Comparative Analysis of Nutritional Quality between Wild and Cultured Takifugu rubripes (Postprint)

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Abstract

This study determined the general nutritional components and muscle amino acid and fatty acid composition of wild 1-year-old, cultured 1-year-old, and cultured 2-year-old tiger pufferfish (Takifugu rubripes) to compare the nutritional quality between wild and cultured tiger pufferfish. Samples included 15 wild 1-year-old tiger pufferfish [body weight 71–139 g, body length 13.4–18.9 cm], 13 cultured 1-year-old tiger pufferfish (fed fresh frozen trash fish) [body weight 90–147 g, body length 14.8–20.4 cm], and 10 cultured 2-year-old tiger pufferfish (fed fresh frozen trash fish) [body weight 578.4–638.7 g, body length 31.3–36.7 cm]. National standard methods were employed to determine the general nutritional components in tissues and the muscle amino acid and fatty acid composition, and the muscle nutritional quality was analyzed. The results showed that whole-body crude protein content was highest in wild 1-year-old tiger pufferfish at 68.07%, which was 32.3% and 20.6% higher than that in cultured 1-year-old and cultured 2-year-old tiger pufferfish, respectively ($P<0.05$), whereas whole-body crude fat content was significantly higher in cultured 1-year-old and cultured 2-year-old tiger pufferfish than in wild 1-year-old tiger pufferfish ($P<0.05$). Muscle crude protein content in cultured 2-year-old tiger pufferfish was significantly higher than that in wild 1-year-old and cultured 1-year-old tiger pufferfish ($P<0.05$), while no significant differences in muscle crude fat and crude ash contents were observed among all samples ($P>0.05$). Liver crude protein and crude fat contents varied considerably among samples; crude protein content decreased significantly in the order of wild 1-year-old, cultured 1-year-old, and cultured 2-year-old ($P<0.05$), while crude fat content exhibited the opposite trend. A total of 18 amino acids were detected in the muscle of both wild and cultured tiger pufferfish, and most amino acids were significantly higher in cultured tiger pufferfish than in wild ones ($P<0.05$). According to amino acid score (ASS) and chemical score (CS), lysine content in tiger pufferfish muscle was relatively abundant, and the essential amino acid index (EAAI) was highest in cultured 2-year-old tiger pufferfish, followed by cultured 1-year-old, and lowest in wild 1-year-old. A total of 19 fatty acids were detected in the muscle of wild and cultured tiger pufferfish, with similar fatty acid composition between wild and cultured groups. No significant differences in saturated fatty acid (SFA) content were found among wild 1-year-old, cultured 1-year-old, and cultured 2-year-old tiger pufferfish muscle ($P>0.05$), whereas polyunsaturated fatty acid (PUFA) content was highest in cultured 2-year-old tiger pufferfish at 51.54%, though differences among samples were not significant ($P>0.05$). The contents of C20:5n-3 (EPA) and C22:6n-3 (DHA) in muscle were both relatively abundant; specifically, DHA content in cultured 1-year-old and cultured 2-year-old tiger pufferfish was 24.7% and 27.2% higher than that in wild 1-year-old tiger pufferfish ($P<0.05$), and EPA+DHA content in cultured 1-year-old and cultured 2-year-old tiger pufferfish was also significantly higher than that in wild 1-year-old tiger pufferfish ($P<0.05$). Therefore, it can be concluded that tiger pufferfish possesses a reasonable nutritional composition, and the nutritional quality of cultured tiger pufferfish is superior to that of wild tiger pufferfish.

Full Text

Comparative Analysis of Nutritional Quality between Wild and Cultured Takifugu rubripes

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Abstract

This study compared the nutritional quality between wild and cultured Takifugu rubripes by analyzing general nutritional components, amino acid composition, and fatty acid composition in the muscle of one-year-old wild, one-year-old cultured, and two-year-old cultured fish. The one-year-old wild group comprised 15 individuals (body weight: 71–139 g, body length: 13.4–18.9 cm) captured from Donggang waters in Dandong through tag-release-recapture. The cultured groups included 13 one-year-old fish (body weight: 90–147 g, body length: 14.8–20.4 cm) and 10 two-year-old fish (body weight: 578–639 g, body length: 31.3-

36.7 cm) obtained from Dalian Tianzheng Industrial Co., Ltd. in Changhai County, all fed with frozen trash fish. Standard national methods were employed to determine tissue nutritional components and muscle amino acid and fatty acid profiles.

