Scientific refutation of traditional Chinese medicine claims about turtles

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Abstract. The Chinese turtle trade is the primary threat to endangered turtle populations throughout Asia, primarily because of the long tradition of consuming turtles in China. Practitioners of Traditional Chinese Medicine (TCM) promote nutritional and medicinal benefits from eating turtles, especially those made from hardshell species. We tested these claims by determining the nutritional value of turtle products (meat, fat and shell) in five species of geoemydid turtle, *Cuora trifasciata*, *C. mouhotii*, *Mauremys mutica*, *M. sinensis* and *Geoemyda spengleri*. Nutritional variables such as the composition of amino acids, fatty acids and mineral elements were analyzed to determine the relative nutritional quality of turtle products. Our study refutes TCM claims about products made from hardshell turtles. Alternative animal products should be substituted to obtain similar minerals, amino acids and fatty acids. Balancing the cultural use of turtles with their conservation status remains a major challenge.

Key words: Asia; Asian turtle crisis; conservation; China; Geoemydidae; nutrition; TCM; turtle trade.

Introduction

Asia has a high diversity of turtle species, but its unique fauna is facing a perilous and uncertain future. The main reason for the Asian turtle survival crisis is Chinese demand for turtle products (van Dijk et al., 2000). In China, turtles are a sought-after delicacy because of widespread popular belief, inspired by Traditional Chinese Medicine (TCM), that turtle meat or shell possesses especially nutritious or curative properties (Lau and Shi, 2000). The demand for these products has fueled a highly profitable captive breeding industry that contributes to the ongoing extirpation of China's wild turtle populations (Shi et al., 2007).

Clearly, the roots of the Chinese demand are deeply ingrained cultural practices as well as the widely held belief about the special qualities afforded hardshell turtle species by TCM. In his Compendium of Materia Medica, Li Shizhen, a noted pharmacologist in the Ming Dynasty (A.D. 1368-1644), states that, turtle helps "repair internal injury caused by overstrain, strengthen the yin and yang" and "replenish vital essence, reduce fever, clam the liver and subdue yang, soften and resolve hard masses". Some species, such as *Cuora trifasciata*, are reported to have additional properties in curing cancer and other hard-to-heal diseases (Li et al., 2000). These claims have led to high prices in markets, which increase as turtles become more and more rare. For example, in 1998 the price of *C. trifasciata* was 320 RMB/kg, but jumped dramatically to 19000 RMB/kg in 2004 (Shi, 2004). On the other hand, turtle meat has been considered as a delicious nutriment with high protein, low fat, and rich in Ca, Fe, animal gum, keratin, and vitamins, since ancient times. For example, "Turtle Bacon Belly" (a Chinese dish) is thought to have the combined flavor and nutrients of beef, mutton, pork, chicken, and fish (Tang and Li, 1999).

This study tests the widely held belief that turtle meat is somehow more nutritious than other common food items. Past research done on the nutritional value of turtles has focused primarily on softshell species (Tang et al., 1998; Niu et al., 1999; Zhan et al., 2000), while analyses of mineral content have only been done on the carapaces of a few species of hard-shell turtles (Wang et al., 1988; Wu and Zhang, 1992; Cui et al., 1997). Li et al. (2000) analyzed the composition and content of amino acids in meat of one species, but their study was based on a single individual and lacked detailed comparisons to other species. We present data on nutritional variables such as the composition and content of fatty acids, amino acids, and minerals in the meat, fat, and shell of five species (*Cuora mouhotii*, *C. trifasciata*, *Geoemyda spengleri*, *Mauremys mutica*, *M. sinensis*) in order to quantify the nutritional value of hardshell turtle products. By doing so we can provide an explicit test of TCM claims about their nutritional value.

Materials and Methods

Most turtle samples used for this study (except *C. trifasciata*) were obtained from local markets in Hainan Province, China (table 1). A turtle farm (Tunchang County,

Table 1. General information on turtles used in this study (mean ± SD).

	M. sinensis	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*
Number of individuals	6	6	3	12	6
Carapace length (mm)	159.3 ± 6.2	165.8 ± 11.0	134.0 ± 5.3	109.8 ± 7.9	108.8 ± 9.5
Body weight (g)	527.0 ± 53.6	712.5 ± 80.2	209.0 ± 10.1	100.3 ± 4.7	171.8 ± 36.3
Source	Farmed	Farmed	Farmed	Wild	Wild

Hainan Province, China; profiled by Shi and Parham, 2001) donated the samples of *C. trifasciata*. Samples of meat, fat, and shell were taken, cut into smaller pieces (approximately 1 × 1 × 1 mm), and divided into two subsamples. The first subsample was dried at 60°C for 2 d, ground to powder, and filtered with a 40-mesh screen for amino acid and mineral analysis, while the second subsample was frozen (−20°C) for fatty acid analysis.

Fatty acid was extracted from the samples with a modified Folch et al. (1957) protocol, using chloroform/methanol (2/1; v/v). Nonadecanoic acid was added as an internal standard. After methylation (NaOH/MeOH followed by HCl/MeOH), fatty acids were analyzed on a gas chromatograph (Shimadzu GC-9A) with a CP-Sil 88 column (50 m × 0.25 mm × 0.2 μm) (Raes et al., 2001). The following temperature program was used: 150°C for 2 min followed by an increase of 1.5°C/min up to 175°C, followed by an increase of 5°C /min to 215°C, and held at this temperature until C22:6 n-3 was detected.

