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Determination of Some Minerals and Heavy Metals in Muscle Tissues of Rainbow Trout, *Onchorhynchus mykiss,* Cultured in Iran

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Abstract: Iran has the first rate of coldwater fish culture in Asia since 2005. Rainbow trout, *Oncorhynchus mykiss* is the main and the first fish in fisheries production of Iran. However, there is a few information about carcass composition of cultured fish in Iran. Rainbow trout samples were collected from six fish fresh and brackish water fish farms of Yazd province during February 2008. Fish muscle samples were freeze in -30°C after being homogenized, till laboratory analyses. Concentrations of metals were analyzed by atomic absorption spectrophotometer after digestion of homogenate muscle tissues with concentrated acid. Measured concentrations of minerals were as: Na 822.58-1052.78ppm, K 12272.75-13715.25ppm, Ca 104.75-171.03ppm, Fe 19-40ppm, Cu 0.044-1.01ppb, Zn 7.02-12.47ppb, Cd 0.879-1.099ppb and Cr 1.895-3.86ppb. Concentrations of studied minerals were not significantly different in fish muscle raised in fresh and brackish water farms. Results showed muscle of cultured *O. mykiss* in Yazd province of Iran were a good source of Na, K and especially Fe, with markedly low concentrations of Cd and Cr, then without any risk for human consumption.

Key words: Mineral · Heavy Metal · Oncorhynchus mykiss · Culture · Yazd · Iran

INTRODUCTION

Oncorhynchus mykiss is the one of the most important cultured fish in the world and the first fish cultured in Iran since 1960 [1]. Iran has one of the highest rates of coldwater fish culture in Asia and the world since 2005 [2]. Rainbow trout, *O. mykiss* is the main and the first fish in aquatic culture production of Iran. Fish culture has developed in Yazd province of Iran since recent decades and the rainbow trout, is the most important fisheries product in the area and the only cultured fish in cold seasons in the most areas of Iran. It is cultured in underground brackish water, because of limited fresh water resources in Yazd province. Culture of *O. mykiss* in underground brackish water is not widely practiced in the world and its brackish water culture mainly limited to pen and cage culture in some countries mainly done in Norway, Finland and Denmark [3-5]. Also, experimental treatments of salinity are used in some studies about carcass quality and proximate composition of rain bow trout cultured in brackish water. Body composition of *O. mykiss* cultured in fresh and brackish water has revealed only little differences [6-8].

Some aspects of *O. mykiss* culture and feeding [9-15] and breeding [16, 17] in underground brackish water of Bafq were studied. Also, concentration of minerals, rare and heavy metals in different tissues of aquatics of Iran [18-35] were studied in recent years. Although this reports mainly indicated levels of minerals closed to desired values and concentrations of heavy metals lower than permissible values in muscle tissue of studied aquatics, but undesirable amounts of metals such as low concentrations of some essential minerals in some marine fish of Caspian sea [30] and high levels of Cadmium in muscle of *Mugil auratus* [22] are reported.

Fish is one of the most important sources of animal protein and other elements for the maintenance of a healthy body. Food scientists recommended people to consume fish but there is a little information about carcass composition and feeding value of cultured fish in Iran.

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Also some people have believed that cultured fish have lower quality than naturally growing fish. In the present study, concentrations of some minerals and rare metals in muscle tissue of cultured *O. mykiss* in Yazd province are examined.

MATERIALS AND METHODS

Six farms of *O. mykiss* with similar culture management were selected in different areas of Yazd provinc at (1) Eslamieh in suburb of Taft, (2&3) Chah-Beigi in suburb of Abar-Kooh, (4) Chah-Afzal in Suburb of Ardakan, (5) Research Station in suburb of Bafq and (6) Behabad. Culture management was similar from the viewpoint of fish nutrition and water management in the selected farms. Stocking of fries were accomplished in early November 2007. Ponds' water salinities were recorded using a WTW salinometer. Main factors of culture such as fish nutrition and water management were considered during the culture period. Fish food prepared from the same factory in all farms.

