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Research Article

Effects of Dietary Histidine Levels on Growth Performance, Feed Utilization, and Expression of Related Genes of Juvenile Hybrid Grouper *Epinephelus fuscoguttatus* ♀×*Epinephelus lanceolatus*♂

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Histidine (His) is a ♀nutritionally significant amino acid and plays a key regulatory role in growth-related cellular functions. The present study was conducted to assess the effects of dietary His levels on hybrid grouper growth performance, feed utilization, and related gene expression. Seven isoenergetic, isoproteic, and isolipidic diets were made up to contain 6.9, 7.2, 8.6, 9.2, 10.7, 11.1, and 11.7 g/kg dietary His levels. The results revealed that protein productive value (PPV), feed efficiency (FE), and weight gain (WG) were increased with an increase in dietary His levels up to 10.7 g/kg. Based on the analysis of the second-order polynomial model, the optimal dietary His level for maximum PPV, WG, and FE was 9.24, 9.62, and 9.47 g/kg, respectively. The gut morphology was affected by various dietary His levels. Furthermore, dietary His levels can affect the relative mRNA levels of growth hormone receptor (GHR1) insulin-like growth factor1 (IGF1), the target of rapamycin (TOR), tumor necrosis factorα (TNF-α), and S6 kinase1 (S6K1). Dietary 9.2 g/kg His revealed a high level of Nrf2 expression in the head kidney. Generally, the optimal dietary His level for the best growth performance of hybrid grouper was found to be 9.62 g/kg dry matter or 21.08 g/kg dietary protein.

1. Introduction

Amino acid research has become increasingly important in recent years as the aquaculture sector moves away from fish meal-based protein feedstuffs [1]. A species-specific understanding of the dietary essential amino acid demands is critical to creating an amino-acid balanced, cost-effective, and ecologically compatible practical diet for intensive aquafarming [2, 3]. Out of the ten required amino acids, histidine (His) is one of the most important in fish diets because it is involved in a variety of activities, such as protein synthesis, tissue development, and repair, as well as the maintenance of osmoregulation [4–6]. Li, Mai, Trushenski, & Wu, [2] reported that His participated in the metabolism of a single carbon unit, which influences protein and DNA synthesis. His is also abundant in hemoglobin protein and has antioxidant potential in the lens, which is important in preventing cataracts in fish [7, 8]. In reactive carbonyl species (RCS), His can play a crucial role in the detoxification and muscle pH buffering [8, 9].

The optimum amount of His in fish feed improves their growth and other biological mechanisms [10]. His supplementation has been shown to increase growth hormone secretion and, as a result, growth [11]. It has also been found that appropriate levels of dietary His can boost the weight gain and feed efficiency of developing grass carp [12]. Because aquatic organisms' capabilities for endogenous His synthesis are limited [10, 13], much research work has been carried out on the dietary His requirements of different fish species, including *Oreochromis niloticus*, *Cirrhinus mrigala*, *Clarias gariepinus; Heteropneustes fossilis*, and *Sciaenop ocellatus* [7, 13–16], with values ranging from 10 to 36 g/kg

dietary protein. The indispensability of His, as well as emerging evidence of its importance in a range of physiological processes, defines it as a unique AA that deserves more research in fish diets.

Histidine has an impact on animals' antioxidant and immunological systems [17–19]. The activity of antioxidant enzymes is directly linked to their mRNA levels and can be regulated by the transcription factor NFE2-related factor 2 (Nrf2), which is a master regulator of the cellular antioxidant response [20, 21]. More research has been done in recent years on the Nrf2 signaling pathway, which regulates aquatic animals' antioxidant capacity [22, 23]. Dietary His supplementation has been shown to mediate associated antioxidant genes via the Nrf2 signaling pathway [24, 25]. To our knowledge, no consistent data has been collected to assess the influence of His on the expressions of antioxidant genes in the hybrid grouper's head kidney.

The target of rapamycin (TOR) is a conserved complex molecule of threonine/serine, phosphate with two distinct structures, TORC1, and TORC2 [26]. The TORC1 is highly complex and conserved among eukaryotes that coordinate metabolism and cellular growth with environmental cues including growth factors and nutrients [27]. TORC1 functions as a target in the TOR signaling pathway in eukaryotic cells, survival, metabolism, and maintaining cell growth, through phosphorylation of downstream translation regulatory factors S6K1 [28–32]. Histidine triggered the mTOR pathway in bovine mammary epithelial cells via modulating the phosphorylation of S6K1 and eIF4E-binding protein (4EBPs) [33]. The GH/IGF-1 system regulates degradation and protein synthesis through PI3K/AKT/TOR signaling pathway [34]. However, few studies on the effects of His on IGF-1 and TOR expression in fish have been conducted.

The *Epinephelus fuscoguttatus* ♀×*Epinephelus lanceolatus*♂ is a highly farmed species in Southeast Asia due to its high economic value. Our previously published research on hybrid grouper has revealed the reference dietary amino acids' (AAs) profile, optimal lipid, protein requirements, and protein: energy ratio [35–37], and also the optimum valine, methionine, threonine, arginine, lysine, leucine, and isoleucine requirements [38–44].

To the best of our knowledge, it is the first attempt and no such study on His nutrition in hybrid grouper is documented yet. Hence, to fill this gap in research, the present study was designed to see how dietary His affected hybrid grouper juveniles' growth performance, feed utilization, and expression of related genes and, hence, to use this data to figure out how much His this fish needs in diets.

2. Materials and Methods

2.1. Ethical Approval. We all authors were aware of and followed the 3Rs rule for this research project. Besides, the use and care of fish species have complied with both the Chinese Animal Management Regulations (2017) and the guidelines of the welfare and ethical committee of Hainan University.

