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The effect of water-borne magnesium on the dietary magnesium requirement of the rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Rainbow trout (*Oncorhynchus mykiss*) (mean initial weight 0.84 g) were fed diets containing graded levels of magnesium (Mg) (78 to 725 μg/g) while being exposed to one of several levels in the rearing water (1.4 to 1000 mg/l). Uptake of Mg from the water, in Mg-deficient fish, was linearly related to the water Mg concentrated. It appears that the fish's Mg requirement can be met from either or both the diet or water. Under the experimental conditions, a water-borne concentration of 46 mg/l was calculated to be sufficient to meet the Mg requirement of the fish fed a Mg-free diet.

Introduction

Fish have the ability to utilize some essential elements directly from their rearing water (Lall 1989; Hepher 1989). However, with the exception of calcium (Ichii and Mugiya 1983), zinc (Spry *et al.* 1988), and selenium (Hodson *et al.* 1986), the relationship between dietary elemental requirement and water concentration has received only cursory examination. Shearer (1989) examined the dietary magnesium (Mg) requirement of the rainbow trout (*Oncorhynchus mykiss*) and concluded that under the experimental conditions used, the requirement was higher than had been previously reported (1400 μg/g *vs.* 500 to 700 μg/g) (Ogino *et al.* 1978; Knox *et al.* 1981). Possible reasons suggested for the observed difference included: differences in the availablity of the dietary Mg sources, differences in the water-borne contribution to the Mg requirement, and differences in diet efficiency. The availability of various dietary Mg sources to fish has since been examined (Dabrowska *et al.* 1989a; Shearer and Åsgård 1990) and does not appear to be a significant factor contributing to reported differences in dietary Mg requirement. The present experiment was designed to examine the effect of water-borne Mg on the dietary Mg requirement of the rainbow trout.

Materials and methods

A 4 × 5 randomized factorial design was used (Table 1). Each treatment was conducted in duplicate. Treatments consisted of one of four dietary levels of Mg (78,193,348, and 725 μg/g) and one of five levels of Mg in the water (1.4,5,20,50, and 1000 mg/l).

Rainbow trout (initial weight 0.4 g) were obtained from a commercial source (Trout Lodge, Summer, WA). The fish were fed a commercial diet (Biodiet Starter, Bioproducts Inc., Warrenton,

Table 1. Experimental design and treatment codes

		Dietary Mg level (μg/g)			
		78	193	348	725
Water Mg concentration (μg/g)	1.4[1]	1	2	3	4
	5[2]	5	6	7	8
	20	9	10	11	12
	150	13	14	15	16
	1000	17	18	19	20

[1]Municipal water contained 1.4 mg/l Mg; [2]total Mg concentration, naturally occurring plus added $MgSO_4$.

OR; 1340 μg/g Mg) for two weeks prior to the start of the experiment. Groups of 20 fish were randomly selected, weighed (mean weight 0.84 g), and placed into plastic buckets containing 8 liters of water. Buckets were placed into a larger tank which acted as a water bath, maintaining temperatures at 14 ± 1°C. Each bucket was aerated and a natural photoperiod (March-April) was used. Fish were poured into a net and were transferred to a clean bucket each morning. Appropriate levels of magnesium sulfate ($MgSO_4$) (ICN Nutritional Biochemicals, Cleveland, OH) were added from a 1:1 (w/w) stock after each water change. Magnesium sulfate was chosen as the Mg source because of its high solubility.

Experimental diets (Table 2) contained graded levels of magnesium oxide (MgO) chosen for its low solubility. Ration levels were adjusted daily based on the results of a previous experiment using a similar diet (Shearer and Åsgård 1990) and predicted growth rates for rainbow trout at 14°C (Austreng *et al.* 1987). A feed efficiency of 140% was assumed. Dietary energy values (gross) of 16.8, 33.5, and 13.4 KJ/g were used for protein, fat, and carbohydrate (NRC 1981). Fish were hand fed the allotted ration or to satiation in up to 10 meals per day for 13 consecutive days and were starved for one day to void the digestive tract prior to sampling for elemental analysis. The weight of any uneaten feed was determined by counting uneaten pellets and multiplying by an average pellet weight. Daily records were kept of uneaten and unfed feed and mortality. Feed efficiency was calculated as weight gain/dry feed ingested.

Table 2. Composition of experimental diets

Ingredient	% of dry weight
Casein, vitamin free	44.0
Gelatin	12.0
Amino acid mix[1]	4.4
Dextrin	6.3
Cod liver oil	22.0
Alpha-cellulose[2]	0.2
Vitamin mix[3]	2.0
Choline chloride (70%)	1.0
Mineral mix[4]	6.2
Ascorbic acid	0.1
Trace mineral solution[5]	–
Magnesium supplement[6]	–
Carboxymethyl cellulose	1.8

[1]As g/kg diet: Arg 11.5, Lys 11.0, Met. 4.0, Leu 9.0, Val 3.2, Thr 5.0; [2]reduced as Mg increased; [3]as reported by Hardy and Shearer (1984); [4]as g/kg diet; $CaHPO_4.2H_2O$, 4.24; KCl, 17.0; NaCl, 2.6; [5]as mg/kg diet: $CoCl_3 \cdot 6H_2O$, 4.0; $CuSO_4 \cdot 5H_2O$, 11.8; $FeSO_4 \cdot 7H_2O$, 115; $MnSO_4 \cdot H_2O$, 32.5; $ZnSO_4 \cdot 7H_2O$, 88; Na_2SeO_3, 4.2; KI, 1.9; [6]Mg added (mg/kg diet as MgO): Diet 1, 2, 3, 4, 5; 0, 125, 250, 400, 1500.

The fish in each bucket were bulk weighed prior to the random selection of fish for elemental analysis on days 0, 14, and 28 ($n = 20$, $n = 7$, $n = 7$). Whole fish and feed samples were dried at 105°C and were ashed at 550°C. Elemental concentrations of fish are reported as μg/g wet and that of feed as μg/g dry. Levels of protein and fat were determined using conventional analysis (AOAC 1975). Elemental analysis followed Shearer (1984). Analysis of variance, multiple mean comparisons (Fisher PLSD), and linear regression were performed using Statview 512+ (Brainpower, Calabrosa, CA). The relationship between water-borne and dietary Mg and whole body Mg concentration was investigated using the GLIM 3.77 software package (Royal Statistical Society).

Results

Diet and water analysis

The experimental diets contained an average of 56.9, 22.3, and 7.3% protein, fat, and ash, respec-

Table 3. Feed consumed in 28 days (g dry weight/fish)[1]

Water Mg concentration (mg/l)	Dietary Mg 78	193	348	725
1.4	0.92^{a}	1.09^{bc}	1.18^{d}	1.16^{cd}
5	1.04^{b}	1.18^{d}	1.14^{bcd}	1.12^{bcd}
20	1.19^{d}	1.12^{bcd}	1.15^{cd}	1.18^{d}
150	1.15^{cd}	1.14^{cd}	1.16^{cd}	1.14^{cd}

[1]Mean not followed by the same letter are significantly different. $p < 0.05$, N = 2; pooled SD = 0.03.

tively. Calculated energy content of the diet was 18.0 MJ/kg diet. Dietary concentrations of the elements examined exceeded established requirements (Lall 1989). The municipal rearing water contained 8.0, 1.4, and 2.2 mg/l of Ca, Mg, and sodium (Na), respectively. The pH in rearing containers varied between 5.8 and 6.2. The maximum ammonia level the fish may have encountered under the present rearing conditions, density, feeding level, *etc.*, was calculated to be less than 0.0038 mg/l which is considered safe by Piper *et al.* (1982). Magnesium concentrations in the rearing water were measured on day 1 of the experiment and were within 5% of required levels.

Feed consumption, growth, and mortality

Feed consumption differed significantly between treatments (Table 3). Fish held in the highest level of water-borne Mg (treatments 17 to 20) consumed considerably less than fish in the other treatments. Fish in treatments 1, 2, and 5 began to show signs of anorexia during the third week of the experiment. Overall feed efficiency (weight gain/feed consumed) did not differ between treatments 1 to 16 and averaged 135% (data not shown).

Final fish weights were related to feed consumption. Fish gained an average of 1.52 g (initial weight 0.84) or an increase of 181%. Mean daily growth rates were 4.8 and 3.6% for the first and second fortnightly periods. Mortalities began to occur in the high water-borne Mg group after day 2, and total mortality was 48% after 14 days. These treatments (17 to 20) were terminated after this sampling. Mortality in the remaining treatments was 5.1% by the end of the experiment and was confined to treatments 1, 2, 5, and 6.

Table 4. Mean fish weights (g) at the end of the experiment (day 28)[1]

Water Mg concentration (mg/l)	Dietary Mg 78	193	348	725
1.4	2.03^{a}	2.31^{abc}	2.71^{ef}	2.46^{bcdef}
5	2.16^{ab}	2.40^{bcdef}	2.35^{abcd}	2.42^{bcdef}
20	2.75^{f}	2.39^{bcde}	2.51^{cdef}	2.50^{bcdef}
150	2.29^{abc}	2.69^{def}	2.58^{cdef}	2.43^{bcdef}

[1]Means not followed by the same letter are significantly different. $p < 0.05$, n = 2; pooled SD = 0.14.

Table 5. Mean whole body Mg concentrations (μg/g wet weight) of the experimental fish after 14 days

Water Mg concentration (mg/l)	Dietary Mg 78	193	348	725
1.4	253^{a}	263^{ab}	295^{cd}	341^{h}
5	274^{ab}	284^{bc}	302^{cde}	329^{fgh}
20	315^{def}	319^{efg}	340^{gh}	346^{h}
150	383^{i}	319^{i}	398^{i}	388^{i}
1000	462^{j}	451^{j}	472^{j}	456^{j}

[1]Means not followed by the same letter are significantly different. $p < 0.05$, N varied from 14 to 21; pooled SD = 24.

Effect of dietary and water-borne Mg on whole body Mg concentration

Initial whole body Mg concentration averaged 342 μg/g. Fish held in water containing 150 or 1000 mg/l had elevated whole body levels after 14 days. Concentrations declined significantly in groups fed less than 725 μg/g and held in low Mg water (Table 5). After 28 days, fish in treatments 1, 5, 9, 15, and 16 had lower Mg levels than fish sampled from these groups after 14 days. All other groups showed an increase in whole body Mg concentration (Table 6). Two-way analysis of variance

Table 6. Mean whole body Mg concentrations (μg/g wet weight) of the experimental fish after 28 days

Water Mg concentration (mg/l)	Dietary Mg			
	78	193	348	725
1.4	247[a]	286[b]	339[cd]	372[def]
5	260[a]	298[b]	354[de]	375[def]
20	312[bc]	371[def]	364[def]	365[def]
150	388[ef]	378[ef]	382[ef]	373[def]

[1]Means not followed by the same letter are significantly different. $p < 0.05$, $N = 9$; pooled SD = 19.

Table 7. The effect of dietary (D) or water-borne (W) Mg on whole body Mg concentration of juvenile Atlantic salmon.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Water	(3)	(23438)		
W_{linear}	1	18217	18217	62.59*
W_{quad}	1	5196	5196	17.85*
W_{cubic}	1	24.34	24.34	0.08
Diet	(3)	(22934)		
D_{linear}	1	18197	18197	62.52*
D_{quad}	1	4721	4721	16.22*
D_{cubic}	1	15.46	15.46	0.05

Starred values are significant ($p < 0.05$).

indicated a significant interaction between dietary and water-borne Mg sources on whole body Mg ($p < 0.007$). Regression analysis indicated that the relationship of whole body Mg to both diet and water-borne Mg is parabolic (Table 7). This effect decreases as the level of water-borne Mg increases (Fig. 1). Examination of the interaction term (water-borne Mg × diet Mg) (Table 8) indicated that the effect of water-borne Mg was similar at all dietary levels since the $W \times D_{linear}$ interaction is significant. This analysis also suggests that, although increasing dietary levels of Mg will increase whole body Mg concentrations (significant $D \times W_{linear}$), this effect is reduced at higher levels (significant $D \times W_{quad}$).

Discussion

The weight increases in treatments 1 to 16 averaged 181% and feed efficiency averaged 135%. Fish growth was therefore only slightly less than predicted by Austreng *et al.* (1987) indicating that the method of rearing did not impart a serious stress on the experimental fish. Normal whole body Mg concentration is size dependent (Shearer 1984) and initial mean Mg concentration of 342 μg/g was normal for fish of this size.

The results of the present study demonstrate that rainbow trout can meet their requirement for Mg (maintain normal whole body levels for fish of this size, about 370 μg/g) (Shearer and Åsgård 1990) from a combination of dietary and water-borne sources. The linear relationship between whole body Mg concentration in the deficient groups (whole body concentration less than 370 μg/g at the end of the experiment) and water-borne Mg concentration suggests that the fish's ability to take up Mg is not related to its state of deficiency. Once the requirement has been met, the fish must prevent further retention of Mg from either the water-borne or dietary source. The response surface plot (Fig. 1) and regression analysis (Tables 7 and 8) illustrate homeostatis once normal body levels have been achieved. Earlier work (Shearer 1989) showed that growth reduction occurred when high levels of Mg (2100 μg/g) are fed in a purified diet. Similar findings were reported by Dabrowska *et al.* (1989b) for tilapia (*Oreochromis niloticus*). The results of the present study also reinforce our earlier conclusion that whole body Mg concentrations are a more sensitive indicator of Mg status than growth (Shearer 1989; Shearer and Åsgård 1990).

High water-borne levels of Mg were detrimental to trout of the size used in this experiment. Fish held in 1000 μg Mg/g water had elevated whole body Mg concentrations and were significantly smaller than fish in the other groups after 14 days. Fish held in 150 μg Mg/g also had elevated whole body concentrations but, at this level, growth was not effected. Miles and Smith (1968) reported a short term (hours) elevation of Mg in salmon smolts when they were placed into saltwater. In our study, small trout after 14 days were unable to adjust to

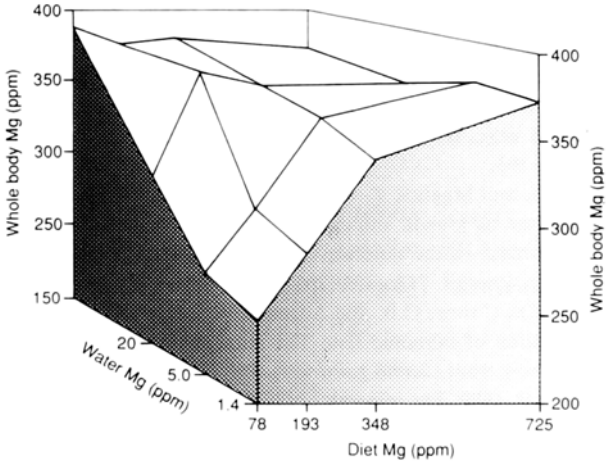

Fig. 1. Calculated water-borne magnesium concentration that would meet the magnesium requirement of rainbow trout fed a magnesium free diet.

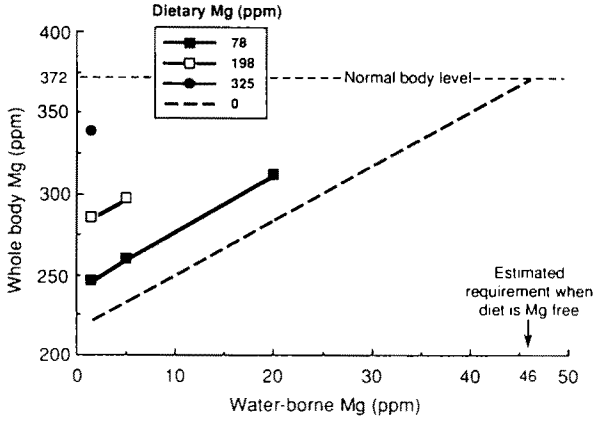

Fig. 2. The relationship between whole body magnesium concentration and water-borne and dietary magnesium in rainbow trout.

Table 8. The interactive effects of dietary (D) or water-borne (W) Mg on whole body Mg concentration of juvenile Atlantic salmon.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Interaction	(9)	(16875)		
$W \times D_{linear}$	3	13433	4477.67	15.38*
$W \times D_{quad}$	3	1859	619.67	2.13
$W \times D_{cubic}$	3	1584	527.67	1.81
Interaction	(9)	(16875)		
$D \times W_{linear}$	3	11761	3820.33	13.47*
$D \times W_{quad}$	3	4956	1652	5.68
$D \times W_{cubic}$	3	158	52.67	0.18
Error	(16)	4657	291.0625	

Starred values are significant ($p < 0.05$).

high water-borne Mg (1000 μg/g) which was near that of seawater (1214 μg/g) (Duxbury 1981).

Using data from day 28, from the treatments where body Mg levels were significantly below normal (less than 354 μg/g; treatments 1, 2, 3, 5, 6, and 9), it is possible to estimate the water-borne Mg concentration that would provide the fish's requirement when they are fed a Mg-free diet under the conditions of the present study. If the dietary Mg contribution to the requirement is linear below 78 μg/g, then the regression line for 0 μg Mg/g in the diet will intercept with the normal whole body Mg concentration at 46 μg/g water-borne Mg (Fig. 2).

If uptake from the water is time dependent, then slower growing fish may be able to meet their requirement at lower water-borne levels if the diet is deficient in Mg. Fish may also be able to meet their Mg requirement entirely from the diet, but this would have to be determined by rearing fish in Mg-free water containing adequate levels of other essential ions to insure fish health. This was not undertaken in the present study.

Fish were able to meet their Mg requirement (maintain whole body levels at approximately 370 μg/g) at a dietary level of 725 μg/g in the present study when water-borne Mg was 1.4 mg/l. This is considerably lower than the 1300 μg/g dietary Mg reported as the requirement of the trout in an earlier study (Shearer 1989). This may be due to a difference in the size of the fish used; larger fish were used to establish the requirement. If the fish's ability to extract the Mg from the water is size related, then the small fish used in the present experiment may have been able to extract more Mg per unit of body mass which would reduce their dietary requirement. In addition, growth rates and feed efficiency were also lower in the present experiment. Both have the effect of reducing the dietary requirement.

In conclusion, our results indicate that rainbow

trout can meet their Mg requirement from either or both dietary or water-borne sources provided the Mg is biologically available. This study also indicates that account must be taken of water-borne Mg when the dietary requirement is being determined. Finally, the fish rearing methods used in this experiment offer an inexpensive alternative to metering chemicals into a flow-through system when dietary water-borne interactions are being examined in small fish.

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