Studies on the nutrition of salmonid fish. The magnesium requirement of rainbow trout (*Salmo gairdneri*)

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1. Rainbow trout (*Salmo gairdneri*) of mean initial weight 35 g were given one of five experimental diets for 20 weeks. The diets contained (g/kg dry diet) 15 calcium, 10 phosphorus and graded levels of magnesium from 0·04 (diet no. 1) to 1·0 (diet no. 5). In a second experiment rainbow trout of mean initial weight 16 g were given one of six experimental diets for 20 weeks. The diets contained (g/kg dry diet): Ca (40), P (30) and levels of Mg from 0·06 (diet no. 6) to 2·0 (diet no. 11).

2. In both experiments weight gains were lowest in those trout given diets containing the basal levels of Mg (diet no. 1 and diet no. 6) but increased with increasing dietary Mg concentration. In neither experiment was there any further increase in weight gain once the Mg concentration reached 0·25–0·5 g/kg dry diet; weight gain reached a plateau at this dietary Mg level.

3. The following trends occurred in serum electrolyte concentrations as dietary Mg increased. Mg increased in both experiments, in Expt 2 it reached a maximum of 1 mmol/l when the diet contained 0·5 g Mg/kg and did not increase further; sodium was positively correlated in both experiments; potassium decreased and in Expt 2 reached a plateau minimum of 1·7 mmol/l at a dietary Mg concentration of 0·5 g/kg; Ca and P altered little in either experiment.

4. In both experiments renal Ca concentrations were greatly increased in trout given diets lacking supplementary Mg; they fell to low levels (3–5 mmol/kg) when diets contained 0·15 g Mg/kg or more. Renal K and P concentrations were negatively correlated with dietary Mg in Expt 2; other electrolytes measured were not altered in concentration by the treatments used.

5. Extracellular fluid volume (ECFV) of muscle was negatively correlated with dietary Mg. In Expt 2 it reached a minimal or normal value at 0·5 g Mg/kg diet and did not decrease further. Muscle Mg concentration increased with diet Mg in both experiments and muscle K concentration was also correlated with diet Mg in Expt 2. These changes were related to the shift in muscle water. In Expt 1, P concentration was decreased with increasing diet Mg but in Expt 2 its concentration increased, these changes may have been connected with the three-fold difference in dietary P in the two experiments.

6. By contrast with skeletal muscle, Mg levels in cardiac muscle increased at low dietary Mg intakes.

7. Concentrations of electrolytes in liver did not alter with the dietary treatments used.

8. The results show that Mg requirement of rainbow trout is met by a diet containing 0·5 g Mg/kg diet.

The effects of magnesium deficiency on rainbow trout (*Salmo gairdneri*) given diets containing different amounts of calcium and phosphorus were recently described (Cowey *et al.* 1977). Some of the pathologies found were similar to those described in carp (*Cyprinus carpio*) by Ogino & Chiou (1976) and later in rainbow trout by Ogino *et al.* (1978), namely poor growth and sluggishness. In addition, calcinosis of kidney and muscle was also demonstrated; electron probe micro-analysis of the renal calculi showed that the deposits contained tricalcium phosphate (Cowey *et al.* 1977).

Since many materials used as components of practical fish diets may contain large amounts of Ca and P, e.g. fish meal, shrimp meal, the present work was undertaken to examine the Mg requirement of rainbow trout given either low or high dietary levels of Ca and P. To this end two experiments were carried out. In the first, diets containing (g/kg dry diet) 15 Ca and 10 P and graded amounts of Mg were given to groups of rainbow trout of mean initial weight 35 g for 20 weeks. In the second, diets containing (g/kg dry diet) 40 Ca and 30 P together with various levels of Mg were given to groups of rainbow trout of mean initial weight 16 g, again for 20 weeks.

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Renal calcinosis, as well as an increase in muscle extracellular fluid volume (ECFV) occurred as a consequence of Mg deficiency in both experiments. An Mg level of 0·5 g/kg dry diet was found to be adequate for rainbow trout in that, in both experiments, weight gain reached a plateau at this concentration and no pathologies or other abnormalities were evident.

