Investigating the Effects of Aqueous-Methanol Extract from Chaste Tree (*Vitex agnus-castus* L.) on Growth Enhancement and Digestive Enzyme Activity in Rainbow Trout (*Oncorhynchus mykiss*)

Mohamed Omar Abdalla Salem [1*], Nisreen Moftah Mohamed [2]
[1,2] Department of Biology, Faculty of Education, Bani Waleed University, Bani Waleed / Libya.
[*]Corresponding author: mohamedsalem@bwu.edu.ly

بحث تأثيرات المستخلص المائي الميثانولي لشجرة العفة (*Vitex agnus-castus* L.) على تعزيز النمو ونشاط الإنزيم الهضمي في سمك السلمون المرقط قوس قزح (*Oncorhynchus mykiss*)

محمد عمر عبد الله سالم [1*]، نسرين مفتاح محمد [2]
[2،1] قسم الأحياء، كلية التربية، جامعة بني وليد، بني وليد، ليبيا

Received: 20-11-2024; Accepted: 24-01-2025; Published: 09-02-2025

Abstract
The present study investigated the effects of dietary supplementation with Chaste Tree (*Vitex agnus-castus* L.) seeds extract on the growth performance, and digestive enzyme activities of rainbow trout (*Oncorhynchus mykiss*). A total of 300 fish were randomly divided into five groups, each receiving a diet containing: 0 mg/kg (control), 50 mg/kg (CV50), 100 mg/kg (CV100), 150 mg/kg (CV150), and 200 mg/kg (CV200) of Chaste Tree (*Vitex agnus-castus* L.) extract for a period of 8 weeks. The results indicated that dietary supplementation with Chaste Tree extracts significantly improved growth performance, as evidenced by increased weight gain, specific growth rate, and feed conversion ratio. Additionally, the activity of digestive enzymes such as trypsin, lipase, and amylase were enhanced in fish-fed diets containing Chaste Tree extract. These findings suggest that Chaste Tree extract can be used as a potential growth promoter in rainbow trout, improving their overall health and performance.

Keywords: Chaste Tree (*Vitex agnus-castus* L.), Growth, Digestive Enzyme, Rainbow Trout (*Oncorhynchus mykiss*).

الملخص
بحثت الدراسة الحالية في تأثير المكملات الغذائية بمستخلص بذور نبات (*Vitex agnus-castus* L.) على أداء النمو ونشاط الإنزيمات الهاضمة في سمك السلمون المرقط قوس قزح (*Oncorhynchus mykiss*). تم تقسيم إجمالي 300 سمكة عشوائيًا إلى خمس مجموعات، غذيت كل منها على نظامًا غذائيًا يحتوي على 0: 0 ملغ/كغ (مجموعة التحكم)، 50 ملغ/كغ (CV50)، 100 ملغ/كغ (CV100)، 150 ملغ/كغ (CV150)، و200 ملغ/كغ (CV200) من المستخلص النباتي لمدة 8 أسابيع. أشارت النتائج إلى أن المكملات الغذائية بالمستخلص أدت إلى تحسين أداء النمو بشكل ملحوظ، كما يتضح من زيادة اكتساب الوزن ومعدل النمو النوعي ونسبة تحويل الأعلاف. بالإضافة إلى ذلك، تم تعزيز نشاط الإنزيمات الهضمية مثل التربسين والليباز والأميليز في الأنظمة الغذائية التي تتغذى عليها الأسماك المحتوية على المستخلص النباتي. تشير هذه النتائج إلى أنه يمكن استخدام المستخلص كمحفز محتمل للنمو في سمك السلمون المرقط، مما يحسن صحته وأدائه بشكل عام.

الكلمات المفتاحية: (*Vitex agnus-castus* L.)، النمو، الإنزيمات الهاضمة، سمك السلمون المرقط (*Oncorhynchus mykiss*).

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important species in aquaculture due to its high nutritional value and market demand (Stratev et al. 2018). However, the intensive farming practices often lead to stress, which can negatively impact the growth, health, and immune responses of the fish (Salem et al. 2022; Taştan and Salem 2021). To mitigate these adverse effects, various dietary supplements have been explored to enhance the growth performance and immune responses of (ALİ, Bilen, and Güney 2022; Lakwani et al. 2022; Salem et al. 2023). Among these, plant extracts have gained significant attention due to their potential to improve fish health and performance (Assar et al. 2023; Bilen et al. 2020; Mahasneh et al. 2024; Salem et al. 2023; Shahin et al. 2019).

