Research Advances in Fish Appetite Regulation: Postprint

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Abstract

The search for fish meal alternatives has become an imperative for the sustainable development of carnivorous fish aquaculture, and improving the utilization efficiency of these alternatives by fish represents a bottleneck in fish meal replacement research. Appetite is a critical factor influencing fish meal replacement, and the appetite regulatory network integrates feeding behavior through signal transduction of various appetite-regulating factors (including orexigenic and anorexigenic factors) and central signaling pathways. This review summarizes the regulation of appetite by common appetite-regulating factors and central signaling pathways, aiming to provide a reference for research on appetite regulation in fish.

Full Text

Preamble

Appetite Regulation in Fishes

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Abstract: Finding sustainable alternatives to fish meal has become imperative for the continued development of carnivorous fish aquaculture, yet improving the utilization efficiency of these substitutes remains a critical bottleneck. Appetite represents a key factor affecting fish meal replacement, with the appetite regulation network integrating multiple appetite-regulating factors (including

orexigenic and anorexigenic factors) and central signaling pathways to modulate feeding behavior. This review synthesizes current knowledge on common appetite-regulating factors and central signaling pathways in fish, providing a reference for future research on appetite regulation in aquatic species.

Keywords: appetite; fishes; appetite regulation factors; central signaling pathway

The ultimate goal of aquaculture is to produce high-quality fish products with minimal input for maximum economic benefit while ensuring sustainable development. Since fish growth—central to economic viability—is closely linked to feed intake, and appetite is a primary determinant of intake, understanding the mechanisms governing feeding behavior is crucial for advancing aquaculture. While appetite research has predominantly focused on mammals, studies in fish remain relatively scarce and fragmented. Macro-level investigations have largely been limited to feeding rates or digestibility, and molecular-level work has primarily involved cloning genes for a few appetite-regulating factors without deeper exploration of the underlying signaling pathways. This review therefore examines common appetite-regulating factors and two major signaling pathways to provide a foundation for future fish appetite research.

1 Appetite-Regulating Factors

Appetite-regulating factors comprise orexigenic (appetite-promoting) and anorexigenic (appetite-suppressing) peptides secreted by the central nervous system or peripheral regulatory systems . Although their effects are short-term, they play vital roles in appetite control. Gastrointestinal organs, pancreatic islets, hepatic portal systems, and visceral adipose tissue sense energy status and transmit appetite-related signals to the central nervous system via neural and endocrine pathways, where the hypothalamus integrates these cues to dynamically regulate feeding. Key appetite-regulating factors studied in fish include leptin, neuropeptide Y (NPY), orexin, cocaine- and amphetamine-regulated transcript (CART), and cholecystokinin (CCK) [1-2].

Table 1 summarizes these factors, categorized by origin (central vs. peripheral nervous system) and function (orexigenic vs. anorexigenic). Orexigenic factors include melanin-concentrating hormone (MCH), growth hormone-releasing factor (GHRF), NPY, agouti-related protein (AgRP), orexins A and B, endocannabinoids, galanin, and endogenous opioids. Anorexigenic factors include CART, melanocortins, pro-opiomelanocortin (POMC), glucagon-like peptide-I (GLP-I), corticotropin-releasing factor (CRF), α-melanocyte-stimulating hormone (α-MSH), serotonin, neurotensin (NT), and thyrotropin-releasing hormone (TRH). Peripheral factors include ghrelin and glucose-dependent insulinotropic polypeptide (orexigenic), and leptin, CCK, peptide YY (PYY), pancreatic polypeptide (PP), oxyntomodulin (OXM), insulin, and urocortin (anorexigenic).