For the amino acid analysis, samples were hydrolyzed in 6 N HCl under a vacuum at 110°C for 24 h (Blackburn, 1968). The hydrolysate was injected into an automatic amino-acid analyzer (Japanese Hitachi 835-50) equipped with an integrator. The tryptophan content was determined in a separate analysis (Hugli and Moore, 1972). The weighed samples were hydrolyzed in 5 N NaOH containing 5% $SnCl_2$ (w/v) for 20 h at 110°C. After hydrolysis, the hydrolysate was neutralized with 6 N HCl and centrifuged, after which the supernatant was subjected to derivatization, as described above.

For the mineral element analysis, samples were mixed with pure nitric acid and perchloric acid, and completely digested in an infrared oven. Once cooled, the samples were diluted to a volume of 25 ml with 2% nitric acid, and measured by iso-ion spectrometry (American Jarrel-ASH Company ICAP-9000) (AOAC, 1990).

Milligrams of essential amino acids (EAA) for every gram of protein (mg/g Pr) were calculated by the equation (amino acid content in dry matter/protein content in dry matter × 1000), and compared to the pattern of relative amino acid recommended by Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) (Jiang and Liu, 1992). Amino acid score (AAS) is used to be an indicator of the actual amounts of individual amino acids in a food, or in the diet relative to the need for the amino acid, so the closer a value is to 100, the better a food is for human beings. The equation for AAS (milligrams of EAA in each gram protein/relative EAA content in FAO/WHO/UNO protein × 100) can be found in Jiang and Liu (1992).

Results

Fatty acids (tables 2, 3)

Thirteen fatty acids were detected, including five saturated fatty acids (SFA) and eight unsaturated fatty acids (USFA). Some dietary SFA are atherogenic, and

Table 2. Composition and content of fatty acids in fat and meat samples of five species of turtle (% Fat). “-” indicates values below the threshold of detection. UFA = Unsaturated fatty acid; TFA = Total fatty acid; PUFA = Polyunsaturated fatty acid; SFA = Saturated fatty acid. The values with different superscripts in the same row indicate significant difference.

Items	Fat					Meat				
	M. sinensis	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*	*M. sinensis*	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*
Lauric acid (C12:0)	0.12 ± 0.01	0.16 ± 0.03	0.15 ± 0.02	-	-	-	-	-	-	-
Myristic acid (C14:0)	3.96 ± 0.06^{a}	3.55 ± 0.19^{a}	2.38 ± 0.20^{b}	0.51 ± 0.01^{c}	3.53 ± 0.51^{a}	5.45 ± 0.21^{a}	2.72 ± 0.16^{b}	2.09 ± 0.15^{c}	2.02 ± 0.10^{c}	3.47 ± 0.58^{b}
Myristoleic acid (C14:1)	0.20 ± 0.03	0.28 ± 0.10	0.41 ± 0.05	0.37 ± 0.05	-	0.51 ± 0.24^{b}	0.37 ± 0.06^{b}	0.35 ± 0.04^{b}	0.31 ± 0.03^{b}	0.95 ± 0.11^{a}
Palmitic acid (C16:0)	17.30 ± 0.26^{a}	19.5 ± 0.26^{b}	16.19 ± 0.12^{a}	20.36 ± 0.54^{b}	20.01 ± 0.51^{b}	24.49 ± 0.54	22.2 ± 0.77	25.26 ± 0.56	21.53 ± 1.18	24.68 ± 2.12
Palmitoleic acid (C16:1)	11.40 ± 0.45	12.9 ± 0.48	9.47 ± 0.08	8.47 ± 0.09	-	11.14 ± 0.40^{a}	10.5 ± 0.73^{a}	5.36 ± 0.30^{b}	4.85 ± 0.43^{b}	9.15 ± 2.30^{a}
Stearic acid (C18:0)	4.12 ± 0.11^{b}	5.88 ± 0.56^{a}	3.51 ± 0.05^{b}	6.46 ± 0.07^{a}	7.15 ± 0.88^{a}	10.01 ± 0.31	11.6 ± 2.03	10.36 ± 0.14	12.33 ± 0.50	15.30 ± 5.25
Oleic acid (C18:1)	38.10 ± 0.42^{c}	38.6 ± 0.43^{c}	43.93 ± 0.54^{b}	51.22 ± 0.17^{a}	35.90 ± 2.04^{c}	34.06 ± 1.26^{c}	37.0 ± 1.38^{a}	39.92 ± 0.18^{a}	38.68 ± 2.06^{a}	32.75 ± 2.60^{c}
Linoleic acid (C18:2)	9.22 ± 0.07^{a}	6.68 ± 0.18^{b}	7.29 ± 0.06^{c}	5.39 ± 0.14^{d}	8.49 ± 0.25^{e}	7.50 ± 0.21^{ab}	6.08 ± 0.13^{bc}	9.16 ± 0.32^{a}	6.53 ± 0.91^{bc}	4.92 ± 1.02^{c}
Linolenic acid (C18:3)	2.38 ± 0.17^{b}	1.10 ± 0.04^{c}	3.60 ± 0.36^{a}	0.74 ± 0.08^{c}	1.21 ± 0.19^{c}	0.62 ± 0.11	0.71 ± 0.06	-	0.06 ± 0.01	1.02 ± 0.52
Arachidic acid (C20:0)	0.10 ± 0.03	0.09 ± 0.07	0.18 ± 0.01	-	-	-	-	-	-	-
Arachidonic acid (C20:4)	0.58 ± 0.13	0.49 ± 0.05	1.84 ± 0.03	-	-	1.88 ± 0.11	1.20 ± 0.32	-	-	-
Eicosapentaenoic acid (C20:5)	1.47 ± 0.12	1.97 ± 0.38	1.16 ± 0.04	-	-	1.31 ± 0.26	1.83 ± 0.10	-	-	-
Docosahexenoic acid (C22:6)	5.39 ± 0.04	3.91 ± 0.38	1.37 ± 0.07	-	-	1.30 ± 0.32	2.61 ± 0.18	-	-	-
UFA/TFA	0.73 ± 0.003^{b}	0.69 ± 0.001^{c}	0.76 ± 0.0028^{a}	0.71 ± 0.005^{bc}	0.60 ± 0.02^{d}	0.59 ± 0.01	0.62 ± 0.01	0.59 ± 0.002	0.58 ± 0.001	0.52 ± 0.07
PUFA	19.04 ± 0.17^{a}	14.15 ± 0.78^{b}	15.26 ± 0.45^{b}	6.13 ± 0.21^{d}	9.70 ± 0.22^{c}	12.61 ± 0.57^{a}	12.43 ± 0.57^{a}	9.16 ± 0.32^{b}	6.57 ± 0.90^{c}	5.60 ± 0.92^{c}
PUFA/SFA	0.74 ± 0.03^{a}	0.49 ± 0.03^{c}	0.68 ± 0.02^{b}	0.23 ± 0.01^{e}	0.32 ± 0.01^{d}	0.32 ± 0.03^{a}	0.34 ± 0.02^{a}	0.24 ± 0.01^{b}	0.19 ± 0.05^{bc}	0.14 ± 0.06^{c}

