Effects of Dietary Lipid Level on Serum Biochemical Indices and Mucin Gene Expression in Juvenile Megalobrama amblycephala Postprint

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Abstract

This study aimed to investigate the effects of different dietary lipid levels on serum biochemical indices and mucin gene expression in juvenile blunt snout bream (Megalobrama amblycephala). A total of 540 juvenile blunt snout bream with an initial body weight of (10.0$±$0.5) g were randomly assigned to 6 groups with 3 replicates per group and 30 fish per replicate, and fed isonitrogenous and isoenergetic experimental diets containing lipid levels of 2.29%, 4.29%, 6.29%, 8.29%, 10.29%, and 12.29%, respectively. After 8 weeks of feeding, growth indices, serum biochemical parameters, and mucin gene expression in skin and intestine were determined. The results showed: 1) The weight gain rate (WG) in the 6.29% and 8.29% groups was significantly higher than in other groups ($P<0.05$), while the feed conversion ratio (FCR) was significantly lower than in other groups ($P<0.05$). 2) The serum cortisol content and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were highest in the 12.29% group, significantly higher than those in the 2.29%, 4.29%, 6.29%, and 8.29% groups ($P<0.05$), but showed no significant difference from the 10.29% group ($P>0.05$). 3) The activities of superoxide dismutase (SOD) and catalase (CAT) and the content of reduced glutathione (GSH) in liver were significantly higher in the 8.29% group than in the 2.29% group ($P<0.05$); glutathione reductase (GR) activity was highest in the 6.29% group, significantly higher than in other groups except the 8.29% group ($P<0.05$); malondialdehyde (MDA) content was lowest in the 6.29% group, significantly lower than in the 2.29% and 12.29% groups ($P<0.05$). 4) The expression of intestinal mucin Muc2 gene in the 12.9% group was significantly higher than in other groups ($P<0.05$); the expression of epidermal mucin Muc5b gene in the 8.29% group showed no significant difference from the 12.29% group ($P>0.05$), but was significantly higher than in other groups ($P<0.05$). 5) The pathogen challenge test showed that mortality rates were higher in the 2.29% and 12.29% groups, and lower in the 6.29% and 8.29% groups, which were significantly lower than in other

groups (P<0.05). In conclusion, under the experimental conditions, dietary lipid levels of 6.29%~8.29% resulted in the highest growth, disease resistance, and epidermal mucin Muc5b gene expression in juvenile blunt snout bream.

Full Text

Dietary Lipid Level Affects Serum Biochemical Indexes and Mucin Gene Expression in Juvenile Blunt Snout Bream (Megalobrama amblycephala)

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Abstract

This study investigated the effects of different dietary lipid levels on serum biochemical indexes and mucin gene expression in juvenile blunt snout bream. A total of 540 juvenile fish with an initial body weight of (10.0$±$0.5) g were randomly divided into six groups, each with three replicates of 30 fish. The groups were fed isonitrogenous and isoenergetic experimental diets containing lipid levels of 2.29%, 4.29%, 6.29%, 8.29%, 10.29%, and 12.29% for eight weeks. Growth performance, serum biochemical indexes, and mucin gene expression in skin and intestine were subsequently measured.

The results showed: (1) Weight gain (WG) in the 6.29% and 8.29% groups was significantly higher than in other groups (P<0.05), while feed conversion ratio (FCR) was significantly lower (P<0.05). (2) Serum cortisol content and alanine transaminase (ALT) and aspartate transaminase (AST) activities were highest in the 12.29% group, significantly exceeding those in the 2.29%, 4.29%, 6.29%, and 8.29% groups (P<0.05), but not significantly different from the 10.29% group (P>0.05). (3) Hepatic superoxide dismutase (SOD), catalase (CAT) activities, and reduced glutathione (GSH) content in the 8.29% group were significantly higher than in the 2.29% group (P<0.05). Glutathione reductase (GR) activity peaked in the 6.29% group, significantly higher than all other groups except the 8.29% group (P<0.05). Malondialdehyde (MDA) content was lowest in the 6.29% group, significantly lower than in the 2.29% and 12.29% groups (P<0.05). (4) Intestinal mucin Muc2 gene expression was significantly higher in the 12.29% group than in all other groups (P<0.05). Skin mucin Muc5b gene expression in the 8.29% group did not differ significantly from the 12.29% group (P>0.05) but was significantly higher than other groups (P<0.05). (5) Pathogen

challenge tests revealed higher mortality in the 2.29% and 12.29% groups, while the 6.29% and 8.29% groups showed significantly lower mortality ($P<0.05$).