2.2. Experimental Diets. A total of seven isoenergetic, isonitrogenous, and isolipidic diets were prepared that comprise graded L-His levels (Table 1). About 456.2 g/kg of dietary protein level and 70 g/kg of dietary crude lipid level were selected by following the previously published protocol [35–37, 39, 41, 42]. The analyzed dietary His concentrations in diets were 6.9, 7.2, 8.6, 9.2, 10.7, 11.1, and 11.7 g/kg dry matter (Table 2). Total AA levels in diets were set by following the previously published work [37], except His (Table 3). The optimal dietary levels of valine, methionine, threonine, arginine, lysine, leucine, and isoleucine requirements have been determined in our previous findings [38–44]. The preparation of experimental diets was carried out as previously described by [40].

2.3. Experimental Procedure. The present study on hybrid grouper was carried out in the commercial hatchery located in Guilinyang, Hainan Island, South China. A total of 315 healthy hybrid grouper juveniles (7.7 ± 0.01 g) were raised in an aerated semi-intensive tank at room temperature ($28.5 \pm 2^{\circ}$C), total NH3 (0-0.22 mg./L), and DO (5.5-6.8 mg./L). The acclimatization and feeding procedures were identical to those used in our previous study [40].

2.4. Sampling and Analysis. At the beginning of the experiment, 10 fish were sampled for the analysis of the initial chemical composition of the whole body. At the end of the growth trial, fish were starved for 14 hours before sampling. After being anesthetized with MS-222 at 0.1 g/L, two fish from each tank were sampled to analyze the proximate composition of the whole body. To determine the somatic indices, three fish from each tank were separately weighed and dissected to obtain visceral tissues after the blood draw. At this dissection, the liver and gut samples were also obtained.

The process of proximate analysis contained crude. protein (N.× .6.25) xbeing estimated by Dumas' method. of combustion employing a rapid MAXN exceeds system (.Elementar.Germany), crude lipid is quantified by ether extraction using Soxtec System (Sox-tec System. HT6., SoX406. Haineng, Shandong., China), and dry matter. determined by heating 2 g samples for three hours at 125°C [45]. The AA composition of experimental diets (Table 2) was analyzed according to our previous procedure [44].

2.5. Gastrointestinal Histology. From the gastrointestinal tract, both foregut and midgut were removed and rinsed with a solution of saline before being treated in Bouin's fixative solution to analyze histology. Samples were embedded in paraffin after repeated dehydration in alcohol. Embedded tissue blocks were cut about 5 μm, and each section was then stained with hematoxylin-eosin and monitored by using light microscopic techniques. The quantitative traits, i.e., the mean microvillus length, fold length/width, and enterocytes length were calculated by following the previously published protocol [46].

2.6. Reverse Transcription and Total RNA Extraction. The total RNA of the pituitary, liver, and head kidney was extracted by following the manufacturer's protocol and using TRIzol Reagent. The amount of RNA in the sample

Table 1: Formulations and analyzed composition of experimental diets (g/kg, DM).

Ingredients	6.9	7.2	8.6	9.2	10.7	11.1	11.7
Peruvian fishmeal (anchovy)[1]	210	210	210	210	210	210	210
Poutry by-product meal[2]	50	50	50	50	50	50	50
Amino acid mixture[3]	252.4	252.4	252.4	252.4	252.4	252.4	252.4
L-histidine	0.00	1.5	3.0	4.5	6.0	7.5	9.0
Alanine	29.2	28.4	27.6	26.8	25.9	25.1	24.3
Chile fish oil (salmon)[4]	42.5	42.5	42.5	42.5	42.5	42.5	42.5
Corn starch	233.7	233.0	232.3	231.6	231.0	230.3	229.6
Vitamin mixture[5]	10	10	10	10	10	10	10
Mineral mixture[6]	5	5	5	5	5	5	5
Cellulose	144.6	144.6	144.6	144.6	144.6	144.6	144.6
Carboxymethyl Cellulose	20	20	20	20.	20.	20	20
Analyzed composition of diets (g/kg, dry weight)[7]							
Dry matter	906	899	895	911	914	888	899
Crude protein	440	444	442	442	443	443	443
Crude lipid	67	67	67	67	67	67	67
Histidine	6.9	7.2	8.6	9.2	10.7	11.1	11.7

[1]Yongsheng Feed Corporation, Binzhou, China; proximate composition (g/kg dry matter): moisture, 85.5; crude protein, 706.9; crude lipid, 95.8 g/kg. [2]American Proteins Inc., USA; proximate composition (g/kg dry matter): moisture, 39.9 g/kg; crude protein, 641.4 g/kg; crude lipid, 146.7 g/kg. [3]Amino acid mixture (g/100 g): L-Arginine,2.34; L-methionine, 0.95; L-threonine, 0.28; L-isoleucine, 1.16; L-leucine, 1.84; L-valine, 0.64; L-aspartic acid, 3.43; L-serine, 1.40; L-glumatic acid, 6.66; glycine, 1.74; L-cystine, 0.55; L-tyrosine, 1.10; L-proline, 1.37; l-lysine sulphate (55% lysine): 1.32; L-phenylalanine, 0.46; [4]Blue Ocean Marine Biological Technology Co.Ltd, Rongcheng, Shandong, China. [5]Vitamin mixture and [6]Mineral mixture [93]. [7]The values indicate the averages of duplicate test diets.

Table 2: Amino acid compositions (g/kg) of experimental test diets (DM basis)[1].