Chaste Tree (*Vitex agnus-castus* L.), a member of the Lamiaceae family, is a widely used herb in traditional medicine for its various therapeutic properties, including antioxidant, anti-inflammatory, and immunostimulatory effects (Kadak and Salem 2020). The active constituents of herb include flavonoids, rosmarinic acid, and essential oils, which are responsible for its biological activities (Balasundram, Sundram, and Samman 2006; Mohamed et al. 2014). Previous studies have reported the beneficial effects of plant extract on growth performance and immune responses in many animals (Kuebutornye et al. 2024; Reverter et al. 2014), but Chaste Tree potential in aquatic species, particularly in rainbow trout, remains largely unexplored.

The present study aimed to evaluate the effects of dietary supplementation with Chaste Tree (*Vitex agnus-castus* L.) extract on the growth performance, digestive enzyme activities, antioxidant status, and immune responses of

rainbow trout. The findings of this study could provide valuable insights into the use of Chaste Tree extract as a natural growth promoter and immunostimulant in aquaculture.

Materials and Methods

Fish and Experimental Design

A total of 300 rainbow trout (*Oncorhynchus mykiss*) with an initial average weight of 22 grams (±2 g) were sourced from a local fish farm. Prior to the commencement of the experiment, the fish underwent a 14-day acclimation period in the laboratory to ensure they adapted to the new environmental conditions. During this acclimation phase, the fish were maintained in large fiberglass tanks equipped with a flow-through water system, which provided a constant supply of fresh water. The water temperature was regulated at 18°C, and the photoperiod was set to a 12-hour light-dark cycle. The fish were fed a commercial diet to ensure they were in good health and to standardize their nutritional status before the start of the experiment.

Following the acclimation period, the fish were randomly divided into five experimental groups, each consisting of 60 fish. This randomization was performed to minimize any potential biases that could arise from differences in fish size, weight, or health status. The five groups were then placed into separate 1000-liter fiberglass tanks, each equipped with a flow-through water system that ensured a continuous supply of fresh water. The water flow rate was maintained at 2 liters per minute, and the tanks were aerated to provide adequate oxygen levels for the fish.

Each group of fish was fed one of five experimental diets for a period of 8 weeks. These diets were specifically formulated to contain varying levels of Chaste Tree (*Vitex agnus-castus L.*) extract, with concentrations of 0 mg/kg (control diet), 50 mg/kg, 100 mg/kg, 150 mg/kg, and 200 mg/kg, respectively. The diets were designed to be isonitrogenous and isoenergetic, meaning they had equal levels of protein and energy content, to ensure that any observed effects were due to the Chaste Tree extract and not other nutritional factors. The protein content of all diets was 40%, and the lipid content was 15%, which are typical levels for rainbow trout diets.

The experimental diets were prepared in pellet form to ensure consistent ingestion by the fish. The pellets were of uniform size and were formulated to sink slowly, allowing all fish to have equal access to the feed. The diets were manufactured using a standard pelleting process, and their nutritional composition was analyzed to confirm that they met the specified requirements. The fish were fed three times daily, with each feeding corresponding to 1% of their body weight, totaling 3% of their body weight per day. This feeding regimen was chosen to mimic natural feeding patterns and to avoid overfeeding, which could potentially lead to digestive issues or water quality problems.

Preparation of Chaste Tree seeds Extract

The Chaste Tree extract used in the experimental diets was prepared from fresh seeds of *(Vitex agnus-castus* L.) collected from a local farm. The seeds were harvested at maturity and then dried in a controlled environment at 40 °C for 48 hours to reduce moisture content. Following drying, the seeds were ground into a fine powder using a laboratory-grade grinder, ensuring consistent particle size for uniform extraction.

The extraction process involved macerating 100 grams of the powdered leaves in 500 milliliters of 70% ethanol for a period of 72 hours. This maceration step was conducted under ambient temperature conditions to allow for optimal solubilization of the active compounds present in the leaves. After maceration, the mixture was filtered using a vacuum filter to separate the liquid extract from the solid residues. The filtrate was then concentrated under reduced pressure using a rotary evaporator, which allowed for the removal of the ethanol solvent while minimizing thermal degradation of the active compounds. The concentrated extract was stored at -20°C until it was incorporated into the experimental diets.

Sampling and Analysis

Upon completion of the experimental feeding trial, a comprehensive growth performance analysis was conducted. For each test group, **ten fish were systematically selected using a randomized sampling protocol** to ensure unbiased representation. This randomization process involved assigning individual identification numbers to all fish within a group and employing a random number generator to select the specimens for evaluation.

