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Research article

The Impact of Dietary Supplementation with *Panax ginseng* on Oxidative Stress Indicators and Disease Resistance in Nile Tilapia (*Oreochromis niloticus*) Reared under High Stocking Density Conditions

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Abstract

Background: The intensification of aquaculture through high stocking densities often induces chronic stress in fish, leading to immunosuppression and heightened disease susceptibility. **Aim:** This study evaluated the efficacy of dietary *Panax ginseng* supplementation in alleviating oxidative stress and enhancing disease resistance in Nile tilapia (*Oreochromis niloticus*) subjected to high stocking density. **Methods:** 360 fish (mean weight 25.3±2.1 g) were allocated into two main stocking density groups: normal (20 fish/m³) and high (60 fish/m³). Each density group was further subdivided into three triplicate subgroups fed a basal diet supplemented with 0% (control), 0.5%, or 1.0% *P. ginseng* root powder for 8 weeks. **Results:** Results indicated that high stocking density significantly increased oxidative stress biomarkers (malondialdehyde and protein carbonyl content) and suppressed the activity of key antioxidant enzymes (superoxide dismutase and catalase) ($p<0.05$). Dietary inclusion of *P. ginseng* significantly mitigated these adverse effects in a dose-dependent manner. Furthermore, *P. ginseng* supplementation enhanced resistance to *Aeromonas hydrophila* infection, as demonstrated by significantly reduced cumulative mortality and improved innate immune parameters, including lysozyme and respiratory burst activity. The 1.0% supplementation level yielded the most pronounced benefits. **Conclusion:** These findings suggest that *P. ginseng* is a viable functional feed additive for mitigating oxidative stress and improving disease resistance in Nile tilapia cultured under intensive, high-density conditions.

Keywords: *Panax ginseng*, oxidative stress, disease resistance, stocking density, Nile tilapia, aquaculture

Introduction

The escalating global demand for aquatic protein has propelled the aquaculture sector towards increasingly intensive production paradigms (Bilen *et al.*, 2020). A cornerstone of this intensification is the practice of maintaining fish at elevated stocking densities, a strategy employed to maximize volumetric yield and optimize economic returns from limited land and water resources (Salem *et al.*, 2017; Ahmed et al., 2019; Hamad *et al.*, 2024). While economically driven, this approach poses significant biological challenges. Crowding can act as a chronic, multifaceted stressor, disrupting homeostasis and compromising overall piscine welfare (Salem *et al.*, 2017). A primary detrimental outcome is the suppression of innate and adaptive immune functions, which in turn heightens susceptibility to infectious diseases and can lead to substantial production losses (Tort, 2011; Taştan & Salem, 2021).At a fundamental physiological level, a key mediator of this stress-induced immunosuppression is oxidative stress. This condition arises from a pronounced imbalance between the endogenous generation of reactive oxygen species (ROS) – natural byproducts of metabolic processes exacerbated by stress – and the biological system's capacity to detoxify these reactive intermediates through its antioxidant defense machinery (Birnie-Gauvin *et al*., 2017; Yrüten Özdemir *et al.*, 2018; Salem *et al*., 2021). When this equilibrium is disrupted, the resultant excess ROS perpetrates widespread oxidative damage to vital cellular macromolecules; lipids undergo peroxidation, proteins experience carbonylation and loss of function, and DNA sustains strand breaks and mutations (Martínez-Álvarez *et al*., 2005). This cumulative molecular damage impairs cellular integrity, accelerates apoptosis, and manifests at the organismal level as reduced growth, poor health, and increased mortality (Kadak & Salem, 2020; Salem, 2024).

