Response of rainbow trout (*Salmo gairdneri*) to increased levels of available carbohydrate in practical trout diets

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1. The physiological response of rainbow trout (*Salmo gairdneri*) reared on different levels of available carbohydrate in practical trout diets having the same levels of energy and nitrogen for 16–24 weeks was determined.

2. Weight gain was significantly reduced in trout reared on the highest level of available carbohydrate, 210 g cerelose (α-glucose)/kg, and there was a significant linear regression (R^2 0·88) of dietary carbohydrate on weight gain.

3. Liver: body-weight values and liver glycogen levels increased in relation to increased dietary carbohydrate.

4. Liver glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) activity increased and liver phosphoenolpyruvate carboxykinase (*EC* 4.1.1.32) activity decreased per kg body-weight of fish with increasing dietary carbohydrate. However, no significant effect was noted on the activity of these liver enzymes above a dietary cerelose level of 140 g/kg.

5. Liver fructose diphosphatase (*EC* 3.1.3.11) activity increased with increasing dietary carbohydrate has been interpreted as meaning a recycling of triosephosphate to glucose-6-phosphate.

6. Dietary carbohydrate level had no significant effect on the liver pyruvate kinase (*EC* 2.7.1.40) activity, the rate of glucose utilization or the percentage conversion of [^{14}C]alanine to glucose in the plasma of trout.

7. The results indicate that rainbow trout have a limited ability to adapt to increased dietary carbohydrate and a level in excess of 140 g/kg of the diet is not efficiently utilized.

Carbohydrates are generally the major source of energy in most domestic animal diets but are considered to be of limited value in the nutrition of carnivorous fish such as the rainbow trout (*Salmo gairdneri*) (Phillips & Brockway, 1959). The rate of aerobic oxidation of glucose by fish is considerably less than that of terrestrial mammals, even allowing for the temperature differential (Lin *et al.* 1978; Cowey & Sargent, 1979). This reduced rate of glucose utilization is related to the inability of fish such as trout to control their blood glucose concentration. The glucose tolerance of rainbow trout is poor indicating that these fish are diabetic-like animals (Palmer & Ryman, 1972). As a consequence, the maximal tolerable level of available carbohydrate for salmonids has been considered to be approximately 200 g/kg diet (Phillips *et al.* 1948; Buhler & Halver, 1961; Luquet, 1971).

Recent studies have indicated that fish such as rainbow trout do have a limited ability to adapt to increased dietary carbohydrate (Cowey, de la Higuera *et al.* 1977; Cowey, Knox *et al.* 1977; Furuichi & Yone, 1980), and that increased levels of dietary carbohydrate can spare dietary protein (Pieper & Pfeffer, 1979; Shimeno *et al.* 1979). As yet, virtually no studies have determined the adaptability of rainbow trout to increased levels of available carbohydrate in diets designed to have the same levels of energy and nitrogen.

This study was conducted to determine: (1) the growth and feed efficiency of rainbow trout reared on practical trout diets having the same levels of energy and N, but containing different levels of available carbohydrate, (2) the effect of increased dietary carbohydrate on the liver enzyme activities of glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49), fructose diphosphatase (*EC* 3.1.3.11) phosphoenolpyruvate carboxykinase (*EC* 4.1.1.32) and pyruvate kinase (*EC* 2.7.1.40), (3) the in vivo utilization of [^{14}C]glucose and [^{14}C]alanine by trout reared on different levels of dietary carbohydrate.

Table 1. *Composition and proximate analyses of the test diets (g/kg)*

Diet no.... Ingredient	1	2	3	4	5
Capelin meal*	380	380	380	380	380
Soya-bean meal	270	270	270	270	270
α-Cellulose†	320	190	170	150	110
Cerelose	0	70	110	140	210
Salmon oil	90	50	45	30	0
Vitamin premix‡	20	20	20	20	20
Mineral premix‡	10	10	10	10	10
Analyses‖					
Protein (%)	40·1	39·6	38·6	39·1	38·3
Lipid (%)	15·9	13·0	11·5	10·1	7·3
Starch (%)	3·3	3·2	3·5	3·2	3·5
Sugar (%)	2·5	7·3	8·7	10·6	18·3

* Capelin meal (crude protein 70%) supplied by Martin's Feed Mill, Elmira Ont., Canada.
† α-Cellulose-(Alpha-floc) Brown Company, Toronto, Canada.
‡ Vitamin and mineral premix as given in Cho *et al.* (1976).
‖ Results are the mean of three samples.

