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Voluntary Feed Intake in Rainbow Trout Is Regulated by Diet-Induced Differences in Oxygen Use[1–3]

Subramanian Saravanan,[4,5] Inge Geurden,[5] A. Cláudia Figueiredo-Silva,[5] Sadasivam Kaushik,[5] Johan Verreth,[4] and Johan W. Schrama[4]*

[4]Aquaculture and Fisheries Group, Wageningen Institute of Animal Sciences, Wageningen University, Wageningen, The Netherlands; and [5]INRA, UR 1067, Nutrition, Metabolism and Aquaculture, Pôle d'Hydrobiologie INRA, St. Pée-sur-Nivelle, France

Abstract

This study investigated the hypothesis that the voluntary feed intake in fish is regulated by diet-induced differences in oxygen use. Four diets were prepared with a similar digestible protein:digestible energy ratio (18 mg/kJ), but which differed in the composition of nonprotein energy source. This replacement of fat (F) by starch (S) was intended to create a diet-induced difference in oxygen use (per unit of feed): diets F30-S70, F50-S50, F65-S35, and F80-S20 with digestible fat providing 28, 49, 65, and 81% of the nonprotein digestible energy (NPDE), respectively. Each diet was fed to satiation to triplicate groups of 20 rainbow trout for 6 wk. As expected, diet-induced oxygen use decreased linearly (R^2 = 0.89; $P < 0.001$) with increasing NPDE as fat. The digestible and metabolizable energy intakes of trout slightly increased with increasing NPDE as fat (i.e., decreasing starch content) (R^2 = 0.30, P = 0.08; and R^2 = 0.34, P = 0.05, respectively). Oxygen consumption of trout fed to satiation declined with increasing dietary NPDE as fat (R^2 = 0.48; P = 0.01). The inverse relation between digestible energy intake of trout and the diet-induced oxygen use (R^2 = 0.33; P = 0.05) suggests a possible role of diet-induced oxygen use in feed intake regulation as shown by the replacement of dietary fat by starch. J. Nutr. 143: 781–787, 2013.

Introduction

In fish, factors influencing feed intake is extensively documented, but the underlying mechanism that regulates feed intake has been less intensively studied compared with mammals (1). It has often been suggested that fish, like other animals, adjust their voluntary feed intake according to the digestible energy content of the diet in order to meet a predefined energy requirement (2–4). However, recent findings in rainbow trout (5–10) and other teleosts (6,11,12) contradict the notion that feed intake is adjusted to have a constant digestible energy intake (DEI)[6]. Similarly, the demand for a target lean growth or protein deposition rather than for a predefined energy requirement has been proposed to regulate feed intake in fish (8,10,13,14). However, several other fish studies did not show an equal protein deposition with satiation feeding (e.g., 7,11). The diet-induced difference in the voluntary intake levels observed in various fish species (7,9,15–17) might be related to the type and level of dietary nonprotein digestible energy (NPDE) source (starch vs. fat) as suggested in mammals [for review, see (18). The mechanism by which the NPDE source affects voluntary feed intake in fish has been, so far, little explored. In terrestrial animals, dietary starch and fat exert their effects on feed intake via feedback mechanisms mediated by circulating glucose (19) (glucostatic regulation) and body fat store (20) (lipostatic regulation), respectively. These chemostatic control mechanisms of feed intake show inconsistent outcomes in fish (7,11). Alternatively, the thermostatic regulation of feed intake in homeotherms proposes the intake to be controlled by the animal's need for body heat and its ability to dissipate the extra heat to the environment generated as a by-product of food processing (21,22). As such, the concept of thermostatic control and its more recent revision, "heat dissipation limit theory (23)," in the feed intake regulation of fish is debatable because of its ectothermic nature. Besides, in homeotherms under thermoneutral conditions, differences in diet-induced thermogenesis are suggested to be involved in the regulation of food intake (24,25). As such, the heat produced by food processing in animals varies with the dietary macronutrient (protein, fat, and starch)

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[3] Supplemental Table 1 is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

[6] Abbreviations used: DEI, digestible energy intake; DM, dry matter; F30-S70, F50-S50, F65-S35, and F80-S20, diet with digestible fat providing 28, 49, 65, and 81% of the nonprotein digestible energy, respectively; NPDE, nonprotein digestible energy.

* To whom correspondence should be addressed. E-mail: johan.schrama@wur.nl.

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composition (26), which implies a difference in the diet-induced oxygen use (per unit of feed). Further, the basic biochemical processes of oxidative nutrient metabolism are analogous between homeotherms and poikilotherms (endotherms and ectotherms). An enhanced oxidative metabolism over the long term has been suggested to impart putative negative effects (e.g., the buildup of reactive oxygen species) on the animal (27,28). In view of the above findings, we previously proposed a role of oxygen consumption in the control of voluntary feed intake in Nile tilapia fed diets highly varying in macronutrient composition (11). In that study, Nile tilapia linearly adjusted the feed intake (DEI) depending on the diet-induced differences in oxygen use and, moreover, had a similar oxygen consumption in some of the diet groups, which suggests that perhaps physiological factors related to oxygen consumption constrained the feed intake.

The present study was designed to test the role of diet-induced differences in oxygen use (O_2 per unit of feed) on the voluntary feed intake in rainbow trout. To this end, the feed intake (DEI) and oxygen consumption were monitored in rainbow trout fed to apparent satiation with diets contrasting in the percentage of NPDE source (starch vs. fat) and with a similar digestible protein:digestible energy ratio. The contrast in fat and starch was expected to create a difference in diet-induced oxygen use. In addition, growth, body composition, nutrient partitioning (nitrogen, fat, and energy balance), and postprandial nutrient (plasma glucose and TGs) concentrations were observed to evaluate their involvement in the regulation of feed intake in rainbow trout.

