Gh., **Zare, M.,** Hamidreza Tabibi, H., Stejskal, V., Caterina Faggio, C., 2023. The synergistic effects of four medicinal plant seeds and chelated minerals on the growth, immunity, and antioxidant capacity of rainbow trout (*Oncorhynchus mykiss*). Fish and Shellfish Immunology 139, 108930. (IF 2022 = 4.7, AIS 2022 = 0.605)

Stejskal, V., Tran, H.Q., Prokesová, **M., Zare,** M., Gebauer, T., Policar, T., Caimi, C., Gai, F., Gasco, L., 2023. Defatted black soldier fly (*Hermetia illucens*) in pikeperch (*Sander lucioperca*) diets: Effects on growth performance, nutrient digestibility, fillet quality, economic and environmental sustainability. Animal Nutrition. 12, 7–19. (IF 2022 = 6.3, AIS 2022 = 1.043)

Zare, M., Esmaeili, N., Paolacci, S., Stejskal, V., 2023. Nettle (*Urtica dioica*) additive as a growth promoter and immune stimulator in fish. Aquaculture Nutrition 2023, 8261473. (IF 2022 = 3.5, AIS 2022 = 0.497)

Zare, M., Heidari, E., Hosseini Choupani, S.M., Akhavan, S.R., Rombenso, A., Esmaeili, N., 2023. The recovery time between early mild stress and final acute stress affects survival rate, growth, immunity, health physiology, and stress response of Oscar (*Astronotus ocellatus*). Animals 13, 1606. (IF 2022 = 3.0, AIS 2022 = 0.498)

Zare, M., Kazempour, M., Hosseini, H., Hosseini Choupani, S.M., Akhavan, S.R., Rombenso, A., Esmaeili, N., 2023. Fish meal replacement and early mild stress improve stress responsiveness and survival of fish after acute stress. Animals 13, 1314. (IF 2022 = 3.0, AIS 2022 = 0.498)

Esmaeili, M., Hosseini, H., **Zare, M.,** Akhavan, S.R., Rombenso, A., 2022. "Early Mild Stress along with Lipid Improves the Stress Responsiveness of Oscar (*Astronotus ocellatus*). Aquaculture Nutrition 2022, 8991678. (IF 2021 = 3.8, AIS 2021 = 0.508)

Hosseini, H., Esmaeili, M., **Zare, M.,** Rombenso, A., 2022. Egg enrichment with n-3 fatty acids in farmed hens in sub-optimum temperature: A cold-temperament additive mix alleviates adverse effects of stress on performance and health. Journal of Animal Physiology and Animal Nutrition 106, 1333–1344. (IF 2021 = 2.7, AIS 2021 = 0.402)

Mahboub, H.H., Rashidian, G., Hoseinifar, S.H., Kamel, S., **Zare, M.,** Ghafarifarsani, H., Algharib, S.A., Moonmanee, T., Van Doan, H., 2022. Protective effects of *Allium hirtifolium* extract against foodborne toxicity of Zinc oxide nanoparticles in Common carp (*Cyprinus carpio*). Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 109345. (IF 2021 = 4.5, AIS 2021 = 0.594))

Rashidian, G., Mahboub, H.H., Hoseinfar, S.H., Ghafarifarsani, H., **Zare, M.,** Punyatong, M., Van Doan, H., 2022. *Allium hirtifolium* protects *Cyprinus carpio* against the detrimental responses mediated by foodborne zinc oxide nanoparticle. Aquaculture 555, 738252. (IF 2021 = 5.1, AIS 2021 = 0.635)

Prokešová, M., Bušová, M., **Zare, M.,** Tran, H.Q., Kučerová, E., Ivanova, A.P., Gebauer, T., Stejskal, V., 2021. Effect of humic substances as feed additive on the growth performance, antioxidant status, and health condition of African catfish (*Clarias gariepinus*, Burchell 1822). Animals 11, 2266. (IF 2020 = 2.8, AIS 2020 = 0.458)

Tran, HQ., Prokešová, M., **Zare, M.,** Gebauer, T., Elia, AC., Colombino, E., Ferrocino, I., Caimi, C., Gai, F., Gasco, L., Stejskal, V., 2021. How does pikeperch *Sander lucioperca* respond to dietary insect meal *Hermetia illucens*? investigation on gut microbiota, histomorphology, and antioxidant biomarkers. Frontiers in Marine Science 8, 680942. (IF 2020 = 4.9, AIS 2020 = 1.368)

Tran, HQ., Prokešová, M., **Zare, M.,** Gebauer, T., Matoušek, J., Ferrocino, I., Gasco, L., Stejskal, V., 2021. Production performance, nutrient digestibility, serum biochemistry, fillet composition, intestinal microbiota and environmental impacts of European perch (*Perca fluviatilis*) fed defatted mealworm (*Tenebrio molitor*). Aquaculture. 547, 2022, 737499. (IF 2020 = 4.2, AIS 2020 = 0.663)

