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Original article

Effect of dietary levels of lipid and carbohydrate on growth performance, body composition, nitrogen excretion and plasma glucose levels in rainbow trout reared at 8 or 18°C

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Summary — Trout reared at 8 or 18°C were fed twice a day almost to satiation with 1 of 3 experimental diets. The diets were formulated to contain the same levels of protein (43%, dry matter (DM) basis) and digestible energy (around 15 kJ/g DM), but different carbohydrate/lipid ratios (30:7 to 23:14). Time-course studies of nitrogen excretion and glycaemia were also carried out. After 12 weeks of feeding, growth, protein retention and body composition were not influenced by the dietary treatment in trout reared at 8°C. At 18°C, the protein retention was not affected by dietary treatment, but the weight gain tended to be higher in trout fed the diet with the lowest carbohydrate/lipid ratio. This result was due to higher body lipid deposition in these trout. Nitrogen excretion was not influenced by dietary treatment, but was higher at 18°C than at 8°C because of a higher feed intake. Glycaemia increased with dietary level of digestible carbohydrate and the highest plasma glucose level was attained later at 8°C in comparison to 18°C.

trout / carbohydrate/lipid ratio / growth performance / glycaemia / temperature

Résumé — Effets du rapport glucides/lipides de l'aliment sur les performances de croissance, la composition corporelle, l'excrétion azotée et le glucose plasmatique chez la truite Arc-en-ciel élevée à 8 ou 18°C. *Des truites Arc-en-ciel élevées à 8 ou 18°C ont été nourries 2 fois par jour à presque satiété avec l'un des 3 régimes expérimentaux. Les régimes ont été formulés pour contenir les mêmes taux de protéines (43%, sur la base de la matière sèche (MS)) et d'énergie digestible (environ 15 kJ/g MS), mais différents rapports glucides/lipides (30/7 à 23/14). Les évolutions dans le temps de l'excrétion azotée et de la glycémie ont aussi été étudiées. Après 12 sem d'alimentation, la croissance, la rétention protéique et la composition corporelle n'ont pas été influencées par le régime alimentaire chez les truites élevées à 8°C. À 18°C, la rétention protéique n'a pas été affectée par le régime alimentaire, mais le gain de poids tendait à être plus élevé chez les truites recevant le régime riche en lipides (16% MS). Ce résultat a été dû à un dépôt plus élevé en lipides chez ces truites. L'excrétion azotée n'a pas été influencée par le régime alimentaire, mais a été plus élevée à 18°C qu'à 8°C*

à cause d'une consommation d'aliment plus importante. Le taux de glucose plasmatique a augmenté avec la quantité de glucides digestibles du régime et le pic de glycémie est apparu plus tard à 8°C qu'à 18°C.

truite / rapport glucides/lipides / performance de croissance / glycémie / température

INTRODUCTION

The high protein requirement for the growth of carnivorous fish gives rise to high food costs and elevated discharges of nitrogenous wastes into the environment.

The protein-sparing effect of lipid in fish is well established (Lee and Putnam, 1973; Reinitz *et al,* 1978; Takeuchi *et al,* 1978; De Silva *et al,* 1991). However, commercial diets containing high levels of lipids have only recently become available, owing to technological advances in fish feed manufacture.

Carbohydrates are a cheaper source of energy than either protein or lipid. Complex carbohydrates, such as crude starch, are poorly digested by fish (Singh and Nose, 1967; Bergot and Brèque, 1983). However, technological treatments of cereals or starch, such as gelatinization or extrusion, have been found to improve the digestibility of starch by cultured fish (Bergot and Brèque, 1983; Takeuchi *et al,* 1990; Jeong *et al,* 1991). Although the protein-sparing effect of the different sources and levels of carbohydrate has been debated (Hilton *et al,* 1987; Higgs *et al,* 1992), evidence suggests that dietary levels of digestible starch improve protein utilization efficiency in rainbow trout held at temperatures between 15 and 18°C (Pieper and Pfeffer, 1980; Kaushik and Oliva-Teles, 1985; Kaushik *et al,* 1989; Kim and Kaushik, 1992). Water temperature influences the utilization of dietary carbohydrate and lipid by rainbow trout; the digestibility of starch has been reported to be low in rainbow trout reared at 8°C relative to those held at 18°C, and utilization of dietary lipid for energy production is depressed by dietary gelatinized starch more strongly at 18°C than at 8°C (Médale *et al,* 1991). Dixon and Hilton (1985) also found that high dietary carbohydrate levels had an adverse effect on liver function. This effect was more pronounced at low temperature. Growth of trout held at 6.5°C was also shown to be depressed when dietary glucose level reaches 15% of the diet (Higgs *et al,* 1992). The aim of this study was to assess the effect of dietary carbohydrate/lipid ratio on nutrient and energy utilization and its consequence on body composition in rainbow trout grown at high (18°C) or low (8°C) temperatures. The effect of dietary carbohydrate level and water temperature on nitrogen excretion and on the time-course of plasma glucose levels was also examined.

