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Analysis and Evaluation of Nutritional Composition of Farmed Male Pufferfish (Takifugu Obscurus)

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Abstract. The nutritional compositions of the main tissues (meat, testis and liver) of farmed Takifugu obscures were investigated. All tissues reported in this study showed high amounts of crude protein in meat (18.44±0.11 g/100g) and testis (17.15±0.15 g/100g), but lower proportions in liver (3.89±0.16g/100g). At the same time, liver (63.86±0.93 g/100g) contained significant amounts of crude fat, while testis (1.82±0.13 g/100g) and meat (1.31 ± 0.21 g/100g) contained lower proportion of crude fat. Concerning to the minerals, K was the most abundant macroelement in meat, testis and liver. At the same time, Zn accounts for the majority of microelement in meat and testis, while Fe does in liver. In addition, amino acids (AA) were rich in testis and meat. The contents of total amino acids (TAA) in meat and testis were 80.08 ±0.75g/100g and 63.33±0.10 g/100g, respectively. The ratios of total essential amino acids (TEAA) to TAA in meat and testis were 40.60% and 39.33%, respectively. The TEAA/TAAs were comparable to the reference values recommended by FAO/WHO. The AA contents of meat were dominated by Glu, testis were dominated by Arg. According to amino acid score (AAS), the first limiting amino acid of meat was Tyr; the first limiting amino acid of testis were (Phe+ Tyr).

Keywords. farmed pufferfish; meat; testis; liver; component; nutritional evaluation

1 Introduction

Pufferfish, also called bubble fish, is a kind of benthic fish living in the warm water. China is the production and marketing power of pufferfish in the world. The annual allowable catch is more than 100,000 tons, accounting for about 70% of the world's total output. 70% to 80% products were exported to Japan and South Korea[1]. In recent years, China has made significant progresses in pufferfish farming industry. A series of the core technologies base on the mechanism of tetrodotoxin (TTX) production was used to control the TTX[2]. Farmed Takifugu obscurus is a unique migration fish of China. It was famous for delicious and nutrient-rich. Yin Ning[3] confirmed that under the condition of freshwater aquaculture, supplemented by certain technical measures, the ovarian and liver of which were non-toxic even the individual Takifugu obscurus have already reached sexual maturity. Hua Yuanyu[4] thoughts that Fugu obscurus, after generations of nurturing by fully freshwater environments, can produce generations with lower toxic level that be safe to eat. Compared with the general fish, pufferfish meat had high protein content, low fat content and rich in essential amino acids and minerals. In addition to meat, sexual gland also had high nutritional value. Testis was rich in protamine, DNA, Zn and Se, and was praised as “beauty milk” in the history. Currently, the study of the nutritional value of the pufferfish meat, testis and liver has not yet completed. The nutritional composition in testis and liver of farmed Takifugu obscurus was investigated in this study, in order to provide a theoretical basis to effectively exploits and utilize of the resources of farmed Takifugu obscurus.

2 Material and methods

2.1 Material

Takifugu obscurus were obtained from the breeding base of Zhongyang Group, Jiangsu in April 2010. 8 male fish (2-year-old) with gonad maturation and uniform size were selected for this research. Their weight and body length were 328±36g and 20±1g, respectively. Try to remove other impurities attach on the tissues. After washed again, the meats were filleted, and homogenized in a meat grinder, and then the mixed ground meats were packaged in market sterilized polythene bags. The testes and livers were packed in the screw bottles, and all the samples were stored at -80°C until analysis. The livers and testes were weight for 43±14g and 20±7g, respectively.

2.2 Analysis of nutritional composition

The moisture, crude protein, crude fat and ash content was determined according to AOAC methods 950.46, 981.10, 948.22, 938.08 (AOAC, 2007)[5]. Amino acid profile was determined according to Adeyeye[6]. Samples were hydrolyzed by 6M HCl, and then determined by an amino acid automatic analyzer (Hitachi L-8800). For tryptophan determination, the method of Wu Fang[7] was used. 5M NaOH which containing 0.5% soluble starch solution was used for hydrolysis. The hydrolysates were determined by fluorescence method (Shimadzu RF-5301pc). The minerals were determined according to Borges[8]. The determination of K, Na, Ca, Mg, Zn, Fe, and Cu was done on a flame atomic absorption spectrophotometer (TAS-990, Beijing Purkinje General Instrument Co., Ltd). Se was determined on an ICP-MS (PE ELAN60000, Beijing Bode HengYue Electronics Co., Ltd.). P was determined on a UV-1100 spectrophotometer (Mei Puda Instrument Co., Ltd., Shanghai, China).

2.3 Evaluation of nutrition value

Evaluation of nutrition value was based on the amino acid score mode proposed by the FAO/WHO in 1991[9]. The amino acid score (AAS), essential amino acid index (EAAI), branched chain amino acids/aromatic value amino acids (TBCAA,/ TArAA)

These values were calculated as follows:

AAS (%) = Test protein amino acid content (g/100g protein)/FAO scoring model amino acid content (g/100g protein) × 100

$$EAAI = \sqrt[n]{\frac{100a}{a_e} \times \frac{100b}{b_e} \times \cdots \times \frac{100\mathrm{h}}{h_e}}$$

Note: n stands for the number of essential amino acids comparing; a, b, c, ..., h are essential amino acids content of test protein (g/100g protein); a_e, b_e, c_e, ..., h_e are essential amino acids content of egg protein (g/100g protein).

2.4 Statistical methods

All analyses were conducted at least in triplicate for each sample. Results were analyzed according to an Analysis of Variance (ANOVA), which were conducted using Excel XP software. The comparison of nutrition qualities of the three tissues had been done. All statistical analyses were performed on SPSS 13.0.

3 Results and discussion

3.1 Proximate composition

Moisture, crude protein and ash contents of liver were 29.81%, 3.89% and 0.64%, respectively. They were significantly lower than that of meat and testis. But, the fat content in liver was 63.86%, much higher than others. TTX exists in puffer as a complex which joints to protein[10], it results in lower protein content in the liver.

The proximate composition of testis was consistent with meat, and can be summarized as high-protein and low-fat. The crude protein content in meat was higher than the testis, while fat and ash content were on the contrary. This result was coincident with the results of blue crab[11]. During maturation, the fish basically had no ingestion. Thus, high fat in the gonad was the results of nutrients accumulation in order to achieve the level for breeding. High protein content in meat, may also be associated with its own genetic characteristics and maternal compensations during fish gonad development[12].

Table 1. Proximate composition in the tissues of farmed Takifugu obscures (g/100g, wet, mean±S.D., n=3)

Tissues	Moisture	Crude protein	Crude fat	Ash
Meat	79.73±0.52a	18.44±0.11a	1.31±0.21b	1.42±0.04b
Testis	79.53±0.90a	17.15±0.15b	1.82±0.13b	2.22±0.08a
Liver	29.81±0.23b	3.89±0.16c	63.86±0.93a	0.64±0.02c

Note: The different small letters in same column indicate statistically significant differences ($p<0.05$).

3.2 Amino acids

Three tissues contained 18 kinds of amino acids in common, including 8 kinds of essential amino acids (EAA) and 10 kinds of non-essential amino acids (NEAA). The highest of total amino acids content was in meat, followed by testis and liver. Glu was the most abundant amino acid in meat, because some of Glu results from the conversion of Gln[10], with Trp being the lowest. In testis, Arg was the most abundant, with the content was 1.5 times higher than meat. The most abundant and least amino acid was also Glu and Trp, respectively.

TEAA (g/100g) of meat, testis and liver was 32.51, 24.91 and 2.21, respectively. The ratio of TEAA/TAA was 40.60%, 39.33% and 39.05%, respectively. The ratio of TEAA/TNEAA was 68.34%, 62.21% and 64.06%, respectively. These results showed that the ratios of TEAA/TAA and TEAA/TNEAA were comparable to the reference values recommended by FAO/WHO.

As far as amino acids were concerned, Asp, Glu, Ala, Gly and Arg were the main factor influence the food flavor, and were called as the umami amino acids. The total umami amino acids (TUAA) content (g/100g) in meat, testis and liver of farmed *Takifugu obscuru* was as high as 47.57, 38.42 and 3.45, respectively. The ratio of TUAA/TAA was 42.11%, 46.12% and 41.76%, respectively. So, the meat, testis and liver of the pufferfish taste very delicious, especially the testis.

TEAA (g/100g protein) in meat, testis and liver were 36.22, 29.61 and 40.60, respectively, all close to or even higher than that recommended by FAO/WHO.

The AAS and EAAI in meat, testis, and liver were calculated, and the results are shown in table 3. From the point of AAS, the first limiting amino acid in meat and testis were Trp and (Phe+Tyr), respectively, while there was no limiting amino acid in the liver. AAS in meat and testis were higher or close to 100, with AAS of liver were higher than 100. AAS of Met+Cys was the highest in all tissues, indicated that sulfur-containing amino acids was rich in all tissues[15]. EAAI is one of the commonly indicator used to evaluate the nutritional value of protein. EAAI of meat, testis and the liver were 71.72, 61.32 and 86.02, respectively.

Table 2. Amino acids composition and content in the tissues of farmed Takifugu obscurus

(%,dry, mean±SD., n = 3)

Amino acids	Meat	Testis	Liver	Amino acids	Meat	Testis	Liver
Asp**	5.92±0.05 a	4.39±0.01b	0.42±0.02c	Lys*	7.79±0.06 a	7.19±0.02 b	0.42±0.03 c
Thr*	3.78±0.05 a	3.01±0.03 b	0.27±0.02c	His	1.99±0.03 a	1.17±0.00 b	0.15±0.01 c
Ser	3.61±0.05	3.14±0.02	0.30±0.02a	Arg**	5.64±0.06 b	8.63±0.01 a	0.39±0.03 c
Glu**	11.97±0.18 a	5.99±0.03 b	0.69±0.04c	Pro	3.69±0.19 a	2.92±0.09 b	0.29±0.02 c
Gly**	5.38±0.04	5.92±0.08	0.52±0.06a	Trp*	0.54±0.02 b	1.06±0.02 a	0.14±0.01 c
Ala**	4.86±0.05 a	4.27±0.01 b	0.35±0.02c	TAA	80.08±0.75 a	63.33±0.10 b	5.66±0.66 c
Cys	1.82±0.01 a	1.19±0.01 b	0.15±0.00c	EAA	32.51±0.30 a	24.91±0.10 b	2.21±0.12 c
Val*c	4.12±0.03 a	3.05±0.01 b	0.30±0.01c	UAA	47.57±0.52 a	38.42±0.11 b	3.45±0.21 c
Met*	3.24±0.04 a	2.07±0.01 b	0.21±0.01c	NEAA	33.72±0.06 a	29.21±0.07 b	2.36±0.15 c
Ile*c	3.48±0.11 a	2.20±0.01 b	0.18±0.01c	TEAA/TAA	40.60	39.33	39.05
Leu*c	6.80±0.05 a	4.35±0.01 b	0.45±0.03c	TEAA/TNEAA	68.34	62.21	64.06
Tyrd	2.74±0.04 a	1.75±0.05 b	0.20±0.01c	TUAA/TAA	42.11	46.12	41.76
Phe*d	2.76±0.05 a	1.99±0.01b	0.23±0.02c				

Note: The different small letters in same column indicate statistically significant differences ($p<0.05$).

TAA: total amino acids; * TEAA: total essential amino acids; TNEAA: total nonessential amino acids;** TUAA: total umami amino acids

It was all close to EAAI of farmed *Takifugu obscurus* (56.02)[16], and freshwater fish like mandarin fish (62.30) and grass carp (60.59)[17].

Table 3. Comparison of AAS and EAAI of farmed Takifugu obscures

EAA	FAO/WHO (1991) (g/100g protein).	AAS		
		Meat	Testis	Liver
Ile	2.8	137	94	116
Leu	6.6	113	79	123
Lys	5.8	148	148	131
Met+Cys[a]	2.5	222	156	260
Phe +Tyr[b]	6.3	96	71	123
Thr	3.4	122	106	143
Trp	1.1	54	115	230
Val	3.5	129	104	155
EAAI	-	71.72	61.32	86.02

[a] Methionine + Cysteine; [b] Phenylalanine + Tyrosine.

FAO/WHO (1991) standard of TEAA contents was 32.0 (g/100g protein)

The contents of TEAA of meat, testis and liver were 36.22, 29.61, 40.60, respectively (g/100g protein)

3.3 Minerals

Table 4. Contents of minerals in the tissues of farmed Takifugu obscures (mg/100g,dry, mean±S.D., n=3)

Mineral contents	Meat	Testis	Liver
K	1462.39±45.07[a]	1534.16±49.76[a]	102.10±11.96[b]
Na	720.81±28.22[b]	862.04±23.76[a]	89.87±8.74[c]
P	302.26±16.34[b]	466.50±4.70[a]	32.03±1.35[c]
Mg	134.79±7.26[a]	75.44±4.37[b]	8.76±0.50[c]
Ca	33.00±5.76[a]	16.63±0.87[b]	6.37±1.15[c]
Fe*	2.88±0.53[b]	6.60±1.29[a]	4.94±0.54[a]
Zn*	7.06±0.60[b]	11.88±0.96[a]	3.68±0.06[c]
Cu*	0.22±0.04[b]	0.24±0.07[b]	0.51±0.03[a]
Se*	0.12±0.00[a]	0.12±0.00[a]	0.001±0.00[b]
K/Na	1.61	1.78	1.14
Ca/P	0.11	0.04	0.20

Note: The different small letters in same column indicate statistically significant differences ($p<0.05$).

As shown in Table 4, mineral content in meat and testis was significantly higher than liver. The most abundant macroelement was K, while the most abundant microelement in meat and testis was Zn, in liver was Fe.

From table 4, three tissues were shown to be one of the good resources of minerals. The characteristic of relatively high amounts of Zn and low proportion of Ca was observed in all tissues. The Zn content in testis was 3 to 4 times higher than that in meat. Hill and Matron put forward that elements with similar properties were antagonistic to each other in its biological function. In terms of fish, the ratio of K/Na and Ca/P were maintained at a constant level. The ratio of K/Na in meat, testis and liver was all greater than 1.0. It indicates that the pufferfish was favorable for hypertension patients. However, the ratio of Ca/P was between 0.04 and 0.23. Generally speaking, the ratio of Ca/P should be less than 0.5. Otherwise, additional supplemental intake of Ca was required[8].

There has TTX in pufferfish body, especially strongest toxicity in liver (toxicity of the edible range). TTX can block Na^+ influx, this may be the reason why the Na content in liver is significantly lower. In addition, minerals involved in detoxification mechanisms, the mineral content is consequently lower the liver[10]. During the period of gonad maturation, gonad and meat will store energy for developing. Thus, mineral content is higher in the meat and testis.

4 Conclusion

In conclusion, meat and testis of farmed *Takifugu obscurus* are good source of protein. Both tissues are rich in protein and amino acids, with TEAA was as high as 80.08±0.75g/100g and 63.33 ± 0.10g/100g, respectively. The ratios of TEAA/TAA were comparable to the reference value recommended by FAO/WHO, and umami amino acids account for the high ratio of the total amino acids. Fat is rich in liver (63.86 ± 0.93g/100g). By the way, liver can be regarded as a good source of minerals, with the rich content and reasonable proportion. K and Zn were rich in meat and testis, while liver is rich in Fe. First limiting amino acid of meat and testis is Trp and Phe+Tyr. Therefore, farmed *Takifugu obscurus* is a kind of delicious and nutritious fish worth studying on the development and utilization.

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