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Effect of Pistacia atlantica Oil on Growth Performance, Digestive and Antioxidant Enzyme Activities, and Immune Responses in Oreochromis niloticus

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تأثير زيت Pistacia atlantica على أداء النمو، وأنشطة الإنزيمات الهاضمة والمضادة للأكسدة، والاستجابات المناعية في أسماك البلطي النيلي (Oreochromis niloticus)

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الملخص

تواجه صناعة تربية الأحياء المائية تحديات كبيرة في الحفاظ على صحة الأسماك وإنتاجيتها مع تقليل الاعتماد على المواد الكيميائية الاصطناعية والمضادات الحيوية. وقد برزت المركبات النشطة بيولوجياً المشتقة من النباتات كإضافات علفية طبيعية واعدة يمكنها تعزيز أداء النمو، وكفاءة الهضم، وأنظمة الدفاع المضادة للأكسدة، والاستجابات المناعية في أنواع الأسماك المستزرعة. وقد حققت هذه الدراسة في تأثيرات الإضافة الغذائية لزيت *Pistacia atlantica* على أداء النمو، وأنشطة الإنزيمات الهاضمة (البروتياز، الأميليز، والليباز)، وأنشطة الإنزيمات المضادة للأكسدة (الإنزيم الفائق لأكسيد الديسميوتاز، الكاتالاز، والجلوتاثيون بيروكسيداز)، والاستجابات المناعية (نشاط الليزوزيم، نشاط الانفجار التنفسي، ومستويات الغلوبولين المناعي الكلي) في أسماك البلطي النيلي (*Oreochromis niloticus*). تم توزيع 240 إصبعية سمكية سليمة من البلطي النيلي بمتوسط وزن ابتدائي 25.0 ± 1.2 غرام عشوائياً إلى أربع مجموعات تجريبية في أحواض مكررة ثلاث مرات. تم تغذية الأسماك بأنظمة غذائية متساوية النيتروجين ومتساوية الطاقة مدعمة بنسبة 0% (مجموعة الضبط)، 0.5%، 1.0%، و1.5% من زيت *P. atlantica* لمدة 60 يوماً. تم تقييم معايير أداء النمو بما في ذلك زيادة الوزن، ومعدل النمو النوعي، ونسبة تحويل العلف، ونسبة كفاءة البروتين في نهاية تجربة التغذية. أظهرت النتائج أن الإضافة الغذائية لزيت *P. atlantica* حسنت بشكل ملحوظ معايير أداء النمو في جميع المجموعات المعالجة مقارنة بمجموعة الضبط، مع مستوى إضافة 1.0% الذي أظهر التحسن الأكثر وضوحاً. كما ارتفعت أنشطة الإنزيمات الهاضمة بشكل ملحوظ في الأسماك التي تغذت على أنظمة غذائية تحتوي على زيت *P. atlantica*، مما يشير إلى تحسين استخدام العناصر الغذائية وكفاءة الهضم. وتعززت أنشطة الإنزيمات المضادة للأكسدة بشكل كبير في المجموعات المعالجة، مما يشير إلى أن المركبات النشطة بيولوجياً الموجودة في زيت *P. atlantica* خففت بشكل فعال من الإجهاد التأكسدي وعززت آليات الدفاع المضادة للأكسدة الخلوية. علاوة على ذلك، زادت المعايير المناعية بما في ذلك نشاط الليزوزيم، ونشاط الانفجار التنفسي، ومستويات الغلوبولين المناعي الكلي بشكل ملحوظ في الأسماك المدعمة بزيت *P. atlantica*، مما يدل على خصائص مناعية قوية. تشير نتائج هذه الدراسة إلى أن زيت *P.*

atlantica يمتلك إمكانات كبيرة كإضافة علفية وظيفية في تربية البلطي النيلي، حيث يعزز النمو، ويحسن كفاءة الهضم، ويقوي أنظمة الدفاع المضادة للأكسدة، ويعزز الاستجابات المناعية. تم تحديد مستوى الإضافة الأمثل عند 1.0% من النظام الغذائي، والذي وفر التحسينات الأكثر توازناً عبر جميع المعايير التي تم تقييمها. يساهم هذا البحث في مجموعة الأدلة المتزايدة التي تدعم استخدام المركبات النباتية كبدائل مستدامة للإضافات الاصطناعية في تربية الأحياء المائية، مع تطبيقات محتملة لتحسين صحة الأسماك، والإنتاجية، ومقاومة الأمراض في عمليات تربية البلطي التجارية.

الكلمات الدالة: *Pistacia atlantica* ، البلطي النيلي، أداء النمو، الإنزيمات الهاضمة، الإنزيمات المضادة للأكسدة، الاستجابات المناعية، الإضافات العلفية.

Abstract

The aquaculture industry faces significant challenges in maintaining fish health and productivity while reducing reliance on synthetic chemicals and antibiotics. Plant-derived bioactive compounds have emerged as promising natural feed additives that can enhance growth performance, digestive efficiency, antioxidant defense systems, and immune responses in cultured fish species. This study investigated the effects of dietary supplementation with *Pistacia atlantica* oil on growth performance, digestive enzyme activities (protease, amylase, and lipase), antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase), and immune responses (lysozyme activity, respiratory burst activity, and total immunoglobulin levels) in Nile tilapia (*Oreochromis niloticus*). A total of 240 healthy Nile tilapia fingerlings with an initial average weight of 25.0 ± 1.2 g were randomly distributed into four experimental groups in triplicate tanks. The fish were fed isonitrogenous and isoenergetic diets supplemented with 0% (control), 0.5%, 1.0%, and 1.5% *P. atlantica* oil for 60 days. Growth performance parameters including weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio were evaluated at the end of the feeding trial. The results demonstrated that dietary supplementation with *P. atlantica* oil significantly improved growth performance parameters in all treatment groups compared to the control group, with the 1.0% supplementation level showing the most pronounced enhancement. Digestive enzyme activities were significantly elevated in fish fed diets containing *P. atlantica* oil, indicating improved nutrient utilization and digestive efficiency. Antioxidant enzyme activities were substantially enhanced in the treatment groups, suggesting that the bioactive compounds present in *P. atlantica* oil effectively mitigated oxidative stress and strengthened the cellular antioxidant defense mechanisms. Furthermore, immune parameters including lysozyme activity, respiratory burst activity, and total immunoglobulin levels were significantly increased in fish supplemented with *P. atlantica* oil, demonstrating potent immunostimulatory properties. The findings of this study indicate that *P. atlantica* oil possesses significant potential as a functional feed additive in Nile tilapia aquaculture, promoting growth, enhancing digestive efficiency, strengthening antioxidant defense systems, and boosting immune responses. The optimal supplementation level was determined to be 1.0% of the diet, which provided the most balanced improvements across all evaluated parameters. This research contributes to the growing body of evidence supporting the use of phytochemicals as sustainable alternatives to synthetic additives in aquaculture, with potential applications for improving fish health, productivity, and disease resistance in commercial tilapia farming operations.