MATERIALS AND METHODS

Experimental diets

Three experimental pelleted diets were formulated to contain the same amounts of crude protein (43–44% dry matter (DM)) but different carbohydrate/lipid ratios. The levels of lipid and carbohydrate and the type of carbohydrate (gelatinized or crude starch) were chosen to obtain diets providing the same amounts of digestible energy (15,5 kJ/g DM). Diet HC contained mainly gelatinized starch as the carbohydrate source and had a low lipid level. Diet MC contained a nearly equal mixture of gelatinized starch and crude starch, and an intermediate level of lipid. Diet LC contained mainly crude starch as the carbohydrate source, and had a high lipid level (table I). Theoretical digestible energy contents of the diets were calculated by addition of the values for individual dietary ingredients. The digestible energy of each ingredient was estimated using

Table I. Ingredients and proximate composition of the experimental diets.

Ingredients (%)	*Diet HC*	*Diet MC*	*Diet LC*
Fish meal [a]	60.1	60.1	60.1
Fish oil [b]	0.0	4.9	9.8
Gelatinized wheat starch	30.1	16.6	0.6
Crude wheat starch	6.8	15.4	26.5
Vitamin mix [c]	1.0	1.0	1.0
Mineral mix [d]	1.0	1.0	1.0
Sodium alginate	1.0	1.0	1.0
Proximate composition			
Crude protein (% DM)	44.1	42.9	42.8
Crude lipid (% DM)	7.0	10.4	14.4
Starch (% DM)	30.1	25.8	22.8
Ash (% DM)	12.1	12.2	12.1
Gross energy (kJ/g DM)	19.1	20.1	21.1

[a] Norwegian herring fish meal (CP: 9.8% DM); [b] acidity value: 6%, stabilized with 200 ppm ethoxyquin, peroxide index < 10, iodine index between 140 and 160; [c] vitamin mix (mg/kg diet): vitamin A acetate, 10 (5 000 IU); DL-cholecalciferol, 20 (2 000 IU); DL-adlpha-tocopherol acetate, 50 (25 IU); menadione, 3.0; thiamin hydrochloride, 7.5; riboflavin, 10; pyridoxin hydrochloride, 7.5; cyanocobalamine, 0.025; nicotinic acid, 100; ascorbic acid, 250; folic acid, 2.5; calcium pantothenate, 25; choline chloride, 1 000; inositol, 500; biotin, 1.2; [d] mineral mix (mg/kg diet): calcium biphosphate•$2H_2O$, 5 000; calcium carbonate, 2 150; sodium chloride, 400; potassium chloride, 900; magnesium hydroxide, 1 240; zinc sulfate•$7H_2O$, 40; manganese sulfate•H_2O, 30; cuprous sulfate•$5H_2O$, 30; cobalt sulfate, 0.2; potassium iodide, 0.4; ferric citrate•$7H_2O$ 200; sodium fluoride, 10.

the known mean values of digestibility and heat of combustion of nutrients (Kim and Kaushik, 1992). Chromic oxide was added to a portion of each diet at a level of 1% as an inert marker for digestibility measurement.

Digestibility trial

Two trials were conducted to study the effect of temperature on digestibility using the same groups of fish in each trial. The 2 trials were performed with an opposite sequence of temperature.

During the first trial, 60 rainbow trout *(Oncorhynchus mykiss)* were randomly allotted to conical bottomed tanks (vol: 60 L) supplied with recirculated water maintained at 8°C (flow rate: 4 L/min). Duplicate groups of 10 trout (mean body weight: 169 g) were fed twice a day (daily ration: 1.3% of body weight) 1 of the experimental diets. After a 5-day acclimation period, the faeces were continuously collected for a period of 9 d through rotative grids according to the procedure described by Choubert *et al* (1982a). Faeces from each tank were recovered and frozen twice a day. Subsequently, the water temperature was progressively increased up to 18°C. During this transition period, which lasted 5 d, the trout were fed the same commercial diet as they were fed before the first trial. The trout (mean body weight: 185 g) were then acclimated to the new temperature and experimental diets for 8 d. They were fed twice a day a daily ration of 1.6% of body weight. Faeces were collected for 9 d.

During a second trial, dietary treatments were tested in triplicate with 17 fish per tank. Daily rations were the same as in the former trial. Digestibility was first measured at 18°C. Trout (mean body weight: 95 g) were acclimated to the respective diets for 6 d before the beginning of faeces collection. Faeces were collected for 9 d.