Water sample was collected from 1m depth of each pond. Fish samples were collected during late February 2008 when farmers catch fish to demand. 9 cultured *O. mykiss* fish were collected from each of selected ponds of the 6 farms to obtain at least 500g muscle tissue [36]. Samples were transited alive to the laboratory. Total length and body weight of each sample was measured. Fish were dissected and the muscle sample was removed from the left side of the fish between the dorsal fin and the operculum. Muscle samples were homogenized using 20000rpm grinder. 100g packages of the samples freezed in -30°C till examinations [37-42].

Pond water samples were analyzed for Na, K, Ca, Fe Cu, Zn, Cd, Mn, Ni, Pb and Cr using flame atomic absorption spectrophotometer and for Al, Ag, Ba and Ti using graphite furnace atomic absorption spectrophotometer, Nova 400. Extraction of metals from ash of fish food and muscle samples accomplished by concentrated acids [43, 44]. Fish food and muscle samples were analyzed for Na, K, Ca, Fe, Cu, Zn. Concentrations of Cd and Cr were also analyzed in fish food and muscle samples because of their relative high concentrations in pond water. Results of metal accumulations in muscles of fish from fresh and brackish water farms were compared by t-test ($p<0.05$). Pearson correlation was used to analyze correlation between total length and body weight of fish and water salinity with concentration of minerals and heavy metals in muscle of studied fish ($p<0.005$).

RESULTS

Fish Culture continued about 4.5 months in different farms. *O. mykiss* samples were collected from three fresh water farms at Eslamieh, Chah-Beigi and Behabad and three brackish water farms at Chah-Afzal, Bafq and Chah-Beigi. Table 1 shows water salinity of the mentioned farms and the total length and weight of fish when sampling at the end of culture period.

Concentration of minerals (ppm) including Na, K, Ca, Fe and Se and rare elements and heavy metals (ppb) including Cu, Zn, Cd, Mn, Ni, Pb, Cr, Al, Ag, Ba and Ti of water in fish culture ponds of different farms are shown in Tables 2.

Concentrations of some main minerals and elements in muscle tissue of examined fish were: Na 329-421 ppm, K 491-549 ppm, Ca 4.19-6.84 ppm, Fe 7.6-16 ppm, Cu 0.044-1.01 ppb, Zn 7.02-12.47 ppb, Cd 0.879-1.099 ppb and Cr 1.895- 3.86 ppb (Table 3).

Concentrations of studied minerals and heavy metals between fish muscle cultured in fresh water farms of Eslamieh, Chah-Beigi (B) and Behabad and brackish water farms of Abar-Kooh, Chah-Afzal and Research Station were not significantly different by t-test ($p>0.05$). Pearson correlations between total length and body weight of fish and water salinity with concentration of minerals and heavy metals in muscle of studied fish were not significantly different ($p<0.005$).

Table 1: Water salinity (ppt), total length (cm) and body weight (g) of cultured O. mykiss collected from earth ponds at different areas of Yazd province, winter 2008. (B: Brackish water, F: Fresh water).

Area	Water salinity	TL±SD	BW±SD
Eslamieh	0.4	24±2.8	160.9±44.03
Chah-Beigi (B)	8.7	29.3±2.9	310.8±82.7
Chah-Beigi (F)	0.3	38.3±2.7	617±32.2
Chah-Afzal	8.5	25.8±2.7	220.5±69.8
Research Station	9.5	26.2±2.7	241.2±75.2
Behabad	0.84	26.2±1.3	233.4±41.7

Table 2: Concentrations of Na, K, Ca, Fe, Se (ppm) and Cu, Zn, Cd, Mn, Ni, Pb, Cr, Al, Ag, Ba and Ti (ppb) in water of O. mykiss culture ponds of different areas in Yazd province, winter 2008.

