Advances and Prospects in Pufferfish Nutrition Research: Postprint

Authors: Liao Zhangbin, Xu Houguo, Wei Yuliang, Liang Mengqing

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

Abstract

Taking Takifugu rubripes and Takifugu obscurus as examples, this paper synthesizes domestic and international research progress on the primary nutrient requirements and requirement characteristics of pufferfish, reviews the requirements or requirement characteristics of pufferfish for proteins and amino acids, lipids and fatty acids, carbohydrates, vitamins, minerals, and other nutrients, proposes prospects for future research, and aims to provide a basis and reference for research and development regarding pufferfish nutritional requirements and specialized formulated feeds for pufferfish.

Full Text

Progress and Outlook in Nutrition Study on Puffers

LIAO Zhangbin[1,2], **XU Houguo**[2], **WEI Yuliang**[2], **LIANG Mengqing**[2,3*]

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

2. Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China

3. Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China

Abstract: Taking *Takifugu rubripes* and *Takifugu obscurus* as representative species, this review synthesizes domestic and international research progress on the nutrient requirements and nutritional characteristics of puffers. The review covers requirements for protein and amino acids, lipids and fatty acids, carbohydrates, vitamins, minerals, and other nutrients, and proposes future

research directions to provide a foundation for puffer nutrition studies and the development of specialized formulated feeds.

Keywords: *Takifugu rubripes*; *Takifugu obscurus*; nutrition; feed

Puffers (Tetraodontiformes: Tetraodontidae) comprise 35 species naturally distributed in the East China Sea, Yellow Sea, and Bohai Sea. Due to their delicious taste, nutritional value, and cultural significance in Chinese cuisine, puffer aquaculture has long occupied an important position in China' s aquaculture industry. The main cultured species are *Takifugu rubripes* and *Takifugu obscurus*, with smaller amounts of *Takifugu flavidus* and *Takifugu bimaculatus* cultured in Fujian and other regions. While artificial propagation and culture techniques are well established, specialized formulated feeds have not been widely adopted. Many regions still rely on direct feeding of fresh trash fish or combinations of soft-shelled turtle/eel feed with fresh fish, leading to high feed conversion ratios and severe water pollution. Following the 2016 joint notice from the Ministry of Agriculture and the China Food and Drug Administration conditionally lifting restrictions on processing and marketing of cultured *T. rubripes* and *T. obscurus*, puffer production and consumption are expected to increase substantially. Consequently, developing specialized formulated feeds has become critical for advancing the puffer aquaculture industry. This review summarizes domestic and international research progress on puffer nutrition (Table 1) to provide a basis for nutrition research and feed development.

1.1 Protein

Protein (amino acids) constitutes the most critical material foundation for fish growth and represents the largest component of aquafeed production costs. As carnivorous-leaning omnivorous fish, puffers have high protein requirements, making research on their protein and amino acid needs essential for developing specialized feeds and reducing production costs.

1.1.1 *Takifugu rubripes*

Research indicates that the optimal dietary crude protein content for 16–50 g *T. rubripes* is 41.08% [1]. Similarly, Kim et al. [2] found that juvenile *T. rubripes* (initial weight 17.05 ± 0.17 g) exhibited optimal growth and physiological performance at 41% crude protein. However, other studies suggest that maximum growth in juvenile *T. rubripes* (initial average weight 18.50 g) requires dietary crude protein levels of (50.0 ± 3.7)% [3], and Yang et al. [4] reported an optimal crude protein level of 50% based on weight gain rate and protein efficiency ratio using casein-based diets.

With global fishmeal production stabilizing while demand and prices continue rising, identifying high-quality protein sources to replace fishmeal has become urgent. Kikuchi et al. [5,6] demonstrated that adding appropriate amounts of mussel extract to defatted soybean meal could replace 30–40% of fishmeal in diets for *T. rubripes* (initial weight 18 g) without negatively affecting growth, feed utilization, or health. Additionally, replacing 40% fishmeal with a combination of 20% defatted soybean meal and 20% mussel extract did not impair growth in juvenile *T. rubripes* (initial average weight 11 g). Other research found that replacing up to 30% of fishmeal with soybean meal did not significantly affect growth in juvenile *T. rubripes* (initial average weight 20.10 g) [7].

