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Changes in integrity of the gill during histidine deficiency or excess due to depression of cellular anti-oxidative ability, induction of apoptosis, inflammation and impair of cell-cell tight junctions related to Nrf2, TOR and NF-κB signaling in fish

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ABSTRACT

This study firstly explored the possible effects of dietary histidine on structural integrity and the related signaling factor gene expression in the gills of fish. Young grass carp (*Ctenopharyngodon idella*) were fed with six diets containing gradual levels of histidine for 8 weeks. The results firstly demonstrated that histidine deficiency caused increases in reactive oxygen species (ROS) contents, and severe oxidative damage (lipid peroxidation and protein oxidation) in the gills of fish, which was partially due to the decreased glutathione (GSH) content and antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-*S*-transferase (GST) and glutathione reductase (GR)]. Further investigations indicated that histidine deficiency caused depressions of those antioxidant enzyme activities are related to the down-regulation of corresponding antioxidant enzyme genes and the related signaling factor Nrf2 mRNA levels. Meanwhile, histidine deficiency induced DNA fragmentation via up-regulation of caspase-3, caspase-8 and caspase-9 expressions that referring to the down-regulation of TOR and S6K mRNA levels. Furthermore, His deficiency down-regulated claudin-b, claudin-c, claudin-3, claudin-12, claudin-15, occludin and ZO-1 transcription in fish gills. These effects were partially related to the up-regulation of pro-inflammatory cytokines, interleukin 1β (IL-1β), interleukin 8 (IL-8), tumor necrosis factor-α (TNF-α) and related signaling factor nuclear factor κB P65 (NF-κB P65) mRNA levels, and the down-regulation of anti-inflammatory cytokines, interleukin 10 (IL-10), transforming growth factor β1 (TGF-β1) and related signaling factor IκBα mRNA levels. Excessive histidine exhibited negative effects that were similar to histidine deficiency, whereas the optimal histidine levels reversed those negative effects. Taken together, our results showed that histidine deficiency or

Abbreviations: ZO-1, zonula occludens-1; TNF-α, tumour necrosis factor α; IL-8, interleukin 8; IL-10, interleukin 10; TGF-β, transforming growth factor β; NF-κB, nuclear factor kappa B; IκB, inhibitor protein-κB; TOR, target of rapamycin; S6K1, ribosome protein S6 kinase 1; ASA, anti-superoxide anion; AHR, anti-hydroxyl radical; SOD1, copper, zinc-superoxide dismutase; GPx, glutathione peroxidase; GSH, glutathione; Nrf2, NF-E2-related factor-2; Keap1, Kelch-like-ECH-associated protein 1; ROS, reactive oxygen species; TJ, tight junction; MDA, malondialdehyde; PC, protein carbonyls; OH, hydroxyl radical; $^1O_2$, singlet oxygen.

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excess impaired the structural integrity of fish gill by disrupted fish antioxidant defenses and regulating the expression of tight junction protein, cytokines, apoptosis, antioxidant enzymes, NF-κB p65, IκBα, TOR, Nrf2, Keap1 and apoptosis-related genes in the fish gills.

1. Introduction

Fish gill is the first organ that exposes to environmental pollutants [1]. The constant exposure of fish gill to their environment typifies it as an immune-competent organ characterized by large mucosal surfaces and the gill-associated lymphoid tissue [2]. However, the structural integrity of fish gill is very easy to be damaged [3]. The damage of fish gill structural integrity often results in impair of immune function, leading to bacterial infection, lamellar fusion and advanced degenerative processes breathing disturbance, which causes poor growth, and even mass mortality in fish [4]. Therefore, it is important to expand our knowledge of how to maintain the structural integrity of fish gills. A few studies, mainly from our laboratory, have shown that nutrients, such as riboflavin [5] and tryptophan [6], could enhance the structural integrity of fish gills, and thus improve the growth performance of fish. Histidine (His) is an essential nutrient for fish [7]. Our previous study observed that histidine deficiency depressed the growth performance in grass crap (*Ctenopharyngodon idella*) [8]. However, little attention has been given to the effects of histidine on the structural integrity of fish gills. It is well known that histidine exists as free form circulating in blood where will be part of tissue utilization [9]. Fish gills are with continual blood flow [10]. Those observations indicated potential effects of histidine on the structural integrity of fish gills, which is an interesting topic to study.

Structural integrity of fish gills is associated with cellular integrity, which can be destructed by cell apoptosis and oxidative damage [11]. Studies observed that apoptosis and oxidative damage could be induced by ROS, and could be inhibited by non-enzymatic antioxidant and antioxidant enzymes in animals [12,13]. Antioxidant enzyme activities are closely related to their mRNA levels which could be regulated by transcription factor NF-E2-related factor-2 (Nrf2) in fish [14,15]. However, information about the effects of histidine on the apoptosis, oxidative damage, ROS, antioxidant ability, and the involved molecular mechanisms in fish gills is scarce. Study *in vitro* showed that histidine is a scavenger singlet oxygen by direct interactions with the imidazole ring [16]. Meanwhile, it has been reported that dietary histidine could increase carnosine content of skeletal muscle in rats [17]. Carnosine could attenuate apoptosis and oxidative damage in a rat experimental subarachnoid hemorrhage model [18]. These observations suggest that histidine may affect apoptosis, oxidative damage, ROS, antioxidant ability, and the involved molecules to influence the cellular integrity of fish gills, which is worthy of investigation.

The structural integrity of fish gills is also associated with the cell-cell structural integrity, linking tightly to the tight junction proteins such as ZO-1, occludin and claudins [19]. Fish gill close interaction with the external environment makes it become a primary site of bacterial infection [20]. The bacterial infection always leads to inflammatory response which is mediated by cytokines in fish [21,22]. Our previous study observed that up-regulation of pro-inflammatory cytokines could cause a tissue damage in fish [23]. TJ proteins also could be impaired by pro-inflammatory cytokines which is regulated by NF-κB and IκB signaling molecules in fish [21,22]. In human coronary arterial endothelial cells (HCAECs), histidine could inhibit pro-inflammatory cytokine interleukin-6 (IL-6) production and NF-κB activation, exhibiting anti-inflammatory actions during endothelial inflammation [24]. However, no reports at present concerned the question that whether histidine can inhibit NF-κB/IκB signaling and pro-inflammatory cytokine production to maintain the cell-cell TJ integrity in animals. Meanwhile, the relationship between histidine and inflammation and the cell-cell structural integrity in fish gills are not clear. These topics are worthy of further research.

This study is a part of a larger study aimed at determining the effects of histidine on fish growth using the same growth trial as our previous study [8]. As mentioned above, gill structural integrity is very important for fish growth. Therefore, here, we aim to explore whether histidine can influence the cell and cell-cell structural integrity of fish gills, and further investigate a possible mechanism that explains how effects of dietary histidine on antioxidant defense system, apoptosis, tight junction proteins, cytokines, and related signaling molecules of fish.

Table 1
Composition and nutrient content of the basal diet.

Ingredients	g/kg	Nutrients content[e]	g/kg
Fish meal	78.0	Crude protein	308.2
Casein	30.0	Crude lipid	46.8
Gelatin	39.9	Histidine	2.0
Amino acid premix[a]	199.9		
Histidine premix[b]	50.0		
Alpha-starch	280.0		
Corn starch	122.1		
Fish oil	22.0		
Soybean oil	18.9		
Vitamin premix[c]	10.0		
Mineral premix[d]	20.0		
$Ca(H_2PO_4)_2$	22.7		
Choline chloride (500 g/kg)	6.0		
Cellulose	100.0		
Ethoxyquin(300 g/kg)	0.5		

[a] Amino acid mix (g/kg): arginine, 12.89 g; isoleucine, 12.69 g; leucine, 20.51 g; lysine, 17.13 g; methionine, 7.78 g; cystine, 0.91 g; phenylalanine, 13.60 g; tyrosine, 10.86 g; threonine, 11.88 g; tryptophan, 3.57 g; valine, 15.33 g; glutamic acid, 32.32 g; glycine, 40.40 g.

[b] L-histidine hydrochloride monohydrate was added to obtain graded level of histidine. Each mixture was made isonitrogenous with the addition of reduced amounts of glycine and compensated with appropriate amounts of corn starch. Per kilogram of histidine premix composition from diet 1 to 6 was as follows (g/kg): L-histidine hydrochloride monohydrate 0.000, 54.89, 109.77, 164.66, 219.54 and 274.42 g; glycine 369.70, 311.11, 252.53, 193.94, 135.35 and 76.77 g; and corn starch 630.30, 634.00, 637.70, 641.40, 645.11 and 648.81 g, respectively.

[c] Per kilogram of vitamin premix (g/kg): retinyl acetate (500,000 IU/g), 0.800 g; cholecalciferol (500,000 IU/g), 0.480 g; D,L-a-tocopherol acetate (50%), 20.000 g; menadione (23%), 0.220 g; thiamine hydrochloride (98%), 0.120 g; riboflavin (80%), 0.990 g; pyridoxine hydrochloride (98%), 0.620 g; cyanocobalamin (1%), 0.100 g; niacin (99%), 2.58 g; D-biotin (2%), 5.000 g; meso-inositol (99%), 52.330 g; folic acid (96%), 0.520 g; ascorhyl acetate (93%), 7.160 g; calcium-D-pantothenate (90%), 2.780 g; corn starch 906.3 g.

[d] Per kilogram of mineral premix (g/kg): $FeSO_4 \cdot H_2O$, 25.00 g; $CuSO_4 \cdot 5H_2O$, 0.60 g; $ZnSO_4 \cdot H_2O$, 4.35 g; $MnSO_4 \cdot H_2O$, 2.04 g; KI, 1.10 g; $NaSeO_3$, 2.50 g; $MgSO_4 \cdot H_2O$, 230.67 g; corn starch 733.74 g.

[e] Crude protein, crude lipid and histidine were measured value.