

## Postprint: Vitamin A Requirement of Largemouth Bass (*Micropterus salmoides*)

**Authors:** Lian Xueyuan, Chen Naisong, Wang Mengle, Yan Chunwei, Ding Guitao

**Date:** 2017-11-08T00:00:00+00:00

### Abstract

To determine the vitamin A requirement in feed for largemouth bass, this study added different amounts (0, 1,000, 2,000, 3,000 and 4,000 IU/kg) of vitamin A acetate to a basal diet to formulate five iso-nitrogenous and iso-energetic diets with different vitamin A levels (measured vitamin A contents were 415, 1,406, 2,755, 3,501 and 4,646 IU/kg, respectively; crude protein 48.47%; gross energy 18.15 kJ/g). The above diets were used to feed largemouth bass with an initial body weight of  $(52.75 \pm 0.12)$  g for 49 d, with 3 replicates per treatment and 30 fish per replicate. The results showed that as dietary vitamin A level increased from 415 IU/kg to 2,755 IU/kg, weight gain rate, specific growth rate, feed efficiency and protein efficiency of the experimental fish increased significantly ( $P < 0.05$ ); however, with further increase in dietary vitamin A level, these indicators showed a plateau trend. As dietary vitamin A level increased from 415 IU/kg to 3,501 IU/kg, hepatic vitamin A content, hepatic total superoxide dismutase activity, serum lysozyme activity, head kidney leukocyte respiratory burst activity, serum complement activity and hemoglobin content increased significantly ( $P < 0.05$ ), while hepatic malondialdehyde content decreased significantly ( $P < 0.05$ ); with further increase in dietary vitamin A level, the magnitude of change in these indicators was small. Different dietary vitamin A levels had no significant effect on serum total protein content and hematocrit of fish ( $P > 0.05$ ). Using weight gain rate and hepatic vitamin A content as evaluation indicators, the broken-line model was employed to determine that the optimal dietary vitamin A requirements for largemouth bass were 2,600 and 3,550 IU/kg, respectively; while using non-specific immunity as the evaluation indicator, the optimal requirement determined through analysis of variance was similar to that obtained using hepatic vitamin A content as the indicator. This study concluded that the optimal dietary vitamin A level for largemouth bass is 2,600–3,550 IU/kg.

## Full Text

### Dietary Vitamin A Requirement of Largemouth Bass (*Micropterus salmoides*)

\*\*LIAN Xueyuan, CHEN Naisong\*, WANG Mengle, YAN Chunwei, DING Guirao\*\*

College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, China

## Abstract

This study evaluated the dietary vitamin A requirement of largemouth bass (*Micropterus salmoides*). Five isonitrogenous (crude protein 48.47%) and isoenergetic (gross energy 18.15 kJ/g) diets were formulated with graded levels of vitamin A acetate (0, 1,000, 2,000, 3,000, and 4,000 IU/kg), resulting in measured dietary vitamin A concentrations of 415, 1,406, 2,755, 3,501, and 4,646 IU/kg. Juvenile largemouth bass with an initial body weight of (52.75±\$0.12) g were fed the experimental diets for 49 days. Each treatment consisted of three replicates with 30 fish per replicate. The results showed that weight gain rate, specific growth rate, feed efficiency, and protein efficiency ratio increased significantly ( $P<0.05$ ) as dietary vitamin A increased from 415 to 2,755 IU/kg, after which these parameters plateaued. Liver vitamin A content, liver total superoxide dismutase activity, serum lysozyme activity, head kidney leucocyte respiratory burst activity, serum complement activity, and hemoglobin content increased significantly ( $P<0.05$ ) as dietary vitamin A rose from 415 to 3,501 IU/kg, while liver malondialdehyde content decreased significantly ( $P<0.05$ ); further increases in dietary vitamin A resulted in minimal changes in these indices. Dietary vitamin A levels had no significant effects on serum total protein content or hematocrit ( $P>0.05$ ). Using broken-line regression analysis with weight gain rate and liver vitamin A content as response criteria, the optimal dietary vitamin A requirements were estimated to be 2,600 and 3,550 IU/kg, respectively. Analysis of non-specific immune parameters yielded a requirement similar to that based on liver vitamin A content. This study concludes that the optimal dietary vitamin A requirement for largemouth bass is 2,600–3,550 IU/kg.