EXPERIMENTAL

General design

Five practical trout diets containing from 0 to 210 g cerelose (α-glucose)/kg (diets 1–5) were fed to triplicate groups of approximately 100 juvenile trout for a period of 24 weeks in water at 15°. Growth factors, carcass and liver compositions, liver enzyme activities and substrate utilization were determined after the trout had been reared on the test diets for 16–24 weeks.

Diet preparation

Five practical trout diets with increasing levels of cerelose (α-glucose), from 210 g/kg, were formulated (Table 1) on the basis of the metabolizable energy (ME) values (37·44 kJ/g fat, 16·74 kJ/g digestible carbohydrate) of Smith (1975). The ω3 fatty acid level of diet 5, the lowest lipid level, was determined to be in excess of 1% of the diet. The diets were processed by steam pelleting in a California Pellet Mill (San Francisco, California, USA). The protein content of the diets was determined by Kjeldahl analysis (N × 6·25) as described by Horwitz *et al.* (1970), the lipid content by the method of Bligh & Dyer (1959) and the starch and sugar content by the method of Clegg (1956).

Supply and maintenance of fish

Rainbow trout were obtained from the Ontario Ministry of Natural Resources, Normandale Hatchery, Turkey Point, Ontario, Canada. After a 2-week acclimation period approximately 100 fish per tank (mean weight 2·9 ± 0·1 g/fish) were transferred to twenty-one rectangular fibre-glass tanks (capacity 60 l). The tanks were aerated and maintained on a one-pass charcoal filter system which had a flow-rate of 2–3 l/min. Water temperature was maintained at 15·6 ± 1·3°, pH varied between 7·4 and 7·8 and dissolved oxygen ranged from 7·7 to 9·4 mg/l throughout the study. The test diets were fed to triplicate groups of approximately 100 fish per tank for 24 weeks. The fish were fed three to four times daily and the feeding rate and size of feed particle adjusted after each 28 d period.

Growth and biochemical assays

Body-weights, feed: gain values and total mortalities were determined after each 28 d period. After 16 weeks approximately twenty fish were randomly selected from each diet group, individually weighed and their livers dissected, weighed, immediately frozen with liquid N_2 and stored at $-20°$ until required for analysis. The livers were assayed for glycogen content by the method of Carroll *et al.* (1956), lipid content by the method of Bligh & Dyer (1959) and the protein content by the method of Horwitz *et al.* (1970). Similarly, carcass samples were freeze-dried and stored at $-20°$. The carcass ash (ignition at 600°), moisture (weight loss at 105° for 24 h) and protein content ($N \times 6·25$) were determined as described by Horwitz *et al.* (1970), and the carcass lipid by the method of Bligh & Dyer (1959).

After 16 weeks, blood was collected from approximately eight fish (fasted 18 h) from each group in heparinized centrifuge tubes after transection of the caudal peduncle, immediately centrifuged at 3500 *g* and the plasma stored at $-20°$ until required for analysis. Plasma glucose was determined by the glucose oxidase method (Glucose Kit no. 510; Sigma Chemical Co., St. Louis, Missouri, USA) and plasma protein by the method of Lowry *et al.* (1951).

Enzyme assays

Enzyme activities were determined in crude liver extracts from fish which had received the various diets for at least 16 weeks. Different fish (eight replicates in each instance) were used for the assay of each enzyme. Glucose-6-phosphate dehydrogenase (GPDH) and pyruvate kinase (PK) are important in the lipogenic and glycolytic pathways respectively, while phosphoenolpyruvate carboxykinase (PEPCK) and fructose diphosphatase (FD) are key enzymes in gluconeogenesis. GPDH was extracted and assayed as described by Freedland (1967), PK by the method of Pogson (1968) and FD by the method of Opie & Newsholme (1967) as modified by Newsholme & Crabtree (1973). PEPCK was assayed in the reverse direction (measuring oxaloacetate synthesis) by the method of Ballard & Hanson (1967) as described by Pogson & Smith (1975). Body- and liver weights were determined for all fish used.

