Scientific Research and Essays Vol. 6(2), pp. 363-368, 18 January, 2011
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE10.797
ISSN 1992-2248 ©2011 Academic Journals

Full Length Research Paper

The concentration of magnesium in the front and rear dorsal ordinary muscles of bluefin tuna, *Thunnus thynnus* L.

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Accepted 11 November, 2010

Concentration of magnesium in dorsal ordinary muscles (Dom) obtained from front (Fdom) and rear (Rdom) muscle samples from wild/fattened female/male bluefin tuna (BFT) in the Turkish Eastern Mediterranean were analyzed and compared. A total of 180 individual that is (90 female and 90 male) samples were investigated. Mean Mg levels in the Fdom and Rdom samples of wild and fattened BFT were found to be as follows: 48.76 and 53.20, 52.69 and 40.29 mg 100 g^{-1} w wt, respectively. The mean Mg values of the Fdom and Rdom samples of 45 wild and 45 fattened individual female BFT were analyzed and found to be as follows: 51.31 and 55.09, 56.46 and 42.33 mg 100 g^{-1} w wt, respectively. The parameters for the Fdom and Rdom of 45 wild and 45 fattened male specimens were determined to be 46.12 and 51.40, 48.92 and 38.26 mg 100 g^{-1} w wt, respectively. Both wild/fattened fish, Mg differences in the Fdom and Rdom tissues and the differences in levels of Mg in the Fdom and Rdom samples of wild vs. fattened female and male samples were significant ($p < 0.05$).

Key words: *Thunnus thynnus,* bluefin tuna, magnesium, muscle, front, rear, wild, fattened.

INTRODUCTION

Magnesium (Mg) is a vital element in terms of maintaining the health of fish. Several metabolic functions in the bodies of fish rely on Mg; it plays a major role in the production and transportation of energy in fish. For example, Mg is an activator of several key enzyme systems, including kinases, mutases, muscle ATPases, alkaline phosphatase, arginase, deoxyribonuclease, and glutamine. Through its role in enzyme activation, Mg and calcium stimulate muscle and nerve contractions; they are also involved in the regulation of intracellular acid-base balance and Mg plays an important role in carbohydrate, protein, and lipid metabolism. Researchers indicate that Mg is important in controlling the muscle contractions (impulses) in fish. Mg is also essential for metabolism in the bones of fish. Mg concentrations in muscle tissue of various fish species are different; high levels are detected in halibut, mackerel, and tuna (Watanabe et al., 1988; NRC, 1993; FAO, 2003; Lim and Klesius, 2003; Nelson and Cox, 2004).

On the other hand, Mg deficiency in fish leads to several signs and pathologies, such as reduced growth, sluggishness, anorexia, convulsions, high mortality, cataracts, calcinosis of the kidneys. The research has mainly involved studies of carp, trout, eel, catfish, sea bass, and sea bream (Ogino and Chiou, 1976; Knox et al., 1981; Gatlin et al., 1982; Shim and Ng, 1988). If excess amounts of Mg are not ingested and assimilated, toxicity may develop.

Mg content is also vital in reference to muscle physiology and activity in bluefin tuna (BFT), *Thunnus thynnus.* After capturing in the wild, BFT are transferred to sea cages in which they are reared for six to eight months. During the fattening period, tuna are fed frozen

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Abbreviations: BFT, Bluefin tuna; **Dom,** dorsal ordinary muscle; **Fdom,** dorsal ordinary muscle obtained from front; **Rdom,** dorsal ordinary muscle obtained from rear.

fish, mainly sardines, mackerels, pilchards, and molluscs. At the conclusion of the rearing time, fish are harvested, chilled, and exported to Japan for sushi. Although, the rearing period of BFT is not long, the weight of the fish increases by 25-35% while they are in cages. Therefore, several factors, such as environmental changes, physical and chemical differences, habitat, reproduction, feeding, food quality and quantity, stocking density, and contaminants affect the health, muscle physiology, and biochemistry of BFT. In addition, the content of lipids, proteins, and carbohydrates and the concentrations of vitamins and minerals are different in various parts of fish bodies. For instance, the lipid content and level of fattiness is higher in the abdominal muscles of fattened fish than in other organs. In addition, the concentrations of the nutrients are different in front (head) and rear (tail) and dorsal and ventral parts of the muscles in bodies of fish (Nakamura et al., 2005, 2006; Percin and Konyalioglu, 2008; Ottolenghi, 2008; Percin et al., 2010).

