# Research Advances on Ghrelin' s Regulation of Feeding in Fish (Postprint)

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## Abstract

Ghrelin, also known as growth hormone-releasing peptide, is a brain-gut peptide that promotes feeding in fish. Studies have reported that fasting or the preprandial state can elevate Ghrelin expression levels in fish, and central or peripheral administration of Ghrelin can increase feed intake. Based on research progress regarding Ghrelin in mammals and fish, this paper elaborates on the structure, tissue distribution, and mechanisms underlying Ghrelin' s regulation of feeding in fish, providing a theoretical foundation for future research and practical applications in fish feeding regulation and growth.

## Full Text

## Preamble

### Research Progress of Ghrelin on Feeding Regulation in Fish Species

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**Abstract:** Ghrelin, also known as growth-hormone-releasing peptide, functions as a brain-gut peptide that promotes feeding in fish. Studies have reported that fasting or pre-feeding conditions can elevate Ghrelin expression levels in fish, while central or peripheral administration of Ghrelin increases feed intake. Based on research progress in mammals and fish, this paper reviews the structure, tissue distribution, feeding regulation, and related mechanisms of Ghrelin in fish, providing theoretical references for future research and production practices related to feeding regulation and growth in fish.

**Keywords:** Ghrelin; growth-hormone-releasing peptide; fish; feeding; appetite factor

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Feeding maintains homeostasis and promotes growth in animals [1]. Feed intake is primarily regulated through interactions between orexigenic and anorexigenic factors (including neuropeptides, monoamines, gastrointestinal peptides, and hormones) in both central and peripheral feeding systems [2]. Ghrelin, also known as growth-hormone-releasing peptide, represents one of the important orexigenic factors in organisms [3]. In 1999, Japanese researcher Kojima et al. [3] identified a 28-amino-acid peptide in rats that stimulates growth hormone (GH) release, naming it growth-hormone-releasing peptide. Subsequent tissue expression analysis revealed its presence in the stomach and hypothalamus. In 2000, Wajnrajch et al. [4] detected Ghrelin expression in the human stomach and hypothalamus, establishing it as a brain-gut peptide [3]. Beyond rats and humans, Ghrelin has also been identified in other mammals [5-7]. As an orexigenic factor, Ghrelin has become a research hotspot in fish feeding-related fields. This paper focuses on elucidating the regulation of feeding by Ghrelin and its mechanisms in fish, aiming to provide theoretical foundations for research and production practices in fish feeding regulation and growth.

### 1.1 Ghrelin Gene Structure

Ghrelin genes have been successfully identified in multiple species. In mammals, the Ghrelin gene typically contains 5 exons and 4 introns (Figure 1 [Figure 1: see original paper]-A). Sequence alignment of genes from humans [4], rats [3], and mice [5] revealed high homology in the 19 bp region of exon 1 and the TATA box-like sequence in the 5' promoter region, suggesting structural conservation of the Ghrelin gene in mammals. In fish, the earliest report came in 2002 when Unniappan et al. [8] cloned the Ghrelin gene in goldfish (*Carassius auratus*) using rapid amplification of cDNA ends (RACE), which contained 4 exons and 3 introns (Figure 1-B). Similar structures have been found in Mozambique tilapia (*Oreochromis mossambicus*) [9], Nile tilapia (*Oreochromis niloticus*) [10], black porgy (*Acanthopagrus schlegeli*) [11], and zebrafish (*Barchydanio rerio*) [12]. However, some fish species such as rainbow trout (*Oncorhynchus mykiss*) [13], channel catfish (*Ietalurus punctatus*) [14], and Atlantic salmon (*Salmo salar*) [15] possess Ghrelin gene subtypes (Ghrelin1 and Ghrelin2) comprising 5 exons and 4 introns, similar to the mammalian Ghrelin gene structure. Whether these differences relate to distinct life habits or biological functions in different fish species requires further investigation.

A: the structure of Ghrelin gene in mammals and a part of fish species including *Oncorhynchus mykiss*, *Ietalurus punctatus* and *Salmo salar*; B: the structure of Ghrelin gene in a part of fish species including *Carassius auratus*, *Oreochromis mossambicus*, *Oreochromis niloticus*, *Acanthopagrus schlegeli* and *Barchydanio rerio*.

**Figure 1 The structure of Ghrelin gene**

### 1.2 Ghrelin Protein Structure

The Ghrelin protein structure comprises a signal peptide, mature peptide, and C-terminal peptide region, with the mature peptide containing 17–28 amino acids. The mature peptide shows high conservation across different species, with serine (Ser) at the third position that can undergo acylation modification, followed by a glycine (Gly)-arginine (Arg)-Arg or Gly-Arg amidation signal structure after the mature peptide. In mammals, Ghrelin protein structures have been identified in humans [4] and rats [3]. In fish, goldfish Ghrelin precursor protein was first discovered to contain 103 amino acids with a 22-amino-acid mature peptide, with the amidation signal located after the 19th amino acid of the mature peptide [8]. Tilapia [9-10], black porgy [11], and zebrafish [12] each contain one Ghrelin protein structure. Rainbow trout and Atlantic salmon possess two Ghrelin protein structures due to having two Ghrelin gene subtypes, with rainbow trout mature peptides containing 24 and 21 amino acids [13], and Atlantic salmon mature peptides containing 23 and 20 amino acids [15]. Although channel catfish also has two Ghrelin gene subtypes, only one Ghrelin protein structure has been identified, with its mature peptide containing 22 amino acids [14]. The first seven amino acids of the mature peptide are highly conserved across species, and the mature peptide region of fish Ghrelin shows relatively high conservation. This conservation may be related to the biological functions of Ghrelin and requires further investigation.

## 2 Ghrelin Tissue Distribution

Ghrelin is widely distributed in both central and peripheral systems across species. In mammals, Ghrelin expression is highest in brain tissue of the central nervous system and is extensively expressed in the stomach and intestine of the peripheral system [3,16-17]. Ghrelin tissue distribution has been reported in various fish species including goldfish [8], Japanese eel (*Anguilla japonica*) [18], black porgy [11], zebrafish [12], *Schizothorax* species [19-20], and red-bellied piranha (*Pygocentrus nattereri*) [21]. Using Northern blot, Unniappan et al. [8] detected Ghrelin mRNA expression in goldfish intestinal tissue, and further RT-PCR analysis revealed the highest expression in the spleen, followed by the intestine, but no expression in the midbrain, hindbrain, pituitary, or other peripheral tissues. Kaiya et al. [18] found through RT-PCR that Japanese eel Ghrelin was expressed in the brain, heart, stomach, intestine, trunk kidney, and head kidney, with the highest expression in the stomach and foregut. Yeung et al. [11] found that black porgy Ghrelin was abundantly expressed only in the stomach. However, Amole et al. [12] discovered Ghrelin distribution in zebrafish brain and hepatopancreas in addition to the stomach. Furthermore, studies have reported higher Ghrelin expression in the brain and intestine of *Schizothorax prenanti* [20] and abundant expression in the intestine of *Schizothorax davidi* [19]. Using quantitative real-time PCR (qRT-PCR), Volkoff [21] found abundant Ghrelin expression in the brain, digestive tract, liver, and spleen of red-bellied piranha.

The widespread expression pattern of Ghrelin across different tissues and species suggests multiple biological functions. Abundant expression in brain tissue may indicate its involvement in central regulation of various life activities, while high expression in the digestive tract may suggest roles in feeding, digestion, and absorption.

### 3 Ghrelin Regulates Fish Feeding

The regulation of feeding function by Ghrelin represents a major research focus, with extensive studies in mammals and some investigations in fish. Research has concentrated on two main aspects: (1) effects of feeding strategies on Ghrelin expression levels, and (2) effects of Ghrelin injection on fish feed intake.

### 3.1 Effects of Feeding Strategies on Ghrelin Expression

Feeding strategies such as pre- and post-feeding conditions and refeeding after fasting can alter Ghrelin expression levels. Researchers have found that orexigenic factors show increased expression before feeding or after fasting, and decreased expression after feeding, while anorexigenic factors display the opposite pattern [22]. In mammals, Ghrelin expression in both central and peripheral tissues decreases after feeding and increases after short-term or long-term fasting, with expression decreasing again upon refeeding after prolonged fasting [23-25]. In fish, Wei et al. [20] found that Ghrelin expression in the brain of *Schizothorax prenanti* significantly decreased at 1.5 and 9.0 h post-feeding, and intestinal Ghrelin expression also significantly decreased at 6 h post-feeding. Similarly, Ghrelin expression significantly decreased at 1 and 3 h post-feeding in gibel carp (*Carassius auratus gibelio*), while significantly increasing after 7 days of fasting [26]. Amole et al. [12] reported that Ghrelin mRNA expression in both brain and intestine of zebrafish significantly increased after 3, 5, and 7 days of fasting, returning to normal feeding group levels upon refeeding.

