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Review

Ghrelin as a Promising Immunostimulant in Aquaculture: Mechanisms and Therapeutic Potential

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ABSTRACT

Ghrelin, a peptide hormone originally known for its role in growth hormone secretion and appetite regulation, is a key immunomodulator in vertebrates, including fish. This hormone and its receptor (GHS-R) are widely expressed in immune cells—T cells, B cells, neutrophils, and macrophages—and tissues. In fish, Ghrelin modulates physiological processes (e.g., reproduction or energy balance) and improves immune defense mechanisms. Studies have demonstrated its ability to promote phagocytic activity, regulate cytokine production, and stimulate antimicrobial peptide production, enhancing resistance to pathogenic infections. Likewise, the development of ghrelin mimetics, like GHRP-6, with a wide range of functionalities, further increases the potential of their use in aquaculture, showing promising results in improving growth and immune responses in fish. Ghrelin and its analogs represent a promising alternative for boosting fish's immune response against pathogens. Thus, understanding Ghrelin's regulatory mechanisms in fish immunity may reveal practical applications for aquaculture practices, helping achieve fish welfare and sustainability.

Keywords: Ghrelin; immune system; fish; growth hormone secretagogue; cytokines; antimicrobial activity

INTRODUCTION

In 1996, a G-protein-coupled receptor was identified, GHS-R1a, back then considered an orphan receptor [1]. The endogenous ligand of this receptor, Ghrelin (GHRL), was reported for the first time in 1999 in rats and human stomachs [2]. This peptide hormone is mainly secreted from the stomach. Still, it's also expressed in other organs such as the brain, pituitary, placenta, ovaries, testes, kidneys, small intestine, pancreas, and lungs [3]. Although its better-known functions are acting as a growth hormone (GH) secretagogue and regulating energy balance and food intake, it plays many roles in all body systems, such as improving cardiac performance, decreasing blood pressure, and inhibiting insulin release [4].

The immune and neuroendocrine systems are intrinsically linked in constant bidirectional communication [5], essential to maintaining homeostasis. The neuroendocrine system has multilevel modulatory properties that affect the functions of the immune system, contributing to both activation and suppression [6]. The crosstalk between the immune system and the endocrine axis that controls appetite and metabolism has gained increasingly more interest. Recent studies have proved that Ghrelin can be crucial in this crosstalk [7, 8, 9, 10]. Thus, this hormone is a central factor that regulates the immune response of various organisms.

This review focuses specifically on Ghrelin's role in fish immunology. Immune cells, like T cells, B cells, and neutrophils, express growth hormone (GH) and Ghrelin and have receptors for these hormones [11]. Ghrelin, the expression of its receptor, and its mRNA are regulated during chronic inflammation, stress, and immune cells exposed to different pathogens [12]. As a growth hormone secretagogue, in increasing GH secretion, Ghrelin modulates cytokine production, enhances B cell maturation and antibody production, and potentiates neutrophile adhesion and monocyte migration, besides having anti-apoptotic effects [13, 14, 15]. As for its effects, Ghrelin attenuates septic shock, has anti-inflammatory effects, modulates phagocytosis, and promotes thymopoiesis [16, 17, 8]. With more and more evidence gathering up on Ghrelin's influence on the immune response of non-mammalian vertebrates, including fish, the purpose of this article is to give an overview of the structure and expression of Ghrelin, its physiological functions, and the relationship between this orexigenic hormone and fish immune system.

Structure of Ghrelin and its receptor

Ghrelin is a 28-amino acid peptide (GSSFLSPEHQKAQQRKESKKPPAKLQPR), with slight variations in length and structure across species, mainly released in the stomach by oxyntic cells [18, 19]. The genomic organization of the ghrelin gene (GHRL) was first described in humans [20]. This gene comprises 3 introns and 4 exons and is located in chromosome 3p25-26 [21]. The goldfish ghrelin gene was the first non-mammalian vertebrate gene to be identified, which shows the same genomic organization described in humans [22]. This peptide hormone is encoded by the ghrelin and obestatin prepropeptide (GHRL) gene and the ghrelin O-acyltransferase (GOAT) then acylates Ghrelin at Ser3 with octanoic acid [18,23]. Once acylated, proghrelin is transferred into the Golgi apparatus and cut into mature ghrelin [24]. There are two forms of Ghrelin in the body: acyl ghrelin and des-acyl ghrelin. The desacylated form is more abundant in blood (90%) than the acylated (10%) [25]. However, des-acyl ghrelin can neither bind GHS-R nor exhibit GH-releasing activity, even though food intake is regulated by it to the same extent as ghrelin [26].
The growth hormone secretagogue receptor's gene is located on chromosome 3q26–q29 [27]. It is alternatively spliced and exists in two isoforms, namely GHS-R1a and GHS-R1b, in most vertebrates [28]. The active ghrelin receptor, known as GHS-R1a, is a G-protein-coupled receptor with seven transmembrane domains; the GHS-R1b modulates the 1a receptor [17]. This type 1b receptor is a COOH-terminal truncated form of the type 1a receptor and is pharmacologically inactive [29].