The aim of the study is to detect concentrations of magnesium, which is essential for growth and muscle metabolism in the front and rear dorsal ordinary muscles and to compare Mg in wild and fattened female and male BFT in Turkish bodies of water in the Eastern Mediterranean.

MATERIALS AND METHODS

Area and sampling

Wild BFT were taken by purse seine around the Levantine Sea, the Bay of Antalya. Fattened BFT were obtained from the cages and a processing plant in Ildir Bay, Cesme-Izmir. BFT had been fattened in cages. The cages were conical in shape and had diameters of 50 m on the surface, 30 m on the bottom, and 30 m in depth. Stock densities in the cages were approximately 6 – 7 kg/m^3 with 3000-4000 fish/cage. BFT were fed *ad libitum* at approximately 10% of their average overall body weight 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*). Muscle samples of BFT were collected from winter to spring.

The physical parameters of the waters, levels of dissolved oxygen, water temperatures, and salinity were measured in both study areas through the use of Oxyguard Handy Gamma (Oxyguard Int. A/S, Denmark). The farming areas contained no pollutants.

After capture by purse seine, the wilds were placed on the deck for 5 to 10 min; during that time, biometric measurements, fork length, total weight, and gender were determined. Then the BFT were slaughtered and gutted. Samples from the front (Fdom) and rear (Rdom) dorsal ordinary muscles were taken and immediately stored in liquid nitrogen. Later, the samples were collected and transferred to the laboratory and washed with distilled water, dried with filter paper, homogenized, packed in polyethylene bags, and stored at -80℃ prior to analysis. The fattened fish were obtained from cages and subjected to the same analysis, after which all samples were dried for 48 h at 110℃.

Digestion procedure and apparatus

The tissues were digested with a mixed solution of perchloric (70%) and nitric (65%) acids (3:7, v/v) and slowly heated to 80℃ until complete digestion. De-ionized 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).

A Flame Atomic Absorption Spectrophotometer (FAAS) with Varian-Spectra-AA 10 plus was used for the analysis of Mg by hollow cathode lamp in completely proper conditions. The standard addition technique, which involved the use of aqueous calibration, was conducted for the measurement of Mg. Adequate volumes of $LaCl_2$ solution (an ionization suppressor) were added to produce 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

Mean differences in Mg levels of Fdom and Rdom samples were shown for wild and fattened female and male BFT, and one-way Analysis of Variance (ANOVA) was conducted on the available data. Minimum (min), maximum (max), median (med) values were measured. Mean and standard error (SE) values were determined and compared using Student's *t* test. Differences were considered significant at $p < 0.05$.

RESULTS

Water quality and biometric measurements of fish

Water quality parameters of dissolved oxygen, temperature, and salinity values were measured in winter and spring. The mean ranges of ten samplings for each season were carried out in Ildir Bay and Antalya Bay. The values were as follows: 8.07–8.28 mg L^{-1}, 15.2–18.9℃, 35.8–37.6% in Ildir Bay, and 7.11–7.75 mg L^{-1}, 16.8–20.4℃, 37.3–38.8% in Antalya Bay. Dissolved oxygen was higher at Ildir Bay than in Antalya Bay, while salinity and temperature were lower in the former than in the latter. According to the physical parameters of the waters, the results were within the tolerated limits for the culture of fish species such as BFT in Ildir Bay, Turkey. The fork length and weight of 90 wild and 90 fattened BFT were determined to be 154. 6 ± 2.3 cm and 55.2 ± 2.4 kg and 159.4 ± 2.6 cm and 58.3 ± 2.9 kg, respectively. Among the wilds, 45 samples were female (155.3 ± 2.1 cm and 56.3 ± 2.7 kg) and 45 samples were male (153.9 ± 2.0 cm and 54.1 ± 2.2 kg). Among the fattened BFT, 45 samples were female (162.0 ± 3.1 cm and 60.0 ± 2.9 kg) and 45 samples were male (156.8 ± 2.3 cm and 56.6 ± 2.6 kg).

Mg content in dorsal ordinary muscles may vary according to size. An effort was made to reduce the possibility of size-related differences by choosing samples with similar fork lengths and weights.

Magnesium concentration in muscle samples

Mg concentrations in the both wild and fattened BFT's Fdom and Rdom samples are shown in Table 1. Mg concentrations were found lower in wild Fdom BFT and in fattened Rdom BFT. The total concentration of Mg in

Table 1. Magnesium concentrations and significance in Fdom and Rdom samples of wild and fattened bluefin tuna (mg 100 g^{-1} w wt).