Materials and Methods

The study was conducted at De Haar vissen, Wageningen University in accordance with the Dutch law on experimental animals and as approved by the Ethical Committee of Wageningen University for animal experiments.

Diets. Four diets, with the aim to have a similar digestible protein: digestible energy ratio (~18 mg/kJ) and contrasting in the percentage of nonprotein energy source (starch vs. fat) were formulated by replacing an iso-energetic (iso-digestible energy) amount of 2 test ingredients: gelatinized maize starch compared with rapeseed oil. The test ingredients were exchanged with the assumption that the digestible energy value of 12.5 g maize starch (87% apparent digestibility coefficient) is equal to that of 5.0 g rapeseed oil (95% apparent digestibility coefficient). To the basal ingredient composition (62.5%), different ratios of the test ingredients (maize starch vs. rapeseed oil) were added. These test ingredients were exchanged iso-energetically to create contrasts in percentage of nonprotein energy (F, fat vs. S, starch) (**Supplemental Table 1**): Diet F30-S70, 0% rapeseed oil + 37.5% maize starch; Diet F50-S50, 5% rapeseed oil + 25% maize starch; Diet F65-S35, 10% rapeseed oil + 12.5% maize starch; and Diet F80-S20, 15% rapeseed oil + 0% maize starch. Due to the difference in energy content (kJ/g) between rapeseed oil and maize starch, an incomplete mass balance of 92.5, 85.0, and 77.5%, respectively occurred to diet F50-S50, F65-S35, and F80-S20. To complete the mass balance to 100%, the amount of both the basal and test ingredients were adjusted in those diets in an equal proportion without affecting the target digestible protein: digestible energy ratio and contrast in nonprotein energy type. Diamol (acid insoluble ash) was added as inert marker to measure the digestibility. The ingredient mixture were extruded (Clextral BC45, twin screw extruder) through a 3-mm die, dried (70°C for 3 h), vacuum coated with oils, and stored at 4°C (Research Diet Service). The analyzed gross and digestible nutrient content of the diets are shown in **Table 1**. The observed digestible protein:digestible energy ratio of diets varied from 18 to 19.8 mg/kJ. The percent fat in the NPDE was 28% in diet F30-S70, 49% in diet F50-S50, 65% in diet F65-S35, and 81% in diet F80-S20.

Fish, housing conditions, and feeding. At the start of the experiment, 240 unfed (feed-deprived for ~36 h) juvenile rainbow trout (*Oncorhynchus mykiss;* supplied by Forrel BV) were individually weighed (under sedation; 2-phenoxy ethanol, 0.25 mL/L water) and randomly distributed among the 12 metabolic tanks (20 fish/tank). Each tank was assigned randomly to 1 of the 4 experimental diets, forming triplicates per diet. The details of the aquatic metabolism unit are described elsewhere (11); in brief, the entire unit was connected to a recirculating aquaculture system with the facilities to collect fish feces and measure the oxygen consumption. Throughout the experiment, the environmental/water quality parameters (means ± SDs) were maintained in optimal conditions for rainbow trout: photoperiod, 12 light:12 dark h; water volume, 150 L/tank; water flow, 7 L/min; water temperature, 13.7 ± 0.1°C; pH, 7.42 ± 0.2; dissolved oxygen of water at the tank inlet, 10.3 ± 0.3 mg/L; conductivity, 2.9 ± 0.2 μS/cm; nitrite, <0.15 mg nitrogen/L; nitrate, <250 mg nitrogen/L; and total ammonia nitrogen, <0.5 mg nitrogen/L. The fish were hand fed with their respective diets twice daily to apparent satiation for 1 h (0900 to 1000 and 1600 to 1700 h). Feed given and uneaten pellets were calculated and registered at each feeding.

TABLE 1 Analyzed nutrient content of the experimental diets[1]

	Diets			
	F30-S70	F50-S50	F65-S35	F80-S20
DM, *g/kg*	947	948	959	954
Crude protein (nitrogen x 6.25), *g/kg DM*	336	363	397	431
Crude fat, *g/kg DM*	71	133	198	283
Starch, *g/kg DM*	493	402	285	164
Ash, *g/kg DM*	60	64	70	71
Gross energy, *kJ/g DM*	19.9	21.4	23.1	25.1
Digestible protein,[2] *g/kg DM*	317	344	377	411
Digestible fat,[2] *g/kg DM*	61	123	186	267
Digestible total carbohydrate,[2] *g/kg DM*	354	298	229	146
Digestible energy,[2] *kJ/g DM*	16.0	18.1	20.3	22.9
NPD, *kJ/g DM*	8.5	10.0	11.3	13.0
Digestible protein:digestible energy ratio, *mg/kJ*	19.8	19.0	18.6	18.0
Fat as NPD, *%*	28	49	65	81

[1] DM, dry matter; F, fat; F30-S70, F50-S50, F65-S35, and F80-S20, diet with fat providing 28, 49, 65, and 81% of the nonprotein digestible energy, respectively; NPD, nonprotein digestible energy; S, starch.
[2] Calculated as product of respective nutrient/energy content in feed and their measured percentage apparent digestibility.

