Aquat. Living Resour. 13 (2) (2000) 107–113

S0990744000001479/FLA

Effect of feeding time on digestibility, growth performance and protein metabolism in the rainbow trout *Oncorhynchus mykiss:* interactions with dietary fat levels

Valérie Bolliet[(a)], Chonlatee Cheewasedtham[(b)], Dominic Houlihan[(b)], Anne Gélineau[(a)], Thierry Boujard[(a*)]

[(a)] *Équipe nutrition, aquaculture et environnement, unité mixte Inra/Ifremer de nutrition des poissons, station d'hydrobiologie Inra, BP 3, 64310 Saint-Pée-sur-Nivelle, France*

[(b)] *Department of Zoology, University of Aberdeen, Tyllidrone avenue, AB9 2TN Aberdeen, Scotland, UK*

Received 6 December 1999; accepted 20 January 2000

Abstract — The effect of feeding time was studied in rainbow trout fed different dietary levels of fat. Fish were fed either 1 h after light on in the morning or 1 h after light off in the evening with a low energy diet (LE, 6 % lipid) or a high energy diet (HE, 23 % lipid). Regardless of the diet, apparent digestibility and post-prandial protein synthesis were higher in fish fed in the morning than in those fed at the beginning of the night. In fish fed the LE diet in the morning, growth performance and nutrient retention efficiency tended to be higher than in those fed at the beginning of the night. In contrast, fish fed the HE diet in the morning had lower protein growth rate, protein content and protein retention efficiency than those fed in the evening. These results suggest that protein metabolism might be involved in the effect of feeding time on growth and that there is an interaction between the time of feeding and dietary level of fat on growth.

Aquaculture / dietary fat level / feeding time / digestibility / protein metabolism

Résumé — Effet de l'heure d'alimentation sur la digestibilité, les performances de croissance et le métabolisme protéique chez la truite arc-en-ciel *Oncorhynchus mykiss :* interactions avec le taux de lipides alimentaires. L'effet de l'heure d'alimentation a été étudié chez la truite arc-en-ciel nourrie à différents taux de lipides alimentaires. Les poissons étaient alimentés soit le matin une heure après le début de la phase d'éclairement, soit le soir une heure après l'extinction de la lumière. Deux aliments étaient utilisés, l'un avait un taux d'énergie bas (LE, 6 % de lipides), l'autre un taux d'énergie élevé (HE, 23 % de lipides). Indépendamment de l'aliment, le coefficient de digestibilité apparente et le taux de synthèse protéique post-prandial était plus élevé chez les poissons nourris le matin que chez ceux nourris le soir. Chez les poissons nourris avec l'aliment LE le matin, la croissance et la rétention des nutriments avaient tendance a être plus élevés que chez ceux nourris en début de nuit. Inversement, les poissons nourris avec l'aliment HE le matin avaient une plus faible croissance protéique, un plus faible taux protéique corporel final et une plus faible efficacité de rétention protéique que ceux nourris le matin. Ces résultats suggèrent que le métabolisme protéique est impliqué dans l'effet de l'heure d'alimentation sur la croissance, et qu'il y a une interaction entre l'heure d'alimentation et le taux de lipides alimentaires sur la croissance.

Aquaculture / taux de lipides alimentaires / heure d'alimentation / digestibilité / métabolisme protéique

1. INTRODUCTION

A number of studies have demonstrated that feeding time affects growth performance in fish [1, 3, 7]. It has been suggested that the optimal feeding time to promote growth might correspond to the natural daily peak of feeding activity in any particular species. Feeding in the rainbow trout, *Oncorhynchus mykiss*, mostly occurs in the light phase with a main peak of feeding activity at dawn [5, 6, 29]. In this species,

* Corresponding author: boujard@st-pee.inra.fr

studies carried out during winter–spring showed that fish fed at dawn had higher weight gain than those fed at midnight [9, 16].

Mechanisms involved in this phenomenon are still unclear. The effect of feeding time on growth has been attributed to different feed intakes and/or different feed conversion efficiency [2, 9, 10, 16, 22, 25, 26]. An increase in conversion efficiencies with respect to feeding time might be due to different processes including better nutrient absorption efficiency, a protein sparing effect due to better utilisation of non-protein energy, or an increase in protein synthesis. Boujard and co-workers [9] demonstrated that rainbow trout fed at midnight needed to eat more than fish fed at dawn to reach a similar final body weight. Fish fed at midnight had a 9 % higher lipid content and a 16 % poorer protein retention than those fed at dawn. In studies performed on the same species, a higher post-prandial RNA concentration in the liver expressed as the RNA to DNA ratio and lower ammonia excretion were reported in fish fed at dawn when compared to those fed at midnight [16, 17]. Taken together, these experiments support the theory that protein metabolism is involved with the effect of feeding time on growth.

