Trace minerals in tilapia fillets: Status in the United States marketplace and selenium supplementation strategy for improving consumer’s health

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Abstract

This goal of this study was to highlight the importance of minerals in the diet of fish for meeting micronutrient requirements in the human diet. First arsenic, calcium, cadmium, copper, iron, molybdenum, magnesium, manganese, sodium, phosphorus, potassium, selenium, and zinc concentrations of twelve commercially available tilapia samples were measured. The nutritional value of fillets in regard to their mineral content were assessed to establish potential health benefits or risks for consumers. The health benefit value of selenium was also calculated. Positive health benefit values indicate that tilapia fillets in the United States marketplace of this study do not pose health risks associated with mercury exposures. Selenium was the trace mineral of interest. After the market study, a seven-week fish feeding trial was conducted to study the influence of organic versus inorganic dietary selenium on Nile tilapia (Oreochromis niloticus). Fish were fed two different diets enriched with the same concentration (0.01g kg⁻¹) of selenium in form of inorganic (sodium selenite) or organic (seleno-L-methionine) selenium in triplicate groups. There were no significant differences between growth and biometrics of fish fed different diets (p>0.05). At the end of trial twelve fish from each treatment were collected. Fillets of fish fed organic selenium had selenium concentrations of 0.55±0.01 μg g⁻¹ which were significantly (p<0.05) higher than fish fed inorganic selenium at levels of 0.22±0.008 μg g⁻¹ or fish samples from the marketplace with a selenium level of 0.2±0.03 μg g⁻¹. Fish fed organic selenium also had significantly higher (p<0.05) plasma and kidney selenium in comparison to fish fed inorganic selenium. No significant differences (p>0.05) were observed in glutathione peroxidase activities in either the plasma or liver of Nile tilapia in the different treatment groups. This study shows that organic selenium is a better option for production of Nile tilapia fillets rich in selenium.
Introduction

Malnutrition is one of the biggest problems facing the world [1]. Fish is a protein rich food that is increasingly favored by consumers. Being packed with nutrients fish can play an incredible role in fighting the malnutrition problem [2, 3]. Fish are not only rich in high quality protein but are also one of the primary sources of Omega 3, polyunsaturated fatty acid (PUFA). Polyunsaturated fatty acid can decrease cardiovascular disease and is essential for brain development. In addition, fish are good sources of highly bioavailable micronutrients such as minerals and vitamins that play important role in human health, growth and development and disease prevention [4]. However, the important role of fish as dietary sources of micronutrients is poorly recognized and under evaluated [5].

With rising pressures on capture fisheries, global fish demand is increasingly being met from aquaculture with aquaculture producing more than half of the fish for human consumption [6]. As the aquaculture industry is growing it is embracing a nutrition sensitive approach and is moving beyond maximizing productivity to improving nutrition and health of the animal. Production of healthier fish with better nutritional quality can in return improve the health of human consumers. Herein the value of aquacultured product was increased by producing the fillets that are fortified with a mineral which enhance the functionality of already healthy product.

Being part of numerous enzymes, minerals participate in many vital biological processes for human and animals. In this context selenium (Se) is one of the most interesting elements. Selenium is the functional component of selenocysteine (Sec), a Se containing amino acid genetically encoded for incorporation into the active sites of unique proteins (selenoproteins) which are expressed in all vertebrates [7]. One of the best studied selenoproteins is glutathione peroxidase (GPX, EC 1.11.1.9) [8]. This enzyme protects the cell membranes against oxidative damage by catalyzing the reactions that are essential for the conversion of hydrogen peroxide and fatty acid hydroperoxides to water and fatty acid. Glutathione peroxidase is measured to evaluate the bioactive status of Se in humans and animal tissues. Selenium is important for various aspects of human health, including optimal immune response, proper thyroid hormone metabolism, cardiovascular health and prevention of neurodegeneration and cancer and antagonism effect against methylmercury and other heavy metal toxicities [9,10,11,12,13].

Selenium is also very important for maintaining the health of fish. Selenium deficiency in fish can compromise the immunity and increase mortality of the animal. Previous studies have provided evidence that farmed fish may require a higher content of Se in their diet, and if it is delivered in an organic form it may better accumulated in tissue, and be more biologically active [14,15].

Due to the beneficial effects of Se for human and animal health this element has been proposed as a valuable resource for including in functional feeds for aquaculture which can promote health and growth of fish as well as improving the quality of their respective fillets for human consumption. When supplementing feed with Se, an important consideration is which form of Se to use [16,17] Supplementation of feed with organic Se can potentially produce fish with higher concentrations of Se for humans to consume.

Therefore, in this study first a small-scale market survey was conducted to evaluate the mineral and heavy metal composition of tilapia fillets available in the U.S. marketplace. The market study aimed to provide information for consumers on potential contribution of consumption of tilapia fillet to mineral contents of their diets. The concentrations of heavy metals were compared to the available fish mineral composition data, standards, and safety limits to evaluate any health risk associated with consumption of this fish regarding heavy metal toxicity. In
addition, Se specific nutritional benefit in relation to mercury (Hg) exposure risk associated with tilapia consumption was assessed by calculating health benefit value of Se (HBV).

After the market study, a fish trial was conducted to evaluate the effect of Se species as functional ingredients in the diet of Nile tilapia, *Oreochromis niloticus*.

The effects of dietary organic and inorganic Se on growth performance, biometrics, plasma and hepatic GPX activity and tissue assimilation of Se was compared to identify species of Se that lead to higher tissue and plasma concentrations. The effect on fish production was evaluated by measuring food conversion ratio (FCR), growth rate, survival, and intestinal microvilli length. The information on Se concentration of tilapia fillets obtained in market study was used to evaluate the success of supplementation of aquafeed with organic and inorganic Se for fortifying Nile tilapia fillets with Se. This was done by comparing the Se concentrations of fillets from market survey to Se concentrations of tilapia fillets from fish trial.