MATERIALS AND METHODS

The experiments were carried out sequentially. In the first experiment, five dietary treatments were used, the intended dietary Ca and P levels being respectively 15 and 10 g/kg diet with five dietary Mg levels. In the second experiment the intended dietary Ca and P levels were 40 and 30 g/kg diet, and six dietary Mg concentrations were used. A dietary Ca:P value of 1·5:1 was used in the diets with a view to the levels of Ca and P that might be obtained in a trout diet when fish meal or shrimp meal for example was used as a main ingredient. Chemical analyses of white fish meal showed Ca and P concentrations (g/kg) of 80 and 35 respectively. Furthermore, analysis of a number of commercial trout pellets has shown Ca:P values ranging from 1:1 to 2:1.

Rainbow trout were obtained from D. M. Brien, Almondbank, Perth; they had been reared on a commercial trout diet. For the first experiment the fish obtained had a mean weight of approximately 35 g, they were given diets 1–5 (Table 1). For the second experiment smaller fish were supplied (mean weight approximately 16 g). They were given diets 6–11 (Table 1). In each experiment the rainbow trout were randomly distributed among circular glass-fibre tanks of diameter 1 m, depth 0·6 m and each containing 400 l water. The water from the tanks was partially recirculated, with a constant 'bleed-in' of fresh tap-water (75 l/tank per h) from the City of Aberdeen domestic supply. The Mg and Ca concentration in the aquarium water was monitored during the experiments; the mean concentrations (mmol/l) found were Mg 0·05 and Ca 0·30. The tanks were housed in an aquarium room with an ambient temperature averaging 15°.

The fish were first given a diet identical to the experimental diet used, but containing (g/kg dry diet) 15 Ca, 10 P and 1 Mg for the first experiment, and 40 Ca, 30 P and 1 Mg for the second experiment.

The trout were given this diet until they appeared to have acclimatized to their surroundings, feeding routine, diet, light etc. Initial weight measurements were then made as previously described (Cowey *et al.* 1977), thereafter the fish were weighed every 4 weeks throughout the experiments which lasted for 20 weeks. The trout were fed to satiation four times daily (6 d/week), food pellets being put sparingly into each tank only so long as they were actively consumed.

The diets used were prepared as before (Cowey *et al.* 1977). The composition of all the diets, including the measured levels of Ca, Mg and P are shown in Table 1. In diets 10 and 11 part of the added Mg was provided as magnesium acetate instead of magnesium sulphate since it was thought that very high levels of sulphate in the diet might be detrimental to the fish.

Chemical methods

Serum from blood obtained from the caudal vein, and samples of kidney, muscle, liver and heart were collected, stored, ashed and analysed as previously (Cowey *et al.* 1977). Analyses were carried out on six fish selected by random methods from each dietary treatment.

The estimate of muscle ECFV used was the chloride space, this was measured by the methods of Manery (1954) adapted as follows: six randomly selected fish from each dietary treatment were anaesthetized with ethyl-m-aminobenzoate, methane sulphonic acid salt (Sigma Chemical Company, Fancy Road, Poole, Dorset) at a concentration of 0·2 g/l. The fish were then injected intravenously with 2 μCi ^{36}Cl (Radiochemical Centre, Amersham,

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

	Expt 1					Expt 2					
Diet no. ...	1	2	3	4	5	6	7	8	9	10	11
Ingredient											
Casein	500	500	500	500	500	500	500	500	500	500	500
Dextrin	100	100	100	100	100	100	100	100	100	100	100
Cod-liver oil	20	20	20	20	20	20	20	20	20	20	20
Soya-bean oil	40	40	40	40	40	40	40	40	40	40	40
Mineral mix*	34·7	34·7	34·7	34·7	34·7	34·7	34·7	34·7	34·7	34·7	34·7
Vitamin mix†	28	28	28	28	28	28	28	28	28	28	28
α-Cellulose	162·8	162·8	162·8	162·8	162·8	85·8	85·8	85·8	85·8	85·8	85·8
Binder‡	50	50	50	50	50	50	50	50	50	50	50
Calcium lactate	64·5	64·5	64·5	64·5	64·5	88·3	88·3	88·3	88·3	88·3	88·3
$CaHPO_4.2H_2O$	0	0	0	0	0	73·3	73·3	73·3	73·3	73·3	73·3
$MgSO_4.7H_2O$	0	1·01	2·53	5·07	10·14	0	2·53	5·07	10·14	15·21	15·21
Magnesium acetate	0	0	0	0	0	0	0	0	0	4·4	8·8
Chemical composition											
Calcium	16·6	14·9	14·5	14·9	14·9	42·7	42·6	42·4	40·9	41·3	41·4
Phosphorus	9·9	10·4	10·2	10·3	10·4	29·2	28·8	27·6	28·9	29·0	28·3
Magnesium	0·04	0·15	0·30	0·56	1·00	0·06	0·28	0·54	0·89	1·36	2·09