Area	Na (ppm)	K (ppm)	Ca (ppm)	Fe (ppm)	Se (ppm)	Cu (ppb)	Zn (ppb)	Cd (ppb)	Mn (ppb)	Ni (ppb)	Pb (ppb)	Cr (ppb)	Al (ppb)	Ag (ppb)	Ba (ppb)	Ti (ppb)
Eslamieh	276	7.75	102.07	0.25	0.10	5.93	<0.01	20.16	4.176	3.86	17.39	39.92	12.33	<0.01	23.5	11.46
Chah-Beigi (B)	4140	37.4	133.53	0.47	2.85	17.41	19.08	53.53	19.13	39.8	70.49	110.5	<0.01	18.97	15.84	27.56
Chah-Beigi (F)	399	27.41	35.34	0.45	1.43	4.38	3.77	33.84	12.93	22.12	37.57	72.39	<0.01	<0.01	<0.01	3.75
Chah-Afzal	3680	3.71	266.85	0.56	3.40	5.09	18.71	54.96	21.04	53.67	95.03	156.2	11.07	26.96	44.08	54.94
Research Station	3300	24.04	218.68	0.82	2.98	15.09	19.59	54.64	22.6	50.13	107.2	151.9	19.058	24.04	0.084	55.1
Behabad	1150	4.95	72.03	0.45	2.11	11.18	10.19	41.56	18.33	36.92	106.5	175.6	<0.01	16.23	19.07	55.22

Table 3: Concentrations of some minerals, rare elements and heavy metals in fish food and muscle tissue of cultured O. mykiss in different fish farms of Yazd province, winter 2008.

Area	Na (ppm)	K (ppm)	Ca (ppm)	Fe (ppm)	Cu (ppb)	Zn (ppb)	Cd (ppb)	Cr(ppb)
Eslamieh	913	13715.25	171.03	21	1.01	12.47	0.879	1.895
Chah-Beigi (B)	936	12272.75	121.38	40	_	8.07	0.879	2.37
Chah-Beigi (F)	1053	12740.83	160.15	27.5	0.98	7.02	0.918	2.672
Chah-Afzal	998	13374.45	129.38	24.3	0.15	8.31	0.966	3.86
Research Station	1040	13251.05	122.95	25	0.46	7.32	1.004	2.646
Behabad	823	13129.05	104.75	19	0.04	7.44	1.099	2.88
Fish food	_	_	6.3	13.3	8.2	149.9	2.479	6.449

DISCUSSION

Favorite amount of sodium in 79g fillet of cultured rainbow trout is 27.7 mg [45]. Human needs 225-500 mg/day sodium [46]. Concentration of sodium in muscle tissue of fish from different farms was 823-1052 ppm wet weight that is desired for feeding (Table 4). Sodium concentration in fresh water of fish culture ponds of Chah-Beigi, Eslamieh and Behabad was lower than brackish water of Chah-Beigi, Bafq and Chah-Afzal. However, it was not considerably different in muscles of fish from different areas.

Aquatic foods are often rich sources of potassium. Human daily need to potassium is about 1000-2000 mg [46]. Favorite amount of potassium in 79g fillet of cultured rainbow trout is 356 mg [45]. Concentration of potassium in muscle tissue of fish from different examined farms was 12273-13715 ppm wet weight that is desired for feeding (Table 4). Higher concentrations of potassium in brackish water ponds than fresh water ponds are expected but it was not considerably different in muscles of fish from different areas.

Ca concentration in muscle tissue of examined fish was 105-171 ppm wet weight. Favorite amount of calcium in 79g fillet of cultured rainbow trout is 52.9 mg [45]. Human daily need to calcium is about 100-1300 mg depending on age, health and body condition [47]. So, cultured fish had lower amounts of calcium from favorite fillets and not desirable for feeding (Table 4). Fish food analysis revealed 0.006 mg/g calcium that is considerably lower than high quality fish food with 1-17 mg/g calcium [48-50]. Calcium concentrations of water in different examined ponds were not at the same level. However, it was not considerably different in muscles tissue of fish from different ponds.

Fe concentrations in examined fish muscle tissue were 19-40 mg/kg wet weight. High quantities of Fe were recorded in all studied farms especially in brackish water ponds. Optimum amount of Fe in a 79g cultured fillet of *O. mykiss* is 0.2 mg [45]. Dietary need of men and women to Fe is 8 and 18 mg/day, respectively [51]. Cultured rainbow trouts of studied farms are good sources of Fe for nutrition (Table 4).

Fish food analysis showed low level about 13mg/kg Fe compared with high quality fish food with 50-60 mg/kg [48, 49]. As fish food had 13 mg/kg Fe that is lower than optimum level of 50-60 mg/kg [48, 49], higher amounts of Fe in cultured fish of brackish water ponds may be concluded from accumulation of Chironomid populations in sediments of these areas [13]. Feeding of rainbow trouts from chironomid larvae especially their nymphs is visible by consideration around the culture ponds. Chironomid larvae live in sediments of many cold waters [52] are rich sources of Fe for aquatics as live food.