**Materials and methods**

**Market study**

**Market samples.** Twelve tilapia samples were purchased from various grocery stores in four different regions of the United States. Samples were collected from the mid-Atlantic (Blacksburg, VA), mid-west (Chicago, IL), northeast (Boston, MA and New York City, NY) and southeast regions (Plano, TX). Data regarding type of culture (wild caught versus farmed raised), country of origins, price, product certification and fish condition were recorded upon purchase (Table 1). Samples were freeze-dried and stored at -20 °C in accordance with United States Environmental Protection Agency (USEPA) guidance for fish tissue analysis [18]. Freeze dried samples were microwave digested and mineral concentrations were measured using the methods below.

**Reagents and material.** Standard solutions for analyzed elements (HPLC grade [S, Ca, Cd, Cu, Fe, Hg, Mb, Mg, Mn, Na, P, Se, and Zn], Sigma Aldrich, St Louis, MO, USA) were used from 1000 mg L\(^{-1}\) stock solution to develop calibration curves. All aqueous solutions of reagents and standards were prepared using ultra-pure water (Milli-RO 12 plus Milli-Q purification system for water, Millipore, Bedford, MA, USA). Trace grade (35% w/v) nitric acid

| Sample | Region      | Super market | Price\(^\ast\) | Farmed | Storage condition | Boneless | Skinless | Whole | Gutted | Certification\(^\ast\) | Origin      |
|--------|-------------|--------------|--------------|--------|------------------|----------|----------|-------|--------|---------------------|-------------|
| T1     | Mid-Atlantic| Kroger       | 5.20         | Yes    | Frozen           | Yes      | No       | Yes   | Yes    | None                | China       |
| T2     | Mid-Atlantic| Walmart      | 3.92         | Yes    | Frozen           | Yes      | No       | Yes   | Yes    | None                | Columbia    |
| T3     | Mid-Atlantic| Walmart      | 7.94         | Yes    | Frozen           | Yes      | No       | Yes   | Yes    | None                | China       |
| T4     | Mid-Atlantic| Food lion    | 4.99         | Yes    | Frozen           | Yes      | No       | Yes   | Yes    | None                | China       |
| T5     | Midwest     | Aldi         | 5.99         | Yes    | Frozen           | Yes      | No       | Yes   | Yes    | Yes                 | China       |
| T6     | Midwest     | Whole food   | 7.98         | Yes    | Fresh            | Yes      | No       | Yes   | Yes    | None                | Ecuador     |
| T7     | Northeast   | Trader joes  | 5.99         | Yes    | Frozen           | Yes      | No       | Yes   | Yes    | None                | Thailand    |
| T8     | Northeast   | Stop and shop| 6.99        | Yes    | Frozen           | Yes      | No       | Yes   | Yes    | Yes                 | China       |
| T9     | Southwest   | Kroger       | 5.99         | Yes    | Frozen           | Yes      | No       | Yes   | Yes    | None                | China       |
| T10    | Southwest   | Market street| 5.99         | No     | Fresh            | Yes      | No       | Yes   | Yes    | None                | Equator     |
| T11    | Southwest   | Market street| 6.99         | Yes    | Fresh            | Yes      | No       | Yes   | Yes    | None                | Mexico      |
| T12    | Southwest   | Tom toms     | 6.99         | Yes    | Fresh            | Yes      | No       | Yes   | Yes    | None                | Costa Rica  |

\(^\ast\)Price is per pound of fillet

\(^\ast\)Certification is a market-based measure for evaluation of aquaculture and capture fisheries in various objects form food safety, quality, and traceability to environmental and social impact.

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Microwave digestion. Samples were digested using a modified method adopted from Olmedo et al. [19]. Briefly, 0.5 g of each sample was weighed into MARS 5 microwave Teflon vessels (CEM Corporation, Matthews, NC, USA) in duplicate, that had been subjected to an anti-static gun. Samples were digested using 8 mL of 35% (w/w) nitric acid and 2 mL of 30% hydrogen peroxide (w/w). Before digestion, the vessels were briefly swirled to mix and allowed to sit open for approximately 10 minutes. The microwave program performed a ramp to 180 °C over 20 minutes and then held at that temperature for 10 minutes before cooling to 50 °C. After digestion, the samples were transferred to polypropylene (PP) tubes and the vessels were quantitatively rinsed using 2% (w/w) nitric acid.

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). The freeze-dried digested samples were diluted to two separate dilutions based on analyte (i.e. mineral) with 2% (w/w) nitric acid prior to analysis. The samples were diluted approximately 200-fold for the higher concentration metals such as Na, K, P, and Ca. To accommodate trace metals, the samples were diluted 10-fold. The dilutions were necessary to reduce the concentrations of acids going on to the instrument as well as to reduce contamination and to allow the analyte concentrations to fall within the calibration range. The calibration standards and ranges for each element are shown in Table 2. Sample acquisition was performed on an Agilent 7900 ICP-MS (Agilent, Santa Clara, CA, USA) using a custom method that utilized the helium mode octopole reaction system (ORS) to reduce and/or remove potential matrix interferences while simultaneously maintaining the lower detection limits needed for the analysis [20]. A certified reference material (DORM-4, National Research Council of Canada) was digested and analyzed along with the samples to ensure proper digestion and analysis.

Health benefit value of selenium (HBV). Health benefit value of Se was used to determine Se-specific nutritional benefits in relation to potential Hg exposure risks presented by tilapia fillets available in the U.S. marketplace. The Hg and Se mass concentrations (parts per million) for each individual fish sample were converted to molar concentrations (μmole kg⁻¹). To determine whether the amounts of Hg and Se present in the fish fillets would potentially result in a Se insufficiency or a net excess, the difference in the molar concentrations of Se and Hg was divided by Se concentration which gives the absolute molar concentration present in the fish fillets. This results in the relative amount of Se available [21].

\[
\text{Relative Se availability} = ([\text{Se}] - [\text{Hg}]) / \text{Se}
\]

To reflect the physiological amount of Se that is potentially provided or lost in respect to sequestration by Hg, the relative amount of Se available was multiplied by the total amount of Hg and Se that was presented in the fish fillets.

\[
\text{HBV} = ([\text{Se}] - [\text{Hg}]) / \text{Se} \times ([\text{Se}] + \text{Hg})
\]

Table 2. Calibration standards and ranges for each analyzed element.

| Metals   | Calibration Concentration (ng mL⁻¹ solution) |
|----------|---------------------------------------------|
| Na, Mg, P, K, Ca, Fe | 10 50 100 500 2,000 5,000 |
| Zn | 1 5 10 50 200 500 |
| Mn, Cu, As, Se, Mo, Cd, Pb | 0.1 0.5 1 5 20 50 |

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Positive HBV means that the fish has Se in molar excess to Hg and thus are unlikely to pose a health risk [21].

