Studies on the nutrition of rainbow trout (*Salmo gairdneri*) Magnesium deficiency: the effect of feeding with a Mg-supplemented diet

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1. For a period of 8 weeks, rainbow trout (*Salmo gairdneri*), mean initial weight 21 g, were given either a low-magnesium or control diet containing 0·03 and 0·58 g Mg/kg diet respectively. Both groups of trout were then given the control diet for a further 11 weeks.

2. Weight gains over the initial 8-week period were lowest in the Mg-deficient trout. Feeding the deficient fish the control diet rapidly improved growth rate until it was the same as that of the control trout.

3. Plasma Mg was significantly lower in the Mg-deficient trout at week 8. Feeding with the control diet for 11 weeks did not increase plasma Mg. Few changes were observed in the plasma concentrations of the other electrolytes.

4. Renal calcium concentrations were unaffected by dietary Mg levels. Similarly, the renal levels of phosphorus, sodium and potassium all fell within the range found in normal rainbow trout.

5. Muscle Mg concentrations were reduced in those trout given the Mg-deficient diet. Feeding with the control diet for a further 11 weeks increased muscle Mg but the level was still significantly lower than that found in trout given the control diet for 19 weeks.

6. The bone ash Mg concentration was significantly lower, and the Ca higher, in the deficient fish at week 8, when compared with the control group.

7. When compared with the value at the start of the experiment, total bone Mg fell slightly in the deficient trout over the initial 8-week period, but increased in the control group of fish. Feeding with the control diet for a further 11 weeks increased total bone Mg in both Mg-deficient trout and control trout.

8. The results show that the Mg deficiency imposed on the rainbow trout was of limited severity and almost complete recovery was obtained when the control diet was fed.

The effects of magnesium deficiency on rainbow trout (*Salmo gairdneri*) have been described in detail in recent years (Cowey *et al.* 1977; Ogino *et al.* 1978; Knox *et al.* 1981*a*). Among the deficiency symptoms were poor growth, loss of appetite, calcinosis of kidney and muscle and a dramatic increase in the muscle extracellular fluid volume.

The present experiment was carried out to examine the efficiency with which mineral levels in soft tissues and bones of Mg-deficient trout were restored to normal levels by feeding a Mg-supplemented diet.

MATERIALS AND METHODS

Rainbow trout of mean weight 21 g were obtained from Dornoch Fisheries, Dornoch, Scotland: they had been reared on a commercial trout diet. The trout were randomly distributed between two circular glass-fibre tanks of diameter 1 m, depth 0·6 m and containing 400 l water (twenty-five fish per tank). The water, obtained from the City of Aberdeen domestic supply, flowed through the tanks at a rate of 20 l/h per tank. During the experimental period the Mg concentration of the water was 0·05 mmol/l and the temperature ranged from 10° at the beginning of the experimental period to 6° at the end.

The composition of the diets used is shown in Table 1; they were prepared as previously described (Knox *et al.* 1981*b*). The trout were given the Mg-supplemented diet until they had acclimatized to their environment, feeding routine and diet (approximately 8 d). Initial weight measurements were then made, fish being weighed individually after being lightly anaesthetized with ethyl-*m*-aminobenzoate, methane-sulphonic acid salt at a concentration

Table 1. *Composition (g/kg dry diet) of the diets given to rainbow trout* (Salmo gairdneri)

Ingredient	Control diet	Magnesium-deficient diet
Casein	500	500
Precooked starch	200	200
Capelin oil	120	120
Vitamin mix*	28	28
Mineral mix†	109·75	109·75
α-Cellulose	30·7	36·3
Ascorbyl palmitate	0·4	0·4
Cystine	5·0	5·0
BHA mix‡	0·5	0·5
Rovimix‡	0·075	0·075
Magnesium sulphate	5·57	0
Measured Mg level (g/kg dry diet)	0·58	0·03

* Adron *et al.* (1976).

† Supplied (/kg dry diet): calcium β-glycerophosphate 92·3 g, KH_2PO_4 6·6 g, $NaH_2PO_4.2H_2O$ 7·5 g, NaCl 2·0 g, $FeSO_4.7H_2O$ 1·0 g, $ZnSO_4.7H_2O$ 0·13 g, $MnSO_4.4H_2O$ 0·13 g, $CuSO_4.5H_2O$ 39 mg, $CoSO_4.7H_2O$ 35 mg, $Al_2(SO_4)_3.16H_2O$ 7·5 mg, KI 7·5 mg.

‡ Knox *et al.* (1982).

of 0·2 g/l (Sigma Chemical Company, Poole, Dorset). The trout were weighed every 2 weeks throughout the experiment which lasted 19 weeks. The fish were given the diets at a level of 20 g diet/kg biomass of trout per d. The amount of food given to each tank was adjusted every 2 weeks in accordance with the measured biomass.

With the resources available it was not possible to replicate the tanks allotted to each diet. However, experience has shown that in the tank system used no difference could be found between trout kept in separate identical tanks, but given the same diet. In an experiment to determine the vitamin E requirement of rainbow trout (Cowey *et al.* 1983) three groups of trout, kept in separate tanks (twenty-five fish/tank), were given the same basal diet for 16 weeks. The mean weights (g) with SEM at week 0 were 14·1 (0·92); 14·9 (1·17) and 14·4 (0·91); at week 16 they were 116·7 (8·58); 106·8 (7·83) and 112·1 (8·31). Analysis of variance (Fisher, 1950) showed no significant difference between the mean weights of the three tanks of trout at the beginning or end of the experimental period. To further minimize any tank effects the fish were returned to a different tank after each bi-weekly weighing; a procedure previously described by Cowey *et al.* (1974).

Chemical methods

Plasma was obtained from blood removed from the caudal vein, the lithium salt of heparin being used as anticoagulant. Kidney and muscle samples were excised from the fish and stored at −20° until required. The remaining whole fish was then dropped into boiling water for 15 min, this facilitated the removal of the flesh adhering to the skeleton. Because of the difficulties encountered in obtaining a complete skeleton the head and tail were removed to leave the vertebral column with its attached neural and haemal spines and ribs. After drying overnight at 100° the skeletons were weighed, ground with a glass mortar and pestle and a sample ashed and analysed. The mineral analysis of all the tissues obtained was carried out as previously described (Cowey *et al.* 1977).

The basal-Mg and control diets were given to the trout for 8 weeks at which time the growth rate in those trout given the basal diet had fallen significantly. At week 8 the mean (with SE) weights of the control and test groups of fish were 51·6 (2·7) g and 40·2 (1·7) g

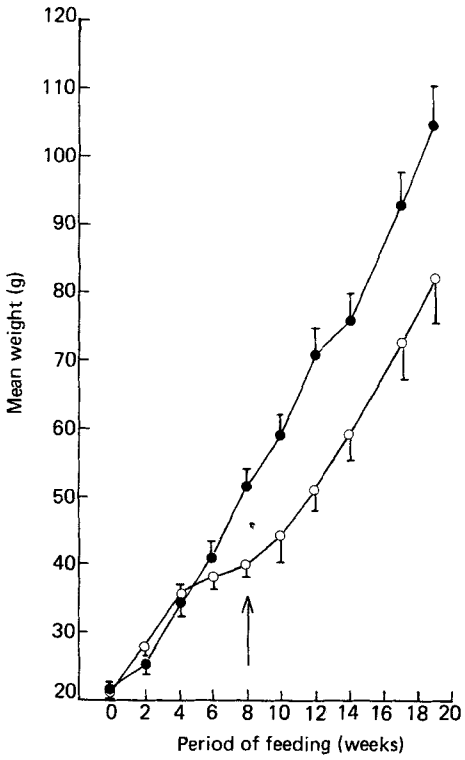

Fig. 1. Growth of rainbow trout given the control diet (●; 580 mg magnesium/kg diet) and the Mg-deficient diet (○; 30 mg Mg/kg diet). ↑, Control diet was fed to the Mg-deficient trout. Points are mean values with their standard errors represented by vertical bars.

($P < 0{\cdot}01$). Ten fish were then randomly selected from each group and tissue samples collected for analysis. The remaining trout were weighed and both groups given the control diet for a further 11 weeks. Samples of tissues were then collected and analysed as before.

The statistical significance of the experimental data was established by Student's t test (Fisher, 1950), differences between treatments were taken as significant at $P < 0{\cdot}01$.

RESULTS

Fig. 1 shows the change in mean weight with time over the experimental period. After 6 weeks the mean weight of the trout given the basal Mg diet was less than that of the control fish and at week 8 was significantly lower ($P < 0{\cdot}01$). Also, over the final 4 weeks of this period there was a noticeable loss of appetite in the deficient group of trout and, between weeks 6 and 8, the deficient group consumed only 12 g diet/kg biomass of fish per d compared with 20 g diet/kg biomass per d in the control group. The food conversion ratios (g body-weight gain: g food intake) for the control and deficient groups of fish for the first 8 weeks of the experiment were 1·07 and 0·69 respectively.

Feeding the Mg-deficient trout with the control diet produced an improvement in growth rate over the final 11-week feeding period, the food conversion ratio being 1·07 in both groups of fish. During this recovery period the water temperature dropped 4° in 2–3 d and remained at 6° thereafter; the amount of food consumed by both groups of fish was the same, 10 g diet/kg biomass per d. The two mortalities among the trout given the basal diet occurred on a day following weighing and may have been associated with the stress involved in weighing, i.e. removal from water, anaesthesia, etc.

Table 2. *Concentrations of minerals in plasma (mmol/l) of rainbow trout* (Salmo gairdneri) *given diets† containing different amounts of magnesium*

(Mean values with their standard errors for ten fish per treatment)

		Magnesium		Calcium		Phosphorus		Sodium		Potassium	
Group	Week	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	8	0·35	0·023	3·73	0·065	3·54	0·136	132·12	2·504	1·55	0·225
	19	0·33	0·038	2·78	0·062	3·32	0·128	129·62	2·957	2·40	0·300
Deficient	8	0·22*	0·012	3·64	0·148	3·06	0·185	122·03*	1·700	3·63	0·779
	19	0·18*	0·021	3·57	0·311	3·03	0·207	126·27	1·150	1·21*	0·184

* Mean values for Mg-deficient group were significantly different from the corresponding control group: $P < 0·01$.

† For details, see Table 1.

Table 3. *Concentrations of minerals in kidneys (mmol/kg wet tissue) of rainbow trout* (Salmo gairdneri) *given diets† containing different amounts of magnesium*

(Mean values with their standard errors for ten fish per treatment)

		Magnesium		Calcium		Phosphorus		Sodium		Potassium	
Group	Week	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	8	6·25	0·089	2·52	0·096	78·82	2·146	45·50	1·670	69·43	1·590
	19	7·23	0·200	3·18	0·226	79·34	1·504	61·99	1·839	78·78	1·235
Deficient	8	6·83*	0·071	2·27	0·095	72·90	1·112	39·81*	0·738	81·53*	2·461
	19	7·26	0·107	3·11	0·354	81·62	4·334	56·65	1·733	88·38	4·259

* Mean values for Mg-deficient group were significantly different from the corresponding control group: $P < 0·01$.

† For details, see Table 1.

The plasma Mg concentration of trout given the diet deficient in Mg for 8 weeks was significantly lower than that of fish given the control diet (Table 2). This difference was still evident after the Mg-deficient fish had been transferred to the control diet and both groups of trout given this diet for a further 11 weeks.

Few changes were observed in the plasma concentrations of other minerals as a consequence of depletion–repletion of Mg. In particular, calcium and phosphorus levels did not alter during either phase. The plasma sodium level was significantly lower in the Mg-deficient fish at 8 weeks than in the control trout. The mean plasma potassium concentration of the Mg-deficient fish at 8 weeks was higher than that of the control trout, the difference was, however, not significant at $P < 0·01$. During the second-half of the experiment the plasma K level in the Mg-deficient trout was significantly lowered both by comparison with the value at week 8 and with the control fish at week 19.

Mean renal levels of Ca (Table 3) in both Mg-deficient and control fish at 8 weeks (and again at 19 weeks) were similar and comparable with the range of values (3·34–6·56 mmol/kg) found earlier (Knox *et al.* 1981*a*) for normal fish.

Although significant differences did occur in renal levels of Mg, Na and K between Mg-deficient and control fish at 8 weeks, these differences were small. The values obtained all fell within the ranges observed in normal trout in the earlier experiment (Knox *et al.* 1981*a*).

