Modeling Fish Growth Using The Concentration Of Metabolites To Regulate Feed Intake And Metabolism

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Keywords: *Fish growth modeling; Feed intake simulation; Rainbow trout*

EXTENDED ABSTRACT

Modeling fish growth to predict fish production is important for planning of aquaculture development. Since growth strongly depends on feed intake, a growth model should be able to predict maximum feed intake of the fish under *ad libitum* feeding. However, the equations for maximum feed intake in the existing fish growth models are still descriptive and do not reflect the underlying mechanisms regulating feed intake.

Feed intake in fish is regulated by a number of factors. Physiologically, effects of metabolites in the blood are very important. The appearance of the metabolites in the blood at a rate greater than that at which they are removed signals satiety. Environmentally, oxygen may be a major determinant of maximum feed intake. The uptake of oxygen for metabolism is limited by the gill surface area and dissolved oxygen concentration in the water. When oxygen supply does not satisfy oxygen demand, fish may stop eating. Oxygen supply and the accumulation of metabolites in the cells and the blood are linked. In any case, inclusion of pools of metabolites in growth models is essential to simulate feed intake regulation.

This study aimed to develop a dynamic explanatory fish growth model which incorporates pools of metabolites as potential regulators of metabolism and feed intake. Initially, we considered only the effect of glucose on feed intake, and parameterized and calibrated the model for rainbow trout (*Oncorhynchus mykiss* Walbaum), as more information related to this species was available.

State variables in the model are pools of metabolites and body constituents, including amino acids, fatty acids, glucose, acetyl CoA and body protein and fat. It was assumed that the conversions between the state variables are influenced by the concentration of the metabolites involved. Concepts of enzyme kinetics were adapted to formulate the equations representing the conversion rates.

Feed intake was modeled based on the glucose static theory, which states that the satiety center in the brain is stimulated by an increase of glucose in the blood, causing a reduction in feed intake. In the model, when glucose concentration is higher than a threshold, fish cease to eat until glucose concentration drops below that point. Growth of the fish was calculated based on the relationship between body weight and protein biomass.

Estimation of the parameters in the rate equations was done indirectly based on general principles of nutrient metabolism and experiments on nutritional physiology of rainbow trout. After parameterization, the model was calibrated with experimental data on growth of rainbow trout under restricted feeding. A computer program was developed in Delphi 7 for the simulations, where the differential equations were solved numerically using the Euler method with a fixed time step of 0.01 day.

Agreement between the simulated and experimental fish weight was assessed based on the magnitude of the relative errors (RE) and the average relative error (ARE), which were calculated as:

$$RE_i = 100 \times \frac{SW_i - EW_i}{\frac{1}{2}(SW_i + EW_i)} \text{ and } ARE = \sum_{i=1}^{n} RE_i$$

where RE_i is the relative error for case i, SW_i and EW_i are the simulated and observed fresh weight of the fish for case i, respectively.

The model predicted fresh weight of the fish with an average relative error of -0.8% (range -18.2% to 17.8%). The parameterization and simulation results showed the gaps in our knowledge about the real values of maximum conversion rates of metabolites and the conditions under which these maximum rates occur. Results of experiments in which the fish are fed *ad libitum* with feed containing various levels of carbohydrates are needed for calibration and validation of the module for feed intake simulation.

1. INTRODUCTION

Modeling fish growth to predict fish production is important for planning of aquaculture development. During the past decade, an explanatory simulation model for fish growth (FGS) was developed at Wageningen University (Machiels 1987; Van Dam 1995). FGS predicts the growth and waste production of various fish species based on the amount and composition of the food and temperature of the water. The success of FGS in growth prediction is attributed to its general, explanatory character. However, the equations for the maximum feed intake of the fish are still descriptive and do not reflect the underlying mechanisms regulating feed intake.

Feed intake in fish is regulated by a number of factors, which can be environmental, social or physiological. Physiologically, the effects of metabolites in the blood are very important (Carter *et al.* 2001). The appearance of metabolites in the blood at a rate greater than that at which they are removed signals satiety, leading to a cessation in feeding. Environmentally, oxygen may be a major determinant of maximum feed intake since there is a lot of experimental evidence for lower feed intake when dissolved oxygen concentration in the water is low. The uptake of oxygen for routine metabolism, food processing and biosynthesis of body constituents is limited by the gill surface area and dissolved oxygen concentration. When oxygen supply does not satisfy oxygen demand, fish may stop eating. At metabolite level, oxygen supply affects the oxidation rates of nutrients and thus affects the accumulation of metabolites in the cells and the blood. In any case, inclusion of pools of metabolites in fish growth models is essential to simulate feed intake regulation.