Sampling and measurements. At the start of Expt., 20 fish were killed (2-phenoxy ethanol, 1.5 mL/L water) and stored at −20°C for the analysis of the initial whole body composition. The entire experiment lasted for 7 wk; during the first 6-wk period (nutrient balance period), feed intake, growth, digestibility, oxygen consumption, and final body composition were determined. After 1 wk of the recovery period (end of 7 wk), blood samples were collected. Fish feces were collected using swirl separator as previously described (11) and stored at −20°C until further analysis. From wk 2 onwards, the water was automatically sampled for a duration of 5 min from the common inlet and outlet of each tank and flushed over the oxygen electrode (WTW-Trioximatic 700 IQ, WTW) to measure dissolved oxygen concentration. The oxygen measurements were performed in a continuous cycle of 2 d (48 h, from 0800 to 0800 h) in a set of 4 tanks consisting of all the dietary treatments. Thus, in 6 d, dissolved oxygen concentrations were measured in all 12 tanks. The oxygen electrode was calibrated once per week. This procedure was repeated until the end of the experiment, which resulted in 5 cycles of 48-h oxygen measurements per tank. At the end of 6 wk, the fish (feed deprived for ~36 h) were individually weighed and, in addition, 6 fish/tank were killed and stored at −20°C for final body composition analysis. The remaining fish in each tank then continued to be fed with their respective diets for 1 wk (recovery period) prior to postprandial blood sampling. After 6 h postfeeding, 5–6 fish from each tank were sampled for blood under sedation. The blood (1 mL) was collected from the caudal part, mixed to 20 μL anticoagulant (potassium oxalate + sodium fluoride), and centrifuged (3000 × *g*, 10 min). The plasma obtained was then stored at −20°C until analysis.

Chemical analyses. Chemical analysis of the feeds, feces, and fish carcasses was done in triplicates for dry matter (DM; ISO 6469/NEN 3332), crude protein (nitrogenx6.25; Kjeldahl, ISO 5983/NEN 3145), crude fat (Soxhlett, ISO-DIS 6492), ash (ISO 5984/NEN 3329), acid-insoluble ash (ISO 5985), and gross energy (adiabatic bomb calorimetry, IKA-calorimeter C 7000). The starch content of the feed was enzymatically determined as glucose (ISO 15914). Plasma glucose and TGs were determined following the protocol provided in the commercial kits, Glucose (RTU no. 61269) and TGs (PAP 150 no. 61236) from Bio-Merieux.

Calculations. The apparent digestibility coefficient (percent) = $[(1 - D_{AIA}/F_{AIA} \times F_N/D_N) \times 100]$, where D_{AIA} and F_{AIA} are the acid insoluble ash content (percent DM) in the diet and feces, respectively, and F_N and D_N are the amount of nutrient in 1 g DM of the feces and diet, respectively. The comparative slaughter method was used to determine the nitrogen [mg nitrogen/$(kg^{0.7} \cdot d)$], fat [g/$(kg^{0.9} \cdot d)$] and energy balance [kJ/$(kg^{0.8} \cdot d)$] as previously described (11). The only exception was the branchial and urinary energy loss, which was calculated as branchial and urinary nitrogen loss × 24.9/1000, where 24.9 is the amount of energy in kJ/g NH_3-N, assuming all nitrogen was lost as ammonia (29). Oxygen consumption of the fish was calculated per tank with the difference in measured concentration of oxygen between inlet and outlet and the rate of water flow in the tank using the formula shown elsewhere (11) without modification.

Statistical analysis. For all dependent variables, the tank was considered as the experimental unit. Data were tested for the relation between the dependent variable (Y; e.g., growth, feed intake, etc.) and the measured percentage of fat in NPDE of the diets as an independent variable (X, %; 28, 49, 65, 81) according to the linear regression model: $Y_i = \alpha + \beta X + \epsilon_i$, where α, β, and ϵ represent intercept, slope, and error term, respectively (i = 1, 2, 3,…12). Data were analyzed using the general linear model procedure in SAS 9.2 (SAS Institute). Linear regression results were reported when $P < 0.1$.

Results

Feed intake. The mean percentage survival of fish, ~93% over all the treatments, was not affected by replacing the nonprotein energy source of the diet ($P = 0.33$). Feed intake was significantly affected by replacing the dietary nonprotein energy source (**Table 2**). The absolute feed intake [g DM/(fish · d)] and feed intake per unit metabolic body weight [g DM/$(kg^{0.8} \cdot d)$] of the trout decreased linearly with increasing percent of fat in NPDE of the diet ($P < 0.001$). The feed intake of trout fed the low-fat diet (F30-S70) was 34% greater than that of the trout fed the high-fat diet (F80-S20).

TABLE 2 Feed intake, growth, and body composition of rainbow trout fed diets with varying amounts of fat and starch for 6 wk[1]

	Diets					Regression analysis		
	F30-S70	F50-S50	F65-S35	F80-S20	Pooled SEM	β (SE)	R^2	*P* value
Initial body weight, *g*	51.3	51.0	50.8	51.3	0.36	—	—	0.92
Final body weight, *g*	134.9	143.4	153.4	160.4	3.28	0.49 (0.08)	0.81	<0.001
Feed intake								
Absolute feed intake, *g DM/(fish · d)*	2.3	2.1	2.0	1.8	0.07	−0.01 (0.002)	0.75	<0.001
Feed intake$_{MBW}$,[2] *g DM/$(kg^{0.8} \cdot d)$*	16.8	15.4	14.0	12.5	0.38	−0.08 (0.009)	0.89	<0.001
Growth								
Absolute, *g/d*	2.0	2.2	2.4	2.6	0.08	0.01 (0.002)	0.81	<0.001
Growth$_{MBW}$,[2] *g/$(kg^{0.8} \cdot d)$*	14.6	15.7	17.0	17.7	0.41	0.06 (0.01)	0.81	<0.001
Feed conversion, *g DM intake/g wet weight gain*	1.16	0.98	0.82	0.70	0.02	−0.01 (0.001)	0.98	<0.001
Final body composition[3]								
DM, *g/kg wet weight*	275	299	307	320	2.8	0.82 (0.08)	0.92	<0.001
Crude protein, *g/kg wet weight*	166	164	158	157	2.5	−0.18 (0.06)	0.50	0.010
Crude fat, *g/kg wet weight*	81	111	123	141	3.1	1.10 (0.08)	0.95	<0.001
Ash, *g/kg wet weight*	22	21	21	20	0.3	−0.04 (0.009)	0.63	0.002
Energy, *kJ/g wet weight*	7.0	8.1	8.6	9.1	0.15	0.04 (0.004)	0.91	<0.001