**Keywords:** largemouth bass (*Micropterus salmoides*); vitamin A requirement; growth performance; non-specific immunity

## Introduction

Vitamin A is a collective term for compounds with retinol biological activity, existing primarily as retinol (vitamin A1) and 3-dehydroretinol (vitamin A2). In animals, vitamin A has three active forms: retinol, retinal, and retinoic acid. Essential for fish as for other vertebrates, vitamin A plays crucial roles in growth, epithelial tissue differentiation, vision, reproduction, and immunity. Vitamin A

deficiency in fish leads to reduced growth, anorexia, fin erosion, hemorrhage at the fin base and orbit, and skeletal deformities. Hybrid striped bass (*Morone chrysops* female  $\times$  *M. saxatilis*) exhibits slow growth, anorexia, and hemorrhage at the skin and fin base when deficient in vitamin A. Rainbow trout (*Oncorhynchus mykiss*) develop gill cover deformities, anemia, and hemorrhage at the fin base and eyes. Yellowtail amberjack (*Seriola lalandi*) shows anemia with reduced erythrocyte counts. However, excessive vitamin A accumulates in liver and other tissues, causing growth depression or toxicity. Japanese flounder (*Paralichthys olivaceus*) fed high-dose vitamin A diets exhibit slow growth and spinal abnormalities, while Atlantic salmon (*Salmo salar*) show increased mortality, reduced growth, and vertebral developmental abnormalities. Therefore, scientific evaluation of appropriate dietary vitamin A levels is of significant theoretical and practical importance.

Largemouth bass (*Micropterus salmoides*) is a typical carnivorous fish and one of China's major freshwater aquaculture species. Previous research on largemouth bass has focused on protein requirements, lipid requirements, energy-to-protein ratios, lysine and methionine requirements, carbohydrate requirements, vitamin C requirements, choline requirements, and selenium requirements. However, no studies have reported the dietary vitamin A requirement for this species. This study employed a dose-response approach using growth performance, body composition, and non-specific immunity as evaluation criteria to determine the optimal dietary vitamin A requirement for largemouth bass, providing a reference for artificial feed formulation.

## Materials and Methods

### 1.1 Experimental Material

Vitamin A acetate was provided by DSM with an effective content of 500,000 IU/g.

### 1.2 Experimental Design

Fish meal and rapeseed oil served as protein and lipid sources, respectively, with  $\alpha$ -starch as carbohydrate source and binder. Crystalline amino acids were supplemented to match the essential amino acid profile of largemouth bass muscle. A basal diet was formulated, and vitamin A acetate was added at 0, 1,000, 2,000, 3,000, and 4,000 IU/kg to create five experimental diets with measured vitamin A concentrations of 415, 1,406, 2,755, 3,501, and 4,646 IU/kg, respectively. The diets were isonitrogenous (crude protein 48.47%) and isoenergetic (gross energy 18.15 kJ/g), designated as A415, A1406, A2755, A3501, and A4646. Diet composition and nutrient levels are shown in Table 1. Chromic oxide ( $\text{Cr}_2\text{O}_3$ ) at 0.5% was included as an indicator for measuring apparent digestibility of nutrients. Zeolite powder was used as filler, and 1% hydrophilic soybean lecithin served as emulsifier.

Feed preparation involved grinding ingredients through an 80-mesh sieve, mixing thoroughly, adding oil, and then water to form a dough. The mixture was extruded through an electric meat grinder to produce 2–4 mm diameter strands, which were frozen and cut into 5–10 mm pellets, then stored at  $-20^{\circ}\text{C}$ .