**Risk and nutritional assessment.** The mineral concentration in a 100 g serving of fish fillet was calculated and converted to wet weight basis by considering the moisture content (range of 74 to 81%) of the samples. For nutritional assessment, the calculated intake of the analyzed essential elements per 100 g serving of tilapia fillets were compared to Dietary Reference Intake (Recommended Daily Allowance and Adequate Index) for children, adult females and adult males aged between 14 and 50 years that is set by the Food and Nutrition Board, Institute of Medicine, United States National Academy of Sciences [22–24].

**Fish trial**

**Fish trial experimental design and diet.** All procedures have been approved by Virginia Tech’s Institute of Animal Care and Use Committee (VT-IACUC-#16–099). A 7-week grow out trial was conducted to evaluate the effect of dietary supplementation of inorganic versus organic Se on Nile tilapia. One hundred and sixty-eight tilapia were acquired from Genetic Spring (Akvaforsk Genetics Center, Miami, FL, USA). After a month of acclimatization, fish with an average weight of 170 ± 2 g. (Mean ± SE) were arbitrarily stocked into six polyethylene tanks (284 L each) in a single recirculating aquaculture system (RAS) at 28 fish per tank. The RAS system was equipped with bead and sand filters for mechanical filtration, bioreactor for nitrification, UV disinfection units, heat exchangers, and distributed diffuse aeration.

Two separate isonitrogenous and isoenergetic diets (Table 3) [25] were prepared. The dry ingredients were mixed followed by the addition of oil and distilled water. The mixture was pelleted (Mill 72A, Lawson Mills Biomass Pellet Solutions, Ebenezer, PEI, Canada) and then dried at room temperature for 24 h. Experimental diet nutritional proximate and trace mineral concentrations for the various diets are presented in Table 4. Feed was then stored in a commercial refrigerator at a temperature between 0–3.5 °C until it was used. Diets were supplemented with 1 mg kg⁻¹ of Se in forms of sodium selenite (2.83 mg kg⁻¹) and seleno-L-methionine (2.19 mg kg⁻¹). Concentration of Se in each diet was measured using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) analysis as described above. Dietary Se concentration were measured to be 2.01 mg kg⁻¹ for the inorganic Se diet and 2.23 mg kg⁻¹ for organic Se diet.

**Table 3. Formulation of basal diet (g kg⁻¹ as is basis).**

| Ingredients          | Inorganic Se supplemented diet | Organic Se supplemented diet |
|----------------------|--------------------------------|-----------------------------|
| Soybean meal         | 526                            | 526                         |
| Dextrinized starch   | 217                            | 217                         |
| Menhaden fish meal   | 117                            | 117                         |
| Soy oil              | 51                             | 51                          |
| CaPO₄, dibasic       | 11                             | 11                          |
| Glycine              | 11                             | 11                          |
| DL-Methionine        | 2                              | 2                           |
| Vitamin premix       | 5                              | 5                           |
| Mineral premix       | 1                              | 1                           |
| Celufil              | 37                             | 37                          |
| CMC                  | 21                             | 21                          |
| Stay-C               | 1                              | 1                           |
| Seleno-L-Methionine  | 0                              | 0.01                        |
| Sodium selenite      | 0.01                           | 0                           |

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Each diet was fed to fish in triplicate tanks. During the trial fish were weighted weekly on a per tank basis. The amount of feed was adjusted based on the weight gain and feed rates (2–3% of fish body weight) were consistent between the two treatment groups. Feeding rate was on a percent body weight per day basis. Feed were loaded on 24 belt feeders (four per tank) to deliver feed hourly over an 18-hour period. Water quality parameters (Table 5) were analyzed using methods adapted from APHA [26] and HACH [27] as follows: Dissolved oxygen and temperature were measured using a meter (YSI ProDO, Cole-Parmer, IL, USA) daily. Alkalinity was also measured daily using a titration method. Nitrite, nitrate, total ammonia nitrogen and pH were measured three times a week. Approximately half of the sump tank (95 liters) water was exchanged daily to prevent potential Se accumulation in the water. Also, the Se content of water in each tank was measured at the beginning, middle and end of the experiment to check for any Se accumulation in the tanks.

Health and production characteristic of fish. Food conversion ratio, survival rates, and weight gain were used to compare the production between the two treatment groups. Survival was assessed daily and FCR was determined by dividing amount of feed provided for consumption by the weight gain of fish on a per tank weekly basis. Weight gain was calculated weekly. Also, the hepatosomatic index (HSI) and viscerosomatic index (VSI) biometrics were assessed for each treatment group at the end of feeding trial.