[1] Values are least-squares mean, $n = 3$. Linear regression results were reported when $P < 0.1$. DM, dry matter; F, fat; F30-S70, F50-S50, F65-S35, and F80-S20, diet with fat providing 28, 49, 65, and 81% of the nonprotein digestible energy, respectively; S, starch.

[2] Expressed in metabolic body weight (MBW), calculated using geometric mean body weight, i.e., $[\sqrt{(\text{final body weight} \times \text{initial body weight})}/1000]^{0.8}$.

[3] Initial body composition of rainbow trout at start of the experiment (g/kg wet weight): DM, 254; crude protein, 156; crude fat, 72; ash, 23; energy 6.4 kJ/g wet weight.

Growth and body composition. Growth performance of the trout and all the measured body composition variables were significantly affected by replacing the nonprotein energy source of the diet (Table 2). The final body weight and growth of trout increased with an increasing percent of fat in NPDE ($P < 0.001$). The growth of trout fed F80-S20 was 21% higher than that in the group fed the F30-S70 diet. The feed conversion ratio decreased linearly from 1.16 to 0.70 with increasing percent fat from 28 to 81% in NPDE of the diet ($P < 0.001$).

The final body DM, crude fat, and energy content increased ($P < 0.001$), whereas the crude protein ($P < 0.01$) and ash ($P = 0.002$) decreased linearly with increasing percent of fat in NPDE of the diet. Compared with the initial body fat content (72 g/kg) of trout, the final body fat content almost doubled (141 g/kg) in the group fed the F80-S20 diet with 81% NPDE as fat. The lowest final body fat content (81 g/kg) was observed in the group that received the F30-S70 diet with 28% NPDE as fat.

Nitrogen, fat, and energy balance. Gross and digestible nitrogen intake were not affected by the replacement of the dietary nonprotein energy source ($P > 0.2$), with intakes of 695 and 659 mg nitrogen/($kg^{0.7} \cdot d$), respectively (**Table 3**). Despite the similar digestible nitrogen intake, the retained nitrogen and retention efficiency increased ($P < 0.01$) with increasing percent of fat in NPDE of the diet. In line with the increasing amount of fat in the diet, the gross and digestible fat intake and fat retention of the trout increased ($P < 0.001$), but the fat retention efficiency decreased linearly ($P < 0.001$). The mean fat retention efficiency increased linearly with increasing fat in NPDE of the diets.

The gross energy intake of the trout tended to decrease linearly ($P = 0.05$), whereas digestible ($P = 0.08$) and metabolizable energy intake ($P = 0.05$) increased with increasing percent of fat in NPDE of the diet; the regression coefficients of digestible and metabolizable energy intake were 0.30 and 0.34 kJ/($kg^{0.8} \cdot$ d per %), respectively. The differences in feed intake or gross energy intake between the dietary treatments did not relate to the growth of trout, but the difference in the DEI of trout was related to the growth ($P = 0.004$) (**Fig. 1***A*). The heat production decreased with increasing fat in NPDE ($P < 0.001$). The higher metabolizable energy intake together with the lower heat production resulted in an increased energy retention and growth (Tables 2 and 3). Similarly, the energy retained as fat and protein increased with increasing fat in NPDE ($P < 0.001$).

Plasma glucose and TGs. At 6 h postfeeding, plasma glucose tended to decrease linearly ($P = 0.09$) and plasma TGs tended to increase linearly ($P = 0.06$) (Fig. 1*B*) with increasing NPDE as fat.

Oxygen consumption and diet-induced oxygen use. The oxygen consumption [mg O_2/(kg · min) and mg O_2/($kg^{0.8}$ · min)] of rainbow trout was linearly affected ($P < 0.01$) by the nonprotein energy source of the diet (**Table 4**). Mean oxygen consumption of rainbow trout decreased with increasing percent inclusion of digestible energy as fat in the diet.

The diet-induced oxygen use (mg O_2/kJ DEI) decreased ($P < 0.001$) with increasing percentage of fat in NPDE (Table 4). The DEI of trout decreased with increasing diet-induced oxygen use ($P = 0.05$) (Fig 1C).

All measured variables were tested for curvilinearity, but for none was the quadratic function significant ($P > 0.10$).