Results showed that the crude protein content in whole body of one-year-old wild T. rubripes was highest at 68.07%, exceeding that of one-year-old and two-year-old cultured fish by 32.3% and 20.6%, respectively ($P<0.05$). Conversely, whole-body crude lipid content was significantly higher in both cultured groups compared to the wild group ($P<0.05$). Muscle crude protein content in two-year-old cultured fish was significantly higher than in one-year-old wild and cultured fish ($P<0.05$), while muscle crude lipid and ash contents showed no significant differences among the three groups ($P>0.05$). Liver crude protein and lipid contents varied substantially, with crude protein decreasing significantly from wild to cultured groups ($P<0.05$) and crude lipid showing the opposite trend.

Both wild and cultured T. rubripes contained 18 amino acids. Most amino acid concentrations in cultured fish muscle were significantly higher than in wild fish. Based on amino acid score (AAS) and chemical score (CS), lysine was relatively abundant in all groups. The essential amino acid index (EAAI) was highest in two-year-old cultured fish, followed by one-year-old cultured fish, and lowest in one-year-old wild fish. Nineteen fatty acids were detected in muscle tissue, with similar composition between wild and cultured fish. Saturated fatty acid (SFA) content showed no significant differences among groups ($P>0.05$). Polyunsaturated fatty acid (PUFA) content was highest in two-year-old cultured fish at 51.54%, though not significantly different among groups ($P>0.05$). Both C20:5n-3 (EPA) and C22:6n-3 (DHA) were abundant. Compared to one-year-old wild fish, DHA content in one-year-old and two-year-old cultured fish increased by 24.7% and 27.2%, respectively ($P<0.05$). EPA+DHA content was also significantly higher in both cultured groups than in wild fish ($P<0.05$). In conclusion, T. rubripes exhibits a reasonable nutritional composition, and cultured fish demonstrate superior nutritional quality compared to their wild counterparts.

Keywords: Takifugu rubripes; amino acids; fatty acids; nutritional quality; evaluation

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Introduction

Takifugu rubripes, belonging to Osteichthyes, Tetraodontiformes, Tetraodontidae, and the genus Takifugu, commonly known as pufferfish, is a benthic carnivorous coastal species. Revered as the "King of Fish," it is prized for its fierce temperament, large size, rapid growth, delicious meat, rich protein content, and abundance of vitamin B1, vitamin B2, and beneficial trace elements. It commands the highest economic value among pufferfish species and is distributed in the Yellow Sea, Bohai Sea, and East China Sea in China, as well as in the

Korean Peninsula and Japan. Due to its unique nutritional value and flavor, T. rubripes has long been a traditional delicacy in China, Japan, and Korea.

Previous research by Gao Lujiao et al. compared nutritional quality under different culture modes and found significant differences. Most cultured T. rubripes are fed fresh trash fish, raising concerns about whether altered food sources and living environments might adversely affect nutritional value—critical for further development of this species. This study provides a comprehensive comparative analysis of general nutritional components and muscle quality between wild and cultured T. rubripes to enhance understanding of its nutritional characteristics, evaluate impacts of artificial culture, and provide theoretical guidance for stage-specific culture practices and formulated feed development.

1.1 Experimental Materials

Experimental fish included one-year-old wild T. rubripes captured in September 2014 from Donggang waters in Dandong through tag-release-recapture (n=15, body weight: 71–139 g, body length: 13.4–18.9 cm). Cultured one-year-old and two-year-old fish (fed frozen trash fish) were purchased in September 2014 from Dalian Tianzheng Industrial Co., Ltd. in Changhai County. The cultured one-year-old group comprised 13 individuals (body weight: 90–147 g, body length: 14.8–20.4 cm), while the two-year-old group included 10 individuals (body weight: 578–639 g, body length: 31.3–36.7 cm). The frozen trash fish contained 73.62% moisture, 56.43% crude protein, 16.87% crude lipid, 13.21% crude ash, and 4.01% crude fiber.