Expression of Ghrelin and GHS-R

Ghrelin expression is tissue-specific, with the highest levels in the stomach and brain [30]. This hormone and its receptors are widely expressed in many brain regions, pituitary, intestine, kidney, thyroid gland, lung, heart, pancreatic islets, ovaries, testis, and sebaceous glands [4]. Even though its better-known effects are being a GH secretagogue and playing an essential part in food intake regulation, it affects the central nervous, cardiovascular, reproductive, and immune systems [17].
Immune cells express both Ghrelin and its receptor. The study carried out by Hattori *et al.* shows that GHS-R and Ghrelin were expressed not only in B cells but also in T cells and neutrophils that did not express substantial GH transcripts, suggesting that ghrelin/GHS-R has unknown biological functions other than enhancing GH secretion in the immune system [11]. Meanwhile, this hormone's expression was shown to be decreased in peritoneal macrophages during HFD intake, and this was associated with increased inflammatory cytokine production, although it exercised an increase in ghrelin expression in these macrophages and reduced inflammatory cytokine production [7]. Ghrelin treatment modulates the secretion of polarizing cytokines of dendritic cells (DCs) during their maturation and thymic DCs [31, 32]. The growth hormone secretagogue receptor's expression is more restricted than its ligand's. However, it is also present in immune cells. The first report of the presence of GHS-R in the immune system was in 2001, in human T cells, B cells, and neutrophils [11]. Later on, macrophages and monocyte-derived dendritic cells have also been shown to express the growth hormone secretagogue receptor [33,34, 35,36].

Ghrelin across different species

Ghrelin is produced across the whole spectrum of vertebrates [37]. This hormone has been identified in several species, including humans, pigs, rats, mice, eels, goldfish, and tilapia [38]. Ghrelin remained a single-gene locus in all vertebrates [39]. However, there are variations in length and structure between these groups.

In mammals, one form of ghrelin peptide and its receptor are present. This is not the case for non-mammalian vertebrates, where multiple forms of ghrelin and ghrelin receptors exist [37]. For instance, Ghrelin's N-terminal 7 amino acids are conserved across teleosts, amphibians, reptiles, and mammals, but specific variations exist, such as the presence of Thr-3 in amphibians [40]. In fish, Ghrelin's sequence seems to be conserved; however, slight differences have been identified. According to Zhong *et al.*, the prepropeptide length ranges from 103 to 108 amino acids, and the similarities between the seven species in that study covered 35.00% to 89.32% [23]. Accordingly, Wang *et al.* stated that, in bighead carp, the ghrelin gene encodes a protein of 106 amino acids [30]. Cod ghrelin, however, has a longer precursor length (122 amino acids) compared to the 102-111 amino acids found in other species; it also contains specific amino acid variations, such as Thr-3, which is also seen in frog ghrelin, and valine as the second amino acid, similar to elasmobranchs [40].

The differences amongst vertebrate groups are also evident in Ghrelin's functions, although its effect as a growth hormone secretagogue seems to be conserved across vertebrates. In mammals like rodents and humans, Ghrelin is considered lypolitic, maintains body adiposity, and regulates glucose metabolism, and its effects in stimulating feeding are well known [41,42]. In contrast, centrally injected Ghrelin inhibits food intake in neonatal chickens and may shift metabolic fuel preference from carbohydrates to lipids [43]. On the other hand, evidence suggests the loss of the ghrelin gene entirely 44 in some avian species, like falcons. Ghrelin was also shown to decrease food intake in juvenile rainbow trout (*Oncorhynchus mykiss*) through the central anorexigenic corticotropin-releasing factor system, although in fish, it's known as a hormone that stimulates feeding [45,46]. In non-mammalian vertebrates, Ghrelin stimulates reproductive functions [47]. Fish ghrelin, specifically, stimulates the release of the luteinizing hormone [48]. This is in contrast to its effect in mammalian species, where ghrelin treatment inhibits GnRH, LH, and FSH secretion at the hypothalamic and pituitary levels [49].

Physiological functions of Ghrelin in fish

Though initially identified for inducing an increased secretion of GH in the stomach, Ghrelin has a broad range of functions on all body systems. The administration of this hormone usually stimulates feeding and an increased body weight in mammals and non-mammals, except birds. Consequently, becoming the first peripheral hormone in being a strong appetite stimulant [46]. It regulates sleep, gastric acid secretion and body temperature [29,50]. It also exhibits hypothalamic activities that result in stimulation of prolactin (PRL) and ACTH secretion, negatively influences the pituitary-gonadal axis at both central and peripheral level, influences behavior, modulates pancreatic exocrine and endocrine function, and affects glucose levels [51]. Likewise, evidence suggests Ghrelin's capacity to suppress inflammation [52]. Amongst its various applications, it improves cardiovascular functions, higher brain functions and prevents neurodegenerative diseases [4].

In fish, Ghrelin regulates diverse physiological processes and influences locomotor activity. This hormone positively affects oocyte maturation, ovarian vitellogenesis, and the number of mature follicles of *Barbus sharpeyi*, while it suppresses reproduction when energy levels are low in *Carassius auratus* [53,54]. Research also revealed that Ghrelin stimulates luteinizing hormone secretion from the pituitary cells of *Cyprinus carpio* and *Carassius auratus* [55,56]. In 2019, a study proved for the first time the effects of an early stimulus of Ghrelin during the embryonic stage of zebrafish, where Ghrelin treated larvae showed higher swimming activity [57]. More evidence of this hormone-modifying locomotor activity was gathered in goldfish (*Carassius auratus*) [58].