In conclusion, under these experimental conditions, dietary lipid levels of 6.29%–8.29% optimized growth, disease resistance, and skin mucin Muc5b gene expression in juvenile blunt snout bream.

Keywords: *Megalobrama amblycephala*; lipid; mucin; Muc5b

Mucins are essential components of mucus, secreted primarily by goblet and Paneth cells in mucosal tissues of the gastrointestinal, respiratory, and urinary tracts. They constitute a critical physical barrier against pathogenic invasion in animals [1-2]. In nature, animals and microorganisms coexist symbiotically, and mucins play a vital role in defending against pathogenic infections [3]. Current research has focused mainly on mammals and poultry, with limited studies on fish mucins. Most fish mucin research has examined components such as lysozyme, immunoglobulins, and antimicrobial peptides in mucus [4]. Compared to terrestrial animals, fish maintain intimate symbiotic relationships with microorganisms in aquatic environments. To prevent pathogenic invasion, fish epidermal surfaces, intestines, gills, and other epithelial tissues are covered by a layer of mucus [5]. Recent studies on terrestrial animals indicate that dietary carbohydrate content affects intestinal mucin secretion, and starvation alters mucin distribution in the small intestine, potentially impacting digestion and immunity [6-7].

Blunt snout bream is an important freshwater aquaculture species, with highest production in Hubei and Jiangsu provinces. In some high-density culture areas, diseases such as mucus reduction and bacterial hemorrhage have sporadically occurred, seriously hindering healthy development of this industry [8-9]. Reduced surface mucus in cultured blunt snout bream may result from environmental stressors (pH, ammonia, or nitrate) or nutritional and seed quality issues. However, research on mucus secretion regulation in fish remains limited, and the causes of reduced surface mucus in blunt snout bream require further investigation. This study examined the effects of different dietary lipid levels on immunity and expression of intestinal mucin Muc2 and epidermal mucin Muc5b genes in juvenile blunt snout bream, aiming to provide theoretical support for disease prevention and control in aquaculture.

1 Materials and Methods

1.1 Experimental Fish and Design

Juvenile blunt snout bream were obtained from the Nanquan Experimental Base of Wuxi Fisheries College, Nanjing Agricultural University, and temporarily held in pond cages. After seven days, 540 healthy fish with uniform size and initial body weight of (10.0$±$0.5) g were randomly allocated into six groups, each with three replicates of 30 fish per cage. The groups were fed isonitrogenous

and isoenergetic diets with lipid levels of 2.29%, 4.29%, 6.29%, 8.29%, 10.29%, and 12.29%. Diet composition and nutrient levels are shown in Table 1 .

1.2 Feeding Management

The culture pond had a water depth of approximately 2.5 m, with cage dimensions of 2 m × 1 m × 1.5 m. Fish were hand-fed four times daily at fixed times and locations at a rate of 3%–5% of body weight, adjusted according to feeding behavior and weather conditions. Feeding continued until satiation without leftover feed. During the eight-week trial, water temperature ranged from 25.5–30.0 °C, pH was 7.1–7.6, ammonia nitrogen was <0.1 mg/L, dissolved oxygen was >5 mg/L, and nitrite was <0.06 mg/L.

1.3 Sample Collection and Processing

After the feeding trial, fish were fasted for 24 h. Fish from each cage were collected, anesthetized with MS-222 (150 mg/L, Sigma), and counted and weighed. Four fish per cage were randomly selected for blood collection via caudal vein using disposable syringes. Body length and weight were measured before rapid dissection on ice. Viscera and liver were separated and weighed. Approximately 0.1 g of liver tissue was immersed in 1 mL RNAiso Plus (TaKaRa) and stored at -80 °C, with remaining liver stored at -20 °C. Intestinal and epidermal tissues were immersed in 1 mL RNAiso Plus and stored at -80 °C. Blood was centrifuged (4 °C, 10,000 r/min, 5 min) and serum stored at -20 °C.

