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Amino Acid and Fatty Acid Composition of Three Strains of Chinese Soft-Shelled Turtle (*Pelodiscus sinensis*)

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HWL and GWZ conceived and designed the study and wrote the manuscript. MMT, LHC and XL performed experimental work. ZL helped in preparation of manuscript.

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Amino acids, Fatty acids, Different strains, *Pelodiscus sinensis*, Chinese soft-shelled turtle.

ABSTRACT

Chinese soft-shelled turtles (*Pelodiscus sinensis*) have been consumed as food and as a medicinal tonic in several countries. The amino acid and fatty acid profiles of muscle and calipash of Huaihe River, Yellow River, and Japanese strains of Chinese soft-shelled turtles were analyzed and compared. Calipash had higher concentrations of flavor amino acids than muscle. Muscle tissue of the Huaihe River strain had the highest concentration of pharmacodynamic amino acids and the highest concentration ($P < 0.05$) of arachidonic acid (females: 109.83 mg/ 100g, males: 110.72 mg/100 g). Concentrations of eicosapentaenoic acid and docosahexaenoic were higher in male muscle than in female muscle. Opposite results were obtained with calipash. The n-3-to-n-6 polyunsaturated fatty acid ratios were within the recommended range in all three strains. Chinese soft-shelled turtle is a healthy food source to humans.

INTRODUCTION

The Chinese soft-shelled turtle, *Pelodiscus sinensis*, belongs to the class Reptilia, the order Testudiformes, and the family Trionychidae, and is native to China, Japan, Thailand, Russia, Vietnam, and Korea (Fritz *et al*., 2010; Lee *et al*., 2006). In China, it is widely distributed through most regions, except in Qinghai and Tibet (Zhang *et al*., 2008). The Chinese soft-shelled turtle has high economical value in China because of its nutrient characteristics and medicinal properties (Lee *et al*., 2006; Li *et al*., 2008, 2011). Recently, soft-shelled turtles have been widely cultured and its production have rapidly increased in China (Zhang *et al*., 2016a, b). In 2015, the total annual production in China, mainly Zhejiang, Hubei, Jiangxi, Anhui and Guangxi provinces, was 344,529 tons (Bureau of Fisheries, Ministry of Agriculture, China, 2017).

There are different strains of Chinese soft-shelled turtles, which have unique morphology and growth characteristics (Li *et al*., 2016; Zhang *et al*., 2016a). The Japanese and Yellow River strains are the most commonly farmed soft-shelled turtles in China. The Japanese strain is widely cultured as a selective-breeding strain in Southeast China due to its rapid growth rate, adequate body shape and color (Zhang *et al*., 2016a). It was developed from the Chinese soft-shelled turtle from Japan and was certified by the Chinese Ministry of Agriculture (No.GS03-001-2007; Zhou *et al*., 2013). The Yellow River strain, which is mainly distributed in the Yellow River basin, has a yellow, large-sized body, an adequate growth rate, and an optimum productivity performance (Zhang *et al*., 2016a). The Huaihe River strain, which is native to the Huaihe River, has adequate growth rates and immune function (Cai *et al*., 2002; Liu *et al*., 2004), and is difficult to find in the wild. However, the Huaihe strain is cultured in some farms. For a sustainable industry of the Chinese soft-shelled turtle, the Huaihe River strain is an important and beneficial germplasm.

The Chinese soft-shelled turtle exhibits considerable sexual dimorphism in body size and growth. Males have higher growth rates than females, which is a bias for male turtles. Most studies have focused on the culture, production technology, and disease control of soft-shelled turtles (Hu *et al*., 2010; Wu *et al*., 2014). The nutritional composition of some strains of Chinese soft-shelled turtles has been investigated (Chen *et al*., 2015; Wang *et al*., 2015). However, no studies have evaluated the nutritional composition of the Yellow River and Huaihe River strains. An understanding of the nutritional value of these strains

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may contribute to the development of novel breeding strategies. Therefore, the objective of this study was to compare the amino acid and fatty acid composition among three strains of Chinese soft-shelled turtles (Japanese, Yellow River, and Huaihe River strains) and between male and female turtles.

MATERIALS AND METHODS

Chinese soft-shelled turtle

Thirty healthy Chinese soft-shelled turtles (two years old) were obtained from Anhui Xijia Agricultural Development Co. Ltd.: five males (580 ± 30 g) and five females (520 ± 16 g) each of Yellow River (YR), Huaihe River (HR), and Japanese (JP) strains. All turtles first received an intraperitoneal injection of pentobarbital (20 mg/kg). Then, the animals were sacrificed by cervical dislocation, and muscle in leg and calipash were removed and transferred to plastic bag. Finally, muscle and calipash samples of same weight from five turtles of different strains were pooled based on gender, respectively. All samples were stored at −40°C. All experimental procedures were performed in accordance with the standards for Animal Care of the Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences (Wuhan, China).

