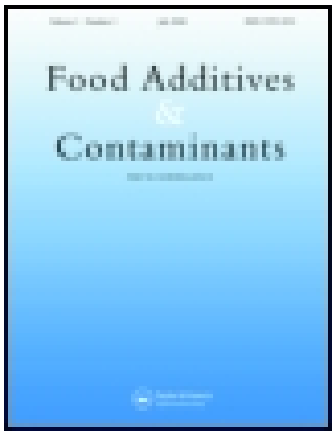

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Trace Elements In Thunnus Thynnus From Mediterranean Sea: Benefit-Risk Assessment For Consumer

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TRACE ELEMENTS IN THUNNUS THYNNUS FROM MEDITERRANEAN SEA AND BENEFIT-RISK ASSESSMENT FOR CONSUMERS

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Abstract

Trace elemental levels were determined by inductively coupled plasma-mass spectrometry in muscle, eggs and sperm of 23 Thunnus thynnus fishes collected from May to August 2013 in the Mediterranean Sea. Zn, Mn, Fe, Cu, Cr, Ni and Se content was compared with Recommended Daily Allowances. Cd, Hg and Pb concentrations were below the maximum limits fixed by the European Legislation. Tuna food safety was evaluated considering Tolerable Weekly Intake or Provisional Tolerable Weekly Intake for As, Hg, Cd and Pb. Only $BMDL_{01}$ data for As and Pb were calculated as established by the Joint FAO/WHO Expert Committed on Food Additives. The daily consumption of fresh tuna ensures a good intake of these elements. None of the tested samples surpassed the European maximum

limits. Cd, Hg and Pb remained within safety margins, while As is slightly higher than the provisional tolerable weekly intake.

Keywords: heavy metals, trace elements, $BMDL_{01}$

INTRODUCTION

Risk benefit assessment is based on the food in total and in particular by the fact that certain foods or food components are of primary importance or at least highly recommended for the health of the population. Elaborated by the European Food Safety Authority (EFSA, 2005), the benefit-risk assessment provides answers based on scientific evidence on the benefit derived from the assumption of a food and on the risk associated with exposure to a particular contaminant in its content. However, there are some factors that may affect this benefit as for fish: nutritionists recommend eating fish as a source of nutrients of high biological value, such as omega-3 fatty acids. Toxicologists recommend caution because fish is an important source of exposure to many contaminants.

Fish represents a powerful model for risk-benefit assessment. In fact, while fish consumption is recommended in balanced diets for all population, on the contrary the aquatic environment can be variously contaminated by anthropic substances present in the water itself and/or in the sediments that can sometimes become concentrated in fish tissues, raising alarm for the safety of the consumer (Loring et al., 2011).

Bluefin tuna, *Thnunnus thynnus* L. 1758, is a long-living and fast-growing pelagic species with spawning migratory behaviour (Vizzini et al., 2010). Bluefin tuna belongs to Scombridae family, a native species of subtropical regions of the Atlantic Ocean, Mediterranean and Black Sea. Worldwide,

bluefin tunas are classified in 3 types: the western Atlantic, the Pacific, the eastern Atlantic ocean and the Mediterranean sea (Majkosky et al., 2007). Eastern Atlantic tunas enter in the Mediterranean sea, where their spawning ground is restricted through the Strait of Gibraltar (Rey et al., 1999). During the migratory session bluefin tuna feed intensively with different nutrients and aquatic species. Bluefin tuna reproduction in the Mediterranean Sea is between May and June, when the tunas reach their spawning grounds, located between Sardinia and Sicily, but also in the Ionian Sea and in central and southern Adriatic.

The high worldwide demand for giant Mediterrran bluefin tuna and the migratory behaviour of the fish dictate careful international management of this species. Japan is the largest importer of tuna from the Mediterranean Sea, including meat, as it contains high levels of fat and is perfect for preparing sushi and sashimi. The edible muscular tissue of tuna species is frequently identifiable by location, muscular structure, lipid content and colour (Nakamura et al., 2005). The most valuable part of muscular structure is produced from the region around the abdominal cavity of the fish. Furthermore some parts of the internal organs of tuna, like eggs and sperm have considerable importance. These products, after being salted and dried through conventional methods, are consumed by different Mediterranean countries as an appetizer or as a sauce for pasta. The muscular tissue, eggs and sperm are an important reservoir for trace elements in bluefin tuna (Licata et al., 2005). Trace elements in tuna are Zn, Mn, Fe, Cu, Cr, Ni, Se and Hg, Cd, Pb and As. In particular the latter can be hazardous contaminants.

