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Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*)

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Abstract – A study was carried out to determine the effect of feeding level and water temperature on growth and feed efficiency, nutrient and energy utilization and waste outputs of rainbow trout. A practical diet was fed to near-satiation to groups of fish reared at 6, 9, 12 and 15 °C. At each temperature, the feed intake of other groups of fish was restricted to about 85 % or 70 % of the amount of feed consumed in the previous week by the fish fed to near-satiation. Total feed intakes over 12 weeks were, on average, 76 % and 65 % of total feed intake of the near-satiety group for R1 and R2, respectively. Reducing the feed allocation resulted in significantly ($P < 0.05$) lower weight gains compared to feeding to near-satiation regardless of the rearing temperature. Feeding level and water temperature had no significant ($P > 0.05$) effect on feed efficiency, carcass composition or efficiencies of digestible nitrogen and digestible energy retention. Increasing temperature resulted in an increase in the apparent digestibility of dietary dry matter, nitrogen and energy ($P < 0.05$). The increase in digestibility of dry matter and nitrogen with increasing temperature resulted in higher estimated total solid and solid nitrogen waste outputs per kg fish produced ($P < 0.05$) at lower water temperatures. Estimated dissolved nitrogen and phosphorus waste outputs (g/kg fish produced) were not affected by the feeding level or water temperature. A highly significant ($P < 0.01$) linear relationship was observed between metabolizable energy (ME) intake above basal metabolism and recovered energy. The efficiency of ME utilization for growth (K_{pf}) was 0.61 and this coefficient was not affected by feed intake or water temperature. Protein and lipid were deposited in a constant ratio (1 kJ protein gain:1.4 kJ lipid gain) regardless of ME intake or water temperature. © Ifremer/Elsevier, Paris

Diet / feeding / temperature / energy / trout / waste

Résumé – Effets de la taille de la ration alimentaire et de la température sur la croissance, l'utilisation des nutriments et de l'énergie, et l'excrétion de déchets par la truite arc-en-ciel (*Oncorhynchus mykiss*). Une étude a été réalisée pour déterminer l'effet de la ration alimentaire et de la température d'élevage sur la croissance, l'utilisation des nutriments et de l'énergie, ainsi que l'excrétion de déchets par la truite arc-en-ciel. Un aliment complet a été donné à satiété à des groupes de poissons élevés à 6, 9, 12 et 15 °C. À chaque température, la prise alimentaire d'autres groupes de poissons était limitée à 85 % (R1) ou 70 % (R2) de la quantité consommée la semaine précédente par les groupes nourris à satiété. Les quantités totales d'aliment consommées par les poissons des groupes R1 et R2 pour les 12 semaines de la période expérimentale se sont élevées respectivement en moyenne à 76 et 65 % de celle consommée par les poissons des groupes nourris à satiété. Une réduction de la ration a amené une réduction significative ($p < 0,05$) du gain de poids des poissons, en comparaison avec les poissons nourris à satiété à toutes les températures. La ration et la température n'ont généralement pas eu d'effet sur le taux d'efficacité alimentaire, la composition corporelle ou l'efficacité de l'utilisation de l'azote et de l'énergie digestibles. Une augmentation de la température a amené une augmentation significative ($p < 0,05$) de la digestibilité apparente de la matière sèche, de l'azote et de l'énergie. L'augmentation de la digestibilité de la matière sèche et de l'azote a amené une réduction significative ($p < 0,05$) de l'excrétion de déchets solides totaux et azotés, avec une augmentation de la température. L'excrétion de déchets azotés soluble ainsi que l'excrétion totale de phosphore n'ont pas été affectées par la température ou la ration. Une relation linéaire significative ($p < 0,01$) a été observée entre la quantité d'énergie métabolisable ingérée au-dessus du niveau de maintenance et la quantité d'énergie corporelle déposée. L'efficacité de l'utilisation de l'énergie métabolique pour la croissance (K_{pf}) était de 0,61 et cet indice n'a pas été affecté par la taille de la ration ou la température. La déposition de protéines et de lipides est demeurée proportionnelle, sans égard à la ration ou à la température d'élevage. © Ifremer/Elsevier, Paris

Aliment / ration / température / énergie / truite / pollution

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1. INTRODUCTION

Feed costs represent a very significant proportion of the production cost in salmonid fish culture. Many fish culture operations have poor feed efficiencies (gain/feed) [18] and this contributes to the high cost of production and often results in significant water pollution. It is necessary to optimize feeding regimes to improve the economical and environmental sustainability of aquaculture.

It is not always clear if low feed efficiencies observed under certain conditions are due to feed wastage or due to a real decrease in the feed utilization efficiency of the fish. The effect of feeding level on the efficiency of feed utilization in rainbow trout and other salmonids is the subject of controversy. It has been suggested that optimum feed efficiency is achieved at feeding levels below that required for maximum growth in salmonids [7, 20, 23]. Other studies suggest that feed efficiency improves to its maximum at moderate feed restriction (e.g. 50 % of maximum ration) and this optimum is maintained up to the ration required for maximum growth of the fish [1, 33]. It has also been suggested that maximum feed efficiency of fish is attained at maximum feed intake and maximum growth [19]. Most of these observations are derived from studies using fixed ration (% live body weight) which may not represent the fish's actual feed requirement or studies conducted under poorly controlled experimental conditions (mechanical distribution of feed, variable temperature, etc.). The effect of feeding level on feed utilization should be re-examined under highly controlled conditions (careful hand-feeding to avoid feed waste, controlled temperature, etc.).

While important from a production point of view, feed efficiency can be a misleading expression of nutrient and energy utilization. Physical quantity of feed used is not a measure of biologically available nutrients and energy supplied to the animal. In addition, weight gain does not always accurately reflect protein, lipid and energy gains since the composition of weight gain is often variable. Protein deposition is associated with substantial water deposition whereas lipid depots contain little water. The ratio between protein and lipid deposition will have an impact on live weight gain and, consequently, feed efficiency.

Being poikilothermic animals, the metabolic rate, growth, energy expenditure and feed intake of fish are highly influenced by water temperature. It is, therefore, important to study how water temperature affects these parameters, as well as to determine the effect of temperature on the efficiency of nutrient and energy utilization. Studies have suggested that temperature can affect the efficiency of energy utilization in salmonids [13]. The effects of water temperature on nutrient and energy utilization should be examined.

The objectives of this experiment were to study the effects of different feeding regimes (satiation vs two lower levels of feeding) and water temperatures on growth, feed efficiency, apparent digestibility, utilization of nitrogen, phosphorus and energy and also waste output of rainbow trout.

