# Advances and Prospects in Pufferfish Nutrition Research: Postprint

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

## Abstract

This paper takes Takifugu rubripes and Takifugu obscurus as examples to synthesize domestic and international research progress on the main nutrient requirements and requirement characteristics of pufferfish, reviews the requirements or requirement characteristics of pufferfish for protein and amino acids, lipids and fatty acids, carbohydrates, vitamins, minerals, and other nutrients, proposes prospects for future related research, and aims to provide a basis and reference for research and development of pufferfish nutritional requirements and specialized compound feeds.

## Full Text

### Progress and Prospects in Pufferfish Nutrition Research

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**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 pufferfish. 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 pufferfish nutritional studies and the development of specialized formulated feeds.

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

Pufferfish belong to the order Tetraodontiformes and family Tetraodontidae, with 35 species naturally distributed in the East China Sea, Yellow Sea, and Bohai Sea waters. Due to their delicious taste, rich nutritional value, and unique cultural significance in Chinese culinary history, pufferfish aquaculture has consistently occupied an important position in China' s aquaculture industry. The main cultured species in China are *Takifugu rubripes* and *Takifugu obscurus*, with smaller quantities of *Takifugu flavidus* and *Takifugu bimaculatus* cultured in Fujian and other regions. While artificial propagation and farming techniques for pufferfish are relatively mature, specialized formulated feeds have not been widely adopted. In some areas, farmers still use raw trash fish directly, or combine soft-shelled turtle and eel formulated feeds with fresh fish. These feeding practices suffer from 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 trading of cultured *T. rubripes* and *T. obscurus*, pufferfish production and consumption are poised for substantial growth. Consequently, developing specialized formulated feeds has become critical for advancing the pufferfish aquaculture industry. This review aims to summarize domestic and international research progress on pufferfish nutrition (Table 1 ) to provide a basis for nutritional 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, pufferfish have high protein requirements. Therefore, research on protein and amino acid requirements is essential for developing specialized feeds and reducing production costs.

**1.1.1 *Takifugu rubripes*** Pufferfish require high dietary protein levels. Research has shown that the optimal crude protein content in feed 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% dietary crude protein. However, other studies suggest that optimal growth in juvenile *T. rubripes* (initial average weight 18.50 g) requires dietary crude protein levels of (50.0 ± 3.7)% [3]. Using casein as the protein source, Yang et al. [4] also identified 50% as the optimal crude protein level based on weight gain rate and protein efficiency.

With global fishmeal production stabilizing while demand and prices continue rising, identifying quality protein sources to replace fishmeal has become an urgent task for aquaculture. In studies with *T. rubripes* (initial weight 18 g), Kikuchi et al. [5-6] found that adding appropriate amounts of mussel extract to defatted soybean meal could replace 30–40% of fishmeal. Using 20% defatted soybean meal and 20% mussel extract as protein sources to replace 40% fishmeal did not negatively affect growth, feed utilization, or health in juvenile *T. rubripes* (initial average weight 11 g). Additional research found that replacing up to 30% of fishmeal with soybean meal did not significantly impact 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]. Similarly, Yang et al. [9] determined the optimal dietary crude protein requirement for juvenile *T. obscurus* (initial weight 23.60 ± 0.27 g) to be 46–49% based on comprehensive evaluation of weight gain rate and protein efficiency. Yoo et al. [10] found that juvenile *T. obscurus* (initial weight 3.43 ± 0.02 g) exhibited good 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 for *T. obscurus* remain scarce. Wang et al. [12] found that when dietary cystine content was 0.20%, the methionine requirement for *T. obscurus* (8.01–24.60 g) was 1.03%.

Research on fishmeal replacement protein sources for *T. obscurus* has identified potential in soybean meal, corn gluten meal, fermented soybean meal, and squid liver powder [13-14]. Zhong et al. [14] found 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 feed 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, body 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 this lipid level yielding the highest antioxidant enzyme activity and healthy liver tissue without pathological changes [16-17]. However, Takii et al. [18] found that 11.50% dietary lipid produced the highest feed efficiency, protein efficiency, and apparent protein retention rate in juvenile *T. rubripes* (approximately 3.70 g).

Regarding fatty acids, Kikuchi et al. [19] conducted nutritional trials with juvenile *T. rubripes* (initial weight 5 g) using eight experimental groups: cod and squid liver oils, three different DHA+EPA gradients, soybean oil (with and without DHA and EPA), and linseed oil (with and without DHA and EPA). Results showed that using only linseed or soybean oil as lipid sources reduced growth, while diets required over 1.50% n-3 long-chain polyunsaturated fatty acids to meet normal growth requirements.

**1.2.2 *Takifugu obscurus*** Yoo et al. [8] found that dietary lipid levels of 7.01–8.98% (using fish oil as the lipid source) produced the highest growth rates in juvenile *T. obscurus* (initial weight 8.32 ± 0.02 g). The same study also 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 use protein or lipids as energy sources, but carbohydrates are also important and more economical energy sources in fish feeds. Inadequate or excessive dietary carbohydrate levels can 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. Rational carbohydrate replacement of lipids can also prevent excessive fat accumulation from high-lipid diets.

Liu et al. [21-24] conducted systematic studies on carbohydrate functions in *T. obscurus* feeds. Research on dextrin levels found that 20–25% dietary dextrin was optimal for juvenile *T. obscurus* (initial weight 10.25 ± 0.51 g) based on comprehensive evaluation of growth, feed utilization, digestive enzyme activity, plasma biochemistry, and cellular immune function [21]. Studies on corn starch found that 23.50% and 22.50% inclusion levels produced the fastest growth and highest feed efficiency, respectively, in juvenile *T. obscurus* (initial weight 7.90 ± 0.20 g) [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 for juvenile *T. obscurus* (initial weight 11.20 ± 0.50 g) [24].