* Supplied (/kg dry diet); Ca $(H_2PO_4).2H_2O$ 24·3 g, $CaCO_3$ 5·2 g, $FeSO_4.7H_2O$ 1·0 g, KCl 2·1 g, NaCl 1·7 g, $Al_2(SO_4)_3.16H_2O$ 6·9 mg, $ZnSo_4.7H_2O$ 139 mg, $CuSO_4.5H_2O$ 34·7 mg, $MnSO_4.4H_2O$ 124·9 mg, KI 6·9 mg, $CoSO_4.7H_2O$ 34·7 mg.

† Supplied (/kg dry diet); riboflavin 200 mg, pyridoxine hydrochloride 40 mg, nicotinic acid 800 mg, calcium pantothenate 280 mg, *myo*-inositol 4 g, biotin 6 mg, pteroylmonoglutamic acid 15 mg, *p*-aminobenzoic acid 400 mg, choline chloride 8 g, ascorbic acid 2 g, DL-α-tocopheryl acetate 400 mg, menaphthone 40 mg, cyanocobalamin 90 μg, thiamin hydrochloride 50 mg.

‡ Edifas; ICI Ltd, Ardrossan, Ayrshire.

Bucks) in 0·1 ml saline solution (9 g sodium chloride/l). After a 5 h equilibration period, to allow complete mixing and penetration of the whole Cl space, the fish were killed by cervical dislocation and samples of skeletal muscle and plasma were collected. A piece of excised muscle (1 g) was rapidly removed and homogenized in 5 ml distilled water and then 1·25 ml trichloroacetic acid (500 g/l; TCA) added to the homogenate. After thorough mixing the homogenate was centrifuged at 2500 *g* for 10 min at room temperature. The total water content of muscle and plasma was measured by drying a known weight of each tissue at 100° for 18 h. The measured muscle water content was then used to calculate the total volume of each muscle homogenate. Portions of the TCA supernatant fraction and plasma from each fish were assayed for ^{36}Cl using a liquid scintillation spectrometer, the amounts of ^{36}Cl being expressed as counts/min per l plasma or counts/min per kg muscle. Then the muscle ^{36}Cl space was calculated for each fish by substituting values in the equation of Manery (1954).

$$\text{Muscle } ^{36}\text{Cl space g water/kg muscle} = \frac{^{36}\text{Clm} \times \text{H}_2\text{Op} \times \text{rCl}}{^{36}\text{Clp}},$$

where ^{36}Clm is muscle ^{36}Cl content (counts/min per kg muscle), ^{36}Clp is plasma ^{36}Cl content (counts/min per l plasma), H_2Op is plasma water content (ml/kg plasma), rCl is Gibbs–Donnan ratio for Cl (0·977), Holmes & Donaldson, 1969.

Correlation coefficients among the experimental results were determined as described by Fisher (1950).

RESULTS

Although the two experiments involved trout of different mean initial weights, and which differed in age by approximately 8 weeks, for ease of presentation, measurements of the same variable are shown together and diets are numbered consecutively.

In both experiments growth and mortality rate were affected by dietary Mg intake (Table 2) the lowest weight gain and the highest rate of mortality occurred in those fish given diets lacking supplementary Mg (diet no. 1 and diet no. 6 containing 0·04 and 0·06 g Mg/kg dry diet respectively). Weight gain reached a maximum, in both experiments, when the concentration of Mg in the diet was 0·25–0·5 g/kg, it did not increase with further rise in dietary Mg up to levels of 1 g/kg (Expt 1) and 2 g/kg (Expt 2). In those trout given diets without supplementary Mg a higher rate of mortality was evident in Expt 2 (those trout given diet no. 6) than in Expt 1 (diet no. 1). This may have resulted either from the smaller size of the fish used in Expt 2 or from the higher dietary Ca and P concentrations used in that experiment.

