Effects of Dietary Histamine Levels on Growth Performance, Serum Biochemical Indices, and Gastrointestinal Mucosal Structure in Yellow Catfish (Pelteobagrus fulvidraco) Postprint

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Abstract

This experiment aimed to investigate the effects of dietary histamine levels on growth performance, serum biochemical indices, and gastrointestinal mucosal structure in yellow catfish. Yellow catfish with an initial average weight of (30.08±0.35) g were used as experimental subjects and fed with tuna fish meal diet (TFM group), white fish meal diet (H0 group, as control), and white fish meal diets supplemented with five histamine levels (H1, H2, H3, H4, and H5 groups). The dietary histamine levels in each group were 53.20, 4.30, 18.00, 56.20, 84.60, 103.50, and 158.90 mg/kg, respectively. The experimental period lasted 60 days. The results showed: 1) The final average weight and specific growth rate of yellow catfish in the H1 group were significantly higher than those in the H0, H2, H3, and H4 groups ($P < 0.05$). The growth rate of yellow catfish exhibited a quadratic functional relationship with dietary histamine levels. The survival rates of yellow catfish in the TFM, H3, H4, and H5 groups were significantly lower than those in the H0, H1, and H2 groups ($P < 0.05$). 2) The total bile acid content in yellow catfish of the H0 group was significantly higher than that in all other groups ($P < 0.05$). There were no significant differences in lateral skin carotenoid and lutein contents among all groups ($P > 0.05$). 3) Through scanning electron microscopy observation of gastric mucosa and transmission electron microscopy observation of intercellular junction structures in intestinal mucosa, it was found that low dietary histamine levels caused no significant damage to gastric mucosa or intestinal tight junctions in yellow catfish, while dietary histamine levels reaching 103.50 mg/kg and above caused severe damage to the tight junction structures between gastric mucosal cells and intestinal mucosal cells in yellow catfish. Therefore, a dietary histamine level of 18.0 mg/kg was beneficial for growth and fish health in yellow catfish, whereas

dietary histamine levels greater than 103.50 mg/kg had significant detrimental effects on physiological health, gastric mucosal cell surface structure, and tight junction structures between intestinal mucosal cells in yellow catfish.

Full Text

Effects of Dietary Histamine Level on Growth Performance, Serum Biochemical Indices, and Gastrointestinal Mucosal Structure of Yellow Catfish (Pelteobagrus fulvidraco)

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Abstract: This experiment investigated the effects of dietary histamine level on growth performance, serum biochemical indices, and gastrointestinal mucosal structure in yellow catfish. Juvenile yellow catfish with an initial average weight of (30.08±0.35) g were fed seven experimental diets: a tuna fish meal diet (TFM group), a white fish meal diet (H0 group, control), and five white fish meal diets supplemented with graded histamine levels (H1, H2, H3, H4, and H5 groups). The dietary histamine concentrations were 53.20, 4.30, 18.00, 56.20, 84.60, 103.50, and 158.90 mg/kg, respectively. The 60-day feeding trial revealed: (1) The final average weight and specific growth rate (SGR) of fish in the H1 group were significantly higher than those in the H0, H2, H3, and H4 groups ($P<0.05$), with SGR showing a quadratic relationship with dietary histamine level. Survival rates in the TFM, H3, H4, and H5 groups were significantly lower than in the H0, H1, and H2 groups ($P<0.05$). (2) Total serum bile acid content in the H0 group was significantly higher than in all other groups ($P<0.05$). No significant differences were observed in carotenoid or lutein content in the lateral skin among all groups ($P>0.05$). (3) Scanning electron microscopy of gastric mucosa and transmission electron microscopy of intestinal tight junctions revealed no significant damage at low dietary histamine levels, whereas severe damage to gastric mucosal cells and tight junctions between intestinal mucosal cells occurred at histamine levels of 103.50 mg/kg and above. These results indicate that a dietary histamine level of 18.0 mg/kg is beneficial for growth and health in yellow catfish, while levels exceeding 103.50 mg/kg cause significant damage to physiological health, gastric mucosal cell surface structure, and intestinal mucosal cell tight junctions.