	Wild (*n* = 90)					T.S.	Fattened (*n* = 90)				
	min	**max**	**med**	**mean**	**s.e.**	**p<0.05**	**min**	**max**	**med**	**mean**	**s.e.**
Fdom	28.12	62.28	55.11	48.76	4.26	*	34.24	66.96	53.57	52.69	5.19
Rdom	41.80	74.33	50.16	53.20	4.98	*	26.81	51.90	40.06	40.29	4.11

T.S.: Table of significance, *: statistically significant (p<0.05).

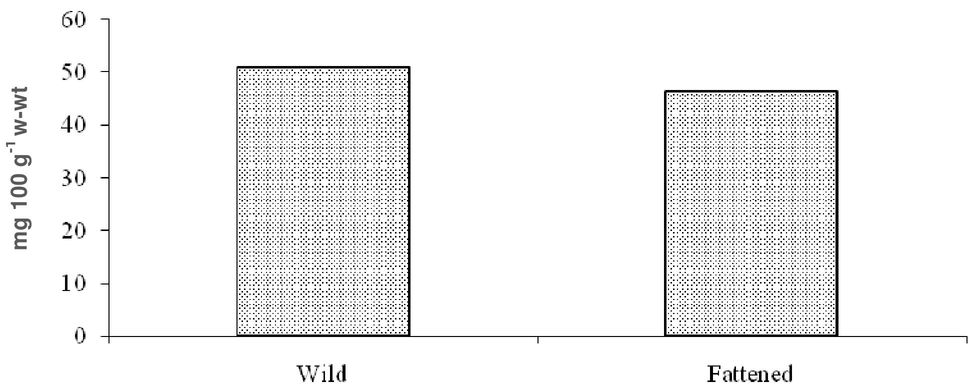

Figure 1. Magnesium concentrations in dorsal ordinary muscle (Dom) samples of wild and fattened bluefin tuna.

Dom samples (Fdom + Rdom) were found higher in wilds than in the fattened fish (Figure 1). Effect of gender on Mg accumulation in Fdom and Rdom in wild and fattened BFT are shown in Table 2 and Figure 2; and significant differences between female and male BFT are indicated in Table 3.

As shown in Tables 2 and 3, Mg levels in both samples of female muscle tissue were higher than in male. The differences were significant between females and males in both wild and fattened Fdom, Rdom samples ($p < 0.05$).

DISCUSSION

Mg is used for energy metabolism, metalloenzymes, and many of the biochemical processes in muscle tissues and nervous system growth, and hormone stimulation (Evans, 1998; Block and Stevens, 2001; Bridges et al., 2002; FAO, 2003; Wu, 2006). In this study, the values of Mg in the Rdom samples were found higher than in the Fdom of wild BFT and Mg concentrations in Fdom and Rdom samples were close to each other. That might be related to swimming activity. Because, BFT swim quickly, and the myoglobulin content in their caudal muscles is high. Thus, increased Mg concentration in muscle tissues might be related to the high myoglobulin content in their caudal muscles, because Mg is used for several enzymatic pathways, metalloenzymes, and biochemical process in muscle tissues.

On the other hand, the muscle activity in Front area (Fdom) is less than in Rear area (Rdom). These effects might decrease the need for Mg in Fdom (Evans, 1998; Block and Stevens, 2001; Bridges et al., 2002; Nelson and Cox, 2004; Nakamura et al., 2006).

In fattened fish, Mg concentration in Fdom was higher than in Rdom. This could be related to the feeding and environmental conditions in the farm-raised fish because they were overfed and had a lack of activity in their cages. These two factors might be lead to obesity and may suppress their ability to exercise and for muscle activity. Thus, the use of Mg in tissues of Fdom could be less or it may be stored in cells. On the contrary, Mg values in Rdom of fattened fish were found to be low. That might be related to cage confinement stress because the weights of fattened fish in cages increased 25-35% by the end of the fattening time and narrow cages might cause the stimulation of corticosteroid hormones, cortisol, testosterone, etc. and increases in reactive oxygen compounds (oxygen radicals). Therefore, the enzymatic reactions in caudal muscles might accelerate the use of Mg to detoxify free radicals (Evans, 1998; Nelson and Cox, 2004; Nakamura et al., 2005; Wu, 2006; Percin and Konyalioglu, 2008).

According to the gender distribution, Mg concentrations in Fdom and Rdom samples of both wild and fattened female tuna were higher than those in the male samples. This could indicate that the use of Mg in the muscle tissues of males is greater than in females, or that females stored more Mg in their muscles. This might be

Table 2. Magnesium concentrations in Fdom and Rdom samples of female and male wild/fattened bluefin tuna (mg 100 g^{-1} w wt).