Further symptoms of Mg deficiency shown by trout were a flaccidity of the muscle and sluggishness (that is they were very easily caught by hand net and removed from the tank at times of weighing). There was little sign of loss of appetite; food consumption was very similar on all dietary treatments during the first 12 weeks of each experiment amounting to 25 g/kg biomass trout per d. Thereafter there was a slight fall in food intake on all treatments but even during weeks 19 and 20 consumption by fish given diet no. 1 (Expt 1) was 18 g/kg biomass trout per d. This compares with values of (g/kg biomass trout per d) 21, 20, 21 and 22 for fish given diet nos. 2–5 respectively. A similar pattern of food consumption was found in Expt 2.

The concentrations of certain minerals in the sera are shown in Table 3. In Expt 1 there was a positive correlation between serum Mg concentration and dietary Mg (r 0·72, n 28, $P < 0·001$). For the smaller fish used in Expt 2 serum Mg increased with dietary Mg to reach a plateau value of 1 mmol/l at a dietary Mg level of 0·54 g/kg, further increase in dietary Mg did not lead to any further increase in serum Mg concentration. In this context it is noteworthy that in Expt 1 the highest serum Mg concentration found was 1·58

Table 2. *Initial numbers of fish, mean initial and final weights (g) and mortalities of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of calcium, magnesium and phosphorus*

	Diet no.*	Initial no. of fish	Mean initial wt (g)	Mean final wt (g)	Mortalities
Expt 1	1	30	33·3	88·6	5
	2	27	35·8	122·2	2
	3	29	35·2	144·1	1
	4	28	34·1	152·3	0
	5	28	35·6	145·4	1
Expt 2	6	32	16·1	46·3	11
	7	30	15·9	64·9	2
	8	31	17·0	67·3	3
	9	31	15·0	52·0	2
	10	32	17·7	59·8	2
	11	31	16·1	54·0	2

* For details, see Table 1.

mmol/l when Mg in the diet was 0·56 g/kg while at the highest dietary Mg level used (1·0 g/kg) serum Mg concentration was 1·35 mmol/l. By comparison with Expt 2 it thus appears likely that had it been possible to include higher dietary Mg levels in Expt 1 these would have shown that, in this experiment also, serum Mg concentration might have reached a plateau value above a certain dietary Mg level.

There was also a positive correlation between serum Na concentration and dietary Mg in both experiments. Serum Na increased from 106 to 154 mmol/l in Expt 1 as dietary Mg rose from 0·04 to 1·0 g/kg diet (r 0·63, n 28, $P < 0·001$). Serum Na concentrations in Expt 2 (r 0·63, n 34, $P < 0·001$) were higher than those found in Expt 1 but the increase with rising dietary Mg was less marked than in Expt 1.

The trends seen in serum K concentrations were the converse of those found with serum Na and Mg. Over the range of dietary Mg concentrations used in Expt 1 serum K was negatively correlated with dietary Mg (r −0·58, n 28, $P < 0·001$). In Expt 2 serum K fell to a concentration of 1·72 mmol/l at a dietary Mg level of 0·54 g/kg. This appeared to be a plateau minimum because serum K concentration did not change further as dietary Mg was raised to 2·0 g/kg.

This elevation of serum K concentration in the Mg-deficient trout is remarkable from a comparative viewpoint in that it contrasts with findings in rats. Elin *et al.* (1971) found no differences in the plasma K levels of Mg-deficient and control rats while George (1976) also using rats found a significant decrease in plasma K in the Mg-deficient animal.

Serum P concentration showed no obvious trend in either experiment with increasing dietary Mg. Serum Ca concentration was also relatively constant in both experiments although trout given diets deficient in Mg (diet no. 1, Expt 1; and diet no. 6, Expt 2) did show slight, but significant, hypercalcaemia. It may be noted that although dietary Ca and P levels differed markedly in the two experiments serum Ca and P levels were generally similar.