1.1.2 *Takifugu obscurus*

For *T. obscurus*, juvenile fish (initial weight 8.56 ± 0.04 g) showed good weight gain, feed efficiency, and specific growth rate when dietary crude protein ranged from 45.90% to 51.60% [8]. Yang et al. [9] similarly reported an optimal dietary crude protein requirement of 46–49% for juvenile *T. obscurus* (initial weight 23.60 ± 0.27 g) based on comprehensive evaluation of weight gain and protein efficiency. Yoo et al. [10] found that juvenile *T. obscurus* (initial weight 3.43 ± 0.02 g) exhibited optimal weight gain, feed efficiency, and specific growth rate at 50% crude protein and 18.84 MJ/kg energy. However, other research indicates that 37–44% crude protein can achieve good growth in juvenile *T. obscurus* (initial weight 12.40 ± 0.11 g) [11]. Currently, basic data on amino acid requirements remain scarce; Wang et al. [12] found that juvenile *T. obscurus* (8.01–24.60 g) require 1.03% methionine when dietary cystine content is 0.20%.

Studies on fishmeal alternatives for *T. obscurus* have identified potential in soybean meal, corn gluten meal, fermented soybean meal, and squid liver powder [13,14]. Zhong et al. [14] reported that replacing 10% of fishmeal with corn gluten meal enhanced lysozyme activity in the head kidney, spleen, and hepatopancreas, as well as c-type lysozyme gene mRNA expression in *T. obscurus* (initial weight 41.26 ± 1.09 g).

1.2 Lipids and Fatty Acids

Lipids serve multiple physiological functions in fish metabolism and are essential nutrients for growth. Certain unsaturated fatty acids (e.g., n-3 and n-6 long-chain polyunsaturated fatty acids) cannot be synthesized by marine fish and must be supplied directly through the diet to ensure healthy growth.

1.2.1 *Takifugu rubripes*

Kikuchi et al. [15] found that dietary lipid levels of 5.90–11.00% supported good growth, condition, and feed utilization in juvenile *T. rubripes* (initial weight 10 g). Similar results showed that 8.93% dietary lipid was most beneficial for growth metabolism in juvenile *T. rubripes* (initial weight 7.71 ± 0.15 g), with the highest antioxidant enzyme activity and healthy, intact liver histology [16,17]. However, Takii et al. [18] reported optimal dietary lipid content of 11.50% for juvenile *T. rubripes* (~3.70 g), yielding maximum feed efficiency, protein efficiency, and apparent protein retention.

Regarding fatty acids, Kikuchi et al. [19] conducted eight dietary treatments with juvenile *T. rubripes* (initial weight 5 g), including cod/squid liver oil, three DHA+EPA gradients, soybean oil (with/without DHA and EPA), and linseed oil (with/without DHA and EPA). Results showed that diets containing only linseed or soybean oil reduced growth, while diets required >1.50% n-3 long-chain polyunsaturated fatty acids to meet normal growth requirements.

1.2.2 *Takifugu obscurus*

Yoo et al. [8] reported that dietary lipid levels of 7.01–8.98% (using fish oil) produced maximum growth in juvenile *T. obscurus* (initial weight 8.32 ± 0.02 g). The same study found that when diets contained 60% fishmeal, soybean and linseed oils could completely replace fish oil without affecting growth in juvenile *T. obscurus* (initial weight 10.30 ± 0.03 g) [20].

1.3 Carbohydrates

Fish primarily utilize protein or lipid as energy sources, but carbohydrates are also important and more economical dietary energy sources. Inadequate or excessive dietary carbohydrate levels adversely affect fish growth and development. Appropriate levels of digestible carbohydrates can reduce crude protein catabolism for energy, directing more protein toward growth while reducing nitrogen excretion pollution. Carbohydrates can also replace some lipids to prevent excessive fat accumulation from high-lipid diets.