Conversion of [¹⁴C]alanine to [¹⁴C]glucose

The percentage conversion of [¹⁴C]alanine to [¹⁴C]glucose was determined by a method similar to that of Cowey, Knox *et al.* (1977). L-[U-¹⁴C]alanine (Amersham Corp., Oakville, Ontario; specific activity 100 mCi/mmol) was diluted with L-alanine to give a solution containing 1 μCi/mmol in 1·0 ml saline (9 g sodium chloride/l). After 20 weeks on the test diets, approximately six fish per group were fasted for 18–20 h and anaesthetized with tricaen methanesulphonate (MS222 Syndel Laboratories, Vancouver, BC) for 1–2 min at a concentration of 0·1 g/l. The trout then received an intraperitoneal injection of the radioactive alanine (20 μCi/kg body-weight) and were returned to the experimental tanks. Time-studies of the formation of radioactive glucose (% administered dose in plasma glucose) in trout reared on either 0 or 210 g cerelose/kg diet indicated that maximum or peak conversion of [¹⁴C]alanine occurred between 30 and 90 min after injection. Therefore, 1 h post-injection, trout were anaesthetized with MS222 and blood collected in sodium-heparinized centrifuge-tubes by amputation of the caudal peduncle. The blood was centrifuged at 3500 *g* for 10 min and a 200–400 μl plasma sample deproteinized by the method of Somogyi (1945) after the addition of 2 vol. distilled water. A 3 ml portion of the protein-free supernatant fraction was then passed through columns (6 × 200 mm) of mixed-bed resin (Amberlite MB3 ion-exchange resin; BDH Chemicals, Toronto, Canada). The column eluate (plus two washings) was concentrated to dryness on a rotary evaporator at 50°, taken up in distilled water and made up to a final volume of 4 ml. The glucose content of the solution was determined by the glucose oxidase method (Glucose Kit no. 510; Sigma

Chemical Co., St Louis, Missouri, USA) and its radioactivity measured by adding a 0·1 ml sample to 10 ml of scintillation fluid (PCS; Amersham Corp.) in a scintillation vial. Radioactivity was determined on a liquid-scintillation spectrometer (Delta 300; Searle Analytical, Toronto, Ontario) with a counting efficiency of 75–79%. In tests of the separation efficiency of the ion-exchange column, less than 2% of [^{14}C]alanine and approximately 100% of [^{14}C]glucose passed through the column.

The amount of [^{14}C]alanine converted to glucose was calculated from the formula of Cowey, Knox et al. (1977) as follows:

$$\begin{array}{l}\text{Amount of dose}\\\text{incorporated}\\\text{into glucose (\%)}\end{array} = \begin{array}{c}\text{body-weight}\\\text{(g)}\end{array} \times \begin{array}{c}\text{glucose diffusion}\\\text{space}\end{array} \times \frac{\begin{array}{c}\text{plasma glucose}\\\text{(disintegrations/ml per min)}\end{array}}{\begin{array}{c}\text{amount of radioactive}\\\text{alanine injected}\\\text{(disintegrations/ml per min)}\end{array}} \times 100$$

The glucose diffusion space constant was assumed to be 13·7% of the body-weight as determined by Cowey, Knox et al. (1977).

