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THE ELEMENTAL COMPOSITION OF OREOCHROMIS NILOTICUS TISSUES.

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ABSTRACT

A number of investigations have been made on the metal requirements for fish diets, but more infromation is needed. Few studies of the tissue metal contents of farmed fish have been made, and in order to extend our knowledge of fish metal utilization the metal contents of some tissues of **Oreoohrmis niloticus** of different ages and sex have been determined. Fishes of circa 4.0, 8.0 and 17.0 cm in standared length were used for the estimation of Ca, P, Mg, K, Na, Zn, Cu, and Fe in skin, muscle and bone. Tissues were dried, digested and all elements excluding P were measyred by atomic absorption. Singificant differences were observed between small and large fish. Some of these differences were as expected, e.g. increase in Ca, P, Mg, in bone with age. However, other significant changes were found. For example, a considerable decrease in zn content of the skin from circa 19.0 mg % dry weight in small fish to 11.6 mg % dry weight in large fish, and of the muscle from 0.7 mg % dry weight to 0.18 mg % dry weight. Other elements such as Fe showed little variation with age. No sex differences were observed.

INTRODUCTION

Requirements of many farmed fish species for various nutrients have been determined and contributed to healthier, more rapidly growing fishes cultured in hatcheries and commercial facilities (Millikin, 1982). However, dietary mineral requirements for such farmed species have received little attention, perhaps because they are difficult to study. Unlike most terrestrial animals, fishes have the ability to absorb some ions not only from their deits but also from the surrounding water. The demand from the aquaculture industry for complete, dry fish diets has greatly increased the interest in the mineral metabolism of fishes. Although little information is available on the complete nutritional requirements, functions, and availability of minerals to fish, most of the published work (some comprehensive reviews may be consulted such as Nose and Arai, 1979; Lall, 1979; Cowey and Sargent, 1979, and Millikin, 1982) is in agreement with the fact that minerals have a marked effect on growth, survival and malformation in farmed species such as common carp, **Cyprinus carpio** (Ogino and Chiou, 1976; Ogino and Takeda, 1976; Sakamoto and Yone,

1978; Pfeffer, 1978 and Satoh et al., 1983), rainbow trout, **Salmo gairdneri** (Ogino and Kamizono, 1975; Ogino and Takeda, 1976; Ogino and Yang, 1978; Ogino et al, 1979; Ketola, 1975; Knox et al., 1981 a,b, and 1982; Satoh et al., 1983 a,b & c, and Yamamoto et al., 1983), channel catfish, **Ictalurus punctatus** (Andrews et al., 1973; Lovell, 1978. Murai et al., 1981; Wilson et al., 1982. and Gatlin et al., 1982), and Red Sea bream, **Chrysophrys major** (Sakamoto and Yone, 1973, 1978 and 1979).

The formulation of an artifical diet requires precise knowledge of the level of the nutrients which will produce optimal growth with an economic cost of production. Recently, there has been an attempt by Tacon and De Silva (1983) to evaluate the mineral composition of commercially available diets and to examine their suitability using criteria for mineral requirements. They found large differences in the mineral composition within and between the different feed categories. The availability of dietary minerals to fish and their evaluation by the growth rate of fish and the chemical analysis of body tissues have been reported in a number of farmed fish species (Pfeffer, 1978) but more information is needed. In order to extend our knowledge of mineral utilization, the mineral contents of **O. niloticus** tissues of different ages and sex have been determined in the present study.

MATERIALS AND METHODS

Oreochromis niloticus were obtained during February and March 1984 from the stocking tanks at Aston University in Birmingham. Fish were of three different sizes (small, medium and large, circa 4, 8, 17 cm respectively), kept in fibre glass tanks, pulmbed with a recirculating system, which included gravel filters and faecal traps. Fish were fed twice daily with commercial trout diets. Fish were selected randomly from each of the three tanks and anaesthetized with benzocaine (1 ml saturated solution of benzocaine in 5 l water). Individual fish were weighed to the nearest 0.01 gm and standard length measured to the nearest 1 mm. Fish were sexed when possible and the state of gonads were recorded. Skin, including scales, and muscle from each fish were removed. The vertebrae and the skull were excised from the carcass after boiling. Samples were wet-weighed individually, dried at 105 $^{\circ}$C over night and then weighed again to estimate the moisture content. The dried skin, muscle and bone were digested in a concentrated nitric acid/ perchloric acid mixture (10 : 3, V/V). A Perkin-Elmer model 373 Atomic Absorption Spectrophotometer was used in accordance with the manfacturer's specifications for the determination of Ca, Na, K, Mg, Fe, Cu, and Zn within the digested samples. For P determination, samples were assayed by the molybdate method (Tauusky and Shorr, 1953). The mineral contents were expressed as gm % dry weight for Ca and P, and mg % dry weight for Na, K, Mg, Fe, Cu, and Zn. All data were subjected to analysis of variance and the statistical significance was established by student's t-test (Fisher, 1950).

