Effects of Vitamin A on Growth, Metabolism, Antioxidant Capacity, and Immune Function in Juvenile Black Carp (Postprint)

Authors: Chen Lian, Wu Chenglong, Zhang Yixiang, Shao Xianping, Chen Shujian, Wang Chunlin, Ye Jinyun

Date: 2018-12-24T00:00:00+00:00

Abstract

This study used juvenile black carp (Mylopharyngodon piceus) as experimental subjects to investigate the effects of six iso-nitrogenous and iso-energetic diets with different vitamin A contents (290, 1,033, 1,734, 3,835, 7,662, and 14,943 IU/kg) on growth, metabolism, antioxidant capacity, and immune capacity of juvenile black carp. A total of 540 juvenile black carp with an initial body weight of (5.35$±$0.16) g were selected and randomly divided into 6 groups, with each group fed one diet, 3 replicates per group, and 30 fish per replicate. The experimental period lasted 60 days. The results showed: 1) With increasing dietary vitamin A content, weight gain rate, specific growth rate, and protein efficiency all exhibited a trend of first increasing and then decreasing, reaching maximum values at 3,835 IU/kg vitamin A. Broken-line model analysis revealed that a dietary vitamin A content of 2,246.64 IU/kg could achieve maximum weight gain rate. 2) As dietary vitamin A content increased from 290 IU/kg to 7,662 IU/kg, the activities of α-amylase, trypsin, chymotrypsin, and elastase in the liver increased significantly ($P<0.05$), and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and total antioxidant capacity (T-AOC) in the liver also showed significant upregulation ($P<0.05$); the contents of glutathione S-transferase (GST) and malondialdehyde (MDA) in the liver exhibited a trend of first decreasing and then increasing with increasing vitamin A content, both reaching minimum values in the 3,835 IU/kg group. 3) Compared with the 290 IU/kg group, serum lysozyme (LYZ) activity and complement 3 (C3) and complement 4 (C4) contents in the 3,835 and 7,662 IU/kg groups increased significantly ($P<0.05$); appropriate levels of dietary vitamin A (1,734, 3,835, and 7,662 IU/kg) could significantly increase the gene expression levels of LYZ, interferon α (INFα), hepcidin (HEPC), natural resistance-associated macrophage protein (NRAMP), C3, and complement 9 (C9) in blood cells (P<0.05). Therefore, appropriate supplementation of vitamin A in diets not only promotes growth and metabolism of juvenile black carp, but also enhances their antioxidant and non-specific immune capacities; the optimal dietary vitamin A content for juvenile black carp is 2,246.64 IU/kg.

Full Text

Effects of Vitamin A on Growth, Metabolism, Antioxidant Capacity and Immunity in Juvenile Black Carp (Mylopharyngodon piceus)

CHEN Lian[1],[2], WU Chenglong[2], ZHANG Yixiang[2], SHAO Xianping[2], CHEN Shujian[1],[2], WANG Chunlin[1], YE Jinyun[2],*

[1]Key Laboratory of Applied Marine Biotechnology of the Ministry of Education, School of Marine Sciences, Ningbo University, Ningbo 315211, China
[2]Zhejiang Provincial Key Laboratory of Aquatic Resources Conservation and Development, School of Life Sciences, Huzhou University, Huzhou 313000, China

Abstract: This study investigated the effects of six dietary vitamin A levels (290, 1,033, 1,734, 3,835, 7,662, and 14,943 IU/kg) on growth, metabolism, antioxidant capacity, and immunity in juvenile black carp (Mylopharyngodon piceus). A total of 540 juvenile black carp with an initial body weight of (5.35$±$0.16) g were randomly divided into six groups, with three replicates per group and 30 fish per replicate. The feeding trial lasted for 60 days. The results showed: (1) With increasing dietary vitamin A content, weight gain rate (WGR), specific growth rate (SGR), and protein efficiency ratio (PER) initially increased and then decreased, reaching maximum values at 3,835 IU/kg vitamin A. Broken-line model analysis indicated that the dietary vitamin A content of 2,246.64 IU/kg yielded the maximum WGR. (2) As dietary vitamin A increased from 290 to 7,662 IU/kg, the activities of α-amylase, trypsin, chymotrypsin, and elastase in the liver increased significantly (P<0.05). Similarly, hepatic superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR) activities, as well as total antioxidant capacity (T-AOC), were significantly up-regulated (P<0.05). Both hepatic glutathione S-transferase (GST) activity and malondialdehyde (MDA) content decreased initially and then increased, reaching minimum values in the 3,835 IU/kg group. (3) Compared with the 290 IU/kg group, serum lysozyme (LYZ) activity and complement 3 (C3) and complement 4 (C4) contents were significantly higher in the 3,835 and 7,662 IU/kg groups (P<0.05). Appropriate dietary vitamin A levels (1,734, 3,835, and 7,662 IU/kg) significantly increased the expression levels of LYZ, interferon α (INFα), hepcidin (HEPC), natural resistance-associated macrophage protein (NRAMP), C3, and complement 9 (C9) genes in blood cells (P<0.05). Therefore, adequate dietary vitamin A not only promotes growth and metabolism but also enhances antioxidant and non-specific immune capacity in juvenile black carp. The optimal dietary vitamin A content for juvenile black carp is 2,246.64 IU/kg.

