Modeling the effects of dietary carbohydrate and ambient oxygen concentration on feed intake and growth in fish

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Modeling the effects of dietary carbohydrate and ambient oxygen concentration on feed intake and growth in fish

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Abstract

Fish production can lead to discharge of wastes and have negative impacts on the environment. It is therefore important to carefully monitor and plan the development of aquaculture. A model that can simulate fish growth on the basis of available fish species and local conditions (like water quality and quantity and quality of fish feed) could therefore be extremely useful. Since growth strongly depends on feed intake, a growth model should be able to predict maximum feed intake of fish under *ad libitum* feeding. This thesis aimed at investigating some potential mechanisms for feed intake regulation and predicting maximum feed intake in the light of these hypothesized mechanisms using a theoretical modeling approach. Among a wide range of factors involved in feed intake regulation, glucose and oxygen are two factors of particular interest. The overall hypotheses were: (1) high blood glucose level has a negative effect on feed intake in fish; and (2) dissolved oxygen concentration of the water is a major determinant of feed intake in fish.

To investigate the effects of blood glucose on feed intake in Nile tilapia (*Oreochromis niloticus*), an experiment was done with feeds containing different combinations of carbohydrate and energy levels. The results showed that fish fed a diet high in carbohydrate but low in energy failed to achieve their digestible energy requirement, suggesting that a high level of blood glucose suppresses feed intake in Nile tilapia. A dynamic explanatory model for fish growth which incorporated metabolite pools as potential regulators of feed intake was then developed. After parameterization and calibration for rainbow trout (*Oncorhynchus mykiss*), the model gave good prediction of fish growth. The results showed that feed intake regulation could be simulated based on the glucose static theory. The parameterization and simulation results showed the gaps in our quantitative knowledge about the intermediary metabolism in fish.

To investigate the effects of dissolved oxygen (DO) concentration and body weight on maximum feed intake and growth of Nile tilapia, three experiments were conducted with different DO levels and body weight classes. The results provide strong support for the theory that limitation of oxygen supply through the gill surface area results in lower feed intake and growth of fish at low DO concentration. The allometric relationship between gill surface area and body weight results in lower relative feed intake, and therefore lower relative growth in bigger fish than in smaller fish. The study also demonstrated that the incipient DO (DO above which feed intake of fish remains unchanged) depends to a large extent on body weight and probably on feed composition.

The short-term effect of DO on *ad libitum* feed intake was then simulated based on the balance between oxygen demand and oxygen supply. The model was calibrated and validated using the experimental data from the present study and some additional data from earlier published experiments. The calibration and validation results showed that the model could predict satisfactorily maximum feed intake and growth of Nile tilapia when DO was limiting. However, feed intake when DO was not limiting was not simulated satisfactorily. This was attributed to the empirical equation for maximum feed intake determined by a factor other than DO.

In the last chapter, the modeling approaches, the role of glucose and oxygen in feed intake regulation, implications for aquaculture management, reliability of data on maximum feed intake and implications for further research are discussed.

Contents

Chapter 1

General introduction

1. Importance of fish growth and feed intake prediction

Fish is an important source of nutrition, with about 1 billion people in the world relying on fish as their main source of animal protein (FAO, 2001) and more than 2.6 billion people with at least 20% of their annual animal protein consumption as fish (FAO, 2007). Worldwide, more than 11 million people are involved in fish farming, which now produces about 43% of the world fish food supply (FAO, 2007). Fish farming has a positive impact on the livelihoods of rural and urban communities. In the tropics, freshwater aquaculture ponds are often part of mixed farming systems. The fish can be fed with crop by-products or the ponds are fertilized with manure or grasses. More intensive forms of aquaculture with higher levels of external nutrient inputs (chemical fertilizers and supplemental feeds) also exist. Ecological and socio-economic studies show that integrating fishponds with other crops or livestock can improve the productivity and profitability of farms without negative impacts on the environment (Prein, 2002). In other words, integrating aquaculture into farm systems can increase the sustainability of such systems. Because of these benefits, integrated agriculture-aquaculture (IAA) systems are being promoted for adoption by large groups of new farmers, not only in Asia but also in Africa. However, there are also risks to this development. Fish production can lead to the discharge of wastes and have negative impacts on the environment (Pullin et al., 1993). It is therefore important to carefully monitor and plan the development and intensification of aquaculture and its integration into farming systems.

For monitoring and planning the development of conventional tropical agriculture, bioeconomic models have been used to calculate the ecological and economic effects of changes in land use. The input-output relationships of crops and livestock are calculated based on factors like soil type, climate, and fertilizer or feeding regime, and then used in linear programming models at the regional level for optimization of land use or for policy evaluation. These models enable policymakers and stakeholders to understand the interactions between the technical, environmental and economic consequences of new technology or new policies (Ruben et al., 2001; Stoorvogel et al., 2001). Some examples of new technology are new crop varieties, irrigation, or increased fertilizer application. Aquaculture can also be seen as a new technology component that may be introduced on farms when the biophysical conditions (climate, water supply, soil type) are favourable.

The technical basis for land use planning models is so-called Technical Coefficient Generators (TCG's): simulation models that calculate input-output relationships at the level of a crop or field (Hengsdijk et al., 1998). With such a model, crop yields can be simulated based on the local climate and soil conditions. With reliable, validated crop models, expensive and time-consuming crop yield experiments for each location can be avoided. Such models also allow calculation of economic and ecological sustainability indicators. If aquaculture technology is to be included in land use planning exercises, a TCG for aquaculture is needed:

a model that can simulate fish production on the basis of local conditions (water quality, availability and quality of fish feed, and the available fish species).

During the past decades, an explanatory simulation model for fish growth (Fish Growth Simulator, FGS) was developed at Wageningen University. FGS predicts the growth and waste production of various fish species based on water temperature and feed composition. Because it is based on the biochemical reaction equations of the intermediary metabolism of fishes, FGS is a general model that can be applied to a wide variety of fish species. Apart from protein and fat deposition in fish biomass, the reaction equations also calculate oxygen consumption, ammonia and carbon dioxide production, and production of organic waste (undigested feed). In this way, FGS links the protein and fat deposition (i.e., growth and body composition), oxygen consumption and waste production of a fish to the amount and composition of the feed consumed. Such a model is extremely useful for aquaculturists. So far, it has been used to simulate the growth of five species: African catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*) and tambaquí (*Colossoma macropomum*) (Machiels and Henken, 1986; van Dam and Penning De Vries, 1995; van der Meer and van Dam, 1998; Verdegem et al., 2000).

The success of FGS to predict the growth of different fish species is attributed to its general, explanatory character. Nevertheless, some parts of the model are still quite descriptive in nature and were adjusted repeatedly in attempts to apply the model to other fish species. Especially the equation for the maximum feed intake of the fish has undergone various revisions. 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. Initially, a table for maximum growth of the African catfish (based on experimental results) was part of the model, and every day the fish would eat until maximum growth for that day was attained (Machiels and Henken, 1986). For Nile tilapia, an equation relating the maximum protein intake rate (in g protein per kg metabolic weight per day) to fish body weight was derived (Verdegem et al., 2000). For simulation of the growth of tambaquí at *ad lib* feeding, the amounts wasted had to be estimated in relation to the feeding level (van der Meer and van Dam, 1998). These equations are, however, only applicable for specific fish species and size and culture conditions. It is important that the model can predict maximum feed intake of a wide range of fish species of different sizes under different conditions. For this, the mechanisms of feed intake regulation must be well understood and integrated into the model.

2. Feed intake regulation in fish

In contrast to mammals, investigations into feed intake regulation in fish are relatively limited. This is probably due to difficulty in measuring feed consumption in fish. Fish are normally housed in group under water and therefore feed is administered to the culture system

rather than to the individual fish. This leads to unavoidable spills, which are difficult to quantify. Feeding fish manually with continuous observation on satiety status and evaluation of feed wastage is very time-consuming and laborious. Automatically monitoring feed waste using on-demand feeders and recording device can be inaccurate due to disintegration of feed. Due to these constraints, most of the theories about the factors governing feed intake in fish are largely based on mammalian data. In both mammals and fish, it has been widely accepted that feed intake is under complex control of many factors. In general, these factors can be physical, physiological, social or environmental. The following provides a brief review of these factors.

2.1. Physical factors

The physical factors controlling feed intake comprise stomach volume and gastric evacuation rate (the decrease in stomach contents per unit time). In mammals, stomach volume plays a role in limiting feed intake. In fish, this is plausible only for species possessing a stomach. Although direct evidence about the cessation of feeding caused by stomach size can hardly be found in fish, a role of stomach volume in restricting feed intake is generally assumed based on the facts that (1) there is a positive relationship between stomach volume and food intake (Pirhonen and Koskela, 2005); and (2) the rate of appetite return is closely related to the rate of gastric evacuation (Brett, 1971; Elliott and Persson, 1978; Grove and Crawford, 1980; Huebner and Langton, 1982; Lee et al., 2000). In this view, it seems impossible to separate physical and physiological effects of stomach fullness on feed intake. The stomach volume can change with changing dietary composition. Fish fed low-energy diets eat more to meet the energy requirement and as a consequence develop a bigger stomach (Ruohonen and Grove, 1996). Because of the plasticity of the stomach, the extent of stomach distention which induces afferent satiety signals is probably involved in the regulation of feeding rather than the physical size of the stomach. It is the pressure of food on not only the stomach but also on the gut walls that signals satiety. Cholecystokinin, a hormone released by the duodenal wall in response to stress caused by entry of chyme inhibits feed intake (Smith and Gibbs, 1975; Lin et al., 2000; Gélineau and Boujard, 2001; Volkoff, 2006). In mammals, the induction of satiety is delayed if the sensory tracts from the mouth are by-passed (Fletcher, 1984). The above-mentioned findings suggest that the stomach fullness must interact with other physiological factors to induce satiety. In fish, it is still not clear to what extent the stomach volume plays a role in the termination of a meal.

Gastric evacuation rate changes the volume of the feed in the gastro-intestinal tract and thus influences the return of appetite. On the other hand, gastric emptying rate is a function of the stomach distention (Jobling, 1981). According to this view, gastric evacuation rate and feed intake are interdependent. Gastric evacuation rate is also influenced by many other factors. Meal size, body size and nutritive and energy density of the chyme are negatively

correlated with the gastric evacuation rate (Swenson and Smith, 1973; Jobling et al., 1977; Grove et al., 1978; Flowerdew and Grove, 1979; Jobling, 1980). On the contrary, temperature and the gastric evacuation rate are directly related (Grove et al., 1978; Santulli et al., 1993).

2.2. Physiological factors

2.2.1. Neural and hormonal systems

The focal region of the brain for studies on neural control of feeding behaviour is the hypothalamus. Most of the experimental work on hypothalamic control of feed intake has been done on rats using either lesions or surgical transection of neural pathways (Kalra et al., 1999b). In the hypothalamic site, the lateral hypothalamus (LH) was hypothesized as the "feeding center" and ventromedial nucleus (VMN) as the "satiety center" (Hetherington and Ranson, 1942; Anand and Brobeck, 1951; Steller, 1954). During the 1990s, discoveries of new orexigenic and anorexigenic signaling molecules and their neuronal production sites and their receptors in the hypothalamus led to new concepts about the hypothalamic pathways and the interconnected circuitry in the central nervous system responsible for feed intake regulation (Elmquist et al., 1999). Apart from LH and VMN, the other sites participating in this circuitry include dorsomedial nucleus (DMN), paraventricular nucleus (PVN), arcuate nucleus (ARC), suprachiasmatic nucleus (SCN) and supraoptic nucleus (SON) in the hypothalamus, paraventricular thalamic nucleus (PVT) in the thalamus, and periaqueductal gray (PAG), locus coeruleus (LC) and raphe nuclei (RN) in the midbrain.

The LH contains neurons which produce two orexigenic neuropeptides, melanocyte-concentrating hormone (MCH) and orexins (Sakurai et al., 1998). The targeted sites for these two neuropeptides include PVT, PAG, RN, LC, SCN, PVN, ARC and SON (Date et al., 1999). Anorexigenic neuropeptides, including cocaine and amphetamine regulated transcript (CART) and corticotrophin releasing factor (CRF), are also found in the LH, which may work against the orexigenic neuropeptides to inhibit feed intake (Lin et al., 2000).

The ARC contains a high density of neurons that produce orexigenic peptides such as neuropeptide Y (NPY), opioids, β-endorphin (β-END), galanin (GAL) and glutamate. α-melanophore-stimulating hormone (α-MSH), an anorexigenic peptide, is also co-produced with β-END in ARC. The terminal fields of these orexigenic and anorexigenic producing neurons extend into various hypothalamic sites, including the VMN, DMN, perifornical hypothalamus (PFH), PVN, and preoptic area (POA) (Kalra et al., 1999b).

The DMN and PVN are other NPY-producing sites. In DMN, the interaction between NPY and leptin is involved in the attenuation or inhibition of feeding by leptin (Kalra et al., 1999b). PVN is one of the important sites for release of orexigenic signals. The release of NPY from PVN may be diminished by the interaction among neurotransmitters/ neuromodulators which results in inhibition of feeding (Kalra et al., 1999b).

The SCN releases anorexigenic signals which hamper feed ingestion. The efferent signals from SCN were found to terminate in VMN, DMN, subparaventricular zone and LH, establishing direct lines of communication between the components of anorexigenic and orexigenic network for control of feeding behaviour (Kalra et al., 1999b).

Different from the other sites in the hypothalamus, VMN is unlikely to produce any orexigenic or anorexigenic substances. However, studies on the disruption of the signalling in VMH have affirmed the role of VMH in appetite regulation (Brobeck, 1946; Shimizu et al., 1987). VMH lesions disrupt signaling differently in each of the orexigenic pathways (Kalra et al., 1997; Jain et al., 1998; Pu et al., 1998; Kalra et al., 1999a). For example, microinjection of colchicine to disrupt neural signaling in the VMN reduced the release of NPY in the ARC and PVN but increased the galaninergic signaling in the hypothalamus (Jain et al., 1998; Pu et al., 1999). It was hypothesized that VMN contains a receptive field for several signaling molecules which control appetite. VMN is the target site for NPY, β-END and CART released from ARC (Finley et al., 1981; Everitt and Hökfelt, 1989; Kristensen et al., 1998). Efferent signals from the VMN to DMN and PVN were demonstrated, supporting the hypothesis that the VMN integrates feeding-regulatory signals and send information to the DMN-PVN axis for the release of orexigenic signals (Bellinger et al., 1986; Bernardis and Bellinger, 1987; Moga and Saper, 1994).

In fish, relatively few studies have been done on neural regulation of feeding. Inferior lobes of the hypothalamus are involved in control of feeding behaviour in bluegill (*Lepomis macrochirus*) (Demski and Knigge, 1971), a cichlid (*Tilapia heudelotti macrocephala*) (Demski, 1973), goldfish (*Carassius auratus*) (Roberts and Savage, 1978) and nurse sharks (*Ginglymostoma cirratum)* (Demski, 1977). It was hypothesized that olfactory input and telencephalon output exert a positive effect on feed intake (Peter, 1979). In addition, input from the optic tectum also evokes feeding behaviour, probably by stimulating the hypothalamus (Peter, 1979). Orexigenic peptides found in fish include GAL, opioids and NPY and anorexigenic peptides include CRF, bombesin and CCK. These peptides act centrally at the hypothalamus to regulate feed intake in the short term. The detailed mechanisms of action of these peptides were presented by Lin et al. (2000) and De Pedro and Björnsson (2001).

There are links between the neural and hormonal systems in the regulation of food intake. Hormones that have simulating effects on feed intake include thyroid hormones (TH), growth hormone (GH) and insulin (short-term effect) and those having inhibitory effects include cholecystokinin (CCK), peptide YY (PYY), glucagon and glucagon-like peptide (GLP), insulin (long-term effect), cortisol, catecholamines and leptin. According to Le-Bail and Boeuf (1997), it is difficult to separate the neurocrine and endocrine effects on feeding. On feed intake control, a hormone could have (1) a direct effect by directly binding to the

receptors on the central nervous system or via activation of vagal afferent neurons (e.g. leptin and CCK); (2) an indirect effect by slowing down the gastric evacuation rate which activates the vagal afferent neurons (e.g. CCK and PYY); (3) an indirect effect by acting directly on the intermediary metabolism via glucose or free fatty acids or amino acids (e.g. insulin and glucagon); and/or (4) and indirect effect by directly or indirectly modifying the secretions of other hormones involved in feed intake regulation (e.g. insulin on GH, PYY on insulin and CCK on insulin and GH). Some of the hormones, like CCK, PYY and glucagon, act as short-term factors which control the initiation, length and feeding rate. On the other hand, the others, like GH, TH and leptin require more time to modify feeding behaviour, thus act as long-term factors (Le-Bail and Boeuf, 1997). Particularly, insulin acts as both short- and long-term effect. The short-term positive effect of insulin is attributed to the fact that an increase in plasma insulin level leads to a rapid reduction in plasma glucose concentration, which stimulates feeding (see 2.2.2). The long-term negative effect of insulin is proposed because (1) insulin is secreted in proportion to adipose tissue mass (similar to leptin; see 2.2.4), given the same blood glucose level (Polonsky et al., 1988; Mommsen and Plisetskaya, 1991; Woods and Seeley, 2000), (2) insulin is present in the brain without corresponding mRNA, suggesting that insulin is produced only in the pancreas and then transported to the brain (Plisetskaya et al., 1993), (3) insulin receptors are present in the brain (Mommsen and Plisetskaya, 1991), and (4) direct administration of insulin into brain suppresses food intake (Chavez et al., 1996; Soengas and Aldegunde, 2004).

2.2.2. Blood metabolites

Glucose

In both mammals and fish, glucose is the main metabolic fuel for the brain. Because its availability is vital for sound functioning of the body, homeostasis of blood glucose is under control of a complex system involved in hormonal and neural mechanisms (Mayer, 1955). It is therefore logical that glucose plays an important role in feed intake regulation, given that feedback mechanisms are pivotal in maintaining the homeostasis. In mammals, the glucostatic theory proposes that glucose receptors in the ventromedial hypothalamus (VMH) increase their rate of firing in response to elevated blood glucose levels, sending satiety signal to the lateral hypothalamus (LH) (Mayer, 1953; 1955). On the contrary, low blood glucose levels were hypothesized to act via the VMH-LH axis, causing disinhibition of the LH to initiate feeding, which serves as a compensatory response to restore glucose homeostasis (Muller et al., 1974). According to this hypothesis, hypothalamic glucoreceptors may be more responsible for the short-term initiation of feeding and prevention of the hypoglycemic effects. However, Russek (1971; 1976) suggested that hepatic glucose receptors rather than the hypothalamus monitor the glucose supply from the intestine and this affects feed intake. Later, he proposed that liver hepatocytes monitor the hepatic carbohydrate reserves rather than the actual glucose supply from the intestine (Russek, 1981a; b). The hypothesis on

hepatic glucostatic food intake regulation was also supported by studies of Novin et al. (1974) and Shurlock and Forbes (1981). Results from some other studies suggest that both hypothalamus and liver are involved in feed intake control in relation to plasma glucose level (Forbes, 1988; 1992).

In contrast to mammals, research on the effects of glucose on feed intake in fish is rare. In addition, observations on feeding behaviour of fish in relation to plasma glucose are often contradictory. For instance, skipjack tuna (*Katsuwonus pelamis*) with elevated blood glucose levels consumed more food than fish with lower blood glucose levels resulting from food deprivation (Magnuson, 1969). In contrast, red piranha (*Rooseveltiella nattereri*) ingested greater meals in response to lower preceding levels of blood glucose (Bellamy, 1968). Lovell (1979) found no correlation between voluntary feed consumption and plasma glucose levels in channel catfish (*Ictalurus punctatus*). On the other hand, Volkoff and Peter (2006) reported that injection of glucose caused reduced feed intake in carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*).

Some authors (e.g. Peter, 1979; Carter et al., 2001) suggested that glucose is unlikely to be a major regulator of feed intake in fish because of low concentrations of glucose receptors in all the tissues and the inability of fish to regulate blood glucose. The latter was attributed to low glucose phosphorylating capacity, which is determined by two enzymes, hexokinase and glucokinase (Wilson, 1994). In mammals, hexokinase is saturated at normal blood glucose levels and inhibited by glucose-6-phosphate whereas activity of glucokinase increases with increasing blood glucose above normal levels, regardless of glucose-6-phosphate concentration (Bender, 2002). In some fish species, prolonged hyperglycaemia was observed after glucose tolerance tests (Palmer and Ryman, 1972; Thorpe and Ince, 1974; Furuichi and Yone, 1981). Tung and Shiau (1991) attributed this phenomenon to the lack of glucokinase based on the saturation characteristics of this enzyme and of hexokinase. Indeed, glucokinase was not detected in rainbow trout (*Oncorhynchus mykiss*) in a study of Cowey et al. (1977). However, Panserat et al. (2000) found high levels of dietary carbohydrate to induce hepatic glucokinase activity in rainbow trout, common carp (*Cyprinus carpio*) and seabream (*Sparus aurata*).

In Nile tilapia (*Oreochromis niloticus*), administration of 2-deoxyglucose, a drug that blocks intracellular glucose utilization and inhibits glycolysis, caused an increase in feed intake (Delicio and Vicentini-Paulino, 1993). In mammals, a reduction in intracellular glucose metabolism stimulated hypothalamic glucoreceptors, leading to increased food intake (Timo-Iaria, 1990). Hence, the effect of 2-deoxyglucose on feed intake in Nile tilapia implies an up-regulation of feed intake by hypoglycemia. Nevertheless, hyperglycemic down-regulation of feed intake in fish still remains questionable.

Amino acids

In mammals, the accumulation of free amino acids from an excess of dietary protein is considered toxic. The deamination of amino acids produces toxic nitrogenous waste and heat, which in mammals could increase body temperature above the normal range in a hot environment (Forbes, 1999). It is therefore logical that the animal stops eating before the toxic threshold of free amino acid is reached. There is evidence about suppressed feed intake caused by increased blood levels of amino acids (Panksepp, 1974; Myers, 1975; Panksepp, 1975; Forbes, 1992). Some studies showed that food consumption may be reduced when feeds contain either very low or high amounts of essential amino acids (Carter et al., 2001). Excessively high concentrations of protein in feeds also causes a decrease in feed consumption, which is attributed to a protective response to avoid harmful consequence of possibly high accumulation of toxic amino acids (de la Higuera, 2001). According to Forbes (1999), reduced consumption of low-protein foods is due to taste and/or other sensory properties of the foods, because direct introduction of a high-protein meal into the stomach by tube had no effect on subsequent food selection whereas a similar meal taken by mouth resulted in subsequent selection of low-protein food. Some amino acids stimulate, whereas others inhibit or have no action on neuronal activity in the lateral hypothalamus (Wayner et al., 1975). However, it is unlikely that there are specific receptors for each amino acid (Forbes, 1992). Unfortunately, effects of each amino acid and the interaction effect among the nutrients on feeding behaviour in fish still remain unexplored.

Fatty acids and glycerol

The role of free fatty acids in feed intake regulation in fish is not clear. In mammals, an increased blood level of fatty acids may inhibit feeding, and low levels may initiate feeding on a short-term basis (Myers, 1975; Panksepp, 1975). However, there has not been any evidence about the receptors for fatty acids so far. On a long-term basis, the release of fatty acids and glycerol from lipolysis into the blood is believed to act as a signal to indicate the size of body fat stores. As a result, feeding behaviour is adjusted based on these signals to maintain the adipose homeostasis (Hirsch, 1972; Mrosovsky and Powley, 1977). The release of glycerol is proportional to the body lipid biomass and the size of adipocytes (Goldrick and McLoughlin, 1970). Glycerol administration suppressed feed intake in rats in a dose-dependent manner (Wirtshafter and Davis, 1977; Booth, 1979). As body-fat-derived glycerol is used either as metabolic fuel or for gluconeogenesis (Bender, 2002, Chapter 5), glycerol might act via oxidation or its influence on plasma glucose concentration to modify food intake. Langhans et al. (1984) injected a high dose of glycerol into rats and found that their food intake was suppressed while plasma glucose and liver glycogen concentrations remained unchanged. In addition, he found that glycerol had no effect on food consumption when a high-protein diet was given; such diet is known to depress the activity of glycerol-3-phosphate dehydrogenase in the liver which would reduce the oxidation rate of glycerol. These findings suggest that it might be the oxidation of glycerol rather than a change in

glucose concentration that plays the main role in inhibiting food intake. In fish, there have been almost no investigations into the effect of glycerol on feed ingestion. Glycerol released from lipolysis in some fish is the main substrate for gluconeogenesis (Plisetskaya, 1980). However, this should not preclude the possibility that glycerol participates in feed intake regulation.

2.2.3. Dietary nutrients and energy

It is widely recognized that animals eat to satisfy their nutrients and energy requirements. As diets are not always in perfect balance, it is important to know how food is regulated with regard to each specific component of food, e.g. protein or energy. Animals fed deficient diets can respond in two opposite ways: (1) increase feed intake to meet the requirement when the deficiency is moderate; or (2) reduce feed intake when the deficiency is great (de la Higuera, 2001). The latter is ascribed to the avoidance of the onset of metabolic disorder after ingesting unbalanced diets.

Many studies have shown that energy intake plays an important role in feed intake regulation in fish. For examples, goldfish and rainbow trout fed diets with kaolin added as an inert material increased feed ingestion to compensate for the low energy content (Rozin and Mayer, 1961; Grove et al., 1978). Jobling and Wandsvik (1983) fed Arctic charr (*Salvelinus alpinus*) with three iso-energetic diets containing different protein and lipid concentrations and found that dietary energy concentrations (total, digestible or metabolisable) were more important than protein or lipid contents in the regulation of feed intake. A study by Morales et al. (1994) indicated that rainbow trout ate to maintain a level of digestible energy intake irrespective of the protein source and level. However, ingestion in order to reach the required energy level could be inhibited by a deficiency of amino acids in the diets (Garcia-Gallego et al., 1998; de la Higuera et al., 1999).

2.2.4. Body composition and the lipostatic theory

Body lipid biomass is thought to be negatively correlated to feed intake. The concepts of lipostatic regulation of feeding were first introduced by Kennedy (1953). According to this model, the size of body fat stores is compared with a set-point in the hypothalamus which results in a long-term regulation of food intake to maintain a target level of adiposity. Since the discovery of the hormone leptin in 1994, many studies on appetite control and especially on obesity have achieved considerable successes, providing an explanation for the regulatory mechanisms underlying the lipostatic model (Hynes and Jones, 2001).

Leptin, a protein coded by the obese gene (*ob*), is secreted mainly by the adipose tissue (Zhang et al., 1994), but also by other tissues like stomach, muscle and placenta (Ahima and Flier, 2000; Ahima et al., 2000). Known as a multifunctional hormone, leptin plays important roles in homeostasis, immune function, reproduction, and predominantly in the regulation of energy balance by inhibiting food intake and increasing energy expenditure (Ahima et al.,

1996; van Dijk, 2001). To undertake the latter role, leptin stimulates lipolysis, promotes fatty acid oxidation and inhibits lipogenesis by suppressing the activity of lipogenic enzymes, which subsequently stimulates the activity of lipolytic enzymes (Hynes and Jones, 2001). Leptin is secreted in proportion to body lipid biomass (Hynes and Jones, 2001) and acts as a regulator of adiposity and energy intake (Johnson et al., 2000). The effect of leptin on food intake is mainly through the central actions by interaction with the hypothalamic peptides. In mammals, leptin receptors have been identified in neurons producing both orexigenic and anorexigenic peptides (Ahima et al., 2000; Williams et al., 2001). Neuropeptide Y (NPY) and orexins are two prominent orexigenic peptides known to be the targets for leptin (see also 2.2.1). In mammals, central administration of leptin depresses expression of NPY and orexins, causing reduced food intake (Lopez et al., 2000; Meister, 2000). On the other hand, leptin and cholecystokinin act synergistically to suppress feed ingestion and reduce body weight (Buyse et al., 2001; Attele et al., 2002).

In fish, there is evidence about the negative effect of adipose tissue on feed intake (Jobling and Miglavs, 1993: *Salvelinus alpinus*; Shearer et al., 1997: *Oncorhynchus tshawytscha*; Johansen et al., 2002; 2003: *Salmo salar*). Together with the detection of leptin in a number of fish species, including largemouth bass (*Micropterus salmoides*), green sunfish (*Lepomis cyanellus*), bluegill (*Lepomis macrochirus*), white crappie (*Pomoxis annularis*), channel catfish (*Ictalurus punctatus*), and rainbow trout (*Oncorhynchus mykiss*) (Johnson et al., 2000), this suggests that the role of body fat in feed intake control in fish is analogous to the mammalian lipostatic model.

2.3. Social factors

The inter-individual variation in feed consumption of a group of fish is mainly caused by social interactions among the individuals. Fish, when housed in groups, often display strong social hierarchies with dominant individuals consuming larger amounts of food than lower-ranking individuals (Stirling, 1977; Webster and Hixon, 2000; Irwin et al., 2002). The social hierarchies also cause stress and thus result in reduced feed intake in the subordinates (Gregory and Wood, 1999). The impact of social interactions on feed intake depends on behavioural characteristics of the species and genetic strains. Within the same species or strain, feeding regime may also modify feed intake of the individuals. When fish are fed with restricted ration, the effect of social interactions is more pronounced than when fed *ad libitum* (McCarthy et al., 1992; Carter et al., 1996). Feeding frequency and feed distribution also affect the inter-individual variation in feed consumption. When feed is unavailable for a certain period of the day, the better competitors that gain early access to food may have digested their previous meal and feed again in the current meal, whereas the individuals having their previous meals later may not regain their appetite before the end of the feeding period (Kestemont and Baras, 2001).

2.4. Environmental factors

2.4.1. Temperature

Temperature is an important controlling factor governing metabolic rate (Fry, 1971), which directly or indirectly influences feeding behaviour (Brett, 1979; Jobling, 1993). Under *ad libitum* feeding, feed intake increases with increasing temperature and reaches a peak before declining precipitously at supra-optimal temperature (Brett, 1979). The explanation for this might be that the increase in metabolic rate with increasing temperature stimulates feeding to meet the energy requirements. However, fish might reach the limitation of the capacity of the respiratory and circulatory systems to deliver oxygen to the highly oxygen-demanding tissues at high temperature. Under this condition, further increase in temperature would suppress appetite (Jobling, 1997).

Temperature also indirectly influences feed intake via its negative effect on the solubility of oxygen in water (Jensen et al., 1993). Thus, a change in temperature may result in a change in the balance between oxygen demand and maximum oxygen supply, determined by the dissolved oxygen concentration. As a consequence, feed intake is modified by a change is oxygen availability for food processing (see 2.4.2).

2.4.2. Oxygen

Low oxygen concentration has a negative effect on fish growth and feed intake (Herrmann et al., 1962; Stewart et al., 1967; Tsadik and Kutty, 1987; Teichert-Coddington and Green, 1993; Papoutsoglou and Tziha, 1996). In fish culture, oxygen can become limiting for feed intake before any other factors and may determine feed consumption to a large extent. This is true both for extensive farm ponds, where oxygen concentrations depend largely on the primary productivity of algae and can show high diel fluctuations, and for intensive aquaculture systems where oxygen needs to be added to the water at a cost.

Total oxygen demand is the oxygen needed to generate the energy for routine metabolism (energy requirement of fish with spontaneous activities under fasting condition), feeding metabolism (energy requirements associated with feed consumption and processing) and biosynthesis. The lower limit of oxygen demand of fish is determined by the routine metabolic rate. The upper limit is usually reached during swimming at maximum sustainable speed, when the fish needs all the oxygen it can consume for aerobic activity (in fact, some fish use anaerobic pathways during burst swimming activity to provide energy for muscle activity). Fishes have to fit all their activities between routine and maximum metabolism: non-routine swimming, feeding, and other behaviour. Under culture conditions (i.e., with high feeding rates and high-protein feeds), oxygen supply may not satisfy oxygen demand. In many benthic fish, demands for feeding metabolism and biosynthesis use up the entire oxygen uptake capacity of the fish (Warren and Davis, 1967). For cod (*Gadus morhua*), it was shown

experimentally that the oxygen consumption can reach maximum values due to feed consumption and the associated metabolic processes alone (Soofiani and Hawkins, 1982).

Maximum oxygen intake by diffusion is limited by the gill surface area of a fish (A), which is allometrically related to body weight W ($A = aW^b$). The value of the exponent b ranges from 0.6 to 0.9, depending on species (Hughes, 1966; Hughes, 1970; Pauly, 1981; 1982). Because gill surface area grows at a slower rate than body mass, the capacity to take in oxygen for processing feed (and therefore the feed intake relative to body size) decreases with increasing body size, theoretically to a point where growth is zero. This is the point where the fish has reached its theoretical asymptotic weight, W_{inf} (Pauly, 1981). Because the relationship between body weight and gill surface area is different in different species of fish, the weight at which oxygen supply balances oxygen demand is different for different species. An analysis of a large number of species showed that a positive relationship between W_{inf} and b exists (Pauly, 1981; 1982). In other words, fish with bigger gills can consume more oxygen and therefore food.