Zare, M., Mirzakhani, M.K., Stejskal, V., 2021. Growth, body proximate composition and selected blood parameters of rainbow trout *Oncorhynchus mykiss* fingerlings fed on a diet supplemented with nettle *Urtica dioica* and tarragon *Artemisia dracunculus*. Aquaculture Research 52, 5691–5702. (IF 2020 = 2.1, AIS 2020 = 0.356)

Zare, M., Tran, H.Q., Prokešová, M., Stejskal, V., 2021. Effects of Garlic *Allium sativum* Powder on nutrient digestibility, haematology, and immune and stress responses in Eurasian perch *Perca fluviatilis* juveniles. Animals 11, 2735. (IF 2020 = 2.8, AIS 2020 = 0.458)

Peer reviewed journals without IF

Zare, M., Khodabandeh, S., Monsef, F., Voshtani, S.S., 2019. Histological and sodium-potassium pump immunolocalization in the intestine of the shrimp *Litopenaeus vannamei*. Journal of Agricultural Science and Technology. 8, 91–97.

International conferences

Zare, M., Tran, H.Q., Prokešová, M., Stejskal, V., 2021. Effects of garlic *Allium sativum* powder on crowding stress response in Eurasian perch *Perca fluviatilis* juveniles. Aquaculture Europe 2022, 27–30 September 2022, Rimini, Italy. (Oral presentation)

Zare, M., Tran, H.Q., Prokešová, M., Stejskal, V., 2021. Effects of garlic *Allium sativum* powder on nutrient digestibility, haematology, and immune and stress responses in Eurasian perch *Perca fluviatilis juveniles*. Aquaculture Europe 2021, Oceans of opportunity, 4–7 October 2021, Madeira, Funchal, Portugal. (Poster presentation)

Prokesova, M., Busova, M., Korytar, T., **Zare, M.,** Tran Quang H., Stejskal V., 2019. Effects of humic substance on growth performance and health status of juvenile *Clarias gariepinus* (Burchell, 1822). In: Book of abstracts. Aquaculture Europe 2019, October 7–10th, Berlin, Germany, pp. 1232–1233. (Poster presentation)

Zare, M., Mirzakhani, M.K., 2017. The Effects of tarragon leaf powder diet (*Artemisia dracunculus*) on growth, body composition and some blood parameters in fingerling rainbow trout *Oncorhynchus mykiss* / ICFAEST 2017: 19th International Conference on Fisheries, Aquaculture Economics and Seafood Trade. Prague, Czech Republic, March, 23-24. (Poster presentation).

Zare, M., Mirzakhani, M. K., 2017. The effects of *Urtica dioica* and *Artemisia dracunculus* Leaf powder on growth, carcass composition and some blood parameters on fingerling rainbow trout (*Oncorhynchus mykiss*) / In: Book of abstracts. 6th International Conference on Healthcare, Environment, Food and Biological Sciences (HEFBS-2017), Istanbul, Turkey, September, 8–10, 2017, p. 105. (Poster presentation).

Zare, M., Mirzakhani, M.K., 2016. The effects of nettle leaf powder diet *Urtica dioica* on growth, carcass composition and some blood parameters in fingerling rainbow trout (*Oncorhynchus mykiss*) / International symposium on fisheries and aquatic sciences, Antalya, Turkey, November 03–05, 2016. (Poster presentation).

Zare, M., Mirzakhani, M.K., 2014. The effect of probiotic (Protexin) and prebiotic (Manan oligosaccharide) as a synbiotic on growth performance, carcass composition and some blood parameters in fingerlings Rainbow trout *Oncorhynchus mykiss* / In: Book of abstracts. International symposium on fisheries and aquatic sciences, Trabzon, Turkey, September 25–27, 2014, p. 314. (Poster presentation).

Zare, M., Khodabandeh, S., Monsef, F., Voshtani, S.S., 2013. Structure and Na^+, K-ATPase immunolocalization in the midgut and hindgut of the shrimp *Litopenaeus Vannamei* / The society for experimental biology (SEB), Valencia, Spain, July 3–6, 2013. (Poster presentation).

National conferences

Zare, M., Khodabandeh, S., Monsef, F., Voshtani, S.S., 2014. The histological of Rectalpad area of intestine in the pacific white shrimp (*Litopenaeus vannamei*) / The 2nd international conference on the persian gulf oceanography and the 10th conference of Iranian marine science and technology, Tehran, Iran, February 18–19. (Poster presentation).