2. MATERIALS AND METHODS

2.1. Fish and experimental conditions

Rainbow trout at 13.3 ± 0.4 g body weight were obtained from the Alma Aquaculture Research Station (Elora, Ontario) and were randomly distributed to 36 tanks divided in four groups of nine tanks each. Each group of nine tanks was maintained at one of four water temperatures, 6 ± 0.8, 9 ± 0.4, 12 ± 0.4 or 15 ± 0.3 °C (mean ± SD). The initial number of fish were 75, 60, 45 and 30, respectively for the different water temperatures. These numbers were determined based on the predicted final body weight of the fish at 12 weeks using a thermal-unit growth coefficient (TGC) = 0.174 % [9] and assuring that the biomass would not exceed a maximum density of 4 kg/50 L (80 $kg{\cdot}m^{-3}$).

The tanks were individually aerated and supplied with water from a recirculation system equipped with a gravel biofilter (20 % make-up water) at a rate of about 3 $L{\cdot}min^{-1}$. Photoperiod was maintained at 12 h light:12 h dark in a windowless laboratory. The animals were kept in accordance with the guidelines of the Canadian Council on Animal Care [8] and the University of Guelph Animal Care Committee. Rainbow trout were acclimatized to experimental conditions for 7 days, during which time they were fed a commercial trout diet (Martin Feed Mills, Elmira, Ontario, Canada).

2.2. Diets and feeding protocol

A practical diet, formulated to contain 460 g of digestible protein (DP) and 20 MJ digestible energy (DE) per kg and all essential nutrients in excess of the levels recommended by the NRC [26] (*tables I* and *II*), was used for all the dietary treatments. Dietary ingredients were obtained from a local feed mill (Martin Mills, Elmira, Ontario, Canada). Acid-washed diatomaceous silica (Celite AW521, Celite Corp., Lompoc, CA, USA) was included in the diet to serve as a digestibility indicator. The diet was mixed using a Hobart mixer (Hobart Ltd, Don Mills, Ontario, Canada), pelleted to appropriate size using a laboratory steam pellet mill (California Pellet Mill Co., San Francisco, CA, USA), dried under forced-air at room temperature for 24 h and then sieved.

Three dietary treatments were each allocated to three blocks of three tanks at each temperature using a complete random block design. All fish were fed to near-satiety during the first week of the experiment. The fish in one tank within each block were hand-fed the diet three times daily to near-satiety (NS) during the next 11 weeks of the trial. The feed intakes of the fish on the restricted feeding regimes were limited to

Table I. Diet formulation.

Ingredients	g/100 g of diet
Fish meal, herring, Canadian East Coast, 68 % crude protein, 10 % ash	40.0
Fish oil	16.0
Corn gluten meal, 54 % crude protein	20.0
Blood meal, AP 301, 84 % crude protein	8.0
Brewer's dried yeast, 41 % crude protein	5.0
Whey, 10 % crude protein	8.0
Celite AW521 [1]	1.0
Vitamin premix [2]	1.0
Mineral premix [3]	1.0

[1] Celite AW521 (acid-washed diatomaceous silica) is a source of acid-insoluble ash.
[2] provides per kg of diet: retinyl acetate (vit. A), 3 000 IU; cholecalciferol (vit. D), 2 400 IU; all-rac-α-tocopheryl acetate (vit. E), 60 IU; menadione sodium bisulfite (vit. K), 1.2 mg; l-ascorbic acid (vit. C), 240 mg; cyanocobalamine (vit. B12), 0.024 mg; d-biotin, 0.168 mg; choline chloride, 2 400 mg; folic acid, 1.2 mg; myo-Inositol, 360 mg; niacin, 12 mg; d-calcium pantothenate, 24 mg; pyridoxine.HCl, 6 mg; riboflavin, 7.2 mg; thiamin.HCl, 1.2 mg.
[3] provides per kg of diet: sodium chloride (NaCl, 39 % Na, 61 % Cl), 3 077 mg; ferrous sulfate ($FeSO_4.7H_2O$, 20 % Fe), 65 mg; manganese sulfate ($MnSO_4$, 36 % Mn), 88.9 mg; zinc sulfate ($ZnSO_4.7H_2O$, 40 % Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25 % Cu), 28 mg; potassium iodide (KI, 24 % K, 76 % I), 33.3 mg.

Table II. Proximate analysis of the experimental diet (on an as-fed basis).

[1] Dry matter, %	94.2
[1] Crude protein, %	48.8
[2] Lipid, %	25.3
[1] Ash, %	7.3
[3] Nitrogen-free extract, %	12.8
[4] Gross energy, $MJ \cdot kg^{-1}$	23.7
[1] Phosphorus, %	1.09
[5] Digestible protein (DP), %	45.8
[5] Digestible energy (DE), $MJ \cdot kg^{-1}$	20.4
DP/DE, $g \cdot MJ^{-1}$	22.4

[1] Dry matter, crude protein, ash and phosphorus were analysed according to standard AOAC methods [2].
[2] Lipid content was determined by the Bligh and Dyer method [4].
[3] Nitrogen-free extract was obtained by difference.
[4] Gross energy was obtained by bomb calorimetry.
[5] Digestible protein (energy) = Σ (% of ingredient inclusion in the diet × crude protein (gross energy) × apparent digestibility of protein (energy) of each ingredient) based on published values [26].

85 % (R1) and 70 % (R2) of the amount consumed by the NS group in the same block during the previous week. The resulting feeding levels expressed as % live body weight·day^{-1} at each temperature for the 12 weeks of the experimental period are presented in *figure 1*. The total actual feed intake over 12 weeks were on average 79, 75, 76, and 74 % of the NS intakes at 6, 9, 12, and 15 °C, respectively, for the R1 group, and 66, 65, 65, and 62 % of the NS intakes at 6, 9, 12, and 15 °C, respectively, for the R2 group, as shown in