Water temperature was then decreased slowly (7 d). Acclimation period to the new temperature (8°C) lasted 13 d. Faeces of trout (mean body weight: 125 g) acclimated to the experimental diets for 7 d were then collected for 9 d.

At the end of each trial, pooled, freeze-dried faecal samples were ground before analyses. Apparent digestibility coefficients (ADC) of nutrients and energy were calculated according to Maynard and Loosli (1969).

Feeding trial

The feeding trials were performed in 2 INRA experimental fish farms which differed in water temperature. The fish farm of Lees-Athas (Pyrénées-Atlantiques) is supplied with mountain spring water at a nearly constant temperature of 8 ± 1°C. Three hundred rainbow trout (mean initial body weight: 56 g) were randomly and equally allotted to 6 separate fibre-glass tanks (water volume 400 L; flow rate: 20 L/min).

The fish farm of Donzacq (Landes) is supplied with water at a constant temperature of 18 ± 1°C drawn from natural springs. Six hundred rainbow trout (mean initial body weight: 53 g) were randomly and equally allotted to 6 individual sand bottomed compartments (water volume: 800 L; flow rate: 240 L/min). Initial density of fish was maintained to be the same in the 2 feeding trials.

Both trials, which lasted 12 weeks, were carried out in winter (January to March), under natural photoperiod. Duplicate groups of fish, from the same strain in both trials, were fed by hand twice a day 1 of the experimental diets to near satiation (visual observation of first feed refusal). They were group-weighed and counted every 3 weeks, and feed intakes were recorded. Specific growth rate was calculated as:

$$SGR = ((\ln BWt\ end - \ln BWt\ start)/83) \times 100$$

Body composition analyses were performed on an initial sample of 10 fish and on groups of 5 fish which were withdrawn from each tank at the end of the experiment and killed after anaesthesia (ethylglycolmonophenylether 1/2 500). Whole bodies were frozen, freeze-dried, ground and pooled for each tank before analyses. Data allowed the calculation of retention efficiencies for protein and energy, which are equal to the ratio (nutrient or energy gained)/ (intake of digestible nutrient or digestible energy).

Estimations of non-faecal energy losses (through gill and urine excretions), metabolizable energy and total energy expenditure (fasting metabolic rate + heat increment of feeding + energy expended for activity) were made from the protein and energy retention according to Cho and Kaushik (1985). These parameters were expressed as percentage of digestible energy intake.

The amounts of digestible protein (DP) and digestible energy (DE) required for the production of 1 kg rainbow trout were also calculated as follows: (DP or DE intake (g) per fish during the period/(FBW – IBW)) x 1 000, where FBW and IBW represent the final and initial mean body weights, respectively.

Chemical analyses

The proximate compositions of the experimental diets (table I) and the chemical composition of the faeces and fish were determined using the following procedures: DM by drying in an oven at 110°C for 24 h, ash by combustion at 550°C in a muffle furnace for 24 h, crude protein (as g N x 6.25) by the Kjeldahl method after an acid digestion, lipid according to Folch *et al* (1957), starch using amylase and glucose oxidase (Thivend *et al,* 1972), gross energy with an adiabatic bomb calorimeter (IKA). Chromic oxide was measured after perchloric acid digestion (Bolin *et al,* 1952).

Nitrogen excretion measurement

Ammonia-nitrogen (NH_4^+-N) excretion was measured first at 18°C. Trout (mean body weight: 70 g) were randomly allotted to 6 tanks (vol: 50 L) supplied with thermoregulated recirculating water (flow rate: 1.7 L/min). Duplicate groups of 12 trout were fed twice a day (daily ration: 1.2% of body weight) one of the experimental diets for 3 weeks. Concentration of NH_4^+-N was measured in the water sampled from each tank and from a standard solution every hour during three 24 h cycles.

Water temperature was then decreased progressively (1.5°C/d) to 8°C. Trout were acclimated to the new temperature for 8 d. They continued to be fed the experimental diets twice a day (daily

ration: 0.8% of body weight). Concentration of NH_4^+-N in water was measured as at 18°C during two 24-h cycles.

Concentrations of NH_4^+-N were measured using an autoanalyser (Alpkem) according to Le Corre and Treguer (1976).

Total nitrogen excretion was estimated to be NH_4^+-N/0.85 (Kaushik, 1980).

Time-course study of plasma glucose

Ninety rainbow trout were randomly allotted to 6 tanks at 8°C and 153 trout to 9 tanks at 18°C. Fish (mean body weight: 120 g) were acclimated to the same tanks as those used for the digestibility trial for 3 weeks before blood sampling. During this period, they were fed twice a day the experimental diets at a ration level of 1.6 and 1.8% BW per day at 8 and 18°C respectively. They were then left unfed for 48 h. Blood samples were collected first on 5 fasted trout under anaesthesia (ethylglycolmonophenylether: 1/4 000) for each dietary treatment (0-sampling time). These trout were then discarded while the others were fed a meal of 1 of the experimental diets at the appropriate feeding level. At each selected time interval (2, 4, 6, 8, 12, 16, 24 and 30 h after feeding), 5–8 fed trout per dietary treatment were randomly removed from the tanks and anaesthetized. Blood was sampled from the caudal vein. A mixture of potassium oxalate 4% and sodium fluoride 4% was used to prevent coagulation and glycolysis. Each trout was sampled once or twice during the experiment. After the first blood sampling, fish were tagged by cutting the adipose fin. The second blood sampling on a same fish occurred at least 12 h after the first one. Blood was immediately centrifuged and plasma kept at 4°C. Plasma glucose analyses were carried out within 4 h following sampling using a glucose analyser (Beckman II, USA).

Statistical analyses

The influence of dietary treatment on apparent digestibility coefficients and on nitrogen excretion was assessed by repeated measure of analysis of variance (Winer, 1971). This test also provided results on the influence of water temperature (repeated measures) and interaction between the 2 treatments. When the influence of the dietary treatment or the interaction was significant, the multiple comparison test of Bonferroni was performed. This test is appropriate when means are dependent unlike the Newman–Keuls' test which is more often used in current statistical analyses (Maxwell, 1980).

Two-way analyses of variance were performed to test the influence of dietary treatment, water temperature and their interaction on plasma glucose for each sampling-time. When a significant effect of the dietary treatment or of the interaction was found, means were compared using the multiple range test of Newman–Keuls.

Because experimental conditions were different between 8 and 18°C feeding trials, one-way analyses of variance were performed at each temperature to assess the influence of dietary treatment on growth performance and body composition. The multiple range test of Newman–Keuls was used to compare means when the effect of the dietary treatment was significant.

Statistical analyses were performed at a significance level of 5% using the SAS package (1987).

RESULTS

The results of the 2 digestibility trials were pooled, since they were not significantly different ($P > 0.05$). Hence, the data of digestibility are presented as the means of 5 values for each diet (table II). ADC of dietary components and energy were significantly lower in trout held at 8°C than in those held at 18°C. The decrease in digestibility was small for protein (on average: 3.3%) but more pronounced for lipid (on average: 8.6%) and starch (on average: 17.3%). At both temperatures, the ADC of starch was the lowest for trout fed the diet containing the highest level of crude starch (diet LC). ADC values of starch were similar for trout fed diets containing mainly gelatinized starch (diets HC and MC) irrespective of the dietary level of crude starch. No significant effect of dietary treatment on ADC of protein and lipid was found ($P > 0.05$ for each trial). The digestibility of energy was

Table II. Effects of treatments (diet: HC, MC, LC; temperature: 8 or 18°C) on apparent digestibility coefficients (ADC) of nutrients and energy, and digestible carbohydrate digestible lipid ratio (DC/DL) of diet for each treatment.

Diet	*Temperature*					
	8°C			*18°C*		
	HC	*MC*	*LC*	*HC*	*MC*	*LC*
ADC*						
Protein	85.3 ^{d}	88.7 ^{cd}	87.2 ^{cd}	88.7 ^{abc}	90.0 ^{a}	89.3 ^{ab}
Lipid	85.4 ^{b}	88.1 ^{b}	86.9 ^{b}	94.0 ^{a}	95.0 ^{a}	93.9 ^{a}
Starch	69.5 ^{b}	63.1 ^{b}	41.2 ^{d}	86.6 ^{a}	84.6 ^{a}	54.6 ^{c}
Energy	74.4 ^{c}	76.1 ^{c}	71.3 ^{c}	83.6 ^{ab}	86.2 ^{a}	80.6 ^{b}
DC/DL	3.5	1.8	0.8	4.3	2.2	0.9

* Data were arcsine transformed for normality before statistical analysis. Values in the same row with different superscript letters are significantly different ($P < 0.05$).

the highest for trout fed the diet containing a mixture of both starch and lipid (diet MC) but the difference was significant ($P < 0.05$) only at 18°C. Consequently, digestible energy supplied by the experimental diets was not the same at both temperatures. Hence, experimental diets were not actually isoenergetic. However, 3 distinct digestible carbohydrate/digestible lipid ratios were obtained for both trials (table II).

Specific growth rates (SGR) were not influenced ($P > 0.05$) by the carbohydrate/lipid ratio of the diet for fish held at 8 or 18°C. However, at 18°C, final body weight tended to be higher ($P < 0.10$) in trout fed the diet with high lipid level (table III). Feed intake, feed gain ratio, and protein retention were not significantly influenced by dietary treatment at either temperature (table III). In contrast, at 18°C, the amounts of digestible protein and digestible energy required for the production of 1 kg of trout were slightly but significantly lower for fish fed diets with high lipid content. At 8°C, the dietary treatment did not influence ($P > 0.05$) these parameters (table III). Whole body protein content were not affected ($P > 0.05$) by dietary treatment at both temperatures (table III). At 8°C, whole body lipid content, and thus, whole body energy contents, were similar ($P > 0.05$) in fish fed the 3 experimental diets. In contrast, at 18°C, whole body lipid and energy content were higher in fish fed the diet with high lipid content (diet LC) (table III). This may be explained by significantly lower energy expenditure and thus, higher energy retention efficiencies for trout fed diet LC relative to those fed diets MC and HC when they were reared at 18°C. These parameters were not affected ($P > 0.05$) by dietary treatment at 8°C (table IV). Estimated values for non-faecal energy losses and for metabolizable energy (ME) were not affected by dietary treatment at either temperature (table IV).

The postprandial pattern of NH_4^+ excretion was similar for the 3 groups of trout at each temperature (fig 1). Excretion peaks were more pronounced in trout held at 18°C than in those held at 8°C. At 18°C, 2 peaks were observed: the first at 13 h and the second at 20 h (*ie* 4 h after the meals). At 8°C,

Table III. Effects of dietary treatment (HC, MC, LC) on intakes, growth performance and body composition of trout (initial mean body weight: 50 g) held at 8 or 18°C for 84 d.

Diet	*Temperature*					
	8°C			*18°C*		
	HC	*MC*	*LC*	*HC*	*MC*	*LC*
Intake (per kg-trout per d)						
Dry matter (g)	9.4	9.2	8.9	13.2	13.1	12.5
Growth performance						
Final body weight (g)	120.4	126.5	123.3	159.6	166.2	174.5
Specific growth rate (%)	0.9	1.0	0.9	1.3	1.4	1.5
Feed gain ratio (%)	1.1	1.0	1.0	1.1	1.1	1.1
Protein retention (%)	44.9	44.7	44.6	43.7	43.9	47.7
DP */kg weight gain (g)	392.7	377.9	374.9	424.8 [a]	418.7 [a]	375.1 [b]
DE **/kg weight gain (kJ)	15.0	15.3	15.2	17.5 [ab]	18.8 [a]	16.9 [b]
Body composition						
Dry matter (% WW***)	25.6	25.8	25.5	29.2	29.7	33.0
Protein (% WW)	17.0	16.8	16.8	18.2	18.2	17.9
Fat (% WW)	6.3	7.0	6.2	7.3 [b]	8.6 [b]	12.3 [a]
Energy (MJ/kg WW)	6.3	6.5	6.2	7.3 [b]	7.5 [b]	8.9 [a]

* Digestible protein intake; ** digestible energy intake; *** wet weight; data expressed as percentages were arcsine transformed for normality before statistical analysis. For each temperature, values in the same row with different superscript letters are significantly different ($P < 0.05$).

Table IV. Effects of dietary treatment (HC, MC, LC) on energy balance (expressed as percentage of digestible energy intake) of rainbow trout held at 8 or 18°C.

Diet	*Temperature*					
	8°C			*18°C*		
	HC	*MC*	*LC*	*HC*	*MC*	*LC*
Non-faecal energy losses	6.0	5.5	5.7	5.5	5.0	4.6
Metabolizable energy	94.0	94.5	94.7	94.5	95.1	95.4
Total energy expenditure	46.8	45.5	47.6	45.0 [a]	47.9 [a]	32.4 [b]
Energy retention	47.2	49.0	46.7	49.5 [b]	47.1 [b]	63.0 [a]

Data were arcsine transformed for normality before statistical analysis. For each temperature, values in the same row with different superscript are significantly different ($P < 0.05$).

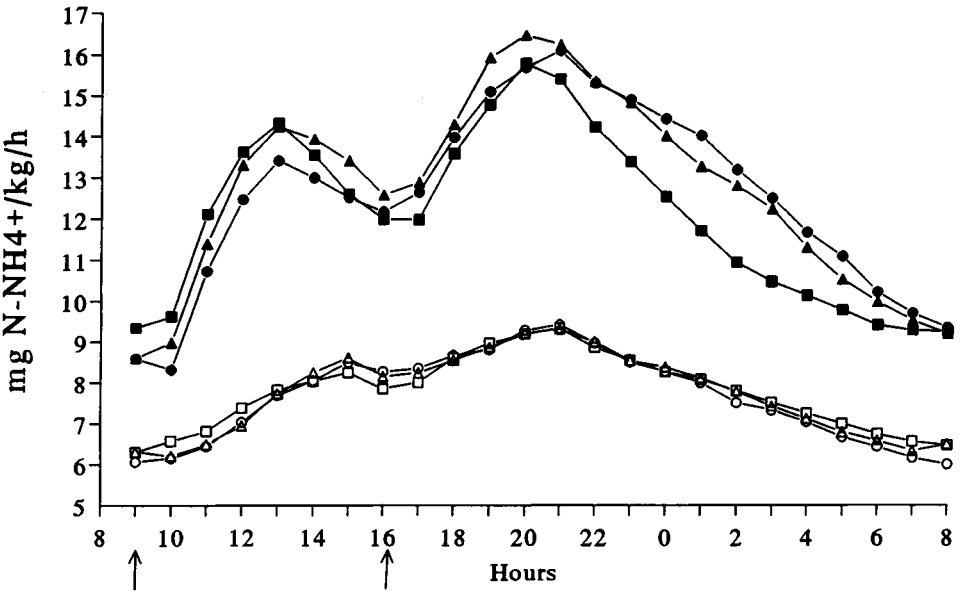

Fig 1. Effects of water temperature and dietary treatment (circles: HC; triangles: MC; squares: LC; closed marks: 18°C trial; open marks: 8°C trial) on time-course of ammonia-nitrogen excretion in rainbow trout. Arrows indicate the times of feeding.

the NH_4^+ excretion increased slowly up to 15 h, decreased slightly between 15 and 16 h, and increased again up to 24 h. The total nitrogen excretion during 24 h was not influenced ($P > 0.05$) by dietary treatment, but it was significantly higher at 18°C than at 8°C: 298 and 185 mg/kg/d respectively. However, expressed as percentage of digestible nitrogen intake, nitrogen excretion was not influenced ($P > 0.05$) by dietary treatment or water temperature, and was on average 52.6%.

At each sampling time, the plasma glucose levels of trout that were sampled twice did not differ from those of trout sampled once only, showing that sampling the same fish twice at 12 h intervals did not affect the glycaemia levels.

Plasma glucose levels of trout increased within the first 2 to 4 h after the meal, irrespective of temperature and diet (fig 2). The peak plasma glucose concentration was higher ($P < 0.05$) in trout fed diets with high digestible carbohydrate level. The daily pattern of glycaemia and the time of attainment of the peak of plasma glucose level were also influenced by water temperature. Peak of plasma glucose level was reached earlier at 18°C than at 8°C for trout fed diets HC and LC. Plasma glucose generally remained high 24 h after the meal in fish fed diets with high digestible carbohydrate levels, at both temperatures. However, plasma glucose returned nearly to the fasting level 30 h after the meal for trout reared at 18°C but not for trout reared at 8°C, when they were fed diet HC. Water temperature also influenced plasma glucose levels of fish but this effect was dependent on the dietary treatment. For trout fed the diet with the high digestible carbohydrate level (diet HC), plasma