Table 3. Composition and content of fatty acids in shells of five species of turtle (% Fat). "-" indicates values below the threshold of detection. UFA = Unsaturated fatty acid; TFA = Total fatty acid; PUFA = Polyunsaturated fatty acid; SFA = Saturated fatty acid. The values with different superscripts in the same row indicate significant difference.

Fatty acid	*M. sinensis*	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*
Lauric acid (C12:0)	-	-	-	-	-
Myristic acid (C14:0)	3.70 ± 0.15^{a}	2.64 ± 0.21^{b}	2.51 ± 0.09^{b}	3.98 ± 0.25^{a}	3.97 ± 0.29^{a}
Myristoleic acid (C14:1)	0.26 ± 0.03^{b}	0.19 ± 0.03^{c}	0.58 ± 0.05^{a}	0.28 ± 0.04^{b}	0.38 ± 0.09^{b}
Palmitic acid (C16:0)	18.63 ± 0.40^{b}	18.99 ± 0.59^{b}	20.57 ± 0.54^{b}	27.29 ± 1.08^{a}	24.90 ± 1.53^{a}
Palmitoleic acid (C16:1)	12.26 ± 0.46^{a}	12.07 ± 0.25^{ab}	8.99 ± 0.26^{c}	5.87 ± 0.29^{d}	10.90 ± 0.52^{b}
Stearic acid (C18:0)	5.36 ± 0.31^{d}	7.88 ± 0.10^{c}	5.59 ± 0.20^{d}	10.80 ± 0.56^{a}	9.34 ± 0.43^{b}
Oleic acid (C18:1)	46.09 ± 0.42^{b}	45.25 ± 0.41^{b}	49.13 ± 0.71^{a}	41.31 ± 1.00^{c}	39.53 ± 0.86^{c}
Linoleic acid (C18:2)	7.65 ± 0.13^{a}	5.69 ± 0.14^{b}	4.50 ± 0.26^{b}	4.92 ± 0.25^{b}	5.45 ± 1.26^{b}
Linolenic acid (C18:3)	0.79 ± 0.09	0.79 ± 0.08	0.68 ± 0.04	-	0.10 ± 0.02
Arachidic acid (C20:0)	0.22 ± 0.07	0.18 ± 0.02	-	-	-
Arachidonic acid (C20:4)	0.55 ± 0.02	0.44 ± 0.03	-	-	-
Eicosapentaenoic acid (C20:5)	0.54 ± 0.05	0.98 ± 0.04	-	-	-
Docosahexenoic acid (C22:6)	0.90 ± 0.10	1.38 ± 0.08	-	-	-
UFA/TFA	0.71 ± 0.00^{a}	0.69 ± 0.01^{a}	0.69 ± 0.01^{a}	0.56 ± 0.01^{b}	0.60 ± 0.01^{b}
PUFA	10.43 ± 0.30^{a}	9.28 ± 0.20^{a}	5.18 ± 0.29^{b}	4.92 ± 0.25^{b}	5.55 ± 1.26^{b}
PUFA/SFA	0.37 ± 0.02^{a}	0.31 ± 0.01^{b}	0.18 ± 0.02^{c}	0.12 ± 0.02^{e}	0.15 ± 0.06^{d}

the presence of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in the diet reduces the level of plasma low-density lipoproteins-cholesterol and also depress the high-density lipoproteins-cholesterol (Mattson and Grundy, 1985). The PUFA/SFA ratio is a very important indicator for evaluating the nutritional value of fatty acids. Research has shown that a PUFA/SFA ratio of 1.0-1.5 in the diet is within the favorable range to reduce the risk of coronary heart disease (CHD) (Kang et al., 2005). In this study, the PUFA/SFA ratio fell within a range of 0.1-0.4 for shell, 0.5-0.7 for meat and 0.2-0.8 for fat, much lower than the standard recommended by Kang et al. (2005). The levels of PUFA in prawn, eel, the Chinese softshell turtle (*Pelodiscus sinensis*), and chicken eggs were higher than the levels found in turtle meat (table 8). In terms of fatty acids, *M. sinensis* and *M. mutica*, both from farms, exhibited the highest levels relative to the other turtles, but overall, the nutritional value was lower than in other readily available products (Institute of Nutrition and Food Hygiene and Chinese Academy of Preventive Medicine, 1991).