Additionally, studies on *Schizothorax davidi* [27], rohu (*Labeo rohita*) [28], and grass carp (*Ctenopharyngodon idellus*) [29] have also found that long-term fasting significantly increases Ghrelin expression, which returns to normal levels after refeeding. These findings demonstrate that Ghrelin functions as an orexigenic factor in fish feeding regulation.

In contrast, reports on Nile tilapia [10], rainbow trout [30], and channel catfish [31] have shown that feeding strategies do not affect Ghrelin expression levels. Given the diverse fish species, taxonomic positions, and feeding patterns, whether Ghrelin functions as a feeding regulator in all fish species requires further investigation.

### 3.2 Effects of Ghrelin Injection on Fish Feed Intake

Central and peripheral Ghrelin injections provide further insights into its regulatory function in feeding. Mammalian studies have found that both central and peripheral Ghrelin injections promote feeding through its receptor, growth hormone secretagogue receptor (GHS-R) [32-36]. Fish studies have yielded similar results. Unniappan et al. [8] reported that intracerebroventricular injection of Ghrelin in goldfish significantly increased feed intake within 1 h. Matsuda et al. [37] found that both intracerebroventricular and intraperitoneal injection of acylated Ghrelin in goldfish significantly increased feed intake within 1 h. Shepherd et al. [38] reported that intravenous Ghrelin injection in rainbow trout significantly increased feed intake.

Miura et al. [39] found that both intraperitoneal and intracerebroventricular injection of acylated Ghrelin significantly increased feed intake in goldfish, while injection of des-acyl Ghrelin produced no significant changes. Tinoco et al. [40] reported that intraperitoneal Ghrelin injection for 7 days significantly increased feed intake in juvenile brown trout. Additionally, Velasco et al. [41] found that Ghrelin injection significantly increased feed intake in rainbow trout after 24 h. Studies on Senegalese sole also demonstrated that Ghrelin promotes feeding [42].

However, some studies have reported different results. Saito et al. [43-44] found that intracerebroventricular injection of various Ghrelin doses in newly hatched chicks caused dose-dependent significant decreases in feed intake within 2 h. Jönsson et al. [30] reported no significant changes in feed intake within 12 h after intraperitoneal Ghrelin injection in rainbow trout. Jönsson et al. [45] also found that long-term (14 days) intraperitoneal Ghrelin injection in juvenile rainbow trout significantly decreased feed intake. In summary, Ghrelin can promote feeding in fish, but effects vary among species, possibly related to drug source, administration method, injection dose, and timing.

### 4 Mechanisms of Ghrelin Regulation of Fish Feeding

The mechanisms underlying Ghrelin' s regulation of feeding remain unclear. Based on existing mammalian and fish studies, this section analyzes the mechanisms from three perspectives: (1) Ghrelin regulation of gastrointestinal motility in fish, (2) the relationship between Ghrelin and gastric digestive factors, and (3) the relationship between Ghrelin and other appetite-regulating factors.

### 4.1 Ghrelin Regulation of Gastrointestinal Motility

Tissue distribution studies have revealed abundant Ghrelin expression in the gastrointestinal tract, while functional studies indicate its involvement in feeding regulation. Given its high structural similarity to motilin [34], some researchers have proposed that Ghrelin regulates feeding by affecting gastrointestinal motility. In mice, GHS-R knockout decreased gastric emptying, while central and peripheral Ghrelin injection promoted gastric emptying and increased gastrointestinal motility through GHS-R. Few studies have investigated Ghrelin's effects on gastrointestinal motility in fish, and no reports have examined its relationship with gastric emptying. Olsson et al. [46] used force displacement transducers in zebrafish to record increased intestinal tonic contraction frequency with increasing Ghrelin concentrations. However, Kitazawa et al. [47] found that Ghrelin did not induce significant gastric or intestinal contractions in rainbow trout, and only caused minor intestinal contractions in goldfish with minimal effect. The regulation of gastrointestinal motility by Ghrelin in fish requires further investigation.