Proximate composition analysis

Moisture, crude protein, crude lipid, and ash content of Chinese soft-shelled turtles were analyzed using methods of the Association of Official Analytical Chemists (AOAC, 1995). Moisture was determined by measuring tissue weight difference after drying to constant weight at 105°C for 24 h. Crude protein was measured by the Kjeldahl method, using a nitrogen-to-protein conversion factor of 6.25. Crude lipid was determined by the Soxhlet method with ether as the extraction solvent. Ash content was measured by weight difference after sample incineration in a muffle furnace at 550°C for 6 h.

Amino acid analysis

Prior to amino acid analysis, muscle and calipash samples were freeze-dried and separately homogenized. Each sample was hydrolyzed with 6 mol L^{-1} HCl at 110°C for 24 h. The hydrolysates were diluted with ultrapure water, dried at 40°C under vacuum in a rotary evaporator to remove HCL, and diluted with buffer (pH 2.2). Finally, muscle and calipash samples were analyzed in an automatic amino acid analyzer (L-8800, Hitachi, Japan). Tryptophan content was not measured.

Fatty acid analysis

Fatty acid analysis of muscle and calipash samples was performed in triplicate. Fatty acid methyl esters (FAMEs) were prepared (GB/T 17376, ISO5509:2000, IDT) and analyzed by capillary gas chromatography using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 7638 series auto-sampler (Agilent, Santa Clara, CA, USA). The column consisted of an HP-FFAP (30 m × 0.25 mm, with 0.25-µm film thickness). The injector and detector temperatures were set at 260 and 280 °C, respectively. The analysis was carried out with a temperature program was 150-210 °C at a rate of 10 °C min^{-1}, 210 °C for 6 min, then 210-230 °C at a rate of 20 °C min^{-1} and 230 °C for 7 min. Nitrogen was used as the gas carrier at a flow rate of 3 ml min^{-1}. The external standard method was used to analyze the fatty acid (cholesterol as the external standard). Fatty acid was quantified by the reference to the cholesterol. Fatty acid composition was presented as mg g^{-1} total fatty acids.

Statistical analysis

All samples were measured in triplicate. Data were presented as mean ± standard deviation (SD) and analyzed by SPSS 18.0 software (Chicago, IL, USA). Differences were calculated using one-way analysis of variance (ANOVA) and Tukey's test. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of muscle and calipash samples based on sex and strain is presented in Table I. The crude protein content in muscle of HR, YR, and JP males were 18.05%, 15.86%, and 17.58%, respectively. Among female turtles, crude protein content was the highest in YR (18.49%) and lowest in HR (17.62%). Crude protein content ranged from 26.93% (JP) to 31.34% (HR) in male calipash and from 21.36% (YR) to 28.75 (JP) in female calipash. Crude lipid content ranged from 0.64% to 1.00% in male muscle, 0.28% to 0.61% in male calipash, 0.49% to 0.62% in female muscle, and 0.30% to 0.50% in female calipash. Crude lipid content was the highest in male muscle of JP (1.00%) and the lowest in male calipash of YR (0.28%). Ash content was approximately 1.00% in muscle and 0.55% in calipash independent of sex or strain.

The Chinese soft-shelled turtle has a high crude protein and a low crude lipid (Table I). The proximate composition of the Chinese soft-shelled turtle in this study was similar to the results obtained in the Florida soft-shelled turtle (*Apalone ferox*) (Wang *et al*., 2015), Qingxi Flower and Japanese strains of the Chinese soft-shelled turtle (Chen *et al*., 2015). Among the strains, muscle had a higher moisture content than calipash, which was in agreement with the results obtained in Qinxi Flower and Japanese strains (Chen *et al*., 2015). Crude protein content was higher in calipash than in muscle among strains and higher in muscle than in calipash of male and female turtles. Furthermore, calipash crude protein was higher in Chinese soft-shelled turtle than that of muscle in gibel carp (Li *et al*., 2016), longsnout catfish (Wang *et al*., 2012), or snakehead fish (Zuraini *et al*., 2006). Crude lipid content was lower in Chinese soft-shelled turtle than in gibel carp (Li *et al*., 2016) or longsnout catfish (Wang *et al*., 2012) and higher than that reported by Chen *et al*. (2015). Ash content was not different among the strains or between male and female turtles. Ash content was approximately 0.5% higher in muscle than in calipash as previously reported (Chen *et al*., 2015). The results revealed that Chinese soft-shelled turtle is high in protein and low in fat.