Discussion

The DEI range in the present study was within that in our previous study with rainbow trout [211–311 kJ/($kg^{0.8} \cdot$ d)], where DEI was found to be significantly increased following the replacement of starch by fat (7). The present data confirm the effect of nonprotein energy source on DEI, although to a lesser degree, because trout in the present study consumed a larger amount (DM) of the less energy-dense (starch) diets than of the high energy-dense (fat) diets. In general, the literature on fish suggests that feed intake is regulated to meet the digestible energy requirement, or in other words, fish maintain a relatively

TABLE 3 Nitrogen, fat, and energy balance of rainbow trout fed diets with varying amounts of fat and starch for 6 wk[1]

	Diets					Regression analysis		
	F30-S70	F50-S50	F65-S35	F80-S20	Pooled SEM	β (SE)	R^2	*P* value
Nitrogen balance								
Gross nitrogen intake, *mg nitrogen/($kg^{0.7} \cdot d$)*	706	700	695	677	19.0	—	—	0.27
Digestible nitrogen intake, *mg nitrogen/($kg^{0.7} \cdot d$)*	667	663	661	645	17.5	—	—	0.38
Retained nitrogen, *mg* nitrogen/*($kg^{0.7} \cdot d$)*	314	331	341	352	7.7	0.72 (0.18)	0.62	0.002
Nitrogen efficiency (retained nitrogen/digestible nitrogen intake), *%*	47	50	52	55	1.1	0.14 (0.03)	0.75	<0.001
Fat balance								
Gross fat intake, *g/($kg^{0.9} \cdot d$)*	1.5	2.6	3.5	4.5	0.09	0.06 (0.002)	0.99	<0.001
Digestible fat intake, *g/($kg^{0.9} \cdot d$)*	1.3	2.4	3.3	4.2	0.07	0.06 (0.002)	0.99	<0.001
Retained fat, *g/($kg^{0.9} \cdot d$)*	1.6	2.7	3.2	3.9	0.12	0.04 (0.003)	0.95	<0.001
Fat efficiency (retained fat/digestible fat intake), *%*	124	110	97	92	2.5	−0.61 (0.06)	0.90	<0.001
Energy balance, *kJ/($kg^{0.8} \cdot d$)*								
Gross energy intake	338	330	322	313	8.3	−0.43 (0.20)	0.33	0.05
DEI	271	279	284	285	6.6	0.30 (0.16)	0.27	0.08
Metabolizable energy intake	260	268	274	276	6.2	0.34 (0.15)	0.34	0.05
Heat production	152	126	108	92	4.5	−1.11 (0.10)	0.92	<0.001
Retained energy	108	143	166	184	4.9	1.45 (0.12)	0.94	<0.001
Retained energy as protein	60	63	64	66	1.4	0.13 (0.03)	0.60	0.003
Retained energy as fat	48	80	102	118	4.5	1.32 (0.11)	0.94	<0.001

[1] Values are least-squares mean, $n = 3$. Linear regression results were reported when $P < 0.1$. DEI, digestible energy intake; F, fat; F30-S70, F50-S50, F65-S35, and F80-S20, diet with fat providing 28, 49, 65, and 81% of the nonprotein digestible energy, respectively; S, starch.

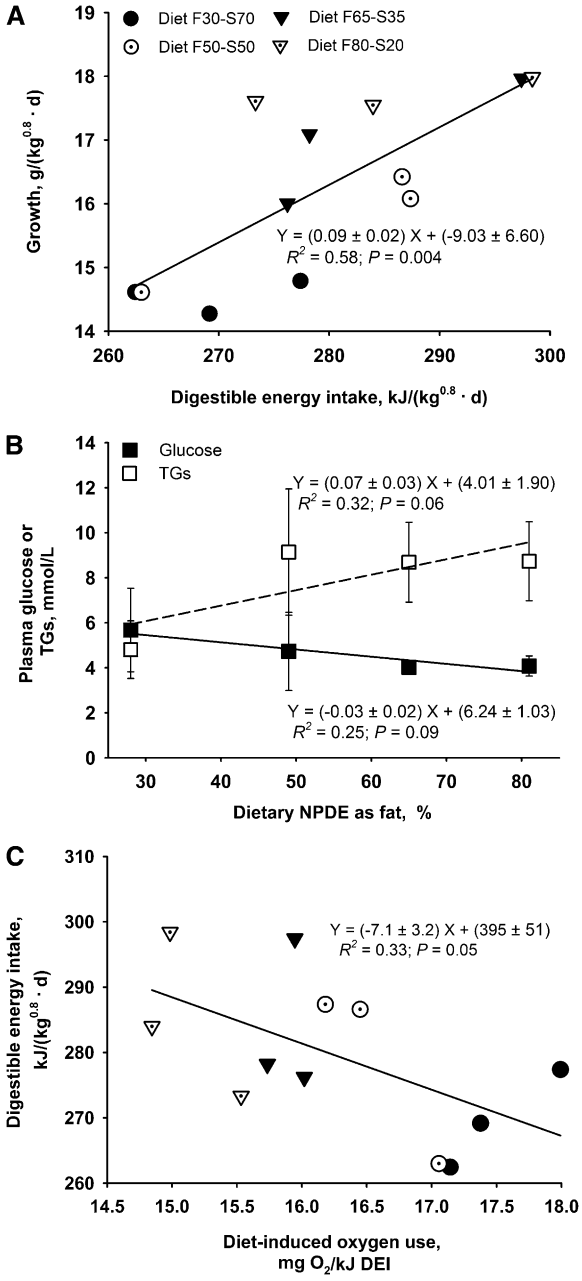

FIGURE 1 Relations between DEI and growth (*A*), percent of dietary NPDE as fat and 6-h postprandial plasma glucose and TG concentrations (*B*), and diet-induced oxygen use and DEI (*C*) in rainbow trout fed diets with varying amounts of fat and starch as NPDE sources for 6 wk. Values are means ± SDs, $n = 3$ (*B*), or in equations, means ± SEs, $n = 12$ (*A,C*). DEI, digestible energy intake; F, fat; F30-S70, F50-S50, F65-S35, and F80-S20, diet with fat providing 28, 49, 65, and 81% of the NPDE, respectively; NPDE, nonprotein digestible energy; S, starch.

constant DEI (30–32). However, the effect of diet on the DEI of fish in the present and also in other studies (7,9,14–17) contradicts the above suggestion.