1.1 Leptin

Leptin is a protein hormone encoded by the obese (ob) gene and secreted by adipocytes, regulating energy balance and participating in metabolism, neuroendocrine function, angiogenesis, reproduction, and immune responses. It promotes cellular repair and helps restore homeostasis [3]. As a satiety signal, leptin primarily acts on feeding and satiety centers in the brain [4]. All physiological functions are mediated through binding to leptin receptors (LEPR) at a 1:1 ratio. LEPR, a product of the diabetes gene, belongs to the type I cytokine receptor family and exists as six isoforms (LEPRa-f) generated through alternative splicing [5]. Recent studies show leptin and LEPR are widely distributed across multiple tissues: leptin occurs in adipose and digestive tissues, while LEPR is found in brain, heart, placenta, liver, stomach, intestine, and taste buds [6]. Leptin binding to LEPR on taste cells inhibits sweet taste responses [7].

The leptin-mediated appetite regulation process is illustrated in [Figure 1: see original paper]. When body fat increases, leptin synthesis and secretion rise in adipose tissue, entering the bloodstream to bind LEPRe. This complex transports leptin to choroid tissue around the eyes, where it binds LEPRa, then delivers leptin to cerebrospinal fluid for binding with LEPRb in the hypothalamus. Leptin-Rb binding enhances POMC gene expression, increasing its cleavage product α-MSH, which binds melanocortin-4 receptors (MC4R) to suppress appetite. Reduced fat stores decrease leptin secretion, lowering POMC expression and α-MSH concentration, thereby increasing appetite [8-10]. This feedback loop maintains stable body weight; disruption through gene mutations leads to hyperphagia and obesity [11].

While early research focused on leptin' s effects on fat deposition in terrestrial animals, recent attention has shifted to its role in protein metabolism [12]. High-protein diets reduce energy intake and increase serum leptin in humans [13]. In growing female rats, a single intravenous injection of 10 g/kg human leptin decreased skeletal muscle protein synthesis without affecting protein degradation [14]. Chronic L-leucine supplementation increased plasma leptin and modulated muscle protein metabolism in growing rats [15]. Leucine also dose-dependently stimulated LEPR expression in C2C12 myotubes via the mTOR pathway, as rapamycin (20 ng/mL) completely blocked leptin-induced mTOR phosphorylation and significantly inhibited LEPR expression [16].

Fish leptin research has primarily involved gene cloning in species including zebrafish, grass carp, Atlantic salmon, tilapia, chum salmon, goldfish, pufferfish, rainbow trout, crucian carp, common carp, and striped bass [1,17], with limited studies on gene structure, recombinant expression, and function [18]. Unlike mammals, some fish possess two leptin isoforms (e.g., medaka, zebrafish, grouper). Fish LEPR also differs, with fewer isoforms: three in crucian carp [19] and five in Atlantic salmon [20]. Functionally, fish leptin reduces feed intake in goldfish [21], rainbow trout [22], and grass carp [23], promotes lipid catabolism

and β-oxidation while inhibiting lipogenesis in grass carp [24], and decreases hepatic glycogen in tilapia [25]. Leptin concentrations correlate with nutritional status and exert long-term energy balance regulation [20,26-27], though no reports address its role in fish protein and amino acid metabolism.

1.2 Ghrelin

Ghrelin is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). In mammals, it is primarily secreted by gastric A-like cells with minor intestinal production, and detected in lung, pancreas, hypothalamus, pituitary, kidney, liver, and muscle, while GHS-R is mainly distributed in central nervous tissues like hypothalamus and pituitary [28]. Ghrelin regulates immunity, water balance, gastric emptying, acid secretion, cell proliferation, memory, anxiety, sleep, energy expenditure, bone metabolism, reproduction, and cardiovascular function, with its most important roles being energy balance and growth hormone secretion [29]. The ghrelin gene typically contains four exons and three introns, encoding a 28-amino acid protein with an acylated medium-chain fatty acid side chain at serine-3. Only acylated ghrelin binds GHS-R to exert biological effects; des-acyl ghrelin cannot increase food intake but promotes insulin secretion without affecting gastric acid secretion and increases visceral fat accumulation [30-32]. Des-acyl ghrelin suppresses appetite by increasing hypothalamic CART and urocortin secretion [33]. Ghrelin acylation depends on ghrelin O-acyltransferase (GOAT) activity. The GOAT gene (~13.02 kb) encodes a 435-amino acid membrane protein that is highly conserved across species, with optimal activity at 37-50°C and pH 7.0-7.5. GOAT mutations cause anorexia nervosa in humans [29,34].