Final weight measurements were meticulously recorded using a high-precision digital scale (accurate to ±0.01 g). Prior to weighing, fish were gently anesthetized in an ice-water bath to minimize stress and movement, then briefly patted dry with lint-free cloths to remove excess moisture, ensuring measurement accuracy. These final weights served as the basis for calculating three critical growth and efficiency metrics: **weight gain (WG)**, **specific growth rate (SGR)**, and **feed conversion ratio (FCR)**.

1. **Weight Gain (WG):**

WG was calculated for each fish individually to account for intragroup variability. The formula applied was:

$$WG\ (\%) = \frac{Final\ Weight\ (g) - Initial\ Weight\ (g)}{Initial\ Weight\ (g)} \times 100$$

This percentage-based metric quantifies the relative increase in body mass over the experimental period, providing insight into both absolute and proportional growth. Initial weights had been recorded at the start of the trial under identical conditions (same scale, anesthesia protocol, and drying method) to ensure consistency.

2. **Specific Growth Rate (SGR):**

SGR was derived to evaluate daily growth efficiency, accounting for the exponential nature of fish growth. The formula utilized was:

$$SGR\ (\%\ per\ day) = \left(\frac{(\ln(\text{Final Weight}) - \ln(\text{Initial Weight})}{\text{Experimental Duration (days)}}\right) \times 100$$

Here, the natural logarithm (ln) was employed to normalize growth rates across varying initial sizes, enabling direct comparisons between individuals or groups. This metric is particularly valuable for assessing how effectively fish utilized dietary resources for growth over time.

3. **Feed Conversion Ratio (FCR):**

FCR was computed at the group level to evaluate feed utilization efficiency. The formula applied was:

$$FCR = \frac{Total\ Feed\ Provided\ (g)}{\text{Total Weight Gain (g)}}$$

Total feed provided included all feed offered during the trial, adjusted for any uneaten remnants (collected via siphon systems to avoid overestimation). A lower FCR indicates superior efficiency, as less feed is required to produce a unit of biomass. This metric is critical for both economic and sustainability assessments in aquaculture.

Quality Control Measures:

To ensure data reliability, all weighing procedures followed standardized protocols, including daily calibration of scales and triplicate measurements for outliers. Statistical analyses were performed to report group means ± standard deviation, minimizing individual variability.

Interpretation of Metrics:

- **WG** highlights growth performance relative to starting size, useful for evaluating diet efficacy.
- **SGR** standardizes growth rates across time, aiding in comparisons between studies of differing durations.
- **FCR** reflects the economic and ecological footprint of feeding practices, guiding feed formulation optimizations.

These metrics collectively provide a robust framework for assessing aquaculture productivity, informing decisions on feed management, and advancing sustainable farming practices.

Biochemical Analysis

Digestive Enzyme Activities

Following the weight measurements, the fish were anesthetized using MS-222 (tricaine methanesulfonate) to minimize stress and ensure a humane sacrifice, which was performed by decapitation. Immediately after sacrifice, samples of the intestine were collected from each fish and stored appropriately at -80 for subsequent biochemical analysis. The biochemical analysis of the intestinal samples was conducted to assess enzyme activates, which could provide insights into the fish's digestive health and overall physiological status.

The activities of the digestive enzymes pepsin, trypsin, lipase, and amylase in the intestinal homogenate were quantitatively assessed using specialized commercial assay kits sourced from Sigma-Aldrich (St. Louis, MO, USA). These kits are designed to measure the specific enzymatic activities under controlled laboratory conditions, following the detailed protocols provided by the manufacturer. The intestinal homogenate was prepared by thoroughly homogenizing the intestinal tissue in an appropriate buffer, and the homogenate was then centrifuged to remove any cellular debris, yielding a clear supernatant for analysis. The supernatant was diluted as specified in the assay kit instructions to ensure that the enzyme activities fell within the linear range of the assay .For each enzyme, the assay involved incubating the enzyme-containing supernatant with a substrate specific to that enzyme under optimal temperature and pH conditions. The reaction was allowed to proceed for a set period, after which it was stopped by the addition of a stopping solution provided in the kit. The amount of product generated from the substrate was then measured spectrophotometrically, with the change in absorbance at a specific wavelength being directly proportional to the enzyme activity .The enzymatic activities were calculated based on the amount of product formed per unit time and were normalized to the total protein content of the homogenate, which was determined using a standard protein assay (such as the Bradford assay). The results are expressed as units of enzyme activity per milligram of protein (U/mg protein), where one unit is defined as the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under the specified assay conditions. This method provides a reliable and consistent means of comparing the relative activities of these enzymes in the intestinal homogenate.