In mammals, the influence of dietary nonprotein energy source on feed (energy) intake has been related to the direct effects of blood glucose (19) and body fat level (20). The nonsignificant relation between DEI and postprandial plasma nutrient concentrations suggests that there were no effects of either postprandial (6 h) plasma glucose ($P > 0.1$) or TG concentration ($P > 0.1$) (data not shown) on the DEI of the trout. The lack of a visible effect of glucostatic feedback on DEI was previously suggested in rainbow trout (7) and Atlantic cod (33). Also, an increased body fat level in the trout fed diets with a high amount of fat did not negatively affect DEI, as would be expected to occur via lipostatic feedback mechanisms (12). On the contrary, our data show a slightly increased DEI with increasing body fat content, in line with other reports in fish (7,11,17,34). This suggests a lesser impact of lipostatic feedback on DEI in the juvenile trout. Similarly, studies in mammals have shown that the intake of dietary fat energy exerts a weaker satiety effect than carbohydrates (35), resulting in a high DEI (18) as also seen in fish (7,10). This difference in the satiety effect between fat and starch has been associated with their postabsorptive metabolic fate, in particular the partitioning between storage and oxidation (36). Irrespective of the fat intake, trout in the present study predominantly deposited dietary fat into body fat with a high-fat retention efficiency (>92%). This confirms the overall low utilization of ingested fat for energy production through oxidation and the large capacity of trout to store body fat without compromising DEI, as previously reported in this species (7,8,34,37) and other fish (38).

The changes in nonprotein energy source altered the diet-induced oxygen use, which increased with increasing dietary starch and decreasing dietary fat, in accordance with our earlier observations in Nile tilapia (9). This may be attributed to the differences in the metabolic use of absorbed glucose and fatty acids, e.g. the synthesis of fat from dietary starch demanding more oxygen than from dietary fat (39). The fat retention efficiency >100% (110–124%) substantiates the occurrence of de novo lipogenesis in trout fed the high-starch diets. This probably contributes to the high oxygen use of starch-rich diets, as seen in tilapia, which displayed even higher fat retentions (>200%). Of interest, the present range in diet-induced oxygen use in trout (15.1–17.5 mg O_2/kJ DEI) is only slightly below the range found in tilapia (16.7–20.5 mg O_2/kJ DEI) despite the overall higher DEI and the differences in metabolic handling of starch in tilapia compared with trout (37).

Our previous studies suggested that the feed intake or DEI in fish can be constrained by a set-point value of oxygen consumption (on a time scale larger than weeks). This is based on the observations in trout and Nile tilapia that the feeding of diets differing in the macronutrient composition resulted in different DEI but with an equal heat production (7,12) or with an equal oxygen consumption (11). The trout in the present study, however, did not consume an equal amount of oxygen. This clearly suggests that oxygen consumption did not impose a physiological constraint on the feed intake or DEI in this study. Yet, DEI was negatively related to the diet-induced oxygen use. This is consistent with our previous data in Nile tilapia, providing further support for a possible role of diet-induced oxygen use in the regulation of feed intake in fish (11). Still, the rate of decrease in DEI per unit increase in diet-induced oxygen use (i.e., slope of line) was 100% greater in tilapia [~14.4 kJ/(kg$^{0.8}$ · d) per diet-induced oxygen use] than in the rainbow trout [~7.1 kJ/(kg$^{0.8}$ · d) per diet-induced oxygen use]. This difference in the slope warrants further confirmation, but may be related to inter-species differences with tilapia handling carbohydrates differently than rainbow trout at a moderately low protein intake (37).

In mammals, under thermoneutral conditions, the consumption of a high-protein diet results in a greater satiety together with the high diet-induced thermogenesis/energy expenditure (40). This increase in satiety effect of a high-thermogenic protein diet at thermoneutral condition is suggested to be due to an

TABLE 4 Oxygen consumption and diet-induced oxygen use in rainbow trout fed diets with varying amounts of fat and starch for 6 wk[1]

	Diets					Regression analysis		
	F30-S70	F50-S50	F65-S35	F80-S20	Pooled SEM	β (SE)	R^2	*P* value
O_2 consumption, *mg O_2/(kg · min)*	5.26	5.10	4.95	4.67	0.11	−0.01 (0.003)	0.61	0.003
O_2 consumption, *mg O_2/(kg$^{0.8}$ · min)*	3.28	3.21	3.13	2.99	0.07	−0.005 (0.002)	0.48	0.013
Diet-induced oxygen use,[2] *mg O_2/kJ DEI*	17.5	16.6	15.9	15.1	0.21	−0.04 (0.005)	0.89	<0.001
Retained energy/oxygen consumed,[3] *J/ mg O_2*	23	31	37	43	0.86	0.37 (0.02)	0.97	<0.001

[1] Values are least-squares mean, n = 3. F, fat; F30-S70, F50-S50, F65-S35, and F80-S20, diet with fat providing 28, 49, 65, and 81% of the nonprotein digestible energy, respectively; S, starch.
[2] Diet-induced oxygen use = O_2 consumption [mg O_2/(kg$^{0.8}$ · d)]/DEI [kJ/(kg$^{0.8}$ · d)].
[3] Retained energy/oxygen consumed = retained energy [J/(kg$^{0.8}$ · d)]/O_2 consumption [mg O_2/(kg$^{0.8}$ · d)].

increase in oxygen consumption and to a lesser extent by the body heat (thermogenesis) (25,41). In the same way, DEI of the trout in the present study reduced with increasing diet-induced oxygen use (per unit of feed). This suggests a possible role for diet-induced oxygen use in the regulation of feed intake/DEI in poikilotherms. In the view of the present results and bearing in mind the key role of oxygen use as the basis of diet-induced thermogenesis, we hypothesize that also in homeotherms in the absence of other potential intake constraints, the DEI is regulated by oxidative metabolism. Similarly, the satiating power of a nutrient has been proposed to be determined by their degree of hepatic oxidative metabolism as outlined in the hepatic oxidation theory in mammals (42).