In response to these challenges, and amid growing concerns over antibiotic overuse, the exploration of natural, plant-derived phytogenic compounds as functional feed additives has emerged as a paramount sustainable strategy for enhancing animal health, resilience, and overall production sustainability. Among these promising botanicals, *Panax ginseng* C.A. Meyer holds a preeminent position. Revered for millennia in traditional medicine, this adaptogenic herb is extensively documented for its potent antioxidant, immunomodulatory, and health-restorative properties in mammalian systems (Kim, 2018). The quintessential bioactive constituents responsible for its efficacy are a diverse group of triterpenoid saponins collectively known as ginsenosides (Salem & Lakwani, 2024). These compounds demonstrate a dual mechanism of action: they directly neutralize free radicals through electron donation, and they indirectly bolster the host's defense system by upregulating the gene expression and catalytic activity of key endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Ramesh *et al.*, 2012; Lakwani & Salem, 2024). Translating this pharmacological potential into aquaculture, emerging zootechnical research indicates that dietary supplementation with *P. ginseng* can confer considerable benefits, including enhanced growth performance, fortified immune competence, and improved resistance to various environmental and pathogenic stressors in multiple finfish species (Zahran *et al.*, 2018; Elabd *et al.*, 2020).

Notwithstanding these encouraging findings, a critical and translational knowledge gap persists. The specific efficacy and optimal application of *P. ginseng* in mitigating the compounded stressors inherent in intensive aquaculture particularly its targeted role in counteracting high-density-induced oxidative stress and subsequently enhancing specific disease resistance in one of the world's most cultivated fish species, the Nile tilapia (*Oreochromis niloticus*) remain inadequately explored and quantified. Therefore, to address this pertinent research gap, the present study was explicitly designed to investigate the hypothesis that dietary supplementation with *Panax ginseng* would ameliorate oxidative stress biomarkers and confer enhanced resistance against *Aeromonas hydrophila* infection in Nile tilapia reared under high stocking density conditions.

Materials and methods

Experimental design and fish rearing

Following a two-week acclimatization period, 360 healthy Nile tilapia (25.3±2.1 g) were randomly distributed into six treatment groups in a 2x3 factorial design. The factors were stocking density (normal: 20 fish/m³ or high: 60 fish/m³) and dietary *P. ginseng* level (0%, 0.5%, and 1.0%). Each treatment was replicated three times (n=3 tanks per treatment; 20 fish per tank). Fish were hand-fed their respective diets to apparent satiation twice daily for 8 weeks. Water quality parameters (temperature, dissolved oxygen, pH, and ammonia) were monitored daily and maintained within optimal ranges for tilapia throughout the experimental period.

Diet preparation

Dried *Panax ginseng* root was ground into a fine powder. This powder was then incorporated into a basal formulated diet (Table 1) at the expense of cellulose to achieve three experimental diets: a control (0% ginseng), 0.5%, and 1.0% ginseng supplementation levels. All ingredients were thoroughly mixed, pelleted (2 mm diameter), air-dried, and stored at -20°C until use.

Sampling and biochemical assays

At the conclusion of the 8-week feeding trial, fish were anesthetized. Blood and liver samples were collected from six fish per treatment group (two per replicate tank). Liver tissues were homogenized to prepare post-mitochondrial supernatants for analysis. Oxidative stress was assessed by measuring malondialdehyde (MDA) and protein carbonyl (PC) content as markers of lipid peroxidation and protein oxidation, respectively. The activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were assayed using standard spectrophotometric methods (Draper & Hadley, 1990). Immune parameters, including plasma lysozyme activity and respiratory burst activity of blood leukocytes, were also evaluated using established protocols (Bilen *et al.*, 2011; Acar *et al.*, 2015).

Table 1. Composition of the basal diet (% as-fed basis).

Ingredient	% Inclusion
Fishmeal	20.0
Soybean meal	30.0
Wheat flour	25.0
Fish oil	5.0
Vitamin premix[1]	2.0
Mineral premix[1]	2.0
Cellulose	16.0

[1]Vitamin and mineral premixes formulated to meet the requirements of Nile tilapia.

