Some effects of vitamin E and selenium deprivation on tissue enzyme levels and indices of tissue peroxidation in rainbow trout (*Salmo gairdneri*)

BY J. G. BELL, C. B. COWEY AND J. W. ADRON

NERC Institute of Marine Biochemistry, St Fittick's Road, Aberdeen AB1 3RA

AND AILEEN M. SHANKS

Marine Laboratory, Victoria Road, Aberdeen AB9 8DB

(*Received 19 December 1983 – Accepted 13 August 1984*)

1. Duplicate groups of rainbow trout (*Salmo gairdneri*) (mean weight 11 g) were given for 40 weeks one of four partially purified diets that were either adequate or low in selenium or vitamin E or both.

2. Weight gains of trout given the dually deficient diet were significantly lower than those of trout given a complete diet or a diet deficient in Se. No mortalities occurred and the only pathology seen was exudative diathesis in the dually deficient trout.

3. There was significant interaction between the two nutrients both with respect to packed cell volume and to malondialdehyde formation in the in vitro NADPH-dependent microsomal lipid peroxidation system.

4. Tissue levels of vitamin E and Se decreased to very low levels in trout given diets lacking these nutrients. For plasma there was a significant effect of dietary vitamin E on Se concentration.

5. Glutathione (GSH) peroxidase (*EC* 1.11.1.9) activity in liver and plasma was significantly lower in trout receiving low dietary Se but was independent of vitamin E intake. The ratios of hepatic GSH peroxidase activity measured with cumene hydroperoxide and hydrogen peroxide were the same for all treatments. This confirms the absence of a Se-independent GSH peroxidase activity in trout liver.

6. Se deficiency did not lead to any compensatory increase in hepatic GSH transferase (*EC* 2.5.1.18) activity; values were essentially the same in all treatments.

7. Plasma pyruvate kinase (*EC* 2.7.1.40) activity increased significantly in the trout deficient in both nutrients. This was thought to be due to leakage of the enzyme from the muscle and may be indicative of incipient (subclinical) muscle damage.

The essentiality of dietary selenium for mammals and its close metabolic relation with vitamin E has been recognized since Schwarz & Folz (1957) first demonstrated that Se could replace vitamin E in the prevention of dietary liver necrosis. Results on this interrelation in fish are limited. Poston *et al.* (1976), using Atlantic salmon (*Salmo salar*) during the first six weeks after yolk sac absorption (mean weight 0·1 g), demonstrated that dietary supplements of both vitamin E and Se were necessary to reduce mortality significantly. In larger fish (0·9 g live weight) both vitamin E and Se were necessary to prevent muscular dystrophy. Hilton *et al.* (1980), using rainbow trout (*Salmo gairdneri*) of 1·28 g mean initial weight, could find no deficiency symptoms at dietary Se levels of 0·07 μg/g with 0·4 μg Se/l of rearing water and in the presence of a dietary vitamin E concentration of 0·4 IU/g. Both experiments were carried out at a water temperature of 14–15°.

The presence of Se-independent glutathione (GSH) peroxidase (*EC* 1.11.1.9) activity has recently been claimed in bullheads (species unspecified) said to be deficient in Se (Heisinger & Dawson, 1983). The possibility exists that the lack of effect of Se deficiency on rainbow trout may result from compensatory increases in such an enzyme. In some mammals, GSH transferase (*EC* 2.5.1.13) activity has been identified as contributing to the Se-independent GSH peroxidase (Lawrence *et al.* 1978). While purified GSH transferase from rainbow trout liver does not have any peroxidase activity, it will partially inhibit malondialdehyde formation by trout liver microsomes in vitro (Bell *et al.* 1984).