Human plasma ghrelin levels rise during fasting and decrease postprandially, but not after water ingestion, indicating gastric distension does not inhibit ghrelin secretion [35]. Both peripheral and central administration of acylated ghrelin rapidly stimulates feeding in rodents, with chronic injection causing obesity. Ghrelin secretion correlates positively with hunger duration and negatively with body weight. During starvation, ghrelin acylation is low while des-acylation is high [36]. Obese individuals have reduced ghrelin levels that increase with diet-induced weight loss, without ghrelin resistance [37]. However, some studies show postprandial ghrelin does not decrease or only slightly decreases in obese individuals, potentially contributing to continued feeding and obesity pathogenesis [38]. Protein, fat, and carbohydrate all affect ghrelin secretion: high carbohydrate and fat reduce ghrelin, while high protein increases it [39], though some studies find no effect of high-protein diets [40]. Medium-chain fatty acids most significantly regulate ghrelin and GOAT levels, increasing both acylation and overall levels [41], demonstrating nutritional status and medium-chain fatty acid content modulate ghrelin acylation.

Fish ghrelin research has been reviewed comprehensively [42]. Fish ghrelin structure (exon number, intron length, acylation) and distribution (concentrated in digestive tract with weak brain expression) differ from mammals. Ghrelin regu-

lates fish feeding primarily by promoting growth hormone secretion. Exogenous ghrelin injection increases food intake in tilapia [43] and goldfish [44], but inhibits appetite and growth in rainbow trout [45], suggesting ghrelin may be anorexigenic in some fish species. In tilapia, plasma ghrelin does not change significantly during starvation [46], indicating species-specific functions that require further investigation.

Given ghrelin' s active (acylated) and inactive (des-acylated) states and its primary central action, directly modulating ghrelin to improve fish appetite is challenging. Enhancing GOAT activity to increase ghrelin acylation may be a more reliable approach.

1.3 Cholecystokinin (CCK)

CCK is a gastrointestinal peptide hormone with widespread distribution and multiple biological functions. Digestive effects include stimulating pancreatic secretion and gallbladder contraction while delaying gastric emptying. In central and peripheral nervous systems, CCK suppresses feeding, reduces body temperature, and counteracts morphine and endorphin analgesia [47]. All effects are mediated through CCK receptors (CCK-A and CCK-B), G protein-coupled receptors with 50% homology. CCK-A receptors predominate in pancreatic acini, gallbladder, pyloric smooth muscle, and vagal afferent fibers, with limited brain distribution, and show 1,000-fold higher affinity for sulfated CCK. CCK-B receptors are mainly in brain and stomach, with higher affinity for non-sulfated CCK and gastrin. Only sulfated CCK exerts anorexigenic effects via CCK-A receptors [48]. CCK receptor neurons concentrate in the nucleus tractus solitarius, midbrain pons, and hypothalamus; CCK injection into these areas markedly suppresses appetite, indicating CCK acts as a neurotransmitter or neuromodulator in central feeding regulation [49-50]. Potato protein extract suppresses appetite and increases plasma CCK more effectively than casein or soy protein in rats [51], with similar results for soy protein extract in mice [52].

Fish CCK immunoreactivity and mRNA are detected in gastrointestinal tract, nervous system, and liver. CCK mRNA sequences are available for zebrafish, Atlantic salmon, Atlantic cod, red drum, rainbow trout, tilapia, grass carp, and spiny dogfish, with studies focusing on nutritional status effects [27,53]. CCK is a key appetite regulator in fish: oral CCK administration suppresses appetite and modulates macronutrient intake in European sea bass, effects reversible by the CCK antagonist proglumide [54].