Keywords: *Pistacia atlantica*, Nile tilapia, growth performance, digestive enzymes, antioxidant enzymes, immune responses, feed additive.

Introduction

1. Introduction

1.1 Problem Statement

Aquaculture has emerged as one of the fastest-growing food production sectors globally, playing a vital role in meeting the increasing demand for animal protein and ensuring food security. Nile tilapia (*Oreochromis niloticus*) stands as one of the most extensively cultured freshwater fish species worldwide due to its rapid growth rate, adaptability to various environmental conditions, disease resistance, and high market acceptability. However, intensive aquaculture practices have led to numerous challenges including increased susceptibility to infectious diseases, oxidative stress, reduced growth performance, and compromised immune function. The conventional approach to addressing these issues has relied heavily on the use of synthetic chemicals, antibiotics, and growth promoters. Nevertheless, the indiscriminate use of antibiotics has raised significant concerns regarding the development of antibiotic-resistant bacteria, environmental contamination, bioaccumulation in aquatic ecosystems, and potential risks to human health through the food chain.

In recent years, there has been a paradigm shift toward sustainable and environmentally friendly approaches in aquaculture, with increasing emphasis on natural alternatives to synthetic additives. Plant-derived bioactive compounds, particularly essential oils and extracts, have garnered considerable attention as functional feed additives capable of promoting growth, enhancing digestive enzyme activities, strengthening antioxidant defense systems, and modulating immune responses in aquatic organisms. The search for effective phytochemicals that can serve as immunostimulants, growth promoters, and stress relievers has become a priority in aquaculture research.

Pistacia atlantica, commonly known as the Atlas pistachio or Mt. Atlas mastic tree, is a member of the Anacardiaceae family and is widely distributed across the Mediterranean region, North Africa, and parts of the Middle East. This plant species has been traditionally utilized in various folk medicine systems for its diverse therapeutic properties, including antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory activities. The essential oil and various extracts derived from different parts of *P. atlantica* have been shown to contain a rich array of bioactive compounds, including monoterpenes, sesquiterpenes, phenolic compounds, flavonoids, tannins, and other secondary metabolites. Despite the extensive documentation of the medicinal properties of *P. atlantica* in mammalian and in vitro systems, there is a notable paucity of research investigating its potential applications in aquaculture, particularly its effects on growth performance, digestive enzyme activities, antioxidant status, and immune responses in commercially important fish species.

1.2 Research Objectives

The primary objectives of this study are:

1. To evaluate the effects of dietary supplementation with *Pistacia atlantica* oil on growth performance parameters including weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio in Nile tilapia (*Oreochromis niloticus*).
2. To assess the impact of *P. atlantica* oil supplementation on digestive enzyme activities including protease, amylase, and lipase in Nile tilapia.
3. To investigate the effects of dietary *P. atlantica* oil on antioxidant enzyme activities including superoxide dismutase, catalase, and glutathione peroxidase in Nile tilapia.
4. To examine the immunomodulatory effects of *P. atlantica* oil supplementation on immune responses including lysozyme activity, respiratory burst activity, and total immunoglobulin levels in Nile tilapia.
5. To determine the optimal dietary supplementation level of *P. atlantica* oil for maximizing growth, digestive efficiency, antioxidant defense, and immune function in Nile tilapia.

1.3 Significance of the Study

This research holds significant scientific and practical implications for the aquaculture industry. From a scientific perspective, this study contributes to the growing body of knowledge regarding the application of phytochemicals in aquaculture and provides comprehensive data on the multiple physiological effects of *P. atlantica* oil in a commercially important fish species. The

investigation of digestive enzyme activities, antioxidant enzyme systems, and immune parameters offers valuable insights into the mechanisms through which plant-derived bioactive compounds exert their beneficial effects on fish health and performance.
From a practical standpoint, the findings of this study have the potential to inform the development of sustainable, natural feed additives that can replace or reduce the reliance on synthetic chemicals and antibiotics in tilapia farming operations. The identification of optimal supplementation levels provides practical guidance for the aquaculture industry in formulating functional feeds that can enhance productivity, improve fish health, and reduce disease susceptibility. Furthermore, the use of *P. atlantica* oil as a feed additive aligns with the growing consumer demand for sustainably produced, chemical-free aquaculture products and supports the transition toward more environmentally responsible aquaculture practices.
The economic implications of this research are also noteworthy, as improved growth performance, enhanced feed efficiency, and reduced disease incidence can translate into significant cost savings and increased profitability for tilapia producers. Additionally, the potential for *P. atlantica* oil to serve as a natural immunostimulant may reduce the need for therapeutic interventions, further contributing to sustainable and cost-effective aquaculture production.

2. Literature Review

2.1 Phytochemical Composition and Bioactive Properties of *Pistacia atlantica*

Pistacia atlantica has been extensively studied for its rich phytochemical composition and diverse biological activities. The essential oil and various extracts obtained from different parts of the plant, including leaves, fruits, galls, and oleoresin, have been shown to contain a wide array of bioactive compounds. Benhammou et al. (2008) investigated the antioxidant and antimicrobial activities of *Pistacia lentiscus* and *Pistacia atlantica* extracts, demonstrating significant free radical scavenging activity and inhibitory effects against various pathogenic microorganisms. The researchers attributed these activities to the presence of phenolic compounds, flavonoids, and tannins in the plant extracts.
Mahjoub et al. (2018) provided a comprehensive review of the traditional uses, phytochemicals, and pharmacological properties of *Pistacia atlantica*. The review highlighted the presence of monoterpenes, sesquiterpenes, triterpenes, phenolic acids, flavonoids, and other secondary metabolites that contribute to the plant's therapeutic potential. The authors documented the traditional use of *P. atlantica* in the treatment of various ailments including digestive disorders, respiratory infections, skin diseases, and inflammatory conditions.
Ahmed et al. (2021) conducted a detailed review of four *Pistacia atlantica* subspecies (atlantica, cabulica, kurdica, and mutica), examining their botany, ethnobotany, phytochemistry, and pharmacology. The review revealed significant variations in chemical composition among the different subspecies, with each exhibiting distinct bioactive profiles and biological activities. The presence of essential oils, phenolic compounds, flavonoids, and other secondary metabolites was consistently reported across all subspecies, with variations in concentration and composition depending on the geographic origin, plant part, and extraction method.
Peksel et al. (2010) evaluated the antioxidant and antiacetylcholinesterase activities of *Pistacia atlantica* leaf extracts, demonstrating potent free radical scavenging activity and enzyme inhibitory effects. The researchers identified several phenolic compounds, including gallic acid, catechin, and various flavonoid derivatives, which were responsible for the observed antioxidant activity. The study suggested that *P. atlantica* leaves could serve as a valuable source of natural antioxidants with potential applications in the prevention of oxidative stress-related disorders.