Table 1. *Composition of the basal diet (g/kg)*

Torula yeast	350
Casein	200
Starch	194·6
Linolenic acid	10
Palmitic acid	90
Amino acid mixture*	80
Vitamin premix†	28
Mineral premix‡	40
Antioxidant mixture§	0·5
Ascorbyl palmitate	0·4
Rovimix A+D‖	0·074

* Supplied (g/kg diet): methionine 8, arginine 8·5, histidine 1·7, isoleucine 2·93, leucine 5·33, lysine 4·57, phenylalanine 3·4, threonine 2·64, tryptophan 0·95, tyrosine 3·49, valine 3·98, glycine 4·34, alanine 1·95, aspartic acid 4·66, cystine 0·19, glutamic acid 13·7, proline 6·32, serine 3·47.

† Supplied (/kg diet): thiamin hydrochloride 50 mg; riboflavin 200 mg, pyridoxine HCl 50 mg, nicotinic acid 750 mg, calcium pantothenate 500 mg, *myo*-inositol 2 g, biotin 5 mg, folic acid 15 mg, choline bitartrate 9 g, ascorbic acid 1 g, menaphthone 40 mg, cyanocobalamin 0·09 mg.

‡ Supplied (g/kg diet): $Ca(H_2PO_4)_2 . H_2O$ 27·6, $CaCO_3$ 2·1, $MgCO_3$ 3·6, $FeSO_4 . 7H_2O$ 1·2, KCl 2·0, NaCl 3·2, $Al_2(SO_4)_3 . 16H_2O$ 0·008, $ZnSO_4 . 7H_2O$ 0·16, $CuSO_4$ 0·04, $MnSO_4 . 4H_2O$ 0·14, KI 0·008, $CoSO_4$ 0·004.

§ Contained (/kg): 200 g butylated hydroxyanisole, 60 g propyl gallate and 40 g citric acid dissolved in 700 g propylene gylcol.

‖ Roche Products Ltd, Dunstable, Beds; supplied 11·1 mg retinol and 185 μg cholecalciferol/kg diet.

To examine this possibility and to explore more fully the interrelation between Se and vitamin E, rainbow trout (of initial mean weight 11 g) were given diets either adequate or low in Se, or vitamin E or both. Afterwards tissue activities of relevant enzymes were assayed and other relevant measurements made.

MATERIALS AND METHODS

Animals and diets

Rainbow trout were obtained from H.I.D.B., Moniack Hatchery, Inverness; they had a mean weight of approximately 10 g and were distributed at random (thirty fish per tank) between eight fibre-glass tanks of diameter 1 m, depth 0·6 m, and containing 500 litres water. The water, from the City of Aberdeen domestic supply, passed through an activated-charcoal filter to the tanks and thence, after passing a faecal trap and biological filters, it was partially recirculated with a constant bleed-in of fresh tap water (1 litre/min per tank); total flow rate to each tank was 10 litres/min. The tanks were housed in an aquarium room and the ambient water temperature averaged 15°. The photoperiod was 12 h light and 12 h dark.

The fish were first weaned from a commercial diet to one of the experimental diets (diet 1, Table 1) and about 1 week later initial weight measurements were made on individual fish that had first been anaesthetized with MS 222 (ethyl *m*-amino benzoate methane sulphonate; Sigma Chemical Co. Ltd, Poole, Dorset; 0·2 g/l). Fish were fed at the rate of 20 g/kg biomass per d (at three or four feeds per day) 6 d each week, any food uneaten from the daily ration was weighed and recorded. Fish were weighed at 28-d intervals, when the ration size was adjusted. The experiment lasted 40 weeks.

The composition of the basal diet, shown in Table 1, was formulated to meet the nutritional requirements of trout other than for vitamin E and Se. The torula yeast was extracted by refluxing with ethanol to lower the vitamin E content. Four diets were formed from the basal diet (Table 2, p. 152) by adding Se as sodium selenite or vitamin E

(DL-α-tocopheryl acetate) as Rovimix E 50. (Roche Products Ltd, Dunstable, Beds). The dry components of the diet were thoroughly mixed in a Hobart commercial mincer, model A200 (Hobart Manufacturing Co. Ltd, London), then formed into a paste by adding 500 ml water/kg powder. The paste was formed into pellets by passing twice through the mincer from which the cutting blades had been removed and using a die with appropriately-sized holes to provide suitable pellets for the fish. After freeze-drying, the pellets were broken into approximately 5-mm lengths. Each of the four diets was given to duplicate groups (tanks) of fish.

Analytical methods

Each of the analytical measurements was made on six individuals from each treatment, three individuals being taken at random from each of the duplicate tanks. Blood samples were take from the caudal vein and muscle samples were always taken from the same place: the anterior, left, dorsal part of the body.

Vitamin E was extracted from the diets as described by McMurray *et al.* (1980) and from fish tissues as described previously (Cowey *et al.* 1981); in both diet and tissue extracts vitamin E was resolved and measured by high performance liquid chromatography (Hung *et al.* 1980) except that detection was by fluorimetry (model LS4 fluorescence spectrometer, Perkin-Elmer, Beaconsfield, Bucks).

Se in rearing-water, diets and fish tissues was measured as described by Hasunuma *et al.* (1982). Erythrocyte fragility measurements were made as described by Draper & Csallany (1969), except that incubations were carried out in a shaking water-bath (60 cycles/min) at 15° for 24 h; every 8 h, tubes were removed from the water-bath and cells thoroughly resuspended by careful shaking. Liver microsomes were prepared as described by Bell *et al.* (1984) and conditions for the NADPH-stimulated oxidation of microsomal lipids were as described by Bell *et al.* (1984).

Enzyme assays were carried out at 20°. For measurement of GSH peroxidase and GSH transferase, liver was homogenized with nine volumes of a solution containing 20 mM-Tris-hydrochloride, pH 7·4, 1 mM-EDTA, 0·5 mM-dithiothreitol and 1% (w/w) triton X-100; the resulting homogenate was used directly for the assays. For GSH peroxidase the rate of NADPH oxidation was followed at 340 nm by the coupled reaction with GSH reductase (NAD(P)H) (*EC* 1.6.4.2). The reaction mixture contained 2 mM-GSH, 0·2 mM-cumene hydroperoxide (or 50 μM-hydrogen peroxide), 0·1 mM-NADPH, 0·5–1·0 units GSH reductase (Boehringer Corporation, London Ltd; 1 unit oxidizes 1 μmol NADPH/min), 1 mM-EDTA, 2 mM-sodium azide and 50 mM-potassium phosphate buffer, pH 7·4. GSH transferase activity was assayed by following the conjugation of GSH with 1-chloro-2,4-dinitrobenzene spectrophotometrically at 340 nm (Habig *et al.* 1974). The assay mixture contained 100 mM-potassium phosphate (pH 6·5), 2 mM-GSH and 1 mM-1-chloro-2,4-dinitrobenzene. Protein was measured by the method of Lowry *et al.* (1951).

Plasma pyruvate kinase (*EC* 2.7.1.40) activity was measured by following the rate of NADH oxidation at 340 nm in the coupled reaction with lactate dehydrogenase (*EC* 1.1.1.27). Final concentrations in the assay were 100 mM-imidazole-HCl, pH 7·4, 70 mM-potassium chloride, 4 mM-magnesium chloride, 2 mM-ADP, 1·5 mM-phosphoenolpyruvate, 0·16 mM-NADH and excess lactate dehydrogenase (5 μl Boehringer hog muscle enzyme, 1 mg protein/ml, in 1 ml total volume).

Histology

At the end of the experiment, small pieces of tissue from six individuals per treatment were fixed in buffered formalin and embedded in paraffin wax. Subsequently sections were prepared. Those from muscle were stained with haematoxylin and eosin; those from liver

Table 2. *Initial and final weights of rainbow* (Salmo gairdneri) *trout given diets† either supplemented with or deficient in vitamin E or selenium for 40 weeks*

(Mean values with their standard errors for thirty fish per tank)