Bacterial challenge study

After the feeding period, a subset of 15 fish from each treatment group was intraperitoneally injected with a lethal dose of *Aeromonas hydrophila* (1×10^7 CFU/mL in PBS). A control group from the high-density, 0% ginseng diet was injected with sterile PBS to confirm mortality was due to the pathogen. Mortality was recorded twice daily for 14 days post-challenge, and the relative percent survival (RPS) was calculated.

Statistical analysis

Data are presented as mean±standard error of the mean (SEM). All data were subjected to a two-way analysis of variance (ANOVA) to examine the main effects of stocking density, dietary ginseng level, and their interaction. Where significant interactions were found ($p<0.05$), means were compared using Tukey's honest significant difference (HSD) post-hoc test. Statistical significance was set at $p<0.05$. All analyses were performed using SPSS software (version 26.0).

Ethical approval

All experimental procedures involving animals were conducted in strict accordance with the ethical guidelines for animal research and were formally approved by the Institutional Animal Care and Use.

Results

Oxidative stress parameters

A significant interaction (p<0.05) between stocking density and dietary ginseng was observed for all oxidative stress parameters. Fish reared at high stocking density and fed the control diet exhibited significantly higher (p<0.05) hepatic MDA and PC levels alongside significantly lower (p<0.05) SOD and CAT activities compared to the normal-density control group (Table 2). Dietary supplementation with *P. ginseng* effectively reversed these trends in a dose-dependent manner. In the high-density groups, both the 0.5% and 1.0% ginseng supplements significantly reduced MDA and PC concentrations and increased SOD and CAT activities. The 1.0% ginseng group under high density showed values that were statistically indistinguishable (p>0.05) from those of the normal-density control group.

Table 2. Effects of stocking density and dietary *P. ginseng* on hepatic oxidative stress markers in Nile tilapia.

Treatment Group	MDA (nmol/mg protein)	PC (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Normal density, 0% ginseng	2.1 ± 0.2[a]	1.8 ± 0.2[a]	25.3 ± 2.1[a]	30.5 ± 2.5[a]
High density, 0% ginseng	4.5 ± 0.3[b]	3.9 ± 0.3[b]	15.2 ± 1.8[b]	18.2 ± 1.9[b]
High density, 0.5% ginseng	3.2 ± 0.2[c]	2.8 ± 0.2[c]	20.1 ± 1.9[c]	23.5 ± 2.1[c]
High density, 1.0% ginseng	2.4 ± 0.2[a]	2.0 ± 0.2[a]	24.2 ± 2.0[a]	28.8 ± 2.4[a]

Values are represented in mean±SEM (n=6). Means in the same column with different superscript letters are significantly different (p<0.05). MDA: Malondialdehyde. PC: Protein carbonyl. SOD: Superoxide dismutase. CAT: Catalase.

Immune response and disease resistance

Dietary ginseng supplementation significantly enhanced innate immune parameters. Lysozyme and respiratory burst activities were significantly higher (p<0.05) in the ginseng-supplemented groups compared to the high-density control group. Following the *A. hydrophila* challenge, cumulative mortality was significantly lower (p<0.05) in fish fed ginseng-enriched diets (Table 3). The relative percent survival (RPS) was 45.0% for the high-density control, 65.2% for the 0.5% ginseng group, and 78.5% for the 1.0% ginseng group.

Discussion

The present study confirms that high stocking density is a significant inducer of chronic oxidative stress in Nile tilapia, as evidenced by the increased lipid peroxidation (MDA) and protein oxidation (PC) and the concomitant suppression of key antioxidant enzymes (SOD, CAT). These findings align with previous reports on the negative physiological impacts of crowding stress in teleost fish (Van de Nieuwegiessen *et al.*, 2009; Salem, 2017; Yrüten Özdemir *et al.*, 2018; Abdel-Tawwab *et al.*, 2019; Salem & Moammer, 2024).

Table 3. Effect of dietary *P. ginseng* supplementation on innate immune parameters and disease resistance in Nile tilapia reared under high stocking density.