Keywords: histamine; growth performance; gastrointestinal mucosa; tight junction; yellow catfish

1.1 Feed Ingredients and Experimental Diets

The tuna fish meal used in this study was produced in Ecuador with the following nutritional composition (dry matter basis): crude protein 74.97%, crude fat 7.88%, crude ash 11.04%, moisture 7.54%, and histamine 226.8 mg/kg. The white fish meal was Russian white fish meal containing crude protein 67.18%, crude fat 9.07%, crude ash 20.31%, moisture 7.44%, and histamine 39.6 mg/kg (dry matter basis). Using white fish meal as the control and based on the nutritional requirements of yellow catfish and practical feed formulation standards, histamine dihydrochloride (C H N ·2HCl, 60.30% histamine content, Sigma-Aldrich, USA) was used as the histamine source to prepare seven experimental diets: white fish meal diet (H0), tuna fish meal diet (TFM), and five white fish meal diets supplemented with graded histamine levels (H1, H2, H3, H4, and H5). The dietary histamine concentrations were 53.20, 4.30, 18.00, 56.20, 84.60, 103.50, and 158.90 mg/kg, respectively. Feed ingredients were ground to pass through a 60-mesh sieve, and histamine was incorporated using the stepwise dilution method to ensure uniform mixing. The mixture was then pelleted into 1.5 mm diameter pellets (2–3 mm length) using a feed pellet mill, air-dried, and stored at 4°C until use. All diets were iso-nitrogenous and iso-lipidic (crude protein 40.28%–40.32%, crude fat 8.21%–8.23%). Biogenic amine types and concentrations in the diets were analyzed by liquid chromatography at the New Hope Liuhe Testing Center. The measured values of cadaverine, spermine, spermidine, putrescine, and histamine are presented in Table 1 .

Table 1 Composition and Nutrient Levels of Experimental Diets (Dry Matter Basis)

Items	Diets
Ingredient	
Tuna fish meal	
White fish meal	
Fine rice bran	
Soybean meal	
Cottonseed meal	
Corn protein powder	
Corpuscle powder meal	
Pork powder meal	
Ca(H PO)	
Zeolite powder	
Wheat	
Soybean oil	
Premix[1]	
Total	
Histamine dihydrochloride (additional addition) (mg/kg)	
Nutrient levels[2]	
Crude protein (CP)	

Items	Diets
Crude fat (EE)	
Ash	
Total phosphorus (TP)	
Biogenic amine (mg/kg)	
Cadaverine	
Spermine	
Putrescine	
Spermidine	
Histamine	

[1]The premix provided the following per kg of diet: Cu 25 mg, Fe 640 mg, Mn 130 mg, Zn 190 mg, I 0.21 mg, Se 0.7 mg, Co 0.16 mg, Mg 960 mg, K 0.5 mg, VA 8 mg, VB 8 mg, VB 8 mg, VB 12 mg, VB 0.02 mg, VC 300 mg, VD 3 mg, VK 5 mg, calcium pantothenate 25 mg, niacin 25 mg, folacin 5 mg, inositol 100 mg.
[2]Nutrient levels were measured values.

1.2 Experimental Design and Management

A total of 420 one-year-old yellow catfish juveniles with uniform size and good health, averaging (30.08±0.35) g, were obtained from Yixing Aquaculture Base in Zhejiang Province. The fish were randomly divided into 7 groups with 3 replicates per group and 20 fish per replicate (cage). Each group was fed one of the experimental diets: tuna fish meal diet (TFM group), white fish meal diet (H0 group, control), or white fish meal diets supplemented with five histamine levels (H1, H2, H3, H4, and H5 groups). The feeding trial lasted 60 days.