3. Aim and outline of the thesis

The mechanisms controlling feeding and satiation in fish are highly complex, multifactorial and still not well understood. Among a wide range of factors involved in feed intake regulation, glucose and oxygen are two factors of particular interest. While in mammals the role of glucose on feed intake is obvious and explained by the glucostatic theory, in fish this role is far less clear. In contrast to glucose, oxygen might play a major role in feed intake regulation in fish, but in mammals this is not the case. Unfortunately, little effort has been devoted to elucidating the role of glucose and oxygen in feed intake regulation in fish. So far, no in-depth studies have been done to investigate the effects of blood glucose on feed intake in fish using a non-invasive approach. Although it is known that low oxygen concentration has a negative effect on fish growth, very few reports in the literature on experimental works directed specifically at the effect of DO on feed intake can be found; moreover, most of these works lack a systematic approach. The mechanisms underlying feed intake regulation in relation to glucose and oxygen still remain to be explored.

In the present study, glucose and oxygen were selected as representatives of physiological and environmental factors, respectively, for studies on feed intake regulation. The general aim of this thesis was to investigate the hypotheses related to glucose and oxygen on feed intake regulation and the possibility to predict maximum feed intake in the light of these hypotheses using a theoretical modeling approach. The overall hypotheses were: (1) high blood glucose level has a negative effect on feed intake in fish; and (2) dissolved oxygen concentration is a major determinant of feed intake in fish.

Chapter 2 investigates the effects of dietary carbohydrate on feed intake in Nile tilapia. It was hypothesized that the use of digestible carbohydrate as a major source of energy would reduce feed intake due to the effect of blood glucose. To test this hypothesis, the maximum feed intake of Nile tilapia fed diets low and high in starch was compared. The formulation of the diets also gave rise to examining the effect of the stomach volume on feed consumption. As the results from Chapter 2 suggested that high dietary starch levels hamper feed intake before the stomach is full, a dynamic simulation model for fish growth and feed intake was developed using the pools of the metabolites as state variables and their concentrations as regulators of the metabolic processes (**Chapter 3**). The metabolite pools (amino acids, fatty acids, glucose and acetyl CoA) were incorporated into the model because they directly or indirectly influence glucose concentration, which controls feed intake. In this model, fish ceased to eat when the glucose concentration exceeded a threshold. Experimental growth data from From and Rasmussen (1984) were used to calibrate the model for rainbow trout.

Chapter 4 and **Chapter 5** investigate the effects of dissolved oxygen (DO) concentration and body weight on maximum feed intake and growth of Nile tilapia. These studies also aimed at obtaining data for calibration of a model simulating the effect of DO on feed intake (**Chapter 6**). In Chapter 4, two weight classes of fish and two DO levels were employed in a single experiment. Since there were only two DO levels in the experiment, Chapter 4 could not indicate whether or not there is one oxygen concentration above which maximum feed intake of fish of any size remains unchanged. Moreover, as energy metabolism was not investigated in Chapter 4, it was unclear how Nile tilapia partitioned its energy budget under different DOs. These issues were studied in Chapter 5, which aimed at determining, for Nile tilapia of different body weights, the incipient DO at which feed intake starts to level off and the effect of DO on energy metabolism. For this, two successive experiments were conducted with two weight classes of Nile tilapia. In each experiment, fish were exposed to four ambient DO concentrations. Based on the findings in Chapter 5, we developed an oxygen limitation module and incorporated it into an existing model (Chapter 6); the core of this model was originally developed by Machiels and Henken (1986) and later revised by van Dam and Penning De Vries (1995) and van Dam and Pauly (1995). In the new oxygen limitation module, a mechanism for short-term feed intake regulation in relation to DO was proposed and tested. The model was calibrated using the data in Chapter 4 and 5 and validated using the data from Kolding et al. (2008).

Chapter 7 concludes the thesis with a general discussion of the results in the light of the original aim of this study. The societal relevance of the thesis' work and issues for further research are also considered.

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Chapter 2

Effects of dietary starch and energy levels on maximum feed intake, growth and metabolism of Nile tilapia, *Oreochromis niloticus*

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Abstract

The aim of this study was to gain insight into how Nile tilapia (*Oreochromis niloticus*) regulate feed and energy intake in response to diets low and high in starch and cellulose. It was hypothesized that high-starch diets would reduce feed intake due to the effect of high blood glucose level, and that stomach volume may limit feed intake of fish fed diets low in energy. Four experimental diets, low starch-no cellulose inclusion, high starch-no cellulose inclusion, low starch-with cellulose inclusion, and high starch-with cellulose inclusion, were formulated. The high-starch diets and diets with cellulose inclusion were 17.5% more energy-diluted than the low-starch diets and diets without cellulose inclusion, respectively. Male tilapia were fed to apparent satiation for six weeks. Feed and digestible energy intake of fish fed diets with cellulose inclusion increased and decreased by 8.3% and 5.5%, respectively, compared to fish fed diets without cellulose inclusion. This suggests the role of stomach volume in restricting feed consumption. Fish fed high-starch diets achieved only 0.5% more feed intake and 13.9% less digestible energy intake than fish fed low-starch diets. The lower increase in feed intake and higher decrease in digestible energy intake of fish fed high-starch diets than of fish fed diets with cellulose inclusion suggests that high blood glucose suppresses feed intake in Nile tilapia. An alternative explanation for the differences in feed and digestible energy intake of fish fed different diets was based on the fact that heat production was not influenced by starch nor cellulose inclusion levels. Thus, under satiation feeding, oxygen uptake capacity may determine feed and digestible energy intake in fish rather than blood glucose or stomach volume.

1. Introduction

Feeding fish to satiation while avoiding feed waste can help maximize production (Brett and Groves, 1979; Elliott, 1994; Sæther and Jobling, 1999; Sun et al., 2006) and mitigate environmental pollution (Persson, 1991; Naylor et al., 2000). This requires knowledge about feed intake regulation. Among a wide range of factors affecting feed intake, nutrient and energy supply (and therefore diet composition) have been shown to be important (Fletcher, 1984; Forbes, 1988; Blundell and Halford, 1994; Woods et al., 1998).

It is likely that the composition of aquaculture feeds will change considerably in the future. Due to the decline in supply of fish meal and fish oil from wild fish (Naylor et al., 2000), plant protein and carbohydrate have been considered as important alternative ingredients for formulated fish feeds. Due to plant characteristics, the replacement of fish meal by plant protein leads to an increase in dietary carbohydrate content. Regardless of the protein source, the use of carbohydrate-rich diets has been considered economical as fish would utilize the inexpensive carbohydrate as a source of energy, thus sparing the absorbed protein for growth. Despite the large number of studies on the effects of dietary carbohydrate levels on fish growth (e.g. Furuichi and Yone, 1980; Shiau and Peng, 1993; Brauge et al., 1994; Peragon et al., 1999; Ali and Al-Asgah, 2001; Keshavanath et al., 2002), information on the relationship between dietary carbohydrate levels and maximum feed intake is scant.

One of the consequences of increasing the carbohydrate content of the diet is an increase in dietary volume, i.e. a decrease in dietary energy concentration. Assuming that fish eat to meet their energy requirement (Rozin and Mayer, 1961; Boujard and Médale, 1994; Morales et al., 1994; Dias et al., 1998), they would compensate a low dietary energy concentration in high-volume, high-carbohydrate diets by ingesting more food. This could lead to a situation in which stomach volume restricts the feed intake before the energy requirement is fulfilled.

Another consequence of higher carbohydrate concentration in the diet may be that blood glucose level increases (Cowey et al., 1977; Brauge et al., 1994). This may also have an effect on feed intake. The glucostatic theory states that glucose receptors in the ventromedial hypothalamus produce hunger or satiation signals in response to below- or above-normal plasma glucose levels (Mayer, 1953, 1955). In mammals, there is ample evidence supporting this role of glucose in feed intake regulation and energetic homeostasis (Russek, 1963; Booth, 1972; Booth and Jarman, 1976; Booth, 1979; Louis-Sylvestre and Le Magnen, 1980; Moran and McHugh, 1981; Shurlock and Forbes, 1981; Niijima, 1983; Ramirez and Friedman, 1983; Campfield and Smith, 1990; Guss et al., 1994; Sakata et al., 1994; Campfield et al., 1996; Rayner et al., 2000). In fish, however, findings on the effects of blood glucose on feed intake are limited and inconsistent. Among those are Bellamy (1968) and Magnuson (1969).

Virtually no studies have been done to investigate the effects of dietary carbohydrate and energy levels on feed intake in Nile tilapia. We hypothesized that the use of digestible carbohydrate as a major source of energy would reduce feed intake in tilapia due to the effect of blood glucose, and that the stomach volume may play a role in regulating feed intake in fish fed diets low in energy. Our objective was to gain insight into how Nile tilapia adjust feed and energy intake in response to diets with different starch (and therefore energy) contents. In order to explain possible differences in feed intake of fish fed different diets, energy metabolism was also examined by calculating energy balance and comparing digestible energy intake and heat production in relation to diet composition.

2. Materials and methods

This experiment was approved by the Ethical Committee for Animal Experiments (DEC) and conducted at the Aquaculture and Fisheries Group of Wageningen University in The Netherlands.

2.1. Experimental diets

Four experimental diets, low starch-no cellulose inclusion (LS-NOC), high starch-no cellulose inclusion (HS-NOC), low starch-with cellulose inclusion (LS-CEL), and high starch-with cellulose inclusion (HS-CEL), were formulated according to a 2 × 2 factorial design (Table 2.1). The first contrast was the inclusion levels of starch in the diets: low starch inclusion (LS) versus high starch inclusion (HS). This contrast was created by exchanging 125 g vegetable oil mixture (soya and palm oil) with 300 g maize flour, which resulted in an equal exchange of calculated energy content and in a contrast in the starch to fat ratios (0.625:1 for LS versus 11.0:1 for HS diets; Table 2.2). Assuming that fish would eat to meet their energy requirements, equal exchange of energy is essential to test the glucostatic hypothesis, since only then will the amount of energy absorbed as glucose differ. The LS and HS diets were formulated to keep the ratios of protein to energy and of premix to energy equal for all the experimental diets. This was done to have similar protein and premix intake if fish would eat similar amount of energy, considering that feed intake would be steered by energy or protein requirement.

Table 2.1. Ingredient composition of the experimental diets (%)

	Diet[1]			
	LS-NOC	**HS-NOC**	**LS-CEL**	**HS-CEL**
Experimental ingredients				
Gelatinized maize flour[2]	12.12	40.00	10.00	33.00
Soya oil[3]	7.575	--	6.25	--
Palm oil[4]	7.575	--	6.25	--
Cellulose	--	--	17.50	17.50
Basal ingredients				
Fishmeal[5]	36.37	30.00	30.00	24.74
Soybean meal[6]	30.31	25.00	25.00	20.62
$CaCO_3$	0.36	0.30	0.30	0.25
$CaPO_4$	0.85	0.70	0.70	0.58
Pellet binder (Durabon)	1.21	1.00	1.00	0.83
Premix[7]	1.21	1.00	1.00	0.83
Diamol[8]	2.42	2.00	2.00	1.65

[1] LS-NOC = Low starch-no cellulose inclusion; HS-NOC = High starch-no cellulose inclusion; LS-CEL = Low starch-with cellulose inclusion; HS-CEL = High starch-with cellulose inclusion.

[2] Suprex Corn < 300 Plata (Article number 15001), produced by Codrico BV, Rotterdam, The Netherlands. This maize flour is pre-gelatinized by extrusion, has a granulation (%) with a size above 300 μm of < 5% and with 85% of the starch being gelatinized. On a dry matter basis it has the following composition: fat 2.5%; starch 85%; crude fibre 0.6%; crude protein 7.0% and ash 0.6% (product information).

[3] Refined soya oil, produced by Romi Smilfood, Heerenveen, The Netherlands.

[4] Refined palm oil , produced by Romi Smilfood, Heerenveen, The Netherlands.

[5] Danish herring meal, bran Skagen FF with a protein content of 70%.

[6] Soybean meal Hipro, produced by Cargill, Amsterdam with a protein content of 47%.

[7] Mineral premix composition (mg kg^{-1} feed): iron (as $FeSO_4 \cdot 7H_2O$), 50; zinc (as $ZnSO_4 \cdot 7H_2O$), 100; cobalt (as $CoSO_4 \cdot 7H_2O$), 2.4; copper (as $CuSO_4 \cdot 5H_2O$), 5; selenium (as Na_2SeO_3), 1; manganese (as $MnSO_4 \cdot 4H_2O$), 25; magnesium (as $MgSO_4 \cdot 7H_2O$), 300; chromium (as $CrCl_3 \cdot 6H_2O$), 1; iodine (as $CaIO_3 \cdot 6H_2O$), 5. Vitamin premix composition (mg kg^{-1} feed): thiamin, 30; riboflavin, 30; nicotinic acid, 200; pantothenic acid, 100; pyridoxine, 30; cyanocaobalamin, 0.05; ascorbic acid, 500; alpha-tocopheryl acetate, 200 IU; folic acid, 15; retinylacetate, 15000 IU; cholecalciferol, 2000 IU; menadione nicotinamide bisulphite (51%), 8; inositol, 200; choline (as choline chloride), 1000; anti-oxidant BHT (E300-321), 100; calcium propionate, 1000.

[8] Diamol GM, Franz Bertram, Hamburg, Germany.

Table 2.2. Calculated nutrient composition of the experimental diets (on a fresh weight basis)

	Diet[1]			
	LS-NOC	**HS-NOC**	**LS-CEL**	**HS-CEL**
Dry matter (g kg^{-1})	890	874	892	878
Crude protein (g kg^{-1})	399	330	329	272
Crude fat (g kg^{-1})	190	33	157	27
Starch (g kg^{-1})	122	363	101	300
Ash (g kg^{-1})	102	85	85	70
Energy (kJ g^{-1})[2]	20.12	16.62	16.60	13.71
Protein/Energy (mg kJ^{-1})[2]	19.75	19.86	19.82	19.84
Starch : Fat ratio	0.625 : 1.0	11.0 : 1.0	0.625 : 1.0	11.1 : 1.0

[1] LS-NOC = Low starch-no cellulose inclusion; HS-NOC = High starch-no cellulose inclusion; LS-CEL = Low starch-with cellulose inclusion; HS-CEL = High starch-with cellulose inclusion.

[2] Cellulosic energy was excluded from the calculation, assuming that cellulose is inert.

Creating the difference in starch level between the diets not only altered the starch level (i.e. starch to fat ratio) but also changed the energy and nutrient (e.g. crude protein) contents of the diets. The HS diets were 17.5% more energy- and protein-diluted than the LS diets. Thus, differences in maximum feed intake on a weight basis between the LS and HS diets could be due to the effect of glucostatic feed intake regulation or the effect of dietary nutrient concentration (volume limitation of feed intake). To quantify the effects of dietary nutrient concentration of the LS and HS diets, a second contrast of cellulose inclusion levels was added to the experimental design, resulting in 0% cellulose-inclusion diets (NOC) and 17.5% cellulose-inclusion diets (CEL). Cellulose was assumed to be inert for the fish, having a 0% digestibility and not affecting the digestibility of the other ingredients.

Diamol (diatomaceous cell powder, product of Franz Bertram, Hamburg, Germany) was added to each diet as an acid insoluble ash (AIA) marker to determine apparent digestibility coefficients (ADC).

2.2. Fish and rearing conditions

Six hundred and forty male tilapia (Swansea Silver) with an average weight (± SD) of 17.0 ± 3.5 g were obtained from a commercial fish farm (Til-Aqua Int.; Velden, Limburg, The Netherlands). At arrival the fish were distributed over 16 120-L rectangular glass tanks at a density of 40 fish $tank^{-1}$. All tanks were connected to a recirculation system equipped with a sedimentation tank, a trickling biofilter, a sump and a pump. From arrival to the start of the experiment (four weeks) the fish were fed a commercial diet (Trouvit, F-1 Pro Aqua Brut 1 mm: 55.0% crude protein, 15.0% crude fat, 0.1% crude fiber and 11.0% ash on a wet weight

basis) using a feeding level of 15 g $kg^{-0.8}$ d^{-1}. Feed was delivered by an automatic feeding belt over a period of eight hours per day (starting at 0900). During the acclimation period, water flow rate through the tanks was maintained at 6 L min^{-1}, temperature at 28°C, DO concentration above 5 mg L^{-1}, photoperiod at 12L:12D, pH between 6.9 and 8.0, NO_2-N below 1 mg L^{-1} and NH_3-N below 0.1 mg L^{-1}.

2.3. Experimental procedures

The same tanks used during the acclimation period were used for the experimental period. After the acclimation period all fish were taken out of the tanks. Then, fish were anesthetized with tricaine methane sulfonate (MS-222; 0.2 g L^{-1} buffered with 0.4 g L^{-1} sodium bicarbonate), weighed individually and randomly allocated to the tanks at a density of 20 fish $tank^{-1}$. The four experimental diets were randomly assigned to the 16 tanks with four replicates (tanks) per diet. Average initial weight (± SD) of the fish in LS-NOC, HS-NOC, LS-CEL and HS-CEL treatments was 51.85 ± 1.56, 52.69 ± 0.86, 50.75 ± 1.41, 52.20 ± 1.16 g, respectively.

Two tanks within each treatment were randomly selected for feces and uneaten feed collection using Choubert collectors (see description in Choubert et al., 1979; 1982). This was done because only eight Choubert collectors were available.

During fish allocation, 20 fish were randomly sampled for initial body composition analysis. These fish were killed with an overdose of MS-222 (0.8 g L^{-1} buffered with 1.6 g L^{-1} sodium bicarbonate), placed in a plastic bag, sealed and stored at -20°C for further processing and analysis.

During the experimental period (six weeks) fish were fed manually to apparent satiation twice per day (starting at 0900 and 1600); each meal lasted for no more than one hour. Every day before feeding, feces collected by the Choubert collector from each tank were transferred into an aluminum box and stored at -20°C for further analysis. Twenty minutes after the end of each meal, uneaten feed from each tank was quantified by counting the flushed-out pellets collected at the associated Choubert collector. To have a representative sample of each diet, 10 grams of each diet was collected daily, pooled and stored at 4°C for further analysis. Water quality was maintained as in the acclimation period.

At the end of the experimental period, fish from each tank were anesthetized with MS-222 (same dose as used for fish allocation) and weighed individually. Five fish from each tank were randomly sampled for final body composition analysis. Procedures for processing of sampled fish were the same as at the start of the experimental period.

Before chemical analysis, the sampled fish were cut into small pieces, which were homogenized by passing them through a 4.5 mm-screen grinder two times. The sampled feed was ground twice using a 1 mm-screen grinder. Samples for dry matter determination were

taken from the homogenates of fish and feed before the remaining materials were freeze-dried. All feces were freeze-dried. Each freeze-dried sample of fish was thoroughly mixed in a blender before further analysis. This was also done for each freeze-dried sample of feed and of feces.

2.4. Analytical procedures

All chemical analyses were done in triplicate. Dry matter content was determined as weight loss after drying the samples for 4 h at 103°C until constant weight (ISO, 1983). Crude protein content was determined using the Kjeldahl method and multiplying nitrogen content by 6.25 (ISO, 1997). Crude fat content was determined after petroleum-ether extraction using a Soxhlett system (ISO, 1999). Gross energy content was determined using a bomb calorimeter (IKA-C7000, IKA-analysentechnik, Weitersheim, Germany). Ash was determined by burning the oven-dried samples in a muffle furnace at 550°C (ISO, 1978). Acid insoluble ash (AIA) was determined by treating the residue obtained after ash determination with hydrochloric acid. This mixture was then filtered to obtain the insoluble residue (ISO, 1981).

2.5. Calculations

Feed intake of the fish expressed as a percentage of body weight (FI_{perc}) and per metabolic weight unit (FI_{MBW}) were calculated as FI_{perc} (% d^{-1}) = $FI/W_{mean} \times 100$ and FI_{MBW} (g $kg^{-0.8}$ d^{-1}) = $FI/(W_{mean}/1000)^{0.8}$, where FI (g d^{-1}) is the average feed intake per fish per day and W_{mean} is the geometric mean body weight, which was calculated as W_{mean} (g) = $\sqrt{W_i \times W_f}$, where W_i and W_f are the initial and final average individual fish weight (g). FI was corrected for the uneaten feed. Because the daily uneaten feed of each tank connected to a Choubert collector was very small (ranging from 1.2% to 2.4% of the daily feed given) and the difference between the daily uneaten feed of the two collector-connected tanks within each treatment was negligible, it was assumed that daily uneaten feed (in percent of daily feed given) was similar for all tanks within each treatment. Thus, the average daily uneaten feed (in percent of daily feed given) of two tanks was used for correction of FI of all the four tanks within each treatment.

Specific growth rates (SGR) were calculated as SGR (% d^{-1}) = $[(\ln W_f - \ln W_i)/t] \times 100$, where t is the experimental duration (days). Growth rates per metabolic weight unit (GR_{MBW}) were calculated as GR_{MBW} (g $kg^{-0.8}$ d^{-1}) = $(W_f - W_i)/(W_{mean}/1000)^{0.8}/t$. Feed conversion ratio (FCR) was calculated as FCR = $FI_{tot}/(W_f - W_i)$, where FI_{tot} (g) is the total feed intake per fish during the experimental period.

Apparent digestibility coefficients of nitrogen and energy in the diets were calculated as $ADC_X = (1 - AIA_{diet}/AIA_{feces} \times X_{feces}/X_{diet}) \times 100$, where X represents nitrogen or energy,

AIA_{diet} and AIA_{feces} are the AIA content (% dry matter) in the diet and feces, respectively and X_{diet} and X_{feces} are the quantity of X in 1 g dry matter of the diet and feces, respectively.

Total digestible nitrogen (DN; mg $fish^{-1}$) was calculated as the product of total gross nitrogen intake (GN; mg $fish^{-1}$) and ADC of nitrogen (in %), where GN was calculated as the product of total feed intake (g $fish^{-1}$) and nitrogen content of the diets (mg g^{-1}). Total fecal nitrogen losses (FN; mg $fish^{-1}$) were calculated as the difference between GN and DN. Total retained nitrogen (RN; mg $fish^{-1}$) was calculated as the difference between the final and initial nitrogen mass (mg $fish^{-1}$). Total branchial and urinary nitrogen losses (BUN; mg $fish^{-1}$) were calculated as the difference between DN and RN. The above-calculated values of GN, FN, DN, BUN and RN were divided by $[t \times (W_{mean}/1000)^{0.8}]$ to be expressed in mg $kg^{-0.8}$ d^{-1}.

Total digestible energy (DE; kJ $fish^{-1}$) was calculated as the product of total gross energy intake (GE; kJ $fish^{-1}$) and ADC of energy (in %), where GE was calculated as the product of total feed intake (g $fish^{-1}$) and energy content of the diets (kJ g^{-1}). Total fecal energy losses (FE; kJ $fish^{-1}$) were calculated as the difference between GE and DE. Total metabolizable energy (ME; kJ $fish^{-1}$) was calculated as the difference between DE and the energy in the total branchial and urinary excretory products (BUE; kJ $fish^{-1}$), which was estimated as BUE = (BUN × 24.9)/1000, where 24.9 is the amount of kJ equivalent to 1 g excreted nitrogen, assuming that all nitrogen is excreted as NH_3-N (Bureau et al., 2002). Total retained energy (RE; kJ $fish^{-1}$) was calculated as the difference between the final and initial energy quantities (kJ $fish^{-1}$). Total heat production (HP; kJ $fish^{-1}$) was calculated as the difference between ME and RE. The above-calculated values of GE, FE, DE, BUE, ME, HP and RE were divided by $[t \times (W_{mean}/1000)^{0.8}]$ to be expressed in kJ $kg^{-0.8}$ d^{-1}.

Note that because ADC was measured for two tanks within each treatment, average ADC of two tanks was used for all the four tanks within the treatment when calculating DN and DE.

2.6. Statistical analysis

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc.). The homogeneity of variances of different groups was checked using Levene's *F* test with PROC ANOVA. Except for RE ($P < 0.05$), the homogeneity assumption was met for all the variables. Log_{10}-transformation of RE, however, satisfied the assumption. Observed variables (with log_{10}-transformation for RE) were subjected to two-way analysis of variance (ANOVA) using Proc GLM with main factors being starch and cellulose inclusion. Normal distribution of the residuals was verified using Kolmogorov-Smirnov's test with PROC UNIVARIATE. Differences among treatment means were considered significant when $P < 0.05$.

3. Results

3.1. Feed intake and growth

Experimental characteristics, feed intake and growth performance of the fish is shown in Table 2.3. Mean initial fish weights were not significantly different ($P > 0.05$) between tanks assigned to high- and low-starch diets and between tanks assigned to diets with and without cellulose inclusion. Mean final fish weights and growth rates of fish fed low-starch diets were significantly higher than of fish fed high-starch diets ($P < 0.001$). There were no effects of cellulose inclusion levels on final fish weight or growth rates ($P > 0.05$). Starch levels did not influence feed intake significantly ($P > 0.1$). There was no effect of cellulose inclusion levels on absolute feed intake ($P > 0.1$). However, FI_{perc} and FI_{MBW} of fish fed diets with cellulose inclusion were significantly higher than of fish fed diets without cellulose inclusion ($P < 0.01$). There were no interaction effects between starch and cellulose inclusion levels on any of the observed variables ($P > 0.05$).

3.2. Body composition

Final body composition of the fish is shown in Table 2.4. Fish fed low-starch diets showed significantly higher dry matter, crude fat and energy contents, but lower ash contents than fish fed high-starch diets ($P < 0.05$). No effect of starch levels on crude protein content was found on a fresh weight basis ($P > 0.5$). On a dry matter basis, however, crude protein contents of fish fed low-starch diets were significantly higher than of fish fed high-starch diets ($P < 0.01$).

Energy contents of fish fed diets without cellulose inclusion were significantly higher than of fish fed diets with cellulose inclusion ($P < 0.05$). Cellulose inclusion levels had no effects on body dry matter, crude fat, crude protein or ash content ($P > 0.1$).

No interaction effects between starch and cellulose inclusion levels on the body contents of any of the components were observed ($P > 0.1$).

3.3. Nitrogen balance

Nitrogen balance data are shown in Table 2.5. Fish fed low-starch diets or diets without cellulose inclusion showed significantly higher GN, DN, BUN and RN than fish fed high-starch diets or diets with cellulose inclusion, respectively ($P < 0.01$). Fecal nitrogen losses of fish fed low-starch diets were significantly lower than of fish fed high-starch diets ($P < 0.01$). Cellulose inclusion levels had no effect on FN expressed in mg $fish^{-1}$ ($P > 0.1$). Fecal nitrogen losses, when expressed in mg $kg^{-0.8}$ d^{-1}, however, were significantly lower in fish fed diets without cellulose inclusion than in fish fed diets with cellulose inclusion ($P < 0.05$). Interaction effects between starch and cellulose inclusion levels were present on FN and RN ($P < 0.05$), but absent on GN, DN and BUN ($P > 0.1$).

Table 2.3. Feed intake and growth performance of Nile tilapia (*Oreochromis niloticus*) in the four dietary groups[1]

	Diet[2]				***P*-value**		
	LS-NOC	**HS-NOC**	**LS-CEL**	**HS-CEL**	Starch	Cellulose	Starch × Cellulose
Growth period (d)	42	42	42	42	--	--	--
No. of fish per tank	20	20	20	20	--	--	--
No. of tanks	4	4	4	4	--	--	--
Survival (%)	100	100	100	100	--	--	--
Initial body weight (g)	51.85 ± 1.56	52.69 ± 0.86	50.75 ± 1.41	52.20 ± 1.16	ns	ns	ns
Final body weight (g)	168.94 ± 6.82	140.22 ± 5.36	157.09 ± 11.05	137.18 ± 5.14	***	ns	ns
Feed intake							
Absolute (g d^{-1})	2.50 ± 0.13	2.39 ± 0.11	2.65 ± 0.24	2.51 ± 0.11	ns	ns	ns
FI_{perc} (% d^{-1})	2.67 ± 0.06	2.78 ± 0.11	2.97 ± 0.18	2.97 ± 0.07	ns	***	ns
FI_{MBW} (g $kg^{-0.8}$ d^{-1})	16.64 ± 0.45	17.02 ± 0.66	18.33 ± 1.19	18.11 ± 0.48	ns	**	ns
Growth							
Absolute (g d^{-1})	2.79 ± 0.14	2.08 ± 0.12	2.53 ± 0.25	2.02 ± 0.11	***	ns	ns
SGR (% d^{-1})	2.81± 0.07	2.33 ± 0.08	2.69 ± 0.14	2.30 ± 0.08	***	ns	ns
GR_{MBW} (g $kg^{-0.8}$ d^{-1})	18.54 ± 0.59	14.84 ± 0.59	17.47 ± 1.17	14.59 ± 0.61	***	ns	ns
FCR	0.90 ± 0.02	1.15 ± 0.04	1.05 ± 0.03	1.24 ± 0.02	***	***	ns

[1] Values represent means ± standard deviation of four replicates (tanks).

[2] LS-NOC = Low starch-no cellulose inclusion; HS-NOC = High starch-no cellulose inclusion; LS-CEL = Low starch-with cellulose inclusion; HS-CEL = High starch-with cellulose inclusion.

Table 2.4. Final body composition on a fresh weight basis of Nile tilapia (*Oreochromis niloticus*) in the four dietary groups[1]

	Diet[2]				***P*-value**[3]		
	LS-NOC	**HS-NOC**	**LS-CEL**	**HS-CEL**	Starch	Cellulose	Starch × Cellulose
Dry matter (g kg^{-1})	299 ± 5	283 ± 4	293 ± 9	282 ± 6	**	ns	ns
Crude fat (g kg^{-1})	96 ± 3	79 ± 5	92 ± 7	75 ± 4	*	ns	ns
Crude protein (g kg^{-1})	164 ± 3	160 ± 4	160 ± 5	162 ± 3	ns	ns	ns
Energy (kJ g^{-1})	7.52 ± 0.11	6.70 ± 0.26	7.11 ± 0.36	6.54 ± 0.19	***	*	ns
Ash (g kg^{-1})	40 ± 1	42 ± 1	39 ± 2	41 ± 1	**	ns	ns

[1] Values represent means ± standard deviation of four replicates (tanks).

[2] LS-NOC = Low starch-no cellulose inclusion; HS-NOC = High starch-no cellulose inclusion; LS-CEL = Low starch-with cellulose inclusion; HS-CEL = High starch-with cellulose inclusion.

[3] The effects of starch and cellulose inclusion levels and the interaction effect between starch and cellulose inclusion levels on body composition on a dry matter basis were exactly similar to those on a fresh weight basis, except for the effect of starch inclusion levels on the crude protein content on a dry matter basis being significant ($P < 0.001$).

Table 2.5. Nitrogen balance of Nile tilapia (*Oreochromis niloticus*) in the four dietary groups[1]

	Diet[2]				***P*-value**		
Parameter[3]	**LS-NOC**	**HS-NOC**	**LS-CEL**	**HS-CEL**	Starch	Cellulose	Starch × Cellulose
Expressed in mg per fish per 42 days							
GN	7323 ± 377	5712 ± 252	6516 ± 600	5174 ± 231	***	**	ns
FN	737 ± 38	914 ± 40	833 ± 77	863 ± 39	**	ns	*
DN	6586 ± 339	4798 ± 211	5683 ± 523	4311 ± 192	***	**	ns
BUN	3480 ± 245	2554 ± 195	2961 ± 368	2096 ± 57	***	**	ns
RN	3106 ± 97	2245 ± 74	2722 ± 231	2215 ± 161	***	*	*
Expressed in mg $kg^{-0.8}$ d^{-1}							
GN	1160 ± 31	969 ± 38	1071 ± 70	888 ± 23	***	**	ns
FN	117 ± 3	155 ± 6	137 ± 9	148 ± 4	***	*	***
DN	1043 ± 28	814 ± 32	934 ± 61	740 ± 19	***	***	ns
BUN	551 ± 25	433 ± 33	487 ± 52	360 ± 8	***	**	ns
RN	492 ± 4	381 ± 6	447 ± 23	380 ± 21	***	*	*

[1] Values represent means ± standard deviation of four replicates (tanks).

[2] LS-NOC = Low starch-no cellulose inclusion; HS-NOC = High starch-no cellulose inclusion; LS-CEL = Low starch-with cellulose inclusion; HS-CEL = High starch-with cellulose inclusion.

3.4. Energy balance

Energy balance data are shown in Table 2.6. Fish fed low-starch diets showed significantly higher GE, FE, DE, BUE, ME and RE than fish fed high-starch diets ($P < 0.001$). Neither starch nor cellulose inclusion levels had effects on HP ($P > 0.1$). Whereas GE and FE were significantly lower, DE, BUE, ME and RE were significantly higher in fish fed diets without cellulose inclusion than in fish fed diets with cellulose inclusion ($P < 0.05$). There were no interaction effects between starch and cellulose inclusion levels on any of the energy balance data ($P > 0.1$), except on FE ($P < 0.05$).