Among the PUFAs, EPA and DHA levels have especially important biological functions. The increased intake of long-chain omega-3 fatty acids (EPA and DHA) can decrease the risk of cardiovascular disease by (1) preventing arrhythmias that can lead to sudden cardiac death, (2) decreasing the risk of thrombosis that can lead to stroke, (3) decreasing serum triglyceride levels, (4) slowing the growth of atherosclerotic plaque, (5) improving vascular endothelial function, (6) lowering blood pressure and (7) decreasing inflammation (Kris-Etherton et al., 2003). Three turtle shells studied here (*C. trifasciata*, *C. mouhotii* and *G. spengleri*) lacked EPA and DHA entirely. In this study, the EPA and DHA levels in the meat of *M. sinensis* and *M. mutica* were 2-5% (table 3), lower than those levels found in prawn and eel (table 8). Also, our study found lower fatty acid concentrations in turtle shells compared to pearl oyster meat by Diao et al. (2000; EPA and DHA levels were 12.6% and 22.1% respectively). Clearly the nutritional value of fatty acids in meat, fat and shell of turtles was lower than that found in other readily available products.

Amino acids (tables 4, 5)

The total amino acid content in meat was 70-80 g/100 g dry weight (DW; table 4). The most abundant amino acid was glutamic acid, with aspartic acid second. The total amount of flavor amino acid (FAA) was about 30 g/100 g DW, and represent about 45% of the total amino acid content. The highest total amino acid content in the shell was found in *G. spengleri* (43.44 g/100 g DW), and the lowest in *C. trifasciata* (28.57 g/100 g DW). The most abundant amino acid was glycine, followed by proline and glutamic acid. The total amount of flavor amino acids was about 20 g/100 g DW, amounting to 50% of the total amino acid content. Among the five species studied here, the levels of amino acids, essential amino acids, and flavor amino acids were significantly lower in *C. trifasciata*. In this study, the levels of total amino acid, flavor amino acid, and essential amino acid in turtle meat were higher than those in turtle shells, as expected. Compared to Najdi-camel meat, the

Table 4. Amino acid content in five species of turtle (g/100 g DW). AA = amino acid. EAA = Essential amino acids (includes Ile, Leu, Lys, Met, Cys, Phe, Tyr, Thr, Val, Trp); FAA = Flavor amino acids (includes Glu, Asp, Gly, Ala, Arg). The values with different superscripts in the same row indicate significant difference.