1.2 Sample Processing

Fish were dissected on ice, and the liver and all muscle tissue from both sides (from behind the head to before the caudal peduncle) were rapidly excised. Samples were rinsed with deionized water, stored at -80°C for later analysis, and two fish from each age group were randomly selected for whole-body nutritional analysis after oven-drying (105°C) and grinding.

1.3 Analytical Methods

1.3.1 General Nutritional Component Determination Moisture content was determined by oven-drying at 105°C (GB 6435-1986). Crude protein was measured using the Kjeldahl method (GB/T 6432-1994). Crude ash was determined by dry ashing at 550°C (GB/T 6438-1992). Crude lipid was extracted using the Soxhlet method (GB/T 5009.6-2003).

1.3.2 Muscle Amino Acid Composition Determination Cysteine was determined by oxidation acid hydrolysis (GB/T 15399-1994) followed by analysis using a Biochrom 20 amino acid analyzer (GB/T 18246-2000). Tryptophan was measured by fluorescence spectrophotometry. The remaining 16 amino acids were analyzed using the Biochrom 20 amino acid analyzer according to GB/T 5009.124-2003.

1.3.3 Muscle Fatty Acid Composition Determination Fatty acid composition was analyzed by gas chromatography. Lipids were extracted from freeze-dried muscle using a modified Folch method. Fatty acid methyl esters were prepared by saponification with 0.5 mol/L KOH-methanol at 70°C for 1 hour, followed by transesterification catalyzed by BF3. Analysis was performed on a Shimadzu GC-9A gas chromatograph equipped with a C-R3A data processor, using a 30 m × 0.25 mm × 0.3 m FFAP antioxidant cross-linked quartz capillary column. The injector temperature was 260°C, detector temperature 230°C, with programmed column heating from 160°C to 230°C at 2°C/min. High-purity nitrogen was used as carrier gas (30 mL/min tail flow), with 1 L injection volume. Fatty acids were identified by comparing retention times with standards, and relative contents were calculated by area normalization. Samples were analyzed in quintuplicate.

1.4 Muscle Nutritional Quality Evaluation

Based on the FAO/WHO (1973) amino acid scoring pattern and the whole egg protein chemical scoring pattern from the Chinese Academy of Preventive Medicine (1991), amino acid score (AAS), chemical score (CS), and essential amino acid index (EAAI) were calculated using the following formulas:

$$\text{AAS} = \frac{\text{Content of amino acid in test protein (mg/g N)}}{\text{Content of same amino acid in FAO/WHO pattern (mg/g N)}}$$

$$\text{CS} = \frac{\text{Content of amino acid in test protein (mg/g N)}}{\text{Content of same amino acid in whole egg protein (mg/g N)}}$$

$$\text{EAAI} = \sqrt[n]{a \times 100 \times b \times 100 \times \cdots \times h \times 100}$$

where n is the number of compared amino acids; $a,\ b,\ \cdots,\ h$ are the contents of each amino acid in the test protein (mg/g N); and $A,\ B,\ \cdots,\ H$ are the contents of each amino acid in whole egg protein (mg/g N). Amino acid content (mg/g N) = (Amino acid content in sample / Crude protein content in sample) × 6.25 × 1000.

1.5 Data Processing

Data are expressed as mean ± standard deviation. Homogeneity of variance was tested using Levene’ s test in SPSS 16.0. If P>0.05, one-way ANOVA was performed; if P$ $0.05, data were transformed to achieve homogeneity before ANOVA. Significance level was set at P<0.05.

2.1 General Nutritional Components in Wild and Cultured Takifugu rubripes

The crude protein, crude lipid, moisture, and ash contents in whole body, muscle, and liver of wild and cultured T. rubripes are shown in Table 1 . Whole-body moisture content was significantly higher in one-year-old wild fish compared to cultured groups (P<0.05). Whole-body crude protein was highest in one-year-old wild fish at 68.07%, exceeding one-year-old and two-year-old cultured fish by 32.3% and 20.6%, respectively (P<0.05). Conversely, whole-body crude lipid was significantly higher in both cultured groups than in wild fish (P<0.05), while ash content showed no significant differences (P>0.05).