4. Discussion

Despite the unclear mechanisms involved in feed intake regulation in fish, dietary energy content has been recognized as a major factor (Fletcher, 1984). This is supported by a large number of studies on different fish species, including Nile tilapia (Kubaryk, 1980), rainbow trout (*Oncorhynchus mykiss*) (Grove et al., 1978; Kaushik and Médale, 1984; Boujard and Médale, 1994; Morales et al., 1994; Yamamoto et al., 2000), Atlantic salmon (*Salmo salar*) (Bendiksen et al., 2002), Artic charr (*Salvelinus alpinus*) (Jobling and Wandsvik, 1983), goldfish (*Carassius auratus*) (Rozin and Mayer, 1961), gilthead seabream (*Sparus aurata*) (Marais and Kissil, 1979), seabass (*Dicentrarchus labrax*) (Dias et al., 1998), turbot (*Scophthalmus maximus*) (Bromley, 1980), channel catfish (*Ictalurus punctatus*) (Page and Andrews, 1973) and mummichog (*Fundulus heteroclitus*) (Weisberg and Lotrich, 1982). The findings from these studies suggest that fish regulate feed intake to maintain a constant level of digestible energy intake, given a specific nutritional state (e.g. body composition) and environmental and social conditions. In this view, digestible energy intake of fish fed different diets should have been the same. Since the energy contents of the high-starch diets were lower than of low-starch diets in the present study, fish fed high-starch diets should have compensated the low dietary energy contents by increasing feed intake. The results, however, showed that starch inclusion levels had no effect on feed intake (in g d^{-1}, % d^{-1} or g $kg^{-0.8}$ d^{-1}), and that DE intake (in kJ $fish^{-1}$ or kJ $kg^{-0.8}$ d^{-1}) of fish fed high-starch diets was significantly lower than of fish fed low-starch diets. Mean FI_{MBW} of fish fed high-starch diets increased by only 0.5% compared with that of fish fed low-starch diets. By comparing mean DE intake of fish fed high-starch diets with that of fish fed low-starch diets, it can be seen that roughly 13.9% of the energy requirement (in kJ $kg^{-0.8}$ d^{-1}) could not be captured by the fish fed high-starch diets. The failure of fish fed high-starch diets to achieve the same DE intake as fish fed low-starch diets was also expressed via their significantly lower RN, RE and growth rates, considering that the protein to energy ratio was the same for all the diets. This failure must have been caused by a certain factor, which prevented further ingestion of food before the requirement for digestible energy was fulfilled.

Table 2.6. Energy balance of Nile tilapia (*Oreochromis niloticus*) in the four dietary groups[1]

	Diet[2]				*P*-value		
Parameter[3]	**LS-NOC**	**HS-NOC**	**LS-CEL**	**HS-CEL**	Starch	Cellulose	Starch × Cellulose
Expressed in kJ per fish per 42 days							
GE	2179 ± 112	1720 ± 76	2333 ± 215	1864 ± 83	***	*	ns
FE	397 ± 20	289 ± 13	715 ± 66	525 ± 23	***	***	*
DE	1782 ± 92	1430 ± 63	1618 ± 149	1339 ± 60	***	*	ns
BUE	86 ± 6	63 ± 5	74 ± 9	52 ± 1	***	**	ns
ME	1696 ± 86	1367 ± 59	1544 ± 141	1287 ± 59	***	*	ns
HP	770 ± 49	777 ± 50	763 ± 44	736 ± 38	ns	ns	ns
RE[4]	926 ± 46	590 ± 69	782 ± 116	550 ± 44	***	*	ns
Expressed in kJ $kg^{-0.8}$ d^{-1}							
GE	345 ± 9	292 ± 11	383 ± 25	320 ± 8	***	***	ns
FE	63 ± 2	49 ± 2	118 ± 8	90 ± 2	***	***	**
DE	282 ± 8	243 ± 9	266 ± 17	230 ± 6	***	*	ns
BUE	14 ± 1	11 ± 1	12 ± 1	9 ± 0	***	**	ns
ME	269 ± 7	232 ± 9	254 ± 16	221 ± 6	***	*	ns
HP	122 ± 5	132 ± 10	126 ± 7	126 ± 5	ns	ns	ns
RE[4]	147 ± 4	100 ± 10	128 ± 15	94 ± 7	***	*	ns

[1] Values represent means ± standard deviation of four replicates (tanks).

[2] LS-NOC = Low starch-no cellulose inclusion; HS-NOC = High starch-no cellulose inclusion; LS-CEL = Low starch-with cellulose inclusion; HS-CEL = High starch-with cellulose inclusion.

[3] GE = Gross energy intake; FE = Fecal energy losses; DE = Digestible energy; BUE = Energy in branchial and urinary excretory products; ME = Metabolizable energy; HP = Heat production; RE = Retained energy.

[4] Values were $\log_{10}$-transformed before statistical analyses.

Changes in body composition, particularly in body fat content, might influence feed intake of the experimental fish. In mammals, a negative effect of body fat content on feed intake was originally articulated by Kennedy (1953), in what is known as the "lipostatic model". According to this model, energy intake is regulated by body fat stores to maintain a "set-point" of body fatness. This regulation is undertaken by leptin, an anorexigenic hormone secreted in proportion to the amount of adipose tissue (Friedman and Halaas, 1998; Hynes and Jones, 2001; Harvey and Ashford, 2003; Neary et al., 2004). Leptin has been found in several fish species (Black, 1988; Johnson et al., 2000) and is suggested to play a role in lipid metabolism and feed intake control in fish (Londraville and Duvall, 2002; Volkoff et al., 2003). In the present study, body fat contents of fish fed high-starch diets were significantly lower than of fish fed low-starch diets. However, the negative feedback of the higher accumulated body fat amount on feed consumption in fish fed low-starch (high-fat) diets was

not explicitly expressed. This rejects the hypothesis that in the present study body fat reserves controlled feed intake, causing lower DE intake of fish fed high-starch diets in comparison with that of fish fed low-starch diets.

In view of the differences in the dietary starch and energy levels, lower DE intake in fish fed high-starch diets could possibly be due to an effect of blood glucose or of stomach fullness and gastric emptying rates. It is, however, important to determine which of these two factors took precedence over the other.

It has been demonstrated that maximum food consumption in fish is closely correlated to stomach fullness and gastric emptying rates (Brett and Higgs, 1970; Adron et al., 1973; Elliott, 1975; Holmgren et al., 1983; Grove et al., 1985). In the present study, although feed intake (FI_{perc} and FI_{MBW}) of fish fed diets with cellulose inclusion were significantly higher than of fish fed diets without cellulose inclusion, DE intake of fish fed diets with cellulose inclusion were significantly lower than of fish fed diets without cellulose inclusion. Quantitatively, mean FI_{MBW} and DE intake (kJ $kg^{-0.8}$ d^{-1}) of fish fed diets with cellulose inclusion increased and decreased by 8.3% and 5.5%, respectively, compared with those of fish fed diets without cellulose inclusion. Referring to the dilution factor of 17.5%, this indicates that fish fed diets with cellulose inclusion compensated only a part of the diluted energy density, suggesting the role of the stomach volume in restricting feed consumption.

The replacement of dietary fat with starch made the high-starch diets 17.5% more diluted than the low-starch diets, which caused an increase in feed intake (g $kg^{-0.8}$ d^{-1}) of 0.5% and a decrease in DE intake (kJ $kg^{-0.8}$ d^{-1}) of 13.9%. The increase in feed intake when diets were diluted by starch was smaller than when diets were diluted by cellulose, although the dilution percentage in these two cases was the same. In line with this, the decrease in DE intake when diets were diluted by starch was larger than when diets were diluted by cellulose. Presuming that stomach volume restricted feed intake of fish fed diets with cellulose inclusion, as argued in the preceding paragraph, this implies that high starch levels hampered feed intake of the fish before their stomachs were full, suggesting the role of blood glucose in feed intake regulation in Nile tilapia. This is in accordance with the observation that feed intake of fish fed HS-NOC diet was numerically lower than of fish fed LS-CEL, although these two diets were isocaloric (Table 2.2).

One striking outcome from the present study is that, among the observed variables for energy balance, only heat production was not affected by starch nor cellulose inclusion levels. According to Bureau et al. (2002), energy utilized for basal metabolism, food ingestion and processing and physical activities is released as heat, which can be estimated by measuring oxygen consumption. Thus, oxygen consumption represents heat production in animals. Unlike terrestrial animals, fish live in water which contains a limited amount of oxygen. Moreover, fish take up oxygen through the gill surface area, which is also limited. As a

consequence, all the processes that need energy depend on the maximum oxygen uptake capacity in fish. This has been demonstrated by a number of studies in which feed intake or scope for activity or for production in fish decreased with decreasing oxygen concentration or reduced gill surface area (Dahlberg et al., 1968; Becker and Fishelson, 1986; Duthie and Hughes, 1987; Tsadik and Kutty, 1987; Buentello et al., 2000; Tran-Duy et al., 2008). Pauly (1981) hypothesized that fish stop eating when oxygen supply does not satisfy oxygen demand. From this point of view, the absence of significant differences in heat production among fish fed different diets in the present study might be due to the fact that under satiation feeding, the fish reached the maximum capacity to take up oxygen for food processing. Thus, lower DE intake in fish fed high-starch diets than in fish fed low-starch diets might not be due to an effect of blood glucose or dietary volume, but might simply reflect the maximum level of oxygen uptake for food consumption and processing. The effect of dissolved oxygen on maximum feed intake, energy metabolism and oxygen consumption in fish, therefore, should be systematically investigated.

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Chapter 3

Modeling fish growth using the concentration of metabolites to regulate feed intake and metabolism: a case study for rainbow trout (*Oncorhynchus mykiss*)

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Abstract

This study aimed at developing 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*), a species for which a considerable quantity of data on feed intake regulation and metabolism 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.

The model predicted fresh weight of the fish with an average relative error of −2.91% (range -32.47% to 26.89%). 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 animal growth can help improve profitability of animal enterprises, enhance understanding about biological systems and determine research priorities (Black, 1988; 1995). In aquaculture, modeling fish growth to predict fish production is important for planning of aquaculture development. During the past decades, 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. Because it is based on the biochemical reaction equations of the intermediary metabolism of fish, FGS is a general model that can in principle be applied to a wide variety of fish species. The success of FGS in growth prediction is attributed to its general, explanatory character. However, the equations for quantifying the maximum feed intake of the fish are, despite various revisions of the model (van der Meer and van Dam 1998; Verdegem et al. 2000), 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 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 evidence for lower feed intake of fish at reduced dissolved oxygen concentrations (Thetmeyer et al., 1999; Buentello et al., 2000; Pichavant et al., 2001). 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 (Pauly, 1981). At the level of metabolites, oxygen supply affects the oxidation rates of nutrients and thus affects the accumulation of metabolites in the cells and the blood.

A dynamic explanatory model for fish growth, which incorporates the regulation of feed intake by blood metabolites should predict the levels of these metabolites in the blood. In the present study, we aimed to develop such a model using metabolite pools (amino acids, glucose, fatty acids and acetyl CoA) which are considered to be essential in the metabolic regulation of growth and potential regulators of feed intake. Such a growth model based on metabolite pools could possibly be expanded in the future to simulate feed intake regulation. In the present study, the model was parameterized and calibrated for rainbow trout (*Oncorhynchus mykiss*), because of the large amount of relevant information available for this species. In line with the glucostatic theory (Mayer, 1953; 1955), 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, we subsequently used the parameterized model to test the potential role of blood glucose on feed intake.

2. Model description

2.1. Conceptualization

The main elements of the model and their relations are depicted in Figure 3.1 using the symbols developed by Forrester (1961). The following assumptions were made:

Digestion and absorption of dietary protein and carbohydrate result in amino acids and glucose, respectively; digestion and absorption of dietary fat result in fatty acids and glycerol. All glycerol arising from the hydrolysis of dietary and body fat is converted to glucose, and all glycerol required for fat synthesis is derived from glucose. Pools of metabolites, including amino acids, glucose, fatty acids and acetyl CoA represent both blood pools and cell pools without discrimination. Acetyl CoA was incorporated in the model since it is an intermediate substrate in the conversion of glucose and amino acids to fatty acids and in the aerobic oxidation of amino acids, glucose and fatty acids (Stryer, 1988).

Body constituents in the model include protein and fat. Glycogen, which accounts for a very small proportion (< 1%) of body weight in fish (From and Rasmussen, 1984; Bureau et al., 2002), was neglected. The amino acid profile of body protein is constant for a given species. Assuming that the biological value of dietary protein is one, the compositions of the amino acid pool and body protein were considered identical. Body fat comprises only tri-oleylglycerol ($C_{57}O_6H_{104}$), since triacylglycerols represent a large proportion of animal lipids (Jobling, 2001) and oleic acid (C18:1) is the most abundant fatty acid in fish (Love, 1980; Henderson and Tocher, 1987). Assuming that dietary fat is made up of fish oil, the fatty acid pool, therefore, comprises only oleic acid. All NADPH required for synthesis of fatty acids was assumed to be generated from the pentose phosphate pathway, in which glucose 6-phosphate originates from glucose.

The energy requirement (metabolic rate) is the sum of routine metabolic rate, feeding metabolic rate and energy required for biosynthesis; routine metabolic rate refers to the energy requirement of fish with spontaneous activities under fasting condition. Energy is supplied by oxidation of acetyl CoA, which is provided by oxidation of amino acids, fatty acids or glucose. These processes are driven by total energy requirement.

Growth of the fish was calculated based on the relationship between body weight and protein biomass. To test the potential role of blood glucose on feed intake, feeding rate of the fish was set at 0 when glucose concentration was higher than a threshold. When glucose concentration was below the threshold, feeding rate was determined based on daily ration. During simulation, at each time step the glucose concentration was checked and compared

with the threshold and feed intake was adjusted accordingly. The model was run with different values of threshold to see how this threshold affected feed intake and growth of fish.

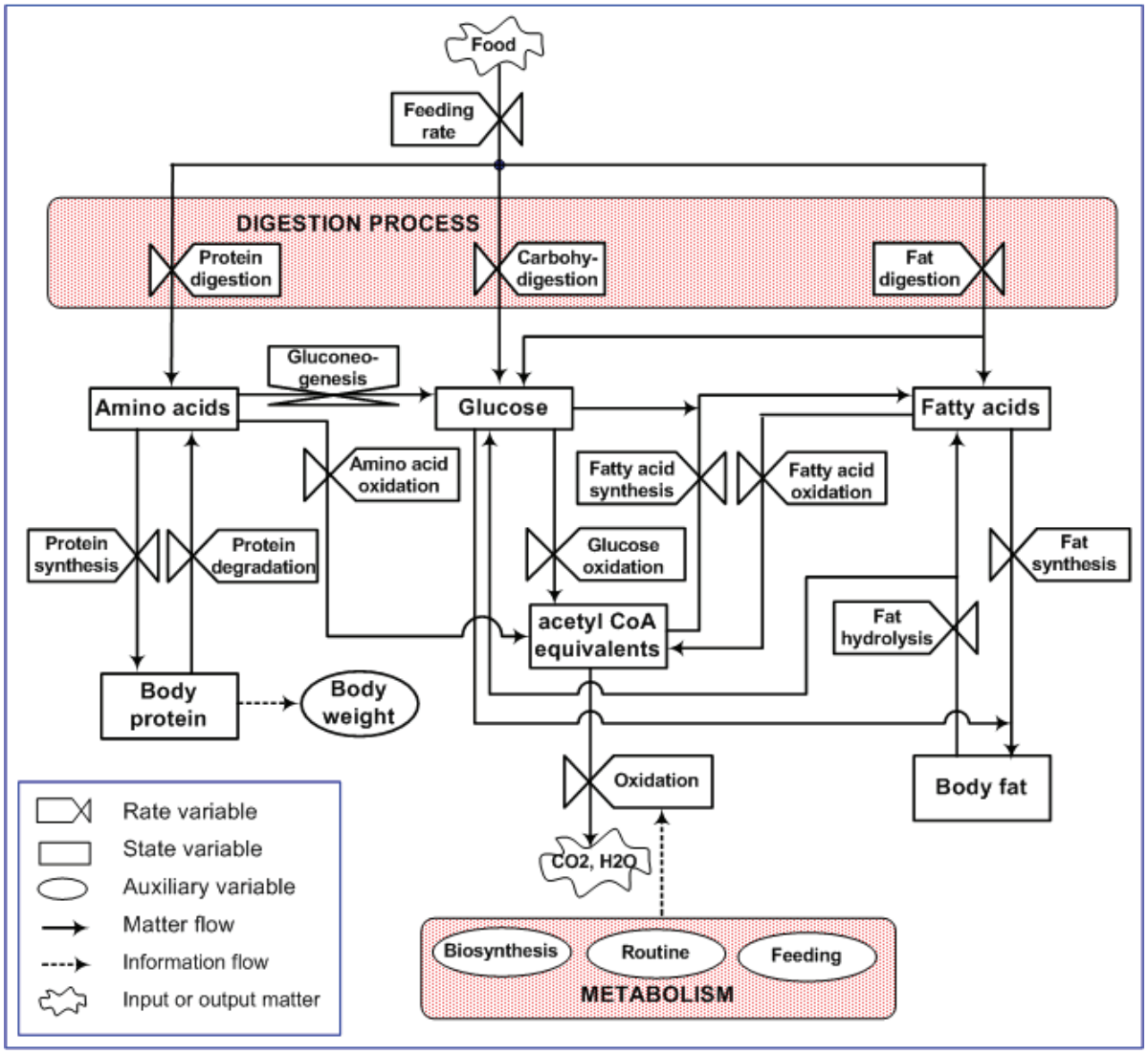

Figure 3.1. Relational diagram of a simulation model for fish growth using the symbols of Forrester (1961).

2.2. Formulation of rates

2.2.1. Conversion rate

In the model, a conversion of state variable Y to Z was designated as Y-Z conversion, with Y as main substrate and Z as product. For a Y-Z conversion, the model calculated two kinds of rates: rate of utilization of Y and rate of production of Z. Concepts of enzyme kinetics were adapted to formulate the mathematical equations representing the utilization rates.

According to the theory about enzyme action and kinetics, the rate of an enzyme-catalyzed reaction increases with increasing substrate concentrations until a maximum rate is reached (Stryer 1988). This concept was applied to the whole pathway of a conversion in the

model, since each single reaction in the pathway is mediated by enzymes. A general equation representing a utilization rate for a conversion of two substrates S_1 and S_2 into one product I was formulated by Gill et al. (1989) as:

$$Ru = \frac{Ru_{max}}{1 + \left(\frac{K_1}{[S_1]}\right)^{T_1} + \left(\frac{K_2}{[S_2]}\right)^{T_2} + \left(\frac{[I]}{J}\right)^{Ti}} \quad \textbf{(3.1)}$$

where Ru = rate of utilization of the main substrate (mmol or g per day), Ru_{max} = maximum utilization 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 which have stimulation effects on the conversion, respectively (mmol per g body weight), [I] = concentration of substrate or product I which has inhibition effect on the conversion (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.

Based on the physiological characteristics of the intermediary metabolism in fish, Equation 3.1 was simplified to the following forms, which were applied to different conversions:

$$Ru = \frac{Ru_{max}}{1 + \frac{K}{[S]}} \quad \textbf{(3.2)}$$

$$Ru = \frac{Ru_{max}}{1 + \frac{K_1}{[S_1]} + \frac{K_2}{[S_2]}} \quad \textbf{(3.3)}$$

$$Ru = \frac{Ru_{max}}{1 + \frac{[I]}{J}} \quad \textbf{(3.4)}$$

$$Ru = \frac{Ru_{max}}{1 + \frac{K}{[S]} + \frac{[I]}{J}} \quad \textbf{(3.5)}$$

Concentrations of the substrates (metabolites) in the model were expressed as mmol substrate per gram body weight, assuming that the metabolites are distributed throughout the entire body. Effects of substrate concentration on the utilization rates (stimulation or inhibition), which determines the application of Equation 3.2-3.5 to the rates, are depicted in Figure 3.2.

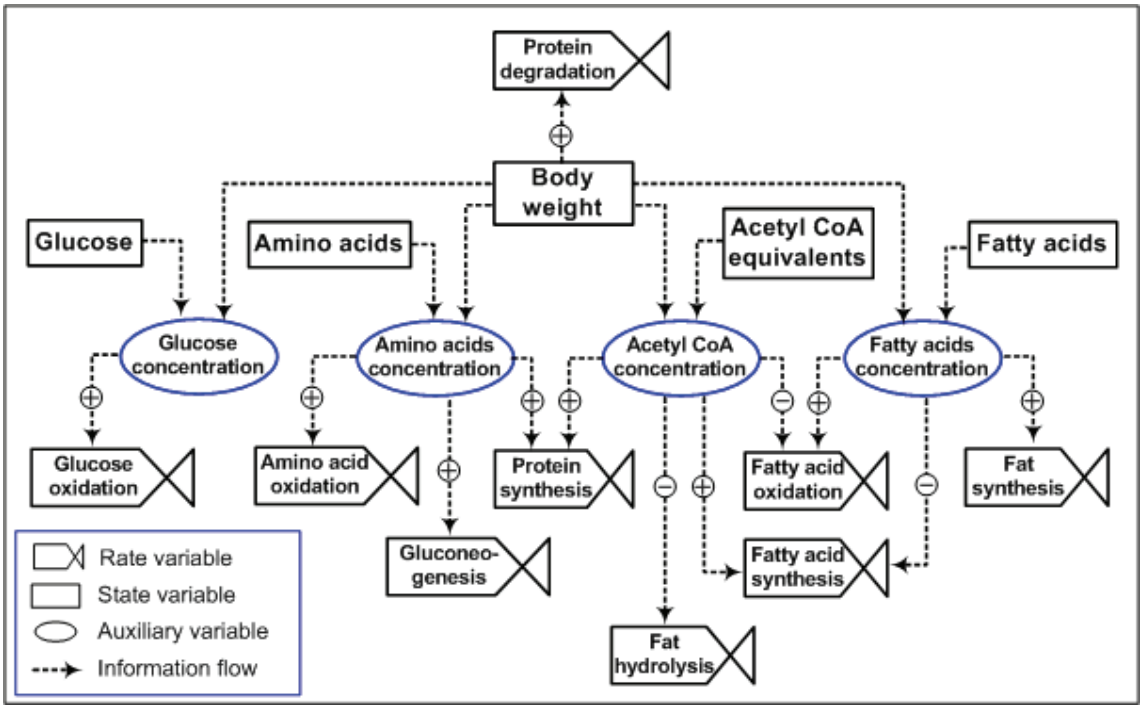

Figure 3.2. Schematic representation of the effects of substrate concentrations on the utilization rates. Flows with plus signs indicate stimulation effect and with minus signs indicate inhibition effect.

The common numerator of the equations representing the maximum utilization rates, Ru_{max}, was quantified by considering that the maximum rate of an enzyme-catalyzed reaction depends on the amount and activity of the enzyme. For a conversion, Ru_{max} was assumed to be proportional to the size of the tissue where the conversion takes place because the size of the tissue is supposed to proportionally affect the amount or activity, or both, of the enzymes located in that tissue. If a conversion takes place throughout a tissue, Ru_{max} was calculated as: $Ru_{max} = Rr_{max} \times$ tissue weight; if the conversion is related to the surface of a tissue, Ru_{max} was calculated as: $Ru_{max} = Rr_{max} \times (\text{tissue weight})^{0.67}$; if the conversion is related to routine metabolism, Ru_{max} was calculated as: $Ru_{max} = Rr_{max} \times (\text{body weight})^{0.8}$, where Rr_{max} represents the maximum relative rate of utilization of the main substrate in the conversion.

The denominators of the equations representing the utilization rates consist of two kinds of terms; the substrate or product concentration is an auxiliary variable which can be calculated from the corresponding state variable and body weight; and the Michaelis-Menten affinity or inhibition is the parameter which needs to be estimated.

Equation 3.2, 3.3, 3.4 or 3.5 were used to calculate the rate of utilization of Y in a Y-Z conversion. If this conversion required participation of substrate X, the rate of utilization of X was calculated as follows:

Rate of utilization of X = Requirement for X per unit of Y × Rate of utilization of Y

Rate of production of Z was calculated as follows:

Rate of production of Z = Yield of Z from one unit of Y × Rate of utilization of Y

When the rate of utilization of the main substrate is quantified, the rates of utilization of the other substrates and of production of the products can be calculated based on the stoichiometric coefficients of the biochemical reaction equations of the conversions (see 3.4).

2.2.2. Metabolic rate

The metabolic rate is the sum of routine metabolic rate, feeding metabolic rate and energy required for biosynthesis. Based on a study of Winberg (1956), routine metabolic rate was calculated as:

$$Mr = Q_{10}^{(T_1-T_2)/10} \times \alpha \times W^{\beta} \quad \textbf{(3.6)}$$

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

Feeding metabolic rate represents energy requirements for feeding activities (capture of food and mechanical work of the gastrointestinal tract) and absorption (transport of the nutrients across the gut mucosa). Since no data are available to quantify energy requirement for feeding activities, the model did not account for this. However, it should be noticed that as a result of protein turnover, the cost for routine metabolism includes part of the total costs for protein synthesis and degradation. Since the models did not partition the total costs for protein synthesis and degradation, the cost for protein turnover in routine metabolism was double-counted. Assuming that this double-counted cost and the costs for feeding activities cancel each other out in the model, no correction was made for ignoring the energy requirements of feeding activities in the total metabolic rate. Energy requirement for absorption was calculated based on the absorption rate, i.e. the rate at which the digested nutrients (amino acids, fatty acids and glucose) pass the gut mucosa, and the transport costs of the nutrients.

Energy requirement for biosynthesis was calculated based on the rates of utilization of amino acids and fatty acids for body protein and fat synthesis, and ATP requirements in these conversions.

2.3. Model variables and parameters

For ease of recognition, we used the following prefixes for variable names and parameters: S_, state variable; R_, rate variable; A_, auxiliary variable; and P_, parameter. The abbreviations with meanings and units in Table 3.1 indicate the entities which form part of the

variable and parameter names. Notations and units of the variables and parameters are shown in Table 3.2.

Table 3.1. Two-letter abbreviations of the entities and their units used in the model

Abbreviation	Entity	Unit
Aa	Amino acids	mmol
Am	Ammonia	mmol
At	ATP	mmol
Ay	Acetyl CoA	mmol
Bw	Body weight	g
Ca	Carbon dioxide	mmol
Cd	Dietary carbohydrate	g
Fa	Fatty acids	mmol
Fb	Body fat	g
Fd	Dietary fat	g
Gl	Glucose	mmol
Ox	Oxygen	mmol
Pb	Body protein	g
Pd	Dietary protein	g

3. Parameterization

3.1. Maximum relative rate of utilization

As shown in the relational diagram (Figure 3.1), the following conversions were considered.

3.1.1. Amino acid-Body protein (protein synthesis)

In fish, it is believed that protein synthesis rates above maintenance ration increase with increasing protein intake (Houlihan et al. 1993). Fauconneau and Arnal (1985) reported that protein synthesis in the whole body of rainbow trout acclimatized at 18^{o}C was higher than that in rainbow trout acclimatized at 10^{o}C. In both cases, fish were fed to satiation with a diet containing 52.5% protein. With estimated protein content being 137.9 g/kg body weight in this experiment, and 8.78 mmol amino acids being required for synthesis of 1 g protein (see 3.4), the relative rate of utilization of amino acids in protein synthesis in rainbow trout at 18^{o}C was estimated to be 0.487 mmol amino acids/g body protein/d. To account for the effects of higher protein intake and temperature on the rate of protein synthesis, this value was arbitrarily increased by 25% before being assigned to $P\_Ur_{Aa,AaPb}$, which was set at 0.609 mmol/g body protein/d.

3.1.2. Body protein-Amino acid (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, rate of utilization of protein in protein degradation ($R\_Ut_{Pb,PbAa}$) was calculated as $R\_Ut_{Pb,PbAa} = P\_Ur_{Pb,PbAa} \times (\text{body protein})^{0.8}$, where $P\_Ur_{Pb,PbAa}$ is the relative rate of protein degradation; $P\_Ur_{Pb,PbAa}$ was set at 0.025 g body protein/(g body protein)$^{0.8}$/d, based on a report of Dobly et al. (2003).

Table 3.2. Notation representing variables and parameters in the model

Notation[a]	Interpretation	Unit[b]
R_Di_x	Digestion rate of x	(g *x*)/d (dry weight)
$R\_Pr_{x,yz}$	Rate of production of x in y-z conversion	(mmol or g *x*)/d
$R\_Ut_{x,yz}$	Rate of utilization of x in y-z conversion	(mmol or g *x*)/d
S_Po_x	Pool of x	mmol or g *x*
A_Co_x	Concentration of x	(mmol x)/g body weight
A_Pi_x	Initial pool of x	mmol or g *x*
$A\_Re_{Ay,yz}$	Requirement for acetyl CoA in y-z conversion	mmol Ay/d
$A\_Um_{x,yz}$	Maximum rate of utilization of x in y-z conversion	(mmol or g *x*)/d
P_Ab_x	Absorption cost for x	mmol ATP/mmol x
P_Ci_x	Initial concentration of x	(mmol x)/g body weight
P_Fr_x	Fractional degradation rate of x	(g x)/(g *x*)$^{0.8}$/d
$P\_Ji_{x,yz}$	Inhibition constant for x in y-z conversion	(mmol x)/g body weight
$P\_Km_{x,yz}$	Affinity constant for x in y-z conversion	(mmol x)/g body weight
$P\_Re_{x,yz}$	Requirement for x in y-z conversion	(mmol *x*)/(mmol or g *y*)
$P\_Ur_{x,yz}$	Maximum relative rate of utilization of x in y-z conversion	(mmol or g *x*)/(g *tissue*)^{k}/d [c]
$P\_Yi_{x,yz}$	Yield of x in y-z conversion	(mmol or g *x*)/(mmol or g *y*)

[a] x, y and z denote the entities of the model. See Table 3.1 for details.

[b] Selection of unit for (mmol or g *x*) or (mmol or g *y*) is based on unit of *x* or *y*. See Table 3.1 for details.

[c] k = 1 if the conversion takes place throughout the tissue; k = 0.8 if the conversion rate is related to routine metabolic rate; k = 0.67 if the conversion rate is related to the surface of the tissue.

3.1.3. Amino acid-Acetyl CoA (amino acid oxidation)

This conversion is likely to take place in the liver since a majority of ammonia is produced in the liver of the fish as a result of amino acid deamination (Jobling 1993). Assuming that the liver biomass is directly proportional to body weight, the maximum relative rate of utilization

of amino acid in amino acid oxidation ($P\_Ur_{Aa,AaAy}$) was expressed in mmol amino acids/g body weight/d. Since amino acids are deaminated in the Aa-Ay conversion, $P\_Ur_{Aa,AaAy}$ can be estimated based on the amount of ammonia excreted after the fish have ingested an extremely large amount of protein, assuming that all nitrogen is excreted as ammonia. Because there is no published data like that in rainbow trout, we used the data reported by Kaushik and de Oliva Teles (1985) in a study in which rainbow trout were fed to satiation diets containing 40-43% protein. The excretion rate of ammonia was found to be in the range of 0.71-1.34 g NH_3/kg body weight/d, equivalent to 0.051 to 0.095 mmol NH_3/g body weight/d. With a yield of 1.28 mmol NH_3 from 1 mmol amino acid in Aa-Ay conversion (see 3.4), the relative rate of utilization of amino acids in Aa-Ay conversion varied from 0.039-0.075 mmol amino acids/g body weight/d. To account for possible higher protein intake, $P\_Ur_{Aa,AaAy}$ was set at 0.094 mmol amino acids/g body weight/d, which was obtained by arbitrarily increasing the upper bound of the above-mentioned range (0.075) by 25%.

3.1.4. Amino acid-Glucose (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 observations of Panserat et al. (2000, 2001a, 2001b) in which the level of some gluconeogenic enzymes was persistently high and the expression of some was independent of the dietary carbohydrate content, we assumed that gluconeogenesis always occurs in rainbow trout and depends only on the amino acid concentration. 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-Acetyl CoA (glucose oxidation)

Lauff and Wood (1996a) found that the use of carbohydrate (sum of glucose, lactate and glycogen) as fuel increased with increasing swimming speeds, and the contribution of carbohydrate as fuel to total oxygen consumption can be up to 38% in rainbow trout swimming at a high speed. The rate of oxygen consumption in rainbow trout swimming at a high speed was estimated to be 0.24 mmol/g body weight/d, based on the results from a study of Kieffer et al. (1998). Assuming that 38% of the oxygen consumption of the fish (Lauff and Wood, 1996a) came from complete oxidation of glucose, the maximum relative rate of utilization of glucose in glucose oxidation ($P\_Ur_{Gl,GlAy}$) was estimated to be 0.015 mmol glucose/g body weight/d (6 mol O_2 is needed for complete oxidation of 1 mol glucose; see 3.4).

3.1.6. Fatty acid-Acetyl CoA (fatty acid oxidation)

It is well-known that dietary fat supply can reduce the oxidation of protein to meet the energy requirement of fish (Watanabe, 1982). This implies that more fats will be oxidized with higher fat ingestion. However, fat ingested in excess of energy demand will be deposited as body fat reserves (Shearer, 1994; Sargent et al., 2002). Because the nutritional conditions under which the maximum rate of fatty acid oxidation occurs were not known, maximum relative rate of fatty acid oxidation ($P\_Ur_{Fa,FaAy}$) was estimated based on routine metabolic rate, assuming that all the energy supply in fasting fish originates from fatty acid oxidation (Dabrowski and Guderley, 2002; Simpskin et al., 2003). Lauff and Wood (1996b) reported that the average rates of utilization of carbon in lipid, protein and carbohydrate oxidation after three days of starvation in rainbow trout were about 45, 11 and 19 µg/g body weight/h, respectively. Using the weight fraction of carbon and the stoichiometry of the oxidation of fatty acids, amino acids and glucose (see 3.4), the routine metabolic rate was estimated to be 1.47 mmol ATP/(g body weight)$^{0.8}$/d. If only fatty acids were oxidized to supply all the energy for routine metabolism, the rate of oxidation of fatty acids would be 0.010 mmol/(g body weight)$^{0.8}$/d. This value was assigned to $P\_Ur_{Fa,FaAy}$.