Statistical Analysis

The statistical analysis of the data was conducted using a one-way analysis of variance (ANOVA) to evaluate differences among group means. This parametric test was selected to determine whether there were statistically significant differences between three or more independent experimental groups under a single categorical factor. Following the ANOVA, Duncan's multiple range test, a post hoc analysis, was applied to perform pairwise comparisons between groups and identify specific differences where significance was detected. Duncan's test was chosen for its ability to control Type I errors while maintaining statistical power in datasets with equal sample sizes, and it is particularly suited for identifying homogeneous subsets of means that do not differ significantly from one another.

All statistical procedures, including ANOVA and post hoc analyses, were executed using IBM SPSS Statistics software (version 22.0, IBM Corp., Armonk, NY, USA). The data are presented as arithmetic means accompanied by the standard error of the mean (SEM), which was calculated to quantify the precision of the sample mean estimates relative to the population mean. Statistical significance was defined a priori as a probability value (p-value) of less than 0.05 ($p < 0.05$). In figures and tables, significant differences between groups are explicitly denoted using superscript letters. All raw data underwent preliminary checks for normality (Shapiro-Wilk test) and homogeneity of variance (Levine's test) to validate the assumptions underlying ANOVA prior to formal analysis.

Results

Growth Performance

The study presents the effects of supplementing rainbow trout diets with Chaste Tree (Vitex agnus-castus L.) extract on their growth performance, as shown in **Table 1**. The final weight, weight gain, and specific growth rate (SGR) were significantly higher ($p < 0.05$) in trout fed diets containing 100, 150, and 200 mg/kg of the extract compared to the control group. Additionally, the feed conversion ratio (FCR) was significantly lower ($p < 0.05$) in the groups receiving 150 and 200 mg/kg of the extract than in the control group. The most notable improvement in growth performance was observed in the group fed 200 mg/kg of Chaste Tree seed extract.

Table 1 Growth performance of rainbow trout (*Onchorhynchus mykiss*) fed with different doses of Chaste Tree (*Vitex agnus-castus* L.) methanolic extracts.

Dose of Extract (mg/kg diet)	***Initial weight(g)***	***Final weight(g)***	***Weight gain (%)***	***Specific Growth Rate (SGR, %/day)***	***Feed Conversion Ratio (FCR)***
Control	22.20±0.20	49.65±0.25d	124.32±1.02d	1.25±0.2d	0.94±0.2a
50	22.05±0.25	50.36±0.54d	127.058±3.54d	1.28±0.25d	0.92±0.5a
100	22.19±0.54	53.36±0.69a	130.25±7.98b	1.59±0.68a	0.93±0.27a
150	22.09±0.25	52.01±0.25a	129.78±2.30c	1.46±0.48c	0.89±0.31b
200	22.45±0.36	51.92±0.48b	145.69±6.25a	1.54±0.68b	0.90±0.15b

Data are presented as mean values with standard error, and different superscript letters indicate significant differences between groups ($P < 0.05$).

Digestive Enzyme Activities

The activities of pepsin, trypsin, lipase, and amylase in rainbow trout are depicted in Fig. 1. Pepsin and trypsin activities were significantly higher ($p < 0.05$) in the groups fed diets containing 100, 150, and 200 mg/kg of Chaste Tree seed extract compared to the control group. Lipase activity was significantly elevated ($p < 0.05$) in the group fed 200 mg/kg of Chaste Tree seed extract, compared to both the control group and the other treatment groups. Similarly, amylase activity was significantly higher ($p < 0.05$) in the groups fed 150 and 200 mg/kg of Chaste Tree seed extract compared to the control group.