This study aimed to develop a dynamic explanatory fish growth model which incorporates pools of metabolites as potential regulators of metabolism and feed intake. Initially, we considered only the effect of glucose on feed intake, and parameterized and calibrated the model for rainbow trout (*Oncorhynchus mykiss* Walbaum), as more information related to nutritional and metabolic aspects of this species was available.

2. MODEL DESCRIPTION

2.1. Model structure

A schematic representation of the model is shown in figure 1 using Forrester's symbols (Forrester 1961). In this representation, a conversion of a substrate to an end product is called a transaction. Main assumptions underlying this representation include:

(1) Biological value of dietary protein equals one.

(2) Tri-oleylglycerol ($C_{57}O_6H_{104}$) represents fish and dietary fat. Fatty acid pool consists of oleic acid (C18:1) since oleic acid is the most abundant fatty acid in fish (Love 1980).

(3) Transaction rates are influenced by the concentration of the substrate(s) and/or product(s) involved. Concepts of enzyme kinetics were adapted to formulate the equations representing the transaction rates.

2.2. Enzyme kinetic approach

According to the theory about enzyme action and kinetics, the rate of an enzyme-catalyzed reaction increases with increasing substrate concentration until a maximal rate is reached (Stryer 1988). This concept was applied to the whole pathway of a transaction, since the reactions in the pathway are mediated by enzymes. A general equation for a transaction rate was formulated as:

$$Rt = \frac{Rm}{1+\left(\frac{K_1}{[S_1]}\right)^{T_1}+\left(\frac{K_2}{[S_2]}\right)^{T_2}+\left(\frac{[I]}{J}\right)^{Ti}} \qquad (1)$$

where Rt = transaction rate (mmol or g per day), Rm = maximal transaction rate (mmol or g per day), K_1 and K_2 = Michaelis-Menten affinity constants for substrates S_1 and S_2, respectively (mmol per g body weight), $[S_1]$ and $[S_2]$ = concentration of substrates S_1 and S_2, respectively (mmol per g body weight), [I] = concentration of substrate or product I that has inhibition effect on the transaction (mmol per g body weight), J = inhibition constant for I (mmol per g body weight), T_1, T_2 and T_i = steepness constants associated with S_1, S_2 and I, respectively.

Depending on physiological characteristics of the metabolism of the fish, (1) was modified to different forms in which the transaction rates could be stimulated by one or two substrates and/or inhibited by one substrate or product.

2.3. Expression of maximal transaction rate

For a single reaction, the maximal reaction rate depends on the amount and activity of the enzyme catalyzing the reaction. For a transaction, the maximal transaction rate (Rm) was assumed to be proportional to the size of the tissue where the transaction takes place because the size of the

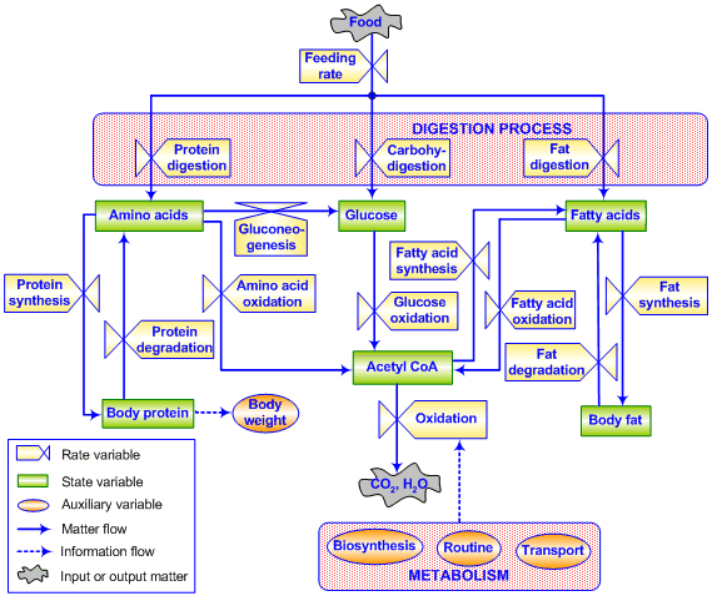

Figure 1. Schematic relational diagram of the model

tissue was supposed to proportionally affect the amount or activity, or both, of the enzymes located in the tissue. If a transaction takes place throughout a tissue, Rm was calculated as: Rm = Rr × tissues weight; if the transaction is related to the surface of a tissue, Rm was calculated as: Rm = Rr × (tissues weight)$^{0.67}$; if the transaction is related to routine metabolism, Rm was calculated as: Rm = Rr × (body weight)$^{0.8}$, where Rr represents the maximal relative rate of the transaction and routine metabolism refers to the metabolism of fish with spontaneous activities under fasting conditions.