3.1.7. Acetyl CoA-Fatty acid (fatty acid synthesis)

According to Henderson and Sargent (1981), the liver is the main site of fatty acid synthesis in rainbow trout. With the same assumption as in 3.1.3, the maximum relative rate of fatty acid synthesis ($P\_Ur_{Ay,AyFa}$) was expressed in mmol acetyl CoA/g body weight/day. Because there has been no direct measurement of the maximum rate of *de novo* fatty acid synthesis, $P\_Ur_{Ay,AyFa}$ was assumed to be the rate at which the fatty acids yielded are equivalent to those contained in the amount of body fat resulted from a maximum rate of fat accretion. Using the experimental data reported by Gélineau et al. (2001), a fat accretion rate of 3.66 g fat/kg body weight/d in rainbow trout was estimated; because the fish were fed to satiation a diet containing very high protein and lipid contents (42.5% and 30.2%, respectively), this rate of fat accretion was assumed to be maximal (Reinitz et al., 1978; Kellems and Sinnhuber, 1982; Jobling et al., 1998; Gélineau et al., 2001). Assuming a degradation rate of 1% of body fat per day (Lauff and Wood, 1996b), $P\_Ur_{Ay,AyFa}$ was set at 0.108 mmol/g body weight/d.

3.1.8. Fatty acid-Body fat (fat synthesis).

The esterification of fatty acids takes place mainly in the adipose tissue. Because the adipose tissue is not as richly vascularized as other tissues (Vernon and Clegg, 1985), 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) (see 3.1.3), maximum relative rate of utilization of fatty acids in fat synthesis ($P\_Ur_{Fa,FaFb}$) was set at 0.523 mmol fatty acids/(g body fat)$^{0.67}$/d.

3.1.9. Body fat-Fatty acid (fat degradation).

This conversion takes place in adipose tissue. As reasoned in 3.1.8, the maximum relative rate of fat degradation (Rr_{FD}) was expressed in g body fat/(g body fat)$^{0.67}$/day. In animals, lipolysis is stimulated in fasting state (Bender, 2002; Jezierska et al., 1982). Thus, $P\_Ur_{Fb,FbFa}$ was estimated based on the depletion of body fat in fasting trout. 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. Initial substrate concentration

No data on metabolite concentration expressed in mmol/g body weight are available in fish. Estimates of normal concentrations of metabolites in blood plasma of rainbow trout were, therefore, used as initial metabolite concentrations.

Using the data reported by Thorpe and Ince (1976), Cowey et al. (1977b) and Yamamoto et al. (2000), plasma amino acid concentrations were found to be in the range of 0.0057-0.0080 mmol/ml. In a review of Plisetskaya (1980), plasma fatty acid concentrations were found to be in the range of 0.00012-0.00160 milli-equivalents/ml. Based on a review of Olsen (1989), plasma glucose concentrations were found to be in the range of 0.0028-0.0056 mmol/ml. In the model, the initial concentrations of amino acids (P_Ci_{Aa}), fatty acids (P_Ci_{Fa}) and glucose (P_Ci_{Gl}) were set at 0.0066, 0.00086 and 0.0042 mmol/g, which are the means of the lower and upper bounds of the corresponding above-mentioned ranges.

For acetyl CoA, no data on plasma concentration is available. The initial pool concentration of acetyl CoA (P_Ci_{Ay}), therefore, was arbitrarily set at 0.0045 mmol/g.

3.3. Affinity and inhibition constants

Affinity and inhibition 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 conversions and characteristics of the equations representing conversion rates. Estimated values of the affinity and inhibition constants are presented in Table 3.3 using the notation presented in Table 3.2.

Table 3.3. Affinity and inhibition constants of the model[a]

y-z conversion	$P\_Km_{y,yz}$	$P\_Km_{Ay,yz}$	$P\_Ji_{z,yz}$	$P\_Ji_{Ay,yz}$
Aa-Ay	0.066	-	-	-
Aa-Gl	0.0025	-	0.0336	-
Aa-Pb	0.0033	0.0023		-
Ay-Fa	0.0180	-	0.0017	-
Fa-Ay	0.0004	-	0.0090	-
Fa-Fb	0.0086	-		
Fb-Fa	-	-		0.0045
Gl-Ay	0.0210	-		-

[a] See Table 3.1 for abbreviations of the entities and Table 3.2 for notation of the parameters.

3.4. Stoichiometric coefficients

The stoichiometric coefficients were determined by establishing an overall reaction equation for each conversion based on the principle described by Penning de Vries et al. (1974) using fundamental biochemical reaction equations available in Lehninger (1975), Schulz (1978), Stryer (1988) and Bender (2002). The following assumptions were made:

(1) NADH and FADH on the right-hand side of the overall reactions, if present, will be re-oxidized in mitochondria; each mole NADH requires 0.5 mole O_2 and is linked to the production of 3 moles ATP; each mole FADH requires 0.5 mole O_2 and is linked to the production of 2 moles ATP. The transport of 1 mole NADH from the cytosol into the mitochondria requires 1 mole ATP, thus the re-oxidation of 1 mole NADH in the cytosol produces 2 moles ATP (Bender, 2002);

(2) One mole GTP is replaced by 1 mole ATP;

(3) Four moles ATP is required for formation of one peptide bond in protein synthesis (Carter and Houlihan, 2001) and one mole ATP is required for breakdown of one peptide bond in protein degradation (Rapoport, 1985).

(4) Ammonia is the only nitrogenous product of amino acid oxidation, since fish excrete a large proportion (can be > 80%) of their nitrogenous wastes as ammonia (Jobling, 1994).

Since there are in total 21 amino acids, the stoichiometric coefficients of the overall reaction equations for amino acid oxidation and gluconeogenesis were determined based on those of the reaction equation for each amino acid being converted to acetyl CoA (Table 3.4) or glucose (see Machiels and Henken, 1986) and the amino acid composition of rainbow trout, which was reported by Wilson and Cowey (1985) (Table 3.4). Mean molecular weight of amino acid (132 g) was calculated also based on the amino acid composition. The

calculation methods can be found in Olsen (1989). The stoichiometric parameters used in the model are presented in Table 3.5 using the notation presented in Table 3.2.

3.5 Metabolic parameters

3.5.1. Routine metabolism

Three parameters in the equation representing routine metabolic rate were Q_{10}, α, and β (see 2.2.2). According to Winberg (1956), Q_{10} depends on water temperature. Based on his suggested values, Q_{10} was set at 2.5 as optimal water temperatures for rainbow trout culture are in the range of 13-18^{o}C (Hardy, 2002). The rate coefficient (α) was set at 2.88 mmol ATP/g$^{0.8}$/day at 20^{o}C (van Dam, 1995). The rate exponent (β) was set at 0.8 (Winberg, 1956).

Table 3.4. Amino acid molecular weight and composition in rainbow trout (*Oncorhynchus mykiss*) and stoichiometric coefficients in the conversion of each amino acid to acetyl CoA (aCoA)

No.	Name	Mol weight (g)	g/100 g amino acids	O2 (mol) (a)	aCoA (mol) (b)	NH3 (mol) (c)	CO2 (mol) (d)	ATP (mol) (e)
1	Alanine	89	6.57	1.0	1	1	1	6
2	Arginine	174	6.41	2.5	1	4	4	15
3	Asparagine	132	0.05	1.0	1	2	2	6
4	Aspartic acid	133	9.94	1.0	1	1	2	6
5	Cysteine	121	0.80	2.0	1	1	1	15
6	Glutamine	146	0.00	2.5	1	2	3	15
7	Glutamic acid	147	14.22	2.5	1	1	3	15
8	Glycine	75	7.76	0.5	1	1	1	3
9	Histidine	155	2.96	2.5	1	3	3	15
10	Hydroxyproline	131	0.00	2.0	2	1	2	11
11	Isoleucine	131	4.34	5.5	1	1	3	31
12	Leucine	131	7.59	0.5	3	1	0	7
13	Lysine	146	8.49	1.0	2	2	0	17
14	Methionine	149	2.28	4.5	1	1	2	11
15	Phenylalanine	165	4.38	4.5	3	1	2	9
16	Proline	115	4.89	3.5	1	1	3	20
17	Serine	105	4.66	0.5	1	1	1	3
18	Threonine	119	4.76	0.5	2	1	1	4
19	Tryptophan	204	0.93	4.0	3	2	4	9
20	Tyrosine	181	3.38	3.5	3	1	2	9
21	Valine	117	5.09	4.0	1	1	2	22

1 amino acid + (a) O_2 → (b) aCoA + (c) NH_3 + (d) CO_2 + (e) ATP

Table 3.5. Values of stoichiometric parameters of the model[a]

y-z conversion	$P\_Yi_{z,yz}$	$P\_Yi_{Gl,yz}$	$P\_Yi_{At,yz}$	$P\_Re_{At,yz}$	$P\_Re_{Gl,yz}$
Aa-Ay	1.46	-	11.72	-	-
Aa-Gl	0.43	-	9.22	-	-
Aa-Pb	0.11	-	-	4.00	-
Pd-Aa	8.78	-	-		-
Pb-Aa	8.78	-	-	8.78	-
Fa-Ay	9.00	-	36.00	-	-
Fa-Fb	0.29	-	-	2.67	0.17
Fb-Fa	3.39	0.57	1.13	-	-
Fd-Fa	3.39	0.57	1.13	-	-
Gl-Ay	2.00	-	12.00	-	-
Cd-Gl	6.17	-	-	-	-
Ay-Fa	0.11	-	-	2.19	0.07
Ay-At	12.00	-	12.00	-	-

[a] See Table 3.1 for abbreviations of the entities and Table 3.2 for notation of the parameters.

3.5.2. Feeding metabolism: absorption and transport costs

The products of fat digestion are mono-acylglycerols and fatty acids (Tocher and Sargent, 1984**)**. The passage of these products across the membrane of the enterocytes occurs by passive diffusion, thus does not require energy. However, after passage across the brush-border, the fatty acids undergo a re-esterification to form tri-acylglycerol in the mucosal cells (Sire et al., 1981). Based on Lehninger (1975), the energy costs of the re-esterification were set at 1.33 mole ATP per mole fatty acid equivalent (1 mole mono-acylglycerol was considered to be equivalent to 1 mole fatty acid).

The product of carbohydrate digestion was assumed to be glucose, as stated in 2.1. The passage of glucose across the membranes of the enterocytes is a Na^+-dependent active process. According to Mandel and Balaban (1981), 1 mole ATP could be used to pump 3 moles Na^+ through the membrane, thus the energy costs for the absorption of glucose were set at 0.33 mol ATP per mole glucose.

The products of protein digestion are amino acids and peptides. The mechanisms of absorption of these products in fish were supposed to be comparable to higher vertebrates (Matthews, 1975a, b; Silk et al., 1985), which can involve Na^+-dependent and Na^+-independent active transport, passive diffusion (Smith, 1970; Cartier et al., 1979) and transport of intact peptides (Ash, 1980; Boge and Rigal, 1981). Because no quantitative information about the proportions of different products of protein digestion is available, we assumed that the total costs for protein absorption were equivalent to the cost for active

transport of a number of individual amino acid molecules equivalent to those in the absorbed products. This assumption was made by considering that the costs for re-absorption of endogenous secreted protein may average out the lower costs for the diffusion, and Na^+-independent and intact peptides transport. Therefore, in analogy to the absorption of glucose, energy costs for the absorption of digested protein were set at 0.33 mol ATP per mol amino acid equivalent.

For transport of nutrients across membranes other than the membranes of the enterocytes, the transport cost was set at 0.33 mol ATP per mol amino acid or glucose using the same above-mentioned approach.

3.6. Other parameters

In the study which generated the dataset used for model calibration (see 4), digestibility of nutrients were not reported. Thus, digestibility was estimated based on other studies on rainbow trout. A review by van Dam and Penning De Vries (1995) showed digestibility of fishmeal and purified proteins ranging from 85 to 90%. Nose (1967) reported digestibility of about 92% for fish meal and soybean meal. Since the fish in the simulation were fed diet containing both fish and soybean meal, protein digestibility was set at 90%. Based on a study of Vandenberg and De La Noüe (2001) in which digestibility of fat ranged from 75 to 92%, fat digestibility in the simulation was set at 80%. For digestibility of carbohydrate, Phillips (1969) reported a value of 57% for cooked starch and of 38% for raw starch. Since the type of the carbohydrate contained in the feed used in the simulation was not reported, carbohydrate digestibility was arbitrarily 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, above which fish stop eating, 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 Borland Delphi 7 (Borland Software Corporation, Texas, USA) 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 restricted 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

and lipid biomass. The agreement was assessed based on the relative error (RE) and the average relative error (ARE), which were calculated as follows:

$$RE_i = 100 \times \frac{SW_i - EW_i}{\frac{1}{2}(SW_i + EW_i)} \quad \textbf{(3.7)}$$

$$ARE = \frac{1}{n}\sum_{i=1}^{n} RE_i \quad \textbf{(3.8)}$$

where RE_i is the relative error for case i, SW_i and EW_i are the simulated and observed values for case i, respectively, and n is the number of cases.

Best agreement between simulated and observed fish weight (Figure 3.3a) and lipid biomass (Figure 3.3b) was achieved with the values of the calibrated parameters in Table 3.6. For fish weight, ARE was −2.91% with REs ranging from -32.47% to 26.89%. For lipid biomass, ARE was −8.26% with REs ranging from -101.27% to 118.80%. The calibration showed that the relative errors for lipid biomass could hardly be improved.

Plots of REs of simulated fish weight against final fish weight and against feeding level are given in Figures 3.4a and 3.4b, respectively. In the former plot, the dots appear to distribute relatively equally at both sides of the X-axis at each weight class (50 g interval), while in the latter plot all the dots at zero feeding level lie below and the other lie slightly more above the X-axis.

Plots of REs of simulated lipid biomass against final fish weight and against feeding level are given in Figure 3.5a and 3.5b, respectively. The dots in both plots do no show a clear bias in distribution in favor of any of the sides of the X-axis.

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.

Table 3.6. Calibrated versus estimated values of some of the parameters in a simulation model for growth of rainbow trout (*Oncorhynchus mykiss*)

Parameter	Estimated value	Calibrated value	Parameter	Estimated value	Calibrated value
$P\_Ur_{Aa,AaPb}$	0.609	1.100	$P\_Ur_{Pb,PbAa}$	0.025	0.030
$P\_Ur_{Aa,AaAy}$	0.094	0.075	$P\_Km_{Aa,AaAy}$	0.066	0.200
$P\_Ur_{Fa,FaFb}$	0.523	0.650	$P\_Km_{Ay,AaPb}$	0.0023	0.0015
$P\_Ur_{Fb,FbFa}$	0.043	0.02	$P\_Km_{Gl,GlAy}$	0.0042	0.0021

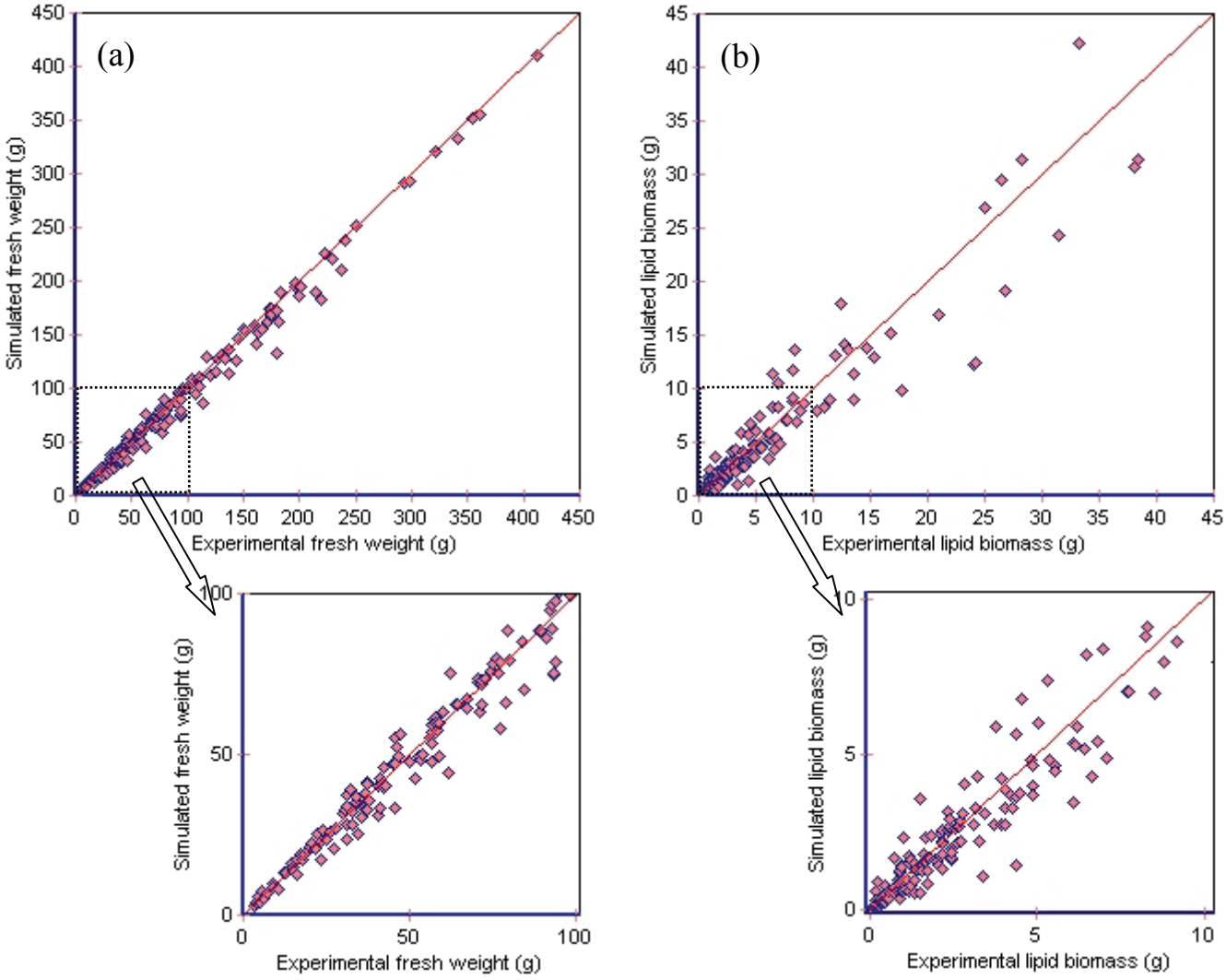

Figure 3.3. Calibration results of the model. The bisector represents best agreement between simulated and observed values. (a) Fish weight; (b) Lipid biomass.

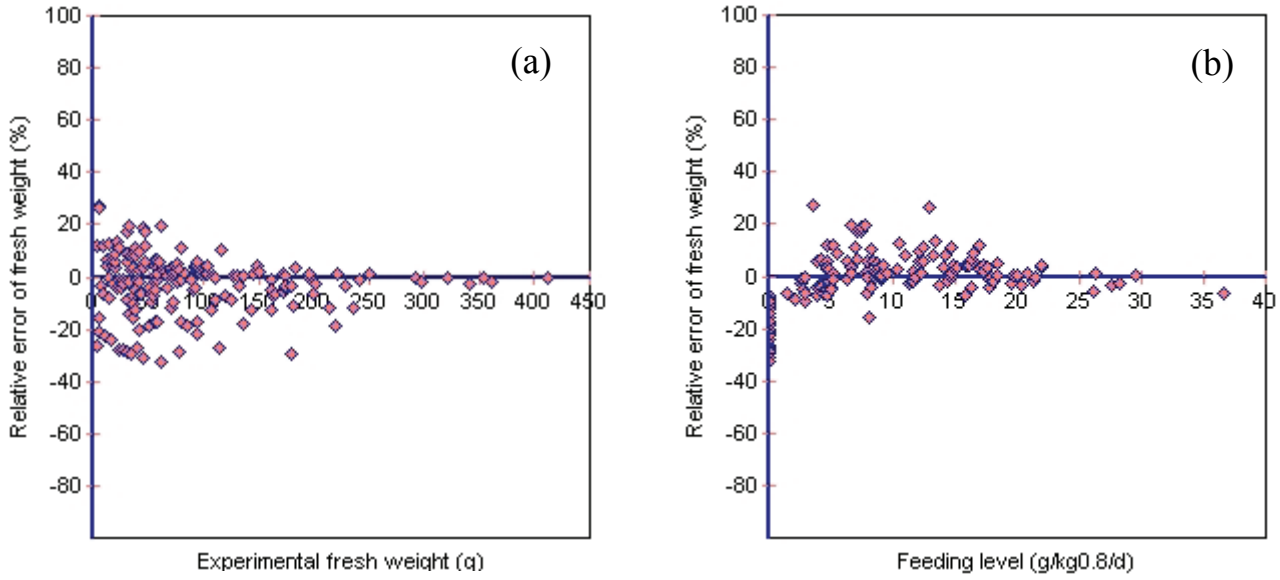

Figure 3.4. Relative errors of simulated fish weight in relation to final fish weight (a) and feeding level (b).

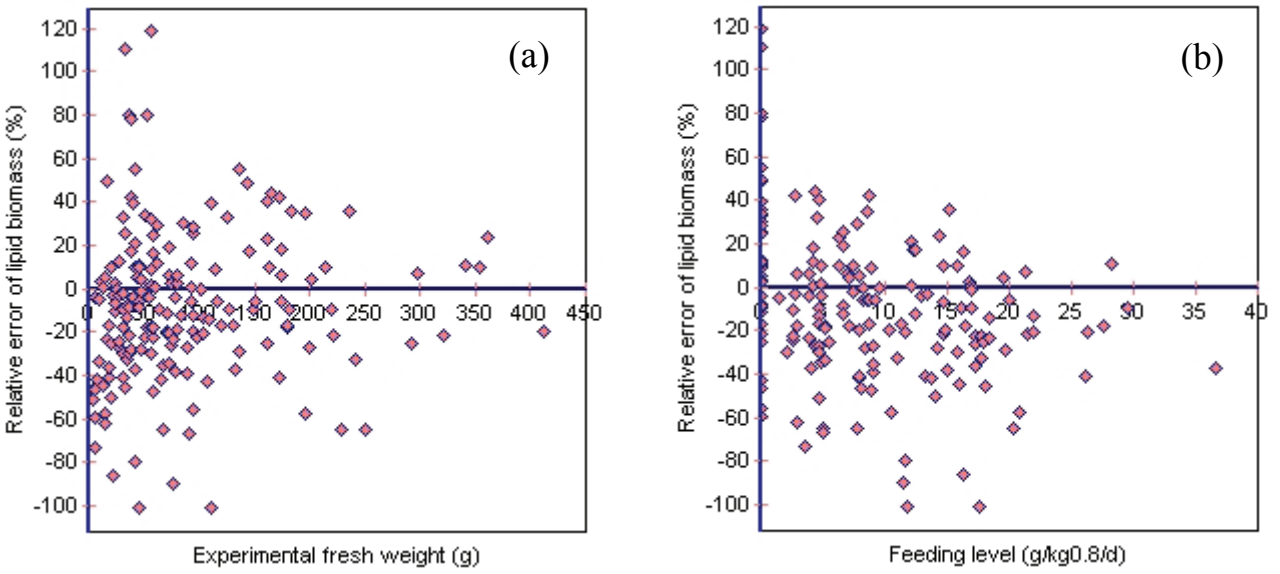

Figure 3.5. Relative errors of simulated lipid biomass in relation to final fish weight (a) and feeding level (b).

5. Discussion

The parameterization showed the gaps in our knowledge about the real values of maximum rates of utilization of the metabolites and the conditions under which these rates occur. The maximum rates were related to the weight of the tissues where the conversions take place. Some conversions occur mainly in the liver, but the liver weight could hardly be quantified at different nutritional states. For instance, the hepatosomatic indices have been shown to vary with varying levels and types of dietary carbohydrates (Kaushik and de Oliva Teles, 1985; Krogdahl et al., 2004). However, there is no published information about the relationship

between the liver and body weight of fish of different sizes fed different diets. Thus, relating the maximum rate of the conversion taking place in the liver and the concentration of the main substrate in that conversion to the body weight is not justified and needs improving.

There is a lack of information about the lower and upper limits of the concentrations of metabolites. Glucose concentration cannot be zero since glucose is the only fuel for the brain but the possible lowest glucose concentration is unknown. Accumulation of free amino acids is known to be toxic but the highest amino acid concentration that the fish can tolerate is still unclear. With these gaps in our knowledge, the models did not constrain the metabolite concentrations, except for glucose. Changing the glucose threshold in the model influences feed intake and growth of the fish.

Because the model was calibrated based on the lowest possible average relative error and the narrowest possible range of relative errors without taking into account whether or not the fish were fasting, the simulated weights of all the fasting fish were underestimated (Figure 3.4b). The calibration failed to obtain at the same time good agreement between simulated and observed fish weight for both fasting and fed fish. When the cases pertaining to fasting fish were excluded from the dataset, best agreement between observed and simulated final fish weights was achieved with $P\_Ur_{Pb,PbAa} = 0.032$ and ARE = 0.26% and REs ranging from -23.42 to 18.55%. This suggests that the model should have a separate module to simulate the weight loss of fasting fish.

The model did not predict lipid biomass satisfactorily. At this stage it is unclear whether the high ARE and wide range of REs were caused by the behavior of the model, by high variation in lipid content of the fish, or by error in analyzing crude fat.

Van Dam and Penning De Vries (1995) parameterized and calibrated the model developed by Machiels (1987) for rainbow trout using the same dataset as used in this study. The calibration results showed that the agreement between simulated (Y) and observed (X) values for fish weight was $Y = 1.03 X – 2.02$ ($R^2 = 0.996$) and for fat percentage $Y = 0.917 X – 0.068$ ($R^2 = 0.665$). In the present study the agreement between simulated (Y) and observed (X) values for fish weight was $Y = 0.97 X + 0.01$ ($R^2 = 0.990$) and for fat percentage $Y = 0.72 X + 1.12$ ($R^2 = 0.495$). It can be seen that while the accuracy of weight prediction of the present model is comparable to that of the model of van Dam and Penning De Vries (1995), the present model appears weaker in predicting lipid biomass.

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 calculating oxygen supply and demand based on gill surface area, dissolved oxygen concentration and stoichiometric parameters for oxygen in all the conversions; and the accumulation of the metabolites based on the balance between the oxygen supply and oxygen demand.

In conclusion, the development of the present model helped to better understand the biochemical pathways of metabolism in fish and to show the gaps in our knowledge about the relationship between the nutritional state and the maximum rate of different conversions. While the model provides potential mechanisms to regulate feed intake, it appears complicated and requires expensive experiments to be calibrated and validated. The high complexity of the model, however, was not compensated by better prediction of body fat in comparison with the previous models. Therefore, at this stage the use of the present model should be restricted to education and scientific (e.g. determination of research priorities) purposes. Data requirements for parameterization of this model are prohibitive for applying it to a wide range of fish species.

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Chapter 4

Effects of oxygen concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia, *Oreochromis niloticus*

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Abstract

Feed intake and satiation in fish are regulated by a number of factors, of which dissolved oxygen concentration (DO) is important. Since fish take up oxygen through the limited gill surface area, all processes that need energy, including food processing, depend on their maximum oxygen uptake capacity. Maximum oxygen uptake capacity relative to body weight in bigger fish is smaller than in smaller fish because the gill surface area is allometrically related to body weight. In this study, effects of DO concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia (*Oreochromis niloticus*) were investigated. Two weight classes of fish (21 g and 147 g) were used. For each class, six tanks were employed of which half were exposed to one of two DO levels (about 3.0 mg L^{-1} and 5.6 mg L^{-1}). Fish were fed to apparent satiation twice per day with a commercial diet. The results showed that (1) feed intake and growth of the fish at high DO level were significantly higher than at low DO level ($P < 0.01$), (2) relative feed intake and growth of small fish were significantly higher than of big fish ($P < 0.001$), and (3) fish at low DO level made no hematological adjustments ($P \geq 0.5$). Data suggest that (1) the limitation of the gill surface area results in lower feed intake and growth of fish at low DO concentration than at high DO concentration and (2) the allometric relationship between the gill surface area and body weight results in lower relative feed intake, which in turn results in lower relative growth in big fish than in small fish.

1. Introduction

Growth prediction, which is crucial for planning of fish production, requires information about feed intake since growth increases with increasing ration (Brett and Groves, 1979; Yoshida and Sakurai, 1984; Klaoudatos and Apostolopoulos, 1986; Cui et al., 1994; Xie et al., 1997). Under *ad libitum* feeding, feed intake prediction still remains a great challenge (Machiels, 1987, van Dam and Pauly, 1995).

Feed intake and satiation in fish are regulated by physiological, social or environmental factors, or by the interaction among them. Despite the recognition of environmental effects on feed intake, dissolved oxygen (DO) concentration has not been investigated systematically as a factor determining feed intake. Fish, like terrestrial animals, need oxygen for maintenance, locomotion, feeding and biosynthesis. In terrestrial animals, oxygen supply from air is hardly ever limiting. Oxygen concentration in water, however, can be 30 times less than in the air. Moreover, oxygen is taken up by fish through the gill surface area, which is limited. Thus, all the processes that need energy, including food processing, depend on maximum oxygen uptake capacity of fish. Pauly (1981) hypothesized that fish stop eating when oxygen supply does not satisfy oxygen demand. He also assumed that maximum oxygen uptake capacity per unit body weight is smaller in big fish than in small fish because gill surface area is allometrically related to body weight, and that therefore maximum feed intake per unit body weight is smaller in big fish than in small fish.

Little experimental work has been done to determine maximum feed intake in fish in relation to DO concentration and body weight. As part of an effort to develop a quantitative model for prediction of fish growth in relation to food quantity and composition and environmental factors (Van Dam & Pauly, 1995; Tran-Duy et al., 2005), we found fewer than a dozen reports in the literature on experimental work directed specifically at the effect of DO on feed intake, of which more than half lacked replication of treatments or statistical analysis of the results. Herrmann et al. (1962) found that food consumption of juvenile coho salmon (*Oncorhynchus kisutch)* within the weight range of 2-15 g remarkably decreased when DO was reduced from 8 mg L^{-1} to lower than 5 mg L^{-1}. According to Tsadik and Kutty (1987), feed ingestion of Nile tilapia (*Oreochromis niloticus*) of 8.1 g decreased 40% when ambient DO fell from 7.0 mg L^{-1} to 1.5 mg L^{-1}. Systematic studies were done on only a few species, namely turbot (*Scophthalmus maximus*) (Pichavant et al., 2000; Pichavant et al., 2001), channel catfish (*Ictalurus punctatus*) (Buentello et al., 2000) and European sea bass (*Dicentrarchus labrax*) (Thetmeyer et al., 1999; Pichavant et al., 2001).

Under hypoxia, fish can utilize several physiological mechanisms to compensate a reduction in oxygen uptake. For instance, carp (*Cyprinus carpio*) can increase breathing frequency (Lomholt and Johansen, 1979; Glass et al., 1990) and trout (*Oncorhynchus mykiss*)

can increase hemoglobin concentration (Soivio et al., 1980). No studies have been done to investigate effect of low DO concentration on hematological parameters of Nile tilapia. Our main objectives were (1) to determine whether maximum feed intake and growth of fish of the same size at low DO concentration are significantly lower than at high DO concentration; (2) to establish a quantitative dataset documenting the effect of DO and body weight on the maximum feed intake of Nile tilapia; and (3) to determine whether Nile tilapia make hematological adjustments at low DO concentrations.

2. Materials and methods

2.1 Fish and rearing conditions

This experiment was conducted at the Faculty of Aquaculture and Fisheries of the Hue University of Agriculture and Forestry in Vietnam. All male tilapia were obtained from a local producer of fingerlings (Cu Chanh fish farm, Huong Thuy, Thua Thien, Vietnam). To obtain two weight classes of fish, fingerlings were purchased at two different dates and reared in 120-L rectangular glass tanks until the start of the experiment. The first group of fish arrived at the university on 7 April 2006 and were kept in six tanks at a density of 134 fish $tank^{-1}$ with an average weight (± SE) of 9.2 ± 0.05 g. The second group arrived on 6 June 2006 and were kept in six other tanks at a density of 117 fish $tank^{-1}$ with an average weight (± SE) of 15.3 ± 0.1 g. All the tanks were connected to a recirculation system equipped with a sedimentation tank (1.6 m^3), a trickling biofilter (2.8 m^3), a sump (2.0 m^3) and pumps. From arrival to the start of the experiment on 20 June 2006 (75 and 14 days for the first and second group, respectively), fish were fed manually twice per day (starting at 8:00 and 16:00) with a commercial diet (Charoen Pokphand floating pellets, 2.5 mm: 17.9 kJ g^{-1}, 94.5% dry matter, 32.0% crude protein, 4.8% crude fat and 10.8% ash on a wet weight basis) using a feeding level of 18 g $kg^{-0.8}$ d^{-1}. During this acclimation period, water flow rate through the tanks was maintained at 6 L min^{-1}, photoperiod at 12L:12D, pH between 6.92 and 7.86, NO_2-N below 1 mg L^{-1}, NH_3-N below 0.1 mg L^{-1}, water temperature (mean ± SD) at 32.3 ± 0.6^oC and DO concentration above 5 mg L^{-1}.