The experiment was conducted in pond net cages at Yixing Aquaculture Base. Experimental cages (1.0 m × 1.5 m × 1.5 m) were placed in a 40 m × 60 m pond supplied with water from Changshan River in Haiyan County. A 1.5 kW paddlewheel aerator operated 12 hours daily. Fish were acclimated to the experimental cages for 2 weeks before the formal feeding trial. Fish were hand-fed twice daily (07:00 and 16:00) at 3%–5% of body weight, with feeding rates adjusted every 10 days based on estimated weight gain. During the trial, water temperature ranged from 24.1 to 36.0°C, dissolved oxygen concentration was >7.0 mg/L, pH was 8.0–8.4, ammonia nitrogen was <0.10 mg/L, nitrite was <0.005 mg/L, and sulfide was <0.05 mg/L.

1.3 Sample Collection and Analysis

At the end of the feeding trial, fish were fasted for 24 hours. The number and total weight of fish in each cage were recorded to calculate survival rate (SR) and specific growth rate (SGR). Three fish were randomly selected from each replicate as whole-body samples for proximate composition analysis. Five fish

per cage were randomly selected for blood collection from the caudal vein. After natural coagulation, blood samples were centrifuged at 3,000 r/min for 10 min at 4°C, and serum from each cage was pooled as one sample, snap-frozen in liquid nitrogen, and stored at -80°C for serum biochemical analysis.

Five fish per cage were dissected to weigh the viscera and hepatopancreas for calculating condition factor (CF), hepatosomatic index (HSI), and viscero-somatic index (VSI). Six fish per group were selected for lateral skin sampling to determine carotenoid and lutein content. Gastric mucosa (from the fundus) and intestinal mucosa (from the mid-intestine) were collected, rinsed with fish physiological saline, and fixed in glutaraldehyde for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) observations of mucosal surface characteristics and intercellular junction structures.

Whole fish samples were homogenized and freeze-dried to constant weight using an LGJ-18B freeze dryer for moisture determination. Crude protein was measured by the Kjeldahl method (GB/T 5009.5-2010), crude fat by Soxhlet extraction (GB/T 14772-2008), and crude ash by the method described in GB/T 5009.4-2010. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein (TP), albumin (ALB), total bile acid (TBA), total cholesterol (TC), and triglyceride (TG) concentrations were determined using an Abbott C8000 automatic biochemical analyzer. Skin carotenoid and lutein contents were analyzed according to AOAC 970.64. Gastric mucosal surface structure was observed by SEM, and intestinal mucosal tight junctions were examined by TEM.

1.4 Calculation Methods

Specific growth rate (%/d) = 100 × (ln Wt -ln Wi) / t
Feed conversion ratio (FCR) = Wa / (W -W)
Protein retention rate (PRR, %) = 100 × (P -P) / Wp
Fat retention rate (FRR, %) = 100 × (F -F) / Wf
Condition factor (%) = 100 × W / L^3
Hepatosomatic index (%) = 100 × Wh / W
Viscero-somatic index (%) = 100 × Wv / W
Carotenoid content (mg/kg) = (A × K × V) / (E × G)
Lutein concentration (mg/kg) = A × 1000 × f / 236 × b × d

Where: Wt and Wi are final and initial average weight (g); t is feeding duration (d); W and W are final and initial total weight (g); Wa is total feed intake (g); P and P are final and initial body protein mass (g); Wp is total protein intake (g); F and F are final and initial body fat mass (g); Wf is total fat intake (g); W is body weight (g); Wv is viscera mass (g); Wh is hepatopancreas mass (g); L is body length (cm); A is absorbance; K is dilution factor; V is extract volume (mL); E is molar extinction coefficient; G is sample weight (g); b is cuvette length (cm); f is instrument error; A is optical density at 474 nm;

d is dilution coefficient.

1.5 Data Processing and Analysis

Data were analyzed by one-way ANOVA using SPSS 18.0, followed by Duncan' s multiple comparison test to identify differences among groups. Results are expressed as means ± standard error. Statistical significance was set at $P<0.05$.