Bassouya et al. (2025) investigated the chemical, mineralogical, and biological properties of *Pistacia atlantica* subsp. *atlantica* essential oils from the Middle Atlas region of Morocco. The study identified numerous volatile compounds, with α-pinene, β-pinene, limonene, and terpinolene being among the major constituents. The essential oil exhibited significant antioxidant activity and antimicrobial effects against various bacterial and fungal strains, supporting the traditional use of the plant for medicinal purposes.

Barrero et al. (2005) analyzed the chemical composition of the essential oils of *Pistacia atlantica* and identified a complex mixture of monoterpenes, sesquiterpenes, and other volatile compounds. The study highlighted the presence of α-pinene, β-pinene, camphene, myrcene, limonene, and other terpenoids that contribute to the characteristic aroma and biological activities of the essential oil.

Hatamnia et al. (2014) examined the antioxidant activity and phenolic profile of different parts of Bene (*Pistacia atlantica* subsp. *kurdica*) fruits. The researchers found that the fruit hulls exhibited the highest phenolic content and antioxidant activity, followed by the kernels and shells. The phenolic compounds identified included gallic acid, catechin, chlorogenic acid, and various flavonoid derivatives, demonstrating the potential of *P. atlantica* fruits as a source of natural antioxidants.

Toul et al. (2017) investigated the antioxidant activity and phenolic profile of different organs of *Pistacia atlantica* subsp. *atlantica* from Algeria. The study revealed that the leaves and fruits contained high levels of phenolic compounds, flavonoids, and tannins, with significant antioxidant activity as measured by DPPH radical scavenging, ABTS radical scavenging, and ferric reducing antioxidant power assays. The researchers suggested that *P. atlantica* could serve as a valuable source of natural antioxidants for various applications.

Gourine et al. (2010) examined the seasonal variation of chemical composition and antioxidant activity of essential oil from *Pistacia atlantica* leaves. The study demonstrated significant seasonal variations in both the chemical composition and antioxidant activity of the essential oil, with the highest antioxidant activity observed during the flowering period. The major compounds identified included α-pinene, β-pinene, limonene, and various oxygenated monoterpenes.

Delazar et al. (2004) conducted GC-MS analysis of the essential oil from the oleoresin of *Pistacia atlantica* var. *mutica*, identifying numerous compounds including monoterpenes, sesquiterpenes, and diterpenes. The study contributed to the understanding of the chemical composition of the oleoresin, which is traditionally used in various medicinal applications.

2.2 Antimicrobial and Immunomodulatory Activities of *Pistacia atlantica*

The antimicrobial and immunomodulatory properties of *Pistacia atlantica* have been extensively documented in various studies. Dalvand et al. (2024) evaluated the antibacterial and antifungal activities of *Pistacia atlantica* and *Pistacia khinjuk*, demonstrating significant inhibitory effects against various pathogenic microorganisms. The researchers attributed the antimicrobial activity to the presence of bioactive compounds including phenolic compounds, flavonoids, and essential oil components.

Salem et al. (2021) investigated the antioxidant enzyme activities and immune responses in rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets supplemented with dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) extracts. The study demonstrated significant enhancements in antioxidant enzyme activities and immune parameters, suggesting that plant-derived bioactive compounds can effectively modulate the immune system and antioxidant defense mechanisms in fish.

Salem et al. (2022) examined the effects of white mustard (*Sinapis alba*) oil on growth performance, immune response, blood parameters, digestive and antioxidant enzyme activities in rainbow trout. The results showed significant improvements in growth performance, digestive enzyme activities, antioxidant status, and immune responses, highlighting the potential of plant oils as functional feed additives in aquaculture.

Salem et al. (2023) investigated the effects of dietary flaxseed (*Linum usitatissimum*) oil supplementation on growth, oxidative stress, immune response, and disease resistance in rainbow trout. The study demonstrated that flaxseed oil supplementation significantly enhanced growth performance, antioxidant enzyme activities, and immune responses, while also improving resistance against bacterial infections.

Taştan and Salem (2021) provided a comprehensive review on the use of phytochemicals as feed supplements in aquaculture, examining their effects on growth, immune response, and antioxidant status of finfish. The review highlighted the potential of various plant-derived compounds, including essential oils, extracts, and bioactive compounds, to serve as effective alternatives to synthetic additives in aquaculture.

2.3 Plant Oils and Extracts in Aquaculture

The use of plant oils and extracts as feed additives in aquaculture has gained considerable attention in recent years. Salem (2026) reviewed the mechanisms of action of plant extracts as alternatives to antibiotics in aquaculture, providing a comprehensive overview of recent studies. The review highlighted the multiple modes of action through which plant-derived compounds exert their beneficial effects, including direct antimicrobial activity, immunomodulation, antioxidant defense enhancement, and growth promotion.

Salem (2023) examined the effects of plant essential oils used as feed supplements in aquaculture on immune response and antioxidant status of finfish. The review documented numerous studies demonstrating the potential of essential oils from various plant species to enhance immune function, strengthen antioxidant defense systems, and improve overall health and performance in cultured fish species.

Amhamed et al. (2023) reviewed the use of plant essential oils in fish aquaculture as growth promoters, highlighting the potential of these natural compounds to enhance growth performance and feed efficiency in various fish species. The review emphasized the importance of identifying optimal supplementation levels and understanding the mechanisms through which essential oils exert their growth-promoting effects.

Ali et al. (2022) investigated the growth promoter, immunostimulant, and antioxidant effects of terebinth (*Pistacia terebinthus*) extract in rainbow trout. The study demonstrated significant improvements in growth performance, immune responses, and antioxidant status, suggesting that *Pistacia* species possess substantial potential as functional feed additives in aquaculture.

Kutluyer et al. (2017) examined the effect of dietary fish oil replacement with plant oils on growth performance and gene expression in juvenile rainbow trout. The study showed that plant oils could effectively replace fish oil in the diet without adversely affecting growth performance, while also influencing gene expression related to fatty acid metabolism.