	Wild (*n* = 90)										**Fattened** (*n* = 90)									
	Female (*n* = 45)					**Male (*n*= 45)**					**Female (*n* = 45)**					**Male (*n* = 45)**				
	min	**max**	**med**	**mean**	**s.e.**	**min**	**max**	**med**	**mean**	**s.e.**	**min**	**max**	**med**	**mean**	**s.e.**	**min**	**max**	**med**	**mean**	**s.e.**
Fdom	29.59	61.30	56.95	51.31	5.12	28.12	62.28	52.40	46.12	4.38	35.57	66.96	54.23	56.46	5.82	34.24	60.74	50.01	48.92	4.67
Rdom	43.87	74.33	52.11	55.09	6.07	41.79	70.69	49.08	51.40	4.83	26.81	51.90	42.91	42.33	4.16	27.55	50.02	39.61	38.26	4.05

related to reproductive hormones (follicular stimulated hormones, luteinized hormones, etc.) or lower levels of stress in females. Researchers have concluded that higher Mg content in tissues may decrease stress levels in fish. On the other hand, male fish might be under more pressure in farmed areas, probably due to the issue of cage confinement. Thus, the use of Mg in muscle tissues of males was higher, and the level was lower than in females (Block and Stevens, 2001; Lim and Klesius, 2003; Wu, 2006; Percin and Konyalioglu, 2008).

According to the sampling areas, Mg values in Fdom and Rdom samples of wild females and males were higher than in fattened females and males (Table 2). Thus, the mean Mg concentration of Dom samples was higher among the wilds (Figure 2). One possible reason might be the reproduction period, gonad development, maturation, and oocyte ovulation in female and male organisms contained Mg in various organs and muscles for enzymatic activities and biochemical reactions for gonad improvement, maturation etc. Another reason for the high level of Mg content in the muscle tissues of wild fish could be related to their life in natural habitats. It may be that lower chronic stress factors affect wild fish. Among the fattened specimens, several environmental differences lead to long-term chronic stress, which could affect their muscle physiology and biochemistry. Thus, the use of essential nutrients such as magnesium, phosphorus, sulphur, etc. In metalloenzymatic reactions of the muscles may be influenced and increased, causing the concentrations of the nutrients to be decreased (Watanabe et al., 1988; Bridges et al., 2002; Block and Stevens, 2001; Wu, 2006; Percin et al., 2010).

Although, tunas are acknowledged to be top-level predators able to concentrate large quantities of metals and represent a dietary source for humans, to our knowledge only few papers have dealt with metal determination in the Mediterranean (Licata et al., 2005; Storelli et al., 2005; Tuzen and Soylak, 2007; Vizzini et al., 2010).

On the other hand, studies on Mg concentration in various fish species is rare and many of them are mainly nutritional and toxicological research commonly on trout, carp, catfish, sea bream and mullets. Knox et al. (1981) found that the range of Mg concentrations in the muscle tissues of rainbow trout is 9.86-13.47 mmol kg^{-1} w wt. Shearer (1989) determined that the Mg concentration in the bodies of rainbow trout was 37 mg 100 g^{-1} wet basis. Uysal et al. (2008) determined the Mg concentrations in the muscles of striped sea bream, gilthead sea bream, grey mullet, and flathead mullet were 304.20, 332.56, 278.60, 295.70 (mg kg^{-1} w wt), respectively. These results were different from our findings, which could be related to the study areas or the fish species.

Percin et al. (2010) detected Mg levels in blood serum samples of wild and fattened BFT of 7.83 and 6.68, (mg 100 ml^{-1}) respectively. In addition, Mg values were found in wild female and male and fattened female and male specimens at 5.92, 8.47, 6.52, and 6.83 (mg 100 ml^{-1}), respectively. In our research, Mg concentrations were detected in muscle samples of all fish (wild/fattened, female/male). Thus, all findings were different from those of Percin et al. (2010), but the values harmonized with each other. Moreover, Hellou et al. (1992) determined that the mean Mg concentration was 680 mgl^{-1}, dry wt in 14 muscle samples of BFT that had been collected from the Virgin Rocks, Newfoundland, Canada. The higher Mg value could be related to the study materials. Hellou et al. (1992) worked on dry samples. Our research, utilized wet muscle samples.