Similarly, Ghrelin modulates energy balance by regulating food intake, body weight, and glucose homeostasis [59]. In juvenile Persian sturgeon (*Acipenser persicus*), ghrelin injection significantly increased GH production and high doses decreased cortisol levels, although there were not significant changes in prolactin levels

[60]. There's also evidence of its effect in the modulation of glucoregulatory machinery, glucose homeostasis in fish, and the regulation of insulin synthesis [61,62]. Likewise, simultaneous ghrelin treatment counteracted the response to increased oleate levels, modulating hypothalamic fatty acid sensing [63]. The study by Salmeron *et al*. confirms this role in regulating lipid metabolism, being an enhancer of lipid turnover in the adipose tissue of rainbow trout [64].

Ghrelin and fish immune system

Besides the previously mentioned effects of Ghrelin, it is a potent immunomodulator with many possible applications in the fish's immune system (**Figure 1**). This hormone is a natural antimicrobial and anti-inflammatory peptide, widely distributed in all body tissues and especially abundant in nonspecific immune organs [4]. Immune system signaling may directly influence the metabolic axis and reciprocally via the expression of several common receptors and cytokine and hormonal ligands; changes in the energy balance and metabolism may exert potent effects on immune function and trafficking [28]. Over the years, accumulating evidence highlights the importance of Ghrelin in regulating inflammation, apoptosis, cytokine production, and phagocytic activity, among other essential aspects of immune response [3,65–68]. In mammalian models, for example, its anti-inflammatory effects have been described [69].