Takii et al. [25] added 10%, 13%, 16%, 19%, and 22% dextrin to experimental diets and found that approximately 16% dextrin was suitable for juvenile *T. rubripes* (initial weight ~10 g), corresponding to about 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 feed.

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

### 1.5 Minerals

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

Studies on *T. rubripes* mineral requirements found that dietary mineral mixtures significantly improved growth and reduced deformities. Furuichi et al. [29] demonstrated that mineral mixture supplementation was necessary even with white fishmeal diets. Calcium supplementation studies by Furuichi et al. [30] showed that appropriate calcium addition to fishmeal diets significantly improved growth. Hossain et al. [31-32] found that supplementing semi-purified diets with 0.10–0.20% calcium met normal growth and skeletal mineralization requirements, while excessive calcium reduced bone zinc content and affected mineralization. Laining et al. [33] found 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 appropriate 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 ratios can serve as indicators for evaluating feed additive effectiveness when basal diets and environmental conditions are consistent. Appropriate attractants in formulated feeds enable fish to feed quickly, reducing feed waste. Compound attractants with multiple components produce synergistic effects, compensating for individual component limitations, improving feed utilization, and enhancing growth and immunity. Qin et al. [36] found that trimethylamine oxide or betaine + alanine + glycine had significant attractant effects on *T. obscurus*. Liang et al. [37] compared attractants for *T. rubripes*, with attractant activity ranking as: clam extract > alanine > histidine > squid visceral fluid > nucleotide (ADP) + alanine > 0.5% betaine > glycine > *Ulva* extract > ADP + glycine + alanine.

Hua et al. [38-39] reported that chitosan (0.2%), probiotics (0.1%), chitosanprobiotic mixtures, or mannan-probiotic mixtures promoted growth, enhanced intestinal amylase activity, and regulated immune function in *T. obscurus*. A recent study showed that 2,000 mg/kg chitosan oligosaccharide promoted growth, improved non-specific immunity and intestinal digestive enzyme activity, and optimized intestinal microflora in *T. rubripes* [40]. Wang et al. [41] demonstrated that injected (intermittent and continuous) and orally administered  -glucan improved non-specific immunity and disease resistance in *T. obscurus*, recommending intermittent administration for production. 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] discovered that caprylic acid in *T. rubripes* feed had anthelmintic effects against monogenean parasites.

### 1.7 Nutrient Digestibility

Data on feed and ingredient digestibility in pufferfish are limited. Wu et al. [44] found that *T. obscurus* had high feed digestibility at 22–23 °C, with lipid digestibility highest, followed by crude protein and carbohydrates. Zhong et al. [45] studied apparent digestibility of several protein ingredients in *T. obscurus*, finding high crude protein and dry matter digestibility for fishmeal, soybean meal, and corn gluten meal (crude protein digestibility: 93.40%, 91.83%, and 94.97%; dry matter digestibility: 88.13%, 85.73%, and 89.25%, respectively). Enzyme supplementation significantly improved crude protein and dry matter digestibility. Yoo et al. [13] reported dry matter apparent digestibility of red fishmeal, white fishmeal, fermented soybean meal, squid liver powder, feather meal, soybean meal, and krill meal as 80%, 78%, 72%, 67%, 56%, 55%, and 54%, respectively, with corresponding crude protein apparent digestibility of 96%, 96%, 93%, 92%, 89%, 88%, and 86%.

### 1.8 Feeding Technology

The optimal growth temperature for pufferfish 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* cultured at approximately 25 °C, fish under 100 g had optimal stocking density of 60 kg/m$^3$ with three daily feedings meeting growth requirements. For fish over 100 g, once-daily feeding at 18 kg/m$^3$ was appropriate [47]. Kang et al. [48] found that once-daily feeding during winter low 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 production, constraining industry development. Despite annual production approaching 30,000 tonnes, surprisingly no large-scale feed manufacturers produce specialized pufferfish feeds. Some farmers use flounder or grouper feeds, or soft-shelled turtle and eel powder feeds mixed with fresh fish, while large enterprises (e.g., Zhongyang Group) have attempted in-house feed production.

Slow development of the pufferfish feed industry stems primarily from: (1) lack of basic nutritional requirement parameters; (2) previous strict government control over pufferfish production and distribution, leaving feed enterprises uncertain about market growth; and (3) entrenched feeding habits among farmers, relatively small production scale, and consequently lower environmental and disease pressures, reducing motivation to change feeding practices.

## 3 Summary and Outlook

In summary, although pufferfish nutrition research has established some foundation, several deficiencies require focused attention: (1) severe lack of data on amino acid, fatty acid, and vitamin requirements; (2) insufficient practical technologies for fishmeal and fish oil replacement that can be rapidly applied in industry; and (3) absence of nutrition studies for different culture modes and growth stages (current research focuses mainly on juveniles of 7-20 g initial weight). Given diverse culture methods (intensive, pond, offshore cage) and land-sea relay characteristics, nutrition and feeding technologies for these systems warrant particular attention.

Meanwhile, constraints on feed industry development are changing. On one hand, conditional lifting of restrictions will accelerate industry growth, prompting small-medium feed enterprises to quickly enter the pufferfish feed market. On the other hand, rising trash fish prices, stricter environmental regulations, and confirmed pathogen transmission and disease incidence from trash fish feeding will inevitably shift farmer feeding strategies toward formulated feeds. In basic research, establishment of a pufferfish nutrition and feed position within the national agricultural industry technology system will gradually advance comprehensive nutritional parameter studies, specialized feed development, and promotion, boosting industry development.

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