2.2. Experimental procedures

The experiment was conducted as a randomized complete block design with three blocks and four treatment combinations within each block and each tank as an experimental unit. Blocks were used because the tanks were installed linearly in one row with one end more exposed to people passing by and occasional daylight than the other. The four treatment combinations comprised two fish weight classes (small and big) and two DO levels (low and high) which were arranged in a 2 × 2 factorial: small fish-low DO (SL), small fish-high DO (SH), big fish-low DO (BL) and big fish-high DO (BH). The small and big fish were those obtained on 6 June 2006 and 7 April 2006 (see 2.1), with average weights (± SE) at the end of the

acclimation period of 20.00 ± 0.33 and 140.87 ± 1.80 g, respectively. Dissolved oxygen concentrations below 3.5 mg L^{-1} were considered low and above 5.0 mg L^{-1} were considered high.

The same tanks used during the acclimation period were used for the experimental period. After the acclimation period, all fish were taken out of the tanks with the small and big fish separate in different basins. Then, fish were anesthetized with benzocaine (standing stock: 100 g benzocaine dissolved in 1 L ethanol 99.8%; dose: 100 mg benzocaine L^{-1} water), weighed individually and randomly allocated to the tanks corresponding to the weight classes (big fish treatment: 9 fish $tank^{-1}$; small fish treatment: 50 fish $tank^{-1}$). Average initial weights (± SE) of the fish in SL, SH, BL and BH treatments were 21.65 ± 0.71, 21.18 ± 0.15, 148.41 ± 3.38 and 144.74 ± 1.56 g, corresponding to stocking densities (± SE) of 9.01 ± 0.29, 8.80 ± 0.06, 11.13 ± 0.23 and 10.90 ± 0.12 kg m^{-3}, respectively.

During fish allocation, 20 fish from each weight class were randomly sampled for hematological parameters and body composition analyses. One ml of blood was collected from the caudal veins of each fish using a heparinized hypodermic syringe. Directly after collection, each blood sample was placed in a cool 2 ml glass tube containing 3 mg Na_2EDTA, gently mixed and immediately stored at -20°C for further analysis. After blood sampling, all fish were killed with an overdose of benzocaine (400 mg L^{-1}), cut into small pieces and ground twice using a meat grinder. Three hundred grams of the fish homogenate from each weight class were placed in a tight plastic bottle and immediately stored at -20°C for further analysis.

Oxygen levels inside the tanks were established by means of aeration and water flow rates through the tanks. Each tank assigned to the high DO level was aerated with two airstones at two diagonally opposite corners. Tanks assigned to the low DO level were not aerated. On the day the fish were allocated to the tanks (20 June), water flow rates were set at 6.6 L min^{-1}. Then, the flow rates in the tanks assigned to the low DO level were gradually reduced to 3.6 L min^{-1} within the next five days (21 to 25 June). As the DO concentrations in the low DO tanks with big fish were about 1 mg L^{-1} higher than in the low DO tanks with small fish between 26 and 30 June, water flow rates in the low DO tanks with big fish were gradually reduced on 1 and 2 July to 2.5 L min^{-1}, at which they were maintained until the end of the experiment. In this way, DO concentrations in the tanks with big and small fish were comparable.

Dissolved oxygen concentrations and temperature at the inlet, inside (central point) and outlet of each tank were measured six times per day, at around 7:30, 9:30, 11:30, 14:00, 15:30 and 17:30 using oxygen meters (WTW 340i; Wissenschaftlich-Technische Werkstätten GmbH, Germany) with membrane-covered galvanic sensors (CellOx® 325). On 30 June, 6 July, 12 July and 18 July, DO concentrations were also measured every 30 minutes from 8:00

to 9:00 and from 16:00 to 17:00, and every hour from 9:30 to 15:30 and from 18:30 to 23:30; and every two hours from 0:30 to 6:30 the next day to assess the diurnal variation in DO concentration. The starting day for these intense DO measurements was selected based on the observation that on that day (four days after the *ad libitum* feeding started; see the following paragraph) feed intake of the fish became stable, i.e. the fish had adapted to the *ad libitum* feeding regime. After the starting day, weekly intervals were selected for the intense DO measurements assuming that fish growth would not affect the diurnal pattern of DO variations within a period of less than one week. Total ammonia (NH_3 + NH_4^+), nitrite and nitrate concentrations were measured twice per day (9:00 and 17:00) at the inlet and outlet of each tank using test kits (Aquamerck; Merck); pH was also measured at the same time and position using a pH meter (MA 130; Mettler Toledo, USA).

Fish were fed manually twice per day (starting at 8:00 and 16:00) with the same diet as used during the acclimation period; each meal lasted for 1 hour. From 21 to 25 June, fish were allowed to adapt to new oxygen conditions and were fed at a feeding level of 8 g $kg^{-0.8}$ d^{-1}. From 26 June to 20 July, fish were fed to apparent satiation.

At the end of the experimental period (21 July), fish from each tank were anesthetized with benzocaine (same dose as used for fish allocation) and weighed individually. Six fish from each tank were randomly selected for hematological parameter analysis. For each tank with small fish, 10 fish were randomly selected for body composition analysis. For each tank with big fish, all nine fish were used for body composition analysis. Procedures for sample preparation were the same as at the start of the experimental period.

2.3. Analytical procedures

Dry matter content was determined as weight loss after drying the samples for 4-6 h at 103^oC until constant weight (ISO, 1983). Crude protein content was determined using the Kjeldahl method and multiplying nitrogen content by 6.25 (ISO, 1997). Crude fat content was determined after petroleum-ether extraction using a Soxhlett system (ISO, 1999).

Hematological parameters for blood samples analysis were red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). These values were determined using an automated hematology analyzer (XT-1800i; Sysmex Coporation, Japan).

2.4. Data analysis

The present study assessed the maximum feed intake of Nile tilapia in relation to DO level and body weight of the fish. Since fish were fed to satiation from day 6 till day 30 of the experiment (*ad libitum* feeding period, ALFP), all the performance parameters (feed intake,

growth rates and FCR) were calculated for this period. Specific growth rates (SGR) were calculated using the following formula (according to Jobling, 1994, chapter nine):

$$SGR\ (\%\ d^{-1}) = [(\ln W_{30} - \ln W_6)/25] \times 100$$

where W_6 and W_{30} are the average fish weight (g) on the day *ad libitum* feeding started (day 6) and at the end of the experimental period (day 30), respectively. W_6 was estimated based on the average fish weight W_1 (g) on the day the fish were allocated to the tanks (day 1), daily feed intake (g) during the first five days after fish allocation and the overall feed conversion ratio (FCR), which was calculated according to Parker (1987) as

$$FCR = FI_{tot}/(W_{30} - W_1)$$

where FI_{tot} (g) is the total feed intake per fish during the experimental period (day 1 till day 30). This estimation of W_6 was based on the assumption that feed conversion ratios during the first five days and during the ALFP (and therefore during the experimental period) were the same. Growth rates per metabolic weight unit (GR_{MBW}) were calculated according to Martins et al. (2005) as

$$GR_{MBW}\ (g\ kg^{-0.8}\ d^{-1}) = (W_{30} - W_6)/(W_{mean}/1000)^{0.8}/25$$

where W_{mean} is the geometric mean body weight (g), which was calculated as

$$W_{mean}\ (g) = \sqrt{W_6 \times W_{30}}$$

Feed intake of the fish expressed as a percentage of body weight (FI_{perc}) and per metabolic weight unit (FI_{MBW}) were calculated as

$$FI_{perc}\ (\%\ d^{-1}) = FI/W_{mean} \times 100 \text{ and}$$

$$FI_{MBW}\ (g\ kg^{-0.8}\ d^{-1}) = FI/(W_{mean}/1000)^{0.8}$$

where FI (g) is the average feed intake per fish over the ALFP per meal or per day (assuming that fish weight is constant from 8:00 to 17:00).

Un-ionized ammonia (NH_3) concentrations were calculated according to Albert (1973) as

$$NH_3 = [NH_3 + NH_4^+]/[1 + 10^{(pKa - pH)}]$$

where pKa was calculated based on the equation developed by Emerson et al. (1975) as

$$pKa = 0.09018 + 2729.92/(T + 273)$$

where T is the water temperature (^oC).

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc.). The homogeneity of variances of different groups was checked using Levene *F* test with PROC ANOVA. Since the homogeneity assumption was met for all the observed variables ($P >$

0.05), mean values of DO concentration, growth, feed intake, body composition and hematological parameters were subjected to two-way analysis of variance (ANOVA) using Proc GLM according to the following model:

$$Y_{ijk} = \mu + S_i + O_j + SO_{ij} + \varepsilon_{ijk}$$

whereY_{ijk} represents the *k*th observation of fish size *i* and oxygen level *j*, μ is the overall mean, S_i is the treatment effect of fish size *i*, O_j is the treatment effect of oxygen level *j*, SO_{ij} is the interaction between fish size *i* and oxygen level *j*, and ε_{ijk} is the error term (SAS Institute Inc., 2004).

Block was excluded from the analysis because there were no effects of block on any of the observed variables. Normal distribution of the residuals obtained from the model was verified using Kolmogorov-Smirnov's test with PROC UNIVARIATE. Except for initial MCH ($P < 0.05$), all the observed variables satisfied the normal distribution assumption ($P > 0.15$). Because logarithmic and square root transformation of the initial MCH did not result in satisfactory assumption, a Kruskal-Wallis one-way ANOVA was performed on this variable with main factor being fish size using PROC NPAR1WAY (SAS Institute Inc., 2004).

Within-day variations in DO concentrations and feed intake (morning versus afternoon feeding) were tested for the effect of fish size, oxygen, time (moment within a day) and their interactions by repeated measures ANOVA using PROC MIXED according to the following model:

$$Y_{ijkm} = \mu + S_i + O_j + SO_{ij} + \varepsilon_{1,ijk} + T_m + ST_{im} + OT_{jm} + SOT_{ijm} + \varepsilon_{2,ijkm}$$

whereY_{ijkm} represents the observation (DO concentration or feed intake) of fish size *i*, oxygen level *j* and tank *k* at time (moment) *m*, μ is the overall mean, S_i is the treatment effect of fish size *i*, O_j is the treatment effect of oxygen level *j*, SO_{ij} is the interaction between fish size *i* and oxygen level *j*, $\varepsilon_{1,ijk}$ is the error term 1, which represents the random effect of tank *k* within fish size *i* and oxygen level *j*, T_m is the effect of time (moment) *m*, ST_{im} is the interaction between fish size *i* and time (moment) *m*, OT_{jm} is the interaction between oxygen level *j* and time (moment) *m*, SOT_{ijm} is the interaction among fish size *i*, oxygen level *j* and time (moment) *m*, and $\varepsilon_{2,ijkm}$ is the error term 2. In this model, effects of fish size, DO level and their interaction were tested against error term 1; effects of time and interactions with time were tested against error term 2 (Kuehl, 2000; SAS Institute Inc., 2004).

Differences among treatment means were considered significant when $P < 0.05$ and not significant (no effect) when $P \geq 0.05$. When $0.05 \leq P < 0.1$, a tendency for significant difference among treatment means was assumed. If significance was detected, multiple comparisons were performed using Tukey adjustment (SAS Institute Inc., 2004).

3. Results

3.1. Dissolved oxygen concentrations and other water quality parameters

Mean DO concentrations inside and at the outlet of the tanks over the ALFP under the four treatment combinations are shown in Table 4.1. Pair-wise comparisons among these values showed that under the same oxygen treatment, DO concentrations in tanks with big fish were significantly higher than in tanks with small fish ($P < 0.001$); however, all the differences were smaller than 0.45 mg L^{-1}. In the same fish weight class, DO concentrations in high DO tanks were significantly higher than in low DO tanks ($P < 0.001$); the differences were in the range of 2.62 - 2.72 mg L^{-1}. There was no interaction effect of fish size and DO level on DO concentrations ($P > 0.5$). Because the DO concentrations measured inside and at the outlet of each tank at the same time were highly correlated under all treatment combinations ($r > 0.98$) and the differences between them were small (95th percentile of 3231 cases being 0.32 mg L^{-1}), only DO concentrations inside the tanks were used for all the following presentations and analyses.

Table 4.1. Mean dissolved oxygen (DO) concentrations (mg L^{-1}) over the *ad libitum* feeding period under the four treatment combinations[1]

	Treatment combination[2]				***P*-value**		
	SL	**SH**	**BL**	**BH**	Size	Oxygen	Size × oxygen
Inside tanks	2.78 ± 0.03	5.46 ± 0.06	3.19 ± 0.01	5.81 ± 0.06	< 0.001	< 0.001	0.539
Outlet of tanks	2.61 ± 0.02	5.28 ± 0.04	2.99 ± 0.01	5.71 ± 0.07	< 0.001	< 0.001	0.545

[1] Values represent means ± SE of three replicates (tanks); each replicate value is the mean of all the data points measured on one tank during the *ad libitum* feeding period.

[2] SL = Small fish-Low DO; SH = Small fish-High DO; BL = Big fish-Low DO; BH = Big fish-High DO. Small and big fish were Nile tilapia *(Oreochromis niloticus*) with individual weights in the range of 21-66 g and of 144-251 g, respectively.

Analysis of mean DO concentrations at six times per day over the whole ALFP and mean DO concentrations at 25 times in four 24-hour periods (Figure 4.1) showed that there were effects of time and interaction effects of time and size and of time and oxygen on the diurnal variations in DO concentrations ($P < 0.001$). There was an abrupt change in DO concentrations from the time just before feeding to the time right after feeding (Figure 4.1); these changes were significantly larger in tanks with small fish than with big fish at the same DO level ($P < 0.05$) and significantly larger in low DO tanks than in high DO tanks in the same weight class ($P < 0.001$). Mean DO concentrations in the tanks with small fish showed a

declining trend throughout the ALFP while DO concentrations in the tanks with big fish declined during the first 2 weeks and were almost constant during the last week (Figure 4.2).

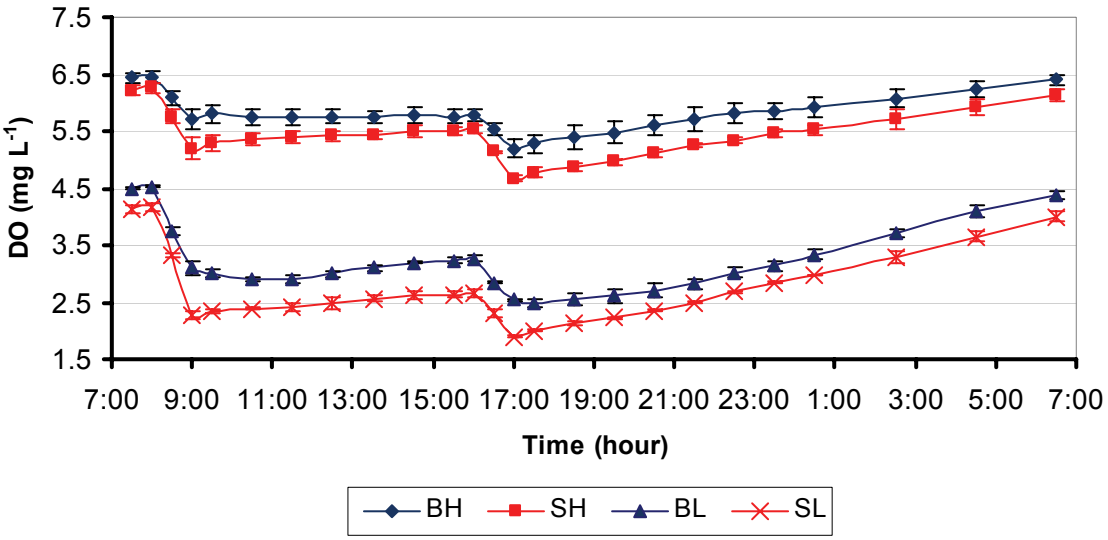

Figure 4.1. Diurnal variations of dissolved oxygen (DO) concentrations under the four treatment combinations. Data points represent means of three replicate values; each replicate value is the mean of four data points measured at the same time in four 24-hour periods. Vertical bars represent two times the standard errors. BH = Big fish-High DO; SH = Small fish-High DO; BL = Big fish-Low DO; SL = Small fish-Low DO. Big and small fish were Nile tilapia (*Oreochromis niloticus*) with individual weight in the range of 144-251 g and of 21-66 g, respectively.

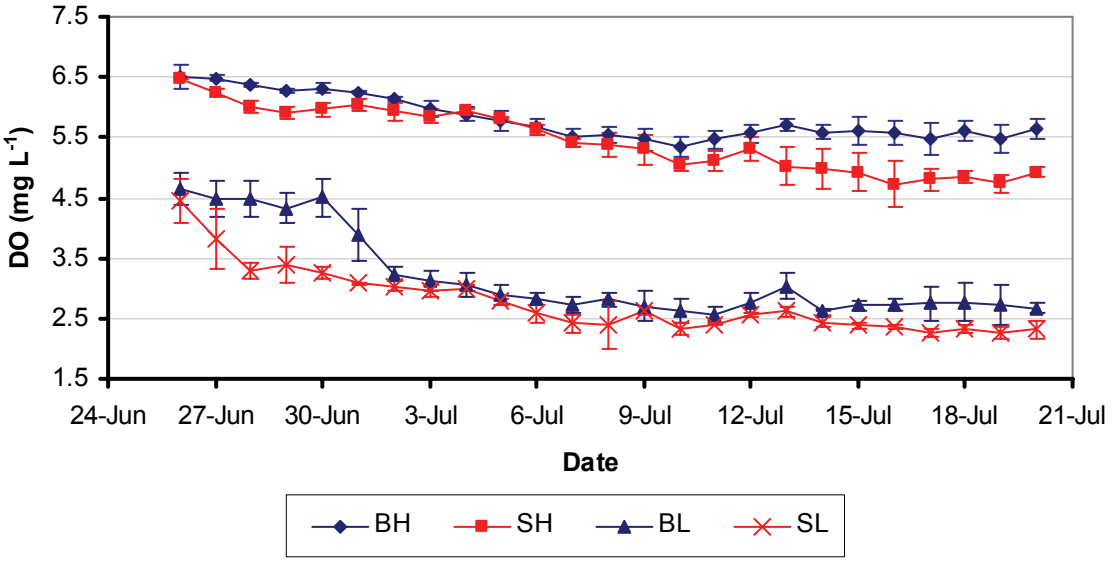

Figure 4.2. Variations in dissolved oxygen (DO) concentrations throughout the *ad libitum* feeding period under four treatment combinations. Data points represent means of three replicate values; each replicate value is the mean of six data points measured during each day. Vertical bars represent two times the standard errors. BH = Big fish-High DO; SH = Small fish-High DO; BL = Big fish-Low DO; SL = Small fish-Low DO. Big and small fish were Nile tilapia (*Oreochromis niloticus*) with individual weights in the range of 144-251 g and of 21-66 g, respectively.

Mean outlet NH_3 concentrations (± SE) over the ALFP under SL, SH, BL and BH treatments were 0.011 ± 0.001, 0.007 ± 0.001, 0.011 ± 0.001 and 0.004 ± 0.001 mg L^{-1}, respectively. NH_3 concentrations in low oxygen tanks were significantly higher than in high oxygen tanks ($P < 0.001$). There was no effect of size ($P = 0.223$) or interaction effect of size and water flow rate ($P = 0.234$) on NH_3 concentrations. Mean outlet NO_2^- concentrations over the ALFP under the four treatment combinations were identical to the inlet NO_2^-, which equaled 0.274 ± 0.194 (SD) mg L^{-1}. Mean outlet NO_3^- concentrations over the ALFP under the four treatment combinations were also identical to the inlet NO_3^-, which equaled 74.5 ± 18.6 (SD) mg L^{-1}. Mean temperature (± SD) of all tanks at both inlet and out let was identical, which equaled 32.7 ± 0.7°C. Mean outlet pH (± SE) over the ALFP under SL, SH, BL and BH treatments were 7.55 ± 0.06, 7.57 ± 0.06, 7.58 ± 0.05 and 7.60 ± 0.07, respectively. No effect of size ($P = 0.651$), water flow rate ($P = 0.734$) or interaction effect of size and water flow rate ($P = 0.951$) on pH was observed.

3.2. Feed intake and growth

Experimental characteristics, feed intake and growth performance of the fish are shown in Table 4.2. Mean initial fish weights were significantly different between weight classes ($P < 0.001$), but not significantly different between low and high DO tanks ($P = 0.307$). Mean final fish weights in high DO tanks, however, were significantly higher than in low DO tanks ($P = 0.004$). Effects of fish size were present in all the responses ($P < 0.001$). Although absolute feed intake and growth rates were higher in big fish, these values were smaller in big fish than in small fish when expressed as percentage of body weight or per metabolic weight unit. Feed conversion ratios (FCR) of big fish were higher than small fish. Oxygen significantly affected feed intake and growth rates ($P < 0.005$) but had no effect on FCR ($P = 0.393$). In the same weight class, feed intake and growth rates of fish in high DO tanks were significantly higher than in low DO tanks ($P < 0.05$). There were no interaction effects of fish size and oxygen on any of the responses ($P > 0.05$), except on the absolute feed intake ($P < 0.05$).

Mean feed intake per fish per meal (g $fish^{-1}$ $meal^{-1}$) and feed intake as percentage of body weight per meal (% $meal^{-1}$) and per metabolic weight unit per meal (g $kg^{-0.8}$ $meal^{-1}$) over the ALFP (Table 4.3) were significantly different between morning and afternoon meals ($P < 0.05$). There was an interaction effect of meal time and oxygen ($P < 0.01$), but no interaction effect of meal time and fish size ($P > 0.05$). Multiple comparisons showed that feed intake in the morning was significantly lower than in the afternoon at high oxygen level ($P < 0.01$). At low oxygen level, however, no effect of meal time on feed intake was found ($P > 0.5$), although feed intake in the morning was numerically higher than in the afternoon (Figure 4.3).

Table 4.2. Feed intake and growth performance of Nile tilapia (*Oreochromis niloticus*) under the four treatment combinations[1]

	Treatment combination[2]				***P*-value**		
	SL	**SH**	**BL**	**BH**	Size	Oxygen	Size × oxygen
Ad libitum feeding period (d)	25	25	25	25			
Number. of fish per tank	50	50	9	9			
Number of tanks	3	3	3	3			
Survival (%)	100	100	100	100			
Initial body weight (g)	21.65 ± 0.71	21.18 ± 0.15	148.41 ± 3.38	144.74 ± 1.56	< 0.001	0.307	0.424
Final body weight (g)	52.61 ± 1.17	65.02 ± 3.64	223.14 ± 5.88	250.51 ± 7.26	< 0.001	0.004	0.176
Feed intake							
Absolute (g fish^{-1} d^{-1})	1.54 ± 0.07	2.15 ± 0.15	4.43 ± 0.09	6.08 ± 0.26	< 0.001	< 0.001	0.012
FI_{perc}(% d^{-1})	4.58 ± 0.21	5.79 ± 0.22	2.44 ± 0.03	3.19 ± 0.09	< 0.001	< 0.001	0.187
FI_{MBW} (g kg$^{-0.8}$ d^{-1})	23.23 ± 0.98	29.94 ± 1.29	17.34 ± 0.23	22.90 ± 1.69	< 0.001	< 0.001	0.537
Growth							
Absolute (g fish^{-1} d^{-1})	1.24 ± 0.07	1.75 ± 0.15	2.99 ± 0.15	4.23 ± 0.23	< 0.001	< 0.001	0.051
SGR (% d^{-1})	3.55 ± 0.18	4.47 ± 0.23	1.63 ± 0.03	2.19 ± 0.07	< 0.001	0.002	0.284
GR_{MBW} (g kg$^{-0.8}$ d^{-1})	18.65 ± 1.02	24.41 ± 1.50	11.68 ± 0.45	15.93 ± 0.64	< 0.001	0.001	0.469
FCR	1.25 ± 0.03	1.23 ± 0.03	1.49 ± 0.04	1.44 ± 0.05	< 0.001	0.393	0.689

[1] Values represent means ± SE over the *ad libitum* feeding period of three replicates (tanks); each replicate value is the mean of the measurements on 50 small fish (those under SL or SH) or 9 big fish (those under BL or BH).

[2] SL = Small fish-Low DO; SH = Small fish-High DO; BL = Big fish-Low DO; BH = Big fish-High DO. Low and high DO were two levels of dissolved oxygen with means around 3.0 and 5.5 mg L^{-1}, respectively.

Table 4.3. Feed intake of Nile tilapia (*Oreochromis niloticus*) in the morning and afternoon under the four treatment combinations[1]

Treatment combination[2]	**SL**		**SH**		**BL**		**BH**	
Meal time	**Morning**	**Afternoon**	**Morning**	**Afternoon**	**Morning**	**Afternoon**	**Morning**	**Afternoon**
Feed intake								
Absolute (g fish^{-1} d^{-1})	0.79 ± 0.03	0.76 ± 0.03	1.04 ± 0.06	1.11 ± 0.08	2.22 ± 0.08	2.21 ± 0.01	2.91 ± 0.15	3.17 ± 0.12
FI_{perc}(% d^{-1})	2.33 ± 0.10	2.25 ± 0.11	2.79 ± 0.08	2.99 ± 0.14	1.22 ± 0.03	1.22 ± 0.03	1.52 ± 0.05	1.67 ± 0.04
FI_{MBW} (g kg$^{-0.8}$ d^{-1})	11.83 ± 0.50	11.40 ± 0.54	14.46 ± 0.49	15.48 ± 0.82	8.68 ± 0.23	8.66 ± 0.16	10.95 ± 0.41	11.95 ± 0.30

[1] Values represent means ± SE over the *ad libitum* feeding period of three replicates (tanks); each replicate value is the mean of the measurements on 50 small fish (those under SL or SH) or 9 big fish (those under BL or BH).

[2] SL = Small fish-Low DO; SH = Small fish-High DO; BL = Big fish-Low DO; BH = Big fish-High DO. Low and high DO were two levels of dissolved oxygen with means around 3.0 and 5.5 mg L^{-1}, respectively.

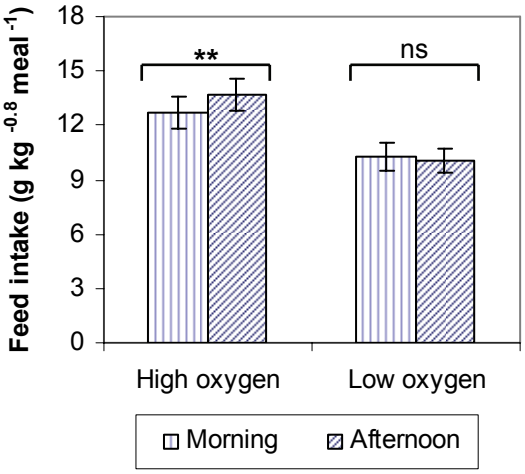

Figure 4.3. Mean feed intake of Nile tilapia (*Oreochromis niloticus*) in the morning and afternoon meals at low (3 mg L^{-1}) and high (5.5 mg L^{-1}) dissolved oxygen levels. Bars represent means of six replicates (tanks); each replicate value is the mean of feed intake during the same time (morning or afternoon meal) over the *ad libitum* feeding period. Error bars represent two times the standard errors. ** means "significantly different with $P < 0.01$" and ns means "not significantly different with $P > 0.05$".

3.3. Body composition

Initial moisture, crude fat and crude protein contents on a fresh weight basis of the small fish were 77.2, 3.9 and 14.4%, respectively, and of the big were 73.2, 8.1 and 14.2%, respectively. Final body composition of the fish is presented in Table 4.4. Both initial and final body composition showed the following trend: crude fat contents in lighter fish were lower than in heavier fish and the opposite occurred with moisture content. Fish size significantly affected final moisture and crude fat contents ($P < 0.02$), but did not affect crude protein content ($P = 0.215$). Final crude fat content in fish at low DO level was significantly lower than at high DO level ($P = 0.029$). There was a tendency that final moisture content in fish at low DO level was higher than at high DO level ($P = 0.073$) and final protein content in fish at low DO level was lower than at high DO level ($P = 0.054$). There were no interaction effects of size and oxygen on moisture, crude protein and crude fat contents ($P > 0.05$), although a tendency on crude fat content was found ($P = 0.058$).

3.4. Hematological parameters

Mean hematological parameters of small fish and big fish at the beginning of the experimental period are given in Table 4.5. No significant difference in any parameter between small and big fish was observed ($P \geq 0.08$). Mean hematological parameters of fish at the end of the experimental period under the four treatment combinations are given in Table 4.6. Except for MCV ($P = 0.147$), there was an effect of fish size on all the parameters ($P < 0.05$), but no effect of oxygen level on any of the parameters ($P > 0.5$). As the fish grew bigger, RCB,

HGB and HCT became significantly higher ($P < 0.001$), while MCH and MCHC became significantly lower ($P < 0.05$). There was no interaction effect of fish size and oxygen level on any of the parameters ($P > 0.5$).

Table 4.4. Final body composition (% fresh weight) of Nile tilapia (*Oreochromis niloticus*) under the four treatment combinations[1]

	Treatment combination[2]				***P*-value**		
	SL	**SH**	**BL**	**BH**	Size	Oxygen	Size × oxygen
Moisture	71.8 ± 0.5	70.9 ± 0.4	70.3 ± 0.7	68.9 ± 0.6	0.011	0.073	0.624
Crude fat	6.9 ± 0.2	8.8 ± 0.6	8.9 ± 0.4	9.1 ± 0.2	0.018	0.029	0.058
Crude protein	15.9 ± 0.3	16.2 ± 0.2	16.0 ± 0.3	16.7 ± 0.1	0.215	0.054	0.370

[1] Values represent means ± SE of three replicates (tanks); each replicate value is a measurement on the homogenate of 10 small fish or 9 big fish.

[2] SL = Small fish-Low DO; SH = Small fish-High DO; BL = Big fish-Low DO; BH = Big fish-High DO. Small and big fish were those with individual weights in the range of 21-66 g and of 144-251 g, respectively. Low and high DO were two levels of dissolved oxygen with means around 3.0 and 5.5 mg L^{-1}, respectively.

Table 4.5. Mean hematological parameters of Nile tilapia (*Oreochromis niloticus*) at the beginning of the experimental period[1]

	Hematological parameter[2]					
Fish size	**RBC ($\times 10^{12}$/L)**	**HGB (g/L)**	**HCT (L/L)**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/L)**
Small	1.878 ± 0.269	76.250 ± 8.012	0.197 ± 0.045	104.330 ± 12.910	40.925 ± 3.217	398.700 ± 62.638
Big	1.853 ± 0.413	79.100 ± 10.223	0.206 ± 0.054	111.470 ± 12.193	44.715 ± 11.724	402.850 ± 96.150
***P*-Value**	0.825	0.333	0.568	0.080	0.665[3]	0.872

[1] Values represent means ± SD of 20 replicates (fish).

[2] RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV= mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

[3] Result from Kruskal-Wallis one-way analysis of variance (see Materials and methods for details).

Table 4.6. Mean hematological parameters of Nile tilapia (*Oreochromis niloticus*) at the end of the experimental period under the four treatments combinations[1]

	Hematological parameter[2]					
Treatment combination[3]	**RBC ($\times 10^{12}$/L)**	**HGB (g/L)**	**HCT (L/L)**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/L)**
SL	1.852 ± 0.069	82.111 ± 3.379	0.208 ± 0.002	112.583 ± 5.807	47.806 ± 3.912	426.333 ± 19.390
SH	1.831 ± 0.140	82.944 ± 1.645	0.208 ± 0.022	112.083 ± 3.394	47.333 ± 4.562	434.667 ± 53.431
BL	2.582 ± 0.026	101.278 ± 2.469	0.311 ± 0.007	120.556 ± 3.785	40.261 ± 1.756	336.500 ± 12.366
BH	2.569 ± 0.022	101.333 ± 1.110	0.308 ± 0.013	118.517 ± 4.560	39.417 ± 0.438	338.944 ± 11.212
***P*-Value**						
Size	< 0.001	< 0.001	< 0.001	0.147	0.039	0.014
Oxygen	0.840	0.853	0.901	0.784	0.839	0.860
Size × Oxygen	0.957	0.871	0.921	0.868	0.954	0.923

[1] Values represent means ± SE of three replicates (tank); each replicate value is the mean of the measurements on six fish.

[2] RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV= mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration;

[3] SL = Small fish-Low DO; SH = Small fish-High DO; BL = Big fish-Low DO; BH = Big fish-High DO. Small and big fish were those with individual weights in the range of 21-66 g and of 144-251 g, respectively. Low and high DO were two levels of dissolved oxygen with means around 3.0 and 5.5 mg L^{-1}, respectively.