Diet...	1				2			
Measured vitamin E content (mg/kg)...	40·6				40·6			
Measured Se content (mg/kg)...	0·869				0·060			
	Tank A		Tank B		Tank C		Tank D	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Mean initial wt (g)	11·3	0·55	11·3	0·55	11·0	0·61	11·0	0·61
Mean final wt (g)	351·8[a]	14·22	357·2[a]	15·64	353·5[a]	15·99	333·9[a,c]	15·99
Feed intake/wt gain	1·54		1·51		1·64		1·59	
Diet...	3				4			
Measured vitamin E content (mg/kg)...	2·14				1·96			
Measured Se content (mg/kg)...	0·877				0·060			
	Tank E		Tank F		Tank G		Tank H	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Mean initial wt (g)	10·9	0·67	10·9	0·67	11·8	0·76	11·8	0·76
Mean final wt (g)	356·7[a]	20·67	292·0[b,c]	15·88	270·5[b]	27·33	277·4[b]	22·96
Feed intake/wt gain	1·55		1·70		2·00		1·78	

[a,b,c] Values in the same row with different superscript letters are significantly different ($P < 0·01$).

† For details of basal diet, see Table 1.

were similarly stained but in addition the Schmorl method for lipofuschin and the nile blue method for lipofuschin (Pearse, 1972) were applied to liver sections.

Statistical analysis

A two-way analysis of variance was carried out to test the main effects of variation in dietary content of vitamin E and Se on growth and a number of other indicies and to examine any interaction of these nutrients. This also enabled any differences between duplicate tanks within treatments to be seen.

RESULTS

Initial and final weights of fish given the four diets are shown in Table 2. Trout given diet 4, depleted of both vitamin E and Se, had significantly lower mean final weights than those given diets 1 (complete diet) or 2 (depleted of Se). For diet 3 (deficient in vitamin E) the duplicate tanks of trout had significantly different mean final weights; for one of these tanks mean final weight was significantly greater than that of trout given the dually deficient diet (diet 4), for the other tank it was not significantly different.

Food utilization measured over the whole period of the experiment tended to be poorer in the deficient dietary treatments. As there were only two observations (one per tank) for

Table 3. *Packed cell volume, erythrocyte fragility and in vitro NADPH-stimulated lipid peroxidation in liver microsomes of rainbow trout* (Salmo gairdneri) *given diets† of different vitamin E and selenium contents*

(Mean values with their standard errors for six fish per treatment (packed cell volume and erythrocyte fragility) or eighteen fish per treatment (lipid peroxidation))

Diet...	1		2		3		4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Packed cell volume (%)	55·67	2·22	54·83	2·54	49·20	1·53	7·33**	1·09
Erythrocyte fragility (% haemolysis)	20·14	1·23	30·9	7·46	21·55	2·14	51·53	15·46
Microsomal lipid peroxidation (nmol malondialdehyde formed/mg protein)	13·98**	2·60	31·53	3·08	40·62	3·83	38·72	2·38

† For details, see Tables 1 and 2.
Significantly different from other treatments: ** $P < 0·01$.

each dietary treatment for each 28 d period, it was not possible to say whether this tendency was significant. No mortalities occurred and no gross pathology was evident in trout given diets 1, 2 and 3. Fish given the dually deficient diet (diet 4), however, all suffered from exudative diathesis, a pale yellow serous fluid filling the body cavity.

No signs of muscle damage could be seen in any of the sections prepared from muscle. In some of the liver sections small foci were found in some of the blood vessel walls which had a positive staining reaction for lipofuschin. Examination of many sections from all treatments showed that these foci were present in all tissues to a similar extent.

There were no differences between the duplicate tanks, for each of the four dietary treatments, for any of the other indices measured (Tables 3–6). There was significant interaction between vitamin E and Se with respect to packed cell volume (Table 3). Consequently, the effects of either nutrient on packed cell volume cannot be considered without reference to the dietary concentration of the other. Packed cell volume values were not significantly affected by giving diets deficient in either vitamin E or Se singly but were reduced to extremely low levels when both nutrients were absent (diet 4).

There was considerable variation in erythrocyte fragility values but there was no interaction between the two nutrients. Microsomal lipid peroxidation values are also shown in Table 3; there was significant interaction between vitamin E and Se with respect to this measurement. Low values were obtained only when the diet was supplemented with both nutrients (diet 1).