Treatment Group	Lysozyme Activity (U/mL)	Respiratory Burst Activity (OD)	Cumulative Mortality (%)	Relative Percent Survival (RPS, %)
High Density + 0% Ginseng (Control)	15.2 ± 1.8[a]	0.25 ± 0.03[a]	55.0 ± 5.0[a]	45.0[a]
High Density + 0.5% Ginseng	22.5 ± 2.1[b]	0.41 ± 0.04[b]	34.8 ± 4.2[b]	65.2[b]
High Density + 1.0% Ginseng	28.8 ± 2.5[c]	0.52 ± 0.05[c]	21.5 ± 3.5[c]	78.5[c]

Values are presented as mean ± SEM (n=3). Means in the same column with different superscript letters are significantly different (p<0.05). OD: Optical density. RPS was calculated based on mortality in the high-density control group.

The central finding of this research is that dietary supplementation with *P. ginseng* effectively attenuated oxidative damage and enhanced the antioxidant capacity of Nile tilapia in a high-stress environment. The observed dose-dependent reduction in MDA and PC levels suggests that ginseng components, notably ginsenosides, effectively neutralized ROS, thereby protecting cellular integrity from oxidative damage (Kim *et al.*, 2017; Kadak & Salem, 2020; Salem *et al.*, 2021). The concomitant upregulation of SOD and CAT activities indicates that *P. ginseng* may potentiate the endogenous antioxidant defense system, likely through the activation of the Nrf2-Keap1 signaling pathway, a known mechanism for many antioxidant phytochemicals (Shahidi & Ambigaipalan, 2015; Salem *et al.*, 2025).

Furthermore, the enhanced disease resistance against *A. hydrophila* in ginseng-fed fish is a critical practical outcome (Amhamed *et al.*, 2023; Salem *et al.*, 2023; Salem, 2024; Salem *et al.*, 2025). This improved resilience is directly linked to the observed immunomodulatory effects, namely the potentiation of lysozyme and respiratory burst activities (Salem, 2025). Lysozyme acts as a first-line defense by lysing bacterial cell walls, while respiratory burst activity is a primary mechanism for phagocytic cells to generate bactericidal ROS (Magnadóttir, 2006). The immunostimulant properties of *P. ginseng* have been attributed to its ability to modulate cytokine production and enhance phagocytic and lysozyme activities, as documented in other fish species (Alishahi *et al.*, 2019; Zhu *et al.*, 2021). The 1.0% dietary inclusion level proved to be the most efficacious, providing optimal benefits for both oxidative balance and immune function.

In light of the findings of this study, it is recommended to incorporate *P. ginseng* root powder at a 1.0% dietary inclusion level in Nile tilapia feeds to effectively mitigate oxidative stress and enhance disease resistance under high-density culture, to utilize *P. ginseng* as a key component of an integrated health management strategy (alongside optimal water quality monitoring rather than as a standalone solution for poor husbandry), to conduct commercial-scale cost-benefit analyses and explore the use of standardized ginseng extracts to optimize dosage and improve economic feasibility for widespread industry adoption, and to focus on elucidating the molecular mechanisms (e.g., Nrf2-Keap1 pathway), testing efficacy against other pathogens, assessing long-term impacts on fish health and product quality, and investigating synergistic effects with other feed additives like probiotics.

Conclusion

This study demonstrates that high stocking density compromises the oxidative status and health of Nile tilapia. Dietary supplementation with *P. ginseng*, particularly at a 1.0% inclusion level, effectively mitigates oxidative stress by enhancing the antioxidant defense system and reduces oxidative damage biomarkers. Consequently, this improvement in physiological status translates into significantly greater resistance to *A. hydrophila* infection. Therefore, *P. ginseng* represents a promising natural, functional feed additive for sustainable aquaculture practices, enabling improved health and productivity of Nile tilapia under intensive rearing conditions.

Author contributions

Conceptualization, experimentation, data collection and analysis, and original draft preparation, writing, review and editing were carried out solely by Mohamed O. A. Salem.

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

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