2.4. Other expressions and assumptions

Substrate concentrations refer to the pool concentrations of metabolites, including amino acids, glucose, fatty acids and acetyl CoA. Assuming that the metabolites are distributed throughout the entire body, concentration of a metabolite y (Co_y) was calculated as: Co_y = (pool of y)/(body weight).

The rates of ATP production from and ATP requirement for all the transactions in the model and ATP requirement for absorption, transport and routine metabolism determined the total metabolic rate, which was met by oxidation of acetyl CoA.

Growth of the fish was calculated based on the relationship between body weight and protein biomass.

2.5. Mechanism for feed intake regulation

Within the scope of this paper, feed intake was determined based on the glucose static theory. According to this theory, the satiety center in the brain is stimulated by an increase of glucose in the blood, causing a reduction in feed intake. In the model, when glucose concentration is higher than a threshold, fish cease to eat until glucose concentration drops below that point.

3. PARAMETERIZATION

3.1. Maximal relative rate of utilization

As shown in the relational diagram (figure 1), the following transactions were considered:

3.1.1. Protein synthesis: In fish, it is believed that protein synthesis rates above maintenance ration increase with increasing protein intake (Houlihan *et al*. 1993). Using the experimental data reported by Fauconneau and Arnal (1985), maximal relative rate of protein synthesis was set at 0.609 mmol amino acids/g body protein/d.

3.1.2. Protein degradation: According to Houlihan *et al*. (1986), fractional rate of protein degradation in rainbow trout decreases with increasing body size. Based on this finding, protein degradation rate (Rt_{PS}) was calculated as: $Rt_{PS} = C_{Pb} \times$ (body protein)$^{0.8}$, where C_{Pb} is the coefficient for protein degradation. C_{Pb} was set at 0.025 g body protein/(g body protein)$^{0.8}$/d, based on a report of Dobly *et al*. (2003).

3.1.3. Amino acid oxidation: this transaction is likely to take place in the liver (Jobling 1993). Because we don't have sufficient data to relate the liver weight to known quantities, maximal relative rate of amino acid oxidation (Rr_{AO}) was expressed in mmol amino acids/g body weight/d. Using the experimental data reported by Kaushik and de

Oliva Teles (1985), Rr_{AO} was set at 0.094 mmol amino acids/g body weight/d.

3.1.4. Gluconeogenesis: In rainbow trout, gluconeogenesis is the main process involved in meeting glucose requirement, where amino acids are the main substrates (Cowey *et al* 1977a, 1977b). Based on the observation of Panserat *et al*. (2001a, 2001b), we assumed that gluconeogenesis always occurs in rainbow trout, regardless of their nutritional status. Hilton and Atkinson (1982) reported that 4-5% of intraperitoneally injected [^{14}C]-alanin was converted to [^{14}C]-glucose after 1 hour. Based on this result, the rate of gluconeogenesis was assigned a value equal to 5% of the amino acid pool per hour.

3.1.5. Glucose oxidation: Based on the respirometric studies of Kieffer *et al*. (1998) and Lauff and Wood (1996a) on fuel use of rainbow trout swimming at high speed, maximal relative rate of glucose oxidation was estimated to be 0.015 mmol glucose/g body weight/d.

3.1.6. Fatty acid oxidation: Because the nutritional conditions under which the maximal rate of fatty acid oxidation occurs were not known, maximal relative rate of fatty acid oxidation (Rr_{FO}) was estimated based on the energy requirement for routine metabolism assuming that all the energy supply comes from fatty acid oxidation. Using data reported by Lauff and Wood (1996b), Rr_{FO} was set at 0.010 mmol fatty acids/(g body weight)$^{0.8}$/d,

3.1.7. Fatty acid synthesis: According to Henderson and Sargent (1981), the liver is the main site of fatty acid synthesis in rainbow trout. For the same reason as stated in 3.1.3, the maximal relative rate of fatty acid synthesis (Rr_{FS}) was expressed in mmol acetyl CoA/g body weight/day. Using the experimental data reported by Gélineau *et al.* (2001) and Lauff and Wood (1996b), Rr_{FS} was set at 0.108 mmol acetyl CoA/g body weight/d.