AA/∑ AA	Dietary his levels (g/kg) 6.9	7.2	8.6	9.2	10.7	11.1	11.7
EAA							
Lysine	21.4	21.3	21.6	20.0	20.4	20.3	21.4
Arginine	33.9	32.9	33.1	33.9	36.5	36.7	34.4
Methionine	14.5	13.2	12.9	14.5	12.9	14.5	12.7
Threonine	10.8	10.2	10.7	11.0	10.8	11.5	10.0
Isoleucine	19.0	18.5	19.2	19.8	19.4	17.1	18.1
Leucine	30.6	31.5	30.9	31.1	30.4	32.8	31.5
Phenylalanine	12.7	11.7	12.2	12.7	11.4	12.2	11.4
Valine	15.4	14.3	15.6	15.0	14.3	13.4	14.3
Histidine	6.9	7.2	8.6	9.2	10.7	11.1	11.7
∑EAA	165.2	160.8	164.8	167.2	166.8	169.6	165.5
NEAA							
Aspartic acid	53.5	52.1	52.1	50.3	50.6	52.9	54.6
Serine	19.4	19.5	20.0	19.7	19.7	20.6	19.3
Glutamic acid	93.1	93.0	90.0	93.8	89.8	82.3	90.8
Glycine	30.4	31.2	29.1	30.0	31.1	29.1	32.6
Alanine	28.7	26.2	26.3	24.9	23.7	22.9	23.0
Cystine	5.0	3.3	5.2	4.6	2.3	6.5	2.7
Tyrosine	16.4	14.4	16.2	16.6	14.9	18.4	13.4
Proline	22.1	21.6	22.2	22.4	23.1	24.6	22.5
∑NEAA	268.6	261.3	261.1	262.3	255.2	257.3	258.9
∑AA	433.8	422.1	425.9	429.5	419.0	426.9	424.4

[1]The values indicate the averages of duplicate test diets.

Table 3: Amino acid composition (g/kg) of the diet ingredients, required supplemental crystalline amino acids in diets, and reference amino acid profile (DM basis)[1].

	Amount in				The reference AA profile[3]
Amino acid	21 g FM[2]	5 g PM	CAA	Total	
EAA					
Lysine	12.2	2.1	7.2	21.6	21.6
Arginine	10.4	2.7	23.4	36.5	36.5
Methionine	4.4	0.6	9.5	14.5	14.5
Threonine	7.1	1.5	2.8	11.5	11.5
Isoleucine	6.9	1.4	11.6	19.8	19.8
Leucine	11.6	2.5	18.4	32.5	32.5
Phenylalanine	6.7	1.4	4.6	12.7	12.7
Valine	7.6	1.6	6.4	15.6	15.6
Histidine	6.1	0.8	6.0	12.9	12.9
NEAA					
Aspartic acid	14.3	2.9	34.3	51.5	51.5
Serine	6.2	1.7	14.0	21.9	21.9
Glutamic acid	20.2	4.7	66.6	91.5	91.5
Glycine	11.2	3.2	17.4	31.9	31.9
Alanine	9.7	2.4	22.1	34.3	34.3
Cystine	1.1	0.4	5.5	6.9	6.9
Tyrosine	5.4	1.0	11.0	17.4	17.4
Proline	7.1	2.3	13.7	23.2	23.2

[1]The values indicate the averages of duplicate test diets. [2]FM: peruvian. fish meal; PM: poultry by-product meal; CAA: crystalline amino acid. [3] The reference amino acid profile reported by. [37], the optimum dietary Arg requirement reported by [41], the optimum dietary Lys requirement reported by Li.et al., [39] the optimum dietary Leu requirement reported by Zhou.et al., [42], the optimum Val requirement reported by [44], the optimum dietary lle requirement reported by [44], the optimum dietary Met reported by Li. et al., [38], and optimum dietary Thr requirement reported by [40].

was measured with NanoDrop® ND-1000 (Thermo Fisher Scientific, America), and the quality and integrity were checked through OD 260/280 and 1% agarose gel electrophoresis. The total RNA was reversely transcribed by the Prime Script™ RT Reagent Kit with gDNA Eraser (Takara, Japan) following the instructions provided by the manufacturer.

2.7. PCR Study of IGF-1, GHR1, S6K1, TOR, TNF-α, Keap1, and Nrf2. The qRT-PCR analysis was performed by using TB Green™ Premix Ex Taq™ II (Takara, Japan) on QuantStudio 6 Flex Real-time PCR systems (Applied Biosystems, Singapore). The primers were mentioned in Table 4 and were designed by using Primer Premier 5.0 (Premier Biosoft, America) according to the sequence of GHR1 (KR269817.1), IGF1 (AY776159.1), TOR (JN850959.1), S6K1 (XM_020085100.1), TNF-α (FJ491411.1), Keap1 (XM_018665037.1), erythroid 2-related Nrf2 (KU892416.1), and β-actin (AY510710.2) in NCBI. The experiment was performed in triplicate, and the relative expression levels of mRNAs were normalized against the expression levels of β-actin mRNA using the comparative CT ($2^{-\Delta\Delta Ct}$) method. The melting curve analysis of amplification products was performed at the end of each PCR experiment to ensure that only one PCR product was present in these reactions.

2.8. Formulas and Statistical Analysis. Using a conventional formula, the following parameters and indices were computed [47]: parameters of growth performance, such as WG and survival; indices of feed utilization, including daily feed intake (DFI) and protein productive values (PPV) and morphology indices, such as HSI CF, and IPF. The following formula was used to calculate the intraperitoneal fat ratio (IPF weight/live weight) × 100) as followed by Turano, Davis & Arnold, [48].

Normality and homoscedasticity of all data were checked before the statistical analysis. The parameters were subjected to a variance (ANOVA) to see if dietary His levels affected the observed responses significantly before further assessment using the regression analysis of orthogonal polynomial contrasts by SPSS 18.0 software, and the *P*-value below 0.05 meant significant. Regression asymptote has been applied to establish hybrid grouper's optimal His requirement.