The purpose of this work is to estimate trace element levels by ICP-MS in tuna

samples and to compare Zn, Mn, Fe, Cu, Cr, Ni and Se content with Recommended Daily Allowance (RDA) values. To ascertain Cd, Hg and Pb concentrations are below the maximum limits as set by European Legislation (European Commission, 2006) and finally, to evaluate tuna food safety considering Tolerable Weekly Intake (TWI) or Provisional Tolerable Weekly Intake (PTWI) for As, Hg, Cd, Pb and BM-DL 0.1 values as established by the Joint FAO/WHO Expert Committee on Food Additives (WHO, 2006; EFSA, 2009) for As and Pb.

MATERIALS AND METHODS

Sample collection

A number of 23 bluefin tuna fishes (13 males and 10 females) were captured from May to August 2013 in the Mediterranean Sea by fishing boat. The specimens had length from 200 to 280 cm and weight from 130 to 290 Kg. From each sample an approximate amount of about 2-3 Kg of muscle tissue from the region around abdominal cavity was taken. From males a small amount of sperm was taken, as well as some eggs from females. Once in the laboratory, samples were frozen at -20°C and stored until analysis.

Instrumentation

Samples were mineralized by means of a closed-vessel microwave digestion system (Ethos 1, Milestone, Bergamo, Italy) equipped with sensors for temperature and pressure control and provided with PTFE vessels capable of withstanding pressures of up to 110 bar. Trace elements in muscle, eggs and fish sperm samples were determined with an Agilent 7500cx (Agilent

Technologies, Santa Clara, CA) ICP-MS spectrometer powered by a 27.12 MHz radiofrequency solid-state generator at 1500W, equipped with a MicroMist glass concentric pneumatic nebulizer coupling with a cooled Scott double pass type spray chamber made of quartz. The ICP torch was a classic Fassel-type with wide diameter (2.5 mm) fitted with a shield torch system. Nickel sampler and skimmer cones of 1.0 mm and 0.4 mm were used. An octopole collision/reaction system with helium gas to minimize polyatomic interferences resulting from plasma and matrix was used (Potortì et al., 2013).

Chemicals and standard solutions

Argon (99.9990%) and helium (99.9995%) gas were supplied by Rivoira gases (Rivoira S.p.A., Milan, Italy). Nitric acid 69% v/v (J.T. Baker, Milan, Italy) and hydrogen peroxide 30% v/v (J.T. Baker, Milan, Italy) were used to digest samples. High purity water with resistivity of 10 MΩ cm (J.T. Baker, Milan, Italy) was used throughout.

Stock standard solutions (1000 mgL^{-1} in 2% nitric acid) of Fe, Zn, Cr, Ni, Cu, Se, Pb and As were purchased from Fluka (Milan, Italy) and Hg, Mn and Cd from Merck (Darmstadt, Germany). Before use, glassware was washed with 5% HNO_3 for at least 12 h, rinsed with ultra-pure water and dried. Also, Re solution of 1000 mgL^{-1} in 2% nitric acid was acquired by Fluka (Milan, Italy) and was used at a concentration of 0.8 mgL^{-1} to verify the digestion of samples and to correct volumetric changes. Stock standard solutions of Sc, Rh and Bi (1000 $mg{\cdot}L^{-1}$ in 2% nitric acid) were purchased from Fluka (Milan, Italy) and used as on-line internal standards (at 1 $mg\ L^{-1}$) to correct instrumental drift and variations due to the matrix.

To tune the instrument, an ICP-MS tuning solution containing 1mg L^{-}

^{1}of ^{7}Li, ^{59}Co, ^{80}Y and ^{205}Tl in 2% HNO_3 was obtained from Agilent (Santa Clara, CA, USA). Standard solutions were prepared from 1000 mg L^{-1} stock solutions of Cr, Mn, Ni, Se, As, Cd, Pb, Hg, Cu, Fe and Zn by adequate dilution with high purity water and used for external calibration.

Fish tissue IAEA-407 (International Atomic Energy Agency, IAEA, Vienna, Austria) was used as certified reference material used for method validation.