1.3.1 Serum Biochemical Index Determination Serum cortisol content was measured using a MAGLUMI1000 automatic chemiluminescence immunoassay analyzer. Serum ALT and AST activities were determined using a Mindray BS-400 automatic biochemical analyzer. Reagent kits were purchased from Shenzhen Mindray Bio-Medical Electronics Co., Ltd.

1.3.2 Liver Antioxidant Index Determination Liver samples were thawed, rinsed with 4 °C physiological saline, blotted dry, and weighed. Tissue was homogenized at a 1:9 ratio. Homogenates were centrifuged (4 °C, 5,000 r/min, 10 min) and supernatants stored at -70 °C. Hepatic MDA and GSH contents and SOD, GR, and CAT activities were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

1.3.3 Total RNA Extraction and cDNA Preparation Samples (<0.1 g) were thoroughly homogenized using a high-throughput tissue grinder (Ningbo Xinzhi Biotechnology Co., Ltd.). Total RNA was extracted following RNAiso Plus protocols. RNA quality and concentration were assessed using NanoDrop 2000 (Thermo Scientific). Samples with OD260/280 ratios of 1.8–2.0 were selected. Genomic DNA was removed using PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa), and 50 ng/ L RNA was reverse-transcribed. cDNA was stored at -20 °C.

1.3.4 Real-Time Fluorescent Quantitative PCR Analysis Gene expression was analyzed by qRT-PCR using primers listed in Table 2 , with β-actin as the reference gene [10]. Primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. qRT-PCR was performed using SYBR Premix Ex Taq™ II (Tli RNaseH Plus) (TaKaRa) on an ABI PRISM 7500 Real-time PCR System, with parameters following Xue et al. [5]. Negative controls used sterile double-distilled water instead of cDNA template. Each sample had three replicates. Gene expression was quantified using the relative standard curve method.

1.4 Challenge Test

Remaining juvenile blunt snout bream were cultured for an additional seven days after sampling. Thirty fish per group were randomly selected and intraperitoneally injected with 0.1 mL of pathogenic *Aeromonas hydrophila* (10^7 CFU/mL) isolated and preserved in our laboratory [11]. Mortality was observed and recorded for 14 days post-challenge.

1.5 Statistical Analysis

Data were analyzed using SPSS 18.0 software. One-way ANOVA and Duncan' s multiple comparison tests were applied. Differences were considered significant at $P<0.05$. Results are expressed as mean ± standard error ($\bar{x}\pm SE$).

2 Results

2.1 Effects of Dietary Lipid Level on Growth Performance

As shown in Table 3 , weight gain (WG), feed conversion ratio (FCR), and condition factor (CF) varied significantly with dietary lipid level ($P<0.05$). WG was significantly higher in the 6.29% and 8.29% groups than in other groups ($P<0.05$). CF was significantly higher in the 8.29% group than in other groups ($P<0.05$). FCR was significantly lower in the 6.29% and 8.29% groups than in other groups ($P<0.05$). Hepatosomatic index (HSI) in the 8.29% group was significantly higher than in the 10.29% group ($P<0.05$). Survival rate and viscerosomatic index (VSI) did not differ significantly among groups ($P>0.05$).

2.2 Effects of Dietary Lipid Level on Serum Biochemical Indexes

Serum cortisol content and ALT and AST activities increased gradually with dietary lipid level (Table 4). The 12.29% group showed the highest values, significantly exceeding those in the 2.29%, 4.29%, 6.29%, and 8.29% groups ($P<0.05$), but not significantly different from the 10.29% group ($P>0.05$).

2.3 Effects of Dietary Lipid Level on Liver Antioxidant Indexes

As dietary lipid level increased from 2.29% to 8.29%, hepatic SOD and CAT activities and GSH content increased, with significantly higher values in the

8.29% group than in the 2.29% group (P<0.05) (Table 5). GR activity peaked in the 6.29% group, significantly higher than all groups except the 8.29% group (P<0.05). MDA content showed a decreasing then increasing trend, reaching its lowest level in the 6.29% group, significantly lower than in the 2.29% and 12.29% groups (P<0.05).