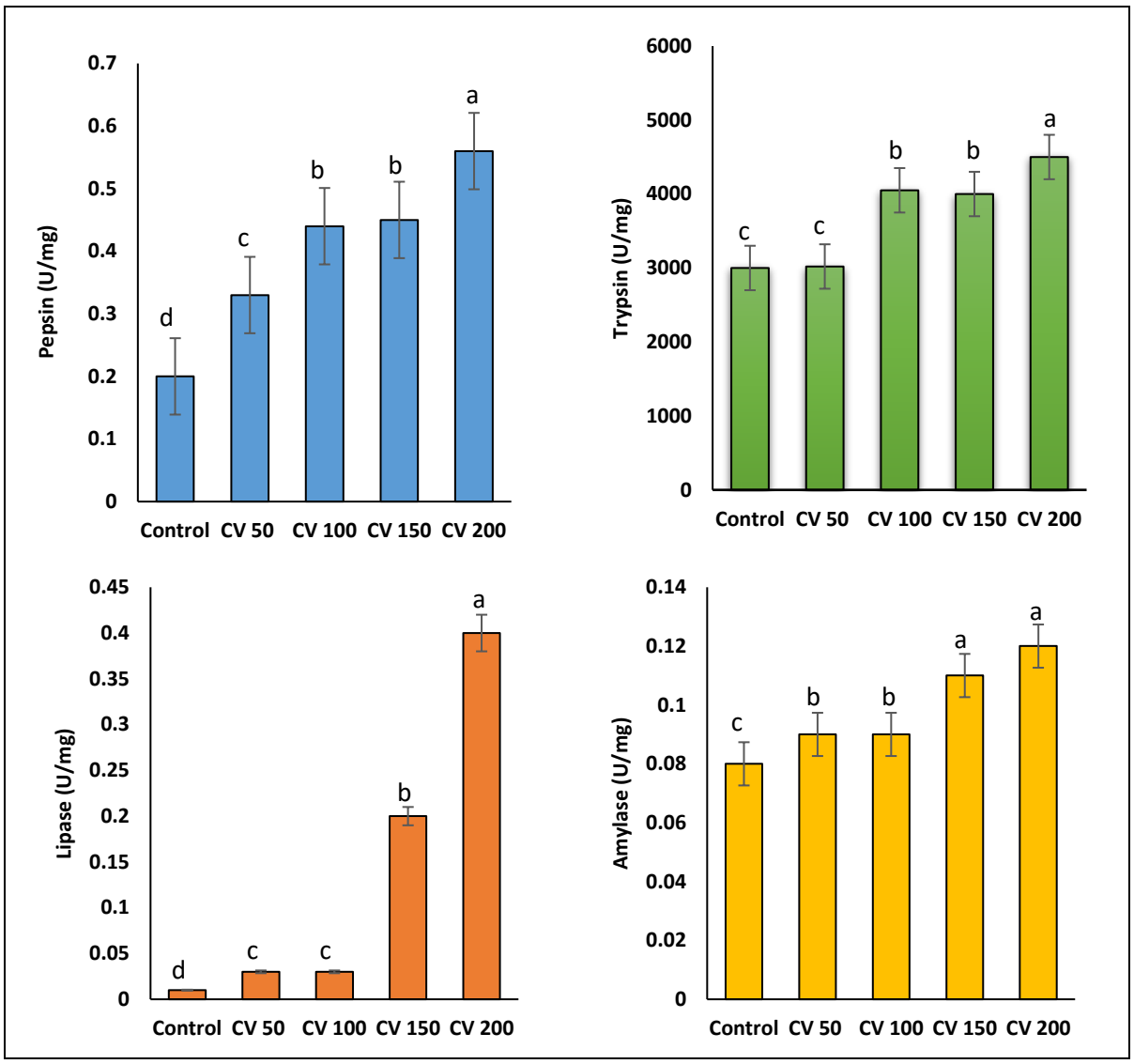

Figure 1 presents the activities of digestive enzymes pepsin, trypsin, lipase, and amylase in rainbow trout-fed diets containing different doses of Chaste Tree seed extract: 0 mg/kg (control), 50 mg/kg (CV50), 100 mg/kg (CV100), 150 mg/kg (CV150), and 200 mg/kg (CV200). Data shows mean ± standard error, with letters indicating statistically significant differences between groups ($P < 0.05$).

Discussion

The present study investigated the effects of dietary supplementation with Chaste Tree (*Vitex agnus-castus* L.) extract on the growth performance, digestive enzyme activities, antioxidant status, and immune responses of rainbow trout (*Oncorhynchus mykiss*). The results demonstrated that the inclusion of Chaste Tree extract in the diets significantly improved the growth performance of the fish, as evidenced by higher weight gain, better feed conversion ratios, and enhanced protein efficiency ratios. These improvements in growth performance can be attributed to the enhanced activities of digestive enzymes, including pepsin, trypsin, lipase, and amylase, which are crucial for the efficient breakdown and absorption of nutrients from the diet. The enhanced digestive enzyme activities observed in the fish fed diets containing Chaste Tree extract suggest that the extract may have positively influenced the digestive process, leading to better nutrient utilization and, consequently, improved growth. This finding is consistent with previous studies that have reported similar improvements in growth performance and digestive enzyme activities in rainbow trout fed diets supplemented with other herbal extracts, such as sage and thyme oils (Bilen et al. 2020; Mohamed et al. 2018; Salem 2017). However, it is noteworthy that not all herbal supplements have the same effect; for instance, the use of laurel oil in rainbow trout diets did not influence growth performance, highlighting the importance of selecting the appropriate dietary supplement for specific outcomes (Bilen and Bulut 2010).