3. Results

3.1. Feed Utilization and Growth Performance. The feed utilization and growth performance of the fish fed a variety of dietary His levels were summarized in Table 5. No significant variation was found in survival among different experimental treatments ($p > 0.05$). Dietary His levels had a substantial impact on the weight gain percentage ($p < 0.05$), with fish fed 10.7 g/kg dietary His having higher WG %, and this parameter was reduced in fish fed dietary His levels above 10.7 g/kg. The PPV and FE exhibited the same trends of variations as WG. When compared to FE, daily feed intake exhibited an inverse relationship. The analysis from a second-order polynomial model showed that the optimal dietary His level for maximum PPV, WG, and FE of the studied species were 9.24, 9.62, and 9.47 g/kg, respectively (Figure 1).

3.2. Hepatosomatic Index and Proximate Composition. Dietary His levels had a significant ($p < 0.05$) effect on the hepatosomatic index (HSI) and IPF ratio (Table 6), both of which were the highest in fish fed 6.9 g/kg dietary His. The condition factor (CF) was not affected by various treatments ($p > 0.05$). Fish fed 11.1 g/kg dietary His showed higher lipid contents in the whole body than fish fed other dietary His. Fish fed 6.9, 7.2, 11.1, and 11.7 g/kg dietary His showed lower protein contents in the whole body and white muscle than fish fed 8.6, 9.2, and 10.7 g/kg dietary His. The moisture contents of white muscle and whole body did not significantly alter with any of the test diets ($p > 0.05$).

3.3. Gut Micromorphology. The gut morphometric traits were affected by various dietary His levels ($p < 0.05$) and were presented in Table 7. Fish fed 6.9, 7.2, 11.1, and 11.7 g/kg dietary His had lower wF, hF, and hMV values in foregut and midgut than fish fed 8.6, 9.2, and 10.7 g/kg dietary His.

Table 4: Quantitative RT-PCR (qPCR) primers.

Purpose	Gene. Name	Accession. No	Primer. Sequence. (5.′-3. ′)
.qPCR.	GHR1[1]	KR269817.1	[8]F: CACAGACTTCTATGCCCAGGT [9]R: GTGTAGCCGCTTCCTTCAG
	IGF-1[2]	AY776159.1	F:TATTTCAGTAAACCAACAGGCTATG R: TGAATGACTATGTCCAGGTAAAGG
	TOR[3]	JN850959.1	F: TCTCCCTGTCCAGAGGCAATAA R: CAGTCAGCGGGTAGATCAAAGC
	S6K1[4]	XM_020085100.1	F: TCCTTCTCCGTCTGTAAACGA R: CATGAACACCTGCTTACCAT
	TNF-α[5]	FJ491411.1	F: GAGGACGGTGGTGTTGGTGG R: TTCTCTTTGGCCTGATTGCG
	Keap1[6]	XM_018665037.1	F: TCCACAAACCCACCAAAGTAA R: TCCACCAACAGCGTAGAAAAG
	Nrf2[7]	KU892416.1	F: TATGGAGATGGGTCCTTTGGTG R: GCTTCTTTTCCTGCGTCTGTTG
	β-Actin	AY510710.2	F:. CTCTGGGCAACGGAACCTCT. R:. GTGCGTGACATCAAGGAGAAGC.

[1]GHR 1: growth hormone. receptor1;[2]IGF 1: insulin-like growth factor1; [3]TOR: target of rapamycin; [4]S6K1: ribosomal protein S6 kinase 1; [5]TNF-α: tumour necrosis factor- α ; [6]Keap1: Kelch sample related protein 1; [7]Nrf2: NFE2-related factor 2; [8]F: forward sequence; [9]R: reverse sequence.

Table 5: Growth and feed utilization of hybrid. grouper juveniles fed various dietary His levels for 8 weeks.

Dietary His levels (g/kg)	WG (%)[1]	DFI[2] (g 100 g fish^{-1} day^{-1})	FE[3]	FCR[4]	PPV[5] %	Survival %
6.9	343	1.84	1.05	0.96	40.63	100
7.2	345	1.78	1.08	0.92	43.41	100
8.6	408	1.73	1.11	0.90	44.79	100
9.2	389	1.77	1.09	0.92	43.77	100
10.7	442	1.64	1.17	0.85	45.88	100
11.1	375	1.80	1.07	0.93	41.66	100
11.7	358	1.84	1.05	0.96	40.65	100
PSE[6]	7.86	0.02	0.01	0.01	0.51	0.00
.Regression.($N = 3$)						
.SOP						
Adj.. R^2	0.551	0.180	0.180	0.180	0.353	
p value	<0.001	0.065	0.065	0.065	0.008	

[1] WG% = Weight gain;[2]DFI = daily feed. intake; [3]FE = feed efficiency; [4]FCR = feed conversion rate; [5]PPV = protein productive value; [6]PSE = pooled standard error of treatment means ($n = 3$); Adj. R^2 = adjusted. R square; SOP. = second order polynomial trend.

3.4. The Relative mRNA Expression Levels of Growth, Protein Synthesis, and Immunity-Related Genes. The relative mRNA levels of hepatic IGF-1 and pituitary GHR1 of the studied species fed 10.7 g/kg dietary His were found higher than those fed with other dietary His levels ($p < 0.05$). For TOR-related signaling molecules, the expression of hepatic S6K1 and TOR was influenced by various dietary His levels (Figure 2). Fish fed 9.2 g/kg dietary His showed higher mRNA levels of TOR than other dietary His levels. Moreover, the group of 9.2 g/kg dietary His had the highest mRNA level of S6K1 among all the studied groups.