To create a strong contrast in the type of nonprotein energy, the diet with the lowest amount of fat had 49% of starch, which is uncommon in the feed for rainbow trout. The general consensus in fish nutrition that carnivorous fish like rainbow trout are glucose intolerant is debatable (43). In the current study, none of the observed variables showed a curvilinear response, which suggests no negative effect of high starch. Also, the postprandial plasma glucose concentrations (4.1–5.8 mmol/L) were well within the range of values (3.8–11 mmol/L) reported in the literature (44,45). Iso-energetic replacement of starch by fat in the diets always coincides with alterations in the dietary energy density. In this study, we chose not to include a dietary filler (e.g., cellulose) because of its possible effect on the feed intake at a high inclusion level (46). Consequently, the dietary concentrations also differed for other nutrients than starch and fat, but their ratios to digestible energy (e.g., digestible protein:digestible energy ratio) were kept comparable between the diets. As for all studies applying changes in the diet composition, the suggested impacts of type of nonprotein energy source in the current study might also be due to other confounding changes in the diets (e.g., nutrient density, protein content, etc.).

In summary, the present study shows that the DEI of trout increased with increasing replacement of dietary starch by fat as nonprotein energy source, but to a lesser extent than previously reported for rainbow trout (7,8,34). In agreement with the observations in Nile tilapia (11), the DEI was inversely related to diet-induced oxygen use, which suggests a possible role of diet-induced oxygen use in feed intake regulation as shown by the replacement of dietary fat by starch.

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Literature Cited

1. Houlihan D, Boujard T, Jobling M. Food intake in fish. Oxford: Wiley-Blackwell; 2001.
2. Yamamoto T, Shima T, Unuma T, Shiraishi M, Akiyama T, Tabata M. Voluntary intake of diets with varying digestible energy contents and energy sources, by juvenile rainbow trout *Oncorhynchus mykiss*, using self-feeders. Fish Sci. 2000;66:528–34.
3. Rasmussen RS, Ostenfeld TH, McLean E. Growth and feed utilisation of rainbow trout subjected to changes in feed lipid concentrations. Aquacult Int. 2000;8:531–42.
4. Kaushik S, Luquet P, Blanc D. Usefulness of feeding protein and non-protein calories apart in studies on energy-protein relationships in rainbow trout. Ann Zootech. 1981;30:3–11.
5. Morales AE, Cardenete G, De la Higuera M, Sanz A. Effects of dietary protein source on growth, feed conversion and energy utilization in rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 1994;124:117–26.
6. Alvarez MJ, Lopez-Bote CJ, Diez A, Corraze G, Arzel J, Dias J, Kaushik SJ, Bautista JM. Dietary fish oil and digestible protein modify susceptibility to lipid peroxidation in the muscle of rainbow trout (*Oncorhynchus mykiss*) and sea bass (*Dicentrarchus labrax*). Br J Nutr. 1998;80:281–9.
7. Saravanan S, Schrama JW, Figueiredo-Silva AC, Kaushik SJ, Verreth JAJ, Geurden I. Constraints on energy intake in fish: the link between diet composition, energy metabolism, and energy intake in rainbow trout. PLoS ONE. 2012;7:e34743.
8. Geurden I, Gondouin E, Rimbach M, Koppe W, Kaushik S, Boujard T. The evaluation of energy intake adjustments and preferences in juvenile rainbow trout fed increasing amounts of lipid. Physiol Behav. 2006;88:325–32.
9. Figueiredo-Silva AC, Saravanan S, Schrama JW, Kaushik S, Geurden I. Macronutrient-induced differences in food intake relate with hepatic oxidative metabolism and hypothalamic regulatory neuropeptides in rainbow trout (*Oncorhynchus mykiss*). Physiol Behav. 2012;106:499–505.
10. Encarnação P, de Lange C, Rodehutscord M, Hoehler D, Bureau W, Bureau DP. Diet digestible energy content affects lysine utilization, but not dietary lysine requirements of rainbow trout (*Oncorhynchus mykiss*) for maximum growth. Aquaculture. 2004;235:569–86.
11. Saravanan S, Geurden I, Figueiredo-Silva A, Kaushik S, Haidar M, Verreth J, Schrama J. Control of voluntary feed intake in fish: a role for dietary oxygen demand in Nile tilapia (*Oreochromis niloticus*) fed diets with different macronutrient profiles. Br J Nutr. 2012;108:1519–29.
12. Tran-Duy A, Smit B, Van Dam AA, Schrama JW. Effects of dietary starch and energy levels on maximum feed intake, growth and metabolism of Nile tilapia, *Oreochromis niloticus*. Aquaculture. 2008;277:213–9.
13. Azevedo PA, Leeson S, Cho CY, Bureau DP. Growth, nitrogen and energy utilization of juveniles from four salmonid species: diet, species and size effects. Aquaculture. 2004;234:393–414.