Table I.- Proximate composition of Chinese soft-shelled turtle strains.

Strain	Tissue	Male				Female			
		Moisture (% WW)	Crude protein (% DW)	Crude lipid (% DW)	Ash (% DW)	Moisture (% WW)	Crude protein (% DW)	Crude lipid (% DW)	Ash (% DW)
HR	Muscle	80.38±0.39	18.05±0.57	0.64±0.16	0.96±0.10	80.21±0.35	17.62±0.06	0.51±0.26	0.88±0.03
	Calipash	66.08±3.80	31.34±2.21	0.40±0.13	0.56±0.03	75.81±1.52	22.28±2.56	0.30±0.12	0.55±0.13
YR	Muscle	79.48±2.51	15.86±1.62	0.56±0.04	0.95±0.15	78.03±0.37	18.49±0.78	0.62±0.17	0.98±0.06
	Calipash	79.96±0.27	27.93±0.48	0.28±0.07	0.57±0.02	70.37±1.26	21.36±1.86	0.42±0.09	0.53±0.10
JP	Muscle	78.74±0.70	17.58±0.07	1.00±0.46	1.01±0.01	78.47±0.89	17.92±0.21	0.49±0.10	1.04±0.04
	Calipash	70.74±2.29	26.93±2.29	0.61±0.31	0.57±0.02	60.05±1.18	28.75±1.68	0.50±0.83	0.51±±0.09

HR, Huaihe River; YR, Yellow River; JP, Japanese; DW, dry weight; WW, wet weight.

Table II.- Amino acids contents (mg/100 mg dry weight) in muscle of Chinese soft-shelled turtle strains.

Amino acid	HR		YR		JP	
	Male	Female	Male	Female	Male	Female
Lysine*❖	9.22±1.52	9.22±1.70	7.77±0.31	8.21±0.65	7.83±0.34	7.68±0.04
Methionine*❖	1.56±0.27	1.46±0.23	1.53±0.20	1.78±0.33	1.72±0.31	1.66±0.16
Threonine*	3.95±0.78	3.73±0.73	3.68±0.06	4.37±0.82	3.87±0.18	3.79±0.12
Valine*	3.82±0.71	3.52±0.81	3.80±0.13	4.17±0.31	3.94±0.27	3.93±0.15
Isoleucine*	3.47±0.96	3.19±1.02	3.46±0.10	3.83±0.27	3.68±0.20	3.60±0.08
Leucine*❖	6.71±1.35	6.23±1.40	6.29±0.15	7.05±0.51	6.69±0.39	6.56±0.24
Phenylalanine*❖	4.40±0.50	4.45±0.76	4.01±0.34	3.92±0.34	3.86±0.22	3.79±0.17
Aspartic acid❖◎	7.68±1.16	7.40±0.96	7.65±0.15	7.34±0.70	8.02±0.34	7.79±0.09
Glutamic acid❖◎	13.84±1.57	13.36±0.96	12.22±0.28	13.70±1.01	12.94±0.74	12.60±0.38
Glycine❖◎	4.55±1.11	4.94±0.75	3.79±0.17	4.04±0.32	4.19±0.23	4.30±0.14
Alanine◎	4.70±1.40	4.60±1.45	4.53±0.14	5.08±0.40	4.89±0.27	4.84±0.17
Histidine*	3.60±0.47	3.65±0.72	3.30±0.33	3.16±0.26	3.08±0.21	3.17±0.08
Arginine*❖	6.33±0.95	6.80±1.07	5.04±0.12	5.72±0.63	5.19±0.26	5.17±0.14
Serine	3.58±0.74	3.53±0.63	3.37±0.05	3.86±0.66	3.55±0.15	3.50±0.08
Cystine	0.64±0.21	0.55±0.24	0.61±0.07	0.61±0.08	0.61±0.08	0.60±0.03
Tyrosine❖	2.26±1.32	2.45±0.66	2.81±0.12	2.84±0.21	2.80±0.10	2.72±0.08
Proline	4.12±0.83	4.86±1.81	3.15±0.12	3.32±0.40	3.23±0.09	3.28±0.19
Total amino acid (TAA)	84.44±9.12	83.92±4.22	77.01±1.11	83.00±6.00	80.08±3.78	78.95±1.31
Essential amino acid (EAA)	36.04±10.41	34.78±2.64	36.78±3.04	33.96±0.50	34.32±0.55	34.98±1.77
Flavour amino acid (FAA)	30.76±4.54	30.30±3.97	28.19±0.70	30.16±1.11	30.05±1.57	29.52±0.71
Pharmacodynamic amino acid (PAA)	56.54±5.53	56.30±1.92	54.60±2.83	51.11±0.58	52.25±0.92	53.24±2.59
EAA/TAA (%)	42.68	41.44	47.76	40.92	42.86	44.30
FAA/TAA (%)	36.43	36.11	36.61	36.34	37.53	37.39
PAA/TAA (%)	66.96	67.09	70.90	61.58	65.24	67.44

HR, Huaihe River; YR, Yellow River; JP, Japanese. *Essential amino acid; ❖Pharmacodynamic amino acid; ◎Flavor amino acid.