The Mg concentration in the muscle of Mg-deficient trout at 8 weeks was significantly

Table 4. *Concentrations of minerals in muscle (mmol/kg wet tissue) of rainbow trout* (Salmo gairdneri) *given diets† containing different amounts of magnesium*

(Mean values with their standard errors for ten fish per treatment)

		Magnesium		Calcium		Phosphorus		Sodium		Potassium	
Group	Week	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	8	12·66	0·252	6·36	1·213	74·93	1·271	11·95	0·400	93·81	1·319
	19	13·22	0·167	4·46	0·849	68·73	1·677	21·13	0·773	101·94	1·247
Deficient	8	8·65*	0·167	11·29	3·110	70·84	2·150	33·81*	2·342	80·49*	2·211
	19	11·19*	0·350	11·73	2·503	74·16	1·386	35·69*	3·151	102·47	2·400

* Mean values for Mg-deficient group were significantly different from the corresponding control group; $P < 0{\cdot}01$.

† For details, see Table 1.

Table 5. *Concentrations of minerals in bone ash (mol/kg ash) of rainbow trout* (Salmon gairdneri) *given diets† containing different amounts of magnesium*

(Mean values with their standard errors for ten fish per treatment)

		Magnesium		Calcium		Phosphorus		Sodium		Potassium	
Group	Week	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	8	0·31	0·013	8·94	0·042	4·89	0·057	0·25	0·008	0·34	0·041
	19	0·24	0·015	8·92	0·085	5·18	0·016	0·24	0·006	0·32	0·011
Deficient	8	0·17*	0·006	9·22*	0·059	4·84	0·046	0·34*	0·010	0·29	0·016
	19	0·15*	0·014	8·81	0·083	5·12	0·054	0·29*	0·011	0·23*	0·009
	0‡	0·44	0·007	8·65	0·055	5·05	0·066	0·34	0·009	0·48	0·009

* Mean values for Mg-deficient group were significantly different from the corresponding control group; $P < 0{\cdot}01$.

† For details, see Table 1.

‡ Minerals in bones of trout at the start of the experiment.

lower than that in the muscle of control trout (Table 4). There was a marked increase in muscle Mg of the deficient fish after 11 weeks of feeding the control diet. Although the muscle Mg level in the deficient trout at week 19 was still less than that found in the controls it had almost reached levels (11·66–13·47 mmol/kg) characteristic of normal fish (Knox *et al.* 1981*a*).

Mean muscle Ca levels were greater in the deficient trout than in the control group fish. However, the differences were not significant. It is noteworthy that in the deficient trout at both weeks 8 and 19 the muscle Ca levels exceeded the range of those already described in trout given adequate dietary Mg (2·42–8·69 mmol/kg wet weight, Knox *et al.* 1981*a*).

At week 8, muscle Na and K levels in deficient trout were significantly different from those found in the control fish, Na being greater and K smaller. During the second period of the experiment (weeks 8–19) K increased in both groups while Na increased only in the control fish.

Changes in the concentrations of minerals in bone ash are shown in Table 5. At week 8 the ash concentrations of Mg, Ca and Na were significantly different between deficient and control trout, Mg being lower, Ca and Na being greater. At week 19, Na was significantly greater in the deficient trout while Mg and K were lower. There was no significant difference between the Ca level in deficient and control fish at week 19.

Table 6. *Total amount of minerals (mmol) in bones of rainbow trout* (Salmo gairdneri) *given diets† containing different amounts of magnesium*

(Mean values with their standard errors for ten fish per treatment)

		Magnesium		Calcium		Phosphorus		Sodium		Potassium	
Group	Week	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	8	0·048	0·0044	1·46	0·142	0·81	0·082	0·046	0·0074	0·054	0·0079
	19	0·084	0·0062	3·19	0·188	1·85	0·114	0·086	0·0062	0·118	0·0083
Deficient	8	0·033	0·0023	1·76	0·100	0·93	0·055	0·065	0·0039	0·055	0·0042
	19	0·057	0·0052	3·35	0·182	1·96	0·123	0·106	0·0072	0·090	0·0083
	0‡	0·039	0·0011	0·77	0·021	0·45	0·023	0·030	0·0010	0·046	0·0012

† For details, see Table 1.
‡ Minerals in bones of trout at the start of the experiment.