2.4 Effects of Dietary Lipid Level on Mucin Muc2 and Muc5b Gene Expression

Intestinal mucin Muc2 and epidermal mucin Muc5b gene expression increased with dietary lipid level (Fig. 1 [Figure 1: see original paper] and Fig. 2 [Figure 2: see original paper]). Muc2 expression was highest in the 12.29% group, significantly higher than in all other groups (P<0.05). Muc5b expression peaked in the 8.29% group, not significantly different from the 12.29% group (P>0.05) but significantly higher than other groups (P<0.05).

2.5 Effects of Dietary Lipid Level on Mortality After *A. hydrophila* Challenge

Mortality was higher in the 2.29% and 12.29% groups and lower in the 6.29% and 8.29% groups, with the latter being significantly lower than other groups (P<0.05) (Fig. 3 [Figure 3: see original paper]).

3 Discussion

Dietary lipid level significantly affects growth, development, and reproduction in aquatic animals, with requirements varying across life stages and seasons. Our eight-week feeding trial demonstrated that juvenile blunt snout bream fed diets containing 6.29%-8.29% lipid exhibited superior growth and lowest mortality after *A. hydrophila* challenge, consistent with Jiang et al. [12]. Our surveys also indicate that commercial feeds for blunt snout bream typically contain approximately 7% lipid.

Stress during culture severely impacts fish growth, health, and disease susceptibility. Cortisol and catecholamine levels are important stress indicators [13]. Elevated cortisol and catecholamine increase blood pressure and glucose while promoting glycogenolysis. Short-term stress enhances immunity, whereas chronic stress causes immunosuppression [14]. Inappropriate stocking density, dissolved oxygen, and water quality impose severe stress on cultured aquatic animals [15-16]. Nutritional deficiencies also induce stress. Vielma et al. [17] reported that long-term feeding of high-carbohydrate diets impairs growth in European whitefish (*Coregonus lavaretus*). Ren et al. [18] found that dietary starch levels exceeding 36.3% inhibit growth and stress resistance in blunt snout bream.

Our results showed that serum cortisol increased gradually with dietary lipid level, peaking at 12.29% lipid. ALT and AST are important transaminases in fish blood. Increased ALT activity indicates muscle or cardiac dysfunction,

while elevated AST activity suggests liver dysfunction [19]. Serum ALT and AST activities increased with dietary lipid level, consistent with findings in tilapia [20]. Both low and high dietary lipid levels reduced disease resistance and increased pathogen-induced mortality.

Mucin Muc2 is predominantly distributed in the intestinal tract, playing crucial roles in nutrient absorption and disease prevention [21-25]. Studies show that dietary β-glucan supplementation increases Muc2 gene expression in chickens and pigs [26-27]. Our results demonstrated that intestinal Muc2 expression increased with dietary lipid level, suggesting that high lipid levels enhance intestinal mucin Muc2 gene expression in juvenile blunt snout bream. Although the specific function of increased intestinal mucin secretion remains unclear, a thickened mucus layer would inevitably affect nutrient absorption. Thus, upregulated intestinal Muc2 expression induced by high dietary lipid may represent a self-regulatory mechanism for nutrient absorption.

Fish epidermal mucus regulates osmotic pressure and defends against pathogen invasion [28-32]. Epidermal mucus contains complex components including mucins, immunoglobulins, lysozyme, and lectins, with mucin Muc5b being the primary structural component [5]. Therefore, Muc5b expression level influences mucus quantity. Our results showed that epidermal Muc5b expression increased then decreased with dietary lipid level, peaking in the 8.29% group. Both low and high dietary lipid levels reduced epidermal mucin expression.

The optimal dietary lipid level for juvenile blunt snout bream is 6.29%-8.29%, similar to commercial feed lipid levels. Therefore, commercial feed lipid content may not be the primary cause of mucus reduction in blunt snout bream.

4 Conclusion

Under the conditions of this study, dietary lipid levels of 6.29%-8.29% optimized growth performance, disease resistance, and epidermal mucin Muc5b gene expression in juvenile blunt snout bream.

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