Table 4: Human daily intake, desired and permissible values for human consumption and reported concentrations of some minerals and heavy metals in muscle tissue of fish from different areas mainly in Iran in ppm wet weight (ww) or dry weight (dw). [A wet weight/dry weight conversion factor of 0.2 is suggested [81]. Conversion factor of wet weight/dry weight in fish of 6 examined areas in the present study was acquired 0.23 to 0.26.].

Optimum of / Fish and area	Na	K	Ca	Fe	Cu	Zn	Cd	Cr	Reference
Daily intake	225-500 mg/day	1000-2000 mg/day	1000-1300 mg/day	8-18 mg/day	0.7-0.9 mg/day	8-12 mg/day	10-20 µg/day	20-35 µg/day	Food & nutrition Board, 1989&2001; Foroozani, 2007
Desired value in edible fish fillet: ww	351	4506	670	2.5					www.nutritiondata.com; Parr, 1988
dw	1755	22530	3350	12.5	4	37			
permissible value in edible fish fillet (dw)					100-350	750	5_10	8	NHMRC,1987; BOE,1991;EEC,1979
Yazd, Culture ponds, *Oncorhynchus mykiss* (ww)	823-1053	12273-13715	105-171	19-40	0.04-1.01 (ppb)	7-12.5 (ppb)	0.88-1.1 (ppb)	1.9-3.9 (ppb)	Present study
Turkey, *Oncorhynchus mykiss* (ww)	255	4120	127	4.2	8.19	5.45	0.01		Celik *et al.*, 2008
Ponds of Guilan (dw):									
Hypophthalmicthys molitrix			110.69		1.74	9.71			Arabani *et al.*, 2010
Ctenopharyngodon idella			89.29		2.26	8.68			
Persian Gulf (dw):									
Selar crumenophthalmus				25.07	15.54	2.31			Shahab Moghadam *et al.*, 2010
Himantura gerrardi				74.78	3.5	875.99			
Gorgan Bay (ww):									
Carp, mullet, kutum							0.014-0.018		Shahryari *et al.*, 2010
Persian Gulf (ww):									
King mackerel					1.84		0.17		Dobaradaran *et al.*, 2010
Tigertooth croaker					1.55		0.23		
Persian Gulf (dw):									
Johnius belangerii				17.5-25.3	6.93-10.5		0.14-0.79		Doraghi *et al.* 2009
Caspian Sea (dw):									
Rutilus frisii kutum	488.17	2174	275	4.49	2.36	5.46		0.78	Pirestani *et al.*, 2009
Liza aurata	650	2201	274	6.16	1.1	7.66		0.16	
Cyprinus carpio	786	2388	966	12.55	2.58	7.44		0.74	
Sander lucioperca	486	2678	635	4.18	2.48	3.34		0.67	
Clupeonella cultriventris caspia	679	1809	828	4.6	1.75	17.23		0.11	
Caspian Sea (dw):									
Mugil auratus				81.11	3.14	43.46			Zeinaly *et al.*, 2009
Rutilus frisi kutum				73.59	3.69	37.99			
Ciprinus carpio				94.78	3.39	73.81			
Persian Gulf (dw):									
Liza dussumieri				26.4-82.6	1.15-3.19	19.72-29.4			Naseri *et al.*, 2006
Caspian Sea (dw):									
Mugil auratus					0.996	14.33	0.321		Amini Ranjbar & Sotoodehnia, 2005
Caspian Sea (ww):									
5 sturgeon species					1.23-1.91	17.95-24.5	0.0015-0.006		Pourang *et al.*, 2005
Caspian Sea (dw):									
2 Sturgeon species					1.46-1.8	26.9-47.4	0.059-0.061		Sadeghi Rad *et al.*, 2005
Persian Gulf (dw):									
Lutjanus coccineus							0.063	0.333	Shahriari, 2005
Otolithes rubber							0.064	0.062	
Persian Gulf (dw):									
Otolithes ruber				4.5-4.6	2.2-3.7	10.7-14.8			Mortezavi, 1999
Pumpus argenteus et al				2.25-2.5	3.1-3.5	14.2-14.7			

Formerly, results of the analysis of brackish water ponds of Research Station in Bafq for minerals showed high level of Fe, too [53].

Cu concentration in muscle tissue of cultured fish varied between 0.001 ppb in brackish water pond of Chah-Beigi to 1.01 ppb in Eslamieh. Dietary need of human to Cu is 7-9 mg/day [51]. Maximum permitted level of Cu in muscle tissue of edible fish is considered from 100 [54] to 350 [55] µg/g dry weight. Concentration of Cu in reference sample of fish muscle tissue provised by International Atomic Energy Agency is 4 mg/g dry weight [57]. (Table 4).

Concentration of Cu in examined pond water was 4.38-17.41 ppb. Maximum permitted level of Cu concentration in pond water at long time exposure of fish is 0.006 mg/lit, however cold water fish can tolerate high concentrations of Cu up to 0.03mg/lit (30ppb) [58]. So, Cu concentration in water of pond cultures is in tolerance range of fish.

Fish food analysis showed 0.008mg/kg Cu that is very lower than its level in a high quality fish food with 1-4 mg/kg Cu [48, 49].

In muscles, gills, livers and bones of five fish species including *Sargus sargus*, *Siganus rivulatus*, *Mugil* *cephalus*, *Caranx crysos* and *Scomberomorus commerson* from Egypt, liver had accumulated the highest level of Cu while the muscle had the lowest. Relatively high liver content that was recorded for *Siganus rivulatus* and *Mugil cephalus* could be related to food habits, where the first is herbivorous while the second is omnivorous. It was also observed that gills accumulate less copper than liver, indicating that the food is the primary path way for uptake of copper in all studied species [39]. Food probably represents a more important source of copper than water and thus burdens in fish can not be consistently related to ambient pollution levels in water [59]. Similar results were also reported by many investigators [18, 60].

Despite the observation that metal levels in invertebrates are frequently higher than in the predator fish [61], researchers [62] showed that liver Cu concentrations were also higher than dietary Cu contents. Higher metal loading to the Río de la Plata in Brazil resulted in a higher metal concentration in the diet of the detritivorous fish *P. lineatus*, but this did not result in a higher tissue metal content and suggests efficient homeostatic mechanisms [38].

Concentration of Zn in muscle tissue of examined fish was between 7.02-12.47 ppb wet weight without considerably difference in studied farms of Yazd province. However, concentration of Zn in brackish water ponds was higher than fresh water ponds. Maximum permitted amount of Zn in muscle tissue of edible fish is considered 750 μg/g dry weight [55]. Concentration of Zn in the reference tissue muscle mentioned by Atomic Energy Agency is 37 mg/kg dry weight [57]. Accumulation of Zn in examined fish was low. Dietary need of human to Zn is 8-12 mg/day [51]. Human receive of Zn must not exceed 45 mg/kg/day [56]. Optimum amount of Zn in a 79g cultured fillet of *O. mykiss* is 0.3 mg [45]. (Table 4).

Fish food analysis showed 0.15mg/kg Zn that is at lower threshold of its level in a high quality fish food with 15-30 mg/kg Zn [48, 49].

Researchers [62] showed that muscle was the poorest indicator of environmental exposure to Cu and Zn.

In a similar study on cultured rainbow trout, *O. mykiss* in Turkey [64], minerals including K, Mg, Na, P and Ca were the most abundant minerals in muscle tissue of fish. Low amounts of rare elements including Co, Cu, Zn and Fe were recorded, too.

Accumulation level of Cd in muscle tissue of cultured *O. mykiss* was 0.879-1.099 ppb wet weight. Maximum permissible content of Cd in muscle tissue of edible fish is considered 5 [54] or 10 [55] μg/g dry weight. The tolerable limit of human for cadmium is 7 ìg/kg bw/week [65] and 10-20 ìg/day [66]. (Table 4).

Concentration of Cd in water of studied ponds was 20.16-54.96 ppb, higher in brackish water ponds but not considerably different in fish muscle of these ponds. Cd residues in fish muscle can not be related to the concentrations in water [39].