Yildiz et al. (2015) investigated the effect of different plant oils on growth performance, fatty acid composition, and flesh quality of rainbow trout. The study demonstrated that various plant oils could be used as alternatives to fish oil in aquaculture diets, with significant effects on growth performance and fatty acid profiles.

2.4 Antioxidant and Immune Responses in Fish

The antioxidant defense system in fish plays a crucial role in protecting cells from oxidative damage caused by reactive oxygen species generated during normal metabolism and in response to various stressors. Salem and Lakwani (2025) investigated the immunomodulatory effects of dietary chaste tree (*Vitex agnus-castus* L.) extract in rainbow trout, demonstrating significant enhancements in immune parameters and antioxidant enzyme activities.

Salem (2026) examined the physiological and immunological responses of Nile tilapia fed diets enriched with *Vitex agnus-castus* extract, documenting significant improvements in growth performance, immune responses, and antioxidant status. The study provided valuable insights into the potential of plant-derived compounds to enhance the health and performance of Nile tilapia.

Salem and Barkah (2025) investigated the effect of dietary supplementation with chaste tree seed extract on the haematological indices of rainbow trout, demonstrating significant improvements in various blood parameters associated with immune function and overall health.

Salem and Mohamed (2025) examined the effects of aqueous-methanol extract from chaste tree on growth enhancement and digestive enzyme activity in rainbow trout, showing significant improvements in growth performance and digestive enzyme activities.

Salem and Osman (2025) reviewed the use of nanoparticles from plant derivatives and their potential usage in aquaculture, highlighting the emerging applications of nanotechnology in enhancing the delivery and efficacy of plant-derived bioactive compounds in fish.

2.5 Digestive Enzyme Activities in Fish

Digestive enzymes play a critical role in the utilization of dietary nutrients in fish. The activities of protease, amylase, and lipase are essential indicators of digestive capacity and nutrient utilization efficiency. Bilen et al. (2019) investigated the effect of lemon balm (*Melissa officinalis*) extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout. The study demonstrated significant enhancements in digestive enzyme activities, suggesting improved nutrient utilization and digestive efficiency.

Salem et al. (2022) reported significant increases in protease, amylase, and lipase activities in rainbow trout fed diets supplemented with white mustard oil, indicating improved digestive function and nutrient absorption. The enhancement of digestive enzyme activities was associated with improved growth performance and feed efficiency.

Salem et al. (2023) observed similar improvements in digestive enzyme activities in rainbow trout fed flaxseed oil-supplemented diets, further supporting the potential of plant oils to enhance digestive function in fish.

Salem and Mohamed (2025) demonstrated significant increases in digestive enzyme activities in rainbow trout fed diets supplemented with chaste tree extract, suggesting that various plant-derived compounds can effectively stimulate digestive enzyme production and activity.

2.6 Growth Performance in Fish

Growth performance is a critical parameter in aquaculture, directly influencing production efficiency and economic viability. Various studies have demonstrated that plant-derived bioactive compounds can significantly enhance growth performance in fish. Salem (2025) investigated the impact of dietary supplementation with *Panax ginseng* on oxidative stress indicators and disease resistance in Nile tilapia reared under high stocking density conditions, demonstrating significant improvements in growth performance and stress resistance.

Salem (2026) reported significant improvements in growth performance parameters in Nile tilapia fed diets enriched with *Vitex agnus-castus* extract, including increased weight gain, enhanced specific growth rate, and improved feed conversion ratio.

Salem and Lakwani (2024) reviewed the effects of using olive tree (*Olea europaea* L.) derivatives as feed additives on growth efficiency, immunological response, and oxidative status in finfish, highlighting the potential of olive-derived compounds to enhance growth performance and overall health in cultured fish species.

Salem and Moammer (2024) examined the potential benefits of *Aloe vera* derivatives in aquaculture, documenting various positive effects on growth performance, immune function, and disease resistance in fish.

2.7 Gaps in Current Knowledge

Despite the extensive documentation of the phytochemical composition and biological activities of *P. atlantica*, and the growing body of evidence supporting the use of plant-derived compounds as feed additives in aquaculture, there are significant gaps in the current knowledge. Specifically, limited research has been conducted on the effects of *P. atlantica* oil on growth performance, digestive enzyme activities, antioxidant enzyme systems, and immune responses in fish. The majority of studies on *P. atlantica* have focused on its chemical composition, antioxidant activity, and antimicrobial properties in vitro or in mammalian systems, with few investigations examining its potential applications in aquaculture.

Furthermore, the optimal dietary supplementation level of *P. atlantica* oil for maximizing growth, digestive efficiency, antioxidant defense, and immune function in fish has not been established. The mechanisms through which *P. atlantica* oil exerts its beneficial effects on fish physiology and performance require further elucidation. Additionally, the potential interactions between *P. atlantica* oil and other dietary components, as well as the effects of long-term supplementation on fish health and product quality, warrant investigation.

This study aims to address these knowledge gaps by comprehensively evaluating the effects of dietary supplementation with *P. atlantica* oil on multiple physiological parameters in Nile tilapia, providing valuable data for the development of sustainable, natural feed additives in aquaculture.

3. Methodology

3.1 Study Population and Sample

A total of 240 healthy Nile tilapia (*Oreochromis niloticus*) fingerlings with an initial average weight of 25.0 ± 1.2 g were obtained from a commercial fish hatchery. The fish were acclimated to laboratory conditions for two weeks prior to the commencement of the feeding trial. During the acclimation period, the fish were fed a commercial basal diet twice daily, and water quality parameters including temperature, dissolved oxygen, pH, and ammonia levels were maintained within optimal ranges for Nile tilapia culture.

3.2 Experimental Design

Following the acclimation period, the fish were randomly distributed into four experimental groups, each with three replicate tanks, resulting in a total of 12 tanks. Each tank contained 20 fish, providing a stocking density of 20 fish per tank. The experimental groups were designated as follows:

- **Group 1 (Control):** Fish fed a basal diet without *P. atlantica* oil supplementation.
- **Group 2 (0.5% PAO):** Fish fed a basal diet supplemented with 0.5% *P. atlantica* oil.
- **Group 3 (1.0% PAO):** Fish fed a basal diet supplemented with 1.0% *P. atlantica* oil.
- **Group 4 (1.5% PAO):** Fish fed a basal diet supplemented with 1.5% *P. atlantica* oil.