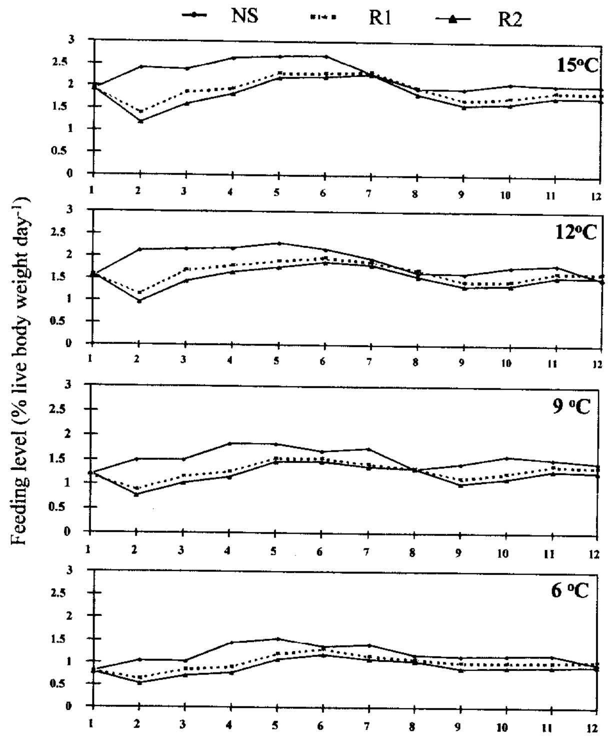


Figure 1. Resulting feeding levels (expressed as % of live body weight) at the four temperatures and three feeding levels.

table III. The fish were weighed every 4 weeks to determine weight gain and feed efficiency (live weight gain:dry feed). Growth rate was calculated using the thermal-unit growth coefficient [9] as follows:

Thermal-unit Growth Coefficient (TGC) =

$$[FBW^{1/3} - IBW^{1/3}] / \Sigma[T \times D] \times 100$$

where: FBW is final body weight (g), IBW is initial body weight (g), T is water temperature (°C) and D is number of days.

At the beginning of the experiment, 25 fish, and at the end, 15, 12, 8 or 5 fish per tank were randomly sampled from each tank at 6, 9, 12 and 15 °C, respectively, to obtain approximately 100 g of fish (wet weight basis) for carcass composition analysis. Fish were cooked in an autoclave (for 10 min. at 110 °C), ground to a slurry, freeze-dried, reground and kept at –20 °C until analysis.

2.3. Digestibility trial

At the end of the feeding trial, fish were maintained in the same aquatic system and hand-fed the experimental diet three times daily to near-satiation. The system was equipped with faeces settling columns (Guelph system) and faecal samples were collected as previously described [14].

Table III. Growth performance and feed efficiency of rainbow trout (IBW = 13.3 g/fish) fed the experimental diet for 12 weeks at 3 feeding levels and 4 water temperatures. $n = 3$ for each feeding level within temperature and for each temperature.

Water temp.	Feeding level	Weight gain (g/fish)	Feed intake[1] (g/fish)	FE[2] (gain:feed)	Thermal-unit Growth Coefficient (TGC) (%)
6 °C	NS	24.8 [a z]	23.0 [a z]	1.15 [a z]	0.188 [a z]
	R1	20.4 [b]	18.2 [b]	1.20 [a]	0.163 [b]
	R2	17.5 [c]	15.3 [c]	1.22 [a]	0.143 [c]
	SEM	0.32	0.16	0.024	0.0016
	HSD	1.60	0.82	0.12	0.0081
9 °C	NS	47.4 [a y]	39.8 [a y]	1.27 [a y]	0.204 [a z]
	R1	37.7 [b]	30.0 [b]	1.34 [a]	0.175 [b]
	R2	32.5 [c]	25.7 [c]	1.34 [a]	0.159 [c]
	SEM	0.58	0.24	0.022	0.0013
	HSD	2.91	1.21	0.11	0.0065
12 °C	NS	71.3 [a x]	62.0 [a x]	1.22 [b y z]	0.192 [a z]
	R1	58.5 [b]	47.4 [b]	1.31 [ab]	0.172 [b]
	R2	49.9 [c]	40.1 [c]	1.32 [a]	0.159 [c]
	SEM	1.23	0.55	0.019	0.002
	HSD	6.21	2.75	0.10	0.010
15 °C	NS	96.8 [a w]	86.3 [a w]	1.19 [a y z]	0.191 [a z]
	R1	74.4 [b]	63.6 [b]	1.25 [a]	0.164 [b]
	R2	63.9 [c]	53.7 [c]	1.26 [a]	0.149 [c]
	SEM	2.06	1.52	0.027	0.0026
	HSD	10.38	7.67	0.12	0.0129

NS = near satiation, R1, R2 = restricted diets. SEM = standard error of mean. HSD = Tukey's honestly significant difference ($P < 0.05$). Means in the same column (within each temperature) with different superscripts (a, b, c) are statistically different ($P < 0.05$). The superscripts w, x, y, z are used for comparing results between temperatures at the NS feeding level ($P < 0.05$). [1] Weight as fed basis, [2] FE = wet weight gain/dry feed.

2.4. Chemical analysis

Diet, faeces, and initial and final carcass samples were analysed in duplicate for dry matter and ash according to AOAC methods [2], for crude protein (%N × 6.25) using a Kjeltech auto-analyzer (Model # 1030, Tecator, Hoganas, Sweden), for lipid using the Bligh and Dyer method [4] and for gross energy (GE) using an adiabatic bomb calorimeter (Model # 1271 EB, Parr Instruments, Moline, IL, USA). The digestion indicator was determined using the acid-insoluble ash (AIA) indicator method [3]. Phosphorus content of the samples was determined by a commercial laboratory (Labstat Inc., Kitchener, Ontario, Canada) according to AOAC method 4.8.14 [2].

2.5. Energy partitioning

The energy partitioning scheme and notation proposed by the NRC [25] was used to describe energy partitioning by the fish. The gross energy intake was calculated based on the heat of combustion of the diet, measured by bomb calorimetry. The digestible (DE) and metabolizable energy (ME) intakes were calculated according to NRC convention [25]. Non-faecal losses (UE + ZE, kJ) were calculated based on estimated dissolved nitrogen losses [11] as follows:

$$(\text{DNI} - \text{RN}) \times 24.9$$

where: DNI is digestible nitrogen intake (g) and RN is retained nitrogen (g).

Basal metabolic rate (HeE) was calculated based on a published equation for salmonids [9]. This equation was derived from measured fasting heat production (HEf) of rainbow trout reared at various temperatures. HEf is expressed as a function of the temperature (T) and their body weight (BW) as follows:

$$\text{HEf} = (-1.04 + 3.26\text{T} - 0.05\text{T}^2)\,(\text{BW}^{0.824})\,\text{D}^{-1}$$

where HEf is fasting heat production in kJ, T water temperature (°C), BW body weight (kg) and D number of days.

Metabolic body weight (MBW) of the fish was calculated for each day of the experimental period (total of 84 days) by recreating their growth curve using the TGC growth model presented earlier. MBW was calculated from the live body weight (LBW) using the scaling factor 0.824 [11], where MBW is equal to the LBW of individual fish in kg to the power 0.824, i.e. $\text{MBW} = \text{LBW kg}^{0.824}$.