Glucose utilization

The rate of glucose utilized in juvenile trout was measured by a method similar to that of Lin et al. (1978). Trout which had been reared on the test diets for approximately 22–24 weeks were fasted for 48 h. Thirty-two trout from each dietary group were anaesthetized with MS222 (0·1 g/l), immediately weighed and injected with [U-^{14}C]glucose (Amersham Corp.; 230 mCi/mmol) at the rate of 2 μCi/kg fish in 100 μl physiological saline. The trout were then placed back in the test tanks and four fish were killed at 25 min and 1, 2, 3, 6, 9, 12 and 24 h post-injection. Blood was collected in heparinized capillary-tubes after amputation of the caudal peduncle and 50 μl blood was then immediately mixed with 0·95 ml distilled water. The blood sample was then deproteinized by the method of Somogyi (1945), centrifuged and the supernatant fraction collected. Supernatant fraction (3 ml) was then passed through the previously-described mixed-bed resin, the column eluate and washings dried under reduced pressure and taken up in 4 ml distilled water. The blood glucose concentration was determined on a portion of the supernatant fraction by the glucose oxidase method. A 0·1 ml portion of the supernatant fraction was added to 10 ml PCS in a scintillation vial and the radioactivity measured with a counting efficiency of approximately 80%. A semilog plot of the glucose specific activity v. time was constructed (Fig. 1) and the rate of decline determined graphically for each dietary group (disintegrations/min per mg glucose per 1 per h).

Statistical analysis

The results were subjected either to linear regression, or analysis of variance and, where applicable, differences determined at the 5% level using Tukey's procedure as described by Steel & Torrie (1960).

RESULTS
Growth, carcass and liver composition

After 16 weeks, trout reared on the highest level of available carbohydrate (diet 5; 210 g/kg) had a significantly lower weight gain than did trout reared on the lowest level of available carbohydrate (diet 1; 0 g/kg) (Table 2). There was also a significant linear regression (R^2 0·88) of available carbohydrate level on the weight gain. No significant difference was detected in the feed:gain values of the trout on the various dietary treatments at this time and mortalities were less than six fish per 10 000 fish days per treatment.

Table 2. *Mean weight gains, feed:gain values and mortalities of rainbow trout* (Salmo gairdneri) *after 16 weeks on the test diets*

(The results are the means of three replicates × 103 fish × four periods and 25118–26904 fish days per treatment (initial body-weight 2·9 g/fish))

Diet no.	1	2	3	4	5	SEM
Gain (kg/100 fish)	6·9[a]	6·3[ab]	6·0[ab]	5·9[ab]	5·1[b]	0·3
Feed:gain	1·1	1·2	1·1	1·2	1·3	0·1
Mortalities*	4·8	3·4	0	1·5	5·9	—

a,b, Values that do not share a common superscript letter are statistically significantly different: $P < 0.05$.
* Based on the number of mortalities per 10000 fish days.

Table 3. *The proximate composition (g/kg) of the final carcasses of rainbow trout* (Salmo gairdneri) *after 16 weeks on the test diets*

(Results are the means of three pooled samples, expressed on a dry matter basis)

Diet no.	1	2	3	4	5	SEM
Protein	483	504	499	517	533	12
Lipid	445	414	421	403	372	13
Moisture	713	701	721	731	737	14
Ash	69	70	72	75	81	3
Energy (kJ/g)	27·6	27·2	26·0	27·0	25·8	1·2

Table 4. *The liver composition (mg/g) and liver:body-weight ratio* (liver weight ÷ body-weight × 100; LBW) *of rainbow trout* (Salmo gairdneri) *after 16 weeks on the test diets*

(Results are the means of three to six samples)

Diet no.	1	2	3	4	5	SEM
Glycogen	28[a]	53[b]	55[b]	66[bc]	93[c]	4·1
Protein	164[a]	144[b]	141[b]	137[b]	132[b]	3·0
Lipid	49	49	53	48	46	3·1
Moisture	750	741	738	724	734	7·4
LBW*	1·4[a]	1·7[ab]	1·8[b]	2·3[bc]	2·6[c]	0·1

a,b,c Values that do not share a common superscript are significantly different: $P < 0.05$.
* Mean of twenty fish based on a wet-weight basis.

The carcass lipid content after 16 weeks decreased with increasing levels of available carbohydrate (Table 3) and there was a significant linear regression (R^2 0·94) of available carbohydrate level on the carcass lipid content. Although both carcass ash and protein content increased with increasing dietary carbohydrate, no significant difference or linear regression was detected in the results.

After 16 weeks, both liver glycogen and liver weight significantly increased while liver protein significantly decreased with increasing dietary carbohydrate (Table 4). No significant differences were detected in the liver lipid and moisture content of the various dietary groups.