3.1.8. Fat synthesis: This transaction takes place mainly in the white adipose tissue. Because the white adipose tissue is not as richly vascularized as other tissues, the metabolic activity per gram of adipose tissue may decrease with increasing adipose size and seems to be related to the adipose surface area. Using the experimental data reported by Gélineau *et al.* (2001), maximal relative rate of fat synthesis was set at 0.523 mmol fatty acids/(g body fat)$^{0.67}$/d.

3.1.9. Fat degradation: this transaction takes place at adipose tissue. As reasoned in 3.1.8, the maximal relative rate of fat degradation (Rr_{FD}) was expressed in g body fat/(g body fat)$^{0.67}$/day. Using the data reported by Jezierska *et al.* (1982), Rr_{FD} was set at 0.043 g body fat/(g body fat)$^{0.67}$/d.

3.2. Affinity, inhibition and steepness constants

Affinity, inhibition and steepness constants were set as fractions or multiples of the initial pool concentration of the associated metabolites. The fractions and multiples were estimated based on priorities and characteristics of the transactions and characteristics of the equations representing transaction rates.

3.3. Initial pool concentration

No data on metabolite pool concentration expressed in mmol/g body weight are available for fish. Estimates of normal concentrations of metabolites in blood plasma of rainbow trout were, therefore, used as initial metabolite pool concentrations. Initial pool concentration of amino acids was set at 0.0066 mmol/g, based on the data reported by Thorpe and Ince (1976) and Yamamoto *et al.* (2000). Initial pool concentration of fatty acids was set at 0.00086 mmol/g, based on the data reported by Plisetskaya (1980). Initial pool concentration of glucose was set at 0.0042 mmol/g, based on a review of Olsen (1989). For acetyl CoA, no data on plasma concentration is available. The initial pool concentration of acetyl CoA, therefore, was arbitrarily set at 0.0045 mmol/g.

3.4. Stoichiometric parameters

The stoichiometric parameters were determined by establishing an overall stoichiometrical equation for each transaction based on the principle described by Penning de Vries *et al.* (1974), using a number of fundamental biochemical reaction equations available in Schulz (1978) and Stryer (1988).

3.5. Energy metabolism

3.5.1. Absorption and transport costs

The products of fat digestion are mono-acylglycerols and fatty acids, which pass the enterocyte membranes by passive diffusion. After passage across the brush-border, the fatty acids undergo a re-esterification to form tri-acylglycerols in the mucosal cells (Sire *et al.* 1981). Using the same methods as described in 3.4, the energy costs of the re-esterification were set at 1.33 mole ATP per mole fatty acid.

The costs for the absorption of 1 mole glucose or amino acid were set at 0.33 mole ATP (Mandel and Balaban 1981).

For the transport of nutrients across a membrane rather than the membranes of the enterocytes, the costs were also set at 0.33 mole ATP per mole amino acid or glucose (Gerrits *et al.* 1997).

3.5.2. Routine metabolism

Based on a study of Winberg (1956), routine metabolic rate was calculated as:

$$Mr = Q_{10}^{(T_1 - T_2)/10} \times \alpha \times W^{\beta} \quad (2)$$

where Mr is the rate of energy consumption (mmol ATP/day), Q_{10} represents the increase in the metabolic rate when the temperature increases by 10^oC, T_1 is the simulation temperature, T_2 is the reference temperature, α is the rate coefficient (mmol ATP/g^{β}/day), W is body weight (g) and β is the rate exponent.

For the simulation, Q_{10} was set at 2, α was set at 2.73 mmol ATP/$g^{0.8}$/day at T_2=20^{o}C and β was set at 0.76 (Winberg 1956).

3.6. Other parameters

For protein digestibility in rainbow trout, a review by Van Dam and Penning De Vries (1995) showed a range of 85-90% for fishmeal and purified proteins. For the simulations, protein digestibility was set at 85%. For fat digestibility, Vandenberg and De La Noüe (2001) found a range of 75-92%. For the simulations, fat digestibility was set at 80%. For carbohydrate digestibility, Phillips (1969) reported values of 90% for sugars (glucose, sucrose), of 57% for cooked starch and of 38% for raw starch. For the simulations, carbohydrate digestibility was set at 40%.