Muscle crude lipid and ash contents did not differ significantly among groups (P>0.05). Muscle moisture was slightly higher in one-year-old cultured fish than in wild and two-year-old cultured fish, but differences were not significant (P>0.05). Muscle crude protein in two-year-old cultured fish was significantly higher than in both one-year-old wild and cultured fish (P<0.05), while one-year-old wild fish had slightly higher muscle crude protein than one-year-old cultured fish without significant difference (P>0.05).

Liver crude protein and lipid contents varied markedly among groups. Crude protein decreased significantly from one-year-old wild to one-year-old and two-year-old cultured fish (P<0.05), while crude lipid showed the opposite trend, increasing significantly in the same order (P<0.05).

2.2.1 Amino Acid Composition in Muscle of Wild and Cultured Takifugu rubripes

The amino acid composition and content in muscle are presented in Table 2 . Eighteen common amino acids were detected, including 8 essential amino acids (EAA), 2 half-essential amino acids (HEAA), and 8 non-essential amino acids (NEAA). Except for histidine and cysteine, the remaining 16 amino acids differed significantly among the three groups. Two EAAs (methionine and threonine) and three NEAAs (glutamic acid, aspartic acid, and glycine) differed significantly between wild one-year-old fish and both cultured groups (P<0.05), but not between the two cultured groups (P>0.05). Five EAAs (leucine, isoleucine, phenylalanine, valine, tryptophan, and lysine), one HEAA (arginine), and two NEAAs (serine and tyrosine) showed no significant differences between wild and one-year-old cultured fish (P>0.05), but differed significantly from two-year-old cultured fish (P<0.05). Total amino acids (TAA), EAA, HEAA, and NEAA contents all differed significantly among groups (P<0.05).

The relative abundance of the 18 amino acids was consistent across all groups, with glutamic acid being the most abundant (12.26%, 13.27%, and 13.49% in wild one-year-old, cultured one-year-old, and cultured two-year-old fish, respectively), followed by aspartic acid, lysine, leucine, arginine, and alanine. Cysteine was the least abundant at 0.50%, 0.48%, and 0.52%, respectively. The EAA/TAA ratios were 41.78%, 41.35%, and 41.64%, while EAA/NEAA ratios were 85.11%, 83.27%, and 84.55% for wild one-year-old, cultured one-year-old, and cultured two-year-old fish, respectively.

2.2.2 Nutritional Quality Evaluation of Muscle from Wild and Cultured Takifugu rubripes

Essential amino acid contents from Table 2 were converted to mg/g N and compared with whole egg protein and FAO/WHO standard patterns to calculate AAS, CS, and EAAI for each group.

As shown in Table 3 , EAA contents increased progressively from wild one-year-old (2,426.11 mg/g N) to cultured one-year-old (2,519.82 mg/g N) and cultured two-year-old fish (2,584.33 mg/g N), all exceeding the FAO/WHO pattern (2,250 mg/g N) but below the whole egg protein pattern (3,059 mg/g N). Lysine contents (542.70, 564.59, and 578.94 mg/g N) exceeded both the FAO/WHO (340 mg/g N) and whole egg protein (441 mg/g N) patterns, indicating that both wild and cultured T. rubripes are highly nutritious.

Table 4 shows that EAA AAS values were near or above 1, and CS values exceeded 0.5 for all groups, with cultured two-year-old fish having the highest values, followed by cultured one-year-old and wild one-year-old fish. This indicates that EAA composition becomes more balanced with age, and cultured fish generally have more balanced and abundant EAAs than wild fish. Based on AAS, the first limiting amino acid was valine, and the second was methionine + cysteine. According to CS, the first limiting amino acid was methionine + cysteine, and the second was valine. EAAI values were 74.96, 77.82, and 79.86 for wild one-year-old, cultured one-year-old, and cultured two-year-old fish, respectively.

2.3 Fatty Acid Composition in Muscle of Wild and Cultured Takifugu rubripes

Muscle fatty acid composition is presented in Table 5 . Wild and cultured T. rubripes showed similar fatty acid profiles. Saturated fatty acid (SFA) content did not differ significantly among groups (P>0.05). Monounsaturated fatty acid (MUFA) content was significantly higher in one-year-old cultured fish than in wild and two-year-old cultured fish ($P<0.05$). Polyunsaturated fatty acid (PUFA) content was highest in two-year-old cultured fish (51.54%), followed by wild one-year-old (50.99%) and cultured one-year-old fish (50.25%), though differences were not significant ($P>0.05$).