Renal Ca levels (Table 4) were elevated by more than three-fold in trout given the basal Mg diet in Expt 1; the kidney Ca concentrations in trout given diets containing increasing amounts of supplementary Mg (0·15–1·0 g/kg) were very constant with a mean value of 3·59 mmol/kg wet weight. The renal Ca level in trout given a diet lacking supplementary Mg in Expt 2 was more than double that found in the corresponding treatment in Expt 1.

Table 3. *Mean concentrations (mmol/l) of calcium, magnesium, phosphorus, sodium and potassium in the sera of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of Ca, Mg and P*

(Mean values with their standard errors for six fish/treatment)

		Ca		Mg		P		Na		K	
	Diet no.*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1	1	4·32	0·14	0·44	0·03	4·89	0·22	106·58	2·13	11·56	1·36
	2	3·93	0·09	0·86	0·06	7·81	0·26	143·82	1·42	8·03	1·08
	3	3·91	0·07	0·95	0·07	7·12	0·20	137·16	1·30	6·39	1·04
	4	3·62	0·08	1·58	0·05	7·63	0·20	126·02	2·48	5·92	0·76
	5	3·70	0·11	1·35	0·05	6·31	0·32	154·56	1·54	3·78	0·51
Expt 2	6	3·88	0·11	0·58	0·07	9·44	0·58	154·59	2·00	10·95	1·13
	7	3·33	0·14	0·88	0·08	6·93	0·17	163·90	2·20	6·57	1·01
	8	3·52	0·19	1·00	0·08	7·48	0·38	165·56	1·99	1·72	0·33
	9	3·24	0·07	1·07	0·04	7·34	0·35	163·08	2·71	2·59	0·54
	10	3·58	0·14	0·96	0·03	7·87	0·30	171·15	1·80	4·02	1·40
	11	3·20	0·09	1·06	0·06	8·42	0·88	171·56	1·99	1·45	0·43

* For details, see Table 1.

Table 4. *Mean concentrations (mmol/kg wet weight) of calcium, magnesium, phosphorus, sodium and potassium in kidneys of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of Ca, Mg and P*

(Mean values with their standard errors for six fish/treatment)

		Ca		Mg		P		Na		K	
	Diet no.*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1	1	12·39	4·23	6·49	0·16	93·76	3·67	66·44	2·90	80·56	1·55
	2	3·85	0·62	5·68	0·13	86·87	1·90	58·19	1·16	74·61	1·60
	3	3·61	0·34	5·07	0·16	83·34	0·97	77·23	2·74	68·74	1·85
	4	3·34	0·17	5·64	0·12	87·80	0·76	69·24	3·45	83·68	1·61
	5	3·56	0·27	6·50	0·15	90·42	1·22	59·49	2·13	77·29	1·98
Expt 2	6	27·52	6·01	6·94	0·20	114·42	6·57	38·30	1·92	88·41	2·45
	7	4·84	0·66	7·02	0·23	82·60	3·44	41·02	1·30	85·67	2·46
	8	6·56	1·37	6·79	0·18	95·36	4·51	41·78	3·25	78·59	3·82
	9	5·73	0·79	7·55	0·19	92·57	1·17	39·42	1·64	82·42	1·22
	10	5·88	1·08	6·08	0·17	86·40	15·14	34·68	1·47	61·05	1·56
	11	4·59	0·30	5·86	0·19	76·62	12·16	33·27	0·99	58·29	2·23

* For details, see Table 1.

Table 5. *Mean concentrations (mmol/kg wet weight) of calcium, magnesium, phosphorus, sodium and potassium in the muscle of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of Ca, Mg and P*

(Mean values with their standard errors for six fish/treatment)

		Ca		Mg		P		Na		K	
	Diet no.*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1	1	15·55[a]	1·72	9·86	0·24	83·67	1·84	37·64[a]	2·04	102·76	2·18
	2	3·63[b]	0·43	10·95	0·23	82·09	1·59	23·42[b]	1·71	100·69	1·58
	3	2·99[b]	0·23	10·87	0·23	81·51	1·33	27·51[b]	1·48	103·28	1·87
	4	2·42[b]	0·15	11·66	0·11	74·92	0·93	20·14[b]	1·13	104·05	2·07
	5	2·40[b]	0·20	12·28	0·24	79·79	0·97	27·54[b]	1·76	98·05	1·14
Expt 2	6	6·15	1·43	10·36	0·44	87·19	1·03	26·48	3·52	88·49	3·30
	7	7·11	2·43	12·30	0·37	96·22	3·31	15·22	1·16	99·09	3·25
	8	5·40	1·29	13·46	0·27	100·88	2·92	12·61	0·48	101·82	5·80
	9	5·06	1·28	13·47	0·34	111·06	3·79	27·41	1·52	115·30	3·14
	10	8·69	2·09	13·25	0·20	104·50	2·18	20·64	1·80	116·60	2·64
	11	7·99	2·18	12·41	0·55	114·32	2·45	22·38	1·21	103·97	4·33