Keywords: vitamin A; Mylopharyngodon piceus; requirement; antioxidant capacity; immune response

---

Vitamin A is an essential fat-soluble nutrient for maintaining fish growth and participates in multiple important physiological processes including cell differentiation, skeletal development, reproduction, and immunity [1-2]. Both deficiency and excess of vitamin A can adversely affect fish growth [3]. When fish diets lack vitamin A or its precursor carotenoids, fish may develop obvious symptoms such as vision impairment, orbital and fin hemorrhage, gill cover deformation, and increased mortality [2-4]. Conversely, excessive vitamin A intake can lead to accumulation in the liver and other adipose tissues [5], causing spinal injuries [6], growth retardation [7], and reduced reproductive capacity [2].

In recent years, optimal dietary vitamin A requirements have been reported for various fish species including grass carp (Ctenopharyngodon idellus) [8], greasy grouper (Epinephelus tauvina) [9], Wuchang bream (Megalobrama amblycephala) [4], Nile tilapia (Oreochromis niloticus) [10], rainbow trout (Oncorhynchus mykiss) [11], and largemouth bass (Micropterus salmoides) [12]. Research has demonstrated that vitamin A plays important roles in visual function, epithelial cell differentiation, skeletal development, lipid metabolism, and immune function in fish [1,12-15]. Like other vertebrates, fish cannot synthesize vitamin A [15], and their requirements are influenced by species, individual size, age, and environmental factors [2]. Therefore, investigating appropriate dietary vitamin A levels is crucial for guiding fish growth, metabolism, and immune function.

Black carp (Mylopharyngodon piceus) is the only carnivorous species among China’ s traditional “four major domestic fish” and is widely cultured due to its nutritional value in strengthening physique, delaying aging, and nourishing qi and stomach [16]. Current nutritional research on black carp has primarily focused on requirements for lipids [17], carbohydrates [18-20], proteins and amino acids [20], and minerals [21], while studies on micronutrient requirements such as vitamins remain scarce. Reports on improving growth and immunity through dietary vitamin supplementation in black carp have mainly involved vitamin C [22], vitamin D [23], and vitamin E [24], with very limited information on vitamin A. This study investigated the effects of different dietary vitamin A levels on growth, metabolism, and antioxidant capacity, as well as immune function, in juvenile black carp to provide reference for vitamin A supplementation in artificial compound feeds and theoretical basis for developing nutritionally complete and cost-effective feeds.

1.1 Experimental Design

A single-factor experimental design was employed. The basal diet was formulated using vitamin-free casein and gelatin as protein sources, dextrin as carbohydrate source, and soybean oil and lecithin as lipid sources, supplemented with amino acid mixtures, mineral premix, and vitamin premix without vitamin A. Diets 1-5 were supplemented with vitamin A at 0, 1,000, 2,000, 4,000, 8,000, and 16,000 IU/kg, respectively. The actual measured contents using high-performance liquid chromatography (Agilent-1100, Agilent, USA) were 290, 1,033, 1,734, 3,835, 7,662, and 14,943 IU/kg. The composition and nutrient levels of experimental diets are shown in Table 1 . Raw materials were ground using a universal grinder and sieved to ensure particle size above 40 mesh. Various ingredients were accurately weighed and thoroughly mixed. The mixed feed ingredients were processed into 1.5 mm diameter strips using a twin-screw extruder, frozen, cut into 2-3 mm pellets, dried in a 40°C hot air oven, and finally sealed in double-layered plastic bags and stored at -20°C.

1.2 Experimental Fish and Management

A total of 540 healthy juvenile black carp with similar size and robust condition, with initial body weight of (5.35$±$0.16) g, were randomly divided into six groups, each with three replicates of 30 fish. The experiment began after a one-week acclimation period. During acclimation, fish were fed the vitamin A-deficient diet (290 IU/kg group) twice daily at 08:00 and 17:00 at 3% of body weight. During the culture period, signs of fish health were observed daily, and feeding behavior and mortality were recorded. The feeding trial lasted for 60 days. Water was exchanged once daily at one-third of the tank volume. Replacement water came from an artificial reservoir aerated for at least one day and was continuously recirculated. Water temperature was maintained at 26-33°C with natural photoperiod, pH at approximately 7.2, and dissolved oxygen above 5.8 mg/L.