Table III.- Amino acids contents (mg/100 mg dry weight) in calipash of Chinese soft-shelled turtle strains.

Amino acid	HR		YR		JP	
	Male	Female	Male	Female	Male	Female
Lysine*❖	3.31±0.09	3.61±0.26	3.80±0.11	3.75±0.32	3.78±0.23	3.51±0.09
Methionine*❖	0.65±0.04[a]	0.78±0.03[ab]	0.81±0.06[b]	0.73±0.04[ab]	0.81±0.07[b]	0.77±0.08[ab]
Threonine*	2.25±0.07	2.54±0.17	2.77±0.58	2.72±0.27	2.63±0.02	2.64±0.07
Valine*	1.87±0.06[a]	2.02±0.03[ab]	2.11±0.19[ab]	2.23±0.19[b]	2.21±0.04[b]	2.21±0.02[b]
Isoleucine*	1.45±0.05[a]	1.56±0.03[ab]	1.63±0.12[ab]	1.70±0.13[b]	1.71±0.03[b]	1.67±0.02[b]
Leucine*❖	2.84±0.10[a]	3.10±0.09[ab]	3.24±0.28[ab]	3.40±0.30[b]	3.37±0.04[b]	3.33±0.01[ab]
Phenylalanine*❖	2.58±0.09	2.85±0.20	3.00±0.10	2.87±0.25	2.89±0.28	2.54±0.02
Aspartic acid⊚❖	5.05±0.16	5.31±0.25	5.18±0.33	5.69±0.46	5.77±0.06	5.54±0.08
Glutamic acid⊚❖	8.47±0.29	9.22±0.27	9.36±0.90	9.87±0.90	9.58±0.05	9.59±0.08
Glycine⊚❖	17.92±0.72	19.69±1.20	19.19±2.56	20.91±2.07	19.48±0.68	20.58±0.31
Alanine⊚	7.14±0.30	7.8±0.44	7.74±1.01	8.24±0.75	7.88±0.22	8.28±0.13
Histidine*	0.97±0.10	1.09±0.23	1.21±0.19	1.09±0.17	1.13±0.16	1.02±0.11
Arginine*❖	6.40±0.23	7.17±0.58	7.21±1.07	7.75±0.96	7.05±0.02	7.19±0.09
Serine	3.70±0.13	4.15±0.22	4.28±0.63	4.40±0.45	4.19±0.03	4.25±0.09
Cystine	0.46±0.01[a]	0.61±0.08[ab]	0.63±0.08[ab]	0.82±0.21[b]	0.65±0.03[ab]	0.74±0.02[b]
Tyrosine⊚	1.16±0.02[a]	1.30±0.15[ab]	1.42±0.06[ab]	1.36±0.10[ab]	1.46±0.15[b]	1.28±0.02[ab]
Proline	9.67±0.32	10.97±0.67	11.25±2.01	11.56±1.41	10.51±0.13	10.13±0.09
Total amino acid (TAA)	75.89±2.05	83.75±3.28	84.84±8.97	89.08±8.85	85.10±0.25	85.27±0.76
Essential amino acid (EAA)	16.58±0.36	18.36±0.76	19.41±1.25	19.57±1.77	19.51±0.70	18.69±0.16
Flavour amino acid (FAA)	38.58±1.46	42.02±1.64	41.47±4.16	44.71±4.13	42.70±0.90	43.99±0.43
Pharmacodynamic amino acid (PAA)	48.40±1.59	53.02±1.72	53.22±4.53	56.33±5.31	54.19±0.08	54.33±0.42
EAA/TAA (%)	21.85	21.92	22.88	21.97	22.93	21.92
FAA/TAA (%)	50.84	50.17	48.89	50.19	50.18	51.59
PAA/TAA (%)	63.78	63.31	63.73	63.24	63.68	63.72

HR, Huaihe River; YR, Yellow River; JP, Japanese. *Essential amino acid; ❖Pharmacodynamic amino acid; ⊚Flavor amino acid. [a,b]Different letters represent significant differences (P < 0.05).