*For details, see Table 1.

a, b, values in the same column in the same experiment with different superscripts were significantly different ($P < 0·01$).

Table 6. *Mean levels (g water/kg wet weight) of total muscle water, extracellular fluid volume and intracellular fluid volume in the muscle of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of calcium, magnesium and phosphorus*

(Mean values with their standard errors for six fish/treatment)

		Total muscle water volume		Muscle extracellular fluid volume		Muscle intracellular fluid volume	
	Diet no.*	Mean	SE	Mean	SE	Mean	SE
Expt 1	1	794·5	4·4	194·1	22·1	600·5	17·8
	2	785·6	3·2	114·6	4·3	671·0	5·6
	3	767·7	2·3	117·5	9·7	650·2	9·8
	4	769·3	1·1	80·0	3·3	689·3	3·2
	5	763·7	0·7	65·8	3·8	697·9	4·0
Expt 2	6	797·9	4·2	179·4	23·5	618·5	20·0
	7	786·7	1·7	83·5	9·0	702·6	8·4
	8	781·3	2·7	69·2	6·0	712·1	6·7
	9	791·9	2·1	53·4	1·0	738·5	8·8
	10	785·2	1·9	68·6	6·3	716·6	6·6
	11	785·2	1·9	72·5	4·5	714·8	2·4

* For details, see Table 1.

Kidney Ca levels of trout given diets with supplementary Mg in Expt 2 tended to be more variable and were higher than those from Expt 1, mean kidney Ca level of these trout was 5·52 mmol/kg wet weight.

Renal P concentrations decreased as dietary Mg increased in Expt 2 (r −0·51, n 34, $P < 0·01$) but this trend was not apparent in Expt 1. A negative correlation between renal K concentration and dietary Mg was apparent in Expt 2 (r −0·84, n 34, $P < 0·001$). Again this trend was absent in Expt 1. In neither experiment were any trends discernible in the renal concentrations of Mg or Na in response to increasing intake of dietary Mg.

Muscle mineral concentrations are shown in Table 5. In Expt 1 muscle P concentration decreased as dietary Mg increased (r −0·41, n 28, $P < 0·01$) while muscle Mg concentration was positively correlated with diet Mg (r 0·66, n 28, $P < 0·01$). No trends were evident in muscle Ca, K or Na concentrations in Expt 1 although in trout given the diet 1, lacking supplementary Mg, both muscle Ca and muscle Na concentrations were significantly greater than for other treatments ($P < 0·01$). In Expt 2 muscle P concentration increased as dietary Mg increased (r 0·71, n 34, $P < 0·001$), muscle K concentration was also positively correlated with diet Mg (r 0·41, n 34, $P < 0·01$). Muscle Mg concentration increased with dietary Mg up to a dietary Mg level of 0·54 g/kg but did not increase further with rising dietary Mg. The inflexion or plateau point in the muscle Mg/diet Mg response relationship occurred at a dietary Mg level of 0·5 g/kg. No trends were evident in muscle Ca or muscle Na concentration in Expt 2.

The distribution of water in the muscle of fish from the two experiments is shown in Table 6. In Expt 1 ECFV was 194 g water/kg muscle when the diet (no. 1) contained no supplementary Mg; ECFV decreased as dietary Mg increased (r −0·73, n 28, $P < 0·001$) until with diet no. 5 (1 g Mg/kg) it had fallen within the normal range at 65·8 g water/kg muscle. ECFV was 179·4 g water/kg muscle in trout given diet no. 6 (Expt 2); in this experiment ECFV fell to a value of 69·2 g water/kg (diet no. 8) but remained sensibly constant at this level as diet Mg increased to 2·0 g/kg.