Sampling. At the end of the 7-week feeding trial, 12 fish from each diet were arbitrarily collected. Fish were netted individually and euthanized with sodium bicarbonate buffered

### Table 4. Experimental diet nutritional proximate and trace mineral concentrations.

| Proximate level (g kg⁻¹)   | Inorganic Se supplemented diet | Organic Se supplemented diet |
|----------------------------|--------------------------------|-----------------------------|
| Crude Protein              | 379                            | 381                         |
| Carbohydrate*              | 281                            | 288                         |
| Ash                        | 86                             | 87                          |
| Total Fat                  | 69                             | 71                          |
| Crude Fiber                | 85                             | 79                          |
| Moisture (%)               | 10                             | 9.4                         |
| Trace mineral level (mg kg⁻¹) |                  |                             |
| Iron                       | 341                            | 348                         |
| Copper                     | 14                             | 14                          |
| Zinc                       | 243                            | 244                         |
| Manganese                  | 67                             | 74                          |
| Selenium                   | 2.0                            | 2.2                         |

*Calculated value (Merrill and Watt, 1973): Carbohydrate = total-(ash+ crude protein + moisture+ total fat)

### Table 5. Mean water quality results for the RAS system during the 7-week trial. Number of sampling events is denoted by n.

| Dissolved Oxygen (mg L⁻¹) as CaCO₃ n = 48 | Alkalinity (mg L⁻¹) n = 48 | Temperature (°C) n = 48 | Nitrate-N (mg L⁻¹), n = 7 | Nitrite-N (mg L⁻¹), n = 21 | Nitrite-N (mg L⁻¹), n = 21 | pH n = 21 | Total ammonia-N (mg L⁻¹), n = 21 | Se Conc. (μg L⁻¹), Day 1, n = 3 | Se Conc. (μg L⁻¹), Day 28, n = 3 | Se Conc. (μg L⁻¹), Day 49, n = 3 |
|-------------------------------------------|-----------------------------|--------------------------|---------------------------|-----------------------------|---------------------------|-----------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 5.3 ± 0.2                                 | 125.5 ± 5.5                 | 29 ± 0.2                 | 11.0 ± 1.7                | 0.08 ± 0.03                 | 0.08 ± 0.03               | 7.78 ±0.05 | 0.3 ± 0.03                        | 0.45 ± 0.01                       | 0.5 ± 0.04                        | 0.54 ± 0.02                       |

Values are mean ± standard errors

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tricaine methanesulfonate solution (MS 222, 250 mg/L, Western Chemical, Ferndale, WI, USA). Weight and length of each fish were measured and recorded before necropsy.

**Plasma collection.** Blood was collected postmortem from the caudal vessels of each fish using a 1mL syringe and 23-gauge needle. Blood samples were placed in ethylenediaminetetraacetic acid (EDTA) coated tubes and centrifuged for 15 minutes at 10,000 x g. After centrifugation, plasma samples were collected and stored at −80 °C until analyzed. Total Se concentration and GPX activities were measured in individual plasma samples.

**Fillet collection.** After blood collection, each fish was filleted by the same person for consistency in sampling. Fillets were weighed for calculation of fillet yield. Fillet samples for total Se concentration were freeze-dried and stored at −20°C. Fillet yield was determined by dividing the fillet weight by the whole weight of each fish.

**Liver, kidney and intestine collection.** The viscera of individual fish were collected and then the liver and the total viscera weights (g) were measured individually. Liver, kidney and intestine samples were collected for microscopy, analysis of GPX activity and Se content of the tissue. Intestinal samples were preserved in 2.5% glutaraldehyde for transmission electron microscopy (TEM). Liver and kidney samples were collected and stored at −80 °C immediately after collection. Hepatosomatic index and VSI were measured by dividing the liver weight and viscera weight by the whole fish weight respectively.

**Total GPx activity measurement.** Liver and plasma GPx activity were measured using a commercial kit (Cayman Chemical, Ann Arbor, MI, USA). This assay is based on the method that measures the decrease in absorbance at 340 nm (A_{340}) while NADPH is oxidizing to NADP⁺. The rate of reduction in A_{340} is directly proportional to GPX activity of -the sample [28,29]. Before analysis, liver samples were homogenized in 10 mL of cold buffer (50 mM Tris HCl, pH 7.5, 5 mM EDTA and 1mM Dithiothreitol (DTT) per gram of tissue. Homogenized samples were centrifuged for 15 minutes at 10,000 x g. The supernatant was collected, and protein concentrations of samples were measured using a bicinchoninic acid (BCA) assay kit (Fisher Scientific, UK). The BCA assay is based on the method from Lowry et al. [30]. Plasma samples were diluted 1:2 with cold buffer (50 mM Tris-HCl, pH 7.6, containing 5 mM EDTA and 1 mg mL⁻¹ of bovine serum albumin (BSA)) prior to analysis.

**Transmission electron microscopy.** Sections of the intestine from each treatment group were preserved in 2.5% glutaraldehyde buffered with 0.1M sodium cacodylate trihydrate. The samples were washed twice in 0.1M Na sodium cacodylate trihydrate for 15 minutes. Washed samples were post-fixed in 1% OsO4 in 0.1M Na cacodylate for 1 hour, washed again 0.1M Na cacodylate twice for 10 minutes, then dehydrated in graded ethanol solutions for 15 minutes at each stage [31]. Propylene oxide was used to further dehydrate the samples. Dried samples were then infiltrated with a 50:50 solution of propylene oxide: Poly/Bed 812 for 24 hours, thereafter with a 100% mixture of Poly/Bed 812 for 12 hours per manufacturer’s instructions (Polysciences Inc., Warrington, PA, USA). Blocks prepared for TEM were sectioned with a diamond knife and ultramicrotome into 90-150nm sections on 200 mesh grids A JEOL 1400 scope (JEOL Ltd., Akishima, Tokyo, Japan) equipped with a Gatan Orius SC 1000 CCD camera (Gatan Inc. Pleasanton, CA, USA) was used to obtain TEM images. Collected images (magnification x 15000) were analyzed using ImageJ (National Institute of Health, USA) [32] and were used to measure microvilli length.

**Statistical assessments.** Statistical analysis was performed using GraphPad Prism for Mac (La Jolla, CA, USA) and JMP 13(SAS institute, Cary, NC, USA). All data were analyzed using t-test to evaluate significant differences among the means. Significant differences were indicated at p<0.05.
Results

Market study

Eleven of the 12 tilapia samples available in U.S. marketplace were farmed raised. China was the main country of the origin (Table 1). Good recovery of analyte was achieved with a combination of HNO₃/H₂O₂ (4:1) mixture for digestion of all the samples using microwave-assisted digestion. The recovery values of DORM-4 reference material were between 87–111% for the elements that were being analyzed. Concentrations of essential and trace minerals and soft electrophiles in tilapia fillets are presented in Fig 1. The major microelements were K, P, Na, Mg and Ca, followed by micronutrients Zn, Fe, As, Se, Cu, Mn, and heavy metals Hg, As, and Cd.