The relative mRNA levels of Keap1 and Nrf2 in the head kidney and hepatic TNF-α were influenced by various experimental treatments (Figure 3). Such levels of Keap1 in fish fed 9.2 g/kg dietary His were observed lower than the other experimental groups. Fish fed 9.2 g/kg dietary His exhibited higher Nrf2 expression than fish fed other dietary His levels ($p < 0.05$). The relative mRNA levels of TNF-α in fish fed 8.6 g/kg dietary His were lowest than those in other experimental groups.

4. Discussion

The present findings revealed that percent weight gain enhanced to a certain point with an increase in dietary His levels (fish fed 10.7 g/kg dietary His) which indicated that deficiency in dietary His may cause poor growth and feed utilization in hybrid grouper. Whereas growth rate performance improved in response to an increase in dietary His level, demonstrating that His is a necessary amino acid for

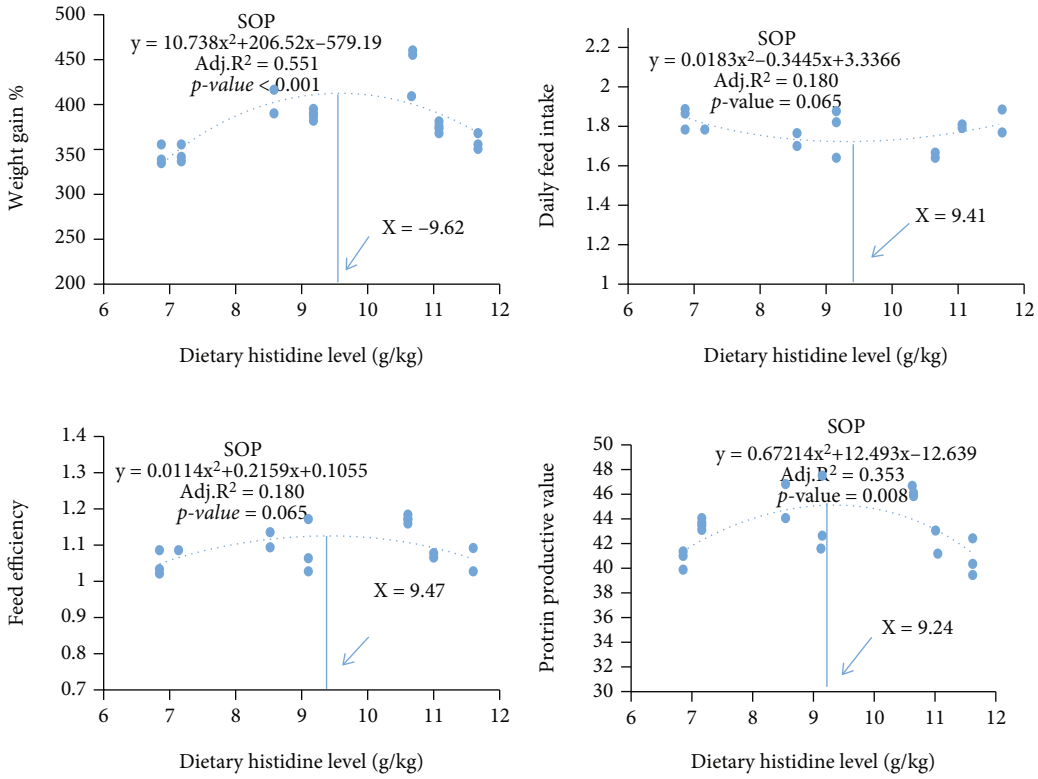

Figure 1: Relationship. of WG, DFI, FE, and PPV with.dietary His levels based on quadratic. regression analysis. SOP.: second-order.polynomial model. Applicable significance levels $p < 0.05$. Adj. R^2 = adjusted. R square.

Table 6: Body condition indices and whole-body and white muscle compositions (fresh-.weight basis, g/kg) of hybrid grouper juveniles fed different dietary His levels for 8 weeks.

	Body condition indices			Whole-body composition g/kg[1]			White muscle composition g/kg		
Dietary His levels (g/kg)	HSI	IPF	CF	Moisture	Protein	Lipid	Moisture	Protein	Lipid
6.9	4.30	1.64	2.81	739.1	162.1	52.6	751.0	191.6	19.4
7.2	4.13	1.55	2.68	746.3	167.3	56.6	755.3	192.1	21.0
8.6	3.99	1.54	2.80	745.2	169.2	57.4	720.2	209.6	25.8
9.2	3.85	1.44	2.83	740.8	168.0	59.1	737.0	200.6	23.3
10.7	3.91	1.30	2.86	740.8	165.4	58.9	764.9	199.2	25.6
11.1	3.94	1.33	3.00	753.4	163.7	63.4	764.7	192.8	21.5
11.7	4.15	1.54	3.05	750.1	163.0	54.3	752.6	191.2	21.1
PSE	0.05	0.04	0.05	0.20	0.06	0.06	0.46	0.16	0.07
Regression ($N = 3$)									
SOP									
Adj. R^2	0.291	0.314	0.152	0.015	0.349	0.550	0.110	0.496	0.339
p value	0.017	0.013	0.088	0.337	0.008	<0.001	0.350	0.001	0.009

[1]Initial.composition. of whole-body (g/kg): protein = 165.4; lipid = 33.5; moisture. = .749.7; HSI =. hepatosomatic-index; IP = intraperitoneal fat. ratio; CF = condition factor; PSE = pooled samples' standard error.

the hybrid grouper. Similar findings have been observed in blunt snout bream [10], yellow croker [49], grass carp [50], stinging catfish [15], and Nile tilapia [16]. To achieve optimal growth of cultured fish, aquatic feeds must have a balanced AA composition [51], and the imbalances of AA reduce the use of dietary protein required for growth. All fish species require amino acids, but the essential His is particularly important [47]. The present study showed that optimal dietary His requirement for the maximum WG% of hybrid grouper is 9.62 g/kg of dry matter (corresponding to 21.08 g/kg of dietary protein). This value was roughly equivalent to that of *Ctenopharyngodon idella* (12 g/kg) [52] and *Oreochromis niloticus* (10 g/kg) [53], but higher than *Clarias gariepinus* (4 g/kg) [13], *Pseudosciaena crocea* (9 g/kg) [49], *Cirrhinus mrigala* (9 g/kg) [14], *Sciaenop ocellatus* (5.9 g/kg) [7], and lower than *Heteropneustes fossilis* (15-16 g/kg)

Table 7: The gut micromorphology of 8-.week-old hybrid grouper juveniles fed various levels of dietary His.

Dietary His levels (g/kg)	Foregut hF (μm)[1]	wF (μm)[2]	hE (μm)[3]	hMV (μm)[4]	Midgut hF (μm)	wF (μm)	hE (μm)	hMV (μm)
6.9	361	83.61	46.33	4.62	365	82.87	45.25	4.42
7.2	405	96.30	46.84	4.86	375	85.61	47.44	4.68
8.6	428	102.83	48.34	5.13	407	91.10	48.44	4.78
9.2	430	90.43	45.57	4.74	401	91.01	45.04	4.75
10.7	416	83.12	43.33	4.33	377	87.12	36.03	4.57
11.1	368	70.21	40.16	4.13	371	83.58	35.63	4.22
11.7	364	68.66	39.52	3.85	359	82.88	36.23	4.12
PSE	7.05	2.84	0.91	0.10	4.49	1.01	1.23	0.06
Regression ($N = 3$)								
SOP								
Adj. R^2	0.700	0.697	0.485	0.794	0.511	0.427	0.715	0.603
p value	<0.001	<0.001	0.003	<0.001	0.002	0.007	<0.001	<0.001

[1]hF = height. of gut fold; [2]wF = width. of gut fold; [3]hE = height of .enterocytes; [4]hMV = height of.microvillus.

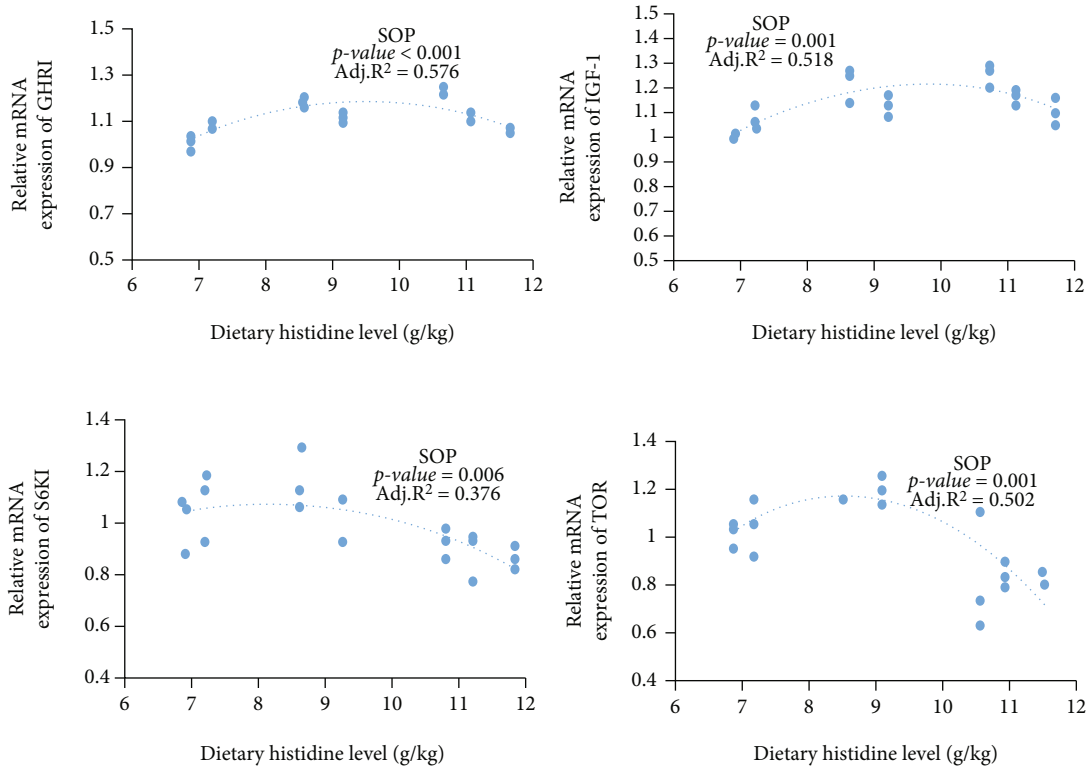

Figure 2: Expression of. GHR1 in pituitary, IGF-1, TOR, and S6K1 in the liver of hybrid. grouper fed various dietary His levels for 8.weeks ($n = 9$). IGF-1: insulin like growth. factor-1; GHR1: growth hormone receptor 1; TOR: target of.rapamycin; S6K1: ribosomal protein S6.kinase 1.

[4]. Disagreements between these studies might be due to a variety of factors, including genetic differences across fish species, environmental conditions throughout the development period, fish age, and size. Slow growth has been well documented in fish-fed low-amino-acid diets as a result of reduced feed intake [47]. However, in this study, fish fed 6.9 g/kg dietary His had higher DFI than fish fed other dietary His levels, indicating that decreased growth in fish fed His-deficient diets was due to a lack of dietary His rather than a lack of feed intake.

The incorporation of necessary amino acids in the diet has been shown to affect somatic indices in fish [54, 55]. The HSI, on the other hand, measures the nutritional status and condition of the fish [56]. In our study, dietary treatments had a significant impact on HSI and IPF ratio. The HSI and IPF were observed to decrease with an increase in

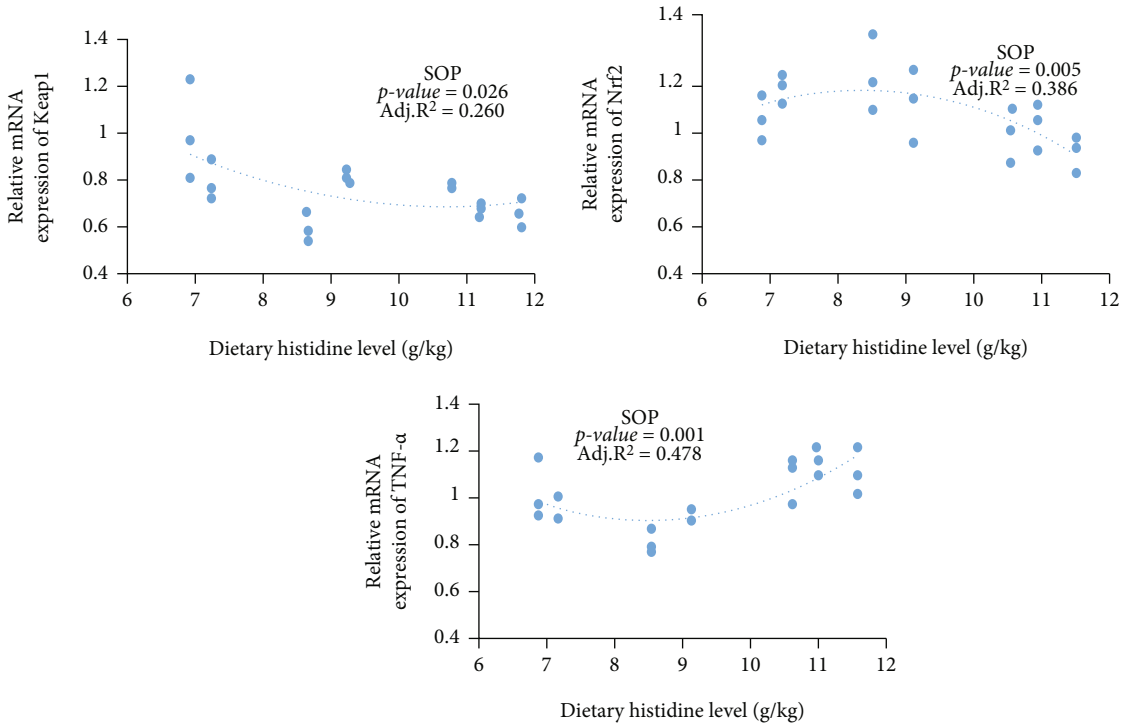

FIGURE 3: Expression. of.Keap1 and .Nrf2 in head.kidney, TNF-α in liver of grouper fed various dietary. His levels. for 8.weeks ($n = 9$). Keap1: Kelch-like ECH-associated protein 1: Nrf2: nuclear-factor-erythroid- (NFE2-) related factor 2; TNF-α: tumour.necrosis. factor-α.

dietary His concentrations up to 10.7 g/kg. However, as the level of dietary His increased from 10.7 g/kg to 11.7 g/kg, the condition factor improved. The high values of HSI and IPF ratio in fish-fed diets with a low level of His (6.9 g/kg) could increase amino acid catabolism and alternatively store energy as fat in fish liver. Furthermore, in blunt snout bream and Nile tilapia the HSI showed no significant differences affected by dietary His levels [10, 16], and differences were found in Catla catla [55].

The higher whole-body and muscle protein contents in fish fed 9.2 and 10.7 g/kg dietary His, as compared with other dietary His levels, suggested that His intake can promote hybrid grouper protein deposition. This is consistent with previous studies by Khan & Abidi [13] and Ahmed & Khan [14], who found that supplementation with L-His resulted in higher body protein levels in African catfish and Indian major carp, respectively. According to Helland & Grisdale-Helland [57], improved amino acid utilization for protein synthesis could account for the favorable effects of optimized His supplementation on body protein growth.

The primary cause of fish growth is nutrient use, which is strongly linked to intestinal growth and development [58, 59]. The dietary amino acids are significantly metabolized in the enterocytes of the gut [60]. However, no previous studies on the effects of dietary His on fish gut morphology has been conducted. Kang et al. [61] found that the intestinal morphology was not significantly affected by His-treated piglets. The dietary His supplementation did not influence the length of the villus and other morphological indexes. But, the morphological results in the foregut and midgut of hybrid grouper revealed that dietary His at 8.6 g/kg, 9.2 g/kg, and 10.7 g/kg enhanced microvillus length, fold length, and width, which may explain some of the fast development of fish given these diets.

In fish nutrition research, the expression of genes has significant potential in identifying genomic responses [62]. For example, IGF-1 is an essential growth factor with anabolic properties that regulates protein synthesis [63, 64]. In the present study, dietary His had a significant effect on the relative expression level of IGF-1 mRNA. Similar results have been reported for blunt snout bream where dietary His levels had a substantial effect on the relative expression level of IGF-1 mRNA [10]. The optimal level of dietary His increased the relative gene expression of IGF-I in juvenile hybrid grouper, indicating His's ability to promote skeletal growth, through the regulation of IGF levels in fish. Similarly, the IGF-1 mRNA levels were found effective in evaluating fish growth rate and the responses to alternative feed nutrition composition [65, 66]. The GHR1 and GHR2 are two clades of GHRs found in teleost fish [67], and they are highly expressed in the liver tissue of fish [68, 69]. The present findings revealed that expression of GHR1 was higher in fish fed 10.7 g/kg His than fish fed other dietary treatments. This indicated that consuming suitable His aroused the mRNA.expression of GHR 1 in hybrid.grouper.