Liu et al. [21–24] systematically investigated carbohydrate functions in *T. obscurus* diets. Studies on dietary dextrin levels in juvenile *T. obscurus* (initial weight 10.25 ± 0.51 g) indicated optimal dextrin levels of 20–25% based on growth, feed utilization, digestive enzyme activity, plasma biochemistry, and cellular immunity [21]. Research on corn starch showed that 23.50% and 22.50% inclusion levels produced the fastest growth and highest feed efficiency, respectively [22]. The amylose/amylopectin (AM/AP) ratio significantly affected growth and digestive enzyme activity, with an optimal ratio of 0.25, close to the average for cereal starches [23]. Using quadratic polynomial regression models for specific growth rate, protein efficiency, and feed efficiency versus carbohydrate/lipid (CHO/L) ratio, the optimal CHO/L range was determined to be 2.01–2.16 [24].

Takii et al. [25] found that the appropriate dextrin level for juvenile *T. rubripes* (initial weight ~10 g) was approximately 16%, corresponding to about 16% carbohydrate content.

1.4 Vitamins

Vitamins are low-molecular-weight organic compounds essential for maintaining and regulating fish metabolism, enhancing immunity, and promoting rapid growth. Although required in small amounts, they must be supplied through the diet.

Kato et al. [26] investigated water-soluble vitamin deficiencies in juvenile *T. rubripes* (average weight 2.90 g) based on deficiency symptoms, growth, mortality, and blood characteristics. *T. rubripes* showed higher requirements for lipid metabolism-related vitamins (choline, niacin, pantothenic acid, vitamin C, and inositol) but lower requirements for protein metabolism-related vitamin B6 compared to other known fish species. Liang et al. [27] found that the optimal dietary vitamin C level for juvenile *T. obscurus* (initial weight 10.28 ± 0.50 g) was 88.60 mg/kg based on growth, immunity, and cold stress resistance. Eo et al. [28] reported that 29 mg/kg vitamin C met normal growth and physiological needs in *T. rubripes*, while 82–160 mg/kg enhanced non-specific immunity.

1.5 Minerals

Minerals are essential nutrients for normal fish growth. Fish can obtain minerals from feed and absorb inorganic salts through gills and skin to compensate for deficiencies. However, research on mineral requirements for puffers remains very limited.

Studies on *T. rubripes* mineral requirements show that dietary mineral mixtures significantly improve growth and reduce deformities. Furuichi et al. [29] found mineral supplementation necessary even with white fishmeal diets. Calcium supplementation studies revealed that adding appropriate calcium to fishmeal diets significantly improved growth [30], though Hossain et al. [31,32] found that 0.10–0.20% calcium in semi-purified diets met normal growth and skeletal mineralization needs, with excessive calcium reducing bone zinc content. Laining et al. [33] reported that a Ca/P ratio of 0.50 (without additional calcium) plus 2,000 U/kg phytase was optimal for juvenile *T. rubripes* (initial weight 9.84 g). Ye et al. [34] demonstrated that dietary phosphorus supplementation improved cell viability, antioxidant capacity, energy production, and lipid transport in *T. obscurus* under cold stress.

1.6 Other Nutrients (Feed Attractants, Immunostimulants, and Exogenous Enzymes)

Liang et al. [35] found that fish RNA/DNA ratio can evaluate feed additive efficacy when basal diets and environmental conditions are consistent. Appropriate attractants enable rapid feeding behavior and reduce feed waste, while compound attractants provide synergistic effects that improve feed utilization, growth, and immunity. Qin et al. [36] reported that trimethylamine oxide or betaine + alanine + glycine showed significant feeding attraction for *T. obscurus*. Liang et al. [37] compared attractant efficacy for *T. rubripes*, ranking them as: clam extract > alanine > histidine > squid viscera fluid > nucleotide (ADP) + alanine > 0.5% betaine > glycine > *Ulva* extract > ADP + glycine + alanine.