Sample preparation

To approximately 0.5 g of muscle, egg and sperm sample, 1 mL of internal standard was added and then digested with 8 mL of HNO_3 (69%, v/v) and 2 mL of H_2O_2 (30%, v/v) in acid-prewashed PTFE vessels. Mineralization was carried out as follows: temperature was increased to 200 °C (5 min hold) for 10 min, and then to 200 °C (5 min hold) for 5 min. After cooling down to room temperature, the digested samples were weighed, quantitatively transferred into pre-cleaned 50 mL volumetric flasks, diluted to mark using deionized water and stored at 4°C. The certified reference material was digested under the same conditions. All determinations were carried out in triplicate.

ICP-MS analysis

The instrument was tuned to achieve the best compromise between higher intensities and lower yields of oxide ions and of doubly charged ions. The ICP-MS conditions were reported in table 1. The instrument is operated in no gas-mode for isotopes Mn, Hg, Pb and Zn and in helium mode for isotopes Cr, Ni, As, Se Cd, Fe and Cu, to remove spectral interferences. As on-line internal standards were used: ^{45}Sc for Cr, Mn, Fe, Ni, Cu, Zn, As, Se; ^{103}Rh for Cd; ^{209}Bi for Hg and Pb. Integration times were 1s/point for Hg, 0.5s/point for As, Cr, Cu, Ni and Se, while 0.1s/point for other elements.

To integrate the peaks, 3 point for each mass and 3 replicate acquisitions were taken.

The effects of $^{40}Ar^{35}Cl$ on ^{75}As, and of $^{44}Ca^{16}O$ and $^{43}Ca^{16}OH$ on ^{60}Ni were checked and the interferences were corrected by elemental interference equations. Also, the isotopic variability in Pb was corrected by elemental interference equation. These equations are reported in various EPA methods (1994) and applied by the instrument software (ICP-MS Chem Station B.03.07) and contain the naturally occurring isotope ratios of elements and allow the subtraction of isobaric or polyatomic interferences.

Statistical method

All statistical calculations were made by SPSS 13.0 software for Windows (SPSS Inc., Chicago, IL, USA). Statistical methods were conducted on two datasets, the former relating to tuna muscle results and the latter relating to gametes results. For both, the starting multivariate matrix was constituted by 23 cases (the different analyzed samples) and 8 variables (Fe, Zn, Cu, Se, Mn, Cr, Hg and As concentrations in analyzed samples). Ni, Cd and Pb were not considered because only few results were above LOQ. Element concentrations were $\log_e$-transformed to reduce the effect of outliers on skewing the data distribution and to bring elemental concentrations within the same range (Škrbic et al., 2010). The datasets were subdivided into groups according to gender or weight of the specimens. The Mann-Whitney U Test was applied to check for significance of differences on elemental concentrations.

RESULTS AND DISCUSSION

Method validation

Comparison between certified and measured data is reported in table 2. Table 3 summarizes the data on linearity, sensitivity, accuracy and precision values of the analytical method employed. The evaluation of the linearity was based on 6 injections of the 4 standard solutions. Good linearity was observed in each concentration range, with R^2=0.9987. The limits of detection (LODs) and of quantification (LOQs) were experimentally calculated as 3.3σ/b and 10σ/b respectively, where σ is the standard deviation of the response of the blanks and b is the slope of the calibration curve (EURACHEM, 2000). LOD values ranged from 0.006 to 0.090 mg/kg, while LOQ values ranged from 0.020 to 0.295 mg/kg. Accuracy and precision were assessed evaluating 6 determinations of the certified reference material. Accuracy was reported as the percent recovery between the value found with the calibration curve and the true value reported in the certified reference material and was between 78,1 and 101,5%. Precision, estimated as Relative Standard Deviation (RSD), was lower or equal to 4,8%.

Trace elements in tuna fish

Concentration ranges and mean values ± SD (mg kg^{-1} ww) of each element in gamete and muscle samples of *Thunnus thynnus* from Mediterranean Sea were reported in table 4, where for convenience the elements were divided into toxic and non toxic ones. European legislation (European Commission 2008) established maximum levels for Pb, Cd and Hg, with values set at 0.3 mg kg^{-1} ww, 0.1 mg kg^{-1} ww and 1 mg kg^{-1} ww, respectively. No tested sample exceeded these limits.

The results provide evidence that in all muscle samples the highest non

toxic metal concentrations were those of Zn (1.983-57.526 mg kg^{-1}), Fe (3.649-21.138 mg kg^{-1}), Se (0.270-1.207 mg kg^{-1}) and Cu (0.158-0.684 mg kg^{-1}), while the highest toxic metal concentrations were those of As (1.336-5.527 mg kg^{-1}) and Hg (0.246-0.714 mg kg^{-1}). The highest non toxic elemental concentrations in eggs were again for Zn (19.465-170.884 mg kg^{-1}), Fe (22.933-102.124 mg kg^{-1}), Se (2.441-8.888 mg kg^{-1}) and Cu (0.805-1.791 mg kg^{-1}), but generally with values higher than those of muscle. The highest toxic elemental concentrations were for As (2.804-61.399 mg kg^{-1}) and Hg (0.044-0.348 mg kg^{-1}). In sperm samples an analogous trend was observed: Zn (1.615-28.709 mg kg^{-1}), Fe (6.056-35.274 mg kg^{-1}), Se (0.443-2.237 mg kg^{-1}) and Cu (0.106-1.015 mg kg^{-1}) were the non toxic elements present at highest concentrations, generally with values higher than in muscle. As (5.425-93.430 mg kg^{-1}) and Hg (0.033-0.260 mg kg^{-1}) were the most abundant toxic elements. All trace elemental concentrations were highest in the gonad samples, except for Hg, Pb and Ni that were highest in muscle. Other authors reported highest concentrations of mercury in muscle (Ray et al., 1984; Barak and Mason, 1990; Goldstein et al., 1996).

As regards Pb levels, in a study reported by Miedico *et al.* 2014 tuna fish was less contaminated, taking into consideration both mean concentration and the significant percentage of blue-fish samples (66.1%) that showed amounts of lead below the LOQ. By comparing these results with those obtained in this study the Pb levels were always below the LOQ. A study of Jinadasa *et al.* 2014 showed relatively high levels of Pb (<0.001-0.91 mg/Kg) in yellowfin tuna than found in this survey (<0.01-0.083 mg/Kg).

Miedico *et al.* 2014 reported that Cd contamination in blue-fish and other seafish was low (mean levels 0.023 and 0.009 mg kg^{-1} respectively), much

lower than the established maximum limits (0.3 mg kg^{-1} for sword fish, 0.1 mg kg^{-1} for different types of blue-fish, such as tuna, mackerel, anchovies and 0.050 mg kg^{-1} for other seafish) as set by the European Commission (2006). The concentration of Cd (<0.001-0.09 mg/Kg) found in tuna muscle samples of Jinadasa *et al.* 2014 were similar to Cd levels reported in this study (0.012-0.025 mg/Kg). Instead, other authors showed Cd concentrations much higher than those found in tuna samples analyzed in this work. In particular, in a study carried out in the Western Indian ocean, comparatively lower Cd concentrations were reported in yellowfin tuna having Cd levels of 1.04 ± 1.09 and 0.25 ± 0.21 mg/kg on dry weight basis, respectively (Kojadinovic et al., 2006). Jaffar *et al.* 1993 reported 0.35 mg/kg Cd in yellowfin tuna of comparable size from Arabian Sea.

Jinadasa *et al*. 2013 reported Hg levels in muscle tissue of Swordfish samples in a range of 0.18- 2.58 mg/kg. Thus, some sensitive birds or mammals might be adversely affected if they consume fish with the highest Hg levels, whereas these levels were similar to other studies worldwide. The results obtained in the work by Jinadasa *et al.* 2013, however, were much higher than mercury concentrations obtained in this work and were always lower than the limits fixed by the European Commission (2006). Chen *et al.* (2007) reported Hg concentrations in Indian Ocean swordfish ranging between 0.06–3.97 mg/kg and 0.08–5.20 mg/kg, respectively, which were much higher than values found in this study. Hg concentrations in this survey (0.246-0.714 mg/Kg) were only a little higher than those reported by another study of Jinadasa *et al.* 2014. These values were similar to other studies in sword-fish and yellowfin tuna from oceans around the world. (Kojadinovic et al., 2006; Adams, 2004).

On average, Cr and Mn levels are in all samples between 0.033 - 0.152 mg kg^{-1} and 0.014 - 0.128 mg kg^{-1}, respectively. Ni, Cd and Pb levels were below the LOQ in 100% of muscle samples from male specimens and in 70-80% of those from female specimens, in 30-60% egg samples, and in 69-100% of sperm samples.

By Mann-Whitney U test was calculated that Zn, Se, Cr and Mn in muscles and Zn, Se, Cr, Mn, Fe, Cu and As in gametes showed significantly different levels between male and female gender at p-level < 0.05. Female specimen showed higher values of Zn, Se and Cr in muscle and of Zn, Se, Cr, Fe and Cu in eggs, while male specimen showed higher values only of Mn in muscle and Mn and As in sperm. Significances of elemental concentration differences between tuna specimen with weight < 200 kg and >200 kg were also estimated by Mann-Whitney U test. The results showed a statistically significant difference at p-level < 0.01 in Zn levels in muscle, which were significantly larger in bigger specimens. Jinadasa *et al.* (2013) also found a positive correlation for Hg between swordfish with weight and length and observed r values of 0.69 and 0.70, respectively, which was similar to another study in this field (Jessica et al. 2006; Chen et al. 2007). Dividing samples between males and females, Mn and Cr showed significant differences according to weight in both genders, while Zn, Fe and Se only showed significant results in males. No significant difference was observed in relation to the weight in egg samples, while in sperm samples Cr was significantly higher in large specimens.

Benefit-risk assessment

The Recommended Dietary Allowance (RDA) in Europe for adults is 10 mg/day for Zn, 14 mg/day for Fe, 1 mg/day for Cu, 2 mg/day for Mn, 0.1

mg/day for Cr and 0.055 mg/day for Se (European Commission 2008). In order not to underestimate the contamination intake, a concentration equal to LOQ was assigned to all samples that showed a non-quantifiable amount of Ni, Cd and Pb. As indicated by a report of the Italian National Institute of Health (2004), the upper-bound approach can be adopted as a protective measure related to health and environment. The contributions at these allowances from presumed ingestion of 200 gram tuna fish muscle per week are reported in table 5, the highest level for Se, with an average of 32%.

PTWI values for Hg, Cd, As and Pb are 0.004, 0.025, 0.015 and 0.025 mg/kg body weight/week, respectively (EFSA, 2004). The samples analyzed do not exceed these values. The EFSA Contaminant Panel noted that the PTWI is no longer appropriate and, in its assessment, shall give particular attention to the most recent data that indicate effects at doses of As and Pb lower than those considered by JECFA. Because of uncertainties in the exposure in key epidemiological studies, the Contaminant Panel identified a range of values for the 95 % lower confidence limit of the benchmark dose of 1 % extra risk ($BMDL_{01}$) for each endpoint. So also in this survey $BMDL_{01}$ values were considered. For As $BMDL_{01}$ values are set between 0.0003 and 0.008 mg/kg bw/day and 0.0015 mg/kg bw/day (EFSA, 2009; EFSA, 2010). As and Pb dietary intake for an adult of 60 Kg from consumption of 200 gram tuna fish muscle per week has been calculated and presented in table 5. Compared to the safety standards the results of the present study show that $BMDL_{01}$ percentages as estimated by mean values are well within the safety limits for Pb, while As exceeds these values with 20-521% of $BMDL_{01}$.

CONCLUSIONS

The risk/benefit assessment of EFSA 2005 recommends and supports the assumption that regular consumption of fish can make a positive contribution to the prevention of cardiovascular disease and the development of the foetus, although in the latter case it has not been established yet the optimal consumption. Fish, however, may contribute significantly to intake of hazardous elements from the environment. The analyzed tuna samples represent a good nutritional source of trace elements. Ingestion of toxic elements studied from tuna fish samples did not present any health risk for the average consumer. Nevertheless, it should be kept in mind that regular or excessive consumption of tuna fish species might exceed the recommended weekly intake (PTWI) or the benchmark dose lower confidence limit ($BMDL_{01}$), which not necessarily exposes a noticeable risk for excessive consumers.

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Table 1 ICP-MS operating conditions.

RF power (W)	1500
plasma gas flow rate (L min^{-1})	15
auxiliary gas flow rate (L min^{-1})	0.9
carrier gas flow rate (L min^{-1})	1.1
helium collision gas flow rate (mL min^{-1})	4
spray chamber T(°C)	2
sample depth (mm)	9
sample introduction flow rate (mL min^{-1})	1
nebulizer pump (rps)	0.1
extract lens (V)	1.5

Table 2 Comparison between analytical results and certified data for reference material Fish tissue IAEA-407.

Element	Certified (mg/kg)	Found (mg/kg)	Recovery (%)
Cr	0.73	0.69	94.5
Mn	3.52	3.57	101.4
Ni	0.60	0.5	86.7
As	12.6	11.3	89.7
Se	2.83	2.65	93.6
Cd	0.189	0.192	101.5
Hg	0.222	0.190	85.6
Pb	0.12	0.10	83.3
Cu	3.28	3.01	91.7
Fe	146	125	85.6
Zn	67.1	52.4	78.1

Table 3 Calibration curve characteristics (y = a + bx), linearity, LOD, LOQ and precision values (n=6).

Element	a intercept	b slope	R^2	LOD (mg/kg)	LOQ (mg/kg)	Precision (RSD %)
Cr	$1.048 \cdot 10^4$	$2.464 \cdot 10^3$	0.9999	0.009	0.031	1.4
Mn	$9.280 \cdot 10^4$	$1.205 \cdot 10^4$	0.9998	0.006	0.020	3.7
Ni	$3.347 \cdot 10^3$	$1.892 \cdot 10^3$	0.9999	0.010	0.032	2.6
As	$1.912 \cdot 10^3$	$2.915 \cdot 10^2$	0.9999	0.015	0.050	1.7
Se	$2.121 \cdot 10^2$	$2.848 \cdot 10^2$	0.9997	0.015	0.050	1.9
Cd	$8.939 \cdot 10^3$	$2.398 \cdot 10^2$	0.9999	0.012	0.038	2.2
Hg	$2.734 \cdot 10^3$	$1.829 \cdot 10^2$	0.9995	0.006	0.020	2.5
Pb	$6.412 \cdot 10^4$	$8.362 \cdot 10^3$	0.9987	0.010	0.032	1.9
Cu	$8.559 \cdot 10^3$	$2.869 \cdot 10^4$	0.9999	0.010	0.032	2.6

Fe	$2.993 \cdot 10^{4}$	$3.804 \cdot 10^{6}$	0,9995	0,090	0,295	4.8
Zn	$2.948 \cdot 10^{3}$	$3.261 \cdot 10^{4}$	0,9993	0,024	0,079	3.4

Table 4 Ranges and mean values (mg kg^{-1} ww) of non toxic and toxic elements in gametes and muscle samples correlated with sex, weight and length of 23 *Thunnus thynnus* fishes collected in the Mediterranean Sea.