4. Discussion

4.1. Effects of DO on feed intake and growth

The high and low DO levels in the experiment were created by applying different aeration regimes and by changing the water flow rates. In addition to affecting the DO levels, the contrast in water flow rates might have affected some of the other water quality parameters which influence the feed intake of fish. However, we think that these effects are negligible. Temperature, nitrite and nitrate concentrations and pH were within the optimal ranges for Nile tilapia (Masser et al., 1999; Popma and Masser, 1999; Ross, 2000). Of all the water quality parameters measured, only NH_3 concentrations were significantly different between low and high oxygen tanks. The highest difference in NH_3 concentrations among the treatments (0.007 mg L^{-1}) was very small and the highest NH_3 concentration (0.011 mg L^{-1}) was far below the lowest level (0.080 mg L^{-1}) which may affect feed intake of Nile tilapia (Shelton and Popma, 2006). El-Shafai et al. (2004) showed that feed intake of Nile tilapia was not influenced by NH_3-N concentrations within the range of 0.004-0.434 mg L^{-1} (corresponding to 0.005-0.527

mg NH_3 L^{-1}); however, for best growth, they recommended an NH_3-N concentration below 0.10 mg L^{-1} (corresponding to 0.12 mg NH_3 L^{-1}). Muir et al. (2000) even considered an NH_3 level below 0.2 mg L^{-1} ideal for flowing-water tilapia culture. However, Soderberg (2006) doubted that the chronic toxicity level of NH_3 might be lower than the level recommended by Muir et al (2000). Based on studies on channel catfish, rainbow trout (*Oncorhynchus mykiss*) and blue tilapia (*Oreochromis aurea*), he estimated chronic toxicity levels of NH_3 ranging from 0.035 to 0.092 mg L^{-1} for tilapias. The lower bound of this range is higher than the highest NH_3 concentration in the present study.

Carbon dioxide is another factor that may affect feed intake but it was not measured in the experiment. Nile tilapia can tolerate CO_2 concentrations above 20 mg L^{-1} (Wedemeyer, 1996) and CO_2 is unlikely to have an adverse effect on fish in intensive culture systems unless its concentration reaches 100 mg L^{-1} (Balarin and Haller, 1982). In our experiment, CO_2 was unlikely to affect feed intake and growth of the fish because: (1) the densities of the fish in the experiment (lower than 12 kg m^{-3} initially) were much lower than the recommended densities of Nile tilapia in the intensive systems (20-25 kg m^{-3} initially for fish of 20-250 g) (Rakocy, 1989); (2) accumulation of CO_2 in the system was counteracted by letting the water go down through the perforated trays on top of the trickling biofilter and by diffused aeration in the sump (see Huguenin and Colt, 2002; chapter 12); and (3) pH in the experiment was high (around 7.6) and thus toxic gaseous CO_2 must be low. The mole fraction of gaseous CO_2 at pH of 7.6 is only 5.6% (calculated based on Henderson-Hasselbach equation; Boyd, 1990). Therefore, the contrast between the DO treatments in the present study was most likely due to real differences in DO concentrations and not to any other water quality parameters.

The results from the present study clearly showed reduced feed intake and growth of Nile tilapia of average weight of 37 and 190 g at DO concentrations of about 2.8 mg L^{-1} and 3.2 mg L^{-1}, respectively. Some investigators have attempted to define an incipient DO concentration at which feed intake and growth of Nile tilapia starts to decline. Reports on incipient DO concentrations for growth of Nile tilapia showed a range from less than 0.8 to 3 mg L^{-1} (Rappaport et al., 1976; Coche, 1977; Melard and Philippart, 1980; Teichert-Coddington and Green, 1993). The lower extreme value (less than 0.8 mg L^{-1}; Teichert-Coddington and Green, 1993) was obtained from an experiment in which there were no significant differences between the yields of Nile tilapia raised in ponds with two aeration regimes, which were established as follows: pond water samples were taken every hour and DO concentrations measured; if DO concentrations were equal or less than critical levels (10% or 30% of saturation), ponds were aerated for one hour. The authors suggested that practical threshold of DO for Nile tilapia was not higher than 10% of saturation (0.8 mg L^{-1} at 26^{o}C; conversion based on Colt (1984)). However, DO variations were not reported and thus actual DO concentrations to which the fish were exposed are unknown.

Based on field observations of Melard and Philippart (1980), where growth rates of Nile tilapia declined at DO concentration of 3 mg L^{-1}, Soderberg (2006) suggested that minimum DO tension for flowing-water tilapia culture should be 60 mmHg, equivalent to 2.9 mg L^{-1} at 32.7^{o}C, the average temperate of the present study (conversion based on Colt, 1984). If oxygen supply to fish is limited by diffusion through the gills, it may not be possible to define one single incipient oxygen concentration below which fish growth starts decreasing. The incipient concentration would depend on the balance between oxygen supply to the fish, determined by the oxygen gradient across the gills, the gill surface area (and therefore body weight) and the blood-water distance; and oxygen demand by the fish, determined by the amount and composition of the feed and the activity level of the fish. In the present study, fish were fed to satiation with a high-protein diet. Their requirements for oxygen may have been higher than fish in natural waters which feed mainly on algae-based materials (Fryer and Iles, 1972; Man and Hodgkiss, 1977; Bowen, 1982; Trewavas, 1983) most likely at a sub-maximum level. For instance, the upper bound of 95% confidence interval of daily feed ingestion of a 200 g Nile tilapia feeding mainly on phytoplankton in Lake George (Uganda) estimated by Moriarty and Moriarty (1973) was about 2.9 g (dry matter), much lower than daily feed intake (4.19 g dry matter) of a 181 g fish (geometric mean) under low oxygen condition in the present study. Thus, under *ad libitum* feeding with a high-protein diet, incipient DO for tilapia may be higher than 3.2 mg L^{-1}, referring to the average DO concentration in tanks with big fish in the present study.

4.2. Effects of body size on feed intake and growth

In accordance with the earlier findings (for examples, see Fänge and Grove (1979) and Jobling (1983)), relative feed intake and growth of small fish were higher than of big fish in the present study. The allometric relationship between growth and body weight in animals has been explained by considering that growth is a result of two counteracting processes: anabolism and catabolism (von Bertalanffy, 1957). Anabolism in a wide range of species has been found to be proportional to a power of body weight smaller than unity (see Glazier, 2005). Catabolism, which occurs in all living cells, was assumed to be directly proportional to the body weight (von Bertalanffy, 1957). Thus, the difference of these two processes must decrease with increasing body weight. The allometric relationship between anabolism and body weight in ectotherms was attributed to the surface area limitation associated with nutrient absorption and gas exchange (Ellenby, 1937; von Bertalanffy, 1957; Whitford and Hutchison, 1967; Hutchison et al., 1968; Ultsch, 1974; 1976) (cited after Glazier, 2005). Pauly (1981) argued that in fish the absorptive area of the gut is unlikely to limit growth for several reasons. Gut surface area is limited only when it is in permanent contact with the ingested food, which is not the case. Also, the relative absorptive area in fish is much more linked to the feeding mode than to growth performance, and long-lasting fat storage in fish

allows them to maintain anabolism long after completion of feeding and nutrient absorption, thus making anabolic processes independent of the gut surface. The results from the present experiment provide additional support for the hypothesis that the body size effect acts through the relative gill surface area.

The effect of body size on feed intake was also expressed through the difference in DO concentrations between tanks with small and big fish. Because we assumed that oxygen supply to the fish is driven by diffusion and therefore by the oxygen concentration gradient across the fish gills, we tried to maintain equal DO concentrations in the tanks with small and big fish within one DO level by regulating the water flow rates through the tanks. Nevertheless, dissolved oxygen concentrations in tanks with small fish were always slightly lower than in tanks with big fish, although water flow rates through tanks with small fish were either higher than or equal to those in tanks with big fish, and the initial biomass in tanks with small fish was smaller than in tanks with big fish (ca. 1.0 kg vs. 1.3 kg $tank^{-1}$, respectively). Because we could not quantify the amount of the oxygen supply by aeration, oxygen consumption of the fish could not be calculated based on the mass balance principle. However, since tanks with small and big fish received water with the same DO concentration at the inlet and were subject to the same aeration regime, lower DO concentrations in tanks with small fish imply that oxygen consumption relative to body weight in small fish was higher than in big fish. This is in accordance with the earlier studies in which fish metabolism was found to be proportional to a power of body weight smaller than unity (Winberg, 1956; Sauders, 1963; Brett, 1965; Glass, 1969; Brett and Glass, 1973; Andrews and Matsuda, 1975; Hölker; 2003). Because fish were fed to satiation in the present study, higher relative oxygen consumption in small fish than in big fish suggests a higher relative oxygen uptake capacity and therefore higher relative feed intake and growth rate in small fish than in big fish. In the present study, FI_{perc} in small fish was almost double that in big fish at the same DO level.

4.3. Feeding rhythm

Although oxygen concentrations in the morning were higher than in the afternoon, feed intake of the fish under high oxygen treatment was lower in the morning than in the afternoon in the present study. Reports on diurnal feeding rhythms of tilapias in natural waters are mixed (Harbott, 1975; Dewan and Saha, 1979; Haroon et al., 1998) and can hardly be used to draw a conclusion about the biological feeding rhythm of the fish, since feeding activity and feed intake are strongly influenced by environmental factors such as DO, temperature, light and food availability (Kestemont and Baras, 2001; Madrid et al., 2001).

Under controlled conditions, a report on feeding activity of Nile tilapia kept at DO above 80% saturation (6.36 mg L^{-1} at 27°C) showed that the fish ate mainly during the day time and the cumulative demand for food between 7:00 and 10:00 was lower than between 16:00 to 19:00 (Toguyeni et al., 1997). Another study showed that the filtration rates of Nile tilapia

between 6:00 and 12:00 were lower than between 14:00 and 20:00. Mean oxygen concentrations in the morning and afternoon were 4.2 and 3.8 mg L^{-1}, respectively (Turker, 2004). These studies showed the same phenomenon as observed in the present study: Nile tilapia at relatively high DO level (above 3.5 mg L^{-1}) consumed more in the afternoon than in the morning, even when the DO concentration in the afternoon was lower than in the morning. At low DO level (below 3.5 mg L^{-1}), fish in the present study consumed slightly more in the morning (at higher DO concentration) than in the afternoon (at lower DO concentration). This shift in feeding rhythm might be related to the limitation of oxygen uptake of the fish when DO concentration drops below a certain level. From a practical point of view, this phenomenon should be elucidated to optimize feeding strategy.

4.4 Hematological parameters

Common responses of fish under hypoxia to safeguard oxygen uptake include increased gill ventilation (Gerald and Cech, 1970; Lomholt and Johansen, 1979; Fernandes and Rantin, 1989) and blood oxygen affinity and/or capacity (Wood and Johansen, 1972; Weber et al., 1976; Soivio et al., 1980; Yamamoto et al., 1985; Nikinmaa, 1992; Nikinmaa and Salama, 1998). Among the responses, increase in hemoglobin concentration is considered to be energetically cost-effective (Weber and Jensen, 1988). The release of red blood cells via spleeny contraction is also employed in fish exposed to severe hypoxia (Yamamoto et al., 1985; Jobling, 1994). According to Weber and Jensen (1988), however, erythropoiesis and hemoglobin synthesis require a long time to complete and can only be involved in long-term adaptation. The absence of differences in hematological parameters between fish at low and high DO levels observed in the present study might be due to short-time exposure of the fish to low DO level, which was likely to be far from severe hypoxia for this species. Fernandes and Rantin (1986) found that high number and length of the gill filaments and high frequency of the secondary lamellae in Nile tilapia allow this species to exchange gas very efficiently. Together with high affinity of its hemoglobin to oxygen (Verheyen et al., 1985), Nile tilapia is very tolerant to very low DO concentration (Welcomme, 1969). Chervinski (1982) supposed that tilapias were able to tolerate DO as low as 1 mg L^{-1}. This value is much lower than the DO concentrations in the low DO treatments in the present study (2.78 and 3.19 mg L^{-1}). It is suggested that lower DO concentrations and longer duration are required to study effect of the hypoxia on hematological parameters of Nile tilapia.

5. Conclusions

The results of the present study showed that feed intake and growth of Nile tilapia of average weight of 37 and 190 g at DO concentration of about 3.0 mg L^{-1} were significantly lower than at DO concentration of about 5.6 mg L^{-1}. Feed intake and growth per body weight unit in fish of 37 g were higher than in fish of 190 g at both DO levels (3.0 and 5.6 mg L^{-1}). This body

size effect is attributed to differences in the relative gill surface area. Fish at high DO level (5.6 mg L^{-1}) consumed more in the afternoon than in the morning while fish at low DO level (3.0 mg L^{-1}) consumed more in the morning than in the afternoon. This indicates a change in feeding rhythm of the fish when the average DO drops below a certain level. Nile tilapia exposed to DO concentration of 3.0 mg L^{-1} for a duration of 25 days made no hematological adjustments. This might be due to short-time exposure of the fish to this DO level, which is likely to be far from severe hypoxia for this species.

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Chapter 5

Feed intake, growth and metabolism of Nile tilapia (*Oreochromis niloticus*) in relation to dissolved oxygen concentration

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Abstract

The objectives of the present study were to determine, for Nile tilapia of different body weights (1) the incipient dissolved oxygen concentration (DO) at which feed intake starts to level off; and (2) the effect of DO on energy metabolism. Two successive experiments were conducted with two weight classes of male Nile tilapia (>200 g, Experiment 1; and < 100 g, Experiment 2). Twelve aquaria were used, which were assigned to four DO levels in each experiment. Ambient DOs (± SE) in Experiment 1 were 1.58 ± 0.05, 2.58 ± 0.08, 4.06 ± 0.10 and 6.08 ± 0.02 mg L^{-1}, and in Experiment 2 were 1.33 ± 0.02, 1.71 ± 0.04, 2.62 ± 0.04 and 3.66 ± 0.05 mg L^{-1}. Fish were fed to apparent satiation for 20 and 24 days in Experiment 1 and 2, respectively. The DO-feed intake curve of fish < 100 g appeared to level off at a DO of 2.6 mg L^{-1}, while that of fish > 200 g continued to go up as DO increases from 2.6 to 6.0 mg L^{-1}. The latter curve suggests that the incipient DO for fish > 200 g must be at least 5 mg L^{-1}.

Fish reduced energy requirement for maintenance as DO declined. The results suggest that a DO reduction causes an increase in the apparent digestibility coefficient (ADC), but further declines in DO to levels below a critical level decrease ADC. This critical level in fish > 200 g was found to be between 2.6 and 1.6 mg L^{-1}, and in fish < 100 g lower than 1.3 mg L^{-1}. Non-fecal energy losses and heat production tended to increase with increasing DOs. The ME:DE ratios were almost constant, which ranged 94-96% in fish > 200 g and 95-97% in fish < 100 g. This suggests that DO does not have a considerable effect on the ME:DE ratio of the diet.

1. Introduction

While the negative effect of dissolved oxygen concentration (DO) on fish growth has been widely demonstrated, little experimental work has been done to determine maximum feed intake in relation to DO. Studies on coho salmon (*Oncorhynchus kisutch*), largemouth bass (*Micropterus salmoides*) and channel catfish (*Ictalurus punctatus*) have shown that DO is unlikely to affect feed conversion efficiency of the fish, except when most of the feed consumed is used for maintenance requirements (Herrmann et al., 1962; Stewart et al., 1967; Andrews et al., 1973). Thetmeyer et al. (1999) found a linear correlation between growth and feed intake in European sea bass (*Dicentrarchus labrax*) exposed to low DOs, suggesting that reduced growth under low DO condition is a direct consequence of reduced feed intake and not related to feed conversion efficiency. From a practical point of view, therefore, regulation of DO in a rearing system to ensure best growth should be based on observation of feed intake.

In a previous study (Tran-Duy et al., 2008), we found that feed intake of Nile tilapia (*Oreochromis niloticus*) at DOs of about 3 mg L^{-1} was significantly lower than at DOs of about 5.6 mg L^{-1}, and that the relative feed intake of fish of 37 g was higher than in fish of 190 g at both DOs. These findings support the hypothesis that feed intake is limited by the oxygen uptake capacity of the fish, which determines the oxygen availability for food processing, and depends on the gill surface area and DO in the water: under low oxygen condition, oxygen uptake by diffusion through the gill surface is limited and the fish stop eating when oxygen supply does not satisfy oxygen demand. Since the gill surface area is allometrically related to body weight, relative oxygen uptake capacity, and therefore relative feed intake are greater in smaller fish than in bigger fish (Pauly, 1981; van Dam and Pauly, 1995).

If oxygen supply to the fish is limited by diffusion through the gills, it is questionable whether maximum feed intake is always greater at higher oxygen concentrations. More likely, there is one oxygen concentration above which maximum feed intake remains unchanged. The latter is commonly referred to in literature as the incipient DO: the concentration at which feed intake and growth of fish start to level off. As the relative oxygen uptake capacity in smaller fish is greater than in bigger fish, the incipient DO of smaller fish would be lower than of bigger fish of the same species under the same rearing conditions. Thus, determining the incipient DO of fish of different weights is of practical importance.

The oxygen uptake capacity of fish has been known to determine the metabolic scope (Priede, 1985). Under low DO condition, the metabolic scope is reduced and all the energy-consuming processes, such as swimming activity and food ingestion, digestion, absorption

and processing, may be modified. It is still unclear how fish regulate these processes in response to a reduction in DO.

In our previous study with Nile tilapia (Tran-Duy et al., 2008) only two oxygen levels were tested, so it was not possible to determine the incipient DOs. Moreover, since energy metabolism was not investigated, it was unclear how Nile tilapia partitioned its energy budget under different DOs. The objectives of the present study were to determine, for Nile tilapia of different body weights (1) the incipient DO; and (2) the effect of DO on energy metabolism.

2. Materials and methods

2.1. Fish and rearing conditions

Two successive experiments were conducted with two weight classes of male Nile tilapia (Swansea Silver) at the Aquaculture and Fisheries Group of Wageningen University in The Netherlands. These experiments were approved by the Ethical Committee for Animal Experiments (DEC).

Four hundred fish with an average weight (± SE) of 130 ± 6.4 g for Experiment 1 and one thousand fish of 25 ± 3.8 g for Experiment 2 were obtained from a commercial fish farm (Til-Aqua Int.; Velden, Limburg, The Netherlands). At arrival, fish were distributed over 16 120-L rectangular glass tanks at densities of 25 and 60 fish $tank^{-1}$ in Experiments 1 and 2, respectively. All tanks were connected to a recirculation system equipped with a sedimentation tank, a trickling biofilter, a sump and a pump. From arrival to the start of the experiments (2 weeks) fish were accustomed to the experimental diet, which we formulated and manufactured in the university's feed mill. The diet (2-mm sinking pellets; analyzed composition: 94.5% dry matter, 19.8 kJ g^{-1}, 33.9% crude protein, 14.0% crude fat, 8.6% ash and 1.7% acid insoluble ash on a wet weight basis) was given to the fish manually twice per day (starting at 09:00 and 16:00) at a feeding level of 18 g $kg^{-0.8}$ d^{-1} in both experiments. Acid insoluble ash (AIA; Diamol; Franz Bertram, Hamburg, Germany) was added to the diet as an inert marker to determine apparent digestibility coefficients (Jobling, 2001). During the acclimation period, water flow rate through the tanks was maintained at 6 L min^{-1}, temperature at 28°C, DO above 5 mg L^{-1}, photoperiod at 12L:12D, pH between 7.0 and 8.0, NH_3-N below 0.1 mg L^{-1} and NO_2-N below 0.5 mg L^{-1}.

2.2. Experimental design

2.2.1. Experimental system

The experimental system (Figure 5.1) consisted of twelve 200-L glass aquaria connected to a recirculation system. All aquaria were isolated in a small area and protected as much as possible from disturbances. The water surface of each aquarium was tightly covered with a floating hard-foam plate to prevent gas exchange between the air and the water. A 3-cm-

diameter opening in each plate at the front side of the aquarium was fixed to a vertical polyethylene pipe to hold a feeding funnel. Water from the outlet of each aquarium passed a 17-L faeces collection tank (AquaOptima AS, Trondheim, Norway; see description in Amirkolaie et al., 2005) before entering a sedimentation tank. After leaving the sedimentation tank, water flowed to the first sump and was pumped to a trickling biofilter, after which it was filtered again by a drum filter (Hydrotech 500®) with an 18-µm screen and collected in a second sump where it was heated by a computer-controlled heater. From there, it was pumped through a cylindrical deoxygenating reactor (Liqui-Cel® Membrane Contactor; Membrana-Charlotte, USA) to remove dissolved oxygen.

After entering the deoxygenating reactor, water flowed into a central distribution tube from where it was forced radially over 300-µm-diameter micro-tubes. The walls of these micro-tubes are made from 100-µm-thick polypropylene membranes which contain micro-pores. The membranes are hydrophobic, so water cannot pass through the micro-pores whereas dissolved oxygen can. A vacuum pump creates low pressure inside the micro-tubes, forcing the oxygen particles from the water into the micro-tubes from where they are released to the atmosphere. In this way, oxygen is extracted from the water as it flows through the reactor.

After deoxygenation, the water was collected in a round 900-L polyethylene tank with four draining tubes at the bottom. Each draining tube led the water to a vertical cylindrical oxygenating reactor where pure oxygen was added to raise the DO to the desired level. From each oxygenating reactor, water flowed into the corresponding block of three aquaria; each aquarium in a block was connected to the common inlet pipe by a 1-m-length plastic tube. The water flow rate through each aquarium was measured with an electronic flow meter (Danfoss Magflow®).

2.2.2. Experimental procedures

After two weeks of acclimatization, fish were anesthetized with tricaine methane sulfonate (MS-222; 0.2 g L^{-1} buffered with 0.4 g L^{-1} sodium bicarbonate), weighed individually and randomly allocated to the 12 aquaria at a density of 20 and 60 fish per aquarium in Experiments 1 and 2, respectively. During fish allocation, 20 fish from each experiment were randomly sampled for initial body composition analysis. These fish were killed with an overdose of MS-222 (0.8 g L^{-1} buffered with 1.6 g L^{-1} sodium bicarbonate), placed in a plastic bag, sealed and stored at -20^{o}C for further processing and analysis.

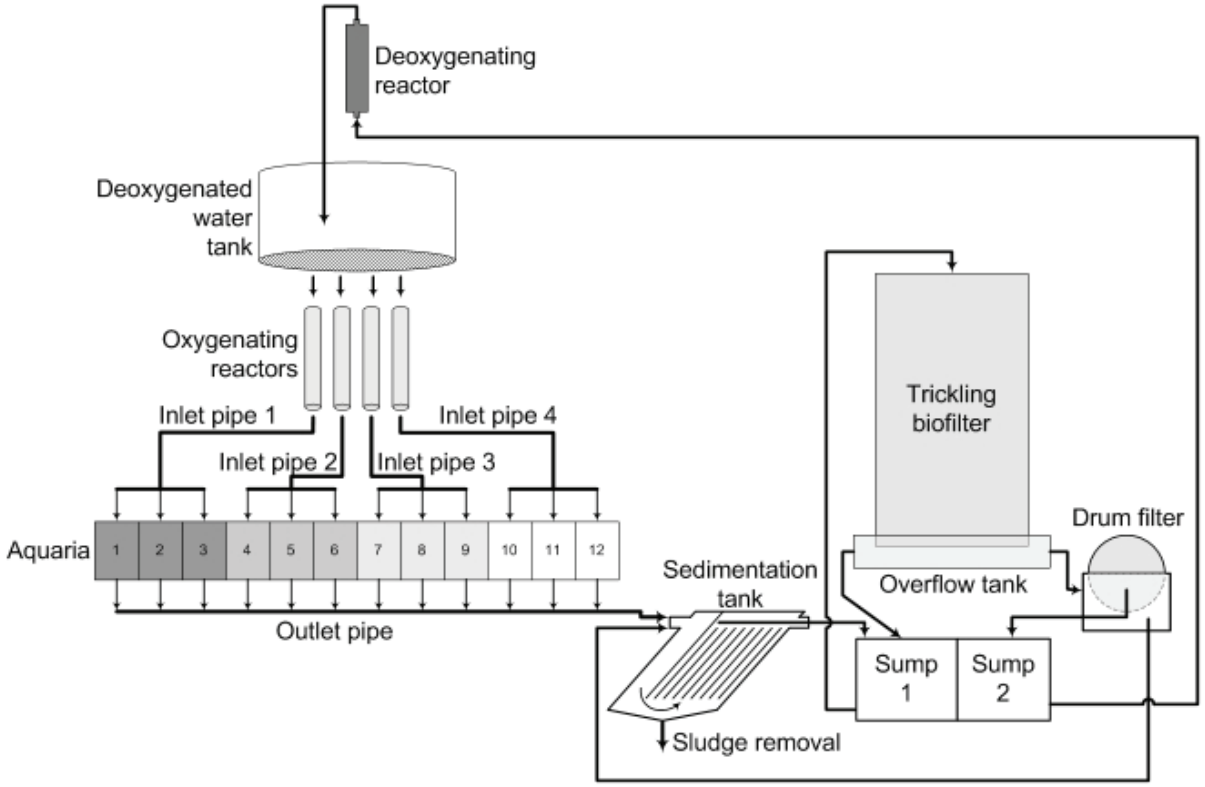

Figure 5.1. Schematic drawing of the experimental system. Arrows indicate water flow.

For each experiment, four DO levels were assigned to the 12 aquaria with three replicates (aquaria) per level. Each level was created by maintaining a constant DO at the common inlet of the three associated aquaria. In Experiment 1, the inlet DOs were set at 3.0, 5.5, 8.0 and 10.5 mg L^{-1}, corresponding to Treatments 1.1, 1.2, 1.3 and 1.4, respectively. The interval of 2.5 mg L^{-1} was employed to create a relatively high contrast in DOs among the treatments. Average initial weights (± SE) of the fish in Treatments 1.1, 1.2, 1.3 and 1.4 were 187.67 ± 0.64, 188.22 ± 0.08, 186.18 ± 1.14 and 187.33 ± 0.81 g, corresponding to stocking densities (± SE) of 18.77 ± 0.06, 18.82 ± 0.01, 18.62 ± 0.11 and 18.73 ± 0.08 kg m^{-3}, respectively. In Experiment 2, the inlet DOs were set at 4.0, 6.0, 8.0 and 10.0 mg L^{-1}, corresponding to Treatments 2.1, 2.2, 2.3 and 2.4, respectively. The average initial weights (± SE) of the fish in Treatments 2.1, 2.2, 2.3 and 2.4 were 52.14 ± 1.08, 51.84 ± 0.19, 52.85 ± 0.60 and 53.14 ± 0.68 g, corresponding to stocking densities (± SE) of 15.64 ± 0.33, 15.55 ± 0.06, 15.85 ± 0.18 and 15.94 ± 0.21 kg m^{-3}, respectively.

As oxygen consumption of fish is proportional to a power of body weight smaller than unity (Winberg, 1956; Sauders, 1963; Glass, 1969; Andrews and Matsuda, 1975; Hölker, 2003), oxygen consumption of an aquarium in Experiment 2 (60 fish of about 52 g initially) was expected to be higher than that of an aquarium in Experiment 1 (20 fish of about 187 g

initially). Moreover, oxygen consumption of each aquarium would increase over time as fish grew bigger. Therefore the lowest inlet DO in Experiment 2 was set at 4.0 mg L^{-1} against 3.0 mg L^{-1} in Experiment 1 to avoid the risk of suffocation in Treatment 2.1, especially towards the end of the experiment. In Experiment 2 an interval of 2 mg L^{-1} (instead of 2.5 mg L^{-1} in Experiment 1) was used, starting from the lowest DO of 4 mg L^{-1}. With an interval of 2.5 mg L^{-1}, the highest inlet DO (11.5 mg L^{-1}) would have been too high to maintain.

When fish were allocated to the aquaria, inlet DO of all the aquaria was kept at 7.8 mg L^{-1}, the same value as the inlet DO of the aquaria used for the acclimation period. From this level, DO at the common inlet of each treatment block was gradually changed to the set-point during the next five days. During the first four days, fish were fed manually twice per day at a feeding level of 13 g $kg^{-0.8}$ d^{-1} in Experiment 1 and 15 g $kg^{-0.8}$ d^{-1} in Experiment 2 with the same diet as used during the acclimation period. This was done to ensure that fish in all aquaria in each experiment consumed the same amount of feed and would therefore have similar weights when the *ad libitum* feeding started on Day 5 (when the designed set-points of the inlet DOs were established). From Day 5, fish were fed manually to apparent satiation twice per day (starting at 09:00 and 16:00) for 16 and 20 days in Experiments 1 and 2, respectively; each meal lasted no longer than one hour. For logistic reasons, the experimental period in Experiment 2 was 24 days against 20 days in Experiment 1.

During the first five days, DO inside each aquarium was measured manually twice per day by inserting an oxygen electrode through the opening on top of the covering plate. Because the differences between DOs inside and at the outlet of each aquarium at the same time were negligible (less than 0.2 mg L^{-1} in all cases), outlet DOs were considered as ambient DOs of the aquaria.

During the *ad libitum* feeding period, feces were collected to determine apparent digestibility. Every day before feeding, feces collected in the settling tank of each aquarium were transferred to an aluminum box and stored at -20^{o}C for further analysis. Every 15 minutes during feeding and 15 minutes after feeding, the flushed-out pellets collected at each settling tank were counted to quantify the uneaten feed of the associated aquarium.

At the end of the experimental period, fish from each aquarium were anesthetized with MS-222 (same dose as used for fish allocation) and weighed individually. Ten fish from each aquarium were randomly sampled for final body composition analysis. Procedures for processing of sampled fish were the same as at the start of the experimental period.

Before chemical analysis, fish samples were cut into small pieces and homogenized by passing them through a 4.5 mm-screen grinder twice. Feed samples were ground twice using a 1 mm-screen grinder. Samples for dry matter determination were taken from the homogenates of fish and feed before the remaining materials were freeze-dried. All feces were freeze-dried.

Each freeze-dried sample of fish, feces and feed was thoroughly mixed in a blender before further analysis.

2.2.3. Water quality maintenance

Maintenance of DOs at the inlets of the aquaria was done by means of hardware and software. Dissolved oxygen concentration of water leaving the deoxygenating reactor was expected to be lower than 3 mg L^{-1}. Pure oxygen was added to the water in each oxygenating reactor with a mass flow controller (Brooks® Smart Mass flow Controller Model 5850S; Brooks Instruments, The Netherlands), which was connected to a microprocessor-based data controller (Brooks® Read Out and Control Electronics Model 0154; Brooks Instruments, The Netherlands). The data controller controlled the mass flow controllers and communicated with a computer via a user interface (Brooks Smart DDE Software; Brooks Instruments, The Netherlands) for setting the DOs at the inlet pipes of the aquaria. The DO at each common inlet pipe of three aquaria was automatically measured using an oxygen meter (WTW 340i; Wissenschaftlich-Technische Werkstätten GmbH, Germany) with a membrane-covered galvanic sensor (CellOx® 325), which was connected to the computer. Every minute the inlet DO measured by each oxygen meter was sent to the computer and compared with the respective set-point of DO. If the actual DO was higher or lower than the set-point, the data controller would instruct the mass flow controller to decrease or increase the oxygen flow into the corresponding oxygenating reactor until the actual DO matched the designed set-point.

Dissolved oxygen concentration, temperature and pH of the water at the outlets of the aquaria were measured using a common sensor (WTW IQ Sensor Net; Wissenschaftlich-Technische Werkstätten GmbH, Germany), which was connected to the computer for data storage. A plastic sampling tube led water sampled at the outlet of each aquarium to the common sensor. Water sampling for each aquarium was done by means of a magnetic valve (ASCO model 24/50 6 W FT; Serial number 48843; ASCO/Joucomatic, Scherpenzeel, The Netherlands), which was opened and closed automatically by the computer. The opening duration was five minutes, of which three minutes were used to flush the remaining water of the previous sampling and two minutes for the current measurement. When the valve of one aquarium was closed, the valve of the next aquarium was opened at the same time. Thus with twelve aquaria, the water quality parameters at the outlet of each aquarium were measured every hour.

During the experiment periods, water flow rate through the aquaria was maintained at 6 L min^{-1}, temperature at 28°C, photoperiod at 12L:12D, pH between 7.5 and 8.0, NH_3-N below 0.1 mg L^{-1} and NO_2-N below 0.5 mg L^{-1}.

2.3. Analytical procedures

All chemical analyses were done in triplicate. Dry matter content was determined as weight loss after drying the samples for 4 h at 103^{o}C until constant weight (ISO, 1983). Crude protein content was determined using the Kjeldahl method and multiplying nitrogen content by 6.25 (ISO, 1997). Crude fat content was determined after petroleum-ether extraction using a Soxhlett system (ISO, 1999). Gross energy content was determined using a bomb calorimeter (IKA-C7000, IKA-analysentechnik, Weitersheim, Germany). Ash was determined by burning the oven-dried samples in a muffle furnace at 550°C (ISO, 1978). Acid insoluble ash was determined by treating the residue obtained after ash determination with hydrochloric acid. This mixture was then filtered to obtain the insoluble residue (ISO, 1981).