Tissue vitamin E levels (Table 4) were in accord with dietary treatment and demonstrated that a deficient state had been reached. For all three tissues a significant effect of dietary vitamin E on tissue vitamin E was evident.

Selenium levels in the three tissues examined were all significantly affected by dietary SE intake (Table 5). For plasma, but not for liver and kidney, there was also a significant vitamin E effect, plasma Se levels being lower in the absence of supplementary dietary vitamin E than in its presence. Tissue Se levels in the depleted state (diets 2 and 4) were of a similar order to those reported by Hilton *et al.* (1980). The lowest dietary Se level used by Hilton *et al.* (1980), 0·07 mg/kg, was also similar to that used in the present study, although water-borne Se in the former study (0·4 μg/l) was greater than that used in the present

Table 4. *Concentrations of vitamin E (μg/ml or g wet tissue) in tissues of rainbow trout* (Salmo gairdneri) *given diets† of different vitamin E and selenium contents*

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

Diet...	1		2		3		4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Whole blood	15·93[a]	2·33	16·02[a]	1·57	2·77[b]	0·49	1·67[b]	0·43
Liver	35·60[a]	2·84	36·79[a]	5·36	3·42[b]	0·53	2·26[b]	0·41
White muscle	6·22[a]	0·59	7·41[a]	0·63	1·28[b]	0·24	0·92[b]	0·32

[a,b] Values in the same row with different superscript letters are significantly different ($P < 0·01$).
† For details, see Tables 1 and 2.

Table 5. *Concentrations of selenium (μg/g or ml wet tissue) in tissues of rainbow trout* (Salmo gairdneri) *given diets† of different vitamin E and Se contents*

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

Diet...	1		2		3		4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma	0·157[a]	0·012	0·038[c]	0·003	0·124[b]	0·005	0·032[c]	0·004
Liver	3·86[a]	0·48	0·29[b]	0·02	2·72[a]	0·31	0·18[b]	0·03
Kidney	1·72[a]	0·15	0·53[b]	0·06	2·80[a]	0·53	0·49[b]	0·05

[a,b,c] Values in the same row with different superscript letters are significantly different ($P < 0·01$).
† For details, see Tables 1 and 2.

experiment (0·04 μg/l). This may mean that uptake of water-borne Se does not contribute greatly to tissue levels. In the deficient state tissue Se levels are, for at least some tissues, similar to those found in mammals. Siddons & Mills (1981) obtained a blood Se concentration of 0·022 μg/ml in Se-deficient calves while Oh *et al.* (1976) reported a concentration of 0·015 μg/ml in Se-deficient lambs. In the kidney of Se-deficient lambs there was 0·233 μg Se/g wet tissue and in the liver 0·049 μg Se/g wet tissue (Oh *et al.* 1976); these values are two- to threefold lower than corresponding values shown in Table 5.

The activities of certain enzymes in liver and plasma are shown in Table 6. There was a significant Se effect on plasma and liver GSH peroxidase activities which were markedly reduced in the Se-deficient state. There was no significant interaction between Se and vitamin E with respect to GSH peroxidase activity. Although there were small differences in mean values for hepatic GSH peroxidase assayed with different substrates, the ratios of activity measured with the two substrates remained virtually constant over the four dietary treatments. This strongly suggests that no Se-independent GSH peroxidase activity is present (nor is it induced in Se deficiency) in trout tissues. Hepatic GSH transferase activity was unchanged in any of the treatments used; this again militates against the presence or induction of a Se-independent peroxidase.

There was significant interaction between vitamin E and Se with respect to plasma pyruvate kinase activity. In the absence of both nutrients activity of the enzyme was significantly elevated. It is presumed that the enhanced activity was due to leakage of the muscle isoenzyme but we were not able to check this electrophoretically.