Health benefit value of selenium. Tilapia fillets had a positive HBV of 11.83 ± 4.95.

Contribution to Dietary Reference Intake (DRI). The contribution of tilapia fillets per 100 g serving to mineral’s DRI is summarized in Table 6 for children and adults. Tilapia fillets provide more than 10% of DRI of P, Se and Na for adult, and more than 10% of DRI of Cu, Mg, P, Se and Na per 100 g of serving for children.

Comparison of mineral composition to USDA data

Concentrations of minerals of tilapia fillet of this study was compared with USDA composition data for raw tilapia fillets which is shown in Table 7. Tilapia fillets from market survey had significantly different concentrations of Ca, Fe, Mg, Mn, K, Na, Cu, and Zn compared to USDA composition data.

Table 6. Contribution (%) of tilapia fillets to dietary reference intake (DRI) of each element for children and adults by gender.

|       | Ca  | Cu  | Fe  | Mg  | Mn  | Mb  | P   | Se  | Zn  | K   | Na  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Children | 0.93 | 13.6 | 2.65 | 17.57 | 0.08 | 1.29 | 31.5 | 68.9 | 7.26 | 8.08 | 10.5 |
| Male   | 0.82 | 6.93 | 2.94 | 5.93  | 0.63 | 0.66 | 17.8 | 39.0 | 1.45 | 6.14 | 12.6 |
| Female | 0.80 | 6.93 | 2.04 | 7.32  | 0.63 | 0.66 | 17.8 | 39.0 | 4.54 | 6.14 | 12.6 |

*Dietary Reference Intake (Recommended Daily Allowance and Adequate Index) for children, adult females and adult males aged between 14 and 50 years is set by the Food and Nutrition Board, Institute of Medicine, United States National Academy of Sciences [22–24].

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Fish trial

Growth performance. Growth performance of fish fed the experimental diets is presented in Table 8. No mortalities were observed during the trial. Fish that were fed organic Se had slight, but insignificantly higher ($p<0.05$) growth and lower FCR than fish fed inorganic Se. There were also no significant differences between the FCR, HSI or VSI of fish fed the different diets.

Se content in fish tissue and plasma. Selenium concentration was significantly higher ($p<0.05$) in plasma, kidney and fillet of fish fed organic Se (Fig 2). However, the concentration of Se was higher in the liver of fish fed inorganic Se. Overall, Se accumulation was higher in the liver and kidney compared to fillets or plasma compared with the fish fed inorganic Se.

Total GPX enzyme activity in plasma and liver. There was no significant difference in total GPX activity in liver or plasma of fish that were fed the different experimental diets (Fig 3).

Microvilli size. There were no significant differences between the microvilli from inorganic and organic Se fed group (Table 9). Representative TEM images of the fish fed organic and inorganic Se is presented in Fig 4.

Comparison of Se content of tilapia fillets from market to tilapia fillets from fish trial. Organic Se fed tilapia had significantly higher ($p<0.05$) concentration of Se in comparison to inorganic fed fish or tilapia samples from commercial markets. There were no significant differences between tilapia samples from the market and inorganic fed fish (Fig 5).

Discussion

Elemental composition of 12 commercially available tilapia samples in U.S. marketplace was analyzed. Among the main elements studied, K had the highest concentration followed by P.

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Table 7. Comparison of mineral concentrations (mg/100 g) of fillets from market study to USDA compositional data for tilapia fillets.

| Mineral | Market Study Data | USDA Composition Data |
|---------|------------------|-----------------------|
| Ca      | 9.2 ± 0.3        | 14                    |
| Fe      | 0.26 ± 0.04      | 0.69                  |
| Mg      | 17 ± 0.7         | 34                    |
| P       | 116 ± 7.8        | 204                   |
| K       | 303 ± 7.6        | 380                   |
| Na      | 125 ± 20         | 56                    |
| Zn      | 0.35 ± 0.2       | 0.41                  |
| Cu      | 0.059 ± 0.006    | 0.075                 |
| Mn      | 0.012 ± 0.0004   | 0.037                 |

Values are mean ± standard errors

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Table 8. Production parameters of Nile tilapia, Oreochromis niloticus, fed inorganic or organic Se for 7 weeks.

| Treatment   | Survival (%) | Weight gain (g) | FCR*       | Weekly weight gain (g) |
|-------------|--------------|-----------------|------------|------------------------|
| Inorganic Se| 100%         | 333±101         | 1.43±0.15  | 41.0±6.7               |
| Organic Se  | 100%         | 362±110         | 1.27±0.08  | 45.0±5.9               |

*FCR: Food Conversion Ratio

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Na, Ca, Mg. Previous study has shown that higher concentration of Na than K in the diet can significantly increase the risk of cardiovascular disease [33]. Concentration of Na was lower than K in all the tilapia fillets samples of the present study demonstrating that these products from marketplace are good choice of a meal for a healthy diet. Total concentrations of As, Cd and Hg were measured and compared to food safety standards showing that the studied fish products do not pose health risks associated with heavy metal exposures. Cadmium concentration in all the samples were below detection limits which agrees with previous research that found only trace amounts of Cd in tissues of aquatic organisms [34]. In nature, As exists in both organic (i.e. arsenobetaine and arsenochloline) and inorganic forms, however in fish tissue it is present mostly in organic forms which are less toxic than the inorganic species [35]. Tilapia fillet total As (organic and inorganic) concentration was significantly lower \((p<0.05)\) than USEPA limit of 1.3 mg kg\(^{-1}\) (wet weight) in freshwater fish for human health protection [36]. Mercury is one of the most toxic heavy metals in the environment. Methylmercury (MeHg) is a contaminant in fish that can pose risks to humans. High MeHg exposures can have severely adverse effects on the central nervous system, particularly the developing brain of a fetus [37]. However, instead of harm, epidemiological studies have consistently found that increasing Hg exposures from maternal consumption of ocean fish during pregnancy result in substantial maternal health benefits and improved neurological outcomes in their children [38–43]. Clearly, it is not the increased Hg exposures that were responsible for these positive health outcomes. Instead, the improved neurological development, motor abilities, verbal intelligence, perception, social behavior, and diminished hyperactivity appear to be due to improved maternal intakes of nutrients such as omega-3 fatty acids and Se which are abundant in ocean fish [44].