2.4. Calculation

The present study assessed the maximum feed intake of Nile tilapia in relation to different DO levels. Since fish were fed to satiation from Day 5 to the end of the experimental period (Day 20 and 24 in Experiments 1 and 2, respectively), all the performance parameters (feed intake, growth rates and FCR) were calculated for this *ad libitum* feeding period (ALFP). Feed intake of the fish expressed as a percentage of body weight (FI_{perc}) and per metabolic weight unit (FI_{MBW}) were calculated as

$$FI_{perc}\ (\%\ d^{-1}) = FI/W_{mean} \times 100 \text{ and}$$

$$FI_{MBW}\ (g\ kg^{-0.8}\ d^{-1}) = FI/(W_{mean}/1000)^{0.8}$$

where FI (g d^{-1}) is the average feed intake over the ALFP per fish per day and W_{mean} is the geometric mean body weight, which was calculated as

$$W_{mean}\ (g) = \sqrt{W_a \times W_f}$$

where W_a and W_f are the average fish weight (g) on the day *ad libitum* feeding started and at the end of the experimental period, respectively. W_a was estimated based on the average fish weight W_i (g) on the day fish were allocated to the aquaria, daily feed intake (g) during Days 1-4 and the feed conversion ratio (FCR), which was estimated as

$$FCR = FI_{tot}/(W_f - W_i)$$

where FI_{tot} (g) is the total feed intake per fish during the experimental period.

In Experiment 1, the average FCR (± SE) over the experimental period in Treatments 1.1, 1.2, 1.3 and 1.4 were 3.41 ± 0.53, 1.32 ± 0.05, 1.25 ± 0.06 and 1.15 ± 0.05, respectively. Pair-wise comparisons showed that there were no significant differences in FCR among the last three treatments ($P > 0.5$; see 2.5), while FCR in Treatment 1.1 was significantly higher than in any of the other treatments ($P < 0.01$). During the experimental period, mean inlet DOs of Treatments 1.2, 1.3 and 1.4 were 5.39-11.74 mg L^{-1}, suggesting that inlet DOs within

this range did not affect FCR. Since during Days 1-4 inlet DOs in Treatment 1.1 were still between 5.5 and 7.8 mg L^{-1}, we assumed that FCR during that period was the same as in the other treatments. Therefore, the average of the average FCR over the experimental period of Treatments 1.2, 1.3 and 1.4 (FCR = 1.24) was used to estimate W_a in all the aquaria. In Experiment 2, the average FCR (± SE) over the experimental period of Treatments 2.1, 2.2, 2.3 and 2.4 was 1.09 ± 0.03, 1.04 ± 0.03, 0.99 ± 0.03 and 1.01 ± 0.01, respectively. As in Experiment 1, the average of the average FCR of Treatments 2.2, 2.3 and 2.4 (FCR = 1.01) was used to estimate W_a in all the aquaria. The feed conversion ratio during the ALFP was then re-calculated as

$$FCR = FI_{adlib}/(W_f - W_a)$$

where FI_{adlib} (g) is the total feed intake per fish during the ALFP.

Specific growth rates (SGR) were calculated using the following formula (Jobling, 1994, Chapter 9):

$$SGR\ (\%\ d^{-1}) = [(\ln W_f - \ln W_a)/t] \times 100$$

where t is the length of the ALFP (days). Growth rates per metabolic weight unit (GR_{MBW}) were calculated as

$$GR_{MBW}\ (g\ kg^{-0.8}\ d^{-1}) = (W_f - W_a)/(W_{mean}/1000)^{0.8}/t$$

Apparent digestibility coefficients (ADC) were calculated as (Bureau et al., 2002):

$$ADC_X = (1 - AIA_{diet}/AIA_{feces} \times X_{feces}/X_{diet}) \times 100$$

where X represents dry matter, crude protein, crude fat or energy, AIA_{diet} and AIA_{feces} are the AIA content (% dry matter) in the diet and feces, respectively and X_{diet} and X_{feces} are the quantity of X in 1 g dry matter of the diet and feces, respectively.

Total digestible nitrogen (DN; mg $fish^{-1}$) was calculated as the product of total gross nitrogen intake (GN; mg $fish^{-1}$) and ADC of nitrogen (in %), where GN was calculated as the product of total feed intake (g $fish^{-1}$) and nitrogen content of the diets (mg g^{-1}). Total fecal nitrogen losses (FN; mg $fish^{-1}$) were calculated as the difference between GN and DN, total retained nitrogen (RN; mg $fish^{-1}$) as the difference between the final and initial nitrogen mass (mg $fish^{-1}$), and total branchial and urinary nitrogen losses (BUN; mg $fish^{-1}$) as the difference between DN and RN. The above-calculated values of GN, FN, DN, BUN and RN were divided by $[t \times (W_{mean}/1000)^{0.8}]$ to be expressed in mg $kg^{-0.8}$ d^{-1}.

Total digestible energy (DE; kJ $fish^{-1}$) was calculated as the product of total gross energy intake (GE; kJ $fish^{-1}$) and ADC of energy (in %), where GE was calculated as the product of total feed intake (g $fish^{-1}$) and energy content of the diets (kJ g^{-1}). Total fecal energy losses (FE; kJ $fish^{-1}$) were calculated as the difference between GE and DE, and total metabolizable

energy (ME; kJ $fish^{-1}$) as the difference between DE and the energy in the total branchial and urinary excretory products (BUE; kJ $fish^{-1}$); the latter was estimated as

$$BUE = (BUN \times 24.9)/1000$$

where 24.9 is the amount of kJ equivalent to 1 g excreted nitrogen, assuming that all nitrogen is excreted as NH_3-N (Bureau et al., 2002). Total retained energy (RE; kJ $fish^{-1}$) was calculated as the difference between the final and initial energy quantities (kJ $fish^{-1}$), and total heat production (HP; kJ $fish^{-1}$) as the difference between ME and RE. Metabolizable energy for maintenance of body weight (ME_{maint}) was estimated by considering that ME is made up of ME_{maint} and metabolizable energy for production (i.e. deposition of body tissues; ME_p); the transformation of ME_p to RE costs some energy, thus the ratio of RE to ME_p is designated as the utilization efficiency of ME_p for RE (K_g) (Heinsbroek, 1987). Therefore, ME_{maint} can be calculated as

$$ME_{maint} = ME - RE/K_g$$

A value of 0.8 for K_g was assumed for Nile tilapia in the present study based on the work of Huisman (1976) on rainbow trout (*Oncorhynchus mykiss*; $K_g = 0.78$) and common carp (*Cyprinus carpio*; $K_g = 0.89$) and Hogendoorn (1983) on African catfish (*Clarias gariepinus*; $K_g = 0.8$). The above-calculated values of GE, FE, DE, BUE, ME, $ME_{maint,}$ HP and RE were divided by $[t \times (W_{mean}/1000)^{0.8}]$ to be expressed in kJ $kg^{-0.8}$ d^{-1}.

2.5. Statistical analysis

Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., 2004). The homogeneity of variances of different groups was checked using Levene's *F* test with PROC ANOVA. Since the homogeneity assumption was met for all variables ($P > 0.05$), mean values of ambient DO, feed intake, growth rates, body composition, digestibility coefficients and energy and nitrogen balance parameters were subjected to one-way analysis of variance (ANOVA) using PROC GLM. Normal distribution of the residuals was verified using Kolmogorov-Smirnov's test with PROC UNIVARIATE. Of all the variables, FCR and ADC of feed, protein and energy in Experiment 1 did not satisfy the normal distribution assumption ($P < 0.01$). Because transformations did not improve this, a Kruskal-Wallis one-way ANOVA was performed on these variables using PROC NPAR1WAY. Differences among treatment means were considered significant when $P < 0.05$ and not significant (no effect) when $P \geq 0.05$. If significance was detected, multiple comparisons were performed using Tukey adjustment.

3. Results

3.1. Dissolved oxygen concentrations

The maintenance of the inlet DO depended on the efficiency of the deoxygenating reactor, the operation of the software and hardware and the diffusion rate of oxygen in the oxygenating reactors. Since this was a complex process, inlet DOs fluctuated around the set-points with means (± SE) over the ALFP in Treatments 1.1, 1.2, 1.3 and 1.4 being 2.78 ± 0.09, 5.39 ± 0.05, 8.13 ± 0.03 and 11.74 ± 0.07 mg L^{-1}, and in Treatments 2.1, 2.2, 2.3 and 2.4 being 5.22 ± 0.01, 6.31 ± 0.01, 7.99 ± 0.00 and 9.87 ± 0.01 mg L^{-1}, respectively. The relatively high deviation of the actual inlet DO from the set-point in Treatment 1.4 (11.74 versus 10.5 mg L^{-1}) was attributed to additional diffusion of pure oxygen into already supersaturated water which did not increase DO immediately. Possibly, this led to situations in which pure oxygen was still added to the reactor while a large amount of oxygen gas had already accumulated. When the actual DO exceeded the set-point, the accumulated oxygen in the reactor would boost the DOs beyond the set-point. The relatively high deviation of the actual inlet DO from the set-point in Treatment 2.1 (5.22 versus 4.0 mg L^{-1}) was due to a reduction in the oxygen removal efficiency of the deoxygenating reactor which could not lower the DO to the expected level.

Mean outlet (ambient) DOs (± SE) over the ALFP in Treatments 1.1, 1.2, 1.3 and 1.4 were 1.58 ± 0.05, 2.58 ± 0.08, 4.06 ± 0.10 and 6.08 ± 0.02 mg L^{-1}, and in Treatments 2.1, 2.2, 2.3 and 2.4 were 1.33 ± 0.02, 1.71 ± 0.04, 2.62 ± 0.04 and 3.66 ± 0.05 mg L^{-1}, respectively. In each experiment, the DOs were significantly different between any pair of the treatments ($P < 0.001$).

3.2. Feed intake and growth (Table 5.1)

Mean initial fish weights were not significantly different among treatments in both experiments ($P > 0.1$). In Experiment 1, mean final fish weights and feed intake increased significantly with each increase in DO ($P < 0.05$). Growth rates were significantly lower in Treatment 1.1 than in 1.2 ($P < 0.001$) and significantly lower in Treatments 1.1. and 1.2 than in 1.3 and 1.4 ($P < 0.01$), but not significantly different between Treatments 1.3 and 1.4 ($P > 0.05$). One tank in Treatment 1.1 had negative growth rates, with SGR = -0.06 % d^{-1} and GR_{MBW} = -0.4 g $kg^{-0.8}$ d^{-1}. This tank was excluded from the calculation of FCR. A Kruskal-Wallis test on FCR in Experiment 1 showed a tendency of significant difference among treatments ($P = 0.058$), in which mean FCR in Treatment 1.1 (6.75) was much higher than in the others (ranging 1.14-1.34). In Experiment 2, mean final body weights, feed intake and growth rates were significantly lower in Treatment 2.1 than in 2.2 ($P < 0.05$) and significantly lower in Treatments 2.1 and 2.2 than in 2.3 and 2.4 ($P < 0.001$), but not significantly different between Treatments 2.3 and 2.4 ($P > 0.5$). Feed conversion ratios were not significantly

different between Treatments 2.1 and 2.2 ($P > 0.1$) and among Treatments 2.2, 2.3 and 2.4 ($P > 0.5$), but significantly higher in Treatments 2.1 than in 2.3 and 2.4 ($P < 0.05$). A plot of feed intake against DO in the two experiments is presented in Figure 5.2.

3.3. Body composition (Table 5.2)

No effects of DO on dry matter, crude protein, crude fat, ash and energy contents of the fish in Experiment 1 were found ($P > 0.1$). In Experiment 2, neither were crude fat and energy contents significantly affected by DO ($P > 0.1$). Dry matter contents were not significantly different among Treatments 2.1, 2.3 and 2.4 ($P > 0.1$) and between Treatments 2.1 and 2.2, but significantly higher in Treatment 2.2 than in 2.3 and 2.4 ($P < 0.05$). Crude protein contents in Treatments 2.2, 2.3 and 2.4 were not significantly different from each other ($P > 0.1$) but they were all higher than in Treatment 2.1 ($P < 0.05$). Ash contents were not significantly different among Treatments 2.1, 2.2 and 2.3 ($P > 0.05$), and between Treatment 2.3 and 2.4 ($P > 0.5$), but significantly higher in Treatments 2.1 and 2.2 than in 2.4 ($P < 0.05$).

3.4. Digestibility (Table 5.3)

In Experiment 1, Kruskal-Wallis tests showed that there was an effect of DO on ADC of dry matter, crude protein and energy ($P < 0.05$). After the *ad libitum* feeding period, priority was given to the AIA analysis (indispensable for ADC calculations) and to crude protein and energy analyses (required for nitrogen and energy balance calculations). Feces remaining after AIA, crude protein and energy analyses in Treatment 1.1 were insufficient for crude fat determination so there were no data for ADC of fat in this treatment. Pair-wise comparisons among Treatments 1.2, 1.3 and 1.4 showed that ADCs of fat significantly decreased with each increase in DO ($P < 0.05$).

In Experiment 2, there was no effect of DO on ADC of dry matter or crude protein ($P > 0.1$). Apparent digestibility coefficients of fat in Treatments 2.1, 2.2 and 2.3 were not significantly different from each other ($P > 0.05$), but they were all significantly higher than in Treatment 2.4 ($P < 0.01$). Apparent digestibility coefficients of energy were not significantly different among Treatments 2.1, 2.2, 2.3 ($P > 0.1$) and between Treatments 2.3 and 2.4 ($P > 0.1$), but significantly higher in Treatments 2.1 and 2.2 than in 2.4 ($P < 0.05$).

3.5. Nitrogen balance (Table 5.4)

In Experiment 1, GN, DN and RN (mg $kg^{-0.8}$ d^{-1}) significantly increased with each increase in DO ($P < 0.05$). Fecal nitrogen losses and BUN followed the same trend: values in Treatments 1.3 and 1.4 were not significantly different ($P > 0.05$), but they were both significantly higher than in Treatments 1.1 and 1.2 ($P < 0.01$), where values in Treatment 1.2 were significantly higher than in 1.1 ($P < 0.05$).

In Experiment 2, GN, FN, DN and RN followed a similar trend as FN and BUN in Experiment 1: values in Treatments 2.3 and 2.4 were not significantly different ($P > 0.1$), but they were both significantly higher than in Treatments 2.1 and 2.2 ($P < 0.01$), where values in Treatment 2.2 were significantly higher than in 2.1 ($P < 0.05$). Branchial and urinary losses were not significantly different between Treatments 2.1 and 2.2 ($P > 0.5$), and between Treatments 2.3 and 2.4 ($P > 0.5$), but significantly lower in Treatments 2.1 and 2.2 than in 2.3 and 2.4 ($P < 0.05$).

3.6. Energy balance (Table 5.5)

In Experiment 1, GE, FE, DE and ME (kJ $kg^{-0.8}$ d^{-1}) significantly increased with each increase in DO ($P < 0.05$) while BUE, HP and RE followed the same trend: values in Treatments 1.3 and 1.4 were not significantly different ($P > 0.05$), but they were both significantly higher than in Treatments 1.1 and 1.2 ($P < 0.05$), where values in Treatment 1.2 were significantly higher than in 1.1 ($P < 0.01$). Ignoring Treatment 1.4, ME_{maint} significantly decreased with each reduction in DO ($P < 0.05$). Metabolizable energy for maintenance of body weight in Treatment 1.4 was not significantly different from that in Treatments 1.2 and 1.3 ($P > 0.05$), but significantly higher than in Treatment 1.1 ($P < 0.001$).

In Experiment 2, GE, FE, DE and ME followed a similar trend as BUE, HP and RE in Experiment 1: values in Treatments 2.3 and 2.4 were not significantly different ($P > 0.1$), but they were both significantly higher than in Treatments 2.1 and 2.2 ($P < 0.001$), where values in Treatment 2.2 were significantly higher than in 2.1 ($P < 0.05$). Energy in branchial and urinary excretory products, HP and RE were not significantly different between Treatments 2.1 and 2.2 ($P > 0.5$), and between Treatments 2.3 and 2.4 ($P > 0.05$), but significantly lower in Treatments 2.1 and 2.2 than in 2.3 and 2.4 ($P < 0.05$). Except that ME_{maint} in Treatment 2.1 was significantly lower than in 2.3 and 2.4, and ME_{maint} in Treatment 2.2 was significantly lower than in 2.4, there were no significant differences between any pairs of treatments ($P > 0.05$).

Table 5.1. Experimental characteristics and feed intake and growth performance of Nile tilapia (*Oreochromis niloticus*) reared at four different ambient dissolved oxygen concentrations in each of two experiments

	Experiment 1				Experiment 2			
Treatment	**1.1**	**1.2**	**1.3**	**1.4**	**2.1**	**2.2**	**2.3**	**2.4**
Ambient DO (mg L^{-1})[1]	**1.58 ± 0.05**	**2.58 ± 0.08**	**4.06 ± 0.10**	**6.08 ± 0.02**	**1.33 ± 0.02**	**1.71 ± 0.04**	**2.62 ± 0.04**	**3.66 ± 0.05**
Growth period (d)	16	16	16	16	20	20	20	20
No of fish per tank	20	20	20	20	60	60	60	60
No of tanks	3	3	3	3	3	3	3	3
Survival (%)	100	100	100	100	100	100	100	100
Initial body weight (g)[2]	195.88 ± 1.19^{a}	197.91 ± 0.18^{a}	195.93 ± 1.20^{a}	197.36 ± 0.78^{a}	57.91 ± 1.18^{a}	57.58 ± 0.21^{a}	58.68 ± 0.66^{a}	59.00 ± 0.74^{a}
Final bodyweight (g)[2]	197.14 ± 1.22^{a}	234.33 ± 2.87^{b}	257.29 ± 5.03^{c}	273.26 ± 3.07^{d}	80.50 ± 0.51^{a}	86.09 ± 0.48^{b}	99.94 ± 1.01^{c}	100.50 ± 0.15^{c}
Feed intake[3]								
FI_{perc} (% d^{-1})	0.60 ± 0.00^{a}	1.43 ± 0.03^{b}	2.13 ± 0.03^{c}	2.33 ± 0.07^{d}	1.85 ±0.04^{a}	2.12 ± 0.04^{b}	2.66 ± 0.05^{c}	2.73 ± 0.05^{c}
FI_{MBW} (g $kg^{-0.8}$ d^{-1})	4.20 ± 0.06^{a}	10.33 ± 0.34^{b}	15.77 ± 0.33^{c}	17.40 ± 0.45^{d}	10.81 ± 0.19^{a}	12.48 ± 0.22^{b}	15.95 ± 0.30^{c}	16.32 ± 0.29^{c}
Growth[3]								
SGR (% d^{-1})	0.04 ± 0.05^{a}	1.06 ± 0.07^{b}	1.70 ± 0.11^{c}	2.03 ± 0.09^{c}	1.65 ± 0.07^{a}	2.01 ± 0.02^{b}	2.67 ± 0.10^{c}	2.67 ± 0.06^{c}
GR_{MBW} (g $kg^{-0.8}$ d^{-1})	0.30 ± 0.36^{a}	7.77 ± 0.55^{b}	12.67 ± 0.78^{c}	15.23 ± 0.69^{c}	9.68 ± 0.40^{a}	11.91 ± 0.12^{b}	16.12 ± 0.64^{c}	16.14 ± 0.37^{c}
FCR[3, 4]	6.75 ± 1.06	1.34 ± 0.06	1.25 ± 0.06	1.14 ± 0.05	1.12 ± 0.03^{a}	1.05 ± 0.03^{ab}	0.99 ± 0.02^{b}	1.01 ± 0.01^{b}

[1] Values represent means ± SE of three replicates (tanks); each replicate value is the mean of all the data points measured on one tank during the *ad libitum* feeding period.

[2] Values represent means ± SE of three replicates (tanks); each replicate value is the mean of the individual measurements on 20 or 60 fish in experiment 1 or 2, respectively.

[3] Except for FCR in treatment 1.1, values represent means ± SE over the *ad libitum* feeding period of three replicates (tanks); each replicate value was calculated based on an individual fish as a mean of one tank.

[4] Value in treatment 1.1 represents mean ± SE of two tanks as one of three replicate tanks had a negative weight gain. A Kruskal-Wallis one-way ANOVA was performed for means in experiment 1, with *P*-value being 0.058.

[a, b, c, d] Means in one row under each experiment lacking a common superscript are significantly different at $P < 0.05$.

Table 5.2. Final body composition (on a fresh weight basis) of Nile tilapia (*Oreochromis niloticus*) reared at four different ambient dissolved oxygen concentrations in each of two experiments

	Experiment 1				Experiment 2			
Treatment	**1.1**	**1.2**	**1.3**	**1.4**	**2.1**	**2.2**	**2.3**	**2.4**
Ambient DO (mg L^{-1})[1]	**1.58 ± 0.05**	**2.58 ± 0.08**	**4.06 ± 0.10**	**6.08 ± 0.02**	**1.33 ± 0.02**	**1.71 ± 0.04**	**2.62 ± 0.04**	**3.66 ± 0.05**
Constituent[2]								
Dry matter (%)	29.9 ± 0.46^{a}	30.2 ± 0.07^{a}	29.7 ± 0.18^{a}	30.4 ± 0.15^{a}	28.9 ± 0.18^{ab}	29.4 ± 0.17^{a}	28.5 ± 0.18^{b}	28.4 ± 0.03^{b}
Crude protein (%)	16.1 ± 0.07^{a}	15.8 ± 0.14^{a}	15.6 ± 0.12^{a}	16.2 ± 0.28^{a}	14.4 ± 0.08^{a}	15.0 ± 0.04^{b}	15.4 ± 0.18^{b}	15.4 ± 0.21^{b}
Crude fat (%)	10.5 ± 0.37^{a}	10.7 ± 0.07^{a}	10.3 ± 0.20^{a}	10.8 ± 0.04^{a}	9.8 ± 0.22^{a}	10.1 ± 0.08^{a}	10.2 ± 0.10^{a}	10.1 ± 0.05^{a}
Ash (%)	3.5 ± 0.13^{a}	3.6 ± 0.04^{a}	3.6 ± 0.06^{a}	3.6 ± 0.07^{a}	3.4 ± 0.05^{a}	3.4 ± 0.07^{a}	3.2 ± 0.03^{ab}	3.2 ± 0.03^{b}
Energy (kJ g^{-1})	7.9 ± 0.17^{a}	7.7 ± 0.08^{a}	7.7 ± 0.03^{a}	7.9 ± 0.11^{a}	7.5 ± 0.03^{a}	7.6 ± 0.08^{a}	7.5 ± 0.09^{a}	7.5 ± 0.02^{a}

[1] Values represent means ± SE of three replicates (tanks); each replicate value is the mean of all the data points measured on one tank during the *ad libitum* feeding period.

[2] Values represent means ± SE of three replicates (tanks); each replicate value is a measurement on the homogenate of 10 fish.

[a, b] Means in one row under each experiment lacking a common superscript are significantly different at $P < 0.05$.

Table 5.3. Apparent digestibility coefficients of dry matter, nutrients and energy of Nile tilapia (*Oreochromis niloticus*) reared at four different ambient dissolved oxygen concentrations in each of two experiments

	Experiment 1				Experiment 2			
Treatment	**1.1**	**1.2**	**1.3**	**1.4**	**2.1**	**2.2**	**2.3**	**2.4**
Ambient DO (mg L^{-1})[1]	**1.58 ± 0.05**	**2.58 ± 0.08**	**4.06 ± 0.10**	**6.08 ± 0.02**	**1.33 ± 0.02**	**1.71 ± 0.04**	**2.62 ± 0.04**	**3.66 ± 0.05**
Parameter (%)[2]								
Dry matter[3]	73.20 ± 2.61	81.76 ± 0.15	81.12 ± 0.06	80.65 ± 0.09	78.80 ± 0.08[a]	78.86 ± 0.26[a]	79.03 ± 0.13[a]	78.90 ± 0.05[a]
Crude protein[3]	88.04 ± 1.83	92.77 ± 0.05	92.40 ± 0.08	92.03 ± 0.04	91.90 ± 0.05[a]	91.88 ± 0.13[a]	92.06 ± 0.07[a]	91.82 ± 0.06[a]
Crude fat	na[4]	96.96 ± 0.09[a]	95.70 ± 0.22[b]	94.74 ± 0.25[c]	95.08 ± 0.21[a]	94.93 ± 0.17[a]	94.44 ± 0.16[a]	93.36 ± 0.11[b]
Energy[3]	83.30 ± 1.70	88.28 ± 0.11	87.56 ± 0.10	86.85 ± 0.13	85.90 ± 0.09[a]	85.81 ± 0.17[a]	85.46 ± 0.16[ab]	85.11 ± 0.12[b]

[1] Values represent means ± SE of three replicates (tanks); each replicate value is the mean of all the data points measured on one tank during the *ad libitum* feeding period.

[2] Values represent means ± SE of three replicates (tanks); each replicate value was calculated based on a pooled sample of feed and of feces collected during the *ad libitum* period.

[3] A Kruskal-Wallis one-way ANOVA was performed for means in Experiment 1 with *P*-values for ADC of dry matter, crude protein and energy all equaling 0.016.

[4] Data were not available since the feces collected from each tank were insufficient for fat content analyses as a result of too low feed intake of fish.

[a, b, c] Means in one row under each experiment lacking a common superscript are significantly different at $P < 0.05$.

Table 5.4. Nitrogen balance of Nile tilapia (*Oreochromis niloticus*) reared at four different ambient dissolved oxygen concentrations in each of two experiments

	Experiment 1				Experiment 2			
Treatment	**1.1**	**1.2**	**1.3**	**1.4**	**2.1**	**2.2**	**2.3**	**2.4**
Ambient DO (mg L^{-1})[1]	**1.58 ± 0.05**	**2.58 ± 0.08**	**4.06 ± 0.10**	**6.08 ± 0.02**	**1.33 ± 0.02**	**1.71 ± 0.04**	**2.62 ± 0.04**	**3.66 ± 0.05**
Parameter (mg $kg^{-0.8}$ d^{-1})[2]								
GN	228 ± 4.5[a]	561 ± 17.5[b]	857 ± 17.6[c]	946 ± 24.4[d]	587 ± 11.7[a]	679 ± 11.4[b]	866 ± 15.9[c]	885.99 ± 16.17[c]
FN	27 ± 3.6[a]	40 ± 1.5[b]	65 ± 2.0[c]	75 ± 1.7[c]	47 ± 1.0[a]	55 ± 1.3[b]	69 ± 1.7[c]	72.43 ± 0.92[c]
DN	201 ± 7.8[a]	521 ± 16.1[b]	792 ± 15.6[c]	871 ± 22.8[d]	540 ± 10.7[a]	624 ± 10.6[b]	797 ± 14.3[c]	813.56 ± 15.30[c]
BUN	168 ± 5.2[a]	319 ± 2.5[b]	488 ± 8.3[c]	446 ± 31.2[c]	339 ± 6.2[a]	333 ± 11.0[a]	384 ± 14.0[b]	400.52 ± 6.87[b]
RN	33 ± 8.1[a]	202 ± 16.7[b]	304 ± 23.0[c]	425 ± 41.1[d]	201 ± 6.0[a]	291 ± 0.9[b]	413 ± 20.0[c]	413.04 ± 21.81[c]

[1] Values represent means ± SE of three replicates (tanks); each replicate value is the mean of all the data points measured on one tank during the *ad libitum* feeding period.

[2] Values represent means ± SE of three replicates (tanks); each replicate value was calculated based on an individual fish as a mean of one tank. GN = Gross nitrogen intake; FN = Fecal nitrogen losses; DN = Digestible nitrogen; BUN = Branchial and urinary nitrogen losses; RN = Retained nitrogen.

[a, b, c, d] Means in one row under each experiment lacking a common superscript are significantly different at $P < 0.05$.

Table 5.5. Energy balance of Nile tilapia (*Oreochromis niloticus*) reared at four different ambient dissolved oxygen concentrations in each of two experiments

	Experiment 1				Experiment 2			
Treatment	**1.1**	**1.2**	**1.3**	**1.4**	**2.1**	**2.2**	**2.3**	**2.4**
Ambient DO (mg L^{-1})[1]	**1.58 ± 0.05**	**2.58 ± 0.08**	**4.06 ± 0.10**	**6.08 ± 0.02**	**1.33 ± 0.02**	**1.71 ± 0.04**	**2.62 ± 0.04**	**3.66 ± 0.05**
Parameter (KJ $kg^{-0.8}$ d^{-1})[2]								
GE	83 ± 1.6^{a}	204 ± 6.4^{b}	312 ± 6.4^{c}	344 ± 8.9^{d}	214 ± 4.3^{a}	247 ± 4.2^{b}	315 ± 5.8^{c}	322 ± 5.9^{c}
FE	14 ± 1.1^{a}	24 ± 0.5^{b}	39 ± 1.0^{c}	45 ± 1.619^{d}	30 ± 0.8^{a}	35 ± 0.6^{b}	46 ± 1.3^{c}	48 ± 1.2^{c}
DE	69 ± 2.7^{a}	180 ± 5.8^{b}	273 ± 5.4^{c}	299 ± 7.3^{d}	184 ± 3.5^{a}	212 ± 3.7^{b}	269 ± 4.5^{c}	274 ± 4.8^{c}
BUE	4 ± 0.1^{a}	8 ± 0.1^{b}	12 ± 0.2^{c}	11 ± 0.8^{c}	8 ± 0.2^{a}	8 ± 0.3^{a}	9 ± 0.4^{b}	10 ± 0.2^{b}
ME	65 ± 2.7^{a}	172 ± 5.9^{b}	261 ± 5.6^{c}	288 ± 7.4^{d}	176 ± 3.4^{a}	204 ± 3.5^{b}	260 ± 4.5^{c}	264 ± 4.9^{c}
HP	55 ± 8.0^{a}	112 ± 6.5^{b}	163 ± 5.6^{c}	158 ± 1.1^{c}	88 ± 1.6^{a}	96 ± 6.9^{a}	124 ± 3.6^{b}	127 ± 2.6^{b}
RE	10 ± 7.0^{a}	60 ± 7.4^{b}	98 ± 7.3^{c}	130 ± 6.7^{c}	88 ± 2.0^{a}	108 ± 3.6^{a}	136 ± 8.1^{b}	137 ± 2.7^{b}
ME_{maint}	52 ± 9.7^{a}	97 ± 7.8^{b}	138 ± 7.0^{c}	126 ± 1.4^{bc}	65 ± 1.3^{a}	69 ± 7.8^{ab}	90 ± 5.6^{bc}	93 ± 2.2^{c}

[1] Values represent means ± SE of three replicates (tanks); each replicate value is the mean of all the data points measured on one tank during the *ad libitum* feeding period.

[2] Values represent means ± SE of three replicates (tanks); each replicate value was calculated based on an individual fish as a mean of one tank. GE = Gross energy intake; FE = Fecal energy losses; DE = Digestible energy; BUE = Energy in branchial and urinary excretory products; ME = Metabolizable energy; HP = Heat production; RE = Retained energy; MEmaint = Metabolizable energy for maintenance of body weight.

[a, b, c, d] Means in one row under each experiment lacking a common superscript are significantly different at $P < 0.05$.

4. Discussion

4.1. Feed intake, growth and feed conversion ratio

The results confirmed the findings in our previous study (Tran-Duy et al., 2008): feed intake and growth of fish at lower DOs were lower than at higher DOs. The variations in feed intake with DO of the big fish (Experiment 1) and small fish (Experiment 2) were different: above DOs of 2.6 mg L^{-1}, the big fish still significantly increased their feed intake while the small fish did not. This implies that maximum oxygen intake can be reached at a lower DO in the small fish than in the big fish, confirming higher relative oxygen uptake capacity at smaller body size.

This is reinforced by the finding that at the same DO levels (2.6 mg L^{-1} in Treatments 1.2 and 2.3; about 1.6 mg L^{-1} in Treatments 1.1 and 2.2), relative feed intake of the small fish was much higher than of the big fish, and that a DO reduction from 2.6 to 1.6 mg L^{-1} reduced feed intake of the big fish dramatically but that of small fish only moderately. The DO-feed intake curve of the big fish is below that of the small fish at DOs below 4 mg L^{-1} and the deviation increases with decreasing DO (Figure 5.2). The DO-feed intake curve of the small fish seems to level off at a DO of 2.6 mg L^{-1}, while that of big fish continues to go up as DO increases from 2.6 to 6.0 mg L^{-1}. It is not clear at which DO the DO-feed intake curve of the big fish starts to plateau, but Figure 5.2 suggests that the incipient DO must be at least 5 mg L^{-1}.

This has an important practical implication: the incipient DO at which feed intake of Nile tilapia <100 g starts to decline is substantially lower than of Nile tilapia >200 g under the same circumstances. As discussed by Tran-Duy et al. (2008), the incipient DO of fish of a given size would be determined by the amount and composition of feed and activity level of the fish. Considering that fish under culture condition spend a relatively small amount of energy for activity (Bureau et al., 2002), feed composition is likely to be the main factor determining the incipient DO of fish fed *ad libitum*. It has been suggested that the increase in oxygen consumption following the ingestion of food was largely caused by increased protein and lipid synthesis (Jobling, 1994, Chapter 8), which are affected by the dietary protein (see Houlihan et al., 1993) and lipid contents (see Watanabe, 1982). According to Ross et al. (1992), post-prandial oxygen consumption of Nile tilapia is principally affected by dietary protein content and much less by dietary lipid. In the light of these findings, dietary protein content appears to be closely correlated with the incipient DO. Van Dam and Pauly (1995) calculated that oxygen demand for ingestion of 1 g of 25% protein feed was 0.172 g, while that of 50% protein feed was 0.268 g. In the present study, fish were fed with a diet containing 33.9% protein while in practice protein contents of diets for grow-out of tilapias

varies from 25 to 35% (Shiau, 2002). It would be useful to examine the feed intake of Nile tilapia fed diets with varying protein levels in relation to DOs, especially for fish >200 g.