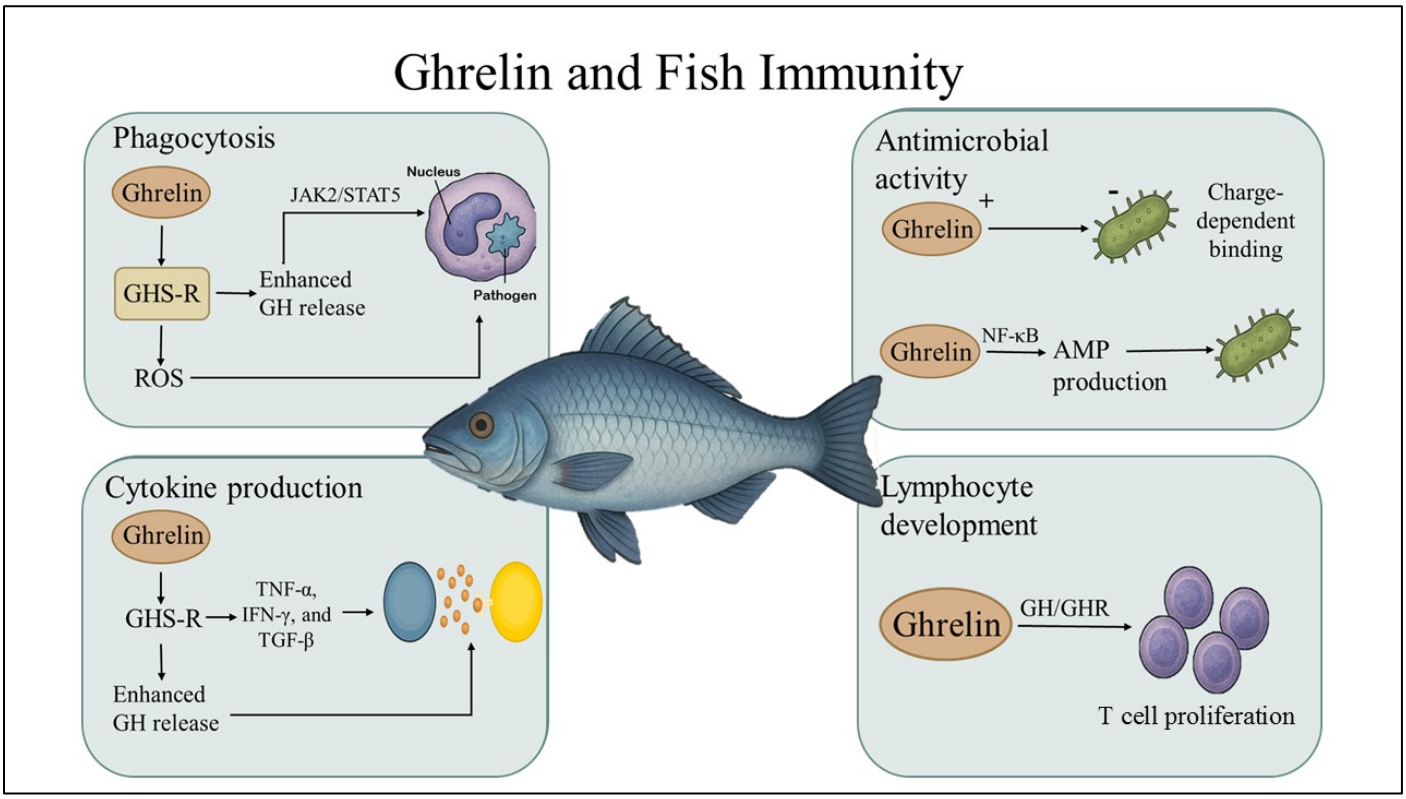

Figure 1. Multifaceted immunomodulatory roles of Ghrelin in teleost fish: Mechanisms and functional outcomes. Ghrelin, acting primarily through its receptor GHS-R, plays diverse roles in fish immunity. It enhances phagocytic activity via the JAK2/STAT5 signaling pathway and promotes reactive oxygen species (ROS) production, aiding pathogen elimination. It also regulates cytokine production, including TNF-α, IFN-γ, and TGF-β, contributing to immune modulation. Additionally, Ghrelin exhibits direct antimicrobial activity through charge-dependent microbial binding and inducing antimicrobial peptide (AMP) production via the NF-κB pathway. Finally, Ghrelin supports lymphocyte development, especially T cell proliferation, through the GH/GHR axis, emphasizing its potential as an immunostimulant in aquaculture.

Ghrelin and phagocytosis

Phagocytosis is a defensive reaction against infection and invasion of the body by foreign substances. In the immune system, phagocytosis is a major mechanism to remove pathogens and/or cell debris [70]. It can interconnect the innate and adaptative immune responses since the pathogen's processing by professional phagocytes is a fundamental stage for antibody production. Evidence shows that Ghrelin is capable of promoting phagocytic activity [71]. However, administering 20 µg/kg of Ghrelin via intraperitoneal injection reduced the increased phagocytic activity induced by acute cold restraint stress (ACRS) in rodents [72]. Additionally, mouse mitochondrial macrophages treated with a 100 ng/mL dose showed a metabolic shift towards oxidative metabolism, which involves ultrastructural and functional protection from inflammatory signals that induce mitochondrial damage [73]. This contrast could be due to dose differences and/or stress conditions, modulating overactivation to prevent immunopathology.

In fish, phagocytic activity is essential as a defense mechanism. As a growth hormone secretagogue, Ghrelin might increase phagocytic activity by increasing GH secretion. Evidence suggests GH's capacity to enhance phagocytosis [74,75]. In a study published in 2020, evaluating Ghrelin's modulation of the immune response against *Aeromonas hydrophila* in juvenile hybrid tilapia, this treatment, via intraperitoneal injection, showed increased phagocytic activity by increasing ROS activity at 5h with doses of 20 ng and 200 ng diluted in 0.1 mL of PBS, being ROS the main mediator of phagocytosis [16]. In an *in vitro* study in *Oncorhynchus mykiss*, using Ghrelin at 10 nM, this orexigenic hormone enhances superoxide production in leukocytes, promoting phagocytosis by binding to GHS-R, activating G-protein-coupled pathways to enhance oxidase-dependent superoxide production while also up-regulating autocrine GH secretion [76], which could amplify immune responses via JAK2/STAT5 signaling.

These studies show that Ghrelin can directly or indirectly regulate phagocytosis, promoting or decreasing it according to the organism's current needs. Interestingly, Ghrelin enhances phagocytic activity in fish, while the results vary in mammals.

Ghrelin and cytokine production

Cytokines are vital mediators that oversee and regulate immune and inflammatory responses via complex networks [77]. Regulating cytokine production is essential for organisms to ensure a balanced immune response, preventing underreaction to pathogens and overreaction. Previous studies have stated that acyl-ghrelin suppresses proinflammatory cytokine expression and/or production [12,78]. Ghrelin reduces the secretion of proinflammatory cytokines such as IL-6 and TNF-α, which could be overexpressed in inflammatory processes [65]. In a study by Beynon *et al.* ghrelin (1, 10, 100 nM) attenuated IL-6 secretion in an LPS challenge in mid-brain dopaminergic neurons, suggesting possible applications in protecting against dopaminergic SN nerve cell damage [79]. In 2021, this hormone appeared as an alternative against the cytokine storm produced by SARS-CoV2 infection, mitigating the uncontrolled cytokine production by up-regulating PPARγ and down-regulating NF-κB expression [80]. Ghrelin treatment also suppressed Th1 (IL-2 and IFN-g) and Th2 (IL-4 and IL-10) cytokines mRNA expression in mice [81].

Fish cytokines are similar to those found in higher vertebrate groups, such as interleukin-1β [82]. Against an *Aeromonas hydrophila* infection in hybrid tilapia, Ghrelin (2.0 ng, 20 ng, and 200 ng diluted in 0.1 mL of PBS) showed regulatory effects on the expression of IL-1β, decreased up-regulated TGF-β expression to normal levels and increased TNF-α expression by up-regulating GH levels [16]. Similar results were obtained in 2011 evaluating GH treatment's effect on TNF-α expression in *Oreochromis niloticus*, showing an increased expression in immune organs with an intraperitoneal injection of 2 mg/g of body weight [83]. In a study with different stimuli (lipopolysaccharide, phytohemagglutinin, or imiquimod), an increased ghrelin expression downregulated the production of cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)- α [84]. A study by Han *et al*. showed Ghrelin, using a dose of 1.25 ng/ µL, regulates cytokine production in fish by a combination of inhibiting certain proinflammatory cytokines (IL-6, TNF-α), inducing others and modulating immune-related gene expressions via two routes: directly mediating cytokine expression and indirectly altering downstream signaling of multifunctional bioregulators (TNF-α, IFN-γ, and TGF-β), simultaneously [8]. These mechanisms collectively contribute to controlling inflammation and maintaining

immune system balance in fish. This shows Ghrelin's prospective uses as a treatment to regulate inflammation via downregulating proinflammatory cytokines and up-regulating regulatory cytokines, acting similarly in mammals and fish.

Ghrelin regulation of lymphocyte development

The thymus is essential for developing, selecting, and maintaining the peripheral T cell pool possessing a broad spectrum of TCR specificities [85]. In a study of old mice injected intraperitoneally with 2 nM of Ghrelin, the treatment did not increase peripheral lymphocytes, but they did exhibit a statistically significant increase in thymic cellularity and differentiation [86]. Dixit *et al.* showed induced thymopoiesis during aging in mice with ghrelin treatment (1.25 µg/h for 2 weeks); the interplay between ghrelin and ghrelin receptor interactions in the thymic compartment and bone marrow stem cell niches leads to rejuvenation of thymic output and improved T cell repertoire in old animals [35]. Ghrelin also plays an important role in reestablishing the proliferation of CD4+ T cells and serves as a promising therapeutic agent in sepsis in humans [87]. Likewise, this hormone modulates Th effector cells in the gut, controlling proliferation and inducing apoptosis [88]. By increasing GH secretion, Ghrelin might indirectly induce this hormone's effects in immune cells, like enhancing B cell development and antibody production [3]. This shows Ghrelin's capacity to regulate immune cell development and proliferation, whether its effect is direct or indirect. When evaluating the impact of somatotrophic axis (GH/GHR) double transgenesis on structural and molecular aspects of the zebrafish immune system, there was a decrease in CD3+ and CD4+ T cells, showing the relevance of this axis for fish immune response [89] and, therefore, the importance of GH on fish lymphocyte development. Further research is needed to understand better how Ghrelin can regulate these aspects of the immune system in fish.

Ghrelin's antimicrobial activity

Ghrelin can inhibit the growth of microorganisms that could harm the organism, also known as antimicrobial activity. Ghrelin is a natural antimicrobial and anti-inflammatory peptide, widely distributed in all body tissues and especially abundant in the proximity to physical barriers such as the stomach, gut, and skin [90]. This hormone shows antimicrobial properties by inhibiting the growth of *Helicobacter pylori* [91]. Both acyl ghrelin and des- acyl ghrelin possess similar antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Ghrelin concentrations equal to, or greater than, 12.5 µg/ml showed significant bacterial killing effects toward these two Gram-negative bacteria, and antimicrobial activity could be derived from AMPs that facilitate their charge-dependent binding to bacteria [92]. In rainbow trout, Ghrelin modulates the expression of antimicrobial peptides' genes; this may result from a combination of ghrelin-mediated functional perturbations of GHS and NF-κB signaling pathways in RT-HKD cells [8], which could lead to increased antimicrobial activity. Ghrelin is also a prospective antimicrobial peptide that can decrease the activity of *Aeromonas hydrophila* [16]. This proves Ghrelin's capacity to modulate antimicrobial activity, whether by acting as an antimicrobial peptide or by regulating the expression of genes related to antimicrobial peptides. These results, gathered with those of previous sections, show that Ghrelin can regulate fish's immune system. This hormone exhibits species-specific immunomodulatory effects, enhancing phagocytic activity, regulating cytokine production, and boosting antimicrobial defenses in teleosts (**Table 1**).

Fish Species	Immune Parameter	Outcome	Ref.
Hybrid *tilapia*	Phagocytic activity	Ghrelin modulates phagocytic activity by increasing reactive oxygen species (ROS) production against *Aeromonas hydrophila*.	16
	Cytokine production	Ghrelin regulates *IL-1β* expression, reduces upregulated *TGF-β* to normal levels, and increases *TNF-α* expression in response to *A. hydrophila*.	
	Antimicrobial activity	Ghrelin decreases the activity of *Aeromonas hydrophila*.	
Oncorhynchus mykiss	Phagocytic activity	Ghrelin enhances superoxide production in leukocytes, promoting phagocytosis.	76

	Cytokine production	Ghrelin regulates cytokine production by inhibiting certain proinflammatory cytokines (*IL-6*, *TNF-α*), inducing others, and modulating immune gene expression via GHS-R pathways.	8
	Antimicrobial activity	Ghrelin modulates the expression of antimicrobial peptide genes.	
Cyprinus carpio	Cytokine production	Increased ghrelin expression downregulates cytokine production, including *IL-1β*, *IL-6*, and *TNF-α*.	84
Danio rerio	Lymphocyte development	Somatotrophic axis (GH/GHR) double transgenesis affects immune structure and function, decreasing *CD3+* and *CD4+* T cells, showing the axis's relevance to immune response.	89

Table 1. Comparative analysis of ghrelin-mediated immune responses across teleost species: Phagocytic activity, cytokine production, and antimicrobial defense.

Molecular mechanisms of ghrelin's immunomodulatory effects

As previously stated here, the immunomodulatory effects of Ghrelin have been extensively documented in fish and other vertebrates, but there are still gaps in knowledge regarding the molecular pathways underlying such effects. The body of evidence is growing, but there is still a lack of complete understanding of these mechanisms, particularly in aquatic species. However, some of these signaling cascades have been identified to some extent (**Figure 2**).

NF-κB Pathways

The NF-κB signaling pathway plays a crucial role in regulating the transcription of cytokine-encoding genes [80]. Likewise, the production of AMPs is often regulated by Toll-like receptors and NOD-like receptors mediated by NF-κB signaling pathways. Notably, Ghrelin has been demonstrated to modulate NF-κB signaling across species, suggesting a conserved immunoregulatory mechanism [8].
NF-κB activity is primarily controlled by a dynamic interplay between its inhibitors (IκB) and IκB kinases (IKK), enabling adaptation to the prevailing circumstances and preventing excessive immune responses [93]. Once activated, the IκB kinase complex phosphorylates IκB proteins, leading to their degradation and NF-κB nuclear translocation for inducing gene expression; Ghrelin suppresses NF-κB by inhibiting NOD2-Rip2 signaling, an upstream activator of this pathway [80]. Meanwhile, the Ghrelin-GH-IGF-I growth axis up-regulates the expression of NF-kB [94]. This shows Ghrelin's dual role in modulating immune responses according to the organism's needs.

Nrf2-Keap1/ARE pathway

ROS is important in stress perception, integration of diverse stress-responsive signaling networks, and activation of animal defense mechanisms, frequently occurring during inflammation in fish [95]. Nrf2, regulated by Keap1, is an important endogenous modulator of ROS overproduction, and Ghrelin has been shown to enhance Nrf2 expression and reduce oxidative damage [80]. In the Nrf2-Keap1/ARE pathway, the nuclear transcription factor Nrf2 translocates into the nucleus to initiate the transcription of antioxidant genes, thereby reducing reactive oxygen species (ROS)-induced cellular damage and maintaining the organism's oxidative-antioxidative equilibrium [96].

JAK2/STAT5 pathway

JAK2/STAT5 signaling intervenes in several immune processes in vertebrates, including respiratory bursts and the expression of proinflammatory cytokines [97]. Ghrelin can act indirectly through the JAK2/STAT5 pathway by inducing an enhanced GH secretion. GH can activate Janus Kinase 2 (JAK2)/transcription factors

STAT5 [94]. Ghrelin binds to its receptor, growth hormone secretagogue receptor 1a (GHSR1a), which is widely expressed in central systems and peripheral organs and stimulates the production of the growth hormone [65]. The JAK2/STAT5 pathway is the principal GH signaling mechanism, leading to transactivation and/or repression of target genes, including IGF-1 [98]. JAK2 initiates signaling upon cytokine receptor activation through auto/transphosphorylation, creating phosphotyrosine docking sites for STAT transcription factors, which SOCS proteins can inhibit. If there's no suppression of JAKS2, STATs are then phosphorylated by JAKs, forming dimers that translocate to the nucleus to regulate target gene expression [99]. Therefore, Ghrelin's ability to modulate immune response is shown by increasing GH secretion.

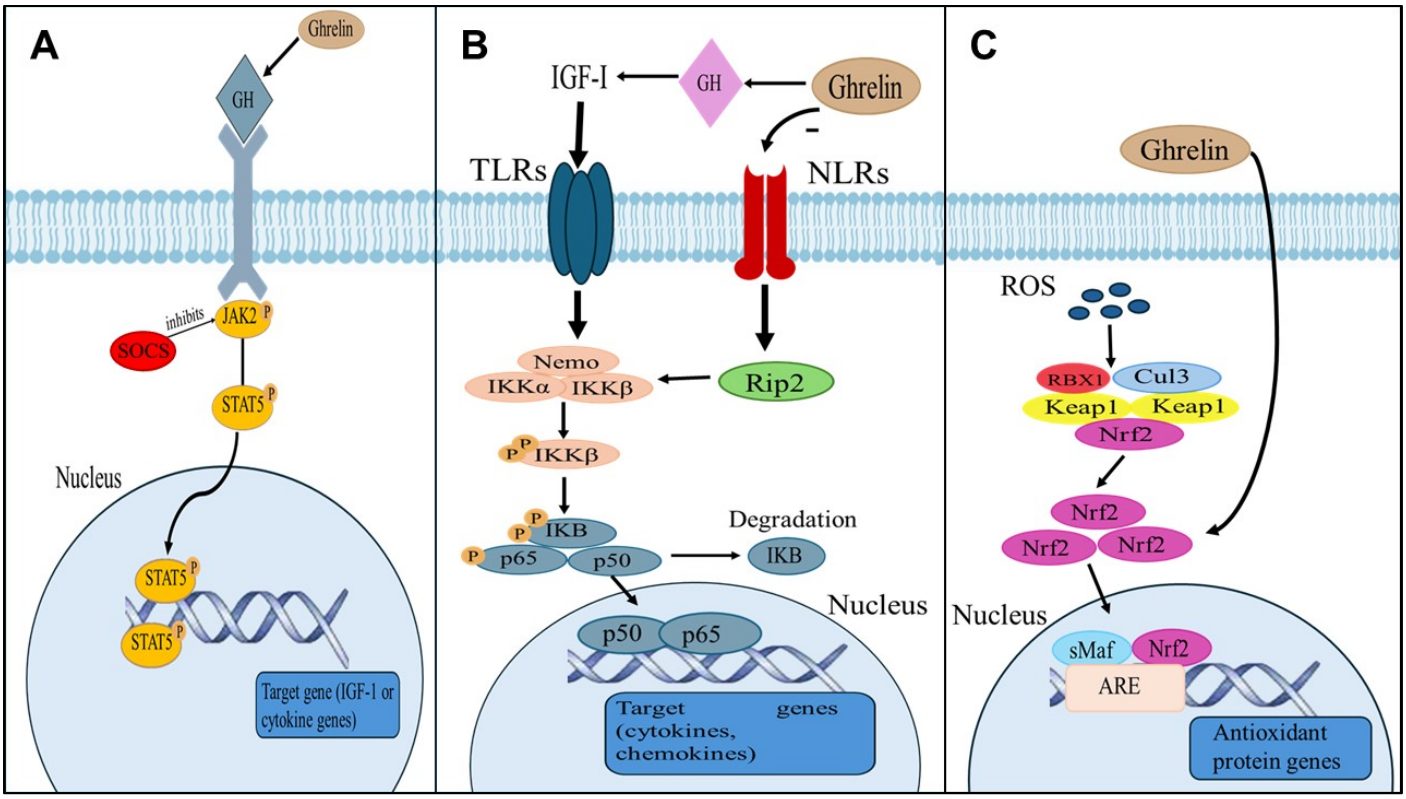

Figure 2. Ghrelin modulates key immune signaling pathways in fish. (A) JAK2/STAT5 pathway: Ghrelin, via growth hormone (GH) stimulation, activates the JAK2/STAT5 axis, leading to phosphorylation of STAT5 and transcription of immune-regulatory genes such as IGF-1 and cytokines. SOCS proteins negatively regulate this pathway, which inhibits JAK2 activity to maintain homeostasis. (B) NF-κB pathway: Ghrelin influences the balance between Toll-like receptors (TLRs) and NOD-like receptors (NLRs), modulating downstream signaling cascades. TLR activation leads to phosphorylation of IKKβ and degradation of IκB, allowing NF-κB (p65/p50) to translocate to the nucleus and induce expression of cytokines and chemokines. Ghrelin also impacts IGF-I levels, linking growth and immune signaling. (C) Nrf2-Keap1/ARE pathway: In response to oxidative stress and reactive oxygen species (ROS), Ghrelin promotes the dissociation of Nrf2 from the Keap1-Cul3 complex. Free Nrf2 translocates into the nucleus where, together with small Maf proteins, it binds to the antioxidant response element (ARE) and drives the expression of antioxidant and cytoprotective genes, reinforcing cellular defense mechanisms.

GHRP-6 and its effects in fish immunity as a mimetic of ghrelin

In 1976, Bowers *et al.* showed the functionality of met-encephalin-derived forms as promoters of GH secretion in rodent's pituitary cells [100]. Even though their signaling pathways were unknown then, this research paved the way for the future development of peptidic growth hormone secretagogues. GHRP-6 (His-(D-Trp)-Ala-

Trp-(D-Phe)-Lys-NH2) is a mimetic of Ghrelin. The peptide was developed in 1984 and proved its possible applications by increasing GH levels with relatively low doses [101].
GHRP-6 reduces the rate of apoptosis in cerebellar cells of aged rats [102]. It can also attenuate doxorubicin (Dox) cytotoxicity and maintain antioxidant cellular defense [103]. On shrimps *Litopenaeus vannamei*, GHRP-6 treatment increased total hemocyte counts, a potential indicator of crustacean immune response [104]. This peptide also possesses anti-inflammatory, antioxidant, and cytoprotective properties [105].
Studies carried out by Martinez *et al.* (2016) demonstrated that GHRP-6 (0.1 µg/gram of body weight) treatment can increase lectin titters and the number of intestinal intraepithelial lymphocytes in *Oreochromis sp.* larvae via oral administration [106]. Likewise, GHRP-6 (0.2 µg/gram of body weight) affects the positive regulation of the transcription levels of three piscidin-like antimicrobial peptides (Oreochromicin I, II and III) and granzyme in a tissue-dependent manner, as well as an improvement in the antimicrobial activity of the serum and the decrease in the bacterial load of *Pseudomonas aeruginosa*, when injected intraperitoneally [107]. Given that dietary supplementation with GHRP-6 (500 µg GHRP-6/kg of feed) improves growth performance in juvenile gilthead sea bream [108], exploring its immunostimulatory potential via oral administration could be of significant interest.
Other analogs of Ghrelin may also serve as treatments to enhance the immune system, growth, and other physiological functions. According to a previous study by Martinez *et al.* (2016), therapy with A228 significantly increased the body weight of tilapia larvae and enhanced superoxide production in tilapia peripheral blood leukocyte cultures [109]. Moreover, the peptide A233 also induces GH secretion, induces superoxide production in tilapia head–kidney leukocyte culture and has antiviral activity [110,111].