Among SFAs, C16:0 was the most abundant (18.78–19.40%) across all groups, with lower levels of C14:0 and C17:0. For MUFAs, C18:1n-9 was predominant, being significantly higher in wild one-year-old fish (11.55%) than in cultured one-year-old (9.75%) and two-year-old fish (9.65%) ($P<0.05$). Regarding PUFAs, both C20:5n-3 (EPA) and C22:6n-3 (DHA) were abundant. DHA content in one-year-old and two-year-old cultured fish was 24.7% and 27.2% higher than in wild one-year-old fish ($P<0.05$), and EPA+DHA content was also significantly higher in cultured groups ($P<0.05$). EPA contents were 8.56%, 8.05%, and 9.15% for wild one-year-old, cultured one-year-old, and cultured two-year-old fish, respectively, without significant differences ($P>0.05$). Wild one-year-old fish had significantly higher C20:4n-6 (arachidonic acid, AA) content than cultured groups (65.5% and 67.7% higher, respectively; $P<0.05$). Except for C16:0, C14:1, C18:2n-6, C20:5n-3, SFA, and PUFA, all other fatty acids differed significantly between wild and one-year-old cultured fish ($P<0.05$). These results demonstrate that living environment, food sources, and activity space substantially influence muscle fatty acid composition.

3.1 Analysis of General Nutritional Components

For most economically important aquatic animals, cultured populations exhibit substantial nutritional differences from wild counterparts due to changes in living environment, food composition, and activity space. This study revealed that whole-body crude protein in one-year-old wild T. rubripes was 32.3% and 20.6% higher than in one-year-old and two-year-old cultured fish, respectively, while crude lipid was 42.5% and 34.3% lower. Similar patterns were observed in liver, where crude protein was 48.6% and 67.9% higher in wild fish than in one-year-old and two-year-old cultured fish, respectively, while crude lipid was 17.4% and 29.3% lower. Muscle crude protein in two-year-old cultured fish was significantly higher than in wild and one-year-old cultured fish ($P<0.05$), while muscle crude lipid showed no significant differences among groups.

The elevated crude lipid content in cultured fish is closely related to culture environment and diet. Wild T. rubripes inhabit vast marine environments with extensive activity ranges and diverse prey, ensuring food freshness while active hunting and environmental stress increase energy expenditure, preventing excessive fat accumulation. Conversely, cultured fish obtain food easily with low foraging costs and have limited activity space, resulting in greater nutrient accumulation. Daniels et al. reported that high-fat diets reduced body protein and increased body lipid in red drum (Sciaenops ocellatus). Liu et al. found that high-fat diets (19.93% crude lipid) significantly increased liver lipid content, hepatosomatic index, and viserosomatic index in topmouth culter (Erythroculter ilishaeformis). Sun et al. demonstrated that hepatosomatic index, viserosomatic index, and whole-body crude lipid in T. rubripes increased significantly with dietary lipid levels. These findings indicate that dietary crude lipid is a key factor causing lipid deposition in fish. In this study, the frozen trash fish fed to cultured T. rubripes contained 16.87% crude lipid, likely contributing to excessive lipid accumulation.

3.2 Analysis of Amino Acid Composition and Nutritional Quality Evaluation

Among all nutrients, protein is paramount, and since proteins comprise amino acids, protein nutrition is essentially amino acid nutrition. The composition and content of amino acids, particularly the eight essential amino acids (EAA) required by humans, determine nutritional value—optimal when matching human requirements. Results showed that TAA (74.72%, 78.13%, and 82.01%) and EAA contents (31.22%, 32.31%, and 34.15%) were very high in all groups, surpassing those reported for Takifugu obscurus, Takifugu ocellatus, and Takifugu xanthopterus. Studies on other species have shown wild fish typically have higher TAA and EAA than cultured fish, attributed to natural prey and balanced nutrition. However, this study found that one-year-old cultured T. rubripes had significantly higher TAA and EAA than wild fish, similar to findings for Yalu River mandarin fish (Simiperca Schezeri Steindachner). This may be because T. rubripes is a fierce carnivore fed frozen trash fish that closely mimics natural prey, with more abundant food ensuring balanced nutrient accumulation.