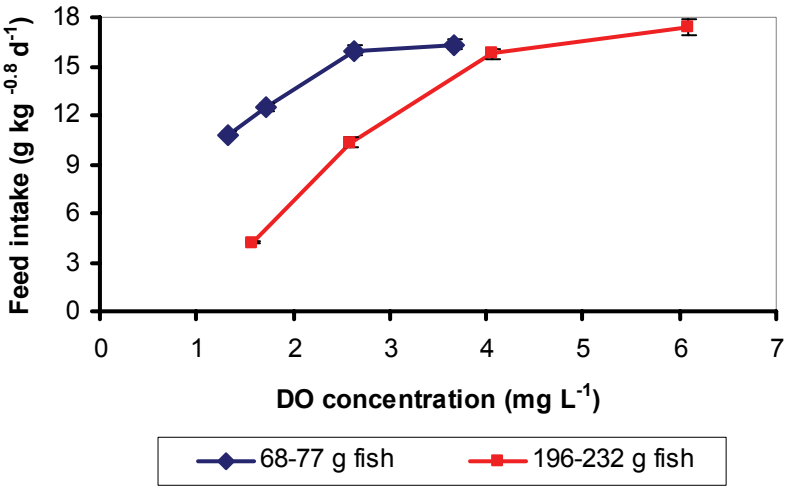

Figure 5.2. Maximum feed intake (g $kg^{-0.8}$ d^{-1}) of Nile tilapia (*Oreochromis niloticus*) of two weight classes (68-77 g and 196-232 g; geometric mean weights) in relation to dissolved oxygen concentrations. Data points represent means of three replicate values; each replicate value was calculated based on an individual fish as a mean of one tank. Vertical bars, which may be obstructed by the symbols, represent two times the standard errors.

In agreement with the observations of Herrmann et al. (1962) on coho salmon, Stewart et al. (1967) on largemouth bass and Andrews et al. (1973) on channel catfish, the present study indicated that FCR was not affected by DO, except when the feed intake was too low. In the big fish, FCR increased only slightly as DO decreased from 6.08 to 2.58 mg L^{-1}. High Pearson's correlation coefficients [N = 9 (tanks)] between FI_{MBW} and GR_{MBW} (r = 0.963), and between FI_{perc} and SGR (r = 0.959) suggest that growth rate of Nile tilapia in the weight range of 200-270 g at DOs not below 2.6 mg L^{-1} is directly proportional to feed intake. Feed conversion ratio was elevated in the big fish at a DO of 1.58 mg L^{-1}, but this was caused not by the low DO itself but by the low feed intake close to the maintenance requirement. In the small fish, the differences in FCR at different DO levels were trivial; together with very high Pearson's correlation coefficients [N = 12 (tanks)] between FI_{MBW} and GR_{MBW} (r = 0.991), and between FI_{perc} and SGR (r = 0.989), this suggests that growth rate of Nile tilapia in the weight range of 58-100 g at DOs not below 1.3 mg L^{-1} is directly proportional to feed intake. Thetmeyer et al. (1999) also found that reduced feed intake was the main cause of reduced growth rate of sea bass of 40-90 g under hypoxic condition (40% saturation, equivalent to 3.5 mg DO L^{-1} at the experimental temperature of 22°C) (conversion based on Colt, 1984).

4.2. Energy metabolism

Fish may respond to hypoxia with activities which affect the energy expenditure. Increased energy demand associated with gill ventilation (Gerald and Cech, 1970; Lomholt and

Johansen, 1979; Rantin et al., 1992; Fernandes and Rantin, 1994) and locomotor activity of escape reactions can occur when oxygen availability is reduced (Jones, 1952; Waller et al., 1997). On the other hand, fish may reduce routine activity to lower the energy demand (Kramer, 1987; Jensen et al., 1993). The results showed that at lower DO, Nile tilapia spent less energy for maintenance of body weight (ME_{maint}) than at higher DO, suggesting that they reduce spontaneous locomotor activity under hypoxia. This agrees with the observation that fish at lower DOs were less active than at higher DOs.

The ingestion of food results in increased activity of digestion (mechanical work of the gastrointestinal tract and synthesis and secretion of digestive enzymes), absorption (active transport of the nutrients across the gut membrane) and post-absorptive nutrient processing (Jobling, 1994, Chapter 8). The rates of digestion and absorption must not exceed the rate of post-absorptive nutrient processing to prevent the accumulation of toxic free amino acids (Jobling, 1981). After the ingestion of food, oxygen consumption remarkably increases quickly and peaks within a few hours (Jobling, 1993). Since the contribution of the motor activity of the gastrointestinal tract to the post-prandial increase in oxygen consumption is small, this suggests that fish avoid nutrient accumulation by processing the absorbed nutrients immediately. Pauly (1981) argued that when DO availability is low, the ingested amino acids can neither be used for protein synthesis or oxidation, nor excreted because all these processes require energy thus oxygen. Therefore, the best way to prevent accumulation of free amino acids in the blood is to cease feeding.

In view of this, there might be two situations that the fish encounter after the cessation of feeding under hypoxia: the rate of oxygen uptake might be (1) just sufficient, or (2) insufficient, for post-absorptive nutrient processing at normal rates of digestion and absorption of the ingested food. In the former case, digestibility would not be affected by the DO. In the latter case, fish must economize on digestion and absorption. This reduces energy expenditure and at the same time limits the accumulation of nutrients in the blood and ensures further processing of the already absorbed nutrients. This might explain why the ADCs of dry matter, crude protein and energy of the big fish decreased considerably at a DO of 1.58 mg L^{-1} while the small fish, with higher relative oxygen uptake capacity, showed no decreases in the ADCs of any of the food components as DO decreased from 3.66 to 1.33 mg L^{-1}. On the contrary, there was a slight increase in the ADC of each of the food components with decreasing DOs in both big fish (except at 1.58 mg L^{-1}) and small fish, which is attributed to the fact that the efficiency of digestion and absorption generally decrease with increasing feed ingestion (Jobling, 1994, Chapter 7). This suggests a dichotomic effect of DO on the ADCs of energy and nutrients in fish: a DO reduction can cause an increase in ADC as a result of reduced feed ingestion, but further declines in DO to levels below a critical level can decrease ADC as a consequence of oxygen shortage for post-absorptive nutrient processing.

Energy losses in the branchial and urinary excretory products and heat production tended to increase with increasing DOs in both big and small fish. This might be because fish at higher DOs ingested more food (and therefore protein) and thus excreted more nitrogen and lost more heat energy associated with the deamination and oxidation of amino acids (Cho and Kaushik, 1985). However, the ratios of BUE to DE and of HP to ME tended to increase with decreasing DOs in the big fish, while in the small fish this tendency was not obvious (Figure 5.3). This suggests that as DO declines a higher proportion of digestible energy is lost through the branchial and urinary excretory products, and a higher proportion of metabolizable energy is lost as heat production in Nile tilapia > 200 g. However, because BUE accounted for very small proportions of DE, variation in BUE as affected by DO did not markedly influence ME as proportions of DE, which ranged from about 94 to 96% in the big fish and from about 95 to 97% in the small fish. According to a number of studies, feeding level is unlikely to affect the ME to DE ratio (see Bureau et al., 2002). In view of this, present findings suggests that feed intake as affected by DO and DO as a factor directly affecting energy metabolism do not have a considerable effect on the ME to DE ratio of the diet.

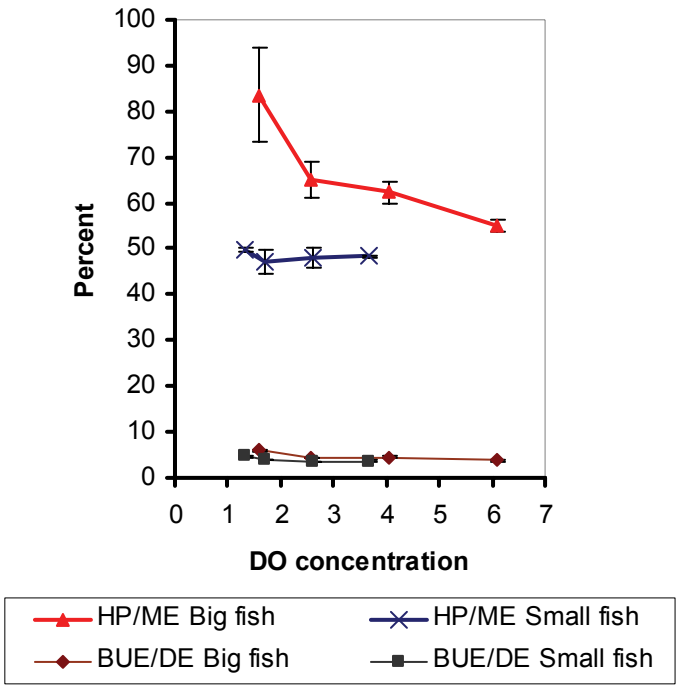

Figure 5.3. Variation in the ratios of BUE to DE and of HP to ME of Nile tilapia (*Oreochromis niloticus*) fed *ad libitum* under different DO concentrations. Data points represent means of three replicate values; each replicate value was calculated based on an individual fish as a mean of one tank. Vertical bars, which may be obstructed by the symbols, represent two times the standard errors. BUE = Energy in branchial and urinary excretory products; DE = Digestible energy; HP = Heat production; ME = Metabolizable energy; Geometric mean weights of big fish and small fish were in the ranges of 196-232 g and 68-77 g, respectively.

5. Conclusions

No single incipient DO for one species exists. It is related to body weight. In the present study, incipient DO for Nile tilapia of 60-100 g was around 2.6 mg L^{-1} and of 200-270 g was unlikely to be below 5 mg L^{-1}. Incipient DO probably also depends on feed composition. Growth rates of fish of 200-270 g and 60-100 g were directly proportional to feed intake at DOs not below 2.6 and 1.3 mg L^{-1}, respectively.

Nile tilapia reduced energy for maintenance of body weight as DO declined. The results suggest that a DO reduction can cause an increase in ADC, but further declines in DO to levels below a critical level can decrease ADC. This critical level in fish > 200 g was found to be between 2.6 and 1.6 mg L^{-1}, and in fish < 100 g lower than 1.3 mg L^{-1}.

Energy losses in the branchial and urinary excretory products and heat production in Nile tilapia tended to increase with increasing DOs. However, the ratios of BUE to DE and of HP to ME tended to increase with decreasing DOs in fish > 200g, while in fish < 100 g this tendency was not obvious. As BUE accounted for very small proportions of DE, the ratios of ME to DE were almost constant, which ranged from about 94 to 96% in fish > 200 g and from about 95 to 97% in fish < 100 g. This suggests that DO does not have a considerable effect on the ME to DE ratio of the diet.

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Chapter 6

Simulation of feed intake regulation and growth in *Oreochromis niloticus* with special reference to ambient oxygen concentration

This chapter was submitted as:
Tran-Duy, A., van Dam, A., Schrama, J.W., 2008. Simulation of feed intake regulation and growth in *Oreochromis niloticus* with special reference to ambient oxygen concentration. Ecological modelling.

Abstract

The overall objective of this study was to model feed intake regulation in fish using recent detailed experimental datasets on the effect of oxygen on feed intake and growth of Nile tilapia (*Oreochromis niloticus*). In the oxygen limitation model, the short-term effect of dissolved oxygen concentration (DO) on *ad libitum* feed intake was simulated based on the balance between oxygen demand (determined by the sum of routine metabolism, feeding metabolism and the energy costs for biosynthesis) and oxygen supply (derived from an allometric relationship with body weight based on gill surface area and Fick's law of diffusion). At each time step, the model compared the oxygen demand related to the processing of one bite of food to the maximum oxygen supply and reduced or increased the size of the next bite accordingly. Fish would stop eating when (1) the food was finished, (2) feeding time was over, or (3) a factor other than DO (formulated as an empirical function of body weight) limited feed intake. The model was calibrated and validated using datasets from experiments in which Nile tilapia of different weights (2-280 g) were fed *ad libitum* at DOs of 1-8 mg L^{-1}. Model performance was evaluated using scenarios with all combinations of two fish sizes (20 and 100 g) and ten constant DOs (1-10 mg L^{-1}). The calibration results showed an average relative error (ARE) of simulated total feed intake of -7.25% with a range of relative errors (RE) of -63.90 to +64.67%. The REs of the simulated fish weight ranged from -47.75% to +18.87% with ARE = -5.05%. Validation results showed an ARE of the simulated final fish weight of 6.93% with a range of RE of -36.17 to +40.45%. Model results provide strong support for the theory that limiting DOs regulate feed intake in fish. However, feed intake in relation to body weight when DO was not limiting was not simulated satisfactorily. This was attributed to the empirical equation for maximum feed intake determined by a factor other than DO. More research into these factors (such as stomach volume, blood metabolites, body composition, water temperature and social interaction) is needed to further elucidate the underlying mechanisms of feed intake regulation.

1. Introduction

As new species are increasingly introduced to aquaculture (FAO, 2007), fish growth models to be used in the planning of aquaculture development should be characterized by high generality. Feed consumption is undoubtedly a required input for any model which claims to give insight into fish growth processes. Under *ad libitum* feeding, prediction of maximum feed intake is therefore indispensable. However, surprisingly little effort has been devoted to the prediction of maximum feed intake under various conditions. This is probably due to the complexity of feed intake regulation in fish. Feed intake and satiation in fish are regulated by physiological, social or environmental factors, or by the interaction among them (Tran-Duy et al., 2005; Chapter 1 and 3 in this thesis). Because the mechanisms underlying this regulation in fish are still not well understood and quantitative information about the interaction of these factors is lacking, incorporating all factors into one model for feed intake regulation is difficult.

Machiels (1987) developed an explanatory simulation model for fish growth (referred to as Fish Growth Simulator 1: FGS1) based on the biochemical reaction equations of the intermediary metabolism of fish. Being based on very basic processes, FGS1 was considered general and applicable to a wide variety of fish species, but no regulatory mechanisms controlling feed intake were included. Under *ad libitum* feeding, feed intake was limited by comparing the predicted growth with the maximum growth observed in experiments with African catfish (*Clarias gariepinus*) of different weights fed with one type of feed at different temperatures. Using this approach, the model was not applicable for *ad libitum* feeding with other feed types and fish species. Van Dam and Penning de Vries (1995) modified FGS1 (resulting in FGS2) and used it to predict growth of Nile tilapia (*Oreochromis niloticus)* and rainbow trout (*Oncorhynchus mykiss)*. While this confirmed the generality of the model, FGS2 still only predicted growth of fish at sub-maximal feeding levels. The model was further extended and applied for predicting growth of tambaquí (*Colossoma macropomum)* (van der Meer and van Dam, 1998) and growth and waste production of Nile tilapia (Verdegem et al., 2000). In the model of van der Meer and van Dam (1998), uneaten feed was estimated in relation to feeding level. In the model of Verdegem et al. (2000), maximum feed intake was determined based on an empirical relationship between protein intake and body weight.

Despite the success of these models in growth prediction, the equations for maximum feed intake were descriptive and not based on knowledge about feed intake regulation. Among the wide range of factors affecting feed intake, dissolved oxygen concentration (DO) is likely to be a major factor. The aerobic metabolic capacity of a fish is primarily limited by the ability of the gills to extract oxygen from the water (Priede, 1985). The difference between the maximum and standard aerobic metabolic rates determines the metabolic scope within which all the energy-demanding processes, including routine metabolism, feeding metabolism and

biosynthesis, must be fit. Fish cease to eat when they cannot take in more oxygen for food processing (Pauly, 1981).

Based on this concept, van Dam and Pauly (1995) developed a module for oxygen limitation and incorporated it into FGS2. In this new model (FGS3), maximum daily feed intake was calculated *a priori* based on the oxygen demand of the fish for ingestion and processing of 1 g feed; and the oxygen supply based on diffusion through the gill surface. Due to a lack of experimental data, FGS3 was calibrated with merely five cases from a study in which only total feed intake of Nile tilapia and average DO over the whole experimental period were reported (Tsadik and Kutty, 1987). Although the model predicted well the total oxygen consumption and final body weight of Nile tilapia (*Oreochromis niloticus*) when feed intake was limited by DO, FGS3 lacked the ability to simulate the short-term effect of DO on maximum feed intake, i.e. the ability to regulate feed intake based on diurnal variation in DO. In addition, the equation for feeding metabolism, which partly determines oxygen demand and therefore feed intake, was descriptive and needed improvement.

Recently, we conducted experiments in which daily maximum feed intake of Nile tilapia and diurnal DOs were measured (Tran-Duy et al., 2008a; Tran-Duy et al., 2008c). These new datasets, apart from confirming the importance of ambient DO concentrations on feed intake in fish, provide an opportunity to revise the oxygen limitation module of FGS3 and re-evaluate the concept of oxygen limitation to maximum feed intake in fish using a mechanism for short-term feed intake regulation. Such a short-term approach also requires a better estimation of the intermediate costs of feeding activity, digestion and absorption, which constitute feeding metabolism. For this, the empirical equation for feeding metabolism in FGS3 needs to be replaced by more mechanistic relationships that apply to a wide range of fish species.

The overall objective of the present study was to improve the oxygen limitation module of FGS3 using the recent detailed experimental datasets on Nile tilapia. This should result in a general model for maximum feed intake in fish in relation to ambient DO concentration that may be applied to other species. Specifically, the objectives were: (1) to reformulate the equation for feeding metabolism based on its underlying mechanisms, (2) to calculate maximum rate of oxygen supply based on the ambient oxygen concentration at each time step, (3) to calculate the rate of oxygen demand based on corresponding feeding rate at each time step, (4) to adjust the feeding rate based on the prior information about the rates of oxygen supply and demand, and (5) to evaluate the performance of the model regarding the effects of DO on maximum feed intake and growth.

2. Materials and methods

2.1. FGS concepts

Extensive descriptions of FGS1, FGS2 and FGS3 can be found in Machiels and Henken, (1986), van Dam and Penning De Vries (1995) and van Dam and Pauly (1995), respectively. State variables in these models are the amounts of amino acids, glucose, body protein and body fat in an individual fish (see also Figure 6.1). In one time step, the following processes take place: (1) all amino acids, which result from protein digestion, are converted to body protein and glucose; (2) all glucose, which results from carbohydrate digestion and gluconeogenesis, is converted to body lipid; and (3) digested lipid goes to body lipid. Total energy requirement (metabolic rate) is calculated as the sum of routine metabolic rate, feeding metabolic rate and energy requirement for biosynthesis. Routine metabolic rate represents the energy requirement of fish with spontaneous activities under fasting condition and is calculated from an allometric relation with body weight (Winberg, 1956). Feeding metabolism is defined as the energy costs of feeding activity, digestion and absorption and is calculated based on the energy content of the feed. Energy cost for biosynthesis is based on the stoichiometry of the reaction equations for protein, fat and glucose synthesis. The total metabolic requirement is met by oxidation of body protein and fat. In FGS1, the proportion supplied by fat oxidation was determined by the ratio of body fat to body protein. In FGS2 and FGS3, this proportion depended on the protein feeding level (in g feed protein [kg body weight]$^{-0.8}$ d^{-1}) and the ratio of dietary protein to energy (in mg protein kJ^{-1}).

In the current study, FGS3 was modified with regard to the calculation of feeding metabolism and oxygen demand and the adjustment of feed intake at each time step. The relational diagram of the new model (hereafter referred to as FGS4) is shown in Figure 6.1.

2.2. Feeding metabolism

Feeding metabolic rate in FGS1 was calculated based on the observation that heat increment above routine metabolism due to feeding accounted for about 15% of gross energy intake, regardless of body weight and ration (Beamish, 1974; Schalles and Wissing, 1976). Based on the biochemistry of protein and fat synthesis, Machiels and Henken (1986) estimated an increase in oxygen consumption of 0.235 g per gram of feed ingested, of which 0.065 g was used for feeding metabolism. Using these estimates, van Dam and Penning De Vries (1995) assumed that feeding metabolism accounted for 30% of the heat increment following the ingestion of a meal. Thus, in FGS2 feeding metabolism was set at 4.5% of the gross energy intake.

To achieve a more explanatory equation in FGS4, feeding metabolism was partitioned into (1) the costs for food capture and handling and motor activity of the gastrointestinal tract, hereafter referred to as *feeding activity*; and (2) the costs associated with absorption and

transport of the nutrients across the cell membranes. The absorption cost of fatty acids was estimated from the biochemistry of re-esterification of the digested fat, which is 1.33 mol ATP per mole fatty acid equivalent; the absorption or transport of 1 mol glucose or amino acid costs 0.33 mol ATP (Tran-Duy et al., 2005, Chapter 3 in this thesis). Feeding activity can then be estimated from the difference between daily feeding metabolism as calculated by FGS2 and the daily costs of absorption and transport. This difference was expressed in percent of daily routine metabolism (FDACPER) and plotted against daily feeding level (FEDLEV; g $kg^{-0.8}$ d^{-1}). Based on the shape of the data points, the relationship between FDACPER and FEDLEV was formulated using linear regression with PROC REG in SAS 9.1 (SAS Institute Inc., 2004):

$$FDACPER = FEDCF1 \times FEDLEV + FEDCF2 \quad \textbf{(6.1)}$$

in which FEDCF1 and FEDCF2 are the slope and intercept, respectively of the regression line.

Based on functional response theory, a curvilinear relationship between FDACPER and FEDLEV may be more likely. As food availability increases, fish increase the activity of food capture and handling and of mechanical digestion, but meanwhile reduce their food searching activity. Consequently, the rate of increase in feeding activity decreases with increasing feeding level. This concept is reminiscent of a Type II functional response (Holling, 1959a; b):

$$FDACPER = \frac{PMAX}{1 + \frac{F}{FEDLEV}} \quad \textbf{(6.2)}$$

where PMAX is the maximum feeding activity (expressed as a percentage of routine metabolism) and F is the feeding level at which feeding activity is half of PMAX. Another potential model for the curvilinear relationship between FDACPER and FEDLEV is the diphasic allometric equation (Koops and Grossman, 1993):

$$FDACPER = b1 \times FEDLEV - (b1\text{-}b2) \times r \times \ln[1 + e^{(FEDLEV\text{-}C)/r}] \quad \textbf{(6.3)}$$

where b1 and b2 are the slopes of the first and second linear segments, r is the smoothness of the transition from the first to the second segment and C is the intersection point.

The parameters for the linear and curvilinear equations were estimated. The equations were then incorporated into FGS3 and tested with experimental datasets (see 2.6.) in order to determine the best fitting equation.

2.3. Oxygen supply and demand

Formulation of oxygen supply and demand in FGS4 was generally identical to FGS3 (van Dam and Pauly, 1995). Oxygen supply was calculated based on Fick's law of diffusion,

assuming that diffusion is the limiting step in the transport of oxygen from the ventilation water to fish tissues. Maximum potential oxygen supply to the fish (O2MAX; g d^{-1}) was formulated as:

$$O2MAX = DP \times \frac{K \times GARF \times GSCF}{WBD} W^{GSEX} \quad \textbf{(6.4)}$$

where DP is the oxygen pressure gradient across the gills (mm Hg), K is Krogh's diffusion constant (g d^{-1} m^{-1} (mm Hg)$^{-1}$), GARF is the fraction of the total gill surface area used for gaseous exchange, GSCF and GSEX are the coefficient and exponent in the allometric relationship between total gill surface area (m^2) and body weight (W; g) and WBD is the anatomical water-blood distance (m).

OLEF, an empirical oxygen limitation factor, was defined as:

$$OLEF = DP \times \frac{K \times GARF \times GSCF}{WBD} \quad \textbf{(6.5)}$$

For a given species, the parameters K, GARF, GSCF and WBD are fixed. Therefore, OLEF depends mainly on DP and thus ambient oxygen pressure (PAMB). O2MAX can then be calculated as

$$O2MAX = OLEF \times W^{GSEX} \quad \textbf{(6.6)}$$

Van Dam and Pauly (1995) found a linear relationship between OLEF and ambient oxygen pressure (PAMB):

$$OLEF = O2CF1 \times PAMB + O2CF2 \quad \textbf{(6.7)}$$

We adopted this relationship for FGS4 and considered O2CF1 and O2CF2 as parameters for calibration.

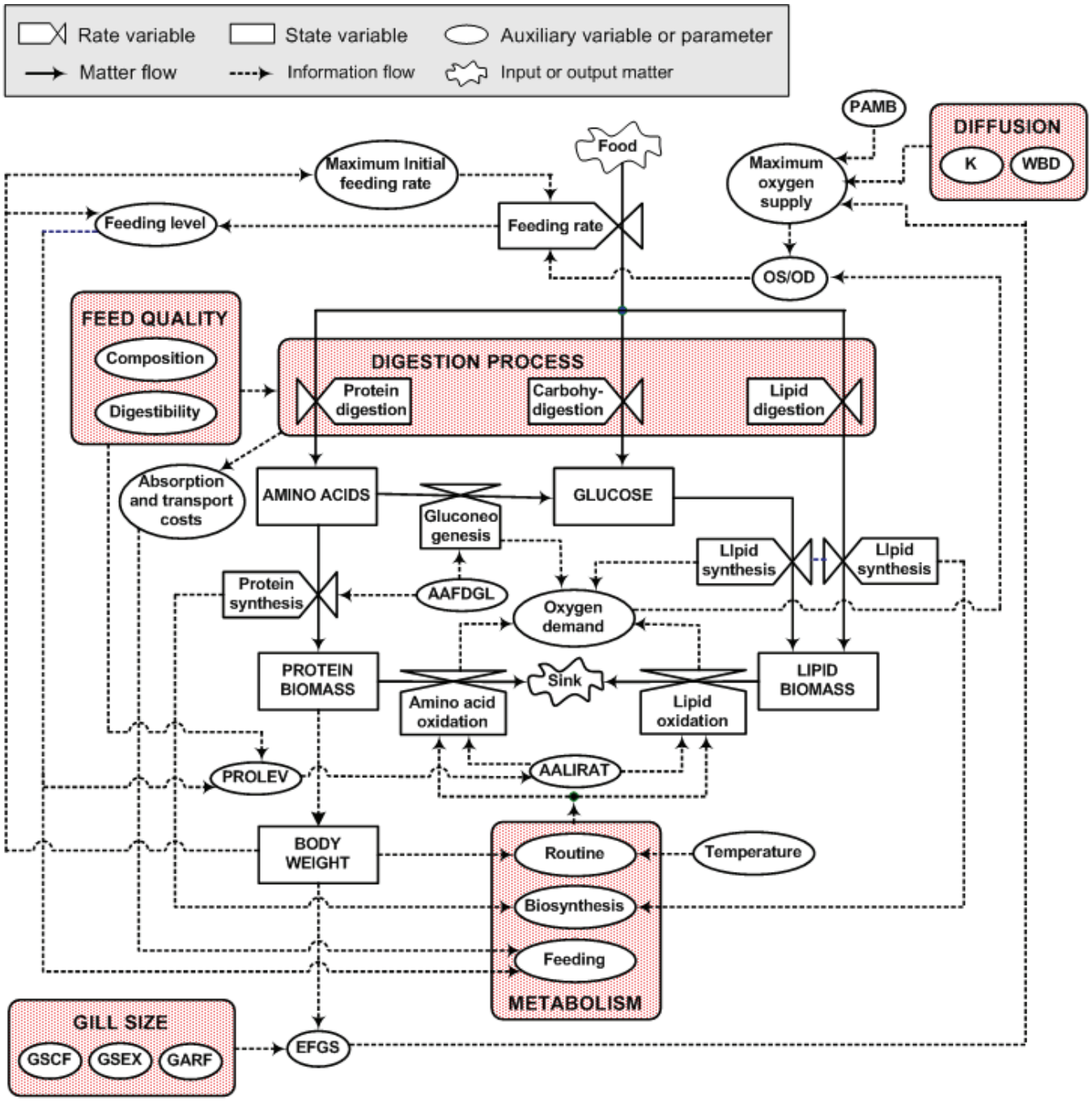

Figure 6.1. Relational diagram of a simulation model for feed intake and growth in fish using the symbols of Forrester (1961). PAMB = driving variable representing the ambient oxygen tension (in mmHg); K and WBD = parameters used to calculate the diffusion rate of oxygen from the water to blood through the gills; OS/OD = auxiliary variable representing the ratio of maximum potential oxygen supply to oxygen demand; AAFDGL = parameter determining the proportion of amino acids used for gluconeogenesis; AALIRAT = auxiliary variable determining the fraction of total energy requirement supplied by fat oxidation; PROLEV = auxiliary variable determining the protein feeding level (in g $kg^{-0.8}$ d^{-1}); GSCF, GSEX and GARF = parameters used to calculate the gill surface area; EFGS = auxiliary variable determining the effective area of the gills that is available for gaseous exchange.

Oxygen demand (O2NEED; g d^{-1}) is determined by the processes that consume oxygen: amino acid (AA) and lipid (TOG) oxidation (AAOX and LIPOX, respectively; g d^{-1}), gluconeogenesis (AAGLUC; g d^{-1}) and lipid synthesis from glucose (GLULIP; g d^{-1}). AAOX and LIPOX were determined based on the total energy requirement, which is the sum of routine metabolism, feeding metabolism and the costs for biosynthesis. AAGLUC was calculated based on the assumption that 5% of the digested protein was converted to glucose. GLULIP was calculated based on the digestion rate of carbohydrate and AAGLUC. The overall reaction equations for the oxygen-demanding processes are as follows (Machiels and Henken, 1986):

$$1\text{ g AA} + 1.25\text{ g } O_2 \rightarrow 1.70\text{ g } CO_2 + 0.17\text{ g } NH_3 + 0.38\text{ g } H_2O + 0.21\text{ mole ATP} \quad \textbf{(6.8)}$$

$$1\text{ g TOG} + 2.9\text{ g } O_2 \rightarrow 2.8\ CO_2 + 1.1\text{ g } H_2O + 0.51\text{ mole ATP} \quad \textbf{(6.9)}$$

$$1\text{ g AA} + 0.68\text{ g } O_2 \rightarrow 0.86\text{ g } CO_2 + 0.17\ NH_3 + 0.12\text{ g } H_2O + 0.53\text{ glucose} + 0.095\text{ mole ATP} \quad \textbf{(6.10)}$$

$$1\text{ g glucose} + 0.22\text{ g } O_2 \rightarrow 0.63\text{ g } CO_2 + 0.29\text{ g } H_2O + 0.29\text{ g TOG} \quad \textbf{(6.11)}$$

Based on Equations 6.8-6.11, O2NEED (in g d^{-1}) was calculated as (van Dam and Pauly, 1995):

$$\text{O2NEED} = \text{AAOX} \times 1.25 + \text{LIPOX} \times 2.9 + \text{AAGLUC} \times 0.68 + \text{GLULIP} \times 0.22 \quad \textbf{(6.12)}$$

2.4. Feed intake regulation

Oxygen demand and feed intake are mutually dependent. Oxygen demand can be calculated only when feed intake is known because (1) feeding metabolism is related to feeding level (see 2.2), (2) the costs for biosynthesis are determined by the digestion rates of protein, lipid and carbohydrate, and (3) the proportion of the total energy requirement supplied by fat oxidation, which determines the oxygen needed for generation of each energy unit, depends on protein feeding level. Conversely, *ad libitum* feed intake is determined by the balance between oxygen demand and oxygen supply.

Due to this inter-dependency, the following mechanism for feed intake regulation was adopted: at the start of the feeding period the fish takes a bite of food. If the oxygen demand related to the processing of this bite is higher or lower than the maximum oxygen supply, the fish reduces or increases the size of the next bite accordingly. The size of a food bite is determined as the product of the instantaneous feeding rate at the time of the bite and the duration of the time step. Then, the oxygen supply/demand ratio (O2MAX/O2NEED) is multiplied with the current feeding rate to obtain the feeding rate of the next time step. After each time step, the cumulative feed intake (CUMFI; g) since the beginning of the feeding period is calculated. A fish stops eating when at least one of the following conditions is met:

(1) The food is finished: CUMFI is greater than the daily ration (RATDAY; g), i.e., under this condition fish are fed restrictively;

(2) Feeding time is over: the value of the time variable exceeds the end of the feeding period, which is determined by the diel feeding rhythms of the fish species, i.e., under this condition the ambient DO concentration limits the maximal feed intake of the fish;

(3) A factor other than ambient DO concentration limits feed intake: CUMFI is greater than the maximum daily feed intake determined by a certain factor other than DO, hereafter referred to as the physiological maximum daily feed intake (PHYFIMAX; g).

These concepts are illustrated in Figure 6.2. Every day, feeding starts at time t_b which is determined by the availability of food and the diel feeding rhythm of the fish species. After t_b, the fish adjusts the feeding rate in each time step based on the information about the feeding rate and the oxygen supply/demand ratio in the previous time step until it stops eating at time t_e. The algorithm for determination of daily feed intake (Figure 6.3) performs a loop for calculation of CUMFI each day, starting from the time when feeding starts (t_b). After each round of calculations (corresponding to one time step), the model checks the three conditions; if one of these is met, the loop ends with the final value of CUMFI as the daily feed intake.

In this approach, feeding rate at the beginning of each feeding period (FEDRTini; g d^{-1}) and the physiological maximum daily feed intake (PHYFIMAX; g) must be determined. Under the conditions that ambient DO concentration does not limit feed intake, an empirical equation was applied to estimate PHYFIMAX. Maximum ingestion rates of the majority of fish species were found to scale in proportion to the body weight raised to the power of 0.6-0.8 (Jobling, 1994). Therefore, PHYFIMAX can be estimated with the following equation:

$$PHYