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# Magnesium Levels in Vital Organs of Bluefin Tuna, *Thunnus thynnus* L., from the Turkish Region of Eastern Mediterranean

[1]Fatih Percin and [2]Ozlem Sogut
[1]Department of Aquaculture, Faculty of Fisheries, Ege University, 35100 Bornova, Izmir, Turkey
[2]Department of Analytical Chemistry, Faculty of Pharmacy, Ege University,
35100 Bornova, Izmir, Turkey

**Abstract:** One of the macro elements, Magnesium (Mg) found in the heart, liver and kidney tissues was analyzed and compared between wild/fattened and female/male Bluefin Tuna (BFT) in the Turkish region of the Eastern Mediterranean. For this purpose, 110 individual (i.e., 55 female and 55 male) samples of wild and fattened tuna were investigated. Mean Mg levels in the heart, liver and kidney tissues of wild BFT were found to be as follows: 42.52, 46.73 and 38.60 mg/100 g w wt. Analogous data for the fattened fish were as follows: 38.06, 49.74 and 41.13 mg/100 g w wt. In comparison of wild with fattened fish, Mg differences in the heart, liver and kidney tissues were significant ($p<0.05$). In terms of gender, mean Mg values in the heart, liver and kidney tissues of 55 wild and 55 fattened individual female BFT were analyzed, respectively to be as follows: 45.12, 49.47, 41.08 and 38.37, 51.02, 44.24 mg/100 g w wt. Additionally, these parameters for the same tissues of 55 wild and 55 fattened male specimens were detected, respectively to be as follows: 39.93, 43.98, 36.12 and 37.74, 48.45, 38.02 mg/100 g w wt. According to gender, differences in levels of Mg in the heart and kidney tissues of wild vs. fattened female samples were significant except for liver tissues ($p<0.01$) however, for male specimens, differences in Mg in the same tissues of wilds vs. females were significant ($p<0.05$).

**Key words:** Bluefin tuna, *Thunnus thynnus,* magnesium, heart, liver, kidney, wild, fattened

## INTRODUCTION

Atlantic Bluefin Tuna (*Thunnus thynnus*) (BFT) which migrates from the Mediterranean to the Atlantic ocean is highly commercial and scientists are interested in studying its development and culture. After capture in the wild, BFT are transferred to towing cages and sent to farms to increase their fat content. In cages, fish are fed fresh or frozen fish food, predominantly round sardines, mackerel, pilchard and mollusks. Fattening time is generally 6-8 months but sometimes takes as long as 10 months. After that BFT are harvested, chilled and exported. Although, the rearing period of BFT is not long compared with that of other aquaculture treatments, the fish are affected by several factors including habitat, reproduction, physical and chemical variables, feeding, food quality, stocking density, quantity and contaminants. Additionally, the weight of this highly active and fast-growing fish increases by 25-35% in cages therefore, changes in environmental conditions and rapid weight gain leads to stress. Thus, these variables suppress the immune system and cause metabolic disorders such as poor growth, cardiovascular disease, hepatic failure, renal impairments etc. (Block and Stevens, 2001; Munday *et al*., 2003; Ottolenghi, 2008; Percin and Konyalioglu, 2008; EFSA, 2009; Percin and Akyol, 2010). In aquaculture treatments, macro elements such as sodium, potassium, chloride, calcium, phosphorus and magnesium are essential for vitality in fish. Serious metabolic diseases arise when these minerals are deficient in the vital organs of fish. Magnesium (Mg) is essential for its fundamental role in enzymatic reactions in intermediary metabolism, skeletal tissue metabolism, osmoregulation, neuromuscular transmission and respiratory system (NRC, 1993; Shriver *et al*., 1994; Hoffman *et al*., 2003; FAO, 2003; Lim and Klesius, 2003). Mg also has biological functions that affect a host's immunological system and the defence mechanisms in homeotherms (Beisel, 1982; Coma, 1991; Lim and Klesius, 2003).

It has been found that Mg participates in numerous reactions relating to immunocompetence such as growth and transformation of lymphocytes, synthesis of immunoproteins and chemotaxis (McCoy and Kenny, 1984; Watanabe *et al*., 1988). The Mg requirement of fish can be met by uptake from the diet or water (NRC, 1993;

**Corresponding Author:** Fatih Percin, Department of Aquaculture, Faculty of Fisheries, Ege University, 35100, Bornova, Izmir, Turkey

FAO, 2003; Lim and Klesius, 2003). On the other hand, signs of magnesium deficiency reported for various fish species include anorexia, sluggishness, reduced growth, reduced tissue Mg content, vertebral deformity, edema, muscle flaccidity, cardiac and liver failure and renal impairments and high mortality (Watanabe *et al*., 1988; Stoskopf, 1993; FAO, 2003; Lim and Klesius, 2003). The heart and vascular system along with respiratory organelles of fish work together to transport gases and ions between the tissues of the body and the environment. While these are distinct systems in the body, their actions are coordinated to optimize those exchange processes. For example, oxygen ($O_2$) is taken up from the environment and delivered to all tissues and in exchange, Carbon dioxide ($CO_2$) and ammonia ($NH_3$) gases are transported from the various tissues of the body and excreted (Leatherland and Woo, 1998) however, stressors in the external environment are associated more with pathological and abnormal conditions inside the body affecting the cardiac and vascular systems.

For example, some causes of cardiovascular disorders are related to unbalanced diets or overfeeding (Leatherland and Woo, 1998; Block and Stevens, 2001). The importance of the liver as a marker for pathological change reflects the central role of teleost hepatic tissues in nutrition, lipid and carbohydrate storage synthesis of proteins and enzymes, fatty acid metabolism and biotransformation and elimination of toxicants and pollutants such as xenobiotics. They accumulate in the liver and hepatocytes biotransform these compounds and transport them to the bile for elimination.

Thus the liver plays an important role in contaminant storage, redistribution, detoxification and transformation and acts as an active site of pathological effects of various metabolic anomalies, defects and toxicity (Tietz *et al*., 1990; Leatherland and Woo, 1998; Licata *et al*., 2005). The kidney has important functions. One is to excrete divalent ions that have been absorbed. The glomerular filtration rate is the major factor controlling urine volume and composition with the exception of divalent ions, creatine and some organic acids are actively secreted by the proximal tubules. The kidneys also play a role in balancing the concentration of $Mg^{+2}$ (Tietz *et al*., 1990).

The fish's urinary function occurs primarily in the caudal kidney which is also responsible for the filtration and excretion of several metabolites and macro and microelements (Stoskopf, 1993; Watanabe *et al*., 1988; Leatherland and Woo, 1998; Hoffman *et al*., 2003). Consequently, limited information is available on magnesium levels in vital organs of fish. Thus, the aim of this study was to determine the patterns of magnesium in the heart, liver and kidney tissues of BFT comparing wild and fattened and female and male fish in the Turkish region of the Eastern Mediterranean.

## MATERIALS AND METHODS

**Studying area:** From the wild area, BFT were captured by purse seine around the Levantine Sea and the Bay of Antalya. The farmed fish were collected from cages in Ildir Bay, Cesme-Izmir. The fattening process takes about 7-8 months. The fish were fed *ad libitum* at approximately 10% of their average overall body weight. Stock densities in cages were approximately 6-7 kg $m^{-3}$ with almost 4000 fish per cage. BFT were fed twice a day with fresh or defrosted food fish such as herring (*Clupea harrengus*), sardines (*Sardina pilchardus*, *Sardinella aurita*), mackerel (*Scomber scombrus*) and squid (*Sephia officinalis*). Tissue samples were collected from winter to spring. Physical and chemical variables (temperature, dissolved oxygen and salinity) of waters in study areas were measured by Oxyguard Handy Gamma (Oxyguard Int. A/S, Denmark). The farming areas contained no pollutants.

**Samples:** Fork length, total weight and gender of all fish were determined. After capture by purse seine, the wilds were placed on the deck for 5-10 min. BFT were then slaughtered and gutted. Heart, liver and kidney tissues were taken and immediately stored in liquid nitrogen. Later, the collecting samples were transferred to the laboratory and washed with distilled water, dried with filter paper, homogenized, packed in polyethylene bags and stored at -80°C prior to analysis. The fattened fish were obtained from the pens and subjected to the same analysis after which all samples were dried for 48 h at 110°C.

**Reagents:** Deionized water from the Milli-Q system (Millipore, MA, USA) was used to prepare all aqueous solutions. The perchloric acid, nitric acid and standard solutions (1000 mg $L^{-1}$) were supplied by Merck (Darmstadt, Germany). Plastics and glassware were cleaned by soaking overnight in a 10% (w/v) nitric acid solution and then rinsed with deionized water. After that a wet sample of 0.5 g of the heart, liver and kidney tissues were dried for 48 h at 110°C.

The samples were digested with a mixed solution of perchloric (70%) and nitric (65%) acids (3:7, v/v) and slowly heated to 80°C until complete digestion. The beaker contents were transferred to a 30 mL plastic measuring bottle with small portions of 1% nitric acid. A blank was also prepared to ensure the same method of determining Mg levels.

**Chemical analysis:** A Flame Atomic Absorption Spectrophotometer (FAAS) with Varian-Spectra-AA 10 plus was used for analysis by hollow cathode lamp of Mg in completely proper conditions. The standard addition technique was used for measurement of Mg where aqueous calibration was conducted. The detection limit was determined to correspond to 3 times the standard deviation of 10 blanks. For determination of Mg, adequate volumes of $LaCl_2$ solution (an ionization suppressor) were added to give a final concentration of 10% (m/v). The detection limit values of Mg in FAAS were found to be between 0.010 and 0.020 mg $L^{-1}$.

**Statistical analysis:** The detected data were subjected to statistical analysis. Correction matrices were produced to examine interrelationships in magnesium concentrations in the heart, liver and kidney samples. Minimum (Min.), Maximum (Max.), Median (Med.) values were given. Mean and Standard Error (SE) value were determined and compared using Student's t-test. Mean differences in magnesium content in the heart, liver and kidney tissues were shown for wild and fattened and for female and male BFT and one-way Analysis of Variance (ANOVA) was conducted on the available data. Differences were considered significant at $p<0.01$ and $p<0.05$.

## RESULTS AND DISCUSSION

**Water quality:** In the study areas of Ildir bay and Antalya bay, temperature, dissolved oxygen and salinity values were measured in winter and early summer. Ten samplings were carried out in each area in each season. The mean ranges of temperature, dissolved oxygen and salinity around the farming area of Ildir bay were detected as follows, respectively: 15.5-18.5°C, 8.10-8.25 mg $L^{-1}$ and 36-37.5%. These parameters around the Antalya bay were found to be as follows, respectively: 17.5-21°C, 7.20-7.50 mg $L^{-1}$ and 37.5-38.5%. According to the results, dissolved oxygen was higher at Ildir bay than at Antalya bay while salinity and temperature were lower. Thus, the results of water quality parameters were within the tolerated limits for culture of fish species such as bluefin tuna and others in Ildir bay, Turkey.

**Physical properties of fish:** The range of fork length and weight of 110 wild and 110 fattened BFT were found to be 151-158 cm and 51-57 kg and 155-162 cm and 54-62 kg, respectively. In wilds, 55 samples were female (154-158 cm and 52-57 kg) and 55 samples were male (151-157 cm and 51-55 kg). Among the fattened fish, 55 samples were female (155-162 cm and 56-62 kg) and 55 samples were male (156-160 cm and 54-59 kg). The wild and farmed fish had nearly identical fork lengths and weights. The fish were specifically chosen and harvested to obtain specimens within a particular age class because magnesium concentrations in vital organs might change with size and age. An effort was made to reduce the possibility of age-related differences by choosing samples with similar fork lengths and weights.