Hua et al. [38,39] reported that dietary chitosan (0.2%), probiotics (0.1%), chitosan-probiotic mixtures, or mannan-probiotic mixtures promoted growth, enhanced intestinal amylase activity, and regulated immune function in *T. obscurus*. Recent research showed that 2,000 mg/kg chitosan oligosaccharides improved growth, non-specific immunity, intestinal digestive enzyme activity, and gut microbiota in *T. rubripes* [40]. Wang et al. [41] demonstrated that intermittent or continuous injection and oral administration of -glucan enhanced non-specific immunity and disease resistance in *T. obscurus*, recommending intermittent administration for practical use. In exogenous enzyme research, Meng et al. [42] found that 1,000–1,500 U/kg phytase promoted growth and improved digestion in juvenile *T. rubripes*. Hirazawa et al. [43] reported that dietary caprylic acid had anthelmintic effects against monogenean parasites.

1.7 Nutrient Digestibility

Data on feed and ingredient digestibility in puffers remain limited. Wu et al. [44] found that *T. obscurus* showed high digestibility for formulated feeds at 22–23 °C, with lipid digestibility highest, followed by crude protein and carbohydrates. Zhong et al. [45] reported high apparent digestibility coefficients for crude protein and dry matter from fishmeal, soybean meal, and corn gluten meal in *T. obscurus* (93.40%, 91.83%, and 94.97% for protein; 88.13%, 85.73%, and 89.25% for dry matter, respectively), with enzyme supplementation significantly improving digestibility. Yoo et al. [13] found dry matter apparent digestibility of red fishmeal, white fishmeal, fermented soybean meal, squid liver powder, feather meal, soybean meal, and krill meal to be 80%, 78%, 72%, 67%, 56%, 55%, and 54%, respectively, with corresponding crude protein digestibility of 96%, 96%, 93%, 92%, 89%, 88%, and 86%.

1.8 Feeding Techniques

The optimal growth temperature for puffers is 18–25 °C. Yang et al. [46] found that juvenile *T. obscurus* (3.45–3.56 g) had an optimal feeding rate of 4% at 23 °C. For *T. rubripes* at ~25 °C, fish <100 g should be stocked at 60 kg/m³ and fed three times daily, while fish >100 g require daily feeding at 18 kg/m³ stocking density [47]. Kang et al. [48] reported that feeding once daily during low winter temperatures provided the best economic returns for winter *T. obscurus* culture.

2 Current Status of Pufferfish Feed Production

Pufferfish nutrition research and specialized feed production in China seriously lag behind aquaculture development, constraining industry advancement. Despite annual production approaching 30,000 tonnes, no large-scale feed manufacturers produce specialized puffer feeds. Some farmers use flatfish or grouper feeds or combinations of soft-shelled turtle/eel powder feeds with fresh fish, while large enterprises (e.g., Zhongyang Group) have attempted in-house feed production.

Slow feed industry development stems from: (1) lack of basic nutritional requirement parameters; (2) previous strict government control over puffer production and distribution, reducing market confidence; and (3) entrenched feeding habits among farmers, relatively small production scale, and manageable environmental/disease pressures, limiting motivation to change feeding practices.

3 Summary and Outlook

Current puffer nutrition research has established some foundation but exhibits several deficiencies requiring focused attention: (1) severe lack of data on amino acid, fatty acid, and vitamin requirements; (2) insufficient practical technologies for fishmeal/fish oil replacement that can be rapidly adopted by industry; and (3) absence of nutrition studies for different culture systems and growth stages (current research focuses on juveniles of 7-20 g). Given diverse culture methods (intensive, pond, offshore cage) and land-sea relay characteristics, nutrition and feeding technologies for different systems warrant particular attention.

Meanwhile, constraints on feed industry development are shifting. Conditional lifting of restrictions will accelerate industry growth, prompting small-medium feed enterprises to enter the puffer feed market. Rising trash fish prices, stricter environmental regulations, and documented pathogen transmission from fresh fish feeding will likely drive farmers toward formulated feeds. With the establishment of the puffer nutrition and feed position in the national agricultural industry technology system, comprehensive research on nutritional requirements and specialized feed development will advance, boosting industry development.

References

[1] WANG S M. Study on optimal dietary protein content for juvenile tiger puffer (*Takifugu rubripes*) [D]. Master' s thesis. Shijiazhuang: Hebei Normal University, 2008: 14-27.

[2] KIM S S, LEE K J. Dietary protein requirement of juvenile tiger puffer (*Takifugu rubripes*) [J]. *Aquaculture*, 2009, 287(1/2): 219-222.

[3] ZHU Q L. Optimal dietary protein content for juvenile tiger puffer (*Takifugu rubripes*) [J]. *Jiangxi Feed*, 2003(3): 41.

[4] YANG Z, YANG J X. Overview of nutritional requirements and feed application for puffers [J]. *Reservoir Fisheries*, 2002, 22(1): 1-2.

[5] KIKUCHI K, FURUTA T. Inclusion of blue mussel extract in diets based on fish and soybean meals for tiger puffer *Takifugu rubripes* [J]. *Fisheries Science*, 2009, 75(1): 183-189.

[6] KIKUCHI K, FURUTA T. Use of defatted soybean meal and blue mussel meat as substitute for fish meal in the diet of tiger puffer, *Takifugu rubripes* [J]. *Journal of the World Aquaculture Society*, 2009, 40(4): 472-482.

[7] LIM S J, KIM S S, KO G Y, et al. Fish meal replacement by soybean meal in diets for tiger puffer, *Takifugu rubripes* [J]. *Aquaculture*, 2011, 313(1/2/3/4):

165–170.

[8] YOO G, YUN H, BAI S C. Optimum dietary protein level in juvenile river puffer *Takifugu obscurus* [J]. *Journal of Fisheries and Marine Sciences Education*, 2014, 26(4): 859–867.

[9] YANG Z, YANG J X. Optimal dietary protein requirement for juvenile obscure puffer (*Takifugu obscurus*) [J]. *Journal of Fisheries of China*, 2003, 27(5): 450–455.

[10] YOO G, BAI S C. Evaluation of the optimum dietary protein to energy ratio in juvenile river puffer *Takifugu obscurus* [J]. *Journal Fisheries and Marine Sciences Education*, 2014, 26(4): 868–876.

[11] YE C X, WU Y L, SUN Z Z, et al. Dietary protein requirement of juvenile obscure puffer, *Takifugu obscurus* [J]. *Aquaculture Research*, 2017, 48(5): 2064–2073.

[12] WANG P P, WANG A L, YE C X, et al. Methionine requirement of juvenile obscure puffer *Takifugu obscurus* [C]//Proceedings of the 9th World Chinese Symposium on Fish Nutrition and Feeding. Xiamen: 9th World Chinese Symposium on Fish Nutrition and Feeding, 2013.

[13] YOO G, BAI S C. Effects of different dietary protein sources on apparent digestibility and growth in juvenile river puffer *Takifugu obscurus* [J]. *Korean Journal of Fisheries and Aquatic Sciences*, 2014, 47(4): 383–389.

[14] ZHONG G F, ZHOU H Q, QIAN X, et al. Effects of fishmeal replacement by corn gluten meal on lysozyme activity and c-type lysozyme mRNA expression in *Takifugu obscurus* [J]. *Journal of Fisheries of China*, 2010, 34(7): 1121–1128.

[15] KIKUCHI K, FURUTA T, IWATA N, et al. Effect of dietary lipid levels on the growth, feed utilization, body composition and blood characteristics of tiger puffer *Takifugu rubripes* [J]. *Aquaculture*, 2009, 298(1/2): 111–117.

[16] SUN Y, JIANG Z Q, LI Y Q, et al. Effects of dietary lipid levels on growth, body composition and blood indices of juvenile tiger puffer *Takifugu rubripes* [J]. *Journal of Tianjin Agricultural University*, 2013, 20(3): 14–18.

[17] SUN Y, JIANG Z Q, LI Y Q, et al. Effects of dietary lipid levels on liver antioxidant enzyme activity and histology of juvenile tiger puffer *Takifugu rubripes* [J]. *Journal of Guangdong Ocean University*, 2013, 33(3): 27–32.