The proportion of protein absorbed compared with that retained as growth varies with the composition of the diet: an increase in dietary level of fat leads to an increase in energy retention and an improvement in protein utilisation for growth [11, 21, 28]. This protein sparing effect of lipids results from an increasing contribution of non-protein energy sources to full energy consumption. The effect of feeding time on the protein sparing effect has never been investigated in fish.

The aim of the current experiment was to investigate the effect of feeding time and the interactions between feeding time and dietary fat level on the apparent digestibility of the nutrients, growth performance, nutrient retention and protein synthesis in rainbow trout. For this purpose, fish were fed either a low energy diet (LE, 6 % lipid) or a high energy diet (HE, 23 % lipid) at two feeding times: 1 h after the onset of light or 1 h after light off. Protein synthesis rates were determined just before and 4 h after feeding.

2. MATERIALS AND METHODS

2.1. Feed preparation and digestibility

Experimental diets were formulated to contain a constant protein level and different proportions of starch and fish oil. A sample of each diet was analysed following usual procedures: dry matter (110 °C for 24 h), protein after acid digestion (Kjeldahl N × 6.25), lipid extraction by petroleum ether in a Soxhlet apparatus after acid hydrolysis, energy using a Gallenkamp adiabatic calorimeter. The ingredients and chemical composition of the diets are summarised in *table I*.

Table I. Ingredients and chemical composition of the experimental diets HE (high energy) and LE (low energy).

Ingredients ($g \cdot kg^{-1}$)	HE	LE
Norvegian herring meal	572	572
Gelatinised starch (wheat)	202	136
Crude starch (wheat)	0	237
Fish oil	186	15
Mineral mix	10	10
Vitamine mix	10	10
Na-alginate	10	10
Chemical composition		
Dry matter (%)	95.4	93.4
Protein (N × 6.25) (% DM*)	44.4	44.5
Fat (% DM*)	22.8	6.5
Gross energy (kJ·g DM*)	23.5	19.5

* DM = dry matter.

Digestibility trials were performed in February on groups of ten rainbow trout (three replicates per treatment, mean individual weight = approximately 100 g) obtained from the Inra experimental fish farm at Donzacq (Landes, France). Groups of fish were stocked in cylindro-conical tanks (60-L capacity with a flow rate of $5\ L \cdot min^{-1}$) supplied with recycled fresh water (temperature, 8 °C).

During 1 week prior to the experiment, trout were adapted to a 10L/14D photoperiod and fed either the high energy (HE) diet or the low energy (LE) diet. Then, fish were fed once a day, either at 09:00 hours (1 h after light onset) or 19:00 hours (1 h after light off) with one of the two experimental diets containing 1 % of chromic oxide as an inert tracer. Faeces were collected and frozen (–20 °C) every day over a 2-week period using a continuous automatic faeces collector [13]. Pooled faeces from each group of fish were freeze-dried prior to analysis of chromic oxide, using the method of Bolin and co-workers [4]. Dry matter, protein, lipid and energy of the faeces were determined as described for the diets. The apparent digestibility coefficients (ADC) of the diets were calculated according to Maynard and Loosly [23]:

$$\text{ADC of dry matter of diet (\%)} = 100 \times [1 - (\text{dietary } Cr_2O_3 \times \text{faecal } Cr_2O_3^{-1})]$$

$$\text{ADC of nutrients and energy of diet (\%)} = 100 \times [1 - (\text{dietary } Cr_2O_3 \times \text{faecal } Cr_2O_3^{-1}) \times (\text{faecal nutrient or energy level} \times \text{dietary nutrient or energy level}^{-1})]$$

and all values were expressed per unit of dry matter.

2.2. Growth experiment

Rainbow trout were obtained from the same experimental fish farm and originated from the same parental

stock as those used in the digestibility trial. After 2 weeks of acclimation, and 1 day without food, fish were anaesthetised, individually weighed and randomly selected to form 12 groups of 30 fish (three replicates per treatment, mean individual weight = 25 ± 7 g). Five fish were killed and frozen (–20 °C) for the estimation of initial whole-body proximate composition.