**Sample analysis:** All measured values are shown in the Table 1-3 the results of magnesium concentrations in the

Table 1: Magnesium levels in heart, liver and kidney tissues of wild and fattened BFT (mg/100 g w wt)

| | Wild (n = 110) | | | | | TS | Fattened (n = 110) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Organs | Min. | Max. | Med. | Mean | SE | p | Min. | Max. | Med. | Mean | SE |
| Heart | 30.46 | 86.51 | 39.92 | 42.52 | 3.95 | * | 32.14 | 42.91 | 39.97 | 38.06 | 2.76 |
| Liver | 22.41 | 89.98 | 40.15 | 46.73 | 4.18 | * | 33.76 | 67.39 | 51.56 | 49.74 | 4.28 |
| Kidney | 20.16 | 83.74 | 34.02 | 38.60 | 3.37 | * | 30.56 | 51.69 | 43.08 | 41.13 | 3.13 |

TS: Table of significance, significantly different: *($p<0.05$)

Table 2: Magnesium levels in heart, liver and kidney tissues of female and male wild/fattened BFT (mg/100 g w wt)

| | Wild (n = 110) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Female (n = 55) | | | | | Male (n = 55) | | | | |
| Organs | Min. | Max. | Med. | Mean | SE | Min. | Max. | Med. | Mean | SE |
| Heart | 31.69 | 86.51 | 43.86 | 45.12 | 4.68 | 30.46 | 78.92 | 36.44 | 39.93 | 3.17 |
| Liver | 24.04 | 89.98 | 45.09 | 49.47 | 4.52 | 22.41 | 84.34 | 38.08 | 43.98 | 3.70 |
| Kidney | 21.50 | 83.74 | 36.32 | 41.08 | 3.57 | 20.16 | 80.49 | 32.05 | 36.12 | 2.41 |
| | Fattened (n = 110) | | | | | | | | | |
| | Female (n = 55) | | | | | Male (n = 55) | | | | |
| Organs | Min. | Max. | Med. | Mean | SE | | | | | |
| Heart | 32.14 | 42.91 | 39.99 | 38.37 | 2.94 | 32.22 | 42.80 | 39.69 | 37.74 | 2.43 |
| Liver | 34.58 | 65.92 | 53.89 | 51.02 | 4.67 | 33.77 | 67.39 | 48.41 | 48.45 | 3.91 |
| Kidney | 30.56 | 51.69 | 45.74 | 44.24 | 3.54 | 33.18 | 50.83 | 40.06 | 38.02 | 2.65 |

Table 3: Table of significance for heart, liver and kidney tissues between female and male wild/fattened BFT

| Organs | $W_{Female}$-$W_{Male}$ | $W_{Female}$-$F_{Female}$ | $F_{Female}$-$F_{Male}$ | $F_{male}$-$W_{Male}$ |
|---|---|---|---|---|
| Heart | * | ** | NS | * |
| Liver | * | NS | * | * |
| Kidney | * | ** | * | * |

Significant differences: ** = $p<0.01$; * = $p<0.05$; N.S.: Not Significant. $W_{female}$ = Wild female, $W_{male}$ = Wild male, $F_{Female}$ = Fattened female, $F_{Male}$ = Fattened male

heart, liver and kidney tissues for the wild and fattened fish are shown in Table 1 those for the wild and fattened female/male specimens in Table 2 and significant differences between female and male BFT are shown in Table 3. As shown in Table 1, Mg concentrations in the liver and kidney tissues of fattened BFT were higher but Mg level was lower in heart tissue among the wild fish. Additionally, the differences were significant when wild and fattened BFT were compared ($p<0.05$). As shown in Table 2 and 3, Mg concentrations in all sampled organs of females were higher than those in males. Among the wild fish, the differences were significant in all sampled tissues between females and males ($p<0.05$). Among fattened fish, Mg differences were significant for liver and kidney tissues except heart tissue ($p<0.05$) between females and males.

On the other hand, comparing the two study areas in the fattened female BFT, Mg concentrations were higher in the liver and kidney tissues but lower in the heart tissue than those of wild females. Additionally, the differences were significant for the heart and kidney tissues ($p<0.01$) except liver tissue ($p>0.05$) between fattened and wild females. Similarly, among the male samples, Mg values were higher in liver and kidney tissues however, they were lower in the heart tissue of fattened fish than in captives. The differences between wild and fattened males were important for all determining tissues ($p<0.05$). The higher Mg concentration in liver and kidney tissue of the fattened BFT might be related to feeding because the fish were fed fish food with high protein and mineral content such as sardine, clupeid, mackerel and squid which are given abundantly. This factor might cause increased liver function because many enzymatic (metalloenzymes) and metabolic (muscle and skeletal growth) activities and biochemical reactions (lipid and carbohydrate metabolism) are carried out in the liver and some of these use Mg in cells. In addition, feeding could increase kidney function because toxic or wasted metabolites and molecules are excreted with some enzymatic reactions using Mg.

Also of all known vertebrate tissues, the kidneys of fish are the major organ of Mg transport (Coma, 1991; Kendrick *et al*., 1992; Leatherland and Woo, 1998; Beyenbach, 2000; FAO, 2003). On the other hand, the lower Mg concentration in heart tissue of fattened fish might be related to stress, perhaps of cage confinement. Thus, it might have an influence on cardiac and vascular systems. Stress rapidly induces corticosteroid hormones. Cortisol and other steroid hormones suppress the immune system of fish therefore magnesium with phosphorus and calcium in heart tissue can be used for enzymatic and biochemical reactions such as phosphohydrolases, phosphotransferases, metalloenzymes and detoxifying free radicals. Thus higher Mg content in tissues may decrease stress (Watanabe *et al*., 1988; Coma, 1991; Kendrick *et al*., 1992; Shriver *et al*., 1994; Block and Stevens, 2001). On the other hand, the higher Mg levels in vital organs of wild and fattened female BFT compared with both male groups might be related to physiology of reproduction and spawning because magnesium, phosphorus and calcium are needed and deposited for use in gonad development, maturation and oocyte ovulation and yolk (Wurst and Stickney, 1989; Tietz *et al*., 1990; Bridges *et al*., 2002; Block and Stevens, 2001). In addition, the higher Mg levels in liver and kidney tissues of fattened female and male BFT might be related to feeding. As indicated, these groups of BFT were fed fish food with high protein and mineral content. On the other hand, the lower concentration of Mg values in the heart tissue of fattened females and males might be related to stress induced by cage confinement as indicated previously (Beisel, 1982; Leatherland and Woo, 1998; Beyenbach, 2000; Block and Stevens, 2001; Bridges *et al*., 2002).

In the literature, the limited information on magnesium concentration in fish, trout, carp, catfish and sea bream is mainly nutritional research (Ogino and Chiou, 1976; Sakamoto and Yone, 1979; Knox *et al*., 1981; Gatlin *et al*., 1982; Shim and Ng, 1988; Shearer, 1989; Reigh *et al*., 1991). Knox *et al*. (1981) are determined the range of Mg concentration in the heart, liver, kidney and muscle tissues of rainbow trout as 3.19-7.86, 6.07-7.43, 5.07-7.55 and 9.86-13.47 mmol $kg^{-1}$ wet weight, respectively. Shearer (1989) found Mg concentration in whole body of rainbow trout as 37 mg/100 g wet basis. For the bluefin tuna, USDA (2009) provided a standard nutrient database reference for Mg level in fresh raw meat (muscle). According to this organisation, Mg level is calculated as 50 mg/100 g wet wt. In the study, mean Mg values in all wild and fattened heart, liver and kidney tissues were found to be, 40.49, 48.24 and 39.87 mg/100 g w wt, respectively. It is concluded that Mg levels in heart liver and kidney tissues were lower than in raw meat (muscle) as indicated by USDA (2009). But the values might change depending on the size and age of fish because the fish we studied had nearly identical fork lengths and weights of 151-162 cm and 51-62 kg, respectively.

According to the literature on age-related differences in BFT, the fish in the study were approximately 6-7 years old (Cort, 1991; Percin and Akyol, 2009; Santamaria *et al*., 2009). Thus the results for Mg values could be applicable only to fish with 151-162 cm fork length and weighing 51-62 kg (at 6 or 7 years).

## CONCLUSION

In this research, the level of magnesium concentration was found in all sampled tissue to be liver >heart>kidney. The highest Mg magnification by the tissues in the study was in the liver because of the central role of hepatocytes in nutrition. Consequently, the study shows Mg levels in vital organs of wild and fattened and female and male bluefin tuna. It emphasizes the need for additional detailed research on Mg burdens in different sizes of Atlantic (*Thunnus thynnus*), Pacific (*Thunnus orientalis*) and Southern (*Thunnus maccoyii*) bluefin tuna species.

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