Mercury concentrations in tilapia fillets analyzed in the market survey were similar to the U.S. FDA report on Hg concentrations of fish and shellfish [45]. The U.S. FDA action level for Hg is 1.0 mg kg\(^{-1}\) (ppm wet weight), but many countries have level of 0.5 mg kg\(^{-1}\) as acceptable
Hg concentration in fish tissue [46,47]. Mercury concentration in all the tilapia fillets in this study were under 0.5 mg kg$^{-1}$.

Recent studies have shown that fish rich in Se provides healthy nutrients while minimizing risks associated with Hg exposure. Therefore, in addition to total Hg measurement, the Hg health risk associated with consumption of tilapia from U.S. marketplace was investigated by measuring HBV. This criterion considers both Se and Hg concentrations and is proposed as a

![Fig 3. Glutathione peroxidase activity of Nile tilapia, Oreochromis niloticus, in (a) plasma and (b) liver supplemented with seleno-L-methionine (organic) and sodium selenite (inorganic) Se in basal diet. Unit (U) is nano moles of NADPH oxidized per milligram of total protein per minute. There were no significant differences ($p>0.05$) in hepatic and plasma activity indicating both forms of Se were sufficient to meet tissue need for maximizing GPX activity.](https://doi.org/10.1371/journal.pone.0217043.g003)

Hg concentration in fish tissue [46,47]. Mercury concentration in all the tilapia fillets in this study were under 0.5 mg kg$^{-1}$.

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Table 9. Biometrics of Nile tilapia, Oreochromis niloticus, fed inorganic or organic Se for 7 weeks.

| Treatment     | HSI$^*$ | VSI$^\dagger$ | Fillet Yield | Microvilli length $^\circ$ (μm) |
|---------------|---------|---------------|--------------|-------------------------------|
| Inorganic Se  | 1.0 ± 0.06 | 6.0 ± 0.2     | 24.0 ± 1.3   | 1.2 ± 0.04                    |
| Organic Se    | 1.0 ± 0.07 | 6.4 ± 0.03    | 26.0 ± 0.9   | 1.3 ± 0.02                    |

Values are mean ± standard errors of 12 samples per diets. Fillets were boneless and skinless.

$^*$HSI: Hepatosomatic Index
$^\dagger$VSI: Visoerosomaltic Index
$^\circ$Refer to Fig 3 for TEM images of microvilli

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Fig 4. Representative transmission electron microscopy (TEM) images of Nile tilapia, *Oreochromis niloticus*, microvilli fed (a) sodium selenite (inorganic Se) and (b) SeMet (organic Se).

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Fig 5. Comparison of Se concentrations (μg g⁻¹ of wet weight) of tilapia fillets from U.S. marketplace to tilapia fillets from fish nutritional trial. Means with different letters were significantly different (p<0.05).

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more reliable risk assessment index in regard to Hg exposures. Tilapia fillets had positive HBV’s which is an indication that health benefits outweigh risks related to Hg exposures.

Mineral contribution to DRI per 100 gram serving for essential and trace minerals calculated. Food that provide more than 10% of a nutrient per serving are considered good source of that nutrient [48]. Tilapia fillets from the U.S. marketplace of this study can provide more than 10% of the select minerals including Se, P for adult and Cu, Mg, P, Se for children and hence are considered good source of these minerals.

Comparison of mineral concentrations in tilapia fillets from marketplace to USDA composition data for raw tilapia fillets showed that tilapia fillets from this market survey had different concentrations of Ca, Cu, Fe, Mg, Mn, K, Na and Zn compared to USDA composition data [49] (Table 7). These differences can be explained by factors such as the geographical variations, types of the diet, the environment that fish were raised in and differences in the methods that has been used to measure the minerals [50].

Diet in the fish trial were supplemented with 1 mg kg$^{-1}$ of organic or inorganic Se. Dietary Se level of 2.06 mg kg$^{-1}$ has been shown to be beneficial for Nile tilapia and this requirement cannot be met by an aquafeed that is not enriched with Se [51]. During the 7-week fish trial fish demonstrated expected performance. Survival was 100% for all the treatment groups with a mean weekly weight gain of 41 and 45 grams for inorganic and organic Se fed fish, respectively.

There were no significant differences in the FCR, weight gain, HSI, VSI, hepatic and plasma GPx activity, or in the intestinal microvilli length of fish from the different treatment groups. Similar results were observed in studies on Crucian carp (Carassius auratus gibelio), juvenile grouper (Epinephelus malabaricus) and rainbow trout (Oncorhynchus mykiss) [52–55]. However, as we hypothesized, feeding the organic form of Se led to a significant increase in Se concentrations observed in kidney, plasma and fillets of the fish. This appears to be due to differences in the absorption, bioaccumulation and bioavailability of different species of Se (organic and inorganic) [56]. Selenomethionine (SeMet), an organic form of Se that was used in this study, is readily absorbed by erythrocytes through the same active mechanism that incorporates methionine (Met), the more abundant sulfur analogue. Since Met and SeMet are incorporated into cellular proteins for repeated protein synthesis cycles until they are eventually degraded several weeks to several months later, organic Se fed in this form tends to be retained for extended periods of time. In contrast, sodium selenite, an inorganic form of Se, is digestively absorbed and rapidly incorporated into Sec. Since Sec cannot be reused in protein synthesis but must instead be actively degraded into inorganic metabolites which are readily excreted when sufficient Se is available, dietary Se provided in the inorganic form is retained to lesser extents in the muscles and is excreted in higher concentrations than organic Se. Because Met and SeMet are immediately incorporated into tissues with high levels of protein synthesis such as skeletal muscles, liver, and kidney, these tissues accumulate greater amounts of Se [57]. Since no significant differences in GPx activity were noted between the two treatment groups, this confirms that the diets were Se-replete.