Amino acids	Meat					Shell				
	M. sinensis	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*	*M. sinensis*	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*
Aspartic acid (Asp)	7.31 ± 0.13^{a}	7.39 ± 0.21^{a}	5.87 ± 0.17^{b}	7.47 ± 0.06^{a}	7.20 ± 0.57^{a}	1.95 ± 0.35^{c}	2.06 ± 0.37^{bc}	1.86 ± 0.19^{c}	2.79 ± 0.08^{ab}	2.91 ± 0.09^{a}
Threonine (Thr)	3.61 ± 0.11^{a}	3.64 ± 0.18^{a}	2.77 ± 0.17^{b}	3.75 ± 0.05^{a}	3.63 ± 0.25^{a}	0.81 ± 0.03^{b}	0.92 ± 0.10^{b}	0.73 ± 0.10^{b}	1.27 ± 0.05^{a}	1.29 ± 0.03^{a}
Serine (Ser)	3.23 ± 0.16^{b}	3.42 ± 0.21^{a}	3.13 ± 0.10^{c}	3.85 ± 0.06^{a}	3.48 ± 0.27^{a}	1.37 ± 0.40^{b}	1.66 ± 0.06^{b}	1.45 ± 0.15^{b}	2.28 ± 0.08^{a}	2.18 ± 0.03^{a}
Glutamic acid (Glu)	12.33 ± 0.19	12.34 ± 0.43	11.77 ± 0.05	12.70 ± 0.08	12.43 ± 0.81	3.25 ± 0.07^{b}	3.47 ± 0.12^{b}	3.19 ± 0.05^{b}	4.64 ± 0.13^{a}	4.62 ± 0.04^{a}
Glycine (Gly)	3.62 ± 0.09^{c}	4.11 ± 0.29^{c}	8.22 ± 0.06^{a}	6.05 ± 0.14^{b}	5.76 ± 0.57^{b}	6.52 ± 0.07^{d}	7.22 ± 0.12^{c}	6.33 ± 0.08^{d}	8.74 ± 0.16^{a}	8.28 ± 0.13^{b}
Alanine (Ala)	4.28 ± 0.05^{c}	4.26 ± 0.08^{c}	5.26 ± 0.08^{a}	4.73 ± 0.03^{b}	4.45 ± 0.22^{bc}	2.58 ± 0.11^{b}	2.38 ± 0.39^{b}	2.47 ± 0.14^{b}	3.37 ± 0.09^{a}	3.46 ± 0.07^{a}
Valine (Val)	5.13 ± 0.08^{a}	4.93 ± 0.23^{a}	2.95 ± 0.05^{b}	3.66 ± 0.12^{c}	3.61 ± 0.23^{c}	1.04 ± 0.08^{ab}	1.41 ± 0.24^{a}	0.81 ± 0.06^{b}	1.24 ± 0.07^{a}	1.37 ± 0.09^{a}
Methionine (Met)	2.03 ± 0.16^{a}	1.97 ± 0.08^{a}	1.34 ± 0.08^{b}	1.94 ± 0.03^{a}	1.94 ± 0.13^{a}	0.26 ± 0.04^{b}	0.27 ± 0.03^{b}	0.20 ± 0.03^{b}	0.36 ± 0.01^{a}	0.40 ± 0.03^{a}
Isoleucine (Ile)	3.62 ± 0.07^{a}	3.56 ± 0.11^{a}	2.88 ± 0.18^{b}	3.67 ± 0.07^{a}	3.54 ± 0.25^{a}	0.57 ± 0.08^{b}	0.65 ± 0.04^{b}	0.49 ± 0.06^{b}	0.87 ± 0.04^{a}	0.94 ± 0.02^{a}
Leucine (Leu)	6.28 ± 0.06^{a}	6.30 ± 0.12^{a}	4.87 ± 0.03^{b}	6.46 ± 0.05^{a}	6.11 ± 0.40^{a}	1.28 ± 0.13^{b}	1.60 ± 0.10^{c}	1.15 ± 0.10^{b}	1.93 ± 0.10^{a}	1.97 ± 0.03^{a}
Tyrosine (Tyr)	3.05 ± 0.08^{a}	3.01 ± 0.18^{a}	2.41 ± 0.25^{b}	3.32 ± 0.06^{a}	3.00 ± 0.20^{a}	1.15 ± 0.08^{b}	2.15 ± 0.46^{a}	1.17 ± 0.20^{b}	2.39 ± 0.14^{a}	2.03 ± 0.06^{a}
Phenylalaninase (Phe)	3.40 ± 0.09^{a}	3.57 ± 0.13^{a}	2.49 ± 0.15^{b}	3.65 ± 0.04^{a}	3.54 ± 0.26^{a}	0.73 ± 0.06^{b}	0.88 ± 0.05^{b}	0.77 ± 0.08^{b}	1.17 ± 0.07^{a}	1.17 ± 0.02^{a}
Lysine (Lys)	5.96 ± 0.09^{a}	5.83 ± 0.04^{a}	5.04 ± 0.19^{b}	5.77 ± 0.09^{a}	5.35 ± 0.39^{ab}	1.04 ± 0.15^{b}	1.03 ± 0.24^{b}	0.99 ± 0.10^{b}	1.59 ± 0.04^{a}	1.57 ± 0.06^{a}
Histidine (His)	1.74 ± 0.03^{ab}	1.86 ± 0.05^{a}	1.28 ± 0.06^{c}	1.64 ± 0.01^{b}	1.47 ± 0.12^{bc}	0.37 ± 0.06^{c}	0.56 ± 0.04^{b}	0.36 ± 0.05^{c}	0.65 ± 0.01^{a}	0.63 ± 0.01^{a}
Arginine (Arg)	4.15 ± 0.13^{ab}	4.23 ± 0.09^{a}	3.30 ± 0.51^{b}	4.14 ± 0.02^{ab}	3.86 ± 0.34^{ab}	2.14 ± 0.47^{ab}	2.39 ± 0.16^{ab}	1.93 ± 0.41^{b}	3.05 ± 0.01^{a}	2.91 ± 0.06^{a}
Proline (Pro)	2.69 ± 0.09^{c}	2.86 ± 0.06^{c}	4.43 ± 0.11^{a}	4.18 ± 0.08^{a}	3.70 ± 0.23^{b}	3.22 ± 0.11^{b}	3.50 ± 0.19^{b}	3.53 ± 0.41^{b}	5.18 ± 0.23^{a}	4.56 ± 0.21^{a}
Tryptophan (Trp)	0.34 ± 0.04^{c}	0.79 ± 0.08^{a}	0.60 ± 0.07^{b}	0.54 ± 0.03^{b}	0.62 ± 0.05^{ab}	0.36 ± 0.04^{bc}	0.35 ± 0.03^{bc}	0.24 ± 0.03^{c}	0.59 ± 0.07^{a}	0.42 ± 0.01^{b}
Cysteine (Cys)	0.88 ± 0.06^{a}	0.86 ± 0.09^{a}	0.63 ± 0.07^{b}	0.87 ± 0.01^{a}	0.86 ± 0.03^{a}	0.33 ± 0.08^{ab}	0.48 ± 0.07^{ab}	0.50 ± 0.09^{b}	0.82 ± 0.04^{a}	0.61 ± 0.04^{ab}
Total of AA	73.6 ± 0.30^{ab}	74.9 ± 0.75^{ab}	69.24 ± 0.56^{b}	78.33 ± 0.65^{a}	74.5 ± 4.76^{ab}	28.97 ± 0.62^{c}	32.98 ± 2.06^{b}	28.17 ± 0.25^{c}	42.93 ± 1.14^{a}	41.32 ± 0.44^{a}
Total of EAA	31.86 ± 0.08^{a}	32.31 ± 0.41^{a}	27.48 ± 0.14^{b}	34.10 ± 0.29^{a}	32.34 ± 2.07^{a}	9.75 ± 0.21^{c}	11.83 ± 0.72^{b}	9.77 ± 0.32^{c}	16.17 ± 0.60^{a}	14.96 ± 0.19^{a}
EAA/AA	43.26 ± 0.10^{a}	43.21 ± 0.39^{a}	39.6 ± 0.16^{b}	43.53 ± 0.10^{a}	43.32 ± 0.40^{a}	33.66 ± 0.49^{c}	35.88 ± 0.10^{b}	34.67 ± 0.82^{bc}	37.65 ± 0.44^{a}	36.21 ± 0.29^{ab}
Total of FAA	31.69 ± 0.14^{a}	32.35 ± 0.64^{a}	34.4 ± 0.37^{b}	35.04 ± 0.29^{a}	33.71 ± 2.22^{a}	16.44 ± 0.17^{b}	17.52 ± 1.14^{b}	15.78 ± 0.10^{b}	22.59 ± 0.44^{a}	22.18 ± 0.27^{a}
FAA/AA	43.03 ± 0.19^{a}	43.1 ± 0.56^{ab}	49.7 ± 0.20^{ab}	44.72 ± 0.10^{b}	45.2 ± 0.38^{ab}	56.80 ± 123^{a}	53.11 ± 0.20^{b}	56.02 ± 0.34^{a}	52.64 ± 0.45^{b}	53.68 ± 0.10^{b}