3.3 Preparation of *Pistacia atlantica* Oil

Pistacia atlantica oil was extracted from the oleoresin of *P. atlantica* trees using the hydrodistillation method. The oleoresin was collected from mature trees and subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours. The essential oil was collected, dried over anhydrous sodium sulfate, and stored in amber glass bottles at 4°C until use. The chemical composition of the oil was determined using Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

3.4 Diet Preparation

Four isonitrogenous and isoenergetic experimental diets were formulated to contain approximately 30% crude protein and 15% crude lipid. The basal diet composition included fish meal, soybean meal, corn gluten, wheat flour, fish oil, soybean oil, vitamin premix, and mineral premix. The experimental diets were prepared by mixing the basal diet ingredients with the appropriate amount of *P. atlantica* oil (0%, 0.5%, 1.0%, or 1.5% of the diet). The diets were pelleted using a laboratory pellet mill, air-dried, and stored in sealed bags at 4°C until feeding.

3.5 Feeding Trial and Management

The feeding trial was conducted for a period of 60 days. Fish were fed the experimental diets twice daily (09:00 and 16:00) at a rate of 3-5% of their body weight per day, adjusted weekly based on the recorded weight gain. Uneaten feed was collected after 30 minutes of feeding to determine the actual feed intake. Water quality parameters were monitored daily and maintained at optimal levels: temperature 28 ± 1°C, dissolved oxygen > 6 mg/L, pH 7.2-7.8, and ammonia < 0.05 mg/L. A 12:12 light-dark photoperiod was maintained throughout the experimental period.

3.6 Sampling Procedures

At the end of the 60-day feeding trial, all fish were weighed individually for growth performance evaluation. After a 24-hour fasting period, fish from each tank were anesthetized using clove oil (50 mg/L). Blood samples were collected from the caudal vein of three fish per tank using heparinized syringes. The blood samples were centrifuged at 3,000 rpm for 10 minutes to obtain plasma, which was stored at -80°C for subsequent immune parameter analysis. Liver and intestinal tissue samples were collected for antioxidant and digestive enzyme activity assays, respectively. Tissue samples were immediately homogenized in appropriate buffers and centrifuged, and the supernatants were stored at -80°C until analysis.

3.7 Growth Performance Parameters

The following growth performance parameters were calculated:

Weight Gain (WG): WG = Final weight - Initial weight

Specific Growth Rate (SGR): SGR (%) = [(ln Final weight - ln Initial weight) / Feeding days] × 100

Feed Conversion Ratio (FCR): FCR = Feed consumed / Weight gain

Protein Efficiency Ratio (PER): PER = Weight gain / Protein consumed

3.8 Digestive Enzyme Activity Assays

Digestive enzyme activities were measured in intestinal tissue homogenates using commercial assay kits.

Protease Activity: Protease activity was measured using the casein hydrolysis method, with one unit of protease activity defined as the amount of enzyme that releases 1 μg of tyrosine per minute per mg protein.

Amylase Activity: Amylase activity was determined using the starch-iodine method, with one unit of amylase activity defined as the amount of enzyme that hydrolyzes 1 mg of starch in 30 minutes per mg protein.

Lipase Activity: Lipase activity was measured using the titrimetric method with olive oil as substrate, with one unit of lipase activity defined as the amount of enzyme that releases 1 μmol of fatty acid per minute per mg protein.

3.9 Antioxidant Enzyme Activity Assays

Antioxidant enzyme activities were measured in liver tissue homogenates using commercial assay kits.

Superoxide Dismutase (SOD) Activity: SOD activity was measured using the nitroblue tetrazolium (NBT) reduction method. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50% per mg protein.

Catalase (CAT) Activity: CAT activity was determined by measuring the decrease in absorbance at 240 nm due to hydrogen peroxide decomposition. One unit of CAT activity was defined as the amount of enzyme that decomposes 1 μmol of hydrogen peroxide per minute per mg protein.

Glutathione Peroxidase (GPx) Activity: GPx activity was measured using the glutathione reductase-coupled method with cumene hydroperoxide as substrate. One unit of GPx activity was defined as the amount of enzyme that oxidizes 1 μmol of glutathione per minute per mg protein.

3.10 Immune Parameter Assays

Immune parameters were measured in plasma samples using commercial assay kits.

Lysozyme Activity: Lysozyme activity was measured using the turbidimetric method with *Micrococcus lysodeikticus* as substrate. One unit of lysozyme activity was defined as the amount of enzyme that causes a decrease in absorbance of 0.001 per minute at 450 nm.

Respiratory Burst Activity: Respiratory burst activity was measured using the nitroblue tetrazolium (NBT) reduction assay, with the results expressed as the optical density at 620 nm per 106 cells.

Total Immunoglobulin (Ig) Level: Total immunoglobulin levels were measured using the turbidimetric method with polyethylene glycol precipitation, with the results expressed as mg/mL.

3.11 Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software (version 26.0). Data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. One-way analysis of variance (ANOVA) was used to determine significant differences among the experimental groups, followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

4. Results

4.1 Growth Performance Parameters

The effects of dietary supplementation with *Pistacia atlantica* oil on growth performance parameters of Nile tilapia are presented in Table 1.

Table 1: Growth Performance Parameters of Nile Tilapia Fed Diets Supplemented with Different Levels of *Pistacia atlantica* Oil

Parameter	Control	0.5% PAO	1.0% PAO	1.5% PAO
Initial Weight (g)	25.3 ± 1.1	24.9 ± 1.3	25.1 ± 1.0	25.2 ± 1.2
Final Weight (g)	68.4 ± 2.3a	81.7 ± 2.8b	96.3 ± 3.1c	89.5 ± 2.9d
Weight Gain (g)	43.1 ± 1.8a	56.8 ± 2.1b	71.2 ± 2.6c	64.3 ± 2.3d
SGR (%/day)	1.67 ± 0.05a	1.98 ± 0.06b	2.27 ± 0.07c	2.11 ± 0.06d
FCR	2.14 ± 0.08a	1.78 ± 0.06b	1.45 ± 0.05c	1.61 ± 0.06d
PER	1.56 ± 0.06a	1.87 ± 0.07b	2.30 ± 0.08c	2.07 ± 0.07d

Values are presented as mean ± SD (n = 9). Different letters in the same row indicate significant differences (p < 0.05).

The results showed that dietary supplementation with *P. atlantica* oil significantly improved all growth performance parameters compared to the control group. The 1.0% PAO group exhibited the highest final weight (96.3 ± 3.1 g), weight gain (71.2 ± 2.6 g), and SGR (2.27 ± 0.07%/day), as well as the lowest FCR (1.45 ± 0.05) and highest PER (2.30 ± 0.08). All treatment groups demonstrated significantly ($p < 0.05$) better growth performance compared to the control group, with a dose-dependent response observed up to the 1.0% supplementation level, beyond which a slight decline in performance was noted.

4.2 Digestive Enzyme Activities

The effects of *P. atlantica* oil supplementation on digestive enzyme activities in Nile tilapia are presented in Table 2.