RESULTS AND DICUSSION

For each group of fish, there were distinct differences regarding standrad length, body weight, age, and moisture content (Table 1). Gonads of large fish were also examined, where sex and state of maturity were determined. All specimens (Table 2) were in the resting stage (II). The following results are based only on individuals of **O. niloticus** examined off the breeding season.

The mean concentration of the mineral studied in the skin, muscle and bone of the different groups of fish (small, medium and large) is shown in Tables 4 and 5. The distribution of these elements within the tissues clearly indicates wide variation in the concentration of each element and these variations are highly significant. Calcium for instance varied between 0.9 gm % in the muscle and 22.1 gm % in bone; P from 1.02 gm % to 10.5 gm %; Mg from 108 mg % to 343 mg %; Zn from 1.98 mg % in muscle to 18.8 mg % in skin. Similarly, the distribution of the major minerals Ca, P, Mg, K, Na varied greatly in the different tissues of rainbow trout and carp (Pfeffer, 1978).

On examining the concentration of the minerals in each tissue, significant differences were found in the different fish groups. Some of these differences were as expected e.g. increase in Ca, P and Mg content with age. Bone calcium of small fish was 16.0 gm % and increased to 20.6 gm % in medium fish and reached its maximum value in large fish, being 22.1 gm %; for P it was 6.0, 10.1 and 10.5 gm %, and for Mg it was 249, 312,and 343 mg % respectively.The same pattern of increase in mineral content was also observed in skin but not quite so in muscles where there was a decrease in Ca, P and Mg content with age (Table 3). These differences may be explained in the light of the function of these elements in each tissue. Ca, P and Mg are known to be closely related in metabolism, particularly in bone and scale formation, while in muscles they participate in other physiological processes such as muscle contraction and maintenance of membrane integrity (Lall, 1979).

Na and K contents in tissues examind displayed a different pattern when compared with Ca, P and Mg contents. There were significant decreases in the concentration of K in the skin, muscle and bone of the fish. Skin K content was found to be higher in small fish than in medium and large fish, being 741, 448 and 245 mg % respectively. Na content exhibited this pattern in skin and muscle but not in bone. In bone there was an increase in Na content with age, being 343, 371 and 515 mg % in small, medium and large fish. However, Love et al. (1968) in their work on the muscle of **Gadus morhua** found that the potassium concentration did not alter according to the size of the fish, but that sodium always decreased as the fish grew.

Information on the availabillity of the major elements in finfish is limited. The availability of P in fish meal is low in **Oreochromis niloticus** when compared with carp and rainbow trout (Watanabe et al., 1980). However,

TABLE 1
Data on fish length (cm), body weight (g), age and moisture content (%) in *Oreochromis niloticus*.

Fish group	Number of Fish	Standard length (cm)		Body weight (g)		Age	Moisture (%)		
		Average	Range	Average	Range		Skin	Muscle	Bone
Small	12	4.1	3.0- 4.9	3.2	1.3- 6.1	Six month	24.2	74.4	70.1
Medium	12	8.0	7.3- 9.2	11.3	8.3- 15.1	Nine month	58.3	77.3	69.6
Large	15	17.7	12.2-19.1	197.6	85.3-276.3	Over One year	64.6	80.9	48.6

TABLE 2
Data on males and females of **Oreochromis niloticus.**

Sex	Male	Female
Number of fish	9	6
Standard length (cm)		
Average	17.7 ± 2.01	16.0 ± 1.7
Range	12.2 - 19.1	12.8 - 18.0
Body weight (g)		
Average	231.1 ± 45.8	121.5 ± 58.4
Range	118.0 - 276.3	85.3 - 190.8

± Standard deviation.
" Gonads of all individuals were in the resting stage (II).

the availability of fish meal P is significantly higher in rainbow trout than carp (Pfeffer, 1978 and Ogino et al., 1979). The difference in the availability of P among various finfish is probably due to the secretion of gastric juices (Yone and Toshima, 1979).

The contents of the trace elements studied (Fe, zn, and Cu) showed different patterns with the age of the fish when compared with the major elements such as Ca, P, and Mg. There were considerable decreases in Zn content of the skin from 18.8 mg % in small fish to 11.6 mg % in large fish, and of the bone from 15.1 to 10.1 mg % and of the muscle from 5.4 to 1.98 mg %. Cu content behaved similarly to Zn in all tissues,e.g. it decreased from 0.72 mg % to 0.18 mg % in muscle. Although there were significant differences in Fe content between small and large fish, the large individual variation in each tissue does not seem to reflect any consistant relationship with the age of the fish, but may reflect the processes including oxidation-reduction activity and electron transport.

Although the mineral requirement of small fish has not been studied in detail, it seems that their requirements for certain minerals, particularly Zn and Cu, is higher than that of adult fish in view of the high metabolic activity and growth in small fish (Tacon and De Silva, 1983). Ogine and Yang (1978) found that when rainbow trout and common carp fingerlings were fed a low of Zn (1 mg/kg dry weight), fish showed poor growth, high mortality, high incidence of cataracts, fine and skin erosion.