Mineral concentrations in cardiac muscle of trout from the two experiments are shown

Table 7. *Mean concentrations (mmol/kg wet weight) of calcium, magnesium, phosphorus, sodium and potassium in the hearts of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of Ca, Mg and P*

(Mean values with their standard errors for six fish/treatment)

		Ca		Mg		P		Na		K	
	Diet no.*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1	1	2·11	0·13	6·36	0·13	58·04	0·69	63·91	1·69	66·72	1·54
	2	2·05	0·17	6·02	0·18	54·16	1·17	59·27	1·83	58·83	1·40
	3	2·34	0·20	5·91	0·14	57·36	0·90	69·93	2·16	67·22	1·29
	4	1·60	0·08	5·54	0·15	54·74	1·20	61·95	1·44	60·98	1·33
	5	2·92	0·19	5·58	0·20	56·04	1·18	63·39	1·67	57·34	1·52
Expt 2	6	6·41	1·59	7·86	1·23	92·41	2·27	52·49	3·06	61·88	2·32
	7	5·42	0·63	6·63	0·26	84·35	2·57	58·29	2·47	61·69	2·40
	8	1·85	0·17	5·35	0·08	68·33	1·22	72·04	4·84	40·84	1·90
	9	2·62	0·32	5·26	0·16	64·42	1·76	47·31	3·79	49·37	1·48
	10	2·96	0·21	3·19	0·24	60·27	2·12	45·06	4·01	41·05	1·04
	11	3·74	0·35	4·17	0·23	63·99	2·05	44·71	3·89	43·24	1·22

* For details, see Table 1.

Table 8. *Mean concentrations (mmol/kg wet weight) of calcium, magnesium, phosphorus, sodium and potassium in the livers of rainbow trout* (Salmo gairdneri) *given diets containing different amounts of Ca, Mg and P*

(Mean values with their standard errors for six fish/treatment)

		Ca		Mg		P		Na		K	
	Diet no.*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Expt 1	1	1·52	0·06	6·07	0·13	92·82	1·71	54·92	0·91	95·10	0·96
	2	1·88	0·14	6·66	0·20	99·67	2·02	38·92	1·07	89·72	1·91
	3	1·39	0·05	6·44	0·15	92·52	2·14	45·41	2·51	100·23	1·75
	4	1·19	0·03	6·53	0·08	92·17	1·63	48·25	1·60	97·58	1·45
	5	1·29	0·09	6·85	0·14	92·87	1·56	47·30	1·44	91·91	1·62
Expt 2	6	1·42	0·13	7·32	0·19	86·62	2·87	37·74	0·57	92·30	2·80
	7	1·44	0·10	7·28	0·25	93·58	1·32	35·98	0·77	93·77	1·47
	8	1·32	0·08	7·10	0·25	96·58	3·76	35·14	1·19	93·97	1·96
	9	1·48	0·17	7·33	0·42	85·90	3·49	30·69	0·64	85·92	5·02
	10	1·54	0·12	7·43	0·60	94·97	4·63	30·99	0·90	93·68	3·64
	11	1·44	0·05	7·38	0·28	98·83	5·27	35·37	1·42	92·32	4·90

* For details, see Table 1.

in Table 7. There was a slight but significant decrease in cardiac muscle Mg concentration as dietary Mg increased (r −0·45, n 28, $P < 0·01$) in Expt 1; no trends were evident in cardiac muscle concentrations of Ca, P, Na or K in Expt 1. Cardiac muscle Mg concentration was also negatively correlated with dietary Mg (r −0·7, n 34, $P < 0·001$) in Expt 2. In this experiment the concentrations of both Ca and P decreased with increasing dietary Mg until the dietary level was 0·56 g Mg/kg thereafter the concentrations of Ca and P remained relatively constant.

Concentrations of Mg, Ca, P, K and Na in the livers of trout from the two experiments are shown in Table 8. In this organ concentrations of all these minerals were relatively constant in both experiments.