Table 6. *Activities (nmol substrate converted or of thioester bond formed/min per mg protein) of certain enzymes in plasma and livers of rainbow trout* (Salmo gairdneri) *given diets† of different vitamin E and selenium contents*

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

Diet...	1		2		3		4	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver glutathione (GSH) peroxidase‡ (*EC* 1.11.1.9)	27·67[a]	1·73	8·18[b]	0·70	26·41[a]	1·28	5·23[b]	0·77
Liver GSH peroxidase§	22·94[a]	2·21	6·84[b]	0·65	19·44[a]	1·89	4·11[b]	0·42
Plasma GSH peroxidase‡	6·32[a]	0·31	2·44[b]	0·4	5·98[a]	0·47	2·09[b]	0·11
Liver GSH transferase (*EC* 2.5.1.18)	96·10	10·47	96·23	7·73	107·16	9·36	95·13	11·20
Plasma pyruvate kinase (*EC* 2.7.1.40)	28·57[a]	8·21	25·20[a]	5·04	60·17[a]	22·79	435·52[b]	136·02

[a,b] Values in the same row with different superscript letters are significantly different: for GSH peroxidase ($P < 0·01$), for plasma pyruvate kinase ($P < 0·05$).

† For details, see Tables 1 and 2.

‡ Cumene hydroperoxide as substrate.

§ Hydrogen peroxide as substrate.

DISCUSSION

It has been apparent from earlier studies (Hung *et al.* 1980; Cowey *et al.* 1981) that, when the diet contains an adequate Se supplement, severe depletions of vitamin E can occur in rainbow trout of 2–10 g or more without clinical signs of disease. It is now evident that the converse is true of Se deficiency in that, in the presence of adequate dietary vitamin E, no gross Se deficiency syndrome occurs, although significant effects of dietary Se on tissue Se concentrations and on tissue GSH peroxidase activities do occur. A significant synergistic interaction between vitamin E and Se was shown in the packed cell volume and NADPH-stimulated microsomal lipid peroxidation values, in addition there was a significant effect of vitamin E on plasma Se concentration.

The absence of any Se-deficiency syndrome is not due to an increase in Se-independent GSH peroxidase activity – no such activity exists in the livers of rainbow trout. Moreover, there was no change in hepatic GSH transferase activity in Se deficiency. Our results on Se-independent GSH peroxidase are wholly at issue with those of Heisinger & Dawson (1983) on black bullhead. Their diet composition, feeding regimen and assay methods are so different from those applied in the present study that comparison between the two would be inappropriate.

It may be noted here that trout liver GSH transferase (in the presence of 1 mM-GSH) inhibited malondialdehyde formation in the NADPH-stimulated microsomal lipid peroxidation system, while trout liver GSH peroxidase failed to do so (Bell *et al.* 1984). In this system GSH was itself inhibitory at 5 mM but not at 1 mM. In addition there is no evidence known to the present authors that the postulated products of GSH peroxidase action on lipid hydroperoxides, monohydroxy polyenoic acids have ever been detected in animal tissues. We infer that GSH peroxidase functions (very efficiently) to remove cytosolic H_2O_2,

a potent inhibitor of superoxide dismutase (*EC* 1.15.1.1). In vitro GSH transferase prevents lipid peroxidation occurring in the microsomal system; whether it performs a similar role in vivo remains to be demonstrated.

It seems unlikely that the absence of pathology observed by Hilton *et al.* (1980) and ourselves on the one hand, and that seen by Poston *et al.* (1976) on the other, are due to differences between species as closely related as *S. gairdneri* and *S. salar*. One possible explanation may be that the diets used by Poston *et al.* (1976) had lower Se concentrations than did those of Hilton *et al.* (1980) or of ourselves. This possibility cannot be resolved as Poston *et al.* (1976) did not measure Se levels in either diets or fish tissues. In addition it has already been noted that plasma and kidney Se levels in our Se-depleted rainbow trout were quite similar to those of Se-deficient lambs; hepatic Se levels were somewhat higher than those of Se-deficient lambs.

Another possibility is that the low level of dietary polyunsaturated fatty acid (10 g linolenic acid/kg) together with synthetic anti-oxidant used by us, compared with 100 g stripped maize oil/kg used by Poston *et al.* (1976), may explain the lack of pathology in our fish. However, this is not consistent with the fact that Hilton *et al.* (1980) used larger quantities of more highly unsaturated fatty acids (150 g salmon oil/kg diet) without synthetic antioxidants.

A further possible explanation is that Poston *et al.* (1976) used very small fish. Vos *et al.* (1981) showed that in ducklings myopathy does not occur if vitamin E depletion is delayed until the birds are 2 weeks of age and provided dietary Se content is not too low (0·074 mg/kg). Under these conditions mortality is very low and growth normal over a 14 week period despite low levels of vitamin E in serum, liver and muscle. The observation is explained by the extremely high rate of growth during the first 2 weeks of age when growth reaches a peak. Similar effects may extend to fish: those used by Poston *et al.* (1976) were 0·1 g in their first experiment and 0·9 g in their second; those used by Hilton *et al.* (1980) were initially 1·3 g.

Large increases in plasma pyruvate kinase activity in vitamin-E-deficient rats have been shown to be due predominantly to the M type isoenzyme (Chen *et al.* 1983). It is likely that the increase in plasma pyruvate kinase in dually deficient trout was also due to leakage of the muscle isoenzyme into the plasma. This probably indicates incipient muscle damage and the observation should be exploited to monitor trout under practical farming conditions for susceptibility to muscle damage.

REFERENCES

Bell, J. G., Cowey, C. B. & Youngson, A. (1984). *Biochimica et Biophysica Acta* **795**, 91–99.
Chen, L. H., Chow, C. K. & Thacker, R. R. (1983). *Nutrition Reports International* **27**, 207–212.
Cowey, C. B., Adron, J. W., Walton, M. J., Murray, J., Youngson, A. & Knox, D. (1981). *Journal of Nutrition* **111**, 1556–1567.
Draper, H. H. & Csallany, A. S. (1969). *Journal of Nutrition* **98**, 390–394.
Duncan, D. B. (1955). *Biometrics* **11**, 1–42.
Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974). *Journal of Biological Chemistry* **249**, 7130–7139.
Hasunuma, R., Ogawa, T. & Kawanishi, Y. (1982). *Analytical Biochemistry* **126**, 242–245.
Heisinger, J. F. & Dawson, S. M. (1983). *Journal of Experimental Zoology* **225**, 325–327.
Hilton, J. W., Hodson, P. V. & Slinger, S. J. (1980). *Journal of Nutrition* **110**, 2527–2535.
Hung, S. S. O., Cho, C. Y. & Slinger, S. J. (1980). *Journal of the Association of Official Analytical Chemists* **63**, 889–893.
Lawrence, R. A., Parkhill, L. K. & Burk, R. F. (1978). *Journal of Nutrition* **108**, 981–987.
Lowry, O. H., Roseburgh, N. J., Farr, A. L. & Randall, R. J. (1951). *Journal of Biological Chemistry* **193**, 265–275.
McMurray, C. H., Blanchflower, W. J. & Rice, D. A. (1980). *Journal of the Association of Official Analytical Chemists* **63**, 1258–1261.
Oh, S.-H., Sunde, R. A., Pope, A. L. & Hoekstra, W. G. (1976). *Journal of Animal Science* **42**, 977–983.

Pearse, A. G. E. (1972). *Histochemistry*, 3rd ed., vol. 2. Edinburgh and London: Churchill Livingstone.
Poston, H. A., Combs, G. F. & Leibovitz, L. (1976). *Journal of Nutrition* **106**, 892–904.
Schwarz, K. & Folz, C. M. (1957). *Journal of the American Chemical Society* **79**, 3293–3298.
Siddons, R. C. & Mills, C. F. (1981). *British Journal of Nutrition* **46**, 345–355.
Vos, J., Hulstaert, C. E. & Molenaar, I. (1981). *Annals of Nutrition and Metabolism* **25**, 299–306.

Printed in Great Britain