2.1 Effects of Dietary Histamine Level on Growth Performance, Body Composition, and Morphological Indices

As shown in Table 2 , dietary histamine level had no significant effect on initial body weight ($P>0.05$). The final body weight and specific growth rate (SGR) of yellow catfish in the H1 group were significantly higher than those in the H0, H2, H3, and H4 groups ($P<0.05$), while no significant differences were observed among other groups ($P>0.05$). Protein retention rate (PRR) and fat retention rate (FRR) in the H1 group were higher than in other groups ($P<0.05$), with no significant differences among the remaining groups ($P>0.05$). Survival rates in the TFM, H3, H4, and H5 groups decreased significantly by 19.17%–24.17% compared with the H0, H1, and H2 groups ($P<0.05$). Due to the pond cage culture system, residual feed could not be collected, resulting in FCR values calculated from actual feed input, which may differ from production data.

No significant differences were observed in condition factor among groups ($P>0.05$). The viscero-somatic index (VSI) in the H1 group was significantly higher than in the TFM, H2, H3, H4, and H5 groups ($P<0.05$), though no significant differences existed among these latter groups ($P>0.05$). The hepatosomatic index (HSI) in the H0 group was significantly higher than in the TFM group ($P<0.05$), with no significant differences among other groups ($P>0.05$). Plotting SGR against dietary histamine level (Figure 1 [Figure 1: see original paper]) revealed a quadratic relationship. These results indicate that tuna fish meal did not significantly differ from white fish meal in terms of growth performance and feed efficiency. Dietary histamine level showed a quadratic effect on growth and feed efficiency, with optimal performance at low histamine concentration (18.00 mg/kg) and inhibitory effects at higher levels. Dietary histamine level had no significant effect on body composition.

Table 2 Effects of Dietary Histamine Level on Growth Performance, Body Composition, and Morphological Indices of Yellow Catfish

Items	Groups
Growth performance (n=3)	
Initial body weight (IBW, g)	29.95±0.10
Final body weight (FBW, g)	56.02±2.74
Specific growth rate (SGR, %/d)	1.04±0.09
Feed conversion ratio (FCR)	3.16±0.31
Protein retention rate (PRR, %)	12.52±6.15

Items	Groups
Fat retention rate (FRR, %)	33.36±19.96
Survival rate (SR, %)	73.33±33.33
Body composition and morphological indices (n=10)	
Moisture (%)	67.22±3.71
Crude protein (%)	17.51±1.04
Crude fat (%)	10.01±2.36
Ash (%)	4.43±0.14
Condition factor (CF)	2.14±0.09
Viscero-somatic index (VSI, %)	6.85±0.50
Hepatosomatic index (HSI, %)	1.65±0.08

In the same row, values with different superscript letters indicate significant differences ($P<0.05$), while values with the same or no superscript letters indicate no significant difference ($P>0.05$). The same applies below.

Figure 1 Relationship between specific growth rate of yellow catfish and dietary histamine level [Figure 1: see original paper]

2.2 Effects of Dietary Histamine Level on Serum Biochemical Indices

As shown in Table 3 , no significant differences were observed in serum total protein or high-density lipoprotein among groups ($P>0.05$). Serum glucose content in the H3 group was significantly higher than in the TFM, H4, and H5 groups ($P<0.05$), while H1 and H2 groups showed slightly lower values than H3 without significant difference ($P>0.05$). Total bile acid content in the H0 group was significantly higher than in all other groups ($P<0.05$), whereas H4 and H5 groups had significantly lower values than other groups ($P<0.05$). No significant differences were found in serum total cholesterol among groups ($P>0.05$). Serum low-density lipoprotein in the TFM, H3, and H4 groups was significantly higher than in the H0, H1, and H5 groups ($P<0.05$). These results suggest that dietary histamine level affected the internal serum environment, with increasing trends in aspartate aminotransferase activity and low-density lipoprotein content indicating potential impacts on hepatopancreatic structure and function. Notably, serum total bile acid content decreased significantly with increasing dietary histamine, suggesting possible impairment of the enterohepatic circulation.

Table 3 Effects of Dietary Histamine Level on Serum Biochemical Indices of Yellow Catfish (n=15)

Items	Groups
Aspartate aminotransferase (AST, U/L)	251±70
Alanine aminotransferase (ALT, U/L)	16.50±15
Total protein (TP, g/L)	45.85±1.6

Items	Groups
Albumin (ALB, g/L)	12.05±1.0
Globulin (GLO, g/L)	33.80±2.6
Glucose (GLU, mmol/L)	7.25±1.20
Total bile acid (TBA, mol/L)	11.90±1.2
Total cholesterol (TC, mmol/L)	10.82±0.2
Triglyceride (TG, mmol/L)	8.00±0.71
High-density lipoprotein (HDL, mmol/L)	2.23±0.21
Low-density lipoprotein (LDL, mmol/L)	4.96±0.59

2.3 Effects of Dietary Histamine Level on Skin Pigment Content

As shown in Table 4 , no significant differences were observed in the two pigment types in the lateral skin among all groups (P>0.05), indicating that neither tuna fish meal nor dietary histamine content significantly affected yellow catfish body color.

Table 4 Effects of Dietary Histamine Level on Skin Pigment Content of Yellow Catfish (Dry Matter Basis, n=6)

Items (mg/kg)	Groups
Carotenoid	8,822.03±2,721.15
Lutein	26.01±6.35

2.4 Scanning Electron Microscopy of Gastric Mucosal Cells

SEM observations of gastric mucosa are presented in Figure 2 [Figure 2: see original paper]. The micrographs revealed mucosal surface structures. In the H0 and H1 groups, gastric mucosal cells appeared normal with clear cell boundaries and tight arrangement. The H2 and H3 groups showed occasional cell rupture at sites indicated by arrows, with other regions remaining relatively intact. In contrast, the TFM, H4, and H5 groups exhibited severe gastric mucosal cell damage. These results demonstrate that high dietary histamine levels (>103.50 mg/kg) caused severe damage to gastric mucosal surface structure, with damage severity increasing with histamine concentration. Although the TFM group showed no significant difference in growth performance compared to the H0 group, fish in this group displayed evident gastric mucosal cell damage.

Figure 2 Scanning electron microscopy of gastric mucosal cells in yellow catfish. A: H0 group; B: H1 group; C: H2 group; D: H3 group; E: H4 group; F: H5 group; G: TFM group. The ← indicates cell damage. Magnification: ×1,500; minimum scale (lower right): 30.0 m [Figure 2: see original paper].

2.5 Transmission Electron Microscopy of Tight Junctions Between Intestinal Mucosal Cells

TEM observations of tight junctions between intestinal mucosal cells are shown in Figure 3 [Figure 3: see original paper]. Intestinal mucosal cells are connected by tight junctions and gap junctions, with tight junctions located near the microvillus end and gap junctions near the basal layer. In the H0 group (white fish meal), intercellular junctions were tightly structured without gaps. The H1 group showed similar junction integrity as H0. Beginning with the H2 group, gaps gradually appeared at both the microvillus and basal ends, indicating damage to intercellular junction structures. The severity of damage, reflected by gap size, increased progressively with dietary histamine level. These findings demonstrate that dietary histamine damaged intestinal mucosal cell tight junctions in a dose-dependent manner.

Figure 3 Transmission electron microscopy of tight junctions between intestinal mucosal cells in yellow catfish. indicates microvillus-end junctions; indicates basal layer-end junctions. Magnification: ×20,000; minimum scale (lower right): 200 nm [Figure 3: see original paper].