The ash concentration of Mg decreased in the control fish over the initial 8 week period at an average rate of 0·016 mol/kg ash per week; during weeks 8–19 this decrease was much smaller, being 0·006 mol/kg ash per week. This suggests that the trout were adjusting from a high Mg intake (the commercial trout diet fed before the experiment contained approximately 2·5 g Mg/kg). In deficient trout the rate of fall of bone ash Mg averaged 0·034 mol/kg ash per week in weeks 0–8. Feeding with the Mg-supplemented diet greatly arrested this decline in bone ash Mg levels to 0·002 mol/kg ash per week. It seems probable that by week 19 the bone ash Mg concentration had ceased to fall.

In the control group of fish, total Mg content of the dried bones increased at an average rate of 1·16 μmol Mg/week during weeks 0–8 and 3·2 μmol/week during the latter period of the experiment (Table 6). On the other hand, the total Mg content of the bones of the deficient group of trout fell at an average rate of 0·69 μmol/week during the first 8 weeks of the experiment, while feeding with the Mg-supplemented diet increased the Mg content of the bones by 2·11 μmol/week during weeks 8–19. Because of the difference in the weights of the fish at weeks 8 and 19 it was inappropriate to apply the Student's *t* test of significance to the results.

DISCUSSION

The poor growth rate in those trout given the basal-Mg diet was similar to the results obtained previously (Knox *et al.* 1981*a*). It is, however, evident from the present experiment that there may be a considerable variation in the time-course at which the symptoms of Mg deficiency appear in trout. The very low concentration of Mg in the present basal diet (approximately half the concentration used previously (Knox *et al.* 1981*a*) may have reduced growth rate but had no effect upon kidney Ca levels. Thus the nephrocalcinosis and hypercalcaemia shown to typify Mg deficiency in trout (Cowey *et al.* 1977; Knox *et al.* 1981*a*) did not develop within the initial 8-week feeding period. MacIntyre & Davidsson (1958) also found that nephrocalcinosis in rats only occurred in those animals suffering from hypercalcaemia.

Plasma and muscle Mg levels were significantly lower in the Mg-deficient fish and changes in the muscle monovalent cation concentrations suggested that the muscle extracellular fluid volume had been increased by Mg deficiency as described previously (Knox *et al.* 1981*a*). Kidney Mg levels were significantly greater in the deficient fish at week 8, previous studies had shown that kidney Mg remained constant after a basal Mg diet had been given for 16 (Cowey *et al.* 1977) or 20 weeks (Knox *et al.* 1981*a*). The evidence would suggest that, in rainbow trout, kidney Mg concentrations are maintained at normal levels during periods

of dietary Mg restriction. The same thing may also be said of rats (Martindale & Heaton, 1964; George & Heaton, 1975), although a recent report has shown that the kidney Mg falls during moderate Mg deficiency (Fischer *et al.* 1981).

Although it was not possible to obtain the complete trout skeleton, the mineral content of the bones collected does give a reasonable indication of the effect dietary Mg restriction had on bone mineral levels. The reduction of total bone Mg content in the deficient trout after 8 weeks suggests that the bone Mg may have been utilized to maintain the soft tissue Mg levels. In rats, similar decreases in bone Mg have been found (Martindale & Heaton, 1964; Ophaug & Singer, 1976) while Rayssiguier & Larvor (1978) have calculated that 20% of the total bone Mg of the rat was lost over a 30 d period when a Mg-deficient diet was given, although total body Mg remained almost constant. Thus, there is a similarity between bone Mg in trout and that in rats in that the bone Mg may be mobilized to meet the needs of soft tissues, under conditions of dietary Mg restriction. However, by comparison with terrestrial vertebrates, the skeleton of trout is a small fraction of the body mass (life in water does not necessitate a large, rigid skeleton to which anti-gravitational muscles can be attached). It is, therefore, unlikely that trout bone can function as a substantial source of mobile Mg.

The Mg deficiency imposed was of limited severity, thus recovery appeared to be almost complete after feeding the control diet (0·58 g Mg/kg dry diet) for 11 weeks. By this time growth rate had recovered, muscle Mg concentration was almost restored and the balance of monovalent cations in the muscle was more normal. A low plasma Mg concentration still persisted in the previously-deficient fish despite the increased Mg intake, suggesting that assimilated Mg was apparently being quickly taken up by the soft tissues. The concentration of Mg in bone ash was also unchanged although the total amount increased. Restoration of bone Mg thus appeared less vital than the recovery of soft tissue Mg levels.

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