1.3 Sample Collection and Analysis

At the end of the feeding trial, feeding was stopped for 24 hours to empty intestinal contents. The total number of fish in each tank was counted and total weight was measured. Five fish were randomly selected from each tank for individual body weight and length measurement. Blood and liver samples were collected by dissection, liver weight was recorded, and samples were immediately frozen in liquid nitrogen and subsequently stored at -80°C.

Crude protein, crude lipid, crude ash, and moisture contents in experimental diets and whole fish were determined as follows: crude protein by Kjeldahl method (kjeltec-2200, FOSS, Denmark); crude lipid by Soxhlet extraction (soxtecTM_{2043}, FOSS, Denmark); moisture by drying at 105°C to constant weight; and crude ash by combustion in a muffle furnace at 550°C for 14 hours. Vitamin A contents in diets and whole fish were determined by high-performance liquid chromatography with two replicates per sample.

Activities of hepatic α-amylase, trypsin, chymotrypsin, elastase, and antioxidant-related enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) were measured using commercial assay kits from Nanjing Jiancheng Bioengineering Institute. Hepatic malondialdehyde (MDA) content, total antioxidant capacity (T-AOC), and serum lysozyme (LYZ), complement 3 (C3), and complement 4 (C4) contents were also determined using commercial kits from the same institute.

1.4.1 Primer Design

Primers for real-time quantitative PCR of non-specific immune genes including LYZ, interferon α (INFα), hepcidin (HEPC), natural resistance-associated macrophage protein (NRAMP), C3, and complement 9 (C9) were designed using Primer 5.0 and Oligo 7.0 software (Table 2). All primers were synthesized by Shanghai BioSune Biotechnology. β-actin was used as the housekeeping gene to verify expression stability across tissues.

1.4.2 Total RNA Extraction

Total RNA from blood cells was extracted using TRIzol reagent (Invitrogen) and quality was assessed using 1% agarose denaturing gel electrophoresis. Complementary DNA (cDNA) was synthesized from 3 g total RNA using PrimeScriptTM reverse transcription kit (TaKaRa, Japan) under conditions of 37°C for 15 min and 85°C for 5 s. The cDNA was stored at -20°C for gene expression analysis.

1.4.3 Real-time Quantitative PCR

Real-time quantitative PCR was performed according to Wu et al. [25] using a CFX96 system (Bio-Rad, USA). The total reaction volume was 25 L, containing 1 L cDNA solution, 12.5 L 2×SYBR Green I Real-time PCR Master Mix (TaKaRa, Japan), 0.2 mol/L forward primer, 0.2 mol/L reverse primer, and water to 25 L. The thermal cycling program was: 95°C for 2 min; followed by 35 cycles of 95°C for 5 s and 59°C for 15 s. Relative gene expression was quantified using the 2-ΔΔCt method [26].

1.5 Calculation Formulas

The following formulas were used to calculate weight gain rate (WGR), specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), hepatosomatic index (HSI), protein efficiency ratio (PER), and condition factor (CF):

WGR (%) = 100 × (final mean weight - initial mean weight) / initial mean weight

SGR (%/d) = 100 × [ln(final mean weight) - ln(initial mean weight)] / experimental days
SR (%) = 100 × (final fish number) / (initial fish number)
FCR = fish weight gain (wet weight) / feed intake
HSI (%) = 100 × liver weight / body weight
CF (g/cm^3) = 100 × final body weight / (final body length)^3
PER (%) = 100 × weight gain (g) / protein intake (g, dry weight)

1.6 Statistical Analysis

All data were analyzed using SPSS 20.0 software by one-way ANOVA. When significant differences were detected, Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as mean ± standard deviation. $P < 0.05$ was considered statistically significant.

2.1 Effects of Vitamin A on Growth Performance and Body Composition

As shown in Table 3 , WGR and SGR of juvenile black carp fed the diet containing 290 IU/kg vitamin A were significantly lower than those of other groups ($P<0.05$). With increasing dietary vitamin A content, WGR, SGR, and PER initially increased and then decreased, reaching maximum values at 3,835 IU/kg vitamin A. HSI decreased initially when dietary vitamin A increased from 290 to 1,734 IU/kg, then gradually increased thereafter. HSI in the 290 IU/kg group was not significantly different from the 1,033 IU/kg group ($P>0.05$) but was significantly higher than other groups ($P<0.05$). Dietary vitamin A levels did not significantly affect CF ($P>0.05$). FCR decreased initially and then increased with increasing dietary vitamin A, reaching the lowest value at 7,662 IU/kg, though no significant differences were observed among groups fed 1,734-14,943 IU/kg vitamin A ($P>0.05$). SR increased initially and then decreased with increasing dietary vitamin A, with no significant differences among groups fed 1,033-14,943 IU/kg ($P>0.05$), and reached the highest value at 7,662 IU/kg.

Dietary vitamin A levels did not significantly affect moisture, crude lipid, or crude ash contents in whole fish body composition ($P>0.05$). Whole-body crude protein content increased initially and then decreased with increasing dietary vitamin A, reaching the highest value in the 7,662 IU/kg group, which was significantly higher than the 290 IU/kg group ($P<0.05$).

Broken-line model analysis of WGR in relation to dietary vitamin A content yielded the following relationships: $y = 0.1043x + 156.54$, $R^2 = 0.9535$; $y = -0.0059x + 404.12$, $R^2 = 0.9705$. Based on this analysis, the optimal dietary vitamin A requirement for juvenile black carp was determined to be 2,246.64 IU/kg (Figure 1 [Figure 1: see original paper]).

2.2 Effects of Vitamin A on Hepatic Metabolic Enzyme Activity and Antioxidant Capacity

Table 4 shows that hepatic α-amylase, trypsin, chymotrypsin, and elastase activities initially increased and then decreased with increasing dietary vitamin A content. α-Amylase and elastase activities reached maximum values in the 3,835 IU/kg group, while trypsin and chymotrypsin activities peaked in the 7,662 IU/kg group. No significant differences were observed among the 3,835 and 7,662 IU/kg groups for these four metabolic enzymes ($P>0.05$).

As shown in Table 5 , hepatic CAT, GPx, and GR activities and T-AOC initially increased and then decreased with increasing dietary vitamin A. T-AOC reached its maximum value at 3,835 IU/kg vitamin A, while CAT, GPx, and GR activities peaked at 7,662 IU/kg. SOD activity showed a continuous increasing trend, reaching the highest value in the 14,943 IU/kg group, which was significantly different from the 290, 1,033, and 1,734 IU/kg groups ($P<0.05$). Hepatic GST activity and MDA content initially decreased and then increased with dietary vitamin A, reaching minimum values in the 3,835 IU/kg group. No significant differences in antioxidant indices were observed between the 3,835 and 7,662 IU/kg groups ($P>0.05$).

2.3 Effects of Vitamin A on Non-specific Immune Capacity

Table 6 shows that serum LYZ, C3, and C4 contents initially increased and then stabilized with increasing dietary vitamin A. Serum LYZ and C3 contents reached maximum values in the 3,835 IU/kg group, while serum C4 content peaked in the 7,662 IU/kg group.

Figure 2 [Figure 2: see original paper] shows that blood cell LYZ gene expression initially increased and then decreased, reaching the highest value in the 3,835 IU/kg group, which was significantly higher than other groups ($P<0.05$), though no significant differences were observed among the 1,734, 7,662, and 14,943 IU/kg groups ($P>0.05$). INFα gene expression peaked in the 1,734 IU/kg group, which was not significantly different from the 3,835 and 7,662 IU/kg groups ($P>0.05$) but was significantly higher than other groups ($P<0.05$). HEPC gene expression did not differ significantly among groups fed 1,734-14,943 IU/kg vitamin A ($P>0.05$), though the highest value occurred in the 1,734 IU/kg group, which was significantly different from the 290 and 1,033 IU/kg groups ($P<0.05$). NRAMP gene expression showed an initial increase followed by a decrease, reaching maximum value in the 1,734 IU/kg group, which was significantly higher than other groups ($P<0.05$). C3 gene expression peaked in the 1,734 IU/kg group, with no significant differences from the 3,835 and 7,662 IU/kg groups ($P>0.05$) but was significantly higher than other groups ($P<0.05$). C9 gene expression in the 290 IU/kg group was significantly lower than other groups ($P<0.05$), reaching the highest value in the 7,662 IU/kg group, though no significant differences were observed among the 1,734, 7,662, and 14,943 IU/kg groups ($P>0.05$).

3.1 Effects of Vitamin A on Growth Performance and Body Composition

The results demonstrate that vitamin A is an essential nutrient promoting growth in juvenile black carp, significantly affecting WGR, SGR, HSI, PER, FCR, and SR, consistent with findings in other fish species [4,8,12,27-28]. Based on WGR using broken-line model analysis, the optimal dietary vitamin A requirement for juvenile black carp was determined to be 2,246.64 IU/kg, which is similar to requirements reported for other carnivorous fish such as greasy grouper [9], rainbow trout [29], largemouth bass [12], Japanese seabass (Lateolabrax japonicus) [30], and Wuchang bream [4]. However, this requirement is significantly higher than that for Amur sturgeon (Acipenser schrencki) [28] but lower than for Japanese flounder (Paralichthys olivaceus) [31] and hybrid tilapia (Oreochromis niloticus × O. aureus) [32], suggesting that vitamin A requirements vary among fish species [12]. The study also found that growth was significantly inhibited when dietary vitamin A was only 290 or 1,033 IU/kg, similar to results in largemouth bass [12], Jian carp (Cyprinus carpio var. Jian) [3], and Japanese flounder [31]. Compared with the adequate vitamin A group (3,835 IU/kg), excessive vitamin A (14,943 IU/kg) significantly inhibited growth, consistent with findings in Japanese flounder [33], orange-spotted grouper (Epinephelus coioides) [27], and red drum (Sciaenops ocellatus) [34]. These results further confirm that both deficiency and excess of dietary vitamin A cause growth inhibition in cultured black carp [3], though the molecular mechanisms underlying this phenomenon require further investigation.

The results also showed that different dietary vitamin A levels did not significantly affect moisture, crude ash, or crude lipid contents in whole fish. Whole-body crude protein content reached maximum value in the 7,662 IU/kg group, which was significantly different from the vitamin A-deficient group but not from other treatment groups. Similar results have been reported in Jian carp [3], largemouth bass [12], and orange-spotted grouper [27]. In these studies, changes in whole-body crude protein content were consistent with PER trends, indicating that vitamin A deficiency or excess reduces protein utilization in juvenile black carp and that vitamin A participates in nutrient metabolism affecting body composition [12,27]. Additionally, whole-body crude lipid content showed an initial increase followed by stabilization with increasing dietary vitamin A, consistent with findings in largemouth bass [12], Japanese seabass [2], and Japanese flounder [31]. However, dietary vitamin A levels did not significantly affect body composition in Wuchang bream [4] or grass carp [5,8], possibly due to species-specific biological differences.

3.2 Effects of Vitamin A on Hepatic Metabolic Enzyme Activity

As the primary organ for storing and metabolizing fat-soluble vitamin A [35], the liver exhibits high metabolic enzyme activity [36], making it an excellent indicator of metabolic status. This study found that hepatic α-amylase activity in juvenile black carp initially increased and then decreased with increasing dietary vitamin A, suggesting that appropriate vitamin A levels improve carbohydrate digestion, absorption, and utilization, consistent with conclusions from higher vertebrate studies [37]. Furthermore, the activities of various proteases in the liver showed trends consistent with whole-body crude protein content and PER, indicating that adequate dietary vitamin A promotes protein digestion, absorption, and utilization, thereby enhancing growth and protein deposition [12,27,31]. Vitamin A deficiency reduced digestive capacity, decreasing nutrient (especially protein) utilization and causing growth inhibition [3]. However, the molecular mechanisms by which vitamin A regulates metabolic enzymes require further investigation.

3.3 Effects of Vitamin A on Hepatic Antioxidant Capacity

Like other aerobic organisms, fish cells are vulnerable to non-specific attack by reactive oxygen species such as superoxide anion and hydrogen peroxide. Fish have evolved a series of antioxidant molecules to defend against oxidative damage [38-39], including non-enzymatic antioxidants (e.g., GSH) and antioxidant enzymes (e.g., CAT, SOD, GST, GPx, and GR). SOD primarily catalyzes the conversion of superoxide anion to hydrogen peroxide, thereby reducing oxidative damage. Consistent with results in red drum [34], grass carp [8], and largemouth bass [12], this study found that SOD activity increased initially and then stabilized with increasing vitamin A, indicating that adequate vitamin A benefits juvenile black carp in resisting oxidative stress [14]. Both CAT and GPx catalyze hydrogen peroxide conversion to harmless water and oxygen molecules. The observed initial increase and subsequent decrease in hepatic CAT, GPx, and GR activities suggest that vitamin A supplementation within a certain range can enhance antioxidant enzyme activities. As a comprehensive indicator of antioxidant capacity, T-AOC showed similar trends to CAT, GPx, and GR activities, indicating that adequate dietary vitamin A can effectively scavenge excess reactive oxygen radicals and promote antioxidant system balance [38]. Hepatic GST activity and MDA content directly reflect the degree of oxidative damage in animal cells. Compared with the vitamin A-deficient group (290 IU/kg), dietary vitamin A at 1,734, 3,835, and 7,662 IU/kg down-regulated hepatic GST activity and MDA content, which increased again in the excess group (14,943 IU/kg), consistent with findings in largemouth bass [12]. These results indicate that adequate vitamin A can alleviate oxidative damage caused by excess reactive oxygen radicals in juvenile black carp cells [38]. Therefore, appropriate dietary vitamin A supplementation can enhance the antioxidant response capacity of juvenile black carp.

3.4 Effects of Vitamin A on Non-specific Immune Capacity

Lysozyme (LYZ) plays an important role in fish non-specific immunity, and its gene expression directly reflects immune status, serving as an important indicator of immune response in aquatic animals [25,28]. In this study, both blood cell LYZ gene expression and serum LYZ content reached maximum values at 3,835 IU/kg vitamin A, consistent with results in tilapia and Jian carp [3,10,12], demonstrating that adequate vitamin A enhances immunity by increasing LYZ gene expression and content. However, Thompson et al. [40] did not observe similar effects in Atlantic salmon (Salmo salar L.), possibly due to species differences. Interferon serves as the first line of defense against pathogens, especially viruses, in higher vertebrates and represents an important defense factor and immune cell activator in lower vertebrates such as fish [41]. High interferon gene expression also reflects strong immune capacity [19]. Combined with the present findings, these results further demonstrate that adequate dietary vitamin A can enhance disease resistance in juvenile black carp by increasing INFα gene expression. NRAMP and HEPC are important antimicrobial peptides and bactericidal agents in animals, playing crucial roles in non-specific immune regulation and enhancing acquired immune defense [42]. NRAMP can activate macrophages to increase production of nitric oxide (NO), an active antimicrobial molecule, thereby enhancing immunity [43]. HEPC can promote macrophage endocytosis and proteolysis of pathogens by binding to iron transport proteins [42]. Therefore, adequate dietary vitamin A can enhance non-specific immune defense in black carp by increasing NRAMP and HEPC gene expression [25]. As important factors in humoral immunity, complement molecules activated through alternative or lytic pathways first bind to foreign bacteria, then promote phagocytosis through corresponding receptors on phagocyte surfaces, implementing lysis and destruction of foreign invaders and enhancing immune defense [44-45]. As key molecules activating the complement system, serum C3 and C4 contents in juvenile black carp were significantly increased by adequate vitamin A, consistent with findings in Wuchang bream [4], Atlantic salmon [40], rainbow trout [29], gilthead seabream (Sparus aurata L.) [46], Japanese flounder [47], and largemouth bass [12]. These consistent effects of vitamin A on non-specific immune indices further demonstrate that adequate dietary vitamin A is important for maintaining health and immune defense in juvenile black carp.

4 Conclusion

1. Dietary supplementation with adequate vitamin A significantly promotes growth and metabolism while enhancing antioxidant capacity and non-specific immunity in juvenile black carp.

2. Broken-line model analysis indicates that the optimal dietary vitamin A content for juvenile black carp is 2,246.64 IU/kg.

References

[1] OLSON J A. Needs and sources of carotenoids and vitamin A[J]. Nutrition Reviews, 1994, 52(2): S67-S73.
[2] National Research Council of the National Academy of Sciences. Nutrient Requirements of Fish and Shrimp[S]. Translated by MAI Kangsen, LI Peng,

ZHAO Jianmin. Beijing: Science Press, 2015.

[3] YANG Qihui, ZHOU Xiaoqiu. Effects of vitamin A deficiency on growth performance and immune function of Jian carp (Cyprinus carpio var. Jian)[J]. Journal of Fishery Sciences of China, 2005, 12(1): 62-67.

[4] LIU B, ZHAO Z X, BROWN P B, et al. Dietary vitamin A requirement of juvenile Wuchang bream (Megalobrama amblycephala) determined by growth and disease resistance[J]. Aquaculture, 2016, 450: 23-30.

[5] JIANG Ming. Studies on vitamin A, D and K requirements of juvenile grass carp (Ctenopharyngodon idellus)[D]. Master' s thesis. Wuhan: Huazhong Agricultural University, 2007.

[6] SALEH G, ELERAKY W, GROPP J M. A short note on the effects of vitamin A hypervitaminosis and hypovitaminosis on health and growth of Tilapia nilotica (Oreochromis niloticus)[J]. Journal of Applied Ichthyology, 1995, 11(3/4): 382-385.

[7] DEDI J, TAKEUCHI T, SEIKAI T, et al. Hypervitaminosis and safe levels of vitamin A for larval flounder Paralichthys olivaceus fed Artemia nauplii[J]. Aquaculture, 1995, 133(2): 135-146.

[8] WU F, ZHU W H, LIU M M, et al. Effects of dietary vitamin A on growth performance, blood biochemical indices and body composition of juvenile grass carp (Ctenopharyngodon idellus)[J]. Turkish Journal of Fisheries and Aquatic Sciences, 2016, 16(2): 339-345.

[9] MOHAMED J S, SIVARAM V, ROY T S C, et al. Dietary vitamin A requirement of juvenile greasy grouper (Epinephelus tauvina)[J]. Aquaculture, 2003, 219(1/2/3/4): 693-701.

[10] GUO R, LIM C, XIA H, et al. Effect of various dietary vitamin A levels on growth performance and immune response of tilapia (Oreochromis niloticus)[J]. Frontiers of Agriculture in China, 2010, 4(4): 507-512.

[11] FALAHATKAR B, LOTFI A, AMLASHI A S, et al. Growth performance, carcass composition, hematological and biochemical indices of rainbow trout, Oncorhynchus mykiss juveniles fed diet containing different levels of vitamin premix[J]. Iranian Society of Ichthyology, 2016, 3(1): 53-63.

[12] LIAN Xueyuan, CHEN Naisong, WANG Mengle, et al. Vitamin A requirement of largemouth bass (Micropterus salmoides)[J]. Chinese Journal of Animal Nutrition, 2017, 29(10): 3819-3830.

[13] TAKEUCHI T, DEDI J, HAGA Y, et al. Effect of vitamin A compounds on bone deformity in larval Japanese flounder (Paralichthys olivaceus)[J]. Aquaculture, 1998, 169(3/4): 155-165.

[14] LIU Mengmei, CHEN Jiaojiao, ZHU Wenhuan, et al. Effects of vitamin A on growth performance and calcium and phosphorus contents in bones of grow-out grass carp (Ctenopharyngodon idellus)[J]. Acta Hydrobiologica Sinica, 2017, 41(1): 101-107.

[15] MAI Kangsen. Aquatic Animal Nutrition and Feed Science[M]. 2nd ed. Beijing: China Agriculture Press, 2011: 149.

[16] CHEN Jianming, SHEN Binqian, PAN Qian, et al. Effects of dietary protein and lipid levels on growth and body composition of large-size black carp (Mylopharyngodon piceus) fingerlings[J]. Acta Hydrobiologica Sinica,

2014, 38(4): 699-705.
[17] WANG Daozun, GONG Xizhang, LIU Yufang. Effects of dietary lipid content on growth of black carp (Mylopharyngodon piceus) fingerlings[J]. Journal of Fisheries of China, 1987, 11(1): 23-28.
[18] WU C L, YE J Y, GAO J E, et al. Effect of varying carbohydrate fractions on growth, body composition, metabolic, and hormonal indices of juvenile black carp, Mylopharyngodon piceus[J]. Journal of the World Aquaculture Society, 2016, 47(3): 435-449.
[19] WU C L, YE J Y, GAO J E, et al. The effects of dietary carbohydrate on the growth, antioxidant capacities, innate immune responses and pathogen resistance of juvenile Black carp Mylopharyngodon piceus[J]. Fish & Shellfish Immunology, 2016, 49: 132-142.
[20] WANG Daozun, SONG Tianfu, DU Hanbin, et al. Effects of dietary protein and carbohydrate contents on growth of black carp (Mylopharyngodon piceus) fingerlings[J]. Journal of Fisheries of China, 1984, 8(1): 9-17.
[21] LENG Xiangjun, WANG Daozun. Study on copper requirement of black carp[C]//Proceedings of the 3rd World Chinese Symposium on Fish and Shrimp Nutrition. Shanghai: China Society of Fisheries, 2007.
[22] HU Yi, HUANG Yun, WEN Hua, et al. Effects of vitamin C on growth, immunity and anti-ammonia stress capacity of juvenile black carp (Mylopharyngodon piceus)[J]. Journal of Fisheries of China, 2013, 37(4): 565-573.
[23] WU Chenglong, YE Jinyun. Effects of vitamin D on growth, antioxidant capacity and non-specific immunity of juvenile black carp (Mylopharyngodon piceus)[C]//Abstracts of the 2015 Annual Meeting of the Chinese Society of Fisheries. Hangzhou: Chinese Society of Fisheries, 2015.
[24] HUANG Yun, HU Yi, WEN Hua, et al. Effects of vitamin E on growth, immunity and anti-ammonia stress capacity of juvenile black carp (Mylopharyngodon piceus)[J]. Acta Hydrobiologica Sinica, 2013, 37(3): 507-514.
[25] WU C L, CHEN L, LU Z B, et al. The effects of dietary leucine on the growth performances, body composition, metabolic abilities and innate immune responses in black carp Mylopharyngodon piceus[J]. Fish & Shellfish Immunology, 2017, 67: 419-428.
[26] LIVAK K J, SCHMITTGEN T D. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method[J]. Methods, 2001, 25(4): 402-408.
[27] HOU Xin. Studies on vitamin A and inositol requirements of orange-spotted grouper (Epinephelus coioides) and tilapia[D]. Master' s thesis. Guangzhou: Sun Yat-sen University, 2009.
[28] GAO Qiang. Studies on vitamin A, C and E requirements of juvenile sturgeon species[D]. Master' s thesis. Chongqing: Southwest Agricultural University, 2005.
[29] THOMPSON I, CHOUBERT G, HOULIHAN D F, et al. The effect of dietary vitamin A and astaxanthin on the immunocompetence of rainbow trout[J]. Aquaculture, 1995, 133(2): 91-102.
[30] ZHANG Lu. Nutritional physiology of several vitamins and protein source development for Japanese seabass and large yellow croaker[D]. PhD thesis.

Qingdao: Ocean University of China, 2006.
[31] HERNANDEZ L H H, TESHIMA S I, ISHIKAWA M, et al. Dietary vitamin A requirements of juvenile Japanese flounder Paralichthys olivaceus[J]. Aquaculture Nutrition, 2015, 11(1): 3-9.
[32] HU C J, CHEN S M, PAN C H, et al. Effects of dietary vitamin A or β-carotene concentrations on growth of juvenile hybrid tilapia, Oreochromis niloticus × O. aureus[J]. Aquaculture, 2006, 253(1/2/3/4): 602-607.
[33] DEDI J, TAKEUCHI T, HOSOYA K, et al. Effect of Vitamin A levels in Artemia nauplii on the caudal skeleton formation of Japanese flounder Paralichthys olivaceus[J]. Fisheries Science, 2011, 64(2): 344-345.
[34] ZHOU Libin, WANG Anli, ZHANG Wei, et al. Effects of dietary vitamin A on growth and immunity of red drum (Sciaenops ocellatus)[J]. Chinese Journal of Animal Nutrition, 2008, 20(4): 482-488.
[35] WEI Yuting. Studies on methionine, arginine, vitamin A and vitamin E requirements of juvenile turbot (Scophthalmus maximus)[D]. Master' s thesis. Qingdao: Ocean University of China, 2010.
[36] YE Yuantu, XUE Min, LIN Shimei, et al. Comparison of protein enzymatic hydrolysis rates in intestine and hepatopancreas of grass carp (Ctenopharyngodon idellus)[J]. Journal of Fishery Sciences of China, 2003, 10(2): 173-176.
[37] SOLIMAN M M, AHMED M M, EL-SHAZLY S A, et al. Effect of vitamin A and E on carbohydrate and lipid metabolism in diet-induced obese wistar rats[J]. Advances in Bioscience and Biotechnology, 2014, 5(1): 41633.
[38] MARTÍNEZ-ÁLVAREZ R M, MORALES A E, SANZ A. Antioxidant defenses in fish: biotic and abiotic factors[J]. Reviews in Fish Biology and Fisheries, 2005, 15(1/2): 75-88.
[39] WANG Fei, LI Xiangfei, LI Pengfei, et al. Effects of dietary vitamin B2 level on growth performance, body composition, antioxidant function and intestinal enzyme activities of juvenile Wuchang bream (Megalobrama amblycephala)[J]. Jiangsu Agricultural Sciences, 2016, 44(5): 319-324.
[40] THOMPSON I, FLETCHER T C, HOULIHAN D F, et al. The effect of dietary vitamin A on immunocompetence of Atlantic salmon (Salmo salar L.)[J]. Fish Physiology and Biochemistry, 1994, 12(6): 513-523.
[41] ZHU L Y, NIE L, ZHU G, et al. Advances in research of fish immune-relevant genes: a comparative overview of innate and adaptive immunity in teleosts[J]. Developmental & Comparative Immunology, 2013, 39(1/2): 39-62.
[42] ZHU S Y, GAO B. Evolutionary origin of β-defensins[J]. Developmental & Comparative Immunology, 2013, 39(1/2): 79-84.
[43] BLACKWELL J M, BARTON C H, WHITE J K, et al. Genetic regulation of leishmanial and mycobacterial infections: the Lsh/Ity/Bcg story continues[J]. Immunology Letters, 1994, 43(1/2): 99-107.
[44] HOLLAND M C H, LAMBRIS J D. The complement system in teleosts[J]. Fish & Shellfish Immunology, 2002, 12(5): 399-420.
[45] AMAR E C, KIRON V, SATOH S, et al. Enhancement of innate immunity in rainbow trout (Oncorhynchus mykiss Walbaum) associated with dietary intake of carotenoids from natural products[J]. Fish & Shellfish Immunology, 2004, 16(4): 527-537.

[46] CUESTA A, ORTUÑO J, RODRIGUEZ A, et al. Changes in some innate defence parameters of seabream (Sparus aurata L.) induced by retinol acetate[J]. Fish & Shellfish Immunology, 2002, 13(4): 279-291.
[47] HERNANDEZ L H H, TESHIMA S I, KOSHIO S, et al. Effects of vitamin A on growth, serum anti-bacterial activity and transaminase activities in the juvenile Japanese flounder, Paralichthys olivaceus[J]. Aquaculture, 2007, 262(2/3/4): 444-450.

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

Source: ChinaXiv —Machine translation. Verify with original.