TABLE 4

Results of the analysis of variance performed on the mean concentration (g % dry weight) of Ca and P in the tissues skin, muscle, and bone) of the different groups of **Oreochromis niloticus.**

Source of variation	d.f.	M.S.	F-value
Fish group (F)	2	8.77	16.41*
Tissues (T)	2	268.77	316.04**
Minerals (M)	1	111.70	205.17**
F X T	4	4.80	8.98*
F X M	2	0.69	1.82
M X T	2	44.92	84.11**
Error	4	0.53	

d.f Degree of freedom
M.S. Mean square.
* Signifficant (P<0.05)
** Signifficant (P<0.01).

The range and mean concentration of the elements in large males and females are presented in Table 6. Statistically significant sex differences were not evident. It should be remembered that all specimens examined were in the resting stage. Therefore, further investigation is needed, particularly during the breeding season. The calcium content of fish scales, which represent 40% of the whole calcium of the body (Simkiss, 1974), was found to decrease in association with the develompent of the ovary. Fouda (1979) found also that the average rate of scale regeneration of the common goby, **Pomatoschistus microps,** increased sharply from 25% to 60% during the breeding season, and in some individuals it reached 90 % of the total scales. The loss of metals in fish scales during the breeding season is compensated for either directly from the fish diet, or indirectly from the other hard tissues (e.g bone) of the fish.

In conclusion, the present study indicates significant differences in the level of metals in the tissues of small and large fish. Thus for each age group of fish and perhaps for each season, there seem to be specific dietary intral requirements which should be considered in formulating fish diets.

TABLE 5

Results of the analysis of variance performed on the mean concentration (mg % dry weight) of the minerals Mg, K, Na, Zn, Cu, Fe in the tissues (skin, muscle and bone) of the different groups of **Oreochromis niloticus.**

Source of variation	d.f.	M.S.	F-value
Fish group (F)	2	12116.7	3.43*
Minerals (M)	5	944915.1	267.46**
Tissues (T)	2	203115.1	57.49**
F X M	10	10492.9	2.97*
M X T	10	416078.8	117.77**
F X T	4	6963.9	1.97
Error	20	353.9	

d.f. Degree of freedom.
M.S. Mean square.
* Significant (P<0.05).
** Significant (P<0.01).

TABLE 6

Range and mean concentration of minerals in males and females of some *Oreochromis niloticus* tissues.

Tissue		Muscle		Skin		Bone	
Sex:		Male	Female	Male	Female	Male	Female
Element							
Ca (g%)	Range	(0.017 - 0.109)	(0.04 - 0.134)	(10.3 - 16.1)	(11.0 - 15.2)	(16.3 - 25.5)	(21.3 - 25.3)
	Mean	0.045 ± 0.03	0.087 ± 0.05	12.4 ± 1.9	12.8 ± 1.6	21.2 ± 3.2	23.4 ± 1.4
P	Range	(0.097 - 1.05)	(0.93 - 1.38)	(4.6 - 7.5)	(5.6 - 6.6)	(8.9 - 11.1)	(10.5 - 11.3)
	Mean	0.99 ± 0.08	1.06 ± 0.19	5.8 ± 1.0	6.2 ± 0.4	10.1 ± 0.7	11.0 ± 0.3
Mg (mg%)	Range	(75 - 114)	(109 - 130)	(185 - 261)	(185 - 250)	(262 - 412)	(340 - 370)
	Mean	101 ± 14.1	118 ± 7.8	(213 ± 29	210 ± 25	339 ± 47	347 ± 13
Na	Range	(115 - 200)	(142 - 180)	(518 - 645)	(565 - 628)	(453 - 650)	(442 - 528)
	Mean	153 ± 28.2	164 ± 14.2	602 ± 33	604 ± 24	538 ± 75	505 ± 35
K	Range	(1049 - 2031)	1109 - 2058)	(155 - 333)	(199 - 250)	(51 - 188)	(56 - 91)
	Mean	1548 ± 349	1700 ± 366	258 ± 78	288 ± 22	124 ± 60	79 ± 14
Fe	Range	(0.3 - 4.0)	(0.25 - 5.0)	(1.5 - 3.5)	(2.0 - 4.1)	(1.6 - 8.6)	(2.3 - 4.5)
	Mean	1.9 ± 1.4	3.1 ± 1.8	2.5 ± 0.6	3.1 ± 0.8	3.7 ± 3	3.7 ± 0.7
Cu	Range	(0.10 - 0.22)	(0.10 - 0.22)	(0.40 - 0.60)	(0.40 - 0.60)	(0.5 - 0.9)	(0.65 - 1.20)
	Mean	0.15 ± 0.05	0.18 ± 0.04	0.48 ± 0.08	0.52 ± 0.08	0.7 ± 0.14	0.9 ± 0.2
Zn	Range	(1.45 - 2.45)	(1.7 - 265)	(8.2 - 14.2)	(9.0 - 12.7)	(7.8 - 10.3)	(10.0 - 12.2)
	Mean	1.83 ± 0.4	2.12 ± 0.3	11.8 ± 2.7	11.1 ± 1.5	9.2 ± 0.9	11.0 ± 0.8

± Standard deviation

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