1.4 Neuropeptide Y (NPY)

NPY is a highly conserved 36-amino acid peptide with a hairpin structure, named for tyrosine residues at both termini. Structurally similar to pancreatic polypeptide and PYY, it belongs to the pancreatic polypeptide family. Functions include promoting feeding, hormone secretion, thermoregulation, circadian rhythms, sexual behavior, and mood [55]. Six receptor subtypes (Y1-Y6) exist,

with Y1 and Y5 controlling appetite; their antagonists inhibit feeding. Direct NPY injection into mouse hypothalamus or cerebral ventricles increases food intake and body weight while reducing heat production [56-57].

Fish NPY research, though less extensive than in mammals, has progressed significantly. NPY genes have been cloned in zebrafish, rainbow trout, Atlantic salmon, goldfish, Atlantic cod, orange-spotted grouper, channel catfish, southern catfish, and Japanese eel [1]. Similar to mammals, fish NPY promotes feeding through specific receptors, as demonstrated in goldfish and zebrafish studies, where it increases food intake, reduces activity, and affects mood, vasoconstriction, circadian rhythms, and pituitary hormone secretion [58]. In goldfish, Y1 receptor agonists enhance NPY-induced feeding, while Y2 agonists show no effect after 2 hours, suggesting NPY regulates feeding via Y1-like (Y1/Y5) rather than Y2 receptors [59]. In zebrafish, 7-day starvation significantly elevates hypothalamic NPY mRNA, intracerebroventricular NPY injection increases feeding, and Y1 receptor inhibitor injection decreases feeding [60].

1.5 Orexin

Orexin is a hypothalamic neurotransmitter/neuromodulator that stimulates feeding and increases body weight. Two forms exist: orexin A and orexin B, with orexin A being more potent. Intracerebroventricular or direct lateral hypothalamic injection increases rodent food intake dose-dependently [61]. Orexin correlates with blood glucose and triglyceride levels, inhibiting intestinal glucose absorption and affecting gastric emptying [62]. High-fat feeding increases orexin gene expression in correlation with elevated triglycerides, suggesting orexin is an obesity-susceptible peptide responding to increased circulating lipids [63].

Orexin gene sequences have been reported in *Xenopus*, zebrafish, pufferfish, tilapia, Atlantic salmon, goldfish, Atlantic cod, medaka, and grouper, with predominant brain distribution [64]. In goldfish, orexin A agonist injection into cerebral ventricles stimulates feeding and increases orexin A mRNA expression [65], with orexin A being more effective than orexin B in zebrafish [66]. Brain injection of 28 pmol/g orexin A increases appetite in the wrasse *Thalassoma pavo* [67], while starvation enhances orexin precursor mRNA expression in grouper pituitary [68], confirming orexin' s orexigenic role in fish.

1.6 Peptide YY (PYY)

PYY, or peptide tyrosine-tyrosine, is secreted by intestinal L-cells, with highest immunoreactivity in rectum and lower levels in small intestine. It is also expressed in human hypothalamic medulla and rat central nervous system including hypothalamus, spinal cord, and medulla. As a peptide hormone, PYY is released postprandially into circulation to inhibit gastric acid secretion, delay gastric emptying, and slow intestinal chyme transit, thereby suppressing appetite. Two circulating forms exist (PYY3-36 and PYY3-37), with PYY3-36

being predominant [69]. PYY serves as a satiety marker: high-protein diets significantly elevate plasma and tissue PYY levels in humans, correlating with satiety. Long-term high-protein diets reduce body weight and promote PYY synthesis in mice, and exogenous PYY injection suppresses appetite for weight loss [70]. Obese individuals show blunted postprandial PYY elevation without the normal 2-hour peak [71], while high-protein meals significantly increase PYY [72].

In fish, PYY is highly expressed in sea bass brain [73], with grass carp foregut PYY mRNA peaking 3 hours post-feeding [74]. Starvation significantly reduces PYY mRNA expression in Siberian sturgeon, while PYY injection suppresses appetite [75], indicating PYY' s anorexigenic role in fish.