Table 2: Digestive Enzyme Activities in Nile Tilapia Fed Diets Supplemented with *Pistacia atlantica* Oil

Enzyme Activity (U/mg protein)	Control	0.5% PAO	1.0% PAO	1.5% PAO
Protease	12.4 ± 1.2a	18.7 ± 1.5b	24.3 ± 1.8c	21.5 ± 1.6d
Amylase	8.6 ± 0.8a	12.3 ± 1.0b	16.8 ± 1.2c	14.9 ± 1.1d
Lipase	5.2 ± 0.5a	7.8 ± 0.7b	10.4 ± 0.9c	9.1 ± 0.8d

Values are presented as mean ± SD (n = 9). Different letters in the same row indicate significant differences (p < 0.05).

Dietary supplementation with *P. atlantica* oil significantly ($p < 0.05$) enhanced digestive enzyme activities in Nile tilapia. The highest enzyme activities were observed in the 1.0% PAO group, with protease activity (24.3 ± 1.8 U/mg protein) being approximately twice that of the control group (12.4 ± 1.2 U/mg protein). Similarly, amylase and lipase activities were significantly elevated in all treatment groups compared to the control, with the 1.0% PAO group showing the most pronounced enhancement. The digestive enzyme activities followed a similar pattern to the growth performance parameters, with an optimal response at the 1.0% supplementation level.

4.3 Antioxidant Enzyme Activities

The effects of *P. atlantica* oil supplementation on antioxidant enzyme activities in Nile tilapia liver tissue are presented in Table 3.

Table 3: Antioxidant Enzyme Activities in Nile Tilapia Fed Diets Supplemented with *Pistacia atlantica* Oil

Enzyme Activity (U/mg protein)	Control	0.5% PAO	1.0% PAO	1.5% PAO
SOD	45.2 ± 3.4a	58.7 ± 4.1b	76.4 ± 5.2c	68.3 ± 4.8d
CAT	32.8 ± 2.6a	43.5 ± 3.2b	55.6 ± 3.8c	50.1 ± 3.5d
GPx	18.3 ± 1.5a	24.6 ± 1.9b	33.7 ± 2.4c	29.8 ± 2.1d

Values are presented as mean ± SD (n = 9). Different letters in the same row indicate significant differences (p < 0.05).

Supplementation with *P. atlantica* oil resulted in significant ($p < 0.05$) increases in antioxidant enzyme activities in Nile tilapia liver tissue. The 1.0% PAO group exhibited the highest SOD (76.4 ± 5.2 U/mg protein), CAT (55.6 ± 3.8 U/mg protein), and GPx (33.7 ± 2.4 U/mg protein) activities, representing increases of 69%, 70%, and 84% compared to the control group, respectively. All supplementation levels significantly enhanced antioxidant enzyme activities, with a clear dose-dependent response up to the 1.0% level, followed by a slight decrease at the 1.5% level.

4.4 Immune Parameters

The effects of *P. atlantica* oil supplementation on immune parameters in Nile tilapia are presented in Table 4.

Table 4: Immune Parameters in Nile Tilapia Fed Diets Supplemented with *Pistacia atlantica* Oil

Immune Parameter	Control	0.5% PAO	1.0% PAO	1.5% PAO
Lysozyme Activity (U/mL)	15.2 ± 1.3a	22.8 ± 1.7b	31.5 ± 2.2c	27.4 ± 2.0d
Respiratory Burst Activity (OD 620 nm)	0.38 ± 0.03a	0.56 ± 0.04b	0.74 ± 0.05c	0.65 ± 0.04d
Total Ig Level (mg/mL)	12.4 ± 1.0a	18.6 ± 1.4b	25.3 ± 1.8c	22.1 ± 1.6d

Values are presented as mean ± SD (n = 9). Different letters in the same row indicate significant differences ($p < 0.05$).

Dietary supplementation with *P. atlantica* oil significantly ($p < 0.05$) enhanced immune parameters in Nile tilapia. The 1.0% PAO group demonstrated the highest lysozyme activity (31.5 ± 2.2 U/mL), respiratory burst activity (0.74 ± 0.05 OD 620 nm), and total Ig levels (25.3 ± 1.8 mg/mL), representing increases of 107%, 95%, and 104% compared to the control group, respectively. All treatment groups showed significantly improved immune responses compared to the control, with the 1.0% supplementation level providing the most pronounced enhancement across all measured parameters.

4.5 Summary of Results

The results of this study demonstrate that dietary supplementation with *Pistacia atlantica* oil significantly improves growth performance, digestive enzyme activities, antioxidant enzyme activities, and immune responses in Nile tilapia. The optimal supplementation level was determined to be 1.0% of the diet, which consistently produced the best results across all evaluated parameters. The dose-dependent response observed in most parameters suggests that the beneficial effects of *P. atlantica* oil are concentration-dependent, with higher supplementation levels (1.5%) potentially inducing adverse effects or reaching a plateau in response.

5. Discussion

5.1 Growth Performance and Digestive Enzyme Activities

The significant improvements in growth performance parameters observed in Nile tilapia fed diets supplemented with *Pistacia atlantica* oil are consistent with previous studies on the effects of plant-derived compounds in aquaculture. The enhancement in weight gain, SGR, and PER, coupled with reduced FCR, indicates that *P. atlantica* oil effectively promotes growth and feed efficiency in Nile tilapia. These findings align with the results of Salem et al. (2022) who reported similar improvements in rainbow trout fed white mustard oil-supplemented diets, and Salem et al. (2023) who observed enhanced growth performance in rainbow trout supplemented with flaxseed oil.

The improved growth performance can be attributed to several factors, including enhanced digestive enzyme activities, improved nutrient utilization, and the potential growth-promoting effects of bioactive compounds present in *P. atlantica* oil. The significant increases in protease, amylase, and lipase activities observed in this study indicate that *P. atlantica* oil stimulates digestive enzyme production and activity, thereby improving the breakdown and absorption of dietary nutrients. This is consistent with the findings of Bilen et al. (2019) who reported enhanced digestive enzyme activities in rainbow trout fed lemon balm extract, and Salem and Mohamed (2025) who observed similar effects in rainbow trout supplemented with chaste tree extract.

The optimal growth performance and digestive enzyme activities observed at the 1.0% supplementation level suggest that there is an optimal concentration of *P. atlantica* oil that maximizes beneficial effects without inducing negative consequences. The slight decline in performance at the 1.5% supplementation level may be attributed to potential toxicity or antinutritional effects of certain bioactive compounds at higher concentrations, as previously reported by Salem et al. (2022) who observed similar dose-dependent responses in rainbow trout fed white mustard oil.

The presence of various bioactive compounds in *P. atlantica* oil, including monoterpenes, sesquiterpenes, phenolic compounds, and flavonoids (Mahjoub et al., 2018; Ahmed et al., 2021), may contribute to the observed improvements in digestive function. These compounds may stimulate digestive enzyme secretion, enhance intestinal absorption, and improve the overall digestive capacity of the fish (Taştan and Salem, 2021). Furthermore, the antioxidant properties of these compounds may protect the gastrointestinal mucosa from oxidative damage, maintaining the structural and functional integrity of the digestive system (Peksel et al., 2010).

5.2 Antioxidant Enzyme Activities

The significant enhancements in antioxidant enzyme activities (SOD, CAT, and GPx) observed in this study indicate that *P. atlantica* oil effectively strengthens the antioxidant defense system in Nile tilapia. These findings are consistent with previous studies on the antioxidant effects of plant-derived compounds in fish. Salem et al. (2021) reported similar increases in antioxidant enzyme activities in rainbow trout fed dandelion and lichen extracts, while Salem (2025) observed enhanced antioxidant status in Nile tilapia supplemented with *Panax ginseng*.

The elevation of antioxidant enzyme activities is likely due to the presence of bioactive compounds in *P. atlantica* oil that act as natural antioxidants or stimulate the endogenous antioxidant defense system. The phenolic compounds, flavonoids, and other secondary metabolites present in *P. atlantica* oil (Hatamnia et al., 2014; Toul et al., 2017) may directly scavenge reactive oxygen species and reduce oxidative stress, thereby upregulating the expression and activity of antioxidant enzymes.

The dose-dependent increase in antioxidant enzyme activities, with optimal effects at the 1.0% supplementation level, suggests that moderate concentrations of *P. atlantica* oil are sufficient to enhance antioxidant defense without causing oxidative stress or toxicity. The slight decrease in antioxidant enzyme activities at the 1.5% supplementation level may indicate that excessive amounts of certain bioactive compounds can induce pro-oxidant effects or overwhelm the cellular antioxidant capacity, as previously suggested by Salem et al. (2023) in their study on flaxseed oil supplementation in rainbow trout.

The enhanced antioxidant defense system is of particular importance in aquaculture, where fish are frequently exposed to various stressors that can induce oxidative stress, including handling, transportation, and high stocking densities (Salem, 2025). The strengthening of the antioxidant system by *P. atlantica* oil supplementation may help fish cope with these stressors, reduce oxidative damage, and maintain overall health and performance.

5.3 Immune Responses

The significant improvements in immune parameters, including lysozyme activity, respiratory burst activity, and total Ig levels, demonstrate that *P. atlantica* oil possesses potent immunostimulatory properties in Nile tilapia. These findings are consistent with previous studies on the immunomodulatory effects of plant-derived compounds in fish. Salem and Lakwani (2025) reported enhanced immune responses in rainbow trout fed chaste tree extract, while Salem (2026) observed similar effects in Nile tilapia supplemented with *Vitex agnus-castus* extract.

Lysozyme is an important component of the innate immune system, acting as a bactericidal enzyme that hydrolyzes the peptidoglycan layer of bacterial cell walls (Salem and Lakwani, 2024). The significant increase in lysozyme activity observed in this study indicates enhanced nonspecific immune defense, which may contribute to improved resistance against bacterial infections. Similarly, the enhanced respiratory burst activity reflects increased production of reactive oxygen species by phagocytic cells, which is crucial for the elimination of pathogens (Salem et al., 2022). The elevated total Ig levels indicate enhanced humoral immune responses, suggesting improved adaptive immunity.

The immunostimulatory effects of *P. atlantica* oil can be attributed to the presence of various bioactive compounds that modulate immune function. Phenolic compounds and flavonoids have been shown to possess immunomodulatory properties, enhancing the activity of immune cells and promoting the production of immune mediators (Taştan and Salem, 2021; Salem, 2023). Additionally, the antioxidant properties of *P. atlantica* oil may indirectly support immune function by reducing oxidative stress, which can impair immune responses (Salem and Moammer, 2024).

The dose-dependent enhancement of immune parameters, with optimal effects at the 1.0% supplementation level, suggests that moderate concentrations of *P. atlantica* oil are sufficient to stimulate immune responses without causing immunosuppression or adverse effects. The slight decline in immune parameters at the 1.5% supplementation level may indicate that higher concentrations of certain bioactive compounds can have inhibitory effects on immune function, as previously reported by Salem et al. (2023) in rainbow trout fed high levels of flaxseed oil.

5.4 Mechanisms of Action

The multiple beneficial effects of *P. atlantica* oil observed in this study can be attributed to several mechanisms of action. The bioactive compounds present in *P. atlantica* oil, including monoterpenes, sesquiterpenes, phenolic compounds, and flavonoids (Bassouya et al., 2025; Barrero et al., 2005), likely act through multiple pathways to enhance growth, digestive function, antioxidant defense, and immune responses.

The digestive enzyme-enhancing effects may be mediated through the stimulation of enzyme secretion, modulation of the gastrointestinal environment, or direct activation of enzyme activity (Salem and Mohamed, 2025). The antioxidant effects are likely due to the direct free radical scavenging activity of phenolic compounds and the upregulation of endogenous antioxidant enzyme systems (Peksel et al., 2010; Gourine et al., 2010). The immunostimulatory effects may involve the activation of immune cells, enhancement of phagocytic activity, and stimulation of immunoglobulin production (Salem and Lakwani, 2025; Salem, 2026).

The optimal effects observed at the 1.0% supplementation level suggest that there is a balance between the beneficial and potentially adverse effects of *P. atlantica* oil. At moderate concentrations, the bioactive compounds exert their beneficial effects through various mechanisms without inducing toxicity or negative side effects. At higher concentrations, however, certain compounds may have pro-oxidant effects, inhibit digestive enzymes, or suppress immune function (Salem et al., 2022).

5.5 Comparison with Previous Studies

The results of this study are consistent with previous research on the effects of plant-derived compounds in aquaculture. The improvements in growth performance and digestive enzyme activities observed in this study are comparable to those reported by Bilen et al. (2019) in rainbow trout fed lemon balm extract, and by Salem et al. (2022) in rainbow trout fed white mustard oil. The enhancement of antioxidant enzyme activities is similar to the findings of Salem et al. (2021) in rainbow trout fed dandelion and lichen extracts, and by Salem (2025) in Nile tilapia supplemented with *Panax ginseng*.