No significant differences were detected in the plasma glucose (range 3·1 to 3·6 mmol/l, SEM 0·2) or plasma protein levels (range 33 to 38 g/l, SEM 1·1) of the rainbow trout reared on the different dietary treatments after 16 weeks.

Table 5. *The activity of liver enzymes of rainbow trout* (Salmo gairdneri) *after 16 weeks on the test diets*

(Results are the means of eight fish)

Diet no.	1	2	3	4	5	SEM
	μmol substrate utilized/min per g wet liver wt					
Glucose-6-phosphate dehydrogenase	2·90[a]	3·50[b]	4·44[bc]	5·42[c]	7·58[d]	0·19
Pyruvate kinase	3·04[a]	4·42[ab]	5·43[b]	3·94[ab]	3·91[ab]	0·24
Fructose diphosphatase	1·80[a]	1·98[a]	2·37[bc]	2·11[ab]	2·52[c]	0·09
Phosphoenolpyruvate carboxykinase	2·31[a]	2·22[a]	1·10[b]	0·58[c]	0·68[c]	0·09
	μmol substrate utilized/min per kg wet body-wt					
Glucose-6-phosphate dehydrogenase	50·6[a]	59·5[a]	96·6[a]	124·5[ab]	201·0[b]	5·1
Pyruvate kinase	43·5[a]	81·0[a]	98·1[a]	91·9[a]	90·7[a]	5·3
Fructose diphosphatase	23·3[a]	30·0[ab]	41·7[b]	41·3[b]	5·61[c]	1·8
Phosphoenolpyruvate carboxykinase	17·8[ab]	22·2[a]	12·9[bc]	7·6[c]	7·9[c]	1·4

a,b,c Values that do not share a common superscript letter are statistically significantly different: $P < 0.05$.

Enzyme activities

All enzymes assayed showed significantly different changes in response to alterations in dietary carbohydrate when activity was expressed per g wet liver (Table 5). GPDH activity increased significantly as dietary carbohydrate increased and dietary fat decreased. PK activity was more constant, but peak activity was observed in fish given diet 3, with a lower activity with diet 1, containing the least carbohydrate. The two gluconeogenic enzymes showed opposite response patterns: whereas PEPCK activity increased significantly as dietary carbohydrate content fell, FD showed a small but significant decrease in activity.

Expression of enzyme activity per unit lower weight was compromised to some extent by the increase in relative liver weight induced by increasing levels of carbohydrate in the diet (Table 4). For this reason, enzyme activities are also reported per kg body-weight. The further variation introduced by this method of expressing values tended to increase the standard deviation of each activity estimate, but significant activity changes were still apparent for all enzymes assayed other than PK (Table 5). This latter enzyme showed a similar trend to that observed on a per liver basis, but the wide variability in the activity of this enzyme seen among fish on all diets precluded observation of any significant change. GPDH, FD and PEPCK showed significant changes in activity expressed per kg body-weight with the same trends as reported per g wet liver, although the correction for liver size and body-weight enhanced the effect of increasing carbohydrate levels on both GPDH and FD activities.

Conversion of [^{14}C]alanine

The amount of [^{14}C]alanine incorporated into glucose in the plasma of the trout tended to decrease with increasing dietary carbohydrate level (Table 6). However, no significant difference or linear regression was detected, due primarily to large variability of the results. Plasma glucose levels of the fish used in the present study were higher than in the same trout sampled after 16 weeks on the test diets.

[^{14}C]glucose utilization

The rate of decline of the glucose specific activity in the trout reared on the different dietary groups did not vary greatly between the different groups, and no significant linear regression

Table 6. *The percentage conversion of* [^{14}C]*alanine to* [^{14}C]*glucose 1 h post injection in the plasma of rainbow trout* (Salmo gairdneri)

(Results are the means of six–eight fish after 20 weeks on the test diets)

Diet no.	1	2	3	4	5	SEM
Percentage converted	3·74	3·03	2·99	2·52	2·52	0·3
Plasma glucose (mmol/l)	4·74	4·42	4·29	4·78	5·40	0·23

Table 7. *The rate of decline in the specific activity of plasma glucose in rainbow trout* (Salmo gairdneri) *after 22 weeks on the test diets*

Diet no.	1	2	3	4	5
Rate of decline	2·0	2·4	2·3	1·7	2·5
(disintegrations/min ($\times 10^4$) per mg glucose per 1 per h)					

was detected in the results (Fig. 1, Table 7). In contrast to the previous [^{14}C]alanine conversion study, plasma glucose levels in the present study were not elevated.

DISCUSSION

The results of this study indicate that rainbow trout have a limited ability to adapt to increased digestible carbohydrate in the diet and a level in excess of 140 g/kg diet is not efficiently utilized. The natural diet of rainbow trout contains little carbohydrate (excluding chitin) and teleologically these fish would not be expected to have developed mechanisms to digest and metabolize dietary carbohydrates efficiently. Thus it is not surprising that the digestibility of complex carbohydrates, such as starch, by salmonids is poor (National Research Council, 1973), that there is an absence of glucokinase in fish livers and that aerobic oxidation of glucose by fish is limited (Lin *et al.* 1978). Despite these observations, the trout in this study did metabolically adapt to increased levels of digestible carbohydrate. The activity of liver GPDH increased while liver PEPCK decreased with increasing dietary carbohydrate (Table 5). However, when enzyme activity was expressed on a body-weight basis, no further increase in GPDH or decrease in PEPCK activities were noted above 140 g cerelose/kg. In addition, there was a significant linear regression (R^2 0·88) of dietary carbohydrate level on weight gain, indicating that increased levels of digestible carbohydrate in the diet depress the growth rate of the trout. It should be noted however, that direct interpretation of the regression analysis is confounded by the greatly depressed weight gain with diet 5 (210 g cerelose/kg) as compared with diet 1 (0 g cerelose/kg; Table 2). There was a significant difference in the weight gain with diet 1 as compared with diet 5 ($P < 0·05$); however, there was no significant difference between these two dietary groups and those given diets 2, 3 and 4. In fact, the weight gains of dietary groups 2, 3 and 4 are essentially the same. Without the benefit of additional treatments, it is difficult to determine if, as indicated by regression analyses, any increase in the level of digestible carbohydrate in the diet will depress the growth rate of the trout. However, in combination with the results of the enzyme studies (Table 5), the growth values indicate that trout are able to adapt to increased levels of digestible carbohydrate in the diet, but that levels in excess of 140 g/kg diet will result in a significant growth depression.

In contrast to PEPCK, FD, the other gluconeogenic enzyme investigated, increased in
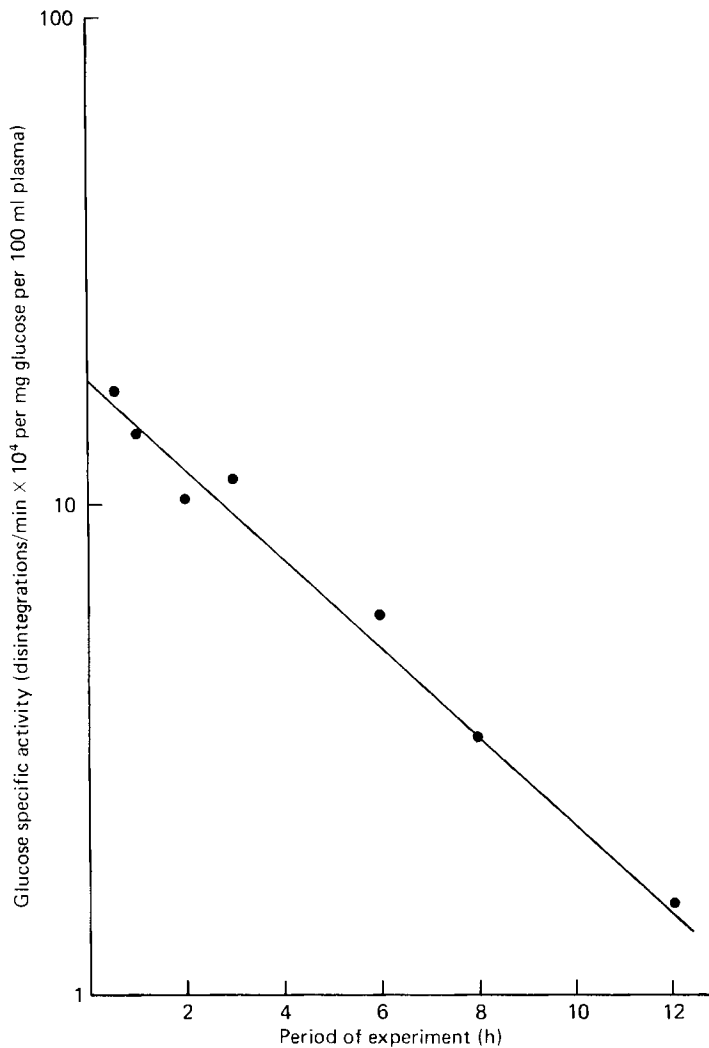
Fig. 1. Decline in glucose specific activity in the plasma of rainbow trout (*Salmo gairdneri*) after 22 weeks on the test diets.