The distribution pattern of Cd in a study on five fish species in Egypt was in the decreasing order of liver > Gills > Bone > Muscle. In other words, the liver seemed to be the organ which accumulates the highest value of Cd. This is in agreement with WHO/IPCS- Environmental Health Criteria of Cd which reported that Cd is stored in the body in various tissues, but the main site of accumulation in aquatic organisms is in the kidney and liver, beside other tissues, notably the gills and exoskeleton [39]. Higher contents of Cd in liver tissue of fish than fish food confirms its bioaccumulation. Bioaccumulation of Cd in different organs of fish especially liver is suggested by many investigators [38, 67, 61].

Concentration of Cr in muscle tissue of the studied fish was 1.895-2.88 ppb wet weight, highly lower than allowed level. Its permissible level in muscle tissue of edible fish is 8μg/g dry weight [36]. Adults need 20-35 μg/day Cr [51]. (Table 4).

Cr contents recorded in bone and liver tissues of five fish species from Egypt were not considerably different with their muscle tissue. Accumulation of Cr in different body tissue of fish is poorly related to their feeding habits [39].

Pattern of rare elements in muscle tissue of rainbow trouts in the present study was as Zn > Cr > Cd > Cu.

Accumulations of rare and heavy metals in muscle tissue of examined fish from different areas of Iran (Table 4) were often closed to desired levels and below the international guidelines for human consumption. High upper concentration of Zn in *Himantura gerrardi* collected from Persian Gulf was attributed to the metabolic characteristics of the species related to bottom living and feeding habits [34].

In an investigation on five fish species in Egypt, concentration levels of Cd, Cr, Cu, Pb and Zn in muscles of were lower than the permissible limits. Metal concentrations were lowest in muscle and highest in gill and liver tissues due to their physiological roles in fish metabolism where target tissues of heavy metals are the metabolically active ones. Therefore, metal accumulation in these tissues is higher compared to muscles where metabolic activity is relatively lower and so there is no risk yet for human consumption of flesh of these fishes. Residues in fish muscle can not often be related to their concentrations in water [39]. Environmental higher metal content resulted in a higher metal concentration in the diet of the detritivorous fish, but this did not result in a higher tissue metal content and suggests efficient homeostatic mechanisms [38].

Generally, low concentrations of heavy metals indicate that the muscle is not an active tissue in accumulating heavy metals as reported by many authors for some fish species [68-72].

Effect of inland groundwater salinity on body composition of some marine species such as milk fish, *Chanos chanos* [73] and mullet, *Mugil cephalus* [74, 75] is previously indicated. Body composition of *O. mykiss* cultured in fresh and brackish water has revealed only little differences [6-8]. Salinity did not affect body composition rainbow trout, *O. mykiss* significantly, except for ash, which was a little higher in salt water than in fresh water [6]. Quality of salmonids is mainly affected by parameters such as feed type, level of dietary intake and growth. Feed composition has a major influence on the proximate composition of salmonids. In particular, whole body lipid as well as the lipid content in the edible fillet is directly related to dietary fat content. Tactics for the rearing of Salmonids for specific purposes should therefore take into consideration the fact that the level of proximate constituents in the whole body as well as the fillet are readily manipulated by feed composition and feeding strategies [76]. Salinity may affect the relative contribution of carrier-mediated and independent uptake to total nutrient absorption in *O. mykiss* [77]. Results of the present study coincide with these finds as concentrations of studied minerals between fish muscle cultured in fresh water and brackish water farms were not significantly different. Also there was not significant correlation between water salinity with concentration of minerals and heavy metals in muscle of studied fish.

As the results of this study showed, fish food used for feeding of cultured fish had very low amounts of Ca, Cu and Zn. High quality fish meal is rich in minerals especially calcium, phosphorus and iron [78, 42]. In addition, fish often obtain majority of rare elements such as Cu and Zn from dietary sources rather than from surrounded water [18, 59, 79, 80].

CONCLUSION

Results of the present study showed muscle of cultured *O. mykiss* in Yazd province of Iran were a good source of Na, K and especially Fe, with markedly low concentrations of Cd and Cr, then without any risk for human consumption. However, much attention needs in feeding management of cultured fish and enrichment of fish food from Ca, Cu and Zn based on definite levels of high quality food.

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