Amino acids

The amino acid (AA) profiles of muscle and calipash samples of Chinese soft-shelled turtle were analyzed on a dry weight basis (Tables II, III). A total of 17 AAs were identified and quantified in muscle and calipash, nine of which were essential amino acids (EAAs). The total amino acids (TAAs) ranged from 77.01 mg/g to 84.44 mg/g in muscle and from 75.89 mg/g to 89.08 mg/g in calipash. There were no differences in the content of the 17 AAs among the three stains ($P > 0.05$); however, there were significant differences in the concentrations of six amino acids (methionine, valine, isoleucine, leucine, cystine, and tyrosine) in calipash. The concentrations of methionine, valine, isoleucine, leucine, and tyrosine in male JP were higher than those in male HR ($P < 0.05$), and the concentrations of valine, isoleucine, leucine, and cystine in female YR were higher than those in male HR ($P < 0.05$). Among the amino acids in muscle, glutamic acid was the most predominant, and cystine was the least predominant in the three strains of Chinese soft-shelled turtle. Glycine was the most prevalent amino acid in calipash.

Flavour amino acids

The nutritional value of Chinese soft-shelled turtle depends on the composition and concentrations of amino acids. The concentration of flavor amino acids (FAAs) determines taste (He *et al*., 2013; Wang *et al*., 2015). Glutamic, aspartic, glycine, and alanine, which are the main FAAs (Fuentes *et al*., 2010), were detected in muscle and calipash samples. FAAs ranged from 28.19 mg/100 mg to 30.76 mg/100 mg in muscle and from 38.58 mg/100 mg to 44.71 mg/100 mg in calipash. The FAA concentrations in calipash or muscle were not different among strains or between males and females ($P > 0.05$). However, the FAA concentrations were lower in muscle than in calipash ($P < 0.05$) and significantly different between males and females ($P < 0.05$). Glycine and alanine confer a sweet taste, and glutamic acid and aspartic acid are the most important amino acids that contribute to palatability. Among the FAAs, glutamic acid was the most predominant in muscle, and glycine was the most predominant in calipash. Furthermore, the concentration of FAAs was approximately 30% of TAAs in muscle and 40% of TAAs in calipash. These results may explain why calipash has a better taste than muscle.

Pharmacodynamic amino acids

Among the strains, nine pharmacodynamic amino acids (PAAs) were present in muscle and calipash: glutamic acid, glycine, methionine, leucine, phenylalanine, aspartic acid, tyrosine, lysine, and arginine. The concentration of PAAs in males and females muscles were 56.54 mg/100 mg and 56.30 mg/100 mg (HR strain), 54.60 mg/100 mg and 51.11 mg/100 mg (YR strain), and 52.25 mg/100 mg and 53.24 mg/100 mg (JP strain), respectively. Glutamic acid was most abundant amino acid and the highest one among all PAAs in muscle. Glycine was the most predominant amino acid in calipash. PAA concentration was the highest in HR muscle and the lowest in HR calipash. The concentration of PAAs in HR muscle was slightly higher than that in YR muscle or JP muscle. The PAA concentrations in muscle and calipash of male and female turtles were not significantly different. However, the average PAA concentration in muscle and calipash were 66.54% and 63.58% of TAA, respectively, which was higher than that of sea cucumber (46.86%; Xiang *et al.*, 2006).

Protein quality

Protein quality was determined by the amino acid composition and concentration of EAAs (Yu *et al.*, 2014). Amino acid scores (AAS) have been used to evaluate protein quality (Zhao *et al.*, 2010). The amino acid concentration was multiplied by 0.625 and compared to the AA amino acid pattern of the FAO/WHO and the AA composition of the egg. AAS and chemical scores (CS) are presented in Supplementary Tables I and II.

The nutritional value of a food is high when AAS and CS are close to 1.00. When compared to the reference amino acid pattern of FAO/WHO (1973), AAS and CS of valine, lysine, isoleucine, methionine, and cysteine in muscle were < 1.00. Phenylalanine and tryptophan had an AAS > 1.00 and a CS < 1.00. Moreover, AAS and CS of leucine were < 1.00, except for female YR (1.00). The results revealed that AAS and CS of muscle were higher than those of calipash. However, these scores were not significantly different between males and females of different strains. These results revealed that Chinese soft-shelled turtle has high quality protein, especially in muscle. Sulfur-containing amino acids (methionine and cysteine) had the lowest AAS and CS in muscle.

Table IV.- Fatty acids contents (mg/100 g dry weight) in muscle of Chinese soft-shelled turtle strains.

Fatty acid	HR		YR		JP	
	Male	Female	Male	Female	Male	Female
C14:0	21.61±4.74^{ab}	16.09±1.92^{a}	37.64±3.12^{d}	21.48±1.35^{ab}	26.61±2.69^{bc}	34.88±5.76^{cd}
C14:1	3.2±0.75	2.69±1.31	3.55±1.53	3.57±0.26	2.34±0.87	3.78±0.63
C15:1	4.88±0.02	5.97±0.99	6.36±0.24	5.67±0.36	4.34±0.92	5.73±1.37
C16:0	447.04±66.45^{ab}	371.98±30.06^{a}	583.85±35.38^{c}	405.29±25.08^{a}	448.41±30.90^{abc}	554.11±81.93^{bc}
C16:1	124.28±25.46^{a}	106.47±15.06^{a}	207.45±17.05^{b}	111.71±6.82^{a}	111.52±8.34^{a}	204.1±34.31^{b}
C17:0	9.83±2.01	8.02±0.59	9.18±0.43	9.05±0.65	7.59±0.23	8.83±1.54
C17:1	4.02±0.76	9.02±8.74	6.29±1.57	5.74±1.27	4.91±0.59	4.24±1.21
C18:0	272.93±37.69^{a}	268.07±12.56^{ab}	259.98±8.76^{ab}	213.37±10.63^{ab}	211.15±10.18^{b}	246.89±33.98^{ab}
C18:1	649.52±118.35^{ab}	574.06±47.10^{a}	969.11±46.31^{c}	596.57±25.62^{a}	566.87±39.16^{a}	862.9±158.34^{bc}
C18:2	335.02±58.69	259.43±38.92	307.80±26.52	243.08±20.83	303.03±19.04	325.41±51.25
C18:3	21.04±2.48^{ab}	17.29±3.25^{a}	26.79±3.66^{bc}	18.04±2.21^{ab}	23.73±2.93^{abc}	31.28±5.05^{c}
C20:1	35.36±5.35^{ab}	23.42±3.30^{a}	39.65±2.67^{b}	23.89±3.94^{a}	33.01±2.50^{ab}	40.54±7.96^{b}
C20:2	8.24±2.26	6.28±1.74	8.47±2.27	7.84±2.79	8.39±0.58	9.97±1.82
C20:3	9.03±1.42^{a}	7.63±0.41^{ab}	7.28±2.03^{ab}	7.73±0.51^{ab}	6.54±0.73^{ab}	5.68±1.22^{b}
C20:4	110.72±27.89^{a}	109.83±19.21^{a}	26.95±5.13^{b}	31.04±4.70^{b}	34.45±2.34^{b}	32.09±5.30^{b}
C20:5	98.71±18.45^{abc}	71.43±16.82^{a}	91.94±17.18^{ab}	96.10±15.09^{ab}	125.22±12.18^{bc}	137.17±16.99^{c}
C22:6	109.2±28.44^{ab}	67.77±16.05^{a}	106.34±16.86^{ab}	112.09±20.40^{abc}	156.79±12.99^{bc}	166.11±22.35^{c}
Saturated fatty acids (SFAs)	751.41±84.88	664.16±44.38	649.19±35.06	890.65±46.92	884.71±122.06	693.76±43.54
Monounsaturated fatty acids (MUFAs)	819.62±149.20	721.62±69.93	747.15±33.72	1232.41±61.38	1121.27±202.88	723.00±47.34
Polyunsaturated fatty acids (PUFAs)	691.95±132.26	539.66±95.11	515.93±62.70	575.57±71.61	707.70±102.47	658.15±47.59
EPA+DHA	207.91±46.81	139.20±32.87	208.20±35.36	198.28±33.61	303.28±38.74	282.01±24.02

HR, Huaihe River; YR, Yellow River; JP, Japanese; EPA, C20:5; DHA, C22:6. [a,b,c,d] Different letters represent significant differences ($P < 0.05$).

AAS of sulfur-containing amino acids of most male and female turtles was the lowest in calipash, except in YR and JP females (AAS of lysine was the lowest). Overall, sulfur-containing amino acids were the limiting amino acids in Chinese soft-shelled turtle, similar to the Florida soft-shell turtle (Chen *et al*., 2015).

Fatty acids

The fatty acid (FA) profiles of the three strains of Chinese soft-shelled turtle are shown in Tables IV and V.

The FA composition included saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). We detected 17 different FAs in muscle (four SFAs and 13 UFAs) and 18 FAs in calipash (five SFAs and 13 UFAs). Moreover, we detected fatty acid C13:0 in calipash but not in muscle and C14:0 in female calipash of the JP strain.

In muscle tissue, MUFAs had the highest concentration (894.18 mg/100 g), followed by SFAs (755.65 mg/100 g), and PUFAs (614.83 mg/100 g). Similar tendencies were observed with calipash; the average concentrations of MUFAs, SFAs, and PUFAs in calipash were 405.31 mg/100 g, 280.50 mg/100 g and 246.22 mg/100 g, respectively. Similar findings were reported by Wang *et al*. (2015). However, our results were different to those reported by Chen *et al*. (2015) in Qingxi Flower strain (SFAs were the highest) and Japanese strain of Chinese soft-shelled turtle (SFAs were the lowest). In our study, there were differences in the concentrations of SFAs, MUFAs, and PUFAs between muscle and calipash. In general, muscle had higher FA concentrations than calipash. Additionally, the concentrations of SFAs, MUFAs, and PUFAs were higher in muscle of males than in muscle of females from HR and JP strains. Opposite results were obtained for the YR strain. For calipash, the level of variation in SFAs, MUFAs, and PUFAs was consistent. FA concentrations in calipash were the highest in JP females and the lowest in HR females.

Table V.- Fatty acids contents ((mg/100 g dry weight)) in calipash of Chinese soft-shelled turtle strains.

Fatty acid	HR		YR		JP	
	Male	Female	Male	Female	Male	Female
C13:0	2.68±0.24[a]	2.46±0.26[a]	2.67±0.42[a]	1.67±0.47[a]	2.15±0.23[a]	3.02±0.35[b]
C14:0	9±3.71	7.55±1.38	6.81±0.89	7.94±2.88	8.93±6.10	23.54±7.02
C14:1	ND	ND	ND	ND	ND	2.99
C15:1	1.69±0.83	1.44±0.69	1.69±0.56	1.69±0.90	1.92±0.78	2.80±0.96
C16:0	131.16±61.38[a]	109.23±20.03[a]	161.35±15.58[a]	148.08±44.54[a]	189.33±98.78[ab]	335.89±68.34[b]
C16:1	36.52±14.96[a]	35.00±4.79[a]	44.17±5.91[a]	52.06±20.02[a]	53.38±36.54[a]	153.55±38.67[b]
C17:0	2.85±0.96[a]	3.02±0.99[a]	4.02±0.83[a]	2.82±0.39[a]	4.12±1.57[a]	7.63±2.12[b]
C17:1	3.53±0.14	2.10±0.19	4.41±3.70	6.16±5.51	6.21±2.00	4.94±0.49
C18:0	66.35±28.00	64.63±12.41	82.21±6.04	71.21±19.28	102.50±57.64	118.17±17.95
C18:1	231.97±79.29[a]	228.38±29.39[a]	270.53±45.28[a]	252.51±30.05[a]	284.85±165.68[a]	627.59±138.60[b]
C18:2	100.5±30.35[ab]	76.17±8.35[a]	117.46±15.06[ab]	86.98±30.05[a]	136.17±68.13[ab]	201.49±37.52[b]
C18:3	5.47±1.72[a]	4.29±1.02[a]	7.69±0.83[a]	5.91±1.80[a]	7.99±4.80[a]	16.94±3.43[b]
C20:1	13.76±2.53[a]	10.13±3.38[a]	14.67±2.06[a]	13.67±4.04[a]	17.67±6.09[a]	31.88±7.47[b]
C20:2	4.32±0.51	4.92±1.40	5.31±1.87	3.63±0,98	5.44±0.91	4.65±1.15
C20:3	2.15±0.23	3.55±0.67	3.45±0.57	2.47±0,93	3.27±1.54	3.4±0.67
C20:4	30.43±5.98	36.39±5.51	33.53±2.51	22.81±4.93	36.76±22.82	31.24±3.04
C20:5	12.24±6.62[a]	13.52±1.10[a]	42.31±4.57[bc]	36.68±8.56[ac]	45.76±18.86[cd]	72.78±13.45[d]
C22:6	19.55±3.66[a]	23.58±4.68[a]	34.35±5.04[a]	27.93±10.46[a]	48.43±24.55[a]	95.42±19.53[b]
Saturated fatty acids (SFAs)	212.05±93.85	186.88±34.23	257.05±23.58	231.72±66.90	307.02±163.94	488.25±94.54
Monounsaturated fatty acids (MUFAs)	287.47±100.13	277.05±37.09	355.47±51.78	326.09±123.14	364.03±209.81	821.75±184.44
Polyunsaturated fatty acids (PUFAs)	174.66±47.49	162.42±21.52	244.11±27.40	186.42±56.60	283.81±141.02	425.92±76.14
EPA+DHA	31.79±10.78	37.10±5.70	76.66±9.54	64.61±18.21	94.19±43.38	168.20±32.45

HR, Huaihe River; YR, Yellow River; JP, Japanese; EPA, C20:5; DHA, C22:6. ND: no detected. [a,b,c,d] Different letters represent significant differences ($P < 0.05$).

Oleic acid (C18:1) and palmitic acid (C16:0) were the primary and secondary SFAs, respectively, in the three strains of Chinese soft-shelled turtle. The most abundant fatty acid was oleic acid (C18:1) ranging from 228.38 mg/100 g to 627.59 mg/100 g in muscle. Additionally, female turtles had higher oleic acid concentrations ($P < 0.05$) than male turtles of the JP strain and lower oleic concentrations than the male turtles of the YR strain ($P < 0.05$). However, there were no significant differences in oleic acid concentrations between males and females of the HR strain ($P > 0.05$). Oleic acid concentration in calipash was higher in JP females than in JP males ($P < 0.05$). Palmitic acid concentrations were the highest in muscle of YR males and in calipash of JP females.

All three strains of Chinese soft-shelled turtle contained arachidonic acid (C20:4), which a precursor of prostaglandin, thromboxane, and leukotrienes (Zuraini *et al*., 2006). These compounds play important roles in blood clotting and wound healing (Zuraini *et al*., 2006). Arachidonic acid concentration was the highest ($P < 0.05$) in muscle of the HR strain (female: 109.83 mg/100 g, male: 110.72 mg/100 g), with no differences in calipash tissue ($P > 0.05$).

Eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) were the major PUFAs in Chinese soft-shelled turtle. It has been reported that EPA and DHA prevent human chronic diseases, such as coronary artery disease, cardiovascular disease, autoimmune diseases and diabetes (Leaf and Webber, 1988; Zuraini *et al*., 2006; Guvenc et al., 2017). EPA concentrations were higher in muscle of JP females than in muscle of HR females or YR males ($P<0.05$), with not significant differences in muscle EPA concentrations between males and females ($P>0.05$). Similar tendencies were observed with DHA. The concentrations of EPA and DHA were higher in muscle of males than in muscle of females. However, the results were inconsistent in calipash. DHA concentrations were higher in calipash of JP females than in calipash of HR and YR females ($P<0.05$).

The PUFA-to-SFA ratio is considered to be a useful indicator of nutritional quality (Steffens and Wirth, 2005). The Department of Health of the United Kingdom (1994) recommends a PUFA-to-SFA ratio of at least 0.45. Ratios < 0.45 may contribute to hypercholesterolemia (Santos-Silva *et al*., 2002). In our study, PUFA-to-SFA ratios in HR, YR, and JP strains were 0.87, 0.72, and 0.88 in muscle, respectively, and 0.85, 0.88, and 0.90 in calipash, respectively. Therefore, the PUFA-to-SFA ratios in muscle and calipash were > 0.45 (approximately twice the recommended value). The PUFA-to-SFA ratios obtained in this study were similar to that reported in snakehead fish (Zuraini *et al*., 2006) and higher than that reported in Florida soft-shelled turtle (Wang *et al*., 2015). The ratio was slightly different between males and females and inconsistent among the different strains. Our results revealed that the Chinese soft-shelled turtle is a healthy food source.

The n-3 PUFA-to-n-6 PUFA ratio is a good indicator of nutritional value (Steffens and Wirth, 2005; Zhao *et al*., 2010). In the human diet, the intake of n-3 fatty acids is lower than that of n-6 fatty acids (Sarma *et al*., 2013). An n-3/n-6 ratio of 0.2 to 1.6 is considered to be optimum (Osman *et al*., 2001; Stanchewa *et al*., 2010). The ratios of the three strains ranged between 0.36 and 0.83 in muscle and between 0.23 and 0.65 in calipash, which were within the recommended range. Therefore, the dietary intake of Chinese soft-shelled turtle would be beneficial to human health. Similar results have been reported in other aquatic animal (Li *et al*., 2016; Wang *et al*., 2012; Zuraini *et al*., 2006). Among the strains, ratios were higher in muscle than in calipash. The highest ratios were obtained with the JP strain, followed by the YR and HR strains. Within the JP strain, females had higher ratios than males. Opposite results were obtained for the YR and HR strains. In calipash, the ratio was higher in females than in males.

CONCLUSIONS

In this study, we analyzed and compared the amino acid and fatty acid concentrations in muscle and calipash of three strains of Chinese soft-shelled turtle. Our findings revealed that the muscle of Chinese soft-shelled turtle has high quality protein and is of better quality than that of calipash. However, calipash has better taste. Chinese soft-shelled turtle is a healthy food source to humans.

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Statement of conflict interest

Authors have declared no conflict of interest.

Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2018.50.3.1061.1069

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