Table 5. Amino acid score (AAS) of essential amino acids in five turtle species. WHO/FAO/UNO recommendations from FAO/WHO/UNO (1985).

Essential amino acid	Meat					Shell					WHO/FAO/UNO recommendation
	M. sinensis	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*	*M. sinensis*	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*	
Ile	119	115	104	118	122	36	40	47	49	61	40
Leu	118	116	100	119	120	46	56	63	62	72	70
Lys	143	137	132	135	134	47	46	69	65	73	55
Met + Cys	109	104	81	103	110	42	52	76	76	74	35
Phe + Tyr	142	141	118	150	150	79	123	124	134	137	60
Thr	119	117	100	121	125	51	56	70	72	83	40
Val	135	127	85	94	100	52	69	62	56	70	50
Trp	45	102	87	70	86	90	85	90	132	107	10

total amino acid content was about 90 g/100 g DW, obviously higher than those in turtle meat (Dawood, 1995).

The essential amino acid composition is one of the most important nutritional qualities of protein (FAO/WHO/UNO, 1985). The nutritional value of a protein depends on its amino acid composition and digestibility. AAS is widely used for evaluating the nutritional quality of protein (Iqbal et al., 2006). AAS provides a method of predicting how efficiently a food protein will be used in meeting human amino acid needs based on its amino acid composition, higher or lower AAS is not better for humans, and AAS of 100 or closer to 100 is best (Jiang and Liu, 1992). In this study, AAS of turtle shells were usually below 100, indicating low nutritional value.

Mineral elements (tables 6, 7)

Calcium and phosphorous levels in the shell of *C. trifasciata* were significantly higher than those in *M. mutica*. However, there was no significant difference in the Ca/P ratio (2-3:1) among the different species (table 6). In other cases, different species contained significantly different composition of mineral elements. For example, silicon level in the shell of *M. sinensis* was approximately three times higher than that in *C. mouhotii*. In addition, the iron content in the shell of *M. mutica* was 3-7 times higher than the other four turtles. Zinc and chromium levels in the shell of *C. trifasciata* were highest among the five species, while copper and manganese content were highest in the shell of *C. mouhotii*, and selenium in *M. sinensis*. The selenium content in the shell was only 0.5-0.8 μg/g DW, and there was no significant difference between these five turtle species.

In meat, *M. sinensis* and *M. mutica* have the highest levels of potassium, while sulphur was highest in *C. trifasciata* and *C. mouhotii*. The content of calcium in the meat of *C. trifasciata* was 7404 μg/g DW, approximately 7 times of that in *M. sinensis* and *M. mutica*. The Ca/P ratio in the meat of *M. sinensis*, *M. mutica*, *C. trifasciata*, *G. spengleri*, and *C. mouhotii* were 1:5, 1:5, 1:1, 1:1, 1:1, respectively. With regard to microelements, the content of iron, manganese and selenium were the highest in the meat of *C. trifasciata*, and lowest in *M. sinensis* (table 7).

In humans, an active calcium (Ca) pumping mechanism prevents the flooding of cells with extracellular calcium. The maintenance of both extracellular and intracellular ions at appropriate levels is critically important, and there are redundant, interacting mechanisms for control of these concentrations. The dietary intake of calcium varies markedly among individuals but usually ranges from 500-1500 mg/d. The recommended dietary allowance for calcium in adults is 1000-1500 mg/d (Goodman, 1988). In order to meet the requirement of calcium, humans should ingest sufficient food with high calcium content. The Ca content of the shells of the five species studied here was rather higher, approximately 0.2 g/g DW. However, the importance of calcium supplementation is not only based on eating foods high in calcium, but other factors affecting the uptake need to be considered. Some contributing aspects are antagonistic factors as well as the ratio of calcium to phos-

Table 6. Mineral element and content in the shell of five species of turtle (μg/g DW). The values with different superscripts in the same row indicate significant difference.