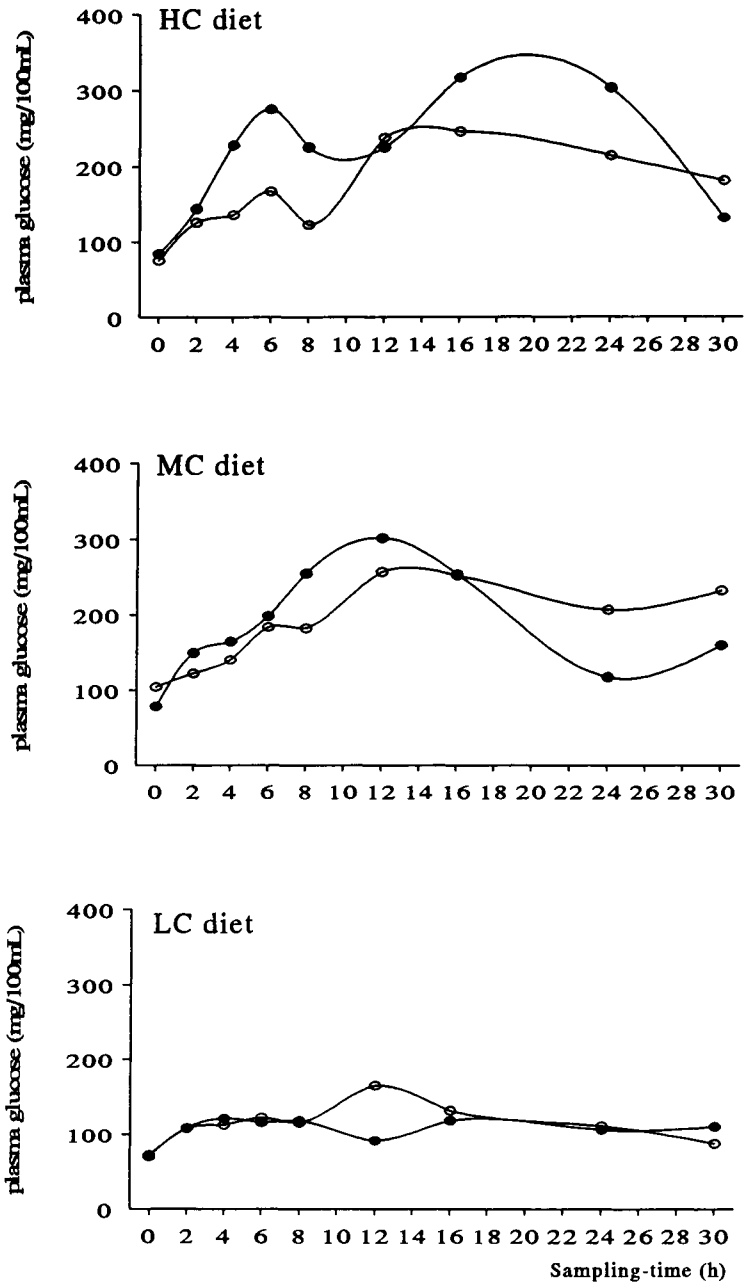

Fig 2. Time-course of plasma glucose in rainbow trout: effect of water temperature for each dietary treatment (HC, MC and LC). Full lines represent the results of the 18°C trial and broken lines, the results of the 8°C trial.

glucose was higher at 18°C than at 8°C between 4 and 24 h ($P < 0.05$ for times 4, 6 and 8 h). Thirty hours after the meal, plasma glucose tended to be lower, although not significantly, in fish held at 18°C than in those reared at 8°C.

DISCUSSION

Nutrient and energy digestibility coefficients were higher in trout reared at high temperature than in those held at low temperature. Médale *et al* (1991) also observed an increase in ADC of crude and gelatinized starch with increased temperature in the same species. These results suggest that the activity of digestive amylase might be higher at high temperature in rainbow trout, as has been shown in roach, *Rutilus rutilus* (Hofer, 1979a). Choubert *et al* (1982b) and Oliva-Teles and Rodrigues (1993) also observed some improvement of the digestibilities of protein and energy by increasing water temperature in the same species. Gastric evacuation rate and total transit rate are increased by increasing temperature in several fish species (Fänge and Grove, 1979) including trout (Fauconneau *et al,* 1983). In another study, some digestive enzyme activities (proteases and amylase) are increased with increasing temperature in the roach (Hofer, 1979a, b). These 2 effects of water temperature are opposed: the first might decrease whereas the second might increase the digestibility of food at high temperature. This opposition might explain why an effect of water temperature on digestibility was not clearly proven in trout (Cho, 1987; Watanabe *et al,* 1989; Médale *et al,* 1991). Dietary treatment, water temperature range and/or genetic stock of trout might interact with the effect of water temperature on digestibility.

Our results showed that protein retention was not influenced by dietary carbohydrate/lipid ratio in trout, irrespective of water temperature. This is consistent with results of previous work where we observed that protein retention was not influenced by carbohydrate/lipid ratio in the diet of trout grown in seawater at 9°C (Brauge *et al,* 1994). Furthermore, in the present work, nitrogen excretion, expressed as percentage of digestible nitrogen intake, was not affected by dietary treatment. Digestible starch therefore seems to be as efficient as lipid in sparing protein. The protein-sparing effect of non-protein energy sources seems to be determined by the amount of digestible energy provided and not by the nature (lipid or carbohydrate) of the energy source in trout. Higgs *et al* (1992) also observed that isonitrogenous (crude protein: 39.5% DM) and isoenergetic (calculated digestible energy: 16.3 kJ/g DM) diets differing by their carbohydrate/lipid ratio (HL, 0.3:17.6; ML, 13.8:13.1; LL, 24.4:7.4, in %DM), with glucose as the carbohydrate source, led to the same protein retention efficiency. However, these authors concluded that trout do not efficiently utilize carbohydrate as an energy source because fish fed diets with a high carbohydrate level had lower food intake and weight gain, which differed from our results. These differences might be due to differences in water temperature, diets, especially carbohydrate source, or degree of satiation (feed intake: 1.0–1.3% of body weight per day in Higgs *et al,* 1992, and 0.8% in our experiment).