The experiment was carried out between December and February (61 days). Each group was maintained in 100-L tanks supplied with river water. The artificial light/dark cycle was 10L/14D (light onset at 08:00 hours) and the water temperature fluctuated between 7 and 12 °C throughout the experiment. Dim red light was used during all periods of darkness.

Groups of fish were fed by hand once a day with one of the two experimental diets either 1 h after light on or 1 h after light off. The tanks were designed so that uneaten pellets were drained off within minutes and collected on a filter. Those pellets were counted every day to calculate the total feed consumption per tank using a mean dry pellet weight. The amount of feed distributed to each tank was adjusted daily by taking, as a reference, the tank showing the lowest consumption on the previous day in order to keep the cumulative feed intake identical in all tanks.

At the end of the trial, fish were individually weighed after 1 day of fasting. Five fish per tank were subsequently killed and frozen for estimation of final whole-body composition. Dry matter, protein, lipid and energy were determined as described for the diets.

Growth performance, body composition and food utilisation were described using the following parameters:

$$\text{feed gain ratio (FGR, dry weight} \cdot \text{dry weight}^{-1}) = \text{feed consumption} \times \text{weight gain}^{-1}$$

$$\text{specific growth rate (SGR)} = 100 \times (\ln(\text{Wf}) - \ln(\text{Wi})) \times \text{days}^{-1}$$

$$\text{protein growth rate (PGR)} = 100 \times (\ln(\text{Wf} \times \text{final protein content per tank}) - \ln(\text{Wi} \times \text{initial protein content}) \times \text{day}^{-1})$$

where Wi is the mean initial weight per tank and Wf is the mean final weight per tank.

$$\text{Protein or energy retention efficiency} = 100 \times ((\text{Wf} \times \%\text{ nutrient of final whole body}) - (\text{Wi} \times \%\text{ nutrient of initial whole body})) \times \text{intake of digestible nutrient}^{-1} \text{ or digestible energy}^{-1}$$

where Wi is the mean initial protein content, and growth heterogeneity was assessed from the ratio between the coefficient of variation of the final and initial distributions of fish body weight (CWf and CWi).

2.3. Protein synthesis

The measurement of fractional rate of protein synthesis was determined both in white muscle and whole body tissues from the incorporation of radioactive phenylalanine using the method described by Garlick and colleagues [15] and detailed in Houlihan and co-workers [19]. The fractional rate of protein synthesis (proportion of total protein synthesis per day) was determined after a single injection of ^{3}H phenylalanine. On the day of injection, six fish from each treatment were randomly selected and injected intraperitoneally at a dose of 1 mL × 100 g^{-1} fish body weight just before feeding time. The same procedure was repeated 4 h after feeding time on nine other fish randomly selected from each treatment. After injection, fish were returned to their tanks and then killed after intervals between 43 and 101 min. The fish were killed by a blow to the head and transection of the spinal cord. White muscle samples of approximately 200–300 mg were taken from the epaxial muscle below the dorsal fin. Both whole bodies and white muscle were frozen in liquid nitrogen and stored at –70 °C until analysis as described by Houlihan and colleagues [20]. Whole body frozen samples were prepared by homogenising the individual bodies in 0.2 M perchloric acid and after centrifugation, 100 mg samples were analysed. Fractional rates of protein synthesis (k_s; %·day^{-1}) were calculated as:

$$k_s = [(S_b \cdot S_a^{-1}) \times (1440 \cdot t^{-1})] \times 100$$

where S_b is the protein-bound phenylalanine-specific radioactivity at time t and S_a is the mean free-pool phenylalanine-specific radioactivity.

2.4. Statistics

Data were analysed by two-way analysis of variance (ANOVA) using GraphPad™ software. Treatment differences were considered significant at $P < 0.05$. If significant, a Bonferroni post-test was applied to test for differences between means. All results are presented as mean ± SD except when stated.

3. RESULTS

3.1. Digestibility

There was no interaction between feeding time and diet on ADC (*table II*). The ADC of the main dietary nutrients and energy tended to be higher in fish fed the HE diet than in those fed the LE diet. However, that trend was significant only for protein and energy. Regardless of the diet, there was a significant effect of feeding time on apparent digestibility of the different

Table II. Apparent digestibility coefficient (ADC) of the experimental diets high and low energy.

	Protein	Energy	Lipid	Dry matter
High energy				
09:00 hours	88.0 ± 0.2	87.0 ± 1.7	88.0 ± 2.9	80.3 ± 1.0
19:00 hours	87.1 ± 0.6	84.2 ± 2.2	83.4 ± 3.2	77.9 ± 1.6
Low energy				
09:00 hours	85.6 ± 0.7	74.6 ± 0.8	86.0 ± 1.7	66.3 ± 0.8
19:00 hours	84.1 ± 1.4	71.8 ± 2.0	80.5 ± 4.6	63.9 ± 1.6
P values				
Interaction	ns	ns	ns	ns
Diet	$P < 0.001$	$P < 0.001$	ns	$P < 0.001$
Feeding time	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

nutrients, ADC being higher in trout fed at 09:00 hours than in those fed at 19:00 hours.

3.2. Growth rates

Mortality was no more than one fish per tank. Fish fed the HE diet had higher weight gain and SGR and a better feed gain ratio than those fed the LE diet (*table III*). There was a significant interaction between feeding time and diet on feed gain ratio, protein growth rate and heterogeneity. In fish fed the HE diet, protein growth rate was significantly higher when fed at 19:00 hours than when fed at 09:00 hours. The highest growth heterogeneity was observed in fish fed at 09:00 hours. Fish fed the LE diet at 09:00 hours had a significantly higher feed gain ratio and PGR than those fed at 19:00 hours (Bonferroni post test, $P < 0.05$).

Regardless of feeding time, protein content was higher and energy and lipid contents were lower in fish fed the LE diet than in those fed the HE diet (*table III*). There was no significant effect of feeding time on proximate body composition of fish fed the LE diet. In contrast, fish fed the HE diet at 09:00 hours had lower protein content than those fed at 19:00 hours. Fish fed both diets at 09:00 hours had higher energy retention than those fed at 19:00 hours. An interaction between feeding time and the diet on protein retention efficiency was observed, the highest value being observed in fish fed at 19:00 hours with the HE diet, whereas fish fed the LE diet at the same time showed the lowest protein retention.

3.3. Protein synthesis

There was a significant linear relationship between fractional rate of protein synthesis (k_s) of the whole body and white muscle for all the data ($P < 0.001$, $r = 0.915$, $n = 60$). This relationship (*figure 1*) can be described by the following equation:

$$Y = 0.257 + 0.940\ (X)$$

where Y is the fractional rate of protein synthesis of white muscle and X is the fractional rate of protein synthesis of whole body tissue. In addition, the mean rate of protein synthesis between whole body

Table III. Feed intake, growth performance whole-body proximate composition and nutrient retention efficiencies in trout fed the high and the energy diets at different times. Nutrient retention efficiency was calculated as a percentage of digestible intake.

	Feed intake (g)	Weight gain (g)	Feed gain ratio	SGR	PGR	CWf/CWi
High energy						
09:00 hours	41.7 ± 1.7	52.3 ± 2.5	0.80 ± 0.02	1.55 ± 0.03	1.74 ± 0.04	1.85 ± 0.31
19:00 hours	41.3 ± 0.5	52.6 ± 0.9	0.79 ± 0.02	1.56 ± 0.03	1.86 ± 0.03	1.10 ± 0.09
Low energy						
09:00 hours	41.3 ± 0.7	43.0 ± 0.4	0.96 ± 0.01	1.36 ± 0.01	1.72 ± 0.02	1.28 ± 0.11
19:00 hours	40.6 ± 1.3	40.2 ± 1.1	1.01 ± 0.01	1.31 ± 0.01	1.68 ± 0.04	1.09 ± 0.17
P values						
Interaction	ns	ns	$P < 0.05$	ns	ns	$P < 0.05$
Diet	ns	$P < 0.001$	$P < 0.001$	$P < 0.001$	ns	$P < 0.05$
Feeding time	ns	ns	ns	ns	ns	$P < 0.005$
	DM (%)	Protein (% DM)	Energy (% DM)	Lipid (% DM)	Protein retention	Energy retention
High energy						
09:00 hours	28.7 ± 0.5	55.9 ± 0.9	27.0 ± 1.0	34.9 ± 2.1	51.5 ± 1.7	59.4 ± 3.1
19:00 hours	28.2 ± 0.5	59.9 ± 0.3	26.0 ± 0.7	32.4 ± 2.1	57.6 ± 1.6	57.6 ± 1.6
Low energy						
09:00 hours	24.0 ± 0.4	74.5 ± 1.1	23.6 ± 0.2	12.2 ± 1.0	52.0 ± 0.8	44.3 ± 2.0
19:00 hours	24.2 ± 0.8	74.1 ± 2.1	23.7 ± 0.3	12.7 ± 1.7	50.9 ± 2.3	38.8 ± 3.1
P values						
Interaction	ns	$P < 0.05$	ns	ns	$P < 0.01$	ns
Diet	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.001$
Feeding time	ns	$P < 0.05$	ns	ns	$P < 0.05$	$P < 0.05$

PGR: protein growth rate; CWf/CWi: ratio between the coefficient of variation of fish body weight.

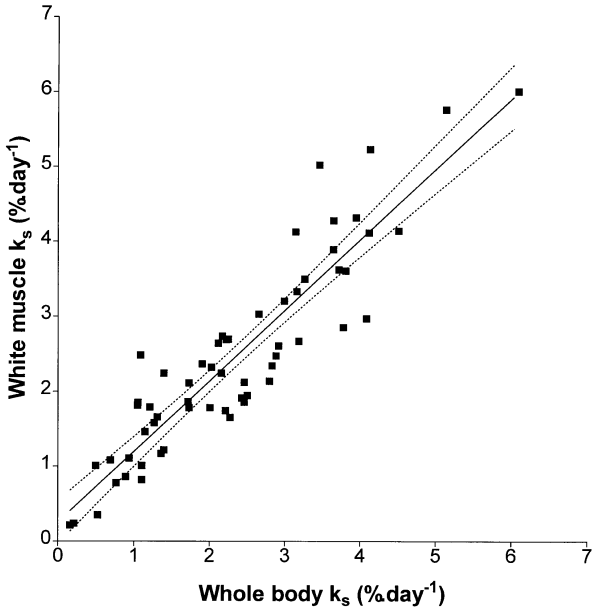

Figure 1. Fractional whole body rates of protein synthesis (K_s, %·day^{-1}) and whole muscle fractional rates of protein synthesis (K_s, %·day^{-1}) for all rainbow trout in the experiment. The solid line is from the regression equation line in the text and the dotted lines are the confidence limit of the regression line.

(2.32 ± 0.17 %·day^{-1}) and white muscle (2.44 ± 0.17 %·day^{-1}) were not statistically different (*t*-test, $P > 0.05$). It was therefore decided to use the fractional rate of protein synthesis of the white muscle to represent protein metabolism of the fish.

Fractional rate of white muscle protein synthesis of fish just before feeding time was not significantly different between treatments (*figure 2a*). However, fish fed high energy diet had a higher fractional protein synthesis rate than those fed the low energy diet after 4 h (*figure 2b*). Regardless of the diet, fish fed at 09:00 hours had a higher rate of protein synthesis that those fed at 19:00 hours.