### 1.3 Experimental Fish and Culture Management

Experimental fish were acclimated for two months in an indoor recirculating aquaculture system at Shanghai Nonghao Feed Co., Ltd., during which they were fed a commercial largemouth bass diet (48% crude protein, 11% crude lipid, 3,150 IU/kg vitamin A). After 24 h starvation, healthy fish of uniform size were selected, weighed, and randomly distributed into 15 fiberglass tanks (800 L capacity) at 30 fish per tank, with three replicates per dietary treatment. Initial body weight was  $(52.75 \pm 0.12)\text{g}$ . Fish were fed to apparent satiety twice daily (08 : 00 and 16 : 00). The recirculating water was filtered through sponge and corals and with UV sterilization. Natural photoperiod was 12 h light/12 h dark. The trial lasted 49 days.

### 1.4 Sampling and Analysis

**1.4.1 Sample Collection** At the start of the experiment, 15 fish were randomly sampled and stored at  $-80^{\circ}\text{C}$  for initial body composition analysis. After two weeks, feces were collected following Lee's method. The collection procedure involved siphoning feces 30 min after feeding, then opening collection devices for 4 h to collect intact fecal strands, which were stored at  $-20^{\circ}\text{C}$ . All fecal samples were subsequently freeze-dried, scales removed, and stored for analysis. At trial termination, fish were starved for 24 h, counted and weighed per tank. Twelve fish were randomly sampled from each tank: five stored at  $-80^{\circ}\text{C}$  for whole-body composition analysis, and seven used for blood collection and dissection. Blood (1 mL) was drawn from the caudal vein with a 2 mL syringe, allowed to clot at  $4^{\circ}\text{C}$  for 4 h, then centrifuged ( $836 \times g$ , 10 min,  $4^{\circ}\text{C}$ ). Serum was stored at  $-80^{\circ}\text{C}$  for immune parameter analysis. After blood collection, liver and viscera were weighed for hepatosomatic index calculation and stored at  $-80^{\circ}\text{C}$  for composition and vitamin A analysis. Dorsal muscle above the lateral line was also stored at  $-80^{\circ}\text{C}$  for composition analysis.

After three additional days, six fish per tank were randomly selected for blood collection (1 mL) into heparinized tubes for hematological analysis. Head kidney was immediately removed for respiratory burst activity measurement.

**1.4.2 Proximate Analysis** Crude protein was determined by Kjeldahl method (Kjeltec-2300, FOSS, Denmark). Moisture content was measured by oven drying at  $105^{\circ}\text{C}$  to constant weight. Crude ash was determined by incineration in a muffle furnace at  $550^{\circ}\text{C}$ . Crude lipid was extracted using chloroform-methanol method. Gross energy was measured by bomb calorimetry (6200, Parr, USA). Chromic oxide content in diets and feces was determined

by Divakaran et al.'s method. Vitamin A in diets and liver was analyzed by high-performance liquid chromatography.

**1.4.3 Immune Parameter Analysis** Serum lysozyme activity was measured by turbidimetry. Serum total protein content was determined by biuret method. Serum complement activity was assayed via the classical pathway. Head kidney leucocyte respiratory burst activity was measured by nitroblue tetrazolium (NBT) reduction. Liver total superoxide dismutase activity and malondialdehyde content were determined using commercial kits from Nanjing Jiancheng Bioengineering Institute.

**1.4.4 Hematological Analysis** Hemoglobin content was measured by cyanmethemoglobin spectrophotometry. Blood was diluted with Natt and Herrick's solution for erythrocyte counting.

## 1.5 Calculation Formulas

- Survival rate (SR, %) = (final fish number / initial fish number) × 100
- Feed intake [FI, g/(fish · d)] = {dry feed intake / [(initial fish number + final fish number)/2]} / trial days
- Weight gain rate (WGR, %) = (final body weight - initial body weight) / initial body weight × 100
- Specific growth rate (SGR, %/d) = (ln final body weight - ln initial body weight) / trial days × 100
- Feed efficiency ratio (FER) = (final body weight - initial body weight) / dry feed intake
- Protein efficiency ratio (PER) = (final body weight - initial body weight) / crude protein intake
- Apparent digestibility coefficient (ADC, %) = [1 - (nutrient content in feces / nutrient content in diet) × (Cr<sub>2</sub>O<sub>3</sub> content in diet / Cr<sub>2</sub>O<sub>3</sub> content in feces)] × 100
- Protein deposition rate (PDR, %) = (body crude protein deposition / crude protein intake) × 100
- Lipid deposition rate (LDR, %) = (body crude lipid deposition / crude lipid intake) × 100
- Hepatosomatic index (HSI, %) = (liver weight / body weight) × 100