Zare, M., Khodabandeh, S., Monsef, F., Voshtani, S.S., 2014. The effects of prebiotic Mannan Oligosaccharide and probiotic Protexin on the ion regulatory function of intestine in the shrimp *Litopenaeus vannamei* / The First national conference on aquaculture, challenges and opportunities, Gorgan, Iran, October 22–23. (Poster presentation).

Zare, M., Khodabandeh, S., Monsef, F., Voshtani, S.S., 2013 The histological examination of intestine in the *Litopenaeus vannamei* / The First national conference on aquatics histology, Khorramshar, Iran, April 11, 2013. (Poster presentation).

Training and supervision plan during study

Name	Mahyar Zare
Research Department	Laboratory of Controlled Reproduction and Intensive Fish Culture of FFPW
Supervisor	Assoc. Prof. Vlastimil Stejskal
Period	27th November 2018 until 14th September 2023

Ph.D. courses	**Year**
Basic scientific communication	2019
Pond aquaculture	2019
Applied hydrobiology	2020
Ichthyology and fish taxonomy	2019
Biostatistics	2020
English language	2023

Scientific seminars	**Year**
Seminar days of FFPW	2019 2020 2021 2022

International conferences	**Year**
Zare, M., Tran, H.Q., Prokešová, M., Stejskal, V., 2021. Effects of garlic *Allium sativum* powder on crowding stress response in Eurasian perch *Perca fluviatilis* juveniles. Aquaculture Europe 2022, 27–30 September 2022, Rimini, Italy. (Oral presentation)	2022
Zare, M., Tran, H.Q., Prokešová, M., Stejskal, V., 2021. Effects of garlic *Allium sativum* powder on nutrient digestibility, haematology, and immune and stress responses in Eurasian perch *Perca fluviatilis* juveniles. Aquaculture Europe 2021, Oceans of opportunity, 4–7 October 2021, Madeira, Funchal, Portugal. (Poster presentation)	2021
Prokesova, M., Busova, M., Korytar, T., **Zare, M.,** Tran Quang H., Stejskal V., 2019. Effects of humic substances on growth performance and health status of juvenile *Clarias gariepinus* (Burchell, 1822). In: Book of abstracts. Aquaculture Europe 2019, October 7–10th, Berlin, Germany, pp. 1232–1233. (Poster presentation)	2019

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Associate professor, Dr. Ilario Ferrocino, Ph.D., Department of Agricultural, Forest, and Food Sciences, University of Turin, Italy. The analysis of the digesta microbiota of rainbow trout and poultry. 16th October to 14th December 2022 (60 days)	2022
Senior researcher Dr. Ardó László Ph.D., The Hungarian University of Agriculture and Life Sciences (MATE), Research Center for Fisheries and Aquaculture (HAKI), Szarvas, Hungary. Determination of digestive and antioxidant enzyme activities, phagocytic activity measurement, growth, and immune-related gene expression. 15th February to 30th June 2023 (136 days)	2023

Pedagogical activities	**Year**
Excursion to visit several innovative fish farms and aquaculture facilities in Czech Republic, Germany, and Poland.	2019
A workshop on haematology and fish blood sampling was held for a group of Yspertal school students from Austria.	2019
Leading the proximate composition analysis (Dry matter, fat, protein, and minerals) of feed and European perch for two summer school students from Austria.	2020

Curriculum vitae

PERSONAL INFORMATION

Name: Mahyar
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EDUCATION

2018 – present Ph.D. student in Fishery, Faculty of Fisheries and Protection of Waters, University of South Bohemia, Ceske Budejovice, Czech Republic
2011–2014 M.Sc., Master of Science, Natural Resource Engineering – Fisheries, Khazar Higher Education Institute, Mahmoud Abad, Mazandaran, Iran
2005–2010 B.Sc., Bachelor of Science, Natural Resource Engineering – Fisheries, Lahijan Branch of the Azad University, Lahijan, Guilan, Iran
2001–2005 Diploma, Rezvan high school, Rezvanshahr, Guilan, Iran

COMPLETED COURSES

04/03–08/03 2019 Basics of scientific communication, Faculty of Fisheries and Protection of Waters, University of South Bohemia, Vodnany, Czech Republic.

RESEARCH STAY AND COLLABORATIONS

16/10–14/12 2022 Associate professor, Dr. Ilario Ferrocino, PhD, Department of Agricultural, Forest, and Food Sciences, University of Turin, Italy. The analysis of the digesta microbiota of rainbow trout and poultry. 16th October to 14th December 2022

15/02–30/06/2023 Senior researcher Dr. Ardó László Ph.D., The Hungarian University of Agriculture and Life Sciences (MATE), Research Center for Fisheries and Aquaculture (HAKI), Szarvas, Hungary. Determination of digestive and antioxidant enzyme activities, phagocytic activity measurement, growth, and immune-related gene expression. 15th February to 30th June 2023