At 18°C, the amounts of digestible protein and energy required per unit weight gain were higher when high digestible carbohydrate level diets (22–26% at 18°C) were provided to trout. This result is probably due to a higher energy expenditure when trout received the high carbohydrate diets. Beamish *et al* (1986) observed an increase in the heat increment of feeding with dietary carbohydrate levels in trout held at 15°C and fed isonitrogenous and isoenergetic diets. In mammals, an increase in dietary carbohydrate levels results in increasing

heat increment of feeding, due to the transformation of dietary glucose into body lipids (Acheson *et al,* 1984).

Lipogenesis from carbohydrate is also effective in rainbow trout and increases with increasing carbohydrate/lipid ratio (Henderson and Sargent, 1981; Brauge *et al,* 1995). Hence, the higher energy expenditure observed in trout held at high temperature and fed the high digestible carbohydrate diet might be due to a higher lipogenesis. This phenomenon might also be due to an influence of high dietary carbohydrate levels on glucose transport and/or excretion as supposed by Beamish *et al* (1986). However, dietary carbohydrate/lipid ratio had no effect on the energy expenditure of trout held at the low temperature of 8°C. Consequently, trout fed the diet with the high lipid level had higher body fat content than those fed the diet with the low lipid level at high temperature, but not at low temperature. Studies have shown that body lipid levels are related to dietary lipid level in fish (Adron *et al,* 1976; Reinitz et Hitzel, 1980; Watanabe, 1982). However, in these studies, an increase in dietary lipid level was associated with an increase in dietary energy levels, whereas, in the present work, the level of digestible energy intake was roughly the same in the 3 groups of fish. Hence, the nature (carbohydrate or lipid) of the non-protein energy source of the diet influences the body lipid composition of trout held at high temperature. However, this effect seems to interact with temperature since we did not observe any difference in body lipid levels of trout held at low temperature. More studies are thus needed to understand the interaction between dietary carbohydrate/lipid ratio and water temperature on energy metabolism of trout.

The ammonia excretion of trout had a typical course after feeding (Rychly and Marina, 1977; Kaushik, 1981). The pattern of nitrogen excretion depends on the species, the size and composition of the meal, and on the water temperature (Kaushik, 1980, 1981; Kaushik and Oliva-Teles, 1985; Cui and Liu, 1990). The effect of water temperature on feed intake (Kaushik, 1981) and on transit rate (Fauconneau *et al,* 1983) may explain, in our experiment, why the effect of the second meal is more obvious at 18°C than at 8°C. The elevated nitrogen excretion of trout reared at 18°C, relative to those held at 8°C, may result from the effect of water temperature on feed intake, since nitrogen excretion was not influenced by water temperature when expressed as percentage of digestible nitrogen intake. This result agrees with Kaushik (1981).

Plasma glucose concentrations were higher in trout fed diets with high digestible carbohydrate levels than in those fed the diet with low digestible carbohydrate level. Similar results have been observed previously in 60–100 g trout held in freshwater at 18 or 12°C (Bergot, 1979; Himick *et al,* 1991) and in 300 g trout held in seawater at 9°C (Brauge *et al,* 1994) with glucose, dextrin or digestible starch as carbohydrate source. Moreover, plasma glucose levels were higher at 18°C compared with those at 8°C during the 10 h after the meal when trout were fed diets with high digestible carbohydrate content. This result may be explained by a higher availability of carbohydrate at high temperatures than at low temperatures when fish were fed diets containing high digestible carbohydrate levels. Indeed, the amounts of digestible carbohydrate ingested by a 100 g fish per meal were higher at elevated temperatures which led to higher plasma glucose levels in trout reared at 18°C than in those reared at 8°C. Finally, increases in the rates of carbohydrate digestion and absorption due to elevated temperatures may explain why the peak of plasma glucose was attained earlier in trout held at high temperature relative to those held at low temperature. Oxidative catabolism of glucose has also been shown

to be higher in another teleost, *Dicentrarchus labrax,* held at a high temperature rather than at a low temperature (Garin, 1984). Such an effect of temperature in rainbow trout might explain the more rapid fall of plasma glucose at high temperature than at low temperature.

CONCLUSION

This study showed that digestible carbohydrates, supplied as gelatinized wheat starch, were as efficient as lipids in maintaining high protein retention in rainbow trout. By contrast, the source of non-protein energy influenced total energy expenditure and lipid deposition at high temperature, but not at low temperature. Temperature also influenced the digestibility of nutrients and energy and time-course of plasma glucose. Although plasma glucose levels were high for trout fed diets with high digestible carbohydrate content, the trout grew well irrespective of dietary treatment at both temperatures. Nitrogen excretion was elevated in trout reared at 18°C because of a higher feed intake relative to trout held at 8°C. Further work is needed to understand the effect of the interaction between temperature and non-protein energy sources on energy and lipid metabolism of trout.

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