[18] TAKII K, UKAWA M, NAKAMURA M, et al. Suitable lipid level in brown fish meal diet for tiger puffer [J]. *Fisheries Science*, 1995, 61(5): 841–844.

[19] KIKUCHI K, FURUTA T, IWATA N, et al. Effect of dietary fatty acid composition on the growth of the tiger puffer *Takifugu rubripes* [J]. *Fisheries Science*, 2011, 77(5): 829–837.

[20] YOO G, BAI S C. Effects of dietary lipid sources and essential fatty acids on the growth and body composition of juvenile river puffer fish *Takifugu obscurus*

[J]. *Korean Journal of Fisheries and Aquatic Sciences*, 2014, 47(4): 390–398.

[21] LIU X H, WANG A L, YE C X, et al. Effects of dietary dextrin levels on growth, digestive enzyme activity and blood biochemical indices of juvenile obscure puffer *Takifugu obscurus* [J]. *Journal of Fisheries of China*, 2013, 37(9): 1359–1368.

[22] LIU X H, YE C X, ZHENG L M, et al. Dietary maize starch influences growth performance, apparent digestibility coefficient, and hepatic enzyme activities of carbohydrate metabolism in obscure puffer, *Takifugu obscurus* (Abe) [J]. *Journal of the World Aquaculture Society*, 2015, 46(1): 102–113.

[23] LIU X H, YE C X, YE J D, et al. Effects of dietary amylose/amylopectin ratio on growth performance, feed utilization, digestive enzymes, and postprandial metabolic responses in juvenile obscure puffer *Takifugu obscurus* [J]. *Fish Physiology and Biochemistry*, 2014, 40(5): 1423–1436.

[24] LIU X H, YE C X, SHEN B D, et al. Effects of dietary carbohydrate/lipid ratio on growth, blood indices, hepatic metabolic enzyme activity and PEPCK gene expression in juvenile obscure puffer *Takifugu obscurus* [J]. *Journal of Fisheries of China*, 2014, 38(8): 1149–1158.

[25] TAKII K, UKAWA M, NAKAMURA M, et al. Suitable sugar level in brown fish meal diet for tiger puffer [J]. *Fisheries Science*, 1995, 61(5): 837–840.

[26] KATO K, ISHIBASHI Y, MURATA O, et al. Qualitative water-soluble vitamin requirements of tiger puffer [J]. *Fisheries Science*, 1994, 60(5): 589–596.

[27] LIANG H Y, YE C X, WANG A L. Effects of vitamin C on growth and immune response of juvenile obscure puffer *Takifugu obscurus* under low temperature stress [C]//Proceedings of the 9th World Chinese Symposium on Fish Nutrition and Feeding. Xiamen: Chinese Fisheries Society, 2013.

[28] EO J, LEE K J. Effect of dietary ascorbic acid on growth and non-specific immune responses of tiger puffer, *Takifugu rubripes* [J]. *Fish & Shellfish Immunology*, 2008, 25(5): 611–616.

[29] FURUICHI M, FURUSHO Y, MATSUI S, et al. Essentiality of mineral mixture supplement to white fish meal diet for tiger puffer [J]. *Journal of the Faculty of Agriculture, Kyushu University*, 1997, 42(1): 77–85.

[30] FURUICHI M, FURUSHO Y, HOSSAIN M A, et al. Essentiality of Ca supplement to white fish meal diet for tiger puffer [J]. *Journal of the Faculty of Agriculture, Kyushu University*, 1997, 42(1): 69–76.

[31] HOSSAIN M A, FURUICHI M. Availability of environmental and dietary calcium in tiger puffer [J]. *Aquaculture International*, 1998, 6(2): 121–132.

[32] HOSSAIN M A, FURUICHI M. Calcium requirement of tiger puffer fed a semi-purified diet [J]. *Aquaculture International*, 1999, 7(5): 287–293.