Comparison of the concentrations of Se in the fillets of market samples and fillets from the fish trial showed that fillets from fish fed organic Se had significantly higher (p<0.05) Se contents. To maintain normal physiological functions, the DRI for adults consume 0.53 μg of Se per day and children (4–8 y) consume 30 μg of Se per day. A 100 g serving of tilapia fillets enriched with organic Se in this study had 0.56 μg of Se which satisfies the recommended intake level per day completely for both children and adult. However, fillets from market study and organic fed fish had 0.22 and 0.2 μg of Se per serving (100 g) that can only satisfy 68% of DRI for children and 38% of DRI for adults.
Thus, this study highlights the importance of production and promoting the consumption of value-added fish fillets for combating micronutrient deficiency. While undernutrition can be improved by increasing energy intake, micronutrient deficiency is of a different nature and result from inadequate quality of diet.

In poorer households and developing countries there are limited affordability, availability and even cultural acceptability of food from animal sources that are main sources of micronutrients. In this respect fish offer an advantage as they are more acceptable, more available and consumed by preference in various regions of the developing world [58]. Therefore, promoting the consumption of value-added fish fillets (i.e. Se enriched) can be used as a whole food approach for solving the problem such as Se deficiency and heavy metal toxicity in developing countries [59].

In conclusion, this study demonstrated that organic Se is a more bioavailable form of Se in Nile tilapia. To produce Se enriched tilapia fillets, organic species of Se should be used by the aquaculture industry as an alternative to inorganic species.

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References

1. IFPRI. Global Nutrition Report: from promise to impact—ending malnutrition by 2030. Washington DC: International Food Policy Research Institute, 2016.

2. HLPE. Sustainable fisheries and aquaculture for food security and nutrition. A report by the High-Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security. Rome: Food and Agriculture Organization, 2014.

3. Béné C., Barange M., Subasinghe R., Pinstrup-Andersen P., Merino G., Hemre, et al. (2015). Feeding 9 billion by 2050—Putting fish back on the menu. Food Security, 7(2), 261–274. https://doi.org/10.1007/s12571-015-0427-z

4. Beveridge M. C. M., Thilsted S. H., Phillips M. J., Metian M., Troell M., & Hall S. J. (2013). Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture. J Fish Biol, 83, 1067–1084. https://doi.org/10.1111/jfb.12187 PMID: 24099563

5. Allison, E. H., Delaporte, A., & Hellebrandt de Silva D. Integrating fisheries management and aquaculture development with food security and livelihoods for the poor. Report submitted to the Rockefeller Foundation, School of International Development, University of East Anglia Norwich, 2013. 124 p.

6. FAO. The State of World Fisheries and Aquaculture. Contributing to food security and nutrition for all. Rome: Food and Agriculture Organization, 2018.

7. Labunskyy VM, Hatfield DL, Gladyshev VN. Selenoproteins: molecular pathways and physiological roles. Physiol Rev. 2014; 94(3):739–77. https://doi.org/10.1152/physrev.00039.2013 PMID: 24987004

8. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science (New York, NY). 1973; 179(4073):588–90. Epub 1973/02/09. PMID: 4686466.

9. Coppingere RJ, Diamond AM. Selenium deficiency and human disease. In: Hatfield DL, editor. Selenium: Its Molecular Biology and Role in Human Health. Boston, MA: Springer US; 2001. p. 219–33.

10. Rayman MP. Selenium and human health. Lancet. 2012; 379(9822):1256–68. https://doi.org/10.1016/S0140-6736(11)61452-9 PMID: 22381456

11. Li GS, Wang F, Kang D, Li C. Keshan disease: an endemic cardiomyopathy in China. Human Pathology. 1985; 16(6):602–9. Epub 1985/06/01. PMID: 3997137.

12. Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. Br Journal Nut. 2001; 85(S2):S67–S74.

13. Ralston NV, Raymond LJ. Dietary selenium’s protective effects against methylmercury toxicity. Toxicology. 2010; 278(1):112–23. https://doi.org/10.1016/j.tox.2010.06.004 PMID: 20561558.

14. Le KT, Fotedar R. Bioavailability of selenium from different dietary sources in yellowtail kingfish (Seriola lalandi). Aquaculture. 2014; 420:57–62.

15. Wang Y, Han J, Li W, Xu Z. Effect of different selenium source on growth performances, glutathione peroxidase activities, muscle composition and selenium concentration of allogynogenetic crucian carp (Carassius auratus gibelio). Anim Feed Science Technol. 2007; 134(3):243–51.

16. Telli GS, Ranzani-Paiva MJT, Dias DD, Sussel FR, Ishikawa CM, Tachibana L. Dietary administration of Bacillus subtilis on hematology and non-specific immunity of Nile tilapia Oreochromis niloticus raised at different stocking densities. Fish & Shellfish Immunology. 2014; 39(2):305–11. https://doi.org/10.1016/j.fsi.2014.05.025 PMID: 24878743

17. Wang C, Lovell RT. Organic selenium sources, selenomethionine and selenoyeast, have higher bioavailability than an inorganic selenium source, sodium selenite, in diets for channel catfish (Ictalurus punctatus). Aquaculture. 1997; 152(1):223–34.