## 1.6 Statistical Analysis

Results are presented as mean ± standard deviation. One-way ANOVA was performed using SPSS 17.0, and significant differences were identified by Duncan's multiple comparison test (P<0.05). Broken-line regression analysis was conducted using NRM software to estimate optimal dietary vitamin A requirement. The model was defined as:  $Y = L - U(R - X_{\{RL\}})$  when  $X \leq R$ , and  $Y = L$  when  $X > R$ , where Y is the response parameter, L and R are coordinates at

the breakpoint (R represents requirement),  $X_{\{RL\}}$  is the independent variable value below R, and U is the slope of the line.

## Results

### 2.1 Effects of Dietary Vitamin A on Growth Performance and Nutrient Utilization

Weight gain rate increased significantly ( $P < 0.05$ ) as dietary vitamin A increased from 415 to 2,755 IU/kg, with no further improvement at higher levels. Specific growth rate, feed efficiency, and protein efficiency ratio showed similar trends (Table 2 and Table 3). Broken-line regression of weight gain rate against dietary vitamin A yielded:  $Y = 111.46 - 0.009 \times (2,600 - X)$  ( $R^2 = 0.765$  when  $X \leq 2,600$  IU/kg;  $Y = 111.46\%$  when  $X > 2,600$  IU/kg), indicating a dietary vitamin A requirement of 2,600 IU/kg for maximal weight gain (Figure 1 [Figure 1: see original paper]). Survival rate and feed intake were not significantly affected by dietary vitamin A ( $P > 0.05$ ) (Table 2). Protein and lipid deposition rates in fish fed 415 IU/kg vitamin A were significantly lower than those fed 1,406–4,646 IU/kg ( $P < 0.05$ ), with no significant differences among the latter groups. The highest protein and lipid deposition rates occurred at 2,755 IU/kg vitamin A (Table 3).

### 2.2 Effects of Dietary Vitamin A on Body Composition

Whole-body crude protein content in fish fed 1,406–4,646 IU/kg vitamin A was significantly higher than in those fed 415 IU/kg ( $P < 0.05$ ). Muscle and viscera crude protein increased significantly with dietary vitamin A from 415 to 4,646 IU/kg ( $P < 0.05$ ). Whole-body crude lipid increased initially then plateaued, reaching maximum at 3,501 IU/kg, significantly higher than at 415 IU/kg ( $P < 0.05$ ). Visceral crude lipid decreased significantly as dietary vitamin A increased from 415 to 4,646 IU/kg ( $P < 0.05$ ), while muscle lipid was not significantly affected ( $P > 0.05$ ). Moisture and ash contents in whole body, muscle, and viscera were not significantly influenced by dietary vitamin A ( $P > 0.05$ ) (Table 4).

### 2.3 Effects of Dietary Vitamin A on Hepatosomatic Index, Liver Vitamin A Content, and Antioxidant Status

Hepatosomatic index was significantly higher in fish fed 415 IU/kg vitamin A than in other groups ( $P < 0.05$ ), with no significant differences among the latter. Liver crude lipid decreased initially then increased, reaching minimum at 3,501 IU/kg, significantly lower than at 415 IU/kg ( $P < 0.05$ ). Liver vitamin A content increased initially then plateaued, while liver total superoxide dismutase activity increased then decreased, both peaking at 3,501 IU/kg. Liver malondialdehyde content decreased then increased, reaching minimum at 3,501 IU/kg (Table 5). Broken-line regression of liver vitamin A content yielded:  $Y = 193.03 - 0.062 \times (3,550 - X)$  ( $R^2 = 0.986$  when  $X \leq 3,550$  IU/kg;  $Y = 193.03 \mu\text{g/g}$  when

$X > 3,550$  IU/kg), indicating a dietary vitamin A requirement of 3,550 IU/kg for maximal liver vitamin A deposition (Figure 2 [Figure 2: see original paper]).

#### 2.4 Effects of Dietary Vitamin A on Non-Specific Immune Parameters

Serum lysozyme activity, head kidney leucocyte respiratory burst activity, and serum complement activity increased initially then plateaued, peaking at 3,501 IU/kg and significantly higher than at 415 IU/kg ( $P < 0.05$ ). Dietary vitamin A had no significant effect on serum total protein content ( $P > 0.05$ ) (Table 6).

#### 2.5 Effects of Dietary Vitamin A on Hematological Indices

Erythrocyte count and hemoglobin content increased initially then plateaued, reaching maximum at 3,501 IU/kg and significantly higher than at 415 IU/kg ( $P < 0.05$ ). Hematocrit was not significantly affected by dietary vitamin A ( $P > 0.05$ ) (Table 7).

### Discussion

#### 3.1 Effects of Dietary Vitamin A on Growth Performance

Vitamin A deficiency commonly causes growth retardation in fish. In this study, fish fed 415 and 1,406 IU/kg vitamin A grew slower than other groups, while weight gain rate peaked at 2,755 IU/kg. Broken-line analysis based on weight gain rate indicated an optimal dietary vitamin A requirement of 2,600 IU/kg for largemouth bass. Similar requirements have been reported for other species: 2,500 IU/kg for Pacific salmon (*Oncorhynchus* spp.), 3,101 IU/kg for greasy grouper (*Epinephelus tauvina*), and 2,000–2,500 IU/kg for rainbow trout. However, requirements of 9,000 IU/kg for Japanese flounder and 879.45 IU/kg for Amur sturgeon (*Acipenser schrenckii*) differ substantially, suggesting species-specific differences. Although vitamin A is essential for growth, excessive levels can be inhibitory, as observed in this study (though not significantly) and reported in rainbow trout, Japanese flounder, and Nile tilapia (*Oreochromis niloticus*). Further research is needed to determine if higher vitamin A levels would significantly impair largemouth bass growth.

#### 3.2 Effects of Dietary Vitamin A on Nutrient Utilization and Body Composition

Feed efficiency increased initially then plateaued with increasing dietary vitamin A, consistent with findings in Japanese flounder and Japanese sea bass (*Lateolabrax japonicus*). This indicates that dietary vitamin A modulates nutrient metabolism, affecting body composition. Protein deposition rate and whole-body crude protein were significantly lower at 415 IU/kg vitamin A, while muscle and viscera crude protein increased with vitamin A level. Similar reductions in protein deposition have been observed in Jian carp (*Cyprinus carpio* var.

Jian) fed vitamin A-deficient diets. Energy metabolism was also affected: whole-body crude lipid increased then plateaued, while liver crude lipid decreased then increased slightly. These patterns align with studies on Japanese sea bass. However, contrasting results exist: dietary vitamin A had no significant effect on body composition of rainbow trout, while body crude lipid decreased significantly in Japanese flounder and greasy grouper. Hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) showed irregular effects on body protein and moisture content.

The liver is the primary organ for vitamin A metabolism and storage, and its vitamin A content is commonly used to evaluate requirements. In this study, liver vitamin A increased with dietary levels up to 3,501 IU/kg but did not increase further. Similar plateaus have been reported in other fish species. However, using liver vitamin A accumulation alone may overestimate the requirement.

### 3.3 Effects of Dietary Vitamin A on Immunity

Serum lysozyme activity, head kidney leucocyte respiratory burst activity, serum complement activity, and liver superoxide dismutase activity increased significantly as dietary vitamin A rose from 415 to 3,501 IU/kg, peaking at 3,501 IU/kg. Erythrocyte count increased significantly from 415 to 2,755 IU/kg but did not increase further. Similar improvements in immune parameters with dietary vitamin A have been reported in red drum (*Sciaenops ocellatus*) and grass carp (*Ctenopharyngodon idella*). Vitamin A deficiency causes anemia in rainbow trout. However, some studies found no significant effects of dietary vitamin A on serum lysozyme in Atlantic salmon or on respiratory burst and complement activities in rainbow trout. Despite these inconsistencies, non-specific immune parameters are valuable criteria for determining vitamin A requirements. The consistent enhancement of immune indices in this study demonstrates that adequate vitamin A is crucial for maintaining health and disease resistance in largemouth bass.