[33] LAINING A, ISHIKAWA M, KYAW K, et al. Dietary calcium/phosphorus ratio influences the efficacy of microbial phytase on growth, mineral digestibility and vertebral mineralization in juvenile tiger puffer, *Takifugu rubripes* [J]. *Aquaculture Nutrition*, 2011, 17(3): 267-277.

[34] YE C X, WAN F, SUN Z Z, et al. Effect of phosphorus supplementation on cell viability, anti-oxidative capacity and comparative proteomic profiles of puffer fish (*Takifugu obscurus*) under low temperature stress [J]. *Aquaculture*, 2016, 452: 200-208.

[35] LIANG M Q, WANG C G, CHEN C, et al. Relationship between growth-promoting effects of several additives and RNA/DNA ratio in tiger puffer *Takifugu rubripes* [J]. *Marine Fisheries Research*, 2001, 22(2): 38-41.

[36] QIN W L, QIN G X, XIE D Y, et al. Comparison of feeding attractants for juvenile obscure puffer *Takifugu obscurus* [J]. *Journal of Aquaculture*, 2013, 34(4): 4-7.

[37] LIANG M Q, YU H, CHANG Q, et al. Feeding attractant activity of different attractants for three fish species [J]. *Journal of Fishery Sciences of China*, 2000, 7(1): 60-63.

[38] HUA X M, ZHOU H Q, ZHANG Y F, et al. Effects of dietary chitosan and probiotics on growth and digestive enzyme activity of juvenile obscure puffer *Takifugu obscurus* [J]. *Acta Hydrobiologica Sinica*, 2005, 29(3): 299-305.

[39] HUA X M, ZHOU H Q, ZHANG D Q, et al. Regulation of immune function by polysaccharides and probiotics in obscure puffer *Takifugu obscurus* [J]. *Journal of Fisheries of China*, 2006, 30(2): 230-235.

[40] SU P, HAN Y, JIANG C, et al. Effects of chitosan-oligosaccharides on growth performance, digestive enzyme and intestinal bacterial flora of tiger puffer (*Takifugu rubripes*) [J]. *Journal of Applied Ichthyology*, 2017, 33(3): 458-467.

[41] WANG Y H, YANG X Y, GUO Z L, et al. Effects of -glucan on non-specific immunity and growth performance of juvenile obscure puffer *Takifugu obscurus* [J]. *Journal of Fishery Sciences of China*, 2013, 20(6): 1247-1256.

[42] MENG X K, SUN Y, QU F, et al. Effects of phytase on growth, digestive enzymes and digestibility in juvenile tiger puffer *Takifugu rubripes* [J]. *Journal of Dalian Ocean University*, 2013, 28(4): 323-328.

[43] HIRAZAWA N, OHTAKA T, HATA K. Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes* [J]. *Aquaculture*, 2000, 188(1/2): 1-13.

[44] WU B Q, SHEN M F, YIN Y, et al. Preliminary study on apparent digestibility of formulated feed in obscure puffer *Takifugu obscurus* [J]. *Journal of Aquaculture*, 2000(1): 22-23.

[45] ZHONG G F, HAN B, HUA X M, et al. Apparent digestibility of protein ingredients and effects of enzymes on digestibility in obscure puffer *Takifugu obscurus* [J]. *Journal of Shanghai Ocean University*, 2012, 21(2): 241-246.

[46] YANG C, YUAN Z G, RUAN C X, et al. Effects of different feeding rates on growth of juvenile obscure puffer *Takifugu obscurus* [J]. *Fujian Fisheries*, 2012, 34(2): 160-162.

[47] KIKUCHI K, IWATA N, KAWABATA T, et al. Effect of feeding frequency, water temperature, and stocking density on the growth of tiger puffer, *Takifugu rubripes* [J]. *Journal of the World Aquaculture Society*, 2006, 37(1): 12-20.

[48] KANG H W, CHO J K, SON M H, et al. Effect of feeding frequency on growth and body composition of juvenile river puffer, *Takifugu obscurus* in winter season [J]. *Journal of Fisheries and Marine Sciences Education*, 2015, 27(3): 718-724.

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