The immunostimulatory effects observed in this study are consistent with the findings of Salem and Lakwani (2025) in rainbow trout fed chaste tree extract, and Salem (2026) in Nile tilapia supplemented with *Vitex agnus-castus* extract. The dose-dependent responses observed in this study, with optimal effects at moderate supplementation levels, are similar to the patterns reported by Salem et al. (2023) in rainbow trout fed flaxseed oil, and by Ali et al. (2022) in rainbow trout fed terebinth extract.

The results of this study extend the existing knowledge by demonstrating the potential of *P. atlantica* oil as a functional feed additive in Nile tilapia. While previous studies have documented the phytochemical composition and biological activities of *P. atlantica* (Mahjoub et al., 2018; Ahmed et al., 2021; Benhammou et al., 2008), and its antimicrobial effects (Dalvand et al., 2024; Hassanzadazar et al., 2021), this study is among the first to comprehensively evaluate its effects on growth, digestive enzyme activities, antioxidant status, and immune responses in a commercially important fish species.

5.6 Implications for Aquaculture

The findings of this study have significant implications for the aquaculture industry. The use of *P. atlantica* oil as a natural feed additive offers a sustainable alternative to synthetic growth promoters and immunostimulants, aligning with the growing demand for environmentally friendly and chemical-free aquaculture products. The multiple beneficial effects of *P. atlantica* oil, including enhanced growth performance, improved digestive efficiency, strengthened antioxidant defense, and boosted immune responses, can contribute to increased productivity, reduced disease incidence, and improved overall health in Nile tilapia farming operations.

The identification of the optimal supplementation level (1.0% of the diet) provides practical guidance for the formulation of functional feeds for Nile tilapia. This information can be directly applied by feed manufacturers and tilapia producers to develop cost-effective and efficacious diets that maximize the benefits of *P. atlantica* oil supplementation.

Furthermore, the potential for *P. atlantica* oil to enhance disease resistance, as suggested by the improved immune parameters, may reduce the reliance on antibiotics and other therapeutic interventions in aquaculture. This is particularly important in the context of increasing concerns about antibiotic resistance and the need for sustainable disease management strategies in aquaculture (Salem, 2026).

5.7 Limitations and Future Research Directions

While this study provides valuable insights into the effects of *P. atlantica* oil in Nile tilapia, certain limitations should be acknowledged. The study was conducted under controlled laboratory conditions, and the results may not fully represent the responses of fish under commercial farming conditions. Additionally, the study evaluated only short-term effects (60 days), and the long-term effects of *P. atlantica* oil supplementation on fish health and product quality warrant investigation.

Future research should focus on elucidating the molecular mechanisms underlying the beneficial effects of *P. atlantica* oil, including gene expression analysis of digestive enzymes, antioxidant enzymes, and immune-related genes. Studies on the effects of *P. atlantica* oil on disease resistance against specific pathogens would provide valuable information for practical applications. Furthermore, investigations on the effects of *P. atlantica* oil supplementation on the gut microbiota composition, fatty acid profile of fish tissues, and sensory quality of the final product would provide a more comprehensive understanding of its potential in aquaculture.

6. Conclusion and Recommendations

6.1 Conclusion

This study investigated the effects of dietary supplementation with *Pistacia atlantica* oil on growth performance, digestive enzyme activities, antioxidant enzyme activities, and immune responses in Nile tilapia (*Oreochromis niloticus*). The results demonstrate that *P. atlantica* oil supplementation at levels of 0.5%, 1.0%, and 1.5% significantly improves all evaluated parameters compared to the control group. The 1.0% supplementation level consistently produced the best results across all parameters, representing the optimal dietary level for maximizing the beneficial effects of *P. atlantica* oil in Nile tilapia.

The significant improvements in growth performance, including increased weight gain, enhanced SGR, improved FCR, and elevated PER, indicate that *P. atlantica* oil effectively promotes growth and feed efficiency in Nile tilapia. The enhancement of digestive enzyme activities (protease, amylase, and lipase) suggests improved nutrient utilization and digestive capacity. The elevation of antioxidant enzyme activities (SOD, CAT, and GPx) indicates strengthened antioxidant defense systems, which may help fish cope with oxidative stress. The enhancement of immune parameters (lysozyme activity, respiratory burst activity, and total Ig levels) demonstrates potent immunostimulatory properties, suggesting improved disease resistance.

The multiple beneficial effects of *P. atlantica* oil can be attributed to the presence of various bioactive compounds, including monoterpenes, sesquiterpenes, phenolic compounds, and flavonoids, which exert their effects through multiple mechanisms including direct antioxidant activity, stimulation of endogenous enzyme systems, modulation of immune function, and enhancement of digestive processes.

The findings of this study contribute to the growing body of evidence supporting the use of phytochemicals as sustainable alternatives to synthetic additives in aquaculture. *Pistacia atlantica* oil shows significant potential as a functional feed additive that can enhance growth, improve digestive efficiency, strengthen antioxidant defense systems, and boost immune responses in Nile tilapia, with practical applications for improving productivity and health in commercial tilapia farming operations.

6.2 Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. **Practical Application:** *Pistacia atlantica* oil should be incorporated into Nile tilapia diets at a level of 1.0% to maximize growth performance, digestive efficiency, antioxidant defense, and immune responses. This supplementation level provides the most balanced improvements across all evaluated parameters and can be directly applied in commercial tilapia farming operations.
2. **Feed Formulation:** Feed manufacturers should consider incorporating *P. atlantica* oil into functional feeds for Nile tilapia, particularly in the context of sustainable and chemical-free aquaculture production. The incorporation of *P. atlantica* oil may reduce the need for synthetic additives and antibiotics while improving fish health and productivity.
3. **Further Research:** Additional studies should be conducted to investigate the long-term effects of *P. atlantica* oil supplementation on fish health, product quality, and economic viability. Research on the effects of *P. atlantica* oil on disease resistance against specific

pathogens, gut microbiota composition, and fatty acid profiles of fish tissues would provide valuable information for practical applications.

4. **Mechanistic Studies:** Elucidation of the molecular mechanisms underlying the beneficial effects of *P. atlantica* oil, including gene expression analysis and signaling pathways, would enhance our understanding of its mode of action and support the optimization of its use in aquaculture.
5. **Commercial Validation:** Field trials under commercial farming conditions should be conducted to validate the findings of this study and assess the practical feasibility and economic benefits of *P. atlantica* oil supplementation in large-scale tilapia production operations.
6. **Regulatory Considerations:** As the use of plant-derived feed additives in aquaculture continues to grow, regulatory frameworks should be developed to ensure the safety, efficacy, and quality of these products. Standardization of *P. atlantica* oil production and quality control measures is essential for its successful commercialization and use in aquaculture.

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