Amino acid composition is also age-related. Fish metabolic patterns, nutritional requirements, and nutrient deposition vary across developmental stages. Studies on different fish species have reported varying trends in TAA and EAA with age. In this study, two-year-old cultured T. rubripes had higher TAA and EAA than one-year-old cultured fish, suggesting greater amino acid requirements at older ages—providing reference for stage-specific feed formulation.

The EAA/TAA ratios (41.78%, 41.35%, and 41.64%) and EAA/NEAA ratios (85.11%, 83.27%, and 84.55%) all meet FAO/WHO criteria for high-quality protein (EAA/TAA 40%, EAA/NEAA $>$ 60%), indicating good amino acid balance. EAAI values (74.96, 77.82, and 79.86) demonstrate that cultured fish have better protein quality than wild fish, with quality improving with age. Lysine, the first limiting amino acid in humans and cereal proteins, was abundant in all groups, offering excellent nutritional balance for cereal-based diets. The main limiting amino acids were valine and methionine + cysteine, which should be considered in feed formulation.

3.3 Analysis of Fatty Acid Composition in Muscle

Lipids serve not only as energy sources but also as essential fatty acid providers. As poikilotherms, fish have species-specific essential fatty acid requirements that vary with age, sex, temperature, and particularly diet. Nutritional quality is importantly judged by highly unsaturated fatty acid (HUFA) content in PUFAs, primarily EPA, DHA, and AA. EPA and DHA reduce blood lipids, decrease vascular fat deposition, improve vascular elasticity, and lower cardiovascular disease risk while also providing anti-aging and brain development benefits.

In this study, wild one-year-old fish had significantly higher C18:1n-9 content than cultured groups, while EPA showed no significant differences among groups. DHA content was highest in two-year-old cultured fish (33.25%), significantly higher than wild one-year-old (24.22%) and cultured one-year-old fish (32.18%). EPA+DHA content followed the same pattern. This aligns with reports on Takifugu obscurus but contrasts with studies on other species showing higher EPA and DHA in wild fish. Research indicates that DHA and EPA are essential fatty acids for marine fish that cannot be fully synthesized and must be obtained from diet. Elevated C18:1n-9 often indicates essential fatty acid deficiency, suggesting that wild T. rubripes may have inadequate DHA due to harsh living conditions, poor foraging, insufficient food intake, and different dietary lipid composition. Cultured fish, with superior environments and abundant food, accumulate more EPA+DHA.

Wild one-year-old fish had 65.5% and 67.7% higher C20:4n-6 (AA) content than cultured groups, similar to findings in large yellow croaker (Pseudosciaena crocea) but contrasting with studies on Chinese sturgeon (Acipenser sinensis) where AA was undetectable in cultured fish. For fish and mammals, AA in membrane phospholipids produces eicosanoids via lipoxygenase and cyclooxygenase pathways, regulating inflammation and lymphocyte function. Under stress, fish require more AA-derived eicosanoids for enhanced cardiovascular and inflammatory responses. Wild T. rubripes in harsh conditions likely have higher AA demands for adaptation. Additionally, eicosanoid production in retinal cells is crucial for normal vision in vertebrates, and elevated AA in wild fish may represent adaptation to deep-water and low-light environments. The high total unsaturated fatty acid content (MUFA+PUFA, averaging 69.2%) in both wild and cultured T. rubripes confirms their rich nutritional value.

Conclusion

Comprehensive evaluation of general nutritional components, amino acids, and fatty acids reveals that both wild and cultured T. rubripes have high protein, low fat, and high EPA+DHA contents, with EAA/TAA and EAA/NEAA ratios exceeding FAO/WHO ideal patterns. Cultured one-year-old and two-year-old fish are nutritionally superior to wild one-year-old fish. Takifugu rubripes is confirmed as a nutritious, high-quality fish worthy of its “King of Fish”reputation, with cultured fish maintaining excellent muscle quality and nutritional balance, representing a premium dietary protein source.

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