The TOR. protein regulates metabolism, cell survival, and growth [70–72]. The fish growth performance can be significantly improved with an increase in the activation of the TOR signaling pathway [73]. However, in our study, the relative gene expression levels of TOR in the liver increase firstly (6.9 g/kg to 9.2 g/kg His) and then decrease (10.7 g/kg to 11.7 g/kg His) with increased dietary His levels.

Similar results have been observed by Jiang et al. [24] who found that the relative gene expression of TOR mRNA in the liver of blunt snout bream and grass carp was increased with an increase in dietary histidine levels (9.9 g/kg) and declined later as the level increased. Moreover, certain amino acids triggered the AA-sensitive TOR signaling pathway [74–77]. The relative gene expression levels of S6K1 in hybrid grouper liver tend to increase at first (6.9 g/kg to 8.6 g/kg His) and then decrease (9.2 g/kg to 1.17 g/kg His) with increased dietary His levels. To the best of our knowledge, no such study was yet documented regarding the effect of His increase on S6K1 mRNA expression in hybrid grouper. On the other hand, Gao et al. [50] and Wilson-Arop et al. [10] have reported the same trend for S6K1 in the bovine mammary epithelial cells and liver of blunt snout bream, respectively. According to Liang et al. [78] and Tu et al. [79], optimum dietary arginine supplementation can increase the level of relative expression of S6K1 mRNA in the livers of blunt snout bream and gibel carp, respectively.

The antioxidation, buffering capacity, and anti-inflammation were the properties of His and His-related compounds [19, 80]. A shortage of His compounds causes cataracts, which is a severe problem in the aquaculture industry [81]. In Atlantic salmon, cataract has been described as due to a His deficiency [82]. This may cause a problem for fish farming with financial losses. Fish with cataracts affect growth rate and are more vulnerable to secondary disease as compared to healthy species [83]. However, in this study, no such cataracts or other signs was detected, which confirmed that lower levels of dietary His were ineffective in inducing cataracts and pathological signs. The transcriptional factor, NFE2-related factor 2, controls the antioxidant stress and Keap1 inhibits Nrf2 nuclear translocation [84]. When Keap1 is exposed to oxidative stress, its capacity to degrade and ubiquitinate Nrf2 is reduced. The binding of Nrf2 to an antioxidant response element regulates a downstream transcription of the antioxidant genes that promote the restoration of body homeostasis [84, 85]. The study of Bae et al. [86] showed that TOR pathways are intended to activate key Nrf2 transcription and Keap1 degradation regulators, thus avoiding oxidative damage to the mice's liver. Similarly, His in the diet regulates the expression of antioxidant enzymes in juvenile *Megalobrama amblycephala* through the Nrf2 signaling pathway [87]. The supplementation of optimal dietary His levels resulted in reduced Nrf2 mRNA expression in the juvenile hybrid grouper head kidney. A similar effect was reported for juvenile Jian carp, where suitable arginine levels produced a decreased mRNA expression of Nrf2 in the gut [73]. Besides, oxidative stress was found to increase Nrf2 transcription in zebrafish [88] and Anguilla anguilla [23]. Further, adequate dietary His level enhanced Keap1 mRNA expression in the head kidney which displayed an opposite pattern with Nrf2 mRNA expression level. According to these findings, an appropriate dietary His level may reduce oxidative stress; on the other hand, an imbalanced His level caused oxidative stress, which might improve head kidney antioxidant enzyme activities and upregulate gene transcriptions via the Nrf2 signaling pathway.

Fish immunity is closely related to inflammation, which is initiated and regulated by inflammatory cytokines [89, 90]. In vertebrates, TNF-α (tumor necrosis factor-α) is a proinflammatory cytokine, that initiates inflammatory processes [91]. In present findings, imbalance dietary His level (6.9, 11.1 g/kg) significantly upregulated proinflammatory cytokine TNF-α mRNA levels in the liver, whereas the appropriate dietary histidine level (9.2/10.7 g/kg) inhibited the mRNA level of TNF-α in the liver, implying that the appropriate dietary His level weakens the inflammatory response of the liver via lowering TNF-α mRNA expression levels. However, Jiang et al. [92] also found that adequate dietary leucine levels decreased tissues' inflammatory responses in grass carp. According to reports, optimal dietary His reduced inflammatory responses in tissues, such as the intestine [87], and gills [24] by downregulating TNF-α gene expression. These findings highlighted that adequate dietary His can reduce the inflammatory response of the liver through the downregulation of mRNA expression of proinflammatory cytokines in hybrid grouper. The precise mechanism through which dietary His can influence cytokine expression in fish remains unidentified, and these topics are worthy of further investigation.

5. Conclusion

The current findings showed that optimal dietary His requirement for a maximum weight gain of hybrid grouper is 9.62 g/kg dry matter, equivalent to 21.08 g/kg dietary protein, which could be used to create amino acid-balanced diets for this species. Dietary His changed the relative gene expression of TOR, IGF, and S6K1, which may activate and modulate the TOR system and enhance TOR-related protein synthesis. Moreover, His supplementation can improve the growth performance, gut morphology, antioxidant status, and immune status of the studied species.

Data Availability

The data used to support the findings of this study are included in the article.

Conflicts of Interest

The authors state that they do not know of conflicting financial or personal interests which would seem to affect the paper.

Authors' Contributions

S. T and M. L conceived the presented idea. M. L and S. T developed the theory, planned the study, and performed the computations. B. Y. and L. G verified the analytical methods. Z. Z., X. W, and W. M engaged in data analysis and amended the manuscript. X. W investigate and supervised the findings of this work and developed the research question. All authors discussed the results and contributed to the final manuscript. Sehrish Taj and Lei Ma contributed equally to this work.

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