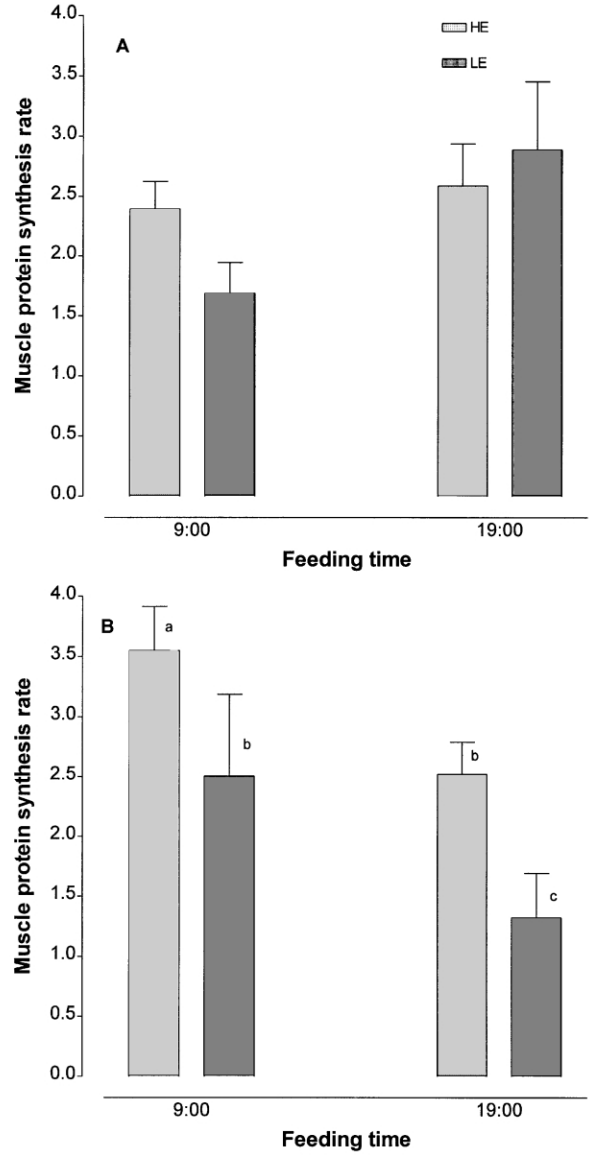

Figure 2. Muscle protein synthesis rate in fish fed at 09:00 hours or 19:00 hours with either the LE (low energy) diet or the HE (high energy) diet: **A)** just before feeding time; **B)** 4 h after feeding time. Data are shown as mean + SEM.

4. DISCUSSION

To the best of our knowledge, the present study provides the first evidence for an effect of feeding time on the apparent digestibility of the main dietary nutrients. Fish fed in the morning had a higher digestibility than those fed 1 h after light off. ADC values were lower than those previously reported for the same diets [8] possibly because of the lower temperature used in the present experiment. The other major result is that fish fed the HE diet showed a better SGR but not a better PGR than those fed the LE diet. The improvement in SGR was due to an increase in fat content. However, it must be remembered that the amount of feed distributed to each tank was calculated by taking, as a reference, the tank showing the lowest consumption rate.

An interaction between feeding time and dietary fat level was observed on feed gain ratio, growth heterogeneity, protein growth rate, protein content and protein retention. Fish fed the LE diet at 09:00 hours tended to have a better growth performance and had a better nutrient retention efficiency than those fed at 19:00 hours. This is consistent with previous findings showing that trout fed at dawn grew better, had higher nutrient retention, higher post-prandial RNA/DNA ratio and a lower ammonia excretion than those fed at

midnight [9, 16, 17]. Regardless of diet, the present results confirm a higher post-prandial protein synthesis rate in fish fed at dawn when compared to those fed 1 h after light off. Such an increase in protein synthesis might be attributed, at least in part, to an increase in protein digestibility leading to a larger pool of free amino acids available for protein synthesis [20].

Surprisingly, fish fed the HE diet at 09:00 hours showed a lower protein retention efficiency than those fed at 19:00 hours despite higher protein digestibility and protein synthesis. This contrasts with results obtained with the LE diet and suggests a higher rate of protein degradation in fish fed at 09:00 hours. One explanation of this result might be that protein retention efficiency in fish fed the HE diet at 09:00 hours has been affected by the high growth heterogeneity. Indeed, it is generally assumed that growth heterogeneity reflects high competition for food that might impose long-term chronic stress on subordinate fish but also on dominant fish which have to maintain their social position. Environmental stress would be expected to increase protein breakdown, leading to a decrease in protein retention and protein growth rate [12, 20].

An increase in dietary fat level led to an overall improvement in wet weight as evidenced by better wet weight/gain and feed/gain ratios. As previously reported, the higher fresh weight/gain in fish fed the HE diet was associated with an increase of energy retention and a higher whole body lipid content [12, 14, 21, 28, 30, 31].

Regardless of the diet, feeding time affected post-prandial protein synthesis in rainbow trout. A post-prandial surge in protein synthesis associated with an increase in oxygen consumption is well known in a number of species (see [18, 24] for a review). Regardless of the diet, such an increase in protein synthesis 4 h after feeding was observed in fish fed at 09:00 hours but not in those fed at 19:00 hours. This is consistent with previous findings showing a higher post-prandial increase in RNA/DNA ratio in the liver and a higher increase in oxygen uptake in fish fed at dawn than in those fed at midnight [16, 17]. Taken together, these results suggest that fish fed at dawn have a higher post-prandial increase in metabolic rate than those fed at night which may lead to a higher amount of protein available for growth. In addition, it has been previously suggested that the effect of feeding time on growth performance might fluctuate depending on season, fish size and ration [1, 3, 27].

In conclusion, we provide the first evidence for an effect of feeding time on the apparent digestibility of nutrients in rainbow trout, the best absorption occurring in fish fed at the beginning of the light phase. The post-prandial protein synthesis was also higher in fish fed in the morning than in those fed at the beginning of the night. These results suggest that rainbow trout are physiologically best prepared to efficiently use nutrients when fed at dawn, i.e. in phase with the natural peak of feeding activity. The present study, showing an interaction between feeding time and dietary levels of fat on growth performance and protein retention efficiency, suggests that the composition of the diet might also affect the effect of feeding time on growth in fish.

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