18. United States Environmental Protection Agency (USEPA), 2000, Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Retrieved form https://www.epa.gov/fish-tech/epa-guidance-developing-fish-advisories

19. Olmedo P, Hernandez AF, Pia A, Fernia P, Navas-Acien A, Gil F. Determination of essential elements (copper, manganese, selenium and zinc) in fish and shellfish samples. Risk and nutritional assessment and mercury-selenium balance. Food and Chemical Toxicology: an international journal published for the British Industrial Biological Research Association. 2013; 62:299–307. Epub 2013/09/07. https://doi.org/10.1016/j.fct.2013.08.076 PMID: 24007738.

20. Ed McCurdy GW, Don Potter. Unmatched Removal of Spectral Interferences in ICP-MS Using the Agilent Octopole Reaction System with Helium Collision Mode, Application note ELakeside Business Park, Cheddle Royal, Cheshire: Agilent, 2006.

21. Ralston NV, Ralston CR, Raymond LJ. Selenium Health Benefit Values: Updated Criteria for Mercury Risk Assessments. Biological Trace Element Research. 2016; 171(2):262–9. https://doi.org/10.1007/s12011-015-0516-z PMID: 26463749.
39. Fish consumption and prenatal methylmercury exposure: cognitive and behavioral outcomes in the
Golding J, Hibbeln JR, Gregory SM, Iles-Caven Y, Emond A, Taylor CM. (2017) Maternal prenatal
exposure to methylmercury from seafood consumption in pregnancy and neurodevelopmental
outcomes in childhood (ALSPAC study): an observational cohort study. Lancet. 369(9561):578–85.
https://doi.org/10.1016/S0140-6736(07)60277-3 PMID: 17307104

40. Julvez J, Méndez M, Fernandez-Barres S, Romaguera D, Vioque J, Llop S, et al. (2016) Maternal
consumption of seafood in pregnancy and child neuropsychological development: a longitudinal
study based on a population with high consumption levels. Am. J. Epidemiol. 183:169–182.
https://doi.org/10.1093/aje/kwv195 PMID: 26740026

41. Llop S, Ballesta F, Murcia M, Forns J, Tardon A, Andiarena A, et al. (2017) Prenatal exposure to
mercury and neuropsychological development in young children: the role of fish consumption. Int. J.
Epidemiol. 46(3):827–838. https://doi.org/10.1093/ije/dyw259 PMID: 27864405
44. Ralston NVC (2018) Effects of Soft Electrophiles on Selenium Physiology. Free Radical Biology and Medicine. (In Press: available on line).

45. FDA 1990–2012, "National Marine Fisheries Service Survey of Trace Elements in the Fishery Resource" Report 1978, "The Occurrence of Mercury in the Fishery Resources of the Gulf of Mexico" Report 2000

46. US Food and Drug Administration (USFDA) 2001. FDA Consumer Advisory. U.S. Food and Drug Administration, Washington, DC (http://www.fda.gov/bbs/topics/ANSWERS/2000/advisory.html

47. de la Guardia M, Garrigues S. Handbook of Mineral Elements in Food Preface. Handbook of Mineral Elements in Food. 2015:Xix–Xix. Book_Doi https://doi.org/10.1002/9781118654316

48. US Food and Drug Administration, Center for Food Safety and Applied Nutrition. "Labeling & Nutrition—Label Claims for Conventional Foods and Dietary Supplements." www.fda.gov/Food/LabelingNutrition/ucm111447.htm.

49. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 28 (slightly revised). Version Current: May 2018. https://ndb.nal.usda.gov/ndb/foods/show/4732?fgcd

50. Schrauzer GN. Nutritional selenium supplements: product types, quality, and safety. J Am Coll Nutr. 2001; 20(1):1–4. Epub 2001/04/11. PMID: 11293463.

51. Lee S, Nambi RW, Won S, Katya K, Bai SC. Dietary selenium requirement and toxicity levels in juvenile Nile tilapia, Oreochromis niloticus. Aquaculture. 2016; 464:153–8

52. KÜÇÜKBAY FZ, Yazlık H, Karaca I, Sahin N, Tuzcu M, Cakmak MN, et al. The effects of dietary organic or inorganic selenium in rainbow trout (Oncorhynchus mykiss) under crowding conditions. Aquacult Nutr. 2009; 15(6):569–76. https://doi.org/10.1111/j.1365-2095.2008.00624.x

53. Lin Y-H. Effects of dietary organic and inorganic selenium on the growth, selenium concentration and meat quality of juvenile grouper Epinephelus malabaricus. Aquaculture. 2014; 430:114–9.

54. Pacitti D, Lawan MM, Sweetman J, Martin SA, Feldmann J, Secombes CJ. Correction: Selenium Supplementation in Fish: A Combined Chemical and Biomolecular Study to Understand Sel-Plex Assimilation and Impact on Selenoproteome Expression in Rainbow Trout (Oncorhynchus mykiss). PloS one. 2016; 11(2):e0144681. Epub 2016/02/11. https://doi.org/10.1371/journal.pone.0144681 PMID: 26863435.

55. Xuxia Zhou YW, Qing Gub, Weifen Li. Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (Carassius auratus gibelio). Aquaculture. 2009; 291:78–81

56. Fairweather-Tait SJ, Collings R, Hurst R. Selenium bioavailability: current knowledge and future research requirements. The Am J Clin Nutr. 2010; 91(5):1484s–91s. Epub 2010/03/05. https://doi.org/10.3945/ajcn.2010.28674J PMID: 20200264.

57. Daniels LA. Selenium metabolism and bioavailability. Biol Trace Elem Res. 1996; 54(3):185–99. Epub 1996/09/01. https://doi.org/10.1007/BF02784430 PMID: 8909692.

58. Gillespie S, van den Bold M. Agriculture, Food Systems, and Nutrition: Meeting the Challenge. Glob Chall. 2017; 1(3), ARTN 1600002 https://doi.org/10.1002/gch2.201600002

59. Chowdhury UK, Biswas BK, Chowdhury TR, Samanta G, Mandal BK, Basu GC, et al. Groundwater arsenic contamination in Bangladesh and West Bengal, India. Environ Health Perspect. 2000; 108(5):393–7. https://doi.org/10.1289/ehp.00108393 PMID: 10811564