### 4.2 Relationship Between Ghrelin and Gastric Digestive Factors

Ghrelin is primarily secreted in the gastrointestinal tract, the main site of digestion. Therefore, Ghrelin may be associated with gastric digestive factors such as gastric acid and digestive enzymes. Masuda et al. [48] found that intravenous Ghrelin injection in rats increased gastric acid secretion. Similarly, Date et al. [49] reported that intracerebroventricular Ghrelin injection dose-dependently increased gastric acid secretion in rats. In contrast, de la Cour et al. [50] found that neither acylated nor des-acyl Ghrelin altered gastric G cell acid secretion in rats. The regulatory effects of Ghrelin on gastric acid secretion require further study. Additionally, appropriate Ghrelin levels can increase pepsin and hepatic lipase activities in mammals. Du et al. [51] reported no significant changes in pepsin activity after treating gastric mucosal cells with Ghrelin for 4 h. However, Du et al. [52-53] found that $1\times10^{3}$ mol/L Ghrelin significantly increased pepsin activity in rat gastric mucosal cells, and intramuscular injection of recombinant Ghrelin in rats significantly enhanced pepsin activity. Nieminen et al. [54] reported that intraperitoneal Ghrelin injection in tundra voles for 4 days significantly increased hepatic lipase activity. No studies have examined Ghrelin' s effects on amylase activity, and research on the relationship between Ghrelin and digestive enzymes in fish remains unexplored.

### 4.3 Interaction Between Ghrelin and Other Appetite-Regulating Factors

Ghrelin is abundantly expressed in the hypothalamic nuclei of the central nervous system and the gastrointestinal tract. As a brain-gut peptide, Ghrelin interacts with various appetite-regulating factors produced in hypothalamic nuclei [neuropeptide Y (NPY)/agouti-related protein (AgRP), proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART), corticotropin-releasing factor (CRF), orexin, and mammalian target of rapamycin (mTOR)] and peptide hormones from peripheral or neuroendocrine systems [leptin, Nesfatin-1, GH, and prolactin (PRL)] to regulate feeding (Figure 2 [Figure 2: see original paper]). Among these, NPY/AgRP, orexin, and GH promote feeding, while POMC/CART, CRF, mTOR, leptin, Nesfatin-1, and PRL inhibit feeding.

**4.3.1 Ghrelin and NPY/AgRP, POMC/CART** Ghrelin may regulate feeding through the NPY/AgRP and POMC/CART signaling pathways. Asakawa et al. [34] demonstrated in mice that Ghrelin promotes feeding through central NPY and its receptor Y1. Miura et al. [55] found in goldfish that intracerebroventricular or intraperitoneal Ghrelin injection significantly increased feed intake and NPY mRNA expression, while co-injection of Ghrelin with the Y1 receptor antagonist BIBP3226 significantly decreased feed intake. Gao et al. [56] showed that feeding Ghrelin-supplemented diets for 8 weeks significantly increased brain NPY mRNA expression in grouper (*Epinephelus coioides*). Ariyasu et al. [57] found that decreased circulating Ghrelin did not significantly alter feed intake or hypothalamic neuropeptide (NPY/AgRP and POMC) expression in mice. Qi et al. [58] reported that chronic overexpression of Ghrelin in the arcuate nucleus increased feed intake and body weight significantly during the first 3 weeks, with no significant change in feed intake from week 4 onward, though body weight remained significantly elevated. After 6 weeks of Ghrelin overexpression, NPY expression in the arcuate nucleus showed no significant change, while POMC expression increased significantly, possibly representing a compensatory mechanism to counteract Ghrelin' s stimulatory effects on feeding. Velasco et al. [59] found that intracerebroventricular Ghrelin injection in rainbow trout significantly decreased hypothalamic POMC and CART expression while significantly increasing NPY and AgRP expression. The mechanisms by which Ghrelin regulates feeding through NPY/AgRP and POMC/CART signaling pathways require further investigation.

**4.3.2 Ghrelin and CRF** Ghrelin may regulate feeding through the CRF signaling pathway. Asakawa et al. [60] reported that intraperitoneal Ghrelin injection in mice significantly increased hypothalamic CRF mRNA expression. Jönsson et al. [45] found that intracerebroventricular Ghrelin injection in juvenile rainbow trout significantly decreased feed intake, which was restored by co-injection with the CRF receptor antagonist ahCRF. However, limited research exists on Ghrelin' s regulation of feeding through the CRF signaling pathway, necessitating further studies.

**4.3.3 Ghrelin and Orexin, mTOR** Ghrelin interacts with orexin and mTOR signaling pathways to regulate feeding. Toshinai et al. [61] reported that Ghrelin induced orexin neuron immunoreactivity in mice. Injection of anti-orexin alone significantly decreased feed intake, while co-injection with Ghrelin resulted in feed intake significantly lower than the Ghrelin-only group but still significantly higher than the control group, indicating that anti-orexin can partially attenuate Ghrelin-induced feeding increase. Miura et al. [62] found that intracerebroventricular injection of the orexin receptor antagonist SB334867 decreased feed intake in goldfish, which returned to normal levels when co-injected with Ghrelin. Additionally, intracerebroventricular Ghrelin injection significantly increased orexin mRNA expression in goldfish diencephalon. Penney et al. [63] reported that intraperitoneal Ghrelin injection significantly increased feed intake within 30 min in cavefish (*Astyanax fasciatus mexicanus*), with significant increases in both mTOR and orexin expression in whole brain. Therefore, both central and peripheral Ghrelin injections increase orexin and mTOR expression, though whether peripheral Ghrelin injection regulates these pathways through feedback mechanisms requires further investigation.