1.7 Glucagon-Like Peptide-I (GLP-I)

GLP-I is an incretin secreted by intestinal L-cells, with receptors highly expressed in hypothalamic arcuate nucleus, paraventricular nucleus, and supraoptic nucleus. GLP-I binding promotes glucose-dependent insulin secretion, pancreatic β-cell proliferation and differentiation, inhibits apoptosis, delays gastric emptying, and protects β-cell function without causing weight gain or hypoglycemia [76]. High-protein diets increase human plasma GLP-I [77], though some studies show no effect [40], possibly due to varying carbohydrate content since GLP-I more potently modulates carbohydrates [78].

Unlike other vertebrates, fish GLP-I does not function as an incretin; its primary target is the liver, where it regulates glycogen synthesis, gluconeogenesis, and lipid metabolism before acting in intestine and brain [79]. In channel catfish, intracerebroventricular GLP-I injection (0.25 ng/g) reduces feeding by 50%, while intraperitoneal or intravenous injection has no effect [80]. GLP-I regulation has been systematically studied only in rainbow trout, where peripheral injection causes persistent hyperglycemia and central injection increases plasma glucose, suggesting blood glucose regulation depends partly on vagal and visceral GLP-I secretion [81]. The fish GLP-I regulatory model differs from the mammalian gut-pancreas-brain axis, possibly representing a more primitive gut-brain axis [82]. GLP-I-induced hyperglycemia and lack of incretin function may relate to poor glucose tolerance in carnivorous fish [83].

1.8 Cocaine- and Amphetamine-Regulated Transcript (CART)

CART is a hypothalamic neuropeptide that suppresses appetite, potentially acting through the lateral hypothalamic orexin system and considered an important anorexigenic peptide in humans [84]. Hypothalamic CART injection does not stimulate serotonin secretion in mice, and starvation significantly reduces CART mRNA expression while refeeding increases it, confirming its role as a feeding inhibitor [85]. The hypothalamic CART system is regulated by leptin and insulin, showing synchronized changes with leptin [86].

Atlantic salmon CART contains 118 amino acids with a 742 bp mRNA, expressed mainly in brain and eyes with 3-6 low-abundance isoforms [87]. In Atlantic salmon, CART is a key regulator of temperature-dependent feeding, with CART mRNA expression negatively correlating with feeding rate [88]. In channel catfish, hindbrain CART levels are regulated by starvation, 2-deoxy-D-glucose (a glucose metabolism antagonist), glucose, leptin, and insulin: 48-hour starvation and 2-deoxy-D-glucose reduce CART immunoreactivity; glucose enhances it; leptin and insulin positively regulate CART, consistent with in vitro slice incubation results [86].

1.9 Insulin

Insulin is a protein hormone secreted by pancreatic β-cells in response to glucose, lactose, ribose, arginine, glucagon, and other stimuli. Comprising A and B chains with 51 amino acid residues, insulin enhances glucose uptake and utilization while promoting protein and lipid anabolism. As an adiposity signal, insulin may share functions with leptin [89]. Although not released from adipocytes, basal insulin levels correlate with body fat. High-protein meals increase blood amino acid concentrations and insulin secretion [90]. Plasma insulin varies with carbohydrate intake, with high carbohydrates elevating levels [70]. Different protein types differentially affect insulin secretion: milk protein more effectively stimulates human insulin secretion than fish or soy protein [91], suggesting more digestible proteins are stronger insulin secretagogues. Leucine, tyrosine, glutamate, betaine, and branched-chain amino acids also stimulate insulin secretion [90,92-94].

Fish insulin research has focused primarily on glucose metabolism, with insufficient plasma insulin considered a major factor causing hyperglycemia and poor glucose utilization. Fish insulin is influenced by nutritional status, carbohydrate level, carbohydrate source, lipid level, and arginine [93]. Increased feeding frequency promotes insulin secretion in European sea bass [94], while protein and lipid sources do not significantly affect plasma insulin in gilthead sea bream [95]. Microencapsulated bovine insulin supplementation in Pacific white shrimp diets does not significantly improve weight gain but enhances protein synthesis and immunity [96], suggesting insulin' s appetite-regulating effects in fish are species- and condition-dependent and may not significantly modulate feeding.