The USDA (2009) provides a standard nutrient database reference for Mg concentrations in muscles of BFT, as 50 mg 100 g^{-1} w wt. In this study, the mean Mg concentration in Fdom and Rdom samples of female and male wild fish were determined to be 51.31; 55.09; 46.12; 51.40 mg 100 g^{-1} w wt, respectively. For the fattened fish, the same parameters were detected as follows:

56.46; 42.33; 48.92; 38.26 mg 100 g^{-1} w wt. The results were determined in the range of the USDA levels except Rdom samples of fattened BFT. This might be related to the feeding habits, types of food, and environmental factors in the cages and the size and age of fish because the fish we examined had nearly identical fork lengths and

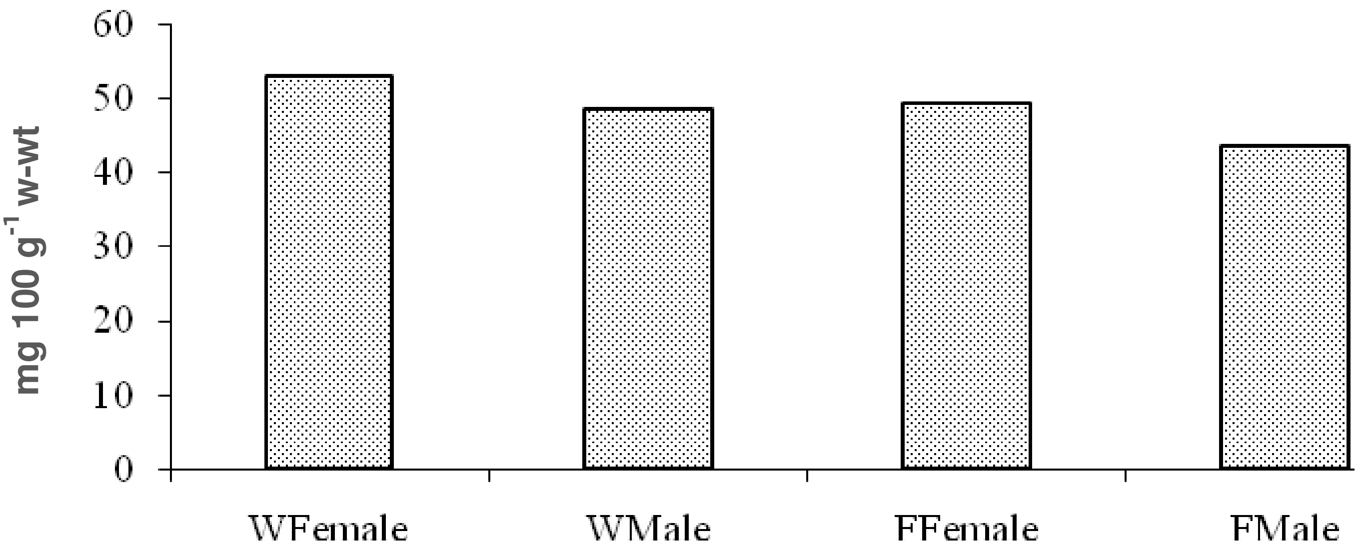

Figure 2. Magnesium concentrations in dorsal ordinary muscle (Dom) samples of female and male bluefin tuna.

Table 3. Significance in magnesium concentrations between female and male wild/fattened bluefin tuna.

	W_{Female}-W_{Male}	W_{Female}-F_{Female}	F_{Female}-F_{Male}	F_{Male}-W_{Male}
Fdom	*	*	*	*
Rdom	*	*	*	*

*: Significance ($p < 0.05$); $\mathbf{W_{Female}}$: Wild female, $\mathbf{W_{Male}}$: Wild male, $\mathbf{F_{Female}}$: Fattened female, $\mathbf{F_{Male}}$: Fattened male.

weights of 150-160 cm and 50-60 kg, respectively. In related literatures on age-related differences in BFT, the fish were approximately 6-7 years old (Cort, 1991; Perçin and Akyol, 2009; Santamaria et al., 2009; Percin and Akyol, 2010). Thus, the results in this research might be applicable only to fish with 150-160 cm fork length and weighing 50-60 kg.

Conclusion

The concentrations of magnesium in Fdom and Rdom samples of all specimens were found to be fattened female Fdom > wild female Rdom > wild male Rdom > wild female Fdom > fattened male Fdom > wild male Fdom > fattened female Rdom > fattened male Rdom. The highest Mg levels in the muscle samples of the study were in Fdom of the fattened female fish. This could be related to the feeding and fattening in cages and the physiology of the female specimens.

As a result, in the study, magnesium concentrations in the Fdom and Rdom samples of wild and fattened and female and male bluefin tuna were determined. In addition, similar studies and examinations should be conducted on Mg burdens in different sizes of other BFT species, *T. thynnus*, *Thunnus maccoyii*, and *Thunnus orientalis*, in different areas.

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