14. Peres H, Oliva-Teles A. Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). Aquaculture. 1999;179:325–34.
15. Da Vies SJ. Comparative performance of juvenile rainbow trout, *Salmo gairdneri* Richardson, fed to satiation with simulated 'standard' and 'high energy' diet formulations. Aquacult Res. 1989;20:407–16.
16. Borges P, Oliveira B, Casal S, Dias J, Conceiçao L, Valente LMP. Dietary lipid level affects growth performance and nutrient utilisation of Senegalese sole (*Solea senegalensis*) juveniles. Br J Nutr. 2009;102: 1007–14.
17. Gélineau A, Corraze G, Boujard T, Larroquet L, Kaushik S. Relation between dietary lipid level and voluntary feed intake, growth, nutrient gain, lipid deposition and hepatic lipogenesis in rainbow trout. Reprod Nutr Dev. 2001;41:487–503.
18. Blundell JE, Lawton C, Cotton J, Macdiarmid J. Control of human appetite: implications for the intake of dietary fat. Annu Rev Nutr. 1996;16:285–319.
19. Mayer J. Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. Ann N Y Acad Sci. 1955;63:15–43.
20. Kennedy GC. The role of depot fat in the hypothalamic control of food intake in the rat. Proc R Soc Lond B Biol Sci. 1953;140:578–96.
21. Brobeck JR. Food intake as a mechanism of temperature regulation. Yale J Biol Med. 1948;20:545.
22. Strominger JL, Brobeck JR. A mechanism of regulation of food intake. Yale J Biol Med. 1953;25:383.
23. Speakman JR, Król E. Limits to sustained energy intake IX: a review of hypotheses. J Comp Physiol B. 2005;175:375–94.
24. Westerterp KR. Diet induced thermogenesis. Nutr Metab (Lond). 2004;1:5.
25. Westerterp-Plantenga MS, Wouters L, Ten Hoor F. Deceleration in cumulative food intake curves, changes in body temperature and diet-induced thermogenesis. Physiol Behav. 1990;48:831–6.
26. Karst H, Steiniger J, Noack R, Steglich H. Diet-induced thermogenesis in man: thermic effects of single proteins, carbohydrates and fats depending on their energy amount. Ann Nutr Metab. 1984;28:245–52.
27. Fridovich I. Oxygen toxicity: a radical explanation. J Exp Biol. 1998;201:1203–9.
28. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. Science. 1996;273:59–63.
29. Bureau DP, Kaushik SJ, Cho CY. Bioenergetics. In: RW Hardy, JE Halver, editors. Fish nutrition. 3rd ed. San Diego: Academic Press; 2002. p. 1–59.
30. Boujard T, Médale F. Regulation of voluntary feed intake in juvenile rainbow trout fed by hand or by self-feeders with diets containing two different protein/energy ratios. Aquat Living Resour. 1994;7: 211–5.
31. Cho CY, Kaushik SJ. Nutritional energetics in fish: energy and protein utilization in rainbow trout (*Salmo gairdneri*). World Rev Nutr Diet. 1990;61:132–72.
32. Lee DJ, Putnam G. The response of rainbow trout to varying protein/energy ratios in a test diet. J Nutr. 1973;103:916.
33. Hemre GI, Lie Ø, Lied E, Lambertsen G. Starch as an energy source in feed for cod (*Gadus morhua*): digestibility and retention. Aquaculture. 1989;80:261–70.
34. Figueiredo-Silva AC, Kaushik S, Terrier F, Schrama JW, Médale F, Geurden I. Link between lipid metabolism and voluntary food intake in rainbow trout fed coconut oil rich in medium-chain TAG. Br J Nutr. 2012;107:1714–25.
35. Rolls BJ, Hammer V. Fat, carbohydrate, and the regulation of energy intake. Am J Clin Nutr. 1995;62:S1086–95.
36. Stubbs RJ. Dietary macronutrients and glucostatic control of feeding. Proc Nutr Soc. 1996;55:467–83.
37. Figueiredo-Silva AC, Saravanan S, Schrama JW, Panserat S, Kaushik S, Geurden I. A comparative study of the metabolic response in rainbow trout and Nile tilapia to changes in dietary macronutrient composition. Br J Nutr. 2013;109:816–26.
38. Schrama JW, Saravanan S, Geurden I, Heinsbroek LTN, Kaushik SJ, Verreth JAJ. Dietary nutrient composition affects digestible energy utilisation for growth: a study on Nile tilapia (*Oreochromis niloticus*) and a literature comparison across fish species. Br J Nutr. 2012;108: 277–89.
39. Reeds PJ, Wahle KWJ, Haggarty P. Energy costs of protein and fatty acid synthesis. Proc Nutr Soc. 1982;41:155–9.
40. Veldhorst M, Smeets A, Soenen S, Hochstenbach-Waelen A, Hursel R, Diepvens K, Lejeune M, Luscombe-Marsh N, Westerterp-Plantenga M. Protein-induced satiety: effects and mechanisms of different proteins. Physiol Behav. 2008;94:300–7.
41. Westerterp-Plantenga MS, Rolland V, Wilson S, Westerterp K. Satiety related to 24 h diet-induced thermogenesis during high protein/carbohydrate vs high fat diets measured in a respiration chamber. Eur J Clin Nutr. 1999;53:495.
42. Allen MS, Bradford BJ. Control of food intake by metabolism of fuels: a comparison across species. Proc Nutr Soc. 2012;71:401–9.
43. Moon TW. Glucose intolerance in teleost fish: fact or fiction? Comp Biochem Physiol B. 2001;129:243–9.
44. Bergot F. Carbohydrate in rainbow trout diets: effects of the level and source of carbohydrate and the number of meals on growth and body composition. Aquaculture. 1979;18:157–67.
45. Kaushik SJ, Oliva-Teles A. Effect of digestible energy on nitrogen and energy balance in rainbow trout. Aquaculture. 1985;50:89–101.
46. Bromley P, Adkins T. The influence of cellulose filler on feeding, growth and utilization of protein and energy in rainbow trout, *Salmo gairdnerii* Richardson. J Fish Biol. 1984;24:235–44.