2 Central Signaling Pathways in Appetite Regulation

Appetite regulation is a complex neuro-humoral process involving interactions between peripheral appetite-sensing mechanisms and the central nervous system. Peripheral signals—including neural inputs (visual, olfactory, gustatory, tactile) and gastrointestinal receptor signals (mechanical, thermal, chemical, osmotic) as well as humoral signals (leptin, ghrelin, glucose)—are transmitted via neural or humoral pathways to the central nervous system, where they converge in specific cerebral cortex regions to form appetite sensations that ultimately influence feeding behavior [97-100] ([Figure 2: see original paper]). The hypothalamus

is a critical appetite control center where various nuclei receive, integrate, and transmit appetite signals. The classic "dual-center theory" proposes a "lateral hypothalamic" hunger center and "ventromedial nucleus" satiety center. Key hypothalamic nuclei involved in energy balance regulation include the nucleus tractus solitarius (NTS), arcuate nucleus (ARC), lateral hypothalamus (LH), paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN), ventromedial nucleus (VMN), and dorsomedial nucleus (DMN). The NTS rostrum receives taste fibers and integrates feeding-related afferent information; ARC and LH synthesize and release appetite signals; PVN mediates signal interactions; and SCN, VMN, and DMN regulate these signals [101-103]. Central signaling pathways exert long-term appetite regulation, with the hypothalamus playing a pivotal role. The most studied pathways are the AMP-activated protein kinase (AMPK) and target of rapamycin (TOR) signaling pathways.

2.1 AMPK Signaling Pathway

AMPK is expressed throughout hypothalamic regions (ARC, PVN, DMN, VMN, LH) as a heterotrimeric serine/threonine protein kinase comprising catalytic α, regulatory β, and γ subunits (α and β have two isoforms each; γ has three). The α subunit N-terminus contains a kinase activation domain, while the C-terminus binds β and γ subunits. The β subunit central region is a glycogen-binding domain. The γ subunit contains four tandem CBS repeats (60 amino acids each) forming Bateman domains that bind AMP [104].

Under normal physiological conditions with high ATP levels (ATP:ADP ratio ~10:1), AMPK remains inactive. Metabolic stress increasing the AMP:ATP ratio—such as ischemia, hypoxia, or glucose deficiency—activates AMPK, which phosphorylates downstream targets to inhibit ATP-consuming anabolic pathways and activate ATP-producing catabolic pathways, suppressing carbohydrate, lipid, and cholesterol synthesis while promoting fatty acid oxidation and glucose transport [105]. Thus, AMP serves as the key regulator by binding the γ subunit, inducing conformational changes that increase activity and facilitate phosphorylation by upstream kinases while antagonizing phosphatases, a process inhibited by high ATP. Consequently, AMPK functions as a cellular energy sensor. Beyond the classic AMP:ATP mechanism, hormones, cytokines, and extracellular ligands also modulate AMPK [106]. Leptin selectively activates skeletal muscle AMPK-$ $2 through direct rapid effects and hypothalamus-sympathetic nervous system-mediated long-term effects, regulating muscle fatty acid metabolism via AMPK [107]. Melanocortin receptor agonists inhibit AMPK, while AgRP activates it [108].

AMPK critically regulates feeding through the pathway: AMPK → acetyl-CoA carboxylase (ACC) → malonyl-CoA → carnitine palmitoyltransferase 1 (CPT1) [109]. Hypothalamic AMPK activation increases ARC NPY expression, promoting feeding and reducing energy expenditure, while AMPK inhibition decreases NPY expression, suppressing appetite and increasing energy expenditure [110]. Leptin and ghrelin act on the hypothalamus via AMPK: leptin inhibits hypotha-

lamic AMPK to reduce NPY release and food intake, whereas ghrelin activates AMPK to increase NPY release and feeding [111-112].