DISCUSSION

It can be concluded from many of the results obtained in Expt 2 that the Mg requirement of rainbow trout in the weight range 16–60 g is satisfied by an Mg concentration in the diet of 0·5 g/kg. At this concentration weight gain, serum Mg and muscle Mg levels reached maximal (plateau) levels, similarly muscle ECFV and serum K reached constant levels and did not decrease further with increasing dietary Mg. Mortalities were few at or above this dietary Mg level nor was there any calcinosis in the kidney.

Several of the results obtained in Expt 1 with trout in the weight range 30–150 g, given diets containing lower levels of Ca and P than those used in Expt 2, also indicate an Mg requirement of 0·5 g/kg diet. Other possible criteria were less conclusive; in particular muscle ECFV, serum K, serum Mg and muscle Mg gave different dose/response relationships than was the case in Expt 2. While these parameters changed in the direction anticipated with increasing dietary Mg concentration they did not reach a constant level. The most obvious explanation for this is that the range of dietary Mg concentrations used in Expt 2 (0·06–2·0 g/kg) was double that in Expt 1 (0·04–1·0 g/kg) so that in the latter experiment it was less easy to see where, with most variables, the limit of response had been reached. Despite the inconclusive nature of the results with muscle ECFV, serum Mg, serum K and muscle Mg other evidence shows that in this experiment also, the Mg requirement is met at a dietary level of 0·5 g/kg. Thus, at or above this concentration, weight gain reached a plateau, few mortalities occurred and there was no renal calcinosis.

While this work was in progress Ogino *et al.* (1978) showed that an Mg concentration of 0·6–0·7 g/kg dry diet was necessary to ensure optimum growth in rainbow trout fry (mean initial weight 0·9 g) given a diet containing approximately 2·8 g Ca and 4·6 g P/kg dry diet. Ogino & Chiou (1976) had earlier shown that the Mg requirement of carp given diets similar to those used with the trout was 0·4–0·6 g/kg.

Studies carried out on guinea pigs and rats (O'Dell *et al.* 1960; McAleese & Forbes, 1961) and on chicks (Nugura & Edwards, 1963) have shown that when dietary Ca or P levels or both were raised the dietary Mg requirement increased. By contrast the results presented in this study do not indicate any increase in Mg requirement as dietary levels of Ca or P are increased.

In both experiments an increase in muscle ECFV was shown to be associated with an inadequate dietary Mg level. This substantiates previous explanations of changes in muscle composition during Mg deficiency (Cowey *et al.* 1977). Much earlier Elin *et al.* (1971) had obtained a similar result using Mg-deficient rats. Although the total muscle water content increased slightly in the Mg-deficient fish, the main factor in the increased ECFV in both experiments appeared to be a shift of water from the intracellular to the extracellular compartment (Table 6). In rats this water shift was associated with a reduction in the concentration of muscle K and an increase in that of muscle Na. Similar trends were evident in muscle K concentration in Expt 2 (although not in Expt 1) however, no significant trends in muscle Na with increasing dietary Mg were evident in either experiment.

The shift in muscle water in Mg-deficient rats also explained the decrease in muscle Mg in this animal for it was shown (Elin *et al.* 1971) that the concentration of Mg in the intracellular fluid (which is rich in Mg) was not significantly different in Mg-deficient and control animals. The trend seen in both the present experiments of a fall in muscle Mg with decreasing dietary Mg may be similarly due to the reduction in intracellular fluid volume. It is noteworthy that Mg concentrations in cardiac muscle showed, in both experiments, opposite trends to that in skeletal muscle, concentrations being maintained during Mg deficiency. Mg concentrations in cardiac muscle are lower than those in skeletal muscle and particular homoeostatic mechanisms may operate to maintain them.

Phosphorus concentrations in muscle showed opposite trends in the two experiments but there is no obvious explanation for this. While it may relate to the higher dietary P concentrations used in Expt 2 those used in Expt 1 are, in fact, greater than levels considered necessary for optimal growth in salmonids (Ketola, 1975; Ogino & Takeda, 1978).

Changes in the Mg, Ca, K and Na concentrations in rat liver as a consequence of Mg deficiency were reported by Martindale & Heaton (1964). However, MacIntyre & Davidsson (1958) and Elin *et al.* (1971) failed to find any changes in the concentrations of these minerals in this tissue as a consequence of Mg deficiency. Our results on trout are consistent with the latter findings in that liver mineral concentrations remained constant in both experiments in response to the treatments used.

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