activity with increased dietary carbohydrate (Table 5). This could permit conversion of triose phosphates to glucose-6-phosphate and enhance oxidation of glucose by the hexose monophosphate pathway, presumably reducing the net flow of glucose through the Embden-Meyerhof pathway of glycolysis. Such a pattern would be consistent with the concomitant increase in GPDH activity already described.

According to Watanabe (1977), optimum dietary levels for salmonids reared at 15° are between 15 and 20% of the diet, depending on the protein level. On this basis, only diet 1 in the present study contains adequate lipid. It is possible that enhanced hexose monophosphate shunt activity in trout reared on low lipid diets may be used for generation of NADPH required for fatty acid synthesis. This implies that a source of acetyl CoA would also be needed for lipid synthesis. However, liver PK activity showed great individual variability and when expressed on a body-weight basis was not affected by the dietary carbohydrate level (Table 5), presumably precluding an extra supply of acetyl CoA by hepatic glycolysis, although conceivably amino acid catabolism could provide this. The decrease in carcass lipid as dietary carbohydrate was increased (Table 3) certainly seems

to indicate that some factor limits the effectiveness of lipogenesis from carbohydrate. Further studies are required to elucidate the effects of increased dietary carbohydrate levels on the alternative pathways of glucose catabolism by trout liver. There may be an optimum value for carbohydrate: lipid in trout diets which would maximize the metabolism of glucose through hepatic glycolysis and the over-all efficiency of dietary glucose use.

Trout reared on high carbohydrate diets have elevated plasma glucose levels for extended periods and have poor glucose tolerance (Palmer & Ryman, 1972). Although Cowey, de la Higuera *et al.* (1977) suggested that a low capacity for glucose phosphorylation is one of the prime reasons for the inability of fish to metabolize glucose rapidly, it is well known that when salmonids are fed on a diet containing high levels of digestible carbohydrate, there is excessive glycogen deposition in the liver. Presumably, therefore, the rate of glucose phosphorylation is greater than the rate of glucose catabolism by the liver. Once formed, liver glucose-6-phosphate may enter several metabolic pathways. Since glycolysis via the Embden–Meyerhof as indicated by PK activity (Table 5) does not seem to be stimulated by increased dietary carbohydrate, enhanced glycogenesis (Table 4) seems a logical accompaniment to the apparent increase in glucose-6-phosphate oxidation through the hexose monophosphate shunt pathway implied by the observed increases in GPDH and FD activities (Table 5).