3.1 Effects of Dietary Histamine Level on Growth Performance, Serum Biochemical Indices, and Skin Pigment Content

Histamine is produced from free histidine through decarboxylation by histidine decarboxylase. After death, fish undergo rigor mortis, softening, autolysis, and spoilage. Softening and autolysis primarily depend on proteolytic enzymes from the digestive system and lysosomes, which hydrolyze proteins to produce free amino acids including histidine. Histidine content varies significantly among fish species, with red-meat fish containing 7–18 mg/g compared to only 0.1 mg/g in white-meat fish. During spoilage, microbial proliferation produces decarboxylase that acts on free histidine, generating substantial histamine. Consequently, red fish meal contains higher histamine levels than white fish meal. In this study, white fish meal and tuna fish meal contained 39.6 and 226.8 mg/kg histamine, respectively.

The added histamine dihydrochloride contained 60.3% histamine. However, some feed components may have interfered with histamine detection, as measured values in Table 1 were lower than theoretical calculations. Histamine is a biologically active biogenic amine that can cause adverse physiological reactions and affect animal growth and health. Different species exhibit varying physiological responses to dietary histamine, with low levels often promoting growth while high levels exert negative effects.

Cruz-Suarez et al. [11] reported that dietary biogenic amines (cadaverine, putrescine, histamine) above 100 mg/kg reduced feed intake, weight gain, and survival in Pacific white shrimp (*Litopenaeus vannamei*). Dietary histamine

at 4,000 mg/kg decreased survival in poultry [12] and mysids [13] (*Neomysis awatschensis* and *N. japonica*). Luo et al. [14] found that histamine did not significantly affect grass carp (*Ctenopharyngodon idella*) survival, possibly because grass carp lack a stomach and histamine receptors, reducing histamine toxicity. Low-dose biogenic amines promoted grass carp growth. Dietary histamine at 4,000 mg/kg inhibited broiler growth and caused gizzard erosion syndrome [12], while the same level reduced feed intake and weight gain in pigs without affecting FCR [15].

In this study, survival rates decreased significantly when dietary histamine exceeded 84.60 mg/kg (H3, H4, H5 groups), likely because yellow catfish, as a stomach-possessing carnivorous species, is more sensitive to histamine stimulation. High histamine levels exerted toxic effects, reducing survival. The TFM group also showed significantly reduced survival, possibly due to higher peroxide values in red fish meal causing fish injury [5], though further investigation is needed.

Low dietary histamine levels have demonstrated growth-promoting effects in various species. Tapia-Salazar et al. [16] reported improved growth in blue shrimp (*Litopenaeus stylirostris*) with appropriate histamine supplementation. Opstvedt et al. [17] observed growth promotion in Atlantic salmon (*Salmo salar*) fed diets with mixed biogenic amines, consistent with results in grass carp [14]. Atlantic halibut [18], turbot [19], and gilthead sea bream [20] showed better growth when fed high-freshness, low-biogenic-amine fish meal compared to medium/low-freshness, high-amine fish meal. In this study, SGR in the H1 group (18.00 mg/kg histamine) was significantly higher than in TFM, H0, H2, H3, H4, and H5 groups, confirming that optimal histamine level (18.00 mg/kg) enhanced yellow catfish growth performance.

Biogenic amines play important roles in nucleic acid function regulation and protein synthesis [21]. Watanabe et al. [22] observed improved protein efficiency in rainbow trout fed 70 mg/kg histamine. Although dietary histamine did not significantly affect PRR, FRR, or main nutrient composition in yellow catfish, the H1 group (18.00 mg/kg histamine) showed the highest protein and fat retention rates, suggesting that appropriate histamine supplementation enhanced nutrient utilization.

HSI and VSI are indicators of fish health, with dietary nutrients typically affecting hepatopancreas development [23]. In this study, dietary histamine did not significantly affect condition factor. The H1 group showed significantly higher VSI than H2, H3, H4, and H5 groups, possibly due to increased visceral fat deposition from higher fat retention. Serum total protein and albumin correlate with fish health and nutritional status [24]. Although no significant differences were observed, H1 group showed the highest serum albumin level, likely because optimal histamine enhanced protein anabolism.

Triglycerides and cholesterol reflect lipid metabolism status [25]. Elevated serum cholesterol indicates hepatic dysfunction and lipid metabolism disorder. HDL

transports cholesterol from peripheral tissues to the liver for excretion, while LDL promotes cholesterol deposition in blood vessels. In this study, no significant differences were observed in HDL, but H1 group showed lower total cholesterol, triglycerides, and LDL, suggesting that appropriate histamine improved lipid metabolism and reduced blood lipids. Serum bile acids are synthesized by the liver; elevated levels indicate hepatocellular damage or bile duct disease [26]. The significantly higher total bile acid content in TFM, H0, H2, H3, and H4 groups compared to H1 suggests potential liver injury.

Blood glucose primarily originates from nutrient digestion and glycogenolysis, serving as an important indicator of carbohydrate metabolism [27]. In this study, serum glucose in histamine-supplemented groups showed a trend of initial increase followed by decrease, suggesting that low histamine levels may maintain relatively high metabolic rates.

Yellow catfish has yellow pigmentation on its lateral sides and abdomen, primarily from carotenoids and lutein. This study found no significant differences in skin pigment content among groups, indicating that dietary histamine levels did not affect body color.

In summary, low dietary histamine level (18.00 mg/kg) positively affected growth, feed efficiency, and health in yellow catfish, while higher levels exerted negative effects. Dietary histamine did not affect skin pigment content under these experimental conditions.

3.2 Effects of Dietary Histamine Level on Gastric Mucosal Surface Structure and Intestinal Mucosal Tight Junctions

Histamine exerts its effects on animals through histamine receptors (HR) in gastrointestinal mucosa, producing widespread physiological or pathological effects [28]. Toxic factors associated with histamine may affect tissue structure and function. SEM observations revealed that high dietary histamine levels (H4, H5 groups) caused obvious gastric mucosal cell damage, including microvillus loss. Fairgrieve et al. [29] and Watanabe et al. [22] reported severe gastric lesions in rainbow trout fed fish meal containing >2,000 mg/kg histamine. This study observed significant gastric damage at 103.50 mg/kg histamine, suggesting species-specific differences in histamine tolerance. Gastric mucosal damage may affect acid secretion and cause regurgitation, though no obvious regurgitation was observed during the trial.

Few studies have examined histamine effects on intestinal mucosa. The intestinal barrier relies on tight junctions and gap junctions between mucosal cells to maintain physical integrity and control permeability. TEM observations revealed that dietary histamine damaged intestinal mucosal cell junctions in a dose-dependent manner. The H4 and H5 groups (>103.50 mg/kg histamine) showed obvious junction damage. Duan et al. [30] reported that histamine reduced barrier function in human keratinocytes, consistent with our findings. Peulen et al. [31] demonstrated that appropriate exogenous spermine could be

rapidly absorbed by intestinal epithelial cells, and Yu et al. [32] reported that 0.10% (1,000 mg/kg) spermine in microdiets promoted intestinal development in half-smooth tongue sole (*Cynoglossus semilaevis*) larvae by increasing microvillus length and mucosal thickness. In this study, dietary spermine content was only 20.00 mg/kg at maximum, far below the 1,000 mg/kg level required for intestinal protection, and thus could not mitigate histamine-induced intestinal damage.

In conclusion, dietary histamine levels below 103.50 mg/kg did not damage gastric mucosal surface structure or intestinal mucosal cell junctions, while levels exceeding this threshold caused significant damage to both gastric mucosal cells and intestinal tight junctions.

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4 Conclusions

1. Dietary histamine level exhibited dose-dependent effects on growth performance and health in yellow catfish. Tuna fish meal and low dietary histamine levels did not significantly affect growth, feed efficiency, or fish health.
2. Dietary histamine levels of 103.50 mg/kg and above caused damage to gastric mucosal cells and tight junctions between intestinal mucosal cells.
3. Serum total bile acid content decreased with increasing dietary histamine level, while dietary histamine did not adversely affect body color.

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