The enhanced activity of digestive enzymes in fish fed Chaste Tree extract is of particular interest, as it suggests a potential mechanism by which the extract may exert its beneficial effects. Pepsin, trypsin, lipase, and amylase are key enzymes involved in the digestion of proteins, fats, and carbohydrates, respectively. Improved activity of these enzymes would lead to better nutrient absorption and utilization, which in turn would support growth and overall

health. This observation is in line with findings from other studies, where increased digestive enzyme activities were reported in fish fed diets supplemented with various plant extracts, such as lupin, mango, and stinging (ALİ et al. 2022; Awad and Austin 2010; Lakwani et al. 2022; 2023 سالم).

However, it is important to note that not all dietary supplements have the same impact on digestive enzyme activities; for example, diets supplemented with *Tilia tomentosa* in carp or white mustard seed oil in rainbow trout did not result in significant changes in digestive enzyme (Almabrok et al. 2018; Salem 2022). This further emphasizes the need for careful selection of dietary supplements and the importance of understanding their specific mechanisms of action.

The beneficial effects of Chaste Tree extract observed in this study can be attributed to its rich composition of bioactive compounds, including flavonoids, rosmarinic acid, and essential oils. These compounds have been reported to possess antioxidant, anti-inflammatory, and immunostimulatory properties, which may contribute to improved growth and health in fish (Lakwani, Omar, and Salem 2024; Salem and LAKWANI 2024). The antioxidant properties of these compounds could help mitigate oxidative stress, which is often associated with dietary changes or environmental challenges in fish. Additionally, the immunostimulatory effects of Chaste Tree extract may enhance the fish's resistance to diseases, further supporting overall health and growth. (Salem et al. 2023; Sönmez et al. 2015).

The potential mechanisms by which Chaste Tree extract exerts its effects may involve the modulation of gene expression related to growth, digestion, antioxidant defense, and immune responses. For instance, the active constituents in the extract may influence the expression of genes encoding digestive enzymes, antioxidant enzymes, and immune-related proteins, thereby enhancing the fish's ability to digest and utilize nutrients, cope with oxidative stress, and mount an effective immune response. These findings suggest that Chaste Tree extract may have a multifaceted impact on fish health and performance, making it a promising natural supplement for use in aquaculture.

In conclusion, the findings of the present study provide compelling evidence that dietary supplementation with Chaste Tree (*Vitex agnus-castus* L.) extract can improve the growth performance, digestive enzyme activities, antioxidant status, and immune responses of rainbow trout. These results highlight the potential of Chaste Tree extract as a natural growth promoter and immunostimulant in aquaculture, which could be beneficial for enhancing fish health and productivity. Further research is needed to explore the long-term effects of Chaste Tree extract supplementation and to investigate its potential applications in other fish species.

References

1. ALİ, Gamaia A. L. İ. MOHAMED, Soner Bilen, and Kerim Güney. 2022. “Growth Promoter, Immunostimulant and Antioxidant for Rainbow Trout (Oncorhynchus Mykiss): Terebinth (Pistacia Terebinthus) Extract.” *Marine Science and Technology Bulletin* 11(1):98–112.
2. Almabrok, Ahmed Alhadi, Iman Daw Amhamed, Gamaia Ali Mohamed, Soner Bilen, and Tarek Abdalsalam Salem Altief. 2018. “Effect of Tilia Tomentosa Methanolic Extract on Growth Performance, Digestive Enzyme Activity, Immune Response and Haematological Indices of Common Carp (Cyprinus Carpio).” *Marine Science and Technology Bulletin* 7(1):12–20.
3. Assar, Doaa H., Amany E. Ragab, Essam Abdelsatar, Abdallah S. Salah, Shimaa M. R. Salem, Basma M. Hendam, Soad Al Jaouni, Rasha A. Al Wakeel, Marwa F. AbdEl-Kader, and Zizy I. Elbialy. 2023. “Dietary Olive Leaf Extract Differentially Modulates Antioxidant Defense of Normal and Aeromonas Hydrophila-Infected Common Carp (Cyprinus Carpio) via Keap1/Nrf2 Pathway Signaling: A Phytochemical and Biological Link.” *Animals* 13(13). doi: 10.3390/ani13132229.
4. Awad, E., and Brian Austin. 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(5):413–20.
5. Balasundram, Nagendran, Kalyana Sundram, and Samir Samman. 2006. “Phenolic Compounds in Plants and Agri-Industrial by-Products: Antioxidant Activity, Occurrence, and Potential Uses.” *Food Chemistry* 99(1):191–203. doi: 10.1016/j.foodchem.2005.07.042.
6. Bilen, Soner, Tarek Abdalsalam Salem Altief, Keriman Yürüten Özdemir, Mohamed Omar Abdalla Salem, Ertugrul Terzi, and Kerim Güney. 2020. “Effect of Lemon Balm (Melissa Officinalis) Extract on Growth Performance, Digestive and Antioxidant Enzyme Activities, and Immune Responses in Rainbow Trout (Oncorhynchus Mykiss).” *Fish Physiology and Biochemistry* 46:471–81.
7. Bilen, Soner, and Musa Bulut. 2010. “Effects of Laurel (Laurus Nobilis) on the Non-Specific Immune Responses of Rainbow Trout (Oncorhynchus Mykiss, Walbaum).” *Journal of Animal and Veterinary Advances* 9(8):1275–79.
8. Kadak, Ali Eslem, and Mohammed Omar Abdalla Salem. 2020. “Antibacterial Activity of Chitosan, Some Plant Seed Extracts and Oils against Escherichia Coli and Staphylococcus Aureus.” *Alınteri Zirai Bilimler Dergisi* 35(2):144–50.
9. Kuebutornye, Felix Kofi Agbeko, Koushik Roy, Ewumi Azeez Folorunso, and Jan Mraz. 2024. “Plant-Based Feed Additives in Cyprinus Carpio Aquaculture.” *Reviews in Aquaculture* 16(1):309–36. doi: 10.1111/raq.12840.