**4.3.4 Ghrelin and Leptin, Nesfatin-1** Few studies have reported on the relationship between Ghrelin and appetite-regulating factors in peripheral tissues. Ghrelin receptors and leptin receptors are co-expressed in over 90% of neurons in the mouse arcuate nucleus [64], and antagonistic effects between leptin and Ghrelin systems have been observed in regulating feeding and energy metabolism in both mammals and fish [65-68]. Some studies have reported that leptin can alter expression of appetite-regulating factors such as AgRP and NPY in patterns opposite to those induced by Ghrelin [69-70]. Additionally, Ghrelin injection significantly increased feed intake in leptin-deficient mice, which returned to control levels when Ghrelin and leptin were co-injected [71]. Kohno et al. [72] also showed that leptin injection could resist Ghrelin-induced feeding increase in mice, with no significant difference in feed intake between the co-injection group and control group. Toshinai et al. [25] found that intraperitoneal leptin injection significantly increased gastric Ghrelin mRNA expression in mice. Furthermore, Shimizu et al. [73] reported that intraperitoneal Nesfatin-1 injection significantly inhibited feeding in mice, while Stengel et al. [74] used confocal microscopy to demonstrate co-localization of Ghrelin and Nesfatin-1 precursor nucleobindin 2 (NUCB2) in gastric X/A-like cells of rats. Therefore, Ghrelin may interact with peripheral appetite-regulating factors such as leptin and Nesfatin-1, though whether they co-regulate feeding in fish and the underlying pathways remain unclear.

**4.3.5 Ghrelin and GH, PRL** Ghrelin injection increases circulating levels of feeding-related hormones such as GH and PRL. Date et al. [75] reported that intracerebroventricular Ghrelin injection in rats increased plasma GH levels. Kaiya et al. [18] found that treating isolated pituitary cells from Japanese eel with Ghrelin (0.1, 1.0, 10.0 nmol) significantly increased GH release at high doses and significantly increased PRL release at all doses. Riley et al. [76] reported that 21 days of intraperitoneal Ghrelin injection in Mozambique tilapia significantly increased pituitary GH mRNA expression. Shepherd et al. [38] also reported that intraperitoneal Ghrelin injection in rainbow trout significantly increased plasma GH levels. Therefore, Ghrelin may regulate feeding by influencing GH and PRL secretion.

In summary, the relationships between Ghrelin and other appetite factors are as follows: (1) Ghrelin can regulate feeding by acting on central nervous system signaling pathways including NPY/AgRP and POMC/CART, CRF, orexin, and mTOR; (2) Ghrelin may interact with peripheral appetite factors such as leptin and Nesfatin-1; and (3) Ghrelin can increase circulating levels of hormones such as GH and PRL.

Ghrelin: growth-hormone-releasing peptide; GHS-R: growth hormone secretagogue receptor; POMC: proopiomelanocortin; NPY: neuropeptide Y; CRF: corticotropin-releasing factor; mTOR: mammalian target of rapamycin; GH:

growth hormone; PRL: prolactin. Down arrow: inhibiting food intake; up arrow: promoting food intake; black font: Ghrelin promotes related gene expression; white font: inconsistent research results or no direct study.

**Figure 2 The mechanisms of Ghrelin regulation on feeding**

### 5 Summary

As a brain-gut peptide, Ghrelin is abundantly expressed in brain tissue of the central nervous system and in the stomach of the peripheral system, representing an important orexigenic factor in both central and peripheral feeding regulation systems. Studies have shown that fasting significantly increases Ghrelin expression in mammals and fish, while central or peripheral Ghrelin injection promotes feed intake. Current research on Ghrelin' s feeding regulation has primarily focused on humans and rats in mammals, and on cyprinids in fish, with limited data available for other fish species. Given that Ghrelin is a research hotspot in fish feeding-related fields, its feeding regulation and mechanisms remain insufficiently understood. Future research should build upon mammalian findings to deeply explore Ghrelin' s feeding regulation mechanisms in different fish species, providing theoretical foundations for fish feeding regulation and production applications.

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*Note: Figure translations are in progress. See original paper for figures.*