Elements	*M. sinensis*	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*
Calcium (Ca)	217400 ± 321^{a}	212000 ± 13584^{a}	223100 ± 5033^{a}	203483 ± 6053^{ab}	183867 ± 6570^{b}
Phosphorous (P)	80270 ± 850^{ab}	80840 ± 189^{ab}	83155 ± 763^{a}	77671 ± 1282^{b}	72187 ± 3268^{c}
Sodium (Na)	7078 ± 14^{a}	6185 ± 92^{b}	6069 ± 49^{b}	5359 ± 42^{c}	4648 ± 124^{d}
Silicon (Si)	7072 ± 33^{a}	6238 ± 43^{b}	2995 ± 75^{c}	2859 ± 68^{c}	2723 ± 280^{c}
Magnesium (Mg)	3407 ± 72^{a}	2657 ± 58^{b}	2876 ± 152^{b}	2781 ± 27^{b}	2685 ± 233^{b}
Sulphur (S)	2262 ± 46^{a}	2905 ± 143^{bc}	2569 ± 186^{ab}	3217 ± 51^{c}	3865 ± 214^{d}
Potassium (K)	1025 ± 41^{bc}	956 ± 16^{bc}	919 ± 75^{c}	1109 ± 53^{b}	1298 ± 51^{a}
Aluminum (Al)	504.00 ± 47.16^{a}	283.60 ± 6.93^{b}	295.10 ± 28.46^{b}	269.07 ± 5.43^{b}	243.03 ± 6.38^{b}
Zinc (Zn)	96.00 ± 14.01^{b}	94.95 ± 0.89^{b}	148.85 ± 21.44^{a}	130.84 ± 18.48^{ab}	112.83 ± 1.93^{ab}
Iron (Fe)	59.32 ± 2.92^{d}	430.00 ± 15.72^{a}	181.55 ± 16.71^{b}	153.98 ± 5.25^{bc}	126.40 ± 21.95^{c}
Manganese (Mn)	2.50 ± 0.55^{a}	3.22 ± 0.03^{ab}	2.92 ± 0.20^{ab}	4.14 ± 0.08^{b}	5.37 ± 0.56^{c}
Chromium (Cr)	0.45 ± 0.09^{ab}	0.56 ± 0.07^{ab}	0.61 ± 0.01^{a}	0.51 ± 0.04^{ab}	0.42 ± 0.04^{b}
Copper (Cu)	0.70 ± 0.04^{d}	1.40 ± 0.20^{b}	1.00 ± 0.02^{c}	1.36 ± 0.15^{b}	1.72 ± 0.01^{a}
Selenium (Se)	0.73 ± 0.03	0.70 ± 0.08	0.57 ± 0.09	0.65 ± 0.06	0.72 ± 0.07

Table 7. Mineral element content in meat of turtles and comparison with other foods (μg/g DW). Items with "*" are from Institute of Nutrition and Food Hygiene and Chinese Academy of Preventive Medicine, 1991. Cells in the table with "-" indicate that the value is below the threshold of detection. The values with different superscript in the same row indicate significant difference.

Items	*M. sinensis*	*M. mutica*	*C. trifasciata*	*G. spengleri*	*C. mouhotii*	Soft shell turtle*	Pork*	Egg*	Carp*
Potassium(K)	9454 ± 150^{a}	$9036 \pm 75a$	5251 ± 58^{b}	5342 ± 39^{b}	4974 ± 843^{b}	3030	3300	600	-
Sulphur(S)	9535 ± 209^{a}	8810 ± 72^{b}	7349 ± 174^{c}	7568 ± 167^{c}	7848 ± 391^{c}	-	-	-	-
Phosphorous(P)	5984 ± 333^{a}	5681 ± 286^{ab}	6001 ± 369^{a}	4231 ± 32^{bc}	3552 ± 954^{c}	31.5	1770	2230	-
Sodium(Na)	2720 ± 61^{a}	3365 ± 77^{a}	2870 ± 63^{a}	2544 ± 96^{a}	5537 ± 762^{b}	2528	110	730	-
Calcium(Ca)	1180 ± 33^{c}	1940 ± 91^{bc}	7404 ± 217^{a}	4198 ± 533^{b}	3265 ± 972^{b}	757	110	520	-
Silicon(Si)	1698 ± 90^{c}	2312 ± 116^{ab}	2737 ± 114^{a}	2043 ± 94^{bc}	1501 ± 442^{c}	82	-	-	-
Magnesium(Mg)	886.50 ± 61.91^{ab}	926.90 ± 25.63^{a}	887.00 ± 20.60^{ab}	987.00 ± 5.69^{a}	776.80 ± 44.55^{b}	105	190	40	-
Aluminum(Al)	148.10 ± 14.38^{b}	200.20 ± 12.43^{b}	396.50 ± 34.80^{a}	178.92 ± 4.67^{b}	154.30 ± 44.69^{b}	5.7	-	-	-
Zinc(Zn)	212.10 ± 3.90^{bc}	206.50 ± 1.16^{bc}	225.80 ± 10.17^{ab}	231.24 ± 7.07^{a}	191.70 ± 16.77^{c}	332	-	-	26.7
Iron(Fe)	119.50 ± 3.47^{c}	140.60 ± 8.5^{bc}	396.60 ± 10.57^{a}	145.35 ± 6.80^{bc}	159.70 ± 11.71^{b}	367	24	39	29.6
Manganese(Mn)	0.93 ± 0.12^{c}	1.27 ± 0.46^{bc}	4.15 ± 0.13^{a}	1.59 ± 0.13^{bc}	1.91 ± 0.21^{b}	1.4	-	-	0.72
Chromium(Cr)	0.29 ± 0.01^{a}	0.55 ± 0.05^{a}	0.62 ± 0.08^{a}	0.44 ± 0.09^{a}	1.91 ± 0.40^{b}	0.7	-	-	0.05
Copper(Cu)	5.37 ± 0.07^{a}	2.90 ± 0.05^{bc}	2.69 ± 0.22^{c}	2.45 ± 0.34^{c}	3.66 ± 0.41^{b}	6.5	-	-	1.45
Selenium(Se)	1.34 ± 0.07^{c}	1.78 ± 0.15^{bc}	3.16 ± 0.07^{a}	1.95 ± 0.17^{b}	2.30 ± 0.29^{b}	5.4	-	-	2.51

Table 8. The PUFA content among other foods (%). Items with "*" were cited from the literature (Institute of Nutrition and Food Hygiene and Chinese Academy of Preventive Medicine, 1991); "-" indicates no available data.