In contrast to the liver enzyme activities, the substrate utilization studies showed little adaptation to increased levels of digestible carbohydrate (Tables 6 and 7). No significant change was noted in the rate of glucose utilization by the trout on the various test diets. Considering that a 48 h fasting period was required before determination of the rate of glucose utilization, it is possible that metabolic changes caused by fasting may have masked the effect of increased dietary carbohydrate on glucose utilization (although this seems unlikely). It is also possible that the lack of difference in the over-all rate of glucose utilization need not rule out that there may have been an increased use in some tissues, e.g. muscle, counterbalanced by a decrease or negligible increase in other tissues. The rates of glucose utilization determined in this study were similar to those in the studies of Cowey, Knox *et al.* (1977) and Lin *et al.* (1978). Aerobic oxidation of glucose is limited in fish and below that of mammals even when compensation is made for temperature differences (Lin *et al.* 1978; Cowey & Sargent, 1979). It has been suggested that this limited glucose utilization is due in part to a lack of insulin secretion with increased glucose loading (Cowey, de la Higuera *et al.* 1977). Histological examination of the pancreatic islets from trout reared on diets 1 and 5 (0 and 210 g cerelose/kg) failed to reveal any difference in the number or size of β cells in the two groups. This is further evidence that glucose does not act as a stimulus for insulin production and secretion in the trout.

The percentage incorporation of [¹⁴C]alanine into plasma glucose in the trout was also unaffected by the dietary carbohydrate level (Table 7). This was surprising since liver PEPCK activity did decline with increasing dietary carbohydrate (Table 5) and previous studies (Cowey, de la Higuera *et al.* 1977) have shown a marked reduction in percentage incorporation of [¹⁴C]alanine into plasma glucose with increased dietary carbohydrate. However, there was a trend toward decreasing conversion of [¹⁴C]alanine with increasing carbohydrate, and the high amount of variability in the results may have nullified any statistical significance. The observed decline in PEPCK activity as dietary carbohydrate increased may have been offset by the concomitant increase observed in the FD activity (Table 5), resulting in no net alteration in gluconeogenic capability. It was noted that the maximum percentage incorporation of [¹⁴C]alanine into plasma glucose occurred within 30–90 min of injection in contrast to 6 h as reported by Cowey, de la Higuera *et al.* (1977). It is difficult to explain why such divergent results should occur in studies with the same species. Further investigation seems necessary. The trout in the alanine incorporation study were also slightly stressed which may have added to the variability of the results. Despite

the lack of statistical support the results of this study tend to confirm the growth and liver enzyme results (Tables 2 and 5), suggesting a limited adaptation of the trout to increased dietary carbohydrate.

Previous studies, based primarily on growth and mortality results, (Phillips *et al.* 1948; Buhler & Halver, 1961; Luquet, 1971) have indicated that the maximum tolerable level of digestible carbohydrate in salmonid diets is approximately 200 g/kg. The results of this study indicated that trout reared on a dietary cerelose level of 210 g/kg showed a significant growth depression (Table 2). Trout reared on this diet did not have significantly higher mortalities than trout reared on the other diets and were therefore presumably in passably good health. However, both liver : body-weight values (LBW) and liver glycogen levels (LG) increased in a linear relation with increased dietary carbohydrate (Table 4). Recent research has indicated that rainbow trout with increased LBW and LG are less tolerant to water-borne copper toxicity than trout with lower LBW and LG (Dixon & Hilton, 1981), and that increased LG can impair liver function in the trout (Hilton & Dixon, 1982). Such clinical diagnostic procedures may prove valuable in re-evaluating the maximal tolerable level for carbohydrate in salmonid diets.

The diets used in this study were formulated to be isonitrogenous and isoenergetic on the basis of the ME values of Smith (1975) for digestible carbohydrate and fat. However, there was a significant growth depression with the highest level of digestible carbohydrate (210 g cerelose/kg) and a significant linear regression (R^2 0·88) of dietary carbohydrate on weight gain (Table 2). It may be noted (Table 1) that the ME of the test diets was kept constant by increasing the levels of α-cellulose and salmon oil as the level of cerelose was decreased. It may be argued that the increase in the level of salmon oil may have been partially responsible for the increase in growth and alteration in enzyme concentrations, and this cannot be completely refuted from the present results. This also suggests that the diets were not in fact isoenergetic and that the ME values for digestible carbohydrate used in this study were too high. A re-assessment of the ME value for digestible carbohydrates in trout would appear to be necessary. This suggests that the ME of digestible carbohydrate for trout is dependent on its level of inclusion in the diet.

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