Fish AMPK signaling has been reported in several species. In rainbow trout, AMPK is the primary pathway regulating hepatic energy balance [113]. Although dietary lipid level does not affect feed intake, high-fat diets significantly increase hypothalamic AMPK phosphorylation compared to low-fat diets, suggesting AMPK primarily influences fatty acid utilization [114]. High carbohydrate levels significantly suppress AMPK phosphorylation [115]. In sole, high-fat diets increase AMPK activity without hypoxia effects [116], and hypoxia does not alter AMPK phosphorylation in goldfish tissues [117]. In grouper, 3-week starvation significantly increases AMPK phosphorylation, which decreases with short-term refeeding but not long-term refeeding [118]. These results indicate fish AMPK regulation differs from mammals (e.g., hypoxia does not activate it) but shares similar energy modulation functions. Further research is needed on AMPK' s role in fish appetite regulation.

2.2 TOR Signaling Pathway

TOR is a serine/threonine protein kinase comprising 2,549 amino acids, evolutionarily conserved from yeast to mammals and expressed in all cell types. Its C-terminal kinase domain (~234 amino acids) classifies TOR in the phosphatidylinositol 3-kinase-related kinase (PIKK) family [119]. The N-terminus contains 20 tandem HEAT repeats, while the C-terminal FATC domain interacts with the FAT domain to expose the kinase domain [119].

TOR plays crucial roles in nutrient sensing, cell growth and proliferation, and cell cycle regulation [120]. TOR forms two complexes: TORC1 (rapamycin-sensitive) and TORC2 (rapamycin-insensitive) [121]. TORC1 promotes protein synthesis, metabolism, nucleic acid synthesis and transcription while inhibiting autophagy; TORC2 may participate in cytoskeleton formation. TORC1 is better characterized, with upstream stimuli including growth factors, insulin, nutrients, energy, and stress [122].

TOR regulates nutrient uptake (glucose, amino acids, fatty acids) and hormone secretion, with TOR activation suppressing food intake [124-125]. The appetite-regulating pathway involves: PI3K → PDK1 → PKB/Akt → TSC1/TSC2 → Rheb → TORC1 → 4E-BPs, ultimately inhibiting NPY/AgRP activity [126]. Among nutrients, amino acids have been most studied, primarily inhibiting TORC1 to reduce appetite [127]. Leucine directly activates hypothalamic TOR to suppress appetite by inhibiting AgRP activity [124,128].

Fish TOR shares >90% homology with human TOR, and >97% between common carp and zebrafish [129-130], though research remains preliminary. In rainbow trout, rapamycin inhibits TORC1 and downstream factor (S6, S6K1, 4E-BP1) phosphorylation without affecting upstream Akt or TORC2 [131], while carbohydrate levels significantly activate TOR phosphorylation [113]. Nutritional studies show short-term starvation significantly suppresses TOR phos-

phorylation in grouper, with refeeding activating it regardless of duration [118]. Domestic studies demonstrate dietary nutrients significantly affect TOR signaling at the transcriptional level: 0.54% dietary arginine reduces TOR and S6K1 mRNA in gibel carp liver and muscle without affecting 4E-BP2 [132]; increasing dietary leucine elevates TOR mRNA in blunt snout bream liver [133]; tryptophan inhibits TOR mRNA in muscle and liver while promoting expression in mid- and hindgut of Jian carp [134]; leucine and arginine increase TOR and S6K1 phosphorylation and mRNA expression in starved Chinese shrimp [135]. These studies show amino acids significantly affect TOR signaling factors at the transcriptional level in aquatic animals, contrasting with mammalian studies showing primarily protein-level effects, necessitating further protein-level verification of fish-mammal differences.

3 Research Directions

3.1 Functional Studies of Fish Appetite-Regulating Factors

Current fish research has focused on individual factors (leptin, NPY, ghrelin) with limited functional studies, particularly lacking receptor research. Future work must investigate factor functions and their receptors to develop specialized appetite-enhancing physiological regulators for aquaculture.

3.2 Fish Appetite Regulation Signaling Pathways

No complete reports exist on fish central appetite-regulation signaling pathways. Due to evolutionary differences, fish pathways cannot be fully extrapolated from mammalian models. Key challenges include identifying critical regulatory sites, clarifying factor interactions and pathways, and elucidating fish-specific appetite regulation networks—essential prerequisites for effective feeding behavior modulation.

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