Sex (n)	*Weight (Kg)*	*Length (cm)*	*Sample*		*Non toxic elements*							*Toxic elements*			
					Zn	*Fe*	*Se*	*Cu*	*Ni*	*Cr*	*Mn*	*As*	*Hg*	*Cd*	*Pb*
F (10)	139-198	221-236	eggs	range	20.264-170.884	43.835-80.093	2.738-6.622	0.805-1.303	0.047-0.303	0.053-0.183	0.014-0.089	3.083-53.730	0.094-0.303	0.018-0.069	<0.010-0.085
				mean values ± SD (n=5)	89.670±72.691	59.807±13.100	4.099±1.621	1.074±0.223	0.131±0.105	0.107±0.054	0.035±0.032	22.443±26.100	0.180±0.082	0.038±0.021	0.037±0.026 (n=3)
			muscle	range	3.875-43.631	7.581-19.417	0.616-1.153	0.187-0.312	<0.010-0.275	0.059-0.109	0.013-0.016	2.317-5.527	0.282-0.578	<0.012-0.025	<0.010-0.014
				mean values ± SD (n=5)	15.003±16.371	13.014±4.334	0.834±0.198	0.258±0.046	0.105±0.148 (n=3)	0.075±0.021	0.014±0.001	3.700±1.339	0.382±0.115	0.025 (n=1)	0.014 (n=1)
	200-250	237-258	eggs	range	19.465-154.316	22.933-102.124	2.441-8.888	0.870-1.791	<0.010-0.088	0.040-0.140	0.043-0.363	2.804-61.399	0.044-0.348	<0.012-0.046	<0.010-0.153
				mean values ± SD (n=5)	121.668±57.350	52.558±30.110	5.334±2.411	1.390±0.383	0.077±0.016 (n=2)	0.074±0.039	0.121±0.136	14.875±26.010	0.191±0.116	0.031±0.016 (n=4)	0.153 (n=1)
			muscle	range	5.182-37.022	3.649-21.020	0.565-0.817	0.158-0.265	<0.010	0.041-0.051	0.023-0.036	2.312-5.288	0.246-0.714	<0.012-0.018	<0.010-0.083
				mean values ± SD (n=5)	13.057±13.447	10.306±6.759	0.705±0.123	0.223±0.042	-- (n=0)	0.047±0.004	0.029±0.006	3.662±1.158	0.491±0.179	0.016±0.004 (n=2)	0.083 (n=1)
M (13)	124-199	221-242	sperm	range	3.308-22.392	6.872-34.287	0.455-1.621	0.221-1.015	<0.010	0.026-0.052	0.012-0.294	5.425-93.430	0.033-0.260	<0.012-0.013	<0.010-0.097
				mean values ± SD (n=8)	12.830±5.302	19.797±9.690	0.785±0.408	0.527±0.249	-- (n=0)	0.039±0.010	0.128±0.110	45.657±25.280	0.134±0.074	0.013 (n=1)	0.073±0.034 (n=2)
			muscle	range	1.983-5.389	6.906-16.765	0.270-0.431	0.195-0.684	<0.010	0.028-0.040	0.032-0.064	1.782-4.031	0.278-0.647	<0.012	<0.010
				mean values ± SD (n=8)	3.368±0.994	9.566±3.148	0.358±0.058	0.293±0.162	-- (n=0)	0.033±0.005	0.049±0.012	2.949±0.748	0.464±0.114	-- (n=0)	-- (n=0)
	209-239	239-261	sperm	range	1.615-28.709	6.056-35.274	0.443-2.237	0.106-0.911	<0.010	0.039-0.303	0.017-0.112	6.152-52.309	0.036-0.244	<0.012-0.025	0.013-0.014
				mean values ± SD (n=5)	11.504±11.137	21.559±13.379	1.065±0.746	0.391±0.319	-- (n=0)	0.152±0.136	0.065±0.033	22.790±21.487	0.146±0.101	0.021±0.003 (n=3)	0.013±0.001 (n=2)
			muscle	range	6.121-57.526	11.593-21.138	0.334-1.207	0.195-0.578	<0.010	0.037-0.088	0.016-0.042	1.336-4.711	0.320-0.606	<0.012	<0.010
				mean values ± SD (n=5)	18.623±21.859	14.406±3.920	0.677±0.331	0.306±0.167	-- (n=0)	0.060±0.023	0.028±0.011	3.008±1.313	0.437±0.110	-- (n=0)	-- (n=0)

Table 5 Mean values for RDA, TWI, PTWI, $BMDL_{01}$ (n=23).

	Non toxic elements							*Toxic elements*			
	Zn	*Fe*	*Se*	*Cu*	*Ni*	*Cr*	*Mn*	*As*	*Hg*	*Cd*	*Pb*
Mean values(mg/kg ww; n=23)	11.320	11.529	0.607	0.273	0.105	0.051	0.033	3.280	0.446	0.019	0.049
Max values(mg/kg ww; n=23)	57.526	21.138	1.207	0.684	0.275	0.109	0.064	5.527	0.714	0.025	0.083
RDA (mg/day)	10	14	0.055	1		0.1	2				
% of RDA estimated by mean value	3%	2%	32%	1%		1%	0.05%				
% of RDA estimated by max value	16%	4%	63%	2%		3%	0.09%				
ML (mg/kg ww)									1	0.1	0.3
TWI or PTWI(mg/kg bw/ week)								0.015	0.004	0.025	0.025
% of TWI or PTWI estimated by mean value								72.9%	37%	2%	0.6%
% of TWI or PTWI estimated by max value								122.8%	60%	3%	1.1%
$BMDL_{01}$ (mg/kg bw/day)								0.0003-0.0080			0.0015
% of $BMDL_{01}$ estimated by mean value								20 – 521%			2%
% of $BMDL_{01}$ estimated by max value								33 – 877%			3%