Using the data from From and Rasmussen (1984), Van Dam and Pennning de Vries (1995) found a linear relationship between protein biomass (P, in g) and body weight (W, in g), which is: P = 0.027 + 0.156 W (R^2 = 0.989; N = 367; W ranging from 2.6-412g). This relationship was used to calculate fish weight in our simulations.

The glucose threshold was arbitrarily set at 0.042 mmol/g, ten times higher than the estimated initial concentration of glucose.

4. MODEL CALIBRATION AND RESULTS

The model was written in Delphi 7 where the differential equations were solved numerically using the Euler method with a fixed time step of 0.01 day.

Experimental data from From and Rasmussen (1984) were used in this study for calibration, which comprise 175 cases with initial fish weights ranging from 3 to 371 g and feeding levels ranging from 0 to 38.6 g/$g^{0.8}$/d. The parameter values were adjusted to obtain good agreement between the simulated and experimental results for final fish weight. The agreement was assessed based on the magnitude of the relative errors (RE) and the average relative error (ARE), which were calculated as follows:

$$RE_i = 100 \times \frac{SW_i - EW_i}{\frac{1}{2}(SW_i + EW_i)} \quad (3)$$

$$ARE = \sum_{i=1}^{n} RE_i \quad (4)$$

where RE_i is the relative error for case i, SW_i and EW_i are the simulated and observed fresh weight of the fish for case i, respectively.

Best agreement between simulated and observed fish weight (figure 2) was achieved with maximal relative rates of amino acid oxidation of 0.075 mmol/g/d, protein synthesis of 0.78 mmol/g/d, fatty acid oxidation of 0.02 mmol/$g^{0.76}$/d and protein degradation of 0.008 g/$g^{0.8}$/d and a glucose threshold of 0.25 mmol/g. The average relative error was −0.8% with the relative errors ranging from -18.2% to 17.8%. Simulated feeding levels ranged from 0 to 38 g/$g^{0.8}$/d. A glucose threshold lower than 0.25 mmol/g reduced feed intake and growth in cases of high feeding level, making RE in these cases more negative. A glucose threshold higher than 0.25 mmol/g did not change the simulation results.

5. DISCUSSION

The parameterization showed the gaps in our knowledge about the real values of maximum conversion rates of metabolites and the conditions under which these maximum rates occur.

Changing the glucose threshold in the model influences feed intake and growth of the fish. The simulation results showed that best agreement between simulated and observed fish weight was obtained when all the feed was consumed and this coincides with the experiments. However, results of experiments in which the fish are fed *ad libitum*

with feed containing various levels of carbohydrates and records of maximum feed intake are needed to calibrate and validate the module for feed intake simulation. If feed intake regulation by glucose concentration does not give good simulated results, effects of other metabolites like amino acids or fatty acids should be investigated. Effects of oxygen on maximum feed intake can also be integrated into the model by establishing an oxygen pool and calculating oxygen supply and demand based on gill surface area, dissolved oxygen concentration and stoichiometric parameters for oxygen in all the transactions.

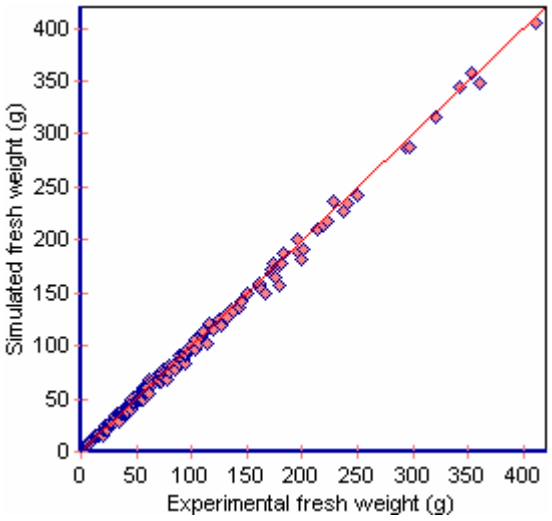

Figure 2. Calibration results of the model. The bisector represents perfect agreement between simulated and observed values.

6. ACKNOWLEDGMENTS

This study was financed by WOTRO (*Netherlands foundation for the advancement of tropical research*). Thanks are given to Dr. M.A.M. Machiels for his valuable comments.

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