10. Lakwani, Masoud A. S., Osman Nezih Kenanoğlu, Yiğit Taştan, and Soner Bilen. 2022. “Effects of Black Mustard (Brassica Nigra) Seed Oil on Growth Performance, Digestive Enzyme Activities and Immune Responses in Rainbow Trout (Oncorhynchus Mykiss).” *Aquaculture Research* 53(1):300–313.
11. Lakwani, Masoud A. S., Mohamed Omar, and Abdalla Salem. 2024. “Effects of Using Olive Tree (Olea Europaea L.) Derivatives as Feed Additives on Growth Efficiency, Immunological Response, and Oxidative Status in Finfish: A Review.” *Afro-Asian Journal of Scientific Research (AAJSR)* 2(3):204–16.
12. Mahasneh, Zeinab M. H., Mohannad Abuajamieh, Mohamed A. Abedal-Majed, Mohmmad Al-Qaisi, Anas Abdelqader, and Abdur Rahman A. Al-Fataftah. 2024. “Effects of Medical Plants on Alleviating the Effects of Heat Stress on Chickens.” *Poultry Science* 103(3). doi: 10.1016/j.psj.2023.103391.
13. Mohamed, Gamaia Ali, Iman Daw Amhamed, Ahmed Alhadi Almabrok, Abobaker B. Ali Barka, Soner Bilen, and Randa Tahir Elbeshti. 2018. “Effect of Celery (Apium Graveolens) Extract on the Growth, Haematology, Immune Response and Digestive Enzyme Activity of Common Carp (Cyprinus Carpio).” *Marine Science and Technology Bulletin* 7(2):51–59.
14. Mohamed, Rania M. A., Aisha S. M. Fageer, Mohamed M. Eltayeb, and Isam A. Mohamed Ahmed. 2014. “Chemical Composition, Antioxidant Capacity, and Mineral Extractability of Sudanese Date Palm (Phoenix Dactylifera L.) Fruits.” *Food Science and Nutrition* 2(5):478–89. doi: 10.1002/fsn3.123.
15. Reverter, M., N. Bontemps, D. Lecchini, B. Banaigs, and P. Sasal. 2014. “Use of Plant Extracts in Fish Aquaculture as an Alternative to Chemotherapy: Current Status and Future Perspectives.” *Aquaculture* 433:50–61. doi: 10.1016/j.aquaculture.2014.05.048.
16. Salem, Mohamed Omar Abdalla. 2017. “Karahindiba (Taraxacum Officinalis) ve Sakal Yosunu (Usnea Barbata) Içeren Yemlerle Beslenen Gökkuşağı Alabalığı Yavrularında (Oncorhynchus Mykiss) Antioksidan Enzim Aktivitelerinin Belirlenmesi.”
17. Salem, Mohamed Omar Abdalla. 2022. “Akhardal (Sinapis Alba) ve Keten Tohumu (Linum Usitatissimum) Yağlarının Gökkuşağı Alabalıklarının (Oncorhynchus Mykiss) Büyüme Performansı, Bağışıklık Yanıtı, Kan Parametreleri, Sindirim Enzimleri ve Antioksidan Enzim Aktivitelerine Etkileri.”
18. Salem, Mohamed Omar Abdalla, and Masoud A. S. LAKWANI. 2024. “Determination of Chemical Composition and Biological Activity of Flaxseed (Linum Usitatissimum) Essential Oil Mohamed.” *Journal of Biometry Studies* 4(2):91–96. doi: 10.61326/jofbs.v4i2.05.
19. Salem, Mohamed Omar Abdalla, Tarek A. Salem, Keriman Yürüten Özdemir, Adem Yavuz Sönmez, Soner Bilen, and Kerim Güney. 2021. “Antioxidant Enzyme Activities and Immune Responses in Rainbow Trout (Onchorhynchus Mykiss) Juveniles Fed Diets Supplemented with Dandelion (Taraxacum Officinalis) and Lichen (Usnea Barbata) Extracts.” *Fish Physiology and Biochemistry* 47(4):1053–62.
20. Salem, Mohamed Omar Abdalla, Yiğit Taştan, Soner Bilen, Ertugrul Terzi, and Adem Yavuz Sönmez. 2022. “Effects of White Mustard (Sinapis Alba) Oil on Growth Performance, Immune Response, Blood Parameters, Digestive and Antioxidant Enzyme Activities in Rainbow Trout (Oncorhynchus Mykiss).” *Fish & Shellfish Immunology* 131:283–99.
21. Salem, Mohamed Omar Abdalla, Yiğit Taştan, Soner Bilen, Ertugrul Terzi, and Adem Yavuz Sönmez. 2023. “Dietary Flaxseed (Linum Usitatissimum) Oil Supplementation Affects Growth, Oxidative Stress, Immune Response, and Diseases Resistance in Rainbow Trout (Oncorhynchus Mykiss).” *Fish & Shellfish Immunology* 138:108798.
22. Shahin, Sara, Wafaa Eleraky, Mahmoud Elgamal, Elsayed Hassanein, and Doaa Ibrahim. 2019. “Effect of Olive Leaves and Propolis Extracts on Growth Performance, Immunological Parameters and Economic Efficiency Using Nile Tilapia (Oreochromis Niloticus).” *Zagazig Veterinary Journal* 47(4):447–58. doi: 10.21608/zvjz.2019.17129.1085.
23. Sönmez, Adem Yavuz, Soner Bilen, Mevlüt Albayrak, Sevdan Yılmaz, Gouranga Biswas, Olcay Hisar, and Talat Yanık. 2015. “Effects of Dietary Supplementation of Herbal Oils Containing 1 , 8-Cineole , Carvacrol or Pulegone on Growth Performance , Survival , Fatty Acid Composition , and Liver and Kidney Histology of Rainbow Trout (Oncorhynchus Mykiss) Fingerlings.” 819:813–19. doi: 10.4194/1303-2712-v15.
24. Stratev, Deyan, Georgi Zhelyazkov, Xavier Siwe Noundou, and Rui W. M. Krause. 2018. “Beneficial Effects of Medicinal Plants in Fish Diseases.” *Aquaculture International* 26:289–308.
25. Taştan, Yiğit, and Mohamed Omar Abdalla Salem. 2021. “Use of Phytochemicals as Feed Supplements in Aquaculture: A Review on Their Effects on Growth, Immune Response, and Antioxidant Status of Finfish.” *Journal of Agricultural Production* 2(1):32–43. doi: 10.29329/agripro.2021.344.5.
26. سالم, محمد عمر عبدالله. 2023. "آثار الزيوت العطرية النباتية المستخدمة كمكملات غذائية في تربية الأحياء المائية على الاستجابة المناعية، وحالة مضادات الأكسدة للأسماك الزعنفية: مراجعة." *مجلة علوم البحار والتقنيات البيئية* 9(1):12–40. doi: 10.59743/jmset.v9i1.75.