Item	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$	$C_{20:5}$ (EPA)	$C_{22:6}$ (DHA)	Total
Chinese soft-shelled turtle*	9.3	4.9	-	1.4	-	15.6
Prawn*	9.0	4.2	-	6.6	4.0	23.8
Eel*	1.9	4.1	1.1	2.6	6.2	15.9
Egg*	14.2	0.1	0.6	-	-	14.9
Pork*	10.3	0.9	0.2	-	-	11.4

phorus (Ca/P). Researches have demonstrated that the optimal ratio of calcium to phosphorus for calcium uptake is 2-1:1 for humans (Chen and Lu, 1989). In the study, the Ca/P ratio in the meat of *M. sinensis* and *M. mutica* was 1:5, resulting in poor uptake. In contrast, the calcium found in the meat of *C. trifasciata* was easily assimilated because of its high content and its optimal Ca/P ratio. Therefore, *C. trifasciata* meat appears to be a good source of calcium, but not when taking cost into account. Due to the rarity and high demand, *C. trifasciata* products sell for USD 2375/kg (Shi, 2004). Cheaper alternatives with similar or better calcium levels exist, such as oral calcium additive or certain vegetables and seafoods.

There is a great deal of evidence indicating that selenium supplementation at high levels reduces the incidence of cancer in animals; more than 60 studies in 20 different animal models of spontaneous, viral, and chemically induced cancers found that selenium supplementation significantly reduced tumor incidence (Rayman and Clark, 2000). Selenium deficiency is a problem in China (Ellis and Salt, 2003) and has often been associated with heart disease, impaired function of the immune system, and enhancement of the virulence or progression of some viral infections (Chen and Lu, 1989; Combs, 1994). Practitioners of TCM have used claims of high selenium to promote the use of turtle products (Li et al., 2000). In our study, selenium levels in the shells of five turtles (0.58-0.73 µg/g DW) were higher than that found in *M. reevesii* (0.15 µg/g DW; Cui et al., 1997), a turtle reputed to be especially healthful by TCM because of its high selenium. The selenium content of turtle meat was the highest in *C. trifasciata*, but this was still less than that found in oysters (3.18 µg/g DW; Wang, 1991) and much lower than found in softshell turtles (5.4 µg/g DW; Institute of Nutrition and Food Hygiene and Chinese Academy of Preventive Medicine, 1991). Clearly, these results do not support the TCM claims that the selenium content of hardshell turtles (especially *M. reevesii* and C. *trifasciata*) is a reason to eat these endangered species (Li et al., 2000).

Discussion

In China, there are approximately 30 indigenous turtle species, among which three species are presumed extinct in the wild (Zhao, 1998; van Dijk et al., 2000). Once extremely common and widespread species, such as *Mauremys reevesii* and

Pelodiscus sinensis, are now very difficult to find in the field (Lau and Shi, 2000) while *Cuora trifasciata* (CITES appendix II) is critically endangered, and still faces intense and targeted harvesting pressure (Shi, 2004). The main reason for the decline of Chinese turtles is that turtles are widely eaten throughout China, fueling a massive trade that threatens all of Asia's turtles (van Dijk et al., 2000; Shi et al., 2007). The impetus behind the demand is that turtles are widely regarded as a delicacy that confers nutritional or medicinal benefits to the consumer.

The nutritional value of fatty acids levels of turtles was much lower than levels found in crab and shellfish (Institute of Nutrition and Food Hygiene and Chinese Academy of Preventive Medicine, 1991; Chen et al., 2006). Moreover, the amino acid scores of essential amino acids found in turtle shell were far from 100, indicating that the amino acids found in turtle shell were difficult for humans to assimilate (FAO/WHO/UNO, 1985). Also, the ratio of calcium to phosphorus in the meat of *M. sinensis* and *M. mutica* was 1:5, which differed greatly from what is required by the human body (Chen and Lu, 1989). In general the selenium content of turtles was not very high as TCM claims.

Practitioners of TCM do not attempt to test the veracity of their own claims. As result, almost all of their recommendations lack an empirical and rational foundation (Zhang, 2006). Nevertheless, turtle jelly, made from the ground up shells of endangered species, has become popular in Hong Kong and several chain stores specializing in this expensive "health food" have opened in the past decade. Our study shows that, where nutritional composition and content are concerned, the human consumption of turtles could be completely substituted by cheaper domestic animals, aquatic animals, or mineral supplements. All of these are widely available in China nowadays, particularly to those able to afford consuming turtles.

The large-scale consumption of turtle products results, in part, from false claims about the nutritional value of turtles. Combating a faith-based misconception with science is an uphill endeavor, but when practitioners of TCM make scientifically testable claims we should be ready to test them in a repeatable framework. Similarly, we openly encourage additional chemical, pharmacological and clinical tests by others to confirm or refute our results. Ideally we would like to see additional studies on other outstanding and potentially false claims of health benefits from using wildlife products made from endangered species.

Conclusion

The main reason for Asian turtle survival crisis is the Chinese demand for turtle products (van Dijk et al., 2000). This demand is fueled by deeply held cultural beliefs, but is promoted by TCM claims of nutritional benefits (Shi, 2004; Li et al., 2000). Our study shows that the same (or better) nutritional benefits of turtles can be obtained with cheaper, common, and less-endangered food sources such as domestic animals. Given the financial and environmental cost of using turtle products, other options for obtaining the same nutrition should be promoted. Future challenges involve additional testing of TCM claims as well as balancing cultural practices with sustaining biodiversity.

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