### 3.4 Factors Affecting Dietary Vitamin A Requirement Estimation

Several factors influence the estimation of optimal dietary vitamin A levels. First, dietary  $\beta$ -carotene can lead to underestimation of vitamin A requirement, as it can be converted to vitamin A in fish. Studies on Nile tilapia show  $\beta$ -carotene conversion to vitamin A1 and dihydroxycarotenoids to vitamin A2, with a conversion ratio of approximately 19:1 in hybrid tilapia. Second, the choice of evaluation criteria affects the estimated requirement. Commonly used criteria include deficiency signs, growth performance, feed efficiency, immunity, and liver vitamin A content. Different criteria often yield different requirements. For Japanese sea bass, requirements based on weight gain rate and liver vitamin A content were 1,934.8 and 3,546.6 IU/kg, respectively. For rainbow trout, requirements based on absence of deficiency signs and weight gain rate were 2,000 and 2,500 IU/kg, respectively. For hybrid striped bass, requirements based on weight gain and liver vitamin A accumulation were 5,850 and 6,970 IU/kg,

respectively. Third, fish size and culture environment affect requirements. Juvenile fish have higher nutrient demands and metabolic rates, requiring more vitamin A than adults. One-year-old Japanese sea bass (28.4–30.2 g) required 4,000–7,000 IU/kg vitamin A, while two-year-old fish (150.1–156.6 g) required 4,000 IU/kg. Intensive culture conditions cause stress, increasing vitamin requirements, whereas semi-intensive or extensive systems provide vitamins from natural food sources.

This study did not examine potential effects of dietary  $\beta$ -carotene, warranting further investigation. Based on comprehensive evaluation of growth performance, feed efficiency, liver vitamin A content, and immunity, the optimal dietary vitamin A requirement for largemouth bass is 2,600–3,550 IU/kg, providing a valuable reference for artificial feed formulation.

## Conclusions

1. Dietary vitamin A level significantly affects growth performance, feed efficiency, protein efficiency ratio, liver vitamin A content, and non-specific immunity in largemouth bass.
2. The optimal dietary vitamin A requirement for largemouth bass is 2,600–3,550 IU/kg.

## References

- [1] HALVER J E. The vitamins[M]//HALVER J E, ed. Fish nutrition. New York: Academic Press, 1972: 29–103.
- [2] OLSON J A. Needs and sources of carotenoids and vitamin A[J]. Nutrition Reviews, 1994, 52(2): S67–S73.
- [3] HERNANDEZ L H H, TESHMA S, ISHIKAWA M, et al. Dietary vitamin A requirements of juvenile Japanese flounder *Paralichthys olivaceus*[J]. Aquaculture Nutrition, 2005, 11(1): 3–9.
- [4] MOHAMED J S, SIVARAM V, ROY T S C, et al. Dietary vitamin A requirement of juvenile greasy grouper (*Epinephelus tawina*)[J]. Aquaculture, 2003, 219(1/2/3/4): 693–701.
- [5] ZHANG L. Nutritional physiology of several vitamins and protein source development for Japanese sea bass and large yellow croaker[D]. PhD thesis. Qingdao: Ocean University of China, 2006: 53–55.
- [6] HEMRE G I, DENG D F, WILSON R P, et al. Vitamin A metabolism and early biological responses in juvenile sunshine bass (*Morone chrysops* × *M. saxatilis*) fed graded levels of vitamin A[J]. Aquaculture, 2004, 235(1/2/3/4): 645–658.
- [7] KITAMURA S, SUWA T, OHARA S, et al. Studies on vitamin requirements

of rainbow trout - : requirement for vitamin A and deficiency symptoms[J]. Nippon Suisan Gakkaishi, 1967, 33(12): 1126-1131.

[8] SHIMENO S. Yellowtail, *Seriola quinqueradiata*[M]//WILSON R P, ed. Handbook of Nutrient Requirements of Finfish. Boca Raton, FL: CRC Press, 1991: 181-191.

[9] 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.

[10] ØRNSRUD R, GRAFF I E, HØIE S, et al. Hypervitaminosis A in first-feeding fry of the Atlantic salmon (*Salmo salar* L.)[J]. Aquaculture Nutrition, 2002, 8(1): 7-13.

[11] TIDWELL J H, WEBSTER C D, COYLE S D. Effects of dietary protein level on second year growth and water quality for largemouth bass (*Micropterus salmoides*) raised in ponds[J]. Aquaculture, 1996, 145(1/2/3/4): 213-223.

[12] ANDERSON R J, KIENHOLZ E W, FLICKINGER S A. Protein requirements of smallmouth bass and largemouth bass[J]. Journal of Nutrition, 1981, 111(6): 750-764.

[13] BRIGHT L A, COYLE S D, TIDWELL J H. Effect of dietary lipid level and protein energy ratio on growth and body composition of largemouth bass, *Micropterus salmoides*[J]. Journal of the World Aquaculture Society, 2005, 36(1): 129-134.

[14] COYLE S D, TIDWELL J H, WEBSTER C D. Response of largemouth bass, *Micropterus salmoides*, to dietary supplementation of lysine, methionine, and highly unsaturated fatty acids[J]. Journal of the World Aquaculture Society, 2000, 31(1): 89-95.

[15] DAIRIKI J K, DOS SANTOS DIAS C T, CYRINO J E P. Lysine requirements of largemouth bass, *Micropterus salmoides*: a comparison of methods of analysis of dose-response trials data[J]. Journal of Applied Aquaculture, 2007, 19(4): 1-27.

[16] GOU Shipan, CHEN Naisong, XU Xiangtai, et al. Effects of dietary digestible starch on growth, body composition and non-specific immune indices of largemouth bass *Micropterus salmoides*[J]. Journal of Fisheries of China, 2015, 39(10): 1499-1510.

[17] YUAN Ruimin. Effects of dietary vitamin C on growth and antioxidant capacity of largemouth bass *Micropterus salmoides*[D]. Master' s thesis. Guangzhou: Sun Yat-sen University, 2013.

[18] ZHOU Ming. Effects of dietary choline and lecithin on growth, body composition and liver of largemouth bass *Micropterus salmoides*[D]. Guangzhou: Sun Yat-sen University, 2007.

- [19] YAO Z, CHEN Y J, LIU Y J, et al. Effect of dietary selenium level on growth performance, body composition and hepatic glutathione peroxidase activities of largemouth bass, *Micropterus salmoides*[J]. Aquaculture Research, 2012, 43(11): 1660-1668.
- [20] CHEN N S, LIANG Q L, XIAO W W, et al. Effects of supplementing EAA to lower protein diets on growth, body composition and immunological index of largemouth bass (*Micropterus salmoides*)[J]. Acta Hydrobiologica Sinica, 2014, 38(2): 262-271.
- [21] LEE S M. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*)[J]. Aquaculture, 2002, 207(1/2): 79-95.
- [22] FOLCH J M S, LEES M M, STANLEY G H S. A simple method for the isolation and purification of total lipides from animal tissue[J]. Journal of the Science of Food & Agriculture, 1957, 226(1): 497-509.
- [23] DIVAKARAN S, OBALDOS L G, FORDTER I P. Note on the methods for determination of chromic oxide in shrimp feeds[J]. Journal of Agricultural Chemistry, 2002, 50(3): 464-467.
- [24] RUSHING L G, COOPER W M, TOMPSON H C. Simultaneous analysis of vitamin A and E in rodent feed by high-pressure liquid chromatography[J]. Journal of Agricultural and Food Chemistry, 1991, 39(2): 296-299.
- [25] SITJÀ-BOBADILLA, MINGARRO M, PUJALTE M J, et al. Immunological and pathological status of gilthead sea bream (*Sparus aurata* L.) under different long-term feeding regimes[J]. Aquaculture, 2003, 220(1/2/3/4): 707-724.
- [26] LU Yongshui, YANG Changguo. Standardization of biuret reaction method for serum total protein determination[J]. Journal of Clinical Laboratory Science, 1990(2): 98-101.
- [27] INGLIS J E, RADZIWONK A, MANIERO G D. The serum complement system: a simplified laboratory exercise to measure the activity of an important component of the immune system[J]. Advances in Physiology Education, 2008, 32(4): 317-321.
- [28] AI Q H, MAI K S, ZHANG L, et al. Effects of dietary beta-1,3 glucan on innate immune response of large yellow croaker, *Pseudosciaena crocea*[J]. Fish & Shellfish Immunology, 2007, 22(4): 394-402.
- [29] BRADFORD M M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding[J]. Analytical Biochemistry, 1976, 72(1/2): 248-254.
- [30] AMOLD J E. Hematology of the sandbar shark, *Carcharhinus plumbeus*: standardization of complete blood count techniques for elasmobranchs[J]. Veterinary Clinical Pathology, 2005, 34(2): 115-123.

- [31] SANDNES K, LIE Ø, WAAGBØ R. Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon, *Salmo salar*[J]. Journal of Fish Biology, 1988, 32(1): 129-136.
- [32] GAO Qiang. Studies on vitamin A, C and E requirements of two sturgeon species[D]. Master's thesis. Chongqing: Southwest Agricultural University, 2005.
- [33] HILTON J W. Hypervitaminosis A in rainbow trout (*Salmo gairdneri*): toxicity signs and maximum tolerable level[J]. Journal of Nutrition, 1983, 113(9): 1737-1745.
- [34] 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, 1998, 64(2): 344-345.
- [35] SALEH G, ERAKY W E, 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, 2007, 11(3/4): 382-385.
- [36] 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.
- [37] 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.
- [38] HU C J, CHEN S M, PAN C H, et al. Effects of dietary vitamin A or  $\beta$ -carotene concentrations on growth of juvenile hybrid tilapia, *Oreochromis niloticus*  $\times$  *O. aureus*[J]. Aquaculture, 2006, 253(1/2/3/4): 602-607.
- [39] HECTOR L, HENANDEZ H, TESHIMA S I, et al. Effects of dietary vitamin A on juvenile bream *Chrysophrys major*[J]. Journal of the World Aquaculture Society, 2005, 35(4): 436-444.
- [40] MOREN M, OPSTAD I, BERNTSEN M H G, et al. An optimum level of vitamin A supplementation for Atlantic halibut (*Hippoglossus hippoglossus*) juveniles[J]. Aquaculture, 2004, 235(1/2/3/4): 587-599.
- [41] 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.
- [42] ZHU Wenhuan. Studies on dietary vitamin A and potassium requirements of two sizes of grass carp (*Ctenopharyngodon idella*)[D]. Master's thesis. Wuhan: Huazhong Agricultural University, 2014.
- [43] THOMPSON I, FLETCHER T C, HOULIHAN D F, et al. The effect of dietary vitamin A on the immunocompetence of Atlantic salmon (*Salmo salar* L.)[J]. Fish Physiology and Biochemistry, 1994, 12(6): 513-523.

[44] KAISUYAMA M, MATSUNO T. Carotenoid and vitamin A metabolism of  $\beta$ -carotene, canthaxanthin, astaxanthin, zeaxanthin, lutein and tunaxanthin in tilapia *Tilapia nilotica*[J]. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 1988, 90(1): 131-139.

[45] PENG Shiming. Nutritional physiology of lipids and vitamin E in black porgy (*Acanthopagrus schlegelii*)[D]. PhD thesis. Shanghai: East China Normal University, 2008.

[46] ZHONG Weiren, ZHANG Shuhua. Studies on vitamin requirements of Japanese sea bass at different growth stages[J]. Journal of Zhejiang Ocean University: Natural Science Edition, 2001, 20(S1): 98-102.

[47] WU Jianbo. Studies on vitamin E deficiency symptoms in juvenile Jian carp[D]. PhD thesis. Chengdu: Sichuan Agricultural University, 2005.

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

*Source: ChinaXiv – Machine translation. Verify with original.*