Future perspectives

Although significant progress has been made in understanding Ghrelin's immunomodulatory roles, key gaps remain in its mechanistic and comparative biology. The influence of Ghrelin in fish lymphocyte development needs further research, as do the molecular mechanisms behind Ghrelin's effects in teleost immunity. Most studies are limited to a single species. A broader focus is needed, especially considering species-specific effects due to different environments and, therefore, possibly, different pathogenic threats. Furthermore, extrapolations from the predominant research in mammals to similar research in fish could be complicated due to evolutionary and physiological divergence. Rather than direct cross-species comparisons, a robust evolutionary framework could better contextualize these differences.
Future studies regarding fish must further clarify Ghrelin's molecular mechanisms regulating immune responses in diverse species. Comparative analyses across taxa might uncover species-specific adaptations, therefore, making tailored ghrelin-based treatments that could be more promising for aquaculture. Direct research into Ghrelin's effects on leukocyte function (e.g., antibody production) and GHS-R1a knockout models (e.g., CRISPR-Cas9 in zebrafish) could clarify receptor-specific versus GH-independent immune modulation, complementing current findings on phagocytosis and cytokine regulation. Transcriptomic profiling of immune cells exposed to acyl/des-acyl ghrelin may reveal novel pathways (e.g., NF-κB, JAK-STAT), while cross-species comparisons (e.g., tilapia vs. gar or tilapia vs. mice) could assess evolutionary conservation of these mechanisms. This could prove conserved immunomodulatory functions of Ghrelin across vertebrates, particularly its anti-inflammatory and antimicrobial properties in fish, and offer translational insights for human medicine—potentially informing therapies for inflammatory diseases, sepsis, or immune aging. Furthermore, its role in lymphocyte development and stress adaptation could bridge comparative immunology and regenerative medicine, where Ghrelin's tissue-protective effects are increasingly explored.
Given aquaculture's challenges, larval immune studies and stress experiments (e.g., Ghrelin's role in crowding-induced immunosuppression) would clarify its potential to enhance resilience, particularly under climate change pressures. Finally, quantifying ghrl expression in the wild versus farmed populations and its possible relation to immune parameters could uncover domestication impacts on immune function. Additionally, research should explore more the applications of novel ghrelin mimetics with improved stability and targeted effects, like GHRP-6. Such multidisciplinary approaches, spanning molecular, ecological, and industry-relevant frameworks, would advance Ghrelin's application as a sustainable alternative to antibiotics.

Future applications in aquaculture should prioritize standardized protocols for ghrelin administration, including intraperitoneal injections (20–200 ng/g body weight, as effective in tilapia), oral delivery similar to that of GHRP-6-supplemented feed (500 µg GHRP-6/kg of feed), or immersion treatments for larvae. Combining ghrelin analogs with probiotics or vaccines could also result in even better results by enhancing fish's immune system. Industry-scale adoption will require cost-effective production of stable mimetics (e.g., GHRP-6), slow-release formulations, and regulatory approval. Pilot trials in commercial species (e.g., salmon, shrimp) must validate these approaches under farm conditions to bridge lab findings to aquaculture in fish farms.

CONCLUSIONS

The production of healthy fish is essential for aquaculture profitability. As the aquaculture industry grows, so do the possibilities for the emergence and spread of infectious diseases. Pathogens cause an estimated annual loss of 6 trillion USD to the aquaculture sector worldwide [112]. The most common treatment for these pathogens is antibiotics. However, antibiotic resistance has become an increasingly pressing matter [113]. This searches for alternative therapies to enhance fish immune response, such as Ghrelin, which is a crucial need.

Ghrelin is a multifunctional peptide hormone, first identified for regulating growth hormone release and appetite. Recent research has revealed its critical part in vertebrate immune system modulation. The wide expression across tissues and receptor presence in immune cells underscore its immunomodulatory capacity. This hormone orchestrates diverse immune functions—from phagocytosis and cytokine balance to thymic development and antimicrobial defense—positioning it as a promising immunotherapeutic agent [3]. This hormone regulates physiological processes in fish while dynamically shaping immune responses [8,16,77,85]. Its effects include augmented phagocytosis, precise cytokine regulation, and antimicrobial peptide synthesis, stimulating host defenses. Its anti-inflammatory actions further highlight a therapeutic potential for immune enhancement. Additionally, ghrelin mimetics (e.g., GHRP-6, A233, A228) could offer an alternative to antibiotics used for aquaculture. For instance, GHRP-6 improves growth metrics, immune performance, and pathogen resistance, acting as both a growth promoter and immunostimulant [106-108].

The ghrelin-immune interplay in fish demands deeper research to unlock its full therapeutic value. Harnessing Ghrelin and its analogs could revolutionize sustainable aquaculture by optimizing fish health and productivity, presenting itself as a sustainable alternative to antibiotics against pathogenic infections. Therefore, considering the current worldwide antibiotic resistance crisis, reducing significant economic losses in the industry and becoming a possible treatment. As evidence accumulates, ghrelin immunology will probably remain an ever-growing area of research, yielding innovations for aquaculture efficiency and animal welfare.

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