

Trace Elements in Cultured Tilapia (*Oreochromis mossambicus*): Results from a Farm in Southern Taiwan

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Received: 20 August 2004/Accepted: 2 November 2004

Fish consumption has been increasing dramatically since 1930s in Taiwan (THA 1998). The production of fishery industry is increasing annually at a rate of about 5% since 1960s (TFA 2002). Currently, the average daily intake of fish by Taiwanese is about 100 g (i.e. 3/week) (THA 1998), which can be defined as heavy fish consumers (Denis et al. 1997; US EPA 1998). In order to satisfy the seafood demand, a fish culture industry based on the culture of fish fry in floating cages has been expanding rapidly during the last several decades. Tilapia is introduced into Taiwan fishery farms in 1966 (Liu 2004); nowadays, the annual production is about 52,000 metric tons and its culture area is more three-tenths of the total fishery farms in Taiwan (Liu 2004). In addition, tilapia grows rapidly and can be harvested in six months, and her filet is also widely favored by Taiwanese. Thus, tilapia is the most important source of fish protein for Taiwanese. Although the benefits of eating fish are well known, the potential health risk owing to the consumption of contaminated fish attracts some concerns recently (Hites et al. 2004). An elevated cancer risk of 5×10^{-4} , because of eating coastal-farmed oysters, is reported (Han et al. 2000) despite of controversies on their assumption of oyster consumption behaviors (Lin and Sun 2001). The health risk regarding consumption of tilapia in arsenic contaminated areas in Taiwan is also estimated between $10^{-6} \sim 10^{-4}$ (Liao and Ling 2003). However, the fish samples analyzed are purchased from market places in these studies (Han et al. 2000; Liao and Ling 2003), which provide very little information about the fish analyzed, for example, where fish grew. In addition, only few metals (Cu, Zn, Cd and As) are studied; and information on other trace elements in fish is scarce in Taiwan. This study aims to evaluate the trace element levels in cultured tilapia from an uncontaminated area in Taiwan.

MATERIALS AND METHODS

A total of 55 tilapia were collected from four floating cages in a fishery farm located at the Southern Taiwan (Pentung County) in March 2003. The ages of these fish were estimated ranging from three to six months. The tilapia were weighed and then classified to ten groups (Table 1). Muscle of five or six similar size fish was pooled together to one sample, and then grounded and refrigerated in a high-density polyethylene bottle for further analysis. Crude lipid fraction of

Table 1. General information on tilapia samples.

Cages	Group	N	Body Weight (g)	Body Length (cm)	Moisture (%)	Crude Lipid in Muscle (%)
A	1	6	61.5±2.7	16.1±1.0	22.6±0.3	3.8±0.2
	2	6	67.3±1.0	16.6±1.2	23±0.5	4.4±0.3
	3	5	70.8±1.1	16.3±0.8	22.7±0.9	4.7±0.1
B	4	5	112.8±4.1	19.8±0.8	22.7±0.9	5.4±0.2
	5	6	123.3±1.6	20.3±0.8	21.4±0.5	3.4±0.2
	6	5	132±2.4	20.9±1.1	20.1±1.0	2.7±0.3
C	7	6	177±2.1	23.7±1.0	21.4±1.8	2.4±0.2
	8	6	186.7±1.0	23.6±0.5	21.5±1.3	6.5±0.7
	9	5	199.6±3.3	24.0±0.2	18.5±0.5	0.8±0.4
D	10	5	236±5.5	24.7±0.4	25.1±0.6	5.0±0.2

fish muscle was determined three times following the method published by AOAC (1995). The procedure as follows: Na_2SO_4 (Analytical grade, Sigma) was used to remove moisture from fish sample, the sample was then refluxed at 60 ~ 68°C for four hours with ethyl ether (Analytical grade, Sigma). Finally, the sample was dried at 98 ~ 100°C for another 60 minutes before weighed. Approximately one gram of tilapia muscle sample was taken and digested with 10 mL of trace-metal free grade nitric acid (J.T. Baker) in microwave digestion bombs (CEM, MDS2000) whose operating conditions were controlled as follows: heating/digestion time: 25 minutes; pressure: 120 psi. The digested samples were then diluted to 25 mL with Milli-Q water. Every digestion batch included one blank sample to minimize possible contamination from reagents and containers. All samples were analyzed three times by using a Perkin-Elmer Elan 5000 Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). The operation conditions were as follows: 1) Carrier gas (argon, 99.999%): 0.8 L/min; 2) Plasma gas (argon, 99.999%): 15 L/min; 3) Auxiliary gas (argon, 99.999%): 0.8 L/min; 4) Pump rate: 1.5 mL/min; 5) Power: 1055 KW. The instrument limits of detection of V, Cr, Mn, Cu, Ni, Zn, As, Se, Ag, Cd and Pb were 0.75, 39.4, 0.48, 2.8, 8.7, 10.5, 3.4, 32.5, 0.73, 8.7 and 19.5 $\mu\text{g/L}$, respectively.

RESULTS AND DISCUSSION

The average concentrations of trace elements in tilapia muscle are presented in Table 2. Our results agree with the reported trace element levels in tilapia (Allinson et al. 2002; Bu-Olayan and Al-Yakoob 1998; Bu-Olayan and Subrahmanyam 1996; Kairu 1999; Rashed 2001a; Rashed 2001b; Wong et al. 2001). The correlation between these metals in muscle tissue was analyzed by using a simple regression model and the results show that only the relationships between Cr and Zn ($R^2 = 0.54$), Mn and Ni ($R^2 = 0.59$), Cd and Zn ($R^2 = 0.48$), Pb and Zn ($R^2 = 0.54$) are significant ($p < 0.05$).

Table 2. Trace element concentration in tilapia ($\mu\text{g/kg}$ wet wt.)

Group	V	Cr	Mn	Cu	Ni	Zn	As	Se	Ag	Cd	Pb
1.00	15.36	1341.24	252.75	1148.12	111.48	12427.38	156.56	406.70	1.78	1.06	34.69
2.00	14.85	1250.23	913.73	1567.71	179.71	17794.52	254.66	346.39	4.64	54.71	535.85
3.00	6.92	497.87	132.29	1304.73	72.26	10779.73	136.57	396.10	3.17	1.91	48.48
4.00	18.07	429.82	209.03	1003.16	93.73	14064.84	194.00	444.00	1.89	7.41	524.04
5.00	12.85	645.05	175.86	5009.80	206.43	10935.11	154.23	344.83	2.46	11.28	71.55
6.00	28.27	459.13	345.67	818.37	209.86	10486.85	189.91	394.84	3.39	3.32	110.20
7.00	11.32	349.13	360.68	519.44	63.16	8191.17	483.47	498.72	3.03	6.74	116.14
8.00	11.29	272.52	1618.15	1005.26	314.36	8926.61	715.34	418.02	7.04	9.26	137.63
9.00	10.67	280.39	178.23	410.86	54.54	6225.13	679.70	447.72	10.04	4.44	70.40
10.00	15.84	446.46	318.02	652.86	105.38	8036.58	226.96	536.19	14.00	10.30	187.02
Average	14.54	597.19	450.44	1344.03	141.09	10786.79	319.14	423.35	5.15	11.04	183.60
Standard Deviation	5.78	384.32	467.00	1335.98	83.80	3353.73	222.60	60.90	4.04	15.74	187.93

Table 3. MRL, RfD, limits of drinking water of trace elements and their estimated daily intake from tilapia

	V	Cr	Mn	Cu	Ni	Zn	As	Se	Ag	Cd
MRL ($\mu\text{g/kg/day}$)	3			20		300	0.3			0.2
RfD ($\mu\text{g/kg/day}$)		3	140		20	300	0.3			0.5
Drinking water ($\mu\text{g/L}$)		100	50	1300	40		10	50	100	
Daily RfD (μg)*		195	9100		1300	19500	19.5			32.5
Estimated daily intake (μg)	1.5	59.7	45.0	134.4	14.1	1078.7	31.9	42.3	0.5	1.1

* Assuming average adult body weight = 65 kg.

Trace elements, which are deemed essential, can be regulated by freshwater fish (Chapman et al. 1996), thus their levels in fish muscle would maintain at constant internal concentrations and do not increase with age. Results reported by Allinson et al. (2002) seems to support this argument because no statistically significant correlations between tilapia length and metals (i.e. As, Ca, Cd, Co, Cu, Fe, Hg, K, Mg, Mn, Na, Sr, and Zn). Our results show a negative correlation ($p < 0.05$) between Cr and tilapia growth ($R^2 = 0.51$ for tilapia weight, $R^2 = 0.57$ for tilapia length), Zn and tilapia growth ($R^2 = 0.60$ for tilapia weight, $R^2 = 0.59$ for tilapia length). It implies that the regulation of Cr and Zn by juvenile tilapia is not as well as adult tilapia. By contrast, the levels of As, Se, and Ag will increase with age. The correlations (R^2) between Se, Ag, and tilapia weight are 0.47 and 0.60, respectively; and those between As, Ag and tilapia length are 0.43 and 0.47, respectively. These findings indicate that an increase of As, Se, and Ag in tilapia muscle with tilapia age could occur. The phenomenon suggests that As, Se and Ag might not act exactly as essential elements in tilapia. The other reason for this disparity may be that the tilapia studied here was cultured in coastal floating cages rather than freshwaters.

The minimal risk levels (MRL), orally reference doses (RfD) and the limits of drinking water of some trace elements complied by US ATSDR are listed in Table 3. The estimated daily uptake of these elements, based on an average daily consumption rate of 100 grams fish muscle, is shown in Table 3. These results show that the daily uptake of toxic metals from fish cultured in uncontaminated areas is much below the MRL or RfD with the exception of As. Among these elements, Cr(III), Mn, Cu, Zn and Se are also deemed essential to human beings (Sizer and Whitney 2003). The adequate daily intakes (AI) of Cr and Mn are 30 and 2000 μg ; and the recommended daily dietary allowances (RDA) of Se, Cu and Zn are 50 μg , 900 μg and 10 mg, respectively (Sizer and Whitney 2003). Thus, the consumption of tilapia would already supply enough amounts of Cr and Se, while the supplies of Mn, Cu and Zn are only one-tenth to one-fourth of their AI or RDA. The public health impact owing to inorganic arsenic in seafood has been attracting some concern in Taiwan (Han et al. 2000; Liao and Ling 2003). Exposure to inorganic arsenic can elevate the risk of lung cancer, skin cancer, bladder cancer, liver cancer, kidney cancer and prostate cancer (ATSDR 2000). The carcinogenic slope factor for ingested inorganic arsenic is 1.5 (mg/kg/day)⁻¹ (ATSDR 2000). Assuming 10% of total As in fish muscle is in inorganic form (Edmonds and Francesconi 1993), the typical risk of cancer owing to consumption of tilapia is estimated as 4.8×10^{-3} . This study clearly calls for a detailed health impact assessment with respect to fish consumption, in particular, the arsenic species in fish tissue in Taiwan.

Acknowledgments. We acknowledge the partial financial support for this work provided by Taiwan National Science Council (Grant #: NSC90-2113-M-264-002; NSC91-2113-M-264-002)

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