2.6. Waste outputs

Total solids, nitrogen and phosphorus (solid and dissolved) waste outputs were calculated using the Biological Method of Predicting Aquaculture Waste Output (BMPAWO) described elsewhere [15, 16]. BMPAWO has been shown to yield reliable estimates of waste output in a series of studies comparing it with direct measurements of solid and dissolved wastes [15, 16].

2.7. Statistical analysis

The effect of feeding level on live body weight gain, carcass composition, nutrient and energy gains and retention efficiencies, energy budget components, and waste outputs were analysed within water temperatures using the SAS General linear model (GLM) procedure [29]. The Tukey's honestly significant difference test [32] with $P < 0.05$ was used to detect significant differences among the means.

Statistical analysis of the apparent digestibility coefficients (ADC) was carried out as a split plot in period using the SAS general linear models (GLM) procedure [29]. The Tukey's honestly significant difference test with $P < 0.05$ was used to detect significant differences among the means of each water temperature [32].

The SAS general linear models (GLM) procedure was used to determine the regression of: a) total retained energy (RE) on metabolizable energy intake minus basal metabolic rate (ME intake – HeE); b) retained energy as lipid (RE_{lipid}) on retained energy as protein ($RE_{protein}$). The following model was used:

$$Y_{ij} = \mu + T_i + \beta 1 \times X_{ij} + \beta 2_i (T_i \times X_{ij}) + \beta 3\, X_{ij}^2 + \varepsilon_{ij}$$

where: μ = intercept, Y_{ij} = j'th observation of the dependent variable in the i'th temperature, X_{ij} = j'th observation of the independent variable in the i'th temperature, $\beta 1$, $\beta 3$ = partial regression coefficient of independent on dependent variable across T_i, $\beta 2_i$ = partial regression coefficient of interaction between independent variable and T_i on dependent variable, ε_{ij} = random error. Non-significant factors ($P > 0.2$) were removed from the model by backward elimination.

3. RESULTS

3.1. Growth and feed efficiency

Fish growth performance and feed efficiency are presented in *table III*. Reducing the amount of feed allocated to the fish caused significantly lower ($P < 0.05$) live weight gains, feed intakes and TGC compared to feeding to near-satiation regardless of rearing temperature. The voluntary feed intake and the body weight gain of the fish fed to near-satiation increased significantly ($P < 0.05$) with increasing water temperature.

Feed efficiency was in general not affected by feeding level or water temperature used. Mortality was less than 5 % for all the dietary treatments and was not significantly affected by feeding level or temperature (results not shown). The TGC did not differ between water temperature providing an indication of the suitability of this growth model.

3.2. Carcass composition

Fish carcass composition is presented in *table IV*. No significant differences between feeding levels were

Table IV. Whole body carcass composition of the fish fed the experimental diet at three feeding levels and four different water temperatures. $n = 3$ tank averages for each feeding level within temperature and for each temperature.

Water Temp.	Feeding Level	Moisture (%)	Crude protein (%)	Lipid (%)	Ash (%)	Gross energy ($kJ \cdot g^{-1}$)	Phosphorus (%)
6 °C	NS	71.0 $^{a\,x}$	15.2 $^{a\,x}$	11.0 $^{a\,y}$	2.3 $^{a\,w}$	8.1 $^{a\,y}$	0.43 $^{a\,w}$
	R1	71.4 ^{a}	15.3 ^{a}	10.4 ^{a}	2.4 ^{a}	7.9 ^{a}	0.41 ^{a}
	R2	71.6 ^{a}	15.9 ^{a}	9.3 ^{a}	2.4 ^{a}	7.7 ^{a}	0.46 ^{a}
	SEM	0.8	0.5	0.3	0.1	0.2	0.024
	HSD	3.8	2.3	1.6	0.6	1.0	0.12
9 °C	NS	70.0 $^{a\,x}$	16.1 $^{a\,w}$	11.1 $^{a\,y}$	2.4 $^{a\,w}$	8.3 $^{a\,y}$	0.45 $^{a\,w}$
	R1	70.8 ^{a}	16.1 ^{a}	10.5 ^{a}	2.3 ^{a}	8.0 ^{a}	0.44 ^{a}
	R2	71.6 ^{a}	13.3 ^{a}	10.2 ^{a}	2.3 ^{a}	7.8 ^{a}	0.44 ^{a}
	SEM	0.4	0.2	0.3	0.1	0.1	0.014
	HSD	2.2	0.8	1.6	0.3	0.6	0.072
12 °C	NS	68.4 $^{a\,y}$	16.6 $^{a\,w}$	12.2 $^{a\,x}$	2.4 $^{a\,w}$	8.9 $^{a\,x}$	0.45 $^{a\,w}$
	R1	69.7 ^{a}	16.2 ^{a}	11.1 ^{a}	2.3 ^{a}	8.3 ^{a}	0.44 ^{a}
	R2	68.4 ^{a}	17.2 ^{a}	11.6 ^{a}	2.3 ^{a}	8.7 ^{a}	0.48 ^{a}
	SEM	0.8	0.6	0.2	0.09	0.2	0.019
	HSD	3.9	3.1	1.3	0.4	0.9	0.095
15 °C	NS	67.2 $^{b\,z}$	16.5 $^{a\,w}$	13.5 $^{a\,w}$	2.3 $^{a\,w}$	9.4 $^{a\,w}$	0.43 $^{a\,w}$
	R1	68.5 ^{ab}	16.2 ^{a}	12.2 ^{b}	2.4 ^{a}	8.8 ^{b}	0.42 ^{a}
	R2	69.6 ^{a}	16.3 ^{a}	11.5 ^{b}	2.3 ^{a}	8.5 ^{b}	0.42 ^{a}
	SEM	0.3	0.2	0.2	0.03	0.1	0.011
	HSD	1.5	1.0	1.0	0.2	0.5	0.06

NS = near satiation, R1, R2 = restricted diets. SEM = standard error of mean. HSD = Tukey's honestly significant difference ($P < 0.05$). Means in the same column (within each temperature) not sharing the same superscript are statistically different ($P < 0.05$). Dry matter, crude protein, ash measured according to standard AOAC methods [2]. Lipid content determined by the Bligh and Dyer method [4] and gross energy by bomb calorimetry. The superscripts w, x, y, z are used for comparing results between temperatures at the NS feeding level ($P < 0.05$).

Table V. Nitrogen, energy, phosphorus and lipid gains and retention efficiencies (REF) of rainbow trout fed the experimental diet for 12 weeks at various feeding levels and rearing temperatures. $n = 3$ tank averages for each feeding level within temperature and for each temperature.

Water Temp.	Feeding level	Nitrogen		Energy		Phosphorus		Lipid	
		gain (g/fish)	REF (% Intake)	gain (kJ/fish)	REF (% intake)	gain (g/fish)	REF (% intake)	gain (g/fish)	REF (% intake)
6 °C	NS	0.62 ^{az}	34.2 ^{ax}	229 ^{az}	42.1 ^{ax}	0.10 ^{az}	39.6 ^{aw}	3.43 ^{az}	59.3 ^{ax}
	R1	0.51 ^{ab}	36.1 ^{a}	186 ^{b}	43.2 ^{a}	0.08 ^{a}	37.8 ^{a}	2.77 ^{b}	60.0 ^{a}
	R2	0.47 ^{b}	39.4 ^{a}	159 ^{b}	44.0 ^{a}	0.08 ^{a}	46.6 ^{a}	2.30 ^{c}	59.3 ^{a}
	SEM	0.02	1.7	5.7	1.5	0.008	3.9	0.08	2.1
	HSD	0.11	8.5	28.8	7.5	0.04	19.7	0.43	10.8
9 °C	NS	1.24 ^{ay}	40.0 ^{aw}	426 ^{ay}	45.1 ^{aw}	0.22 ^{ay}	49.6 ^{aw}	6.03 ^{ay}	60.1 ^{ax}
	R1	1.00 ^{b}	42.8 ^{a}	329 ^{b}	46.3 ^{a}	0.16 ^{b}	50.3 ^{a}	4.63 ^{b}	61.2 ^{a}
	R2	0.82 ^{c}	41.0 ^{a}	277 ^{c}	45.5 ^{a}	0.14 ^{b}	49.2 ^{a}	3.90 ^{b}	60.2 ^{a}
	SEM	0.02	0.9	9.3	1.4	0.009	2.5	0.20	2.6
	HSD	0.11	4.7	46.7	7.1	0.05	12.8	0.98	13.1
12 °C	NS	1.93 ^{ax}	39.8 ^{aw}	673 ^{ax}	45.8 ^{aw}	0.31 ^{ax}	46.8 ^{aw}	9.57 ^{ax}	61.0 ^{ax}
	R1	1.54 ^{ab}	41.6 ^{a}	519 ^{b}	46.2 ^{a}	0.25 ^{a}	48.8 ^{a}	7.20 ^{b}	60.1 ^{a}
	R2	1.41 ^{b}	45.5 ^{a}	472 ^{b}	49.7 ^{a}	0.24 ^{a}	55.2 ^{a}	6.60 ^{b}	65.1 ^{a}
	SEM	0.08	2.1	19.4	1.5	0.02	3.0	0.25	1.9
	HSD	0.41	10.4	98.0	7.6	0.08	15.4	1.24	9.4
15 °C	NS	2.58 ^{aw}	38.5 ^{aw}	956 ^{aw}	46.7 ^{aw}	0.41 ^{aw}	43.0 ^{aw}	14.1 ^{aw}	64.9 ^{aw}
	R1	1.96 ^{b}	39.6 ^{a}	692 ^{b}	46.1 ^{a}	0.31 ^{b}	44.1 ^{a}	9.9 ^{b}	62.0 ^{a}
	R2	1.71 ^{b}	40.6 ^{a}	579 ^{b}	45.4 ^{a}	0.26 ^{b}	44.7 ^{a}	8.2 ^{b}	60.3 ^{a}
	SEM	0.07	1.1	26.2	1.5	0.02	2.2	0.4	2.2
	HSD	0.33	5.5	132.1	7.5	0.09	11.2	2.1	11.1

NS = near satiation, R1, R2 = restricted diets. SEM = standard error of mean; HSD = Tukey's honestly significant difference ($P < 0.05$). Means in the same column (within each temperature) with different superscripts (a, b, c) are statistically different ($P < 0.05$). The superscripts w, x, y, z are used for comparing results between temperatures at the NS feeding level ($P < 0.05$).

observed with respect to the levels of moisture, crude protein, lipid, ash, gross energy or phosphorus at 6, 9 and 12 °C. However, at all the rearing temperatures, lipid content of the carcass tended to be higher in fish fed to near-satiation, compared to the fish fed at lower feed allocations, although this effect was only statistically significant at 15 °C. Gross energy content was not significantly affected by feeding level at 6, 9, and 12 °C. However, at 15 °C, the NS feeding level was associated with a significantly ($P < 0.05$) higher energy content compared to the reduced feed allocations. Furthermore, at 15 °C, the fish fed at NS had a significantly ($P < 0.05$) lower moisture content than those at the R2 feeding level. Water temperature of NS fish significantly ($P < 0.05$) influenced carcass lipid, gross energy and moisture. Lipid content and consequently gross energy contents increased with increasing water temperature. Conversely, the moisture content decreased with increasing water temperature. Crude protein content of the fish reared at 6 °C was significantly lower ($P < 0.05$) than that of fish reared at higher temperatures.

3.3. Energy and nutrient gains and retention efficiencies

Nitrogen, energy, and lipid gains were significantly higher ($P < 0.05$) for NS fish at all the rearing temperatures compared to the fish fed at lower feed allocations (*table V*). On the other hand, nitrogen, energy and lipid retention efficiencies (% total intake) were not influenced by feeding levels or temperatures. Phosphorus gains and retention efficiency (% of P intake) were not significantly affected by feeding level ($P > 0.05$) at the water temperatures of 6 and 12 °C. However, higher phosphorus gains were observed for the NS fish at 9 and 15 °C. Nitrogen, energy and phosphorus retention efficiencies (% digestible intake) were not significantly ($P < 0.05$) affected by feeding level at all the water temperatures (*table VI*). The NS fish showed a significant increase ($P < 0.05$) in energy and nutrient gains with increasing water temperature (*table V*). Nitrogen and energy retention efficiencies (% gross intake) were significantly lower at 6 °C. However, the digestible nitrogen and digestible energy retention efficiencies were higher at 9 °C compared with the other water temperatures (*table VI*). Lipid retention efficiencies (% gross intake) were significantly higher at 15 °C compared with the other water temperatures.

3.4. Apparent digestibility

The apparent digestibility coefficients of dry matter, crude protein and gross energy increased significantly ($P < 0.05$) with an increase in water temperature (*table VII*). Some differences were observed between the apparent digestibility of phosphorus at the various temperatures ($P < 0.05$).

Table VI. Nitrogen, energy and phosphorus retention efficiencies (REF) as percentage of their digestible intakes of rainbow trout fed the experimental diet for 12 weeks at various feeding levels and rearing temperatures. $n = 3$ tank averages for each feeding level within temperature and for each temperature.

Water Temp.	Feeding Level	Nitrogen REF (% DNI)	Energy REF (% DEI)	Phosphorus REF (% DPI)
6 °C	NS	42.3 $^{a\,x}$	56.9 $^{a\,x}$	63.9 $^{a\,w}$
	R1	44.5 ^{a}	58.4 ^{a}	61.0 ^{a}
	R2	48.7 ^{a}	59.4 ^{a}	75.2 ^{a}
	SEM	2.1	2.0	6.3
	HSD	10.6	10.1	31.7
9 °C	NS	47.1 $^{a\,w}$	62.6 $^{a\,w}$	72.9 $^{a\,w}$
	R1	50.3 ^{a}	64.4 ^{a}	74.0 ^{a}
	R2	48.3 ^{a}	63.3 ^{a}	72.3 ^{a}
	SEM	1.1	1.9	3.7
	HSD	5.4	9.7	18.8
12 °C	NS	46.3 $^{a\,wx}$	57.2 $^{a\,x}$	69.8 $^{a\,w}$
	R1	48.3 ^{a}	57.7 ^{a}	72.8 ^{a}
	R2	52.9 ^{a}	62.2 ^{a}	82.3 ^{a}
	SEM	2.4	1.9	4.6
	HSD	12.2	9.5	22.9
15 °C	NS	43.3 $^{a\,wx}$	55.0 $^{a\,x}$	68.3 $^{a\,w}$
	R1	44.5 ^{a}	54.2 ^{a}	70.1 ^{a}
	R2	45.6 ^{a}	53.5 ^{a}	70.9 ^{a}
	SEM	1.2	1.8	3.5
	HSD	6.1	8.8	17.7

NS = near satiation, SEM = standard error of mean; R1, R2 = restricted diets. DNI = digestible nitrogen intake, DEI = digestible energy intake, DPI = digestible phosphorus intake. HSD = Tukey's honestly significant difference ($P < 0.05$). Means in the same column (within each temperature) with different superscripts (a, b, c) are statistically different ($P < 0.05$). The superscripts w, x, y, z are used for comparing results between temperatures at the NS feeding level ($P < 0.05$).

Table VII. Apparent digestibility coefficients (ADC) of the experimental diet calculated from samples of two collection periods ($n = 6$ faecal samples for each water temperature).

Water temp.	Dry matter (%)	Crude protein (%)	Phosphorus (%)	Gross energy ($kJ \cdot g^{-1}$)
6 °C	73 ^{d}	81 ^{c}	62 ^{b}	74 ^{d}
9 °C	77 ^{c}	85 ^{b}	68 ^{a}	78 ^{c}
12 °C	79 ^{b}	86 ^{b}	67 ^{a}	81 ^{b}
15 °C	82 ^{a}	89 ^{a}	63 ^{b}	85 ^{a}
SEM	0.5	0.5	0.5	0.5
HSD	2.2	2.4	2.2	2.3

SEM = standard error of mean ; HSD = Tukey's honestly significant difference ($P < 0.05$). Within the same column, values not sharing a common superscript are significantly different.

3.5. Waste outputs

Feeding level did not influence total solid wastes, solid and dissolved nitrogen waste and solid and dissolved phosphorus wastes (g/kg fish produced) at 6, 9 or 15 °C (*table VIII*). At 12 °C, slightly higher total solid wastes and solid nitrogen wastes were obtained at the NS feeding level.

The comparison of waste outputs between temperatures for NS fish shows that higher temperatures were associated with lower total solid wastes, and solid nitrogen waste. At the NS feeding levels, dissolved nitrogen waste was significantly higher ($P < 0.05$) at 15 vs 9 °C. Solid phosphorus waste was lower at 9 and 12 °C than at 6 and 15 °C. Water temperature did not significantly affect dissolved phosphorus waste at this feeding level.

3.6. Efficiency of metabolizable energy utilization for growth

Figure 2 shows the relationship between recovered energy (RE) and the metabolizable energy intake above basal metabolism (ME intake – HeE) both expressed as kJ per kg metabolic body weight. A highly significant linear regression ($R^2 = 0.96$) was observed between (ME intake – HeE) and RE and no quadratic effect was observed. Water temperature had no significant effect on this regression as the interaction of the effects of (ME intake – HeE) vs RE and

Table VIII. Total solids, nitrogen and phosphorus wastes by rainbow trout fed the experimental diet for 12 weeks (g/kg fish produced). n = 3 tank averages for each feeding level within temperature and for each temperature.

Water temp.	Feeding level	Total solid wastes	Nitrogen		Phosphorus	
			solid	dissolved	solid	dissolved
6 °C	NS	245 [a w]	13.7 [a w]	33.8 [a wx]	3.8 [a w]	2.3 [a w]
	R1	234 [a]	13.2 a	31.1 [a]	3.7 [a]	2.4 [a]
	R2	229 [a]	12.9 [a]	29.2 [a]	3.6 [a]	1.4 [a]
	SEM	4.5	0.2	1.2	0.08	0.37
	HSD	22.6	1.2	6.1	0.39	1.86
9 °C	NS	182 [a x]	10.5 [a x]	29.5 [a x]	2.9 [a x]	1.7 [a w]
	R1	172 [a]	9.9 [a]	26.2 [a]	2.8 [a]	1.5 [a]
	R2	171 [a]	9.9 [a]	27.2 [a]	2.7 [a]	1.6 [a]
	SEM	2.8	0.2	1.0	0.05	0.2
	HSD	13.9	0.8	5.0	0.2	1.2
12 °C	NS	172 [a x]	9.5 [a y]	31.5 [a wx]	3.1 [a x]	1.9 [a w]
	R1	161 [ab]	8.9 [ab]	28.2 [a]	2.9 [a]	1.6 [a]
	R2	159 [b]	8.8 [b]	25.4 [a]	2.9 [a]	1.0 [a]
	SEM	2.5	0.1	1.6	0.05	0.3
	HSD	12.5	0.7	8.1	0.26	1.4
15 °C	NS	151 [a y]	7.7 [a z]	35.2 [a w]	3.6 [a w]	1.9 [a w]
	R1	145 [a]	7.3 [a]	33.0 [a]	3.5 [a]	1.8 [a]
	R2	143 [a]	7.2 [a]	31.9 [a]	3.4 [a]	1.7 [a]
	SEM	2.8	0.1	1.3	0.07	0.2
	HSD	14.1	0.7	6.5	0.36	1.2

NS = near satiation, R1, R2 = restricted diets. SEM = standard error of mean. HSD = Tukey's honestly significant difference ($P < 0.05$). Means in the same column (within each temperature) not sharing the same superscripts (a, b, c) are different ($P < 0.05$). The superscripts w, x, y, z are used for comparing results between temperatures at the NS feeding level ($P < 0.05$).

temperature was not significant ($P = 0.58$). The regression coefficient of the regression across temperatures, i.e. the efficiency of ME utilization for growth (K_{pf}), was 0.61. The relationship between RE and ME, both as kJ/kg MBW was also linear ($R^2 = 0.98$, data not shown), indicating no temperature dependence and also supporting the fact the HeE calculated was a good estimation of the basal metabolism at all the water temperatures used. A constant ratio was observed between the energy recovered as lipid and the energy recovered as protein (*figure 3*) and this did not depend on the ME intake or the water temperature. For every kJ of energy

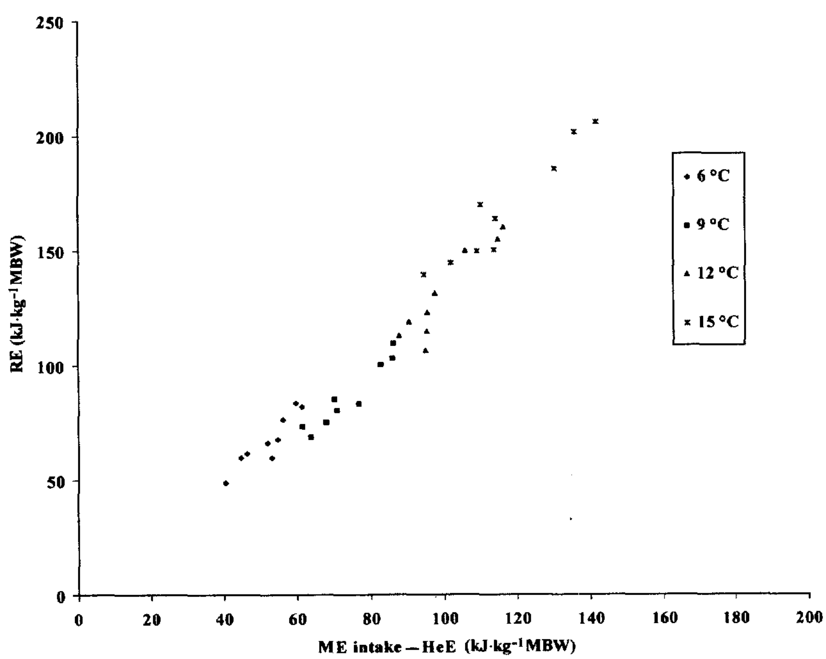
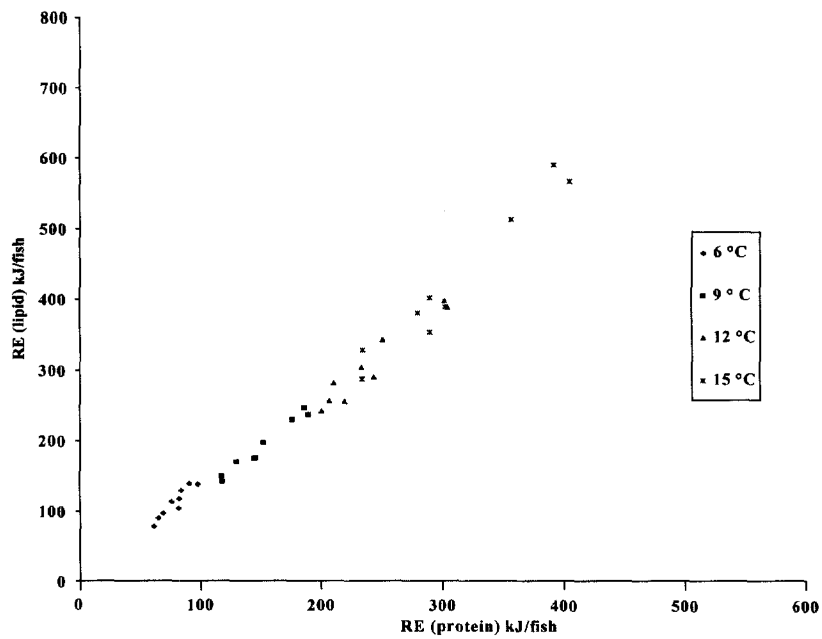


Figure 2. The relationship between recovered energy (RE) and ME intake above basal metabolism (HeE) at water temperatures of 6, 9, 12, and 15 °C is:
$y = 0.61x + 16.61$, $P < 0.0001$, $R^2 = 0.96$,
n = 36 tank averages (experimental units)
where y = RE ($kJ \cdot kg^{-1}$ MBW), x = ME intake – HeE ($kJ \cdot kg^{-1}$ MBW), MBW = metabolic body weight ($kg^{0.824}$), RE is recovered energy, ME is metabolizable energy intake and HeE is basal metabolism.

Figure 3. The relationship between energy retention as lipid on retained energy as protein at water temperatures of 6, 9, 12, and 15 °C is:
$y = 1.39x - 11.40$, $P < 0.0001$, $R^2 = 0.98$,
$n = 36$ tank averages (experimental units)
where y = RE (lipid) (kJ/fish), x = RE (protein) (kJ/fish) and RE is recovered energy.



retained as protein, 1.39 kJ of energy was deposited as lipid.

4. DISCUSSION

Feeding levels employed in this study (see *figure 1*) had little impact on feed efficiency regardless of water temperature. These results are in agreement with a study involving Atlantic salmon [33] which suggested that there was no difference in feed efficiency of fish fed at 50, 75 or 100 % of the ration required for maximum growth. The results are also in agreement with another study [1] which showed that feed restriction (achieved through a decrease in the reward level with a demand feeder) had no affect on the feed efficiency of rainbow trout. These results are, however, contrary to the results of a number of studies [7, 20, 34] which suggested that maximum feed efficiency was obtained only at feeding levels below that required for maximum growth. The results from the present study are also in contradiction to a recent hypothesis suggesting that maximum feed efficiency is attained at maximum feed intake [19]. Water temperature had very little effect on feed efficiency and this is in agreement with the results of the study by Alanara [1] which showed no difference in the feed efficiency of rainbow trout reared at 5 or 15 °C and fed using demand feeders.

The significant decrease in the apparent digestibility coefficient of dry matter, nitrogen and energy with decreasing temperature in the present study is in agreement with the results of Choubert et al. [17] who observed a significant increase of apparent digestibility of dry matter, nitrogen and energy for trout when water temperature increased from 10 to 18 °C. It is also in agreement with results from a recent study in our laboratory (Harris et al., unpubl. data) which showed a significant decrease of protein and energy digestibility with decreasing water temperature from 15 to 7.5 °C. Results from another laboratory using a different faeces collecting system [36, 37] also suggest that apparent digestibility of nitrogen and energy by rainbow trout increase with increasing water temperature (from 5 to 15 °C). These observations are in disagreement with the results of Cho and Kaushik [11] who did not observe any effect of temperature on apparent digestibility of protein, lipid and energy for rainbow trout between 9 and 18 °C. The comparisons between results on the pattern of ADCs of nutrients and energy with changes in water temperature must be interpreted carefully. Different results from the literature on this subject could have been caused by different dietary compositions of the experimental diets, mainly different amounts of undigestible or low digestible components, such as raw starch. Also the amount of anti-nutritional factors or the technological processes used in the processing of ingredients or the faeces collection method used could also have caused these differences. The reduction in apparent digestibility with decreasing water temperature in this study could be due, in part, to experimental (methodological) errors. Such errors are associated with differences in feed intake and consequently faeces production between the fish at the four different water temperatures. Other factors, such as the reduction in activity of digestive enzymes at low temperature or progressively greater endogenous gut losses proportionally per g of diet could also be responsible for the decrease in apparent digestibility with the decrease in water temperature. It seems plausible that the effect of water temperature on apparent digestibility is the result of an effect of feed intake rather than an effect of water temperature itself. Feed intake of fish decreased significantly with a decrease in temperature. At low feed intake, endogenous gut losses are believed to represent a greater proportion of faecal waste. This effect would result in a decrease in the apparent but not the 'true' digestibility of the diet. This hypothesis requires further investigation.

Increasing dry matter and lipid content of Atlantic salmon fingerling with increasing feed intake has previously been reported [33]. The results from this study on protein and lipid deposition clearly show that this increase in lipid content of fish at higher feeding level or higher growth rate is not due to enhanced deposition of lipid compared to that of protein. Rather, it is presumably a result of the low lipid and relatively high protein contents of the carcass of small fish. Proportionally constant protein and lipid depositions will result in little change in the protein content (expressed as %) of the fish carcass and very significant increase in the lipid content [31]. Similar proportionality of protein and lipid deposition at increasing feed intakes has been reported in domestic animals [5, 6].

Total solids, solid and dissolved nitrogen and phosphorus waste outputs (g/kg fish produced) were little affected by feeding level. These results contradict a published simulation model [19]. This simulation model predicted that feed restriction would result in significant increase in N and P waste outputs compared to feeding to near-satiation [19]. Incorrect assumptions used by the simulation model, such as a decrease in the feed efficiency with decreasing ration and constant body composition of fish regardless of body size explain the discrepancy between the results from the simulation model [19] and the findings from the present study.

Water temperature had a significant effect on total and nitrogen solid waste outputs because of a decrease in the apparent digestibility of dry matter and nitrogen with a decrease in temperature and feed intake. Dissolved nitrogen and phosphorus waste outputs were, in most cases, not affected by feeding level or by water temperature. These results are in accordance with the results of a study with brook trout (*Salvelinus fontinalis*) [27] who did not show a significant effect of temperature on the metabolism of dietary phosphorus and the results from other studies [22, 24, 35] who showed that water temperature did not significantly affect the dissolved nitrogen waste output per kg weight gain of rainbow trout.

The utilization of ME for gain (K_{pf}) was 0.61. This value is very similar to that found in common carp (*Cyprinus carpio* L.) [30] for which a K_{pf} between 0.60 and 0.80 was observed. Using multiple regression, partial efficiencies of ME utilization for protein deposition (K_p) of 0.56 and lipid deposition (K_f) of 0.72 were calculated for common carp [30]. This K_p value is significantly higher than that observed in mammals [21, 28]. In the present study, it was not possible to use multiple regression to calculate partial efficiency for protein and lipid deposition since protein-energy and lipid-energy deposition were highly correlated. Accurate estimation of K_p and K_f may require the use of diets with different protein and lipid contents in an attempt to achieve differences in protein and lipid deposition rates.

Increasing water temperature from 6 to 15 °C increased the energy retention efficiency from 42 to 47 % of IE for fish fed to near-satiation in this study. This is in agreement with the results from a number of studies [10, 12, 13] who reported that increasing the water temperature from 7.5 to 20 °C increased energy retention efficiency. In those studies, this increase in gross energy retention efficiency was attributed to a decrease in the energy losses as HiE (% GE intake). Increasingly higher HiE (based on g oxygen consumption/kg biomass per day) were measured for rainbow trout as water temperature increased from 7.5 to 15 °C [12]. In the present study, the decrease in gross energy retention efficiency at lower temperatures appears to be related to a decrease in apparent digestibility of dietary energy rather than to an increase in HiE since efficiency of ME utilization for growth (and probably the DE utilization for growth) was not affected by water temperature. No attempt was made in the present study to directly estimate HiE. Whether the increase in gross energy retention efficiency with increasing temperature is related to an improvement of apparent digestibility of energy or a reduction of HiE remains unclear and deserves further study.

5. CONCLUSION

Decreasing feeding level and water temperature resulted in a lower body weight gain. Feed efficiency, carcass composition and nitrogen and energy retention efficiencies were, in most cases, unaffected by the feeding level. Increasing water temperature resulted in an increase in the apparent digestibility of dry matter, nitrogen and energy of the diet. The gross energy retention efficiency was significantly lower at 6 °C compared with other temperatures. The increase in digestibility of dry matter and nitrogen with increasing temperature resulted in lower total solid and solid nitrogen waste outputs per kg fish produced at higher water temperatures. Dissolved N and P waste outputs (g/kg fish produced) were, in almost every case, not affected by feeding level or water temperatures. The efficiency of ME utilization for growth (K_{pf}) was 0.61 and this coefficient was not affected by feeding level or water temperature.

Fish consuming more feed either as a result of an increase in water temperature or as a result of increasing feed allocation grew faster and appeared to utilize digestible nutrients with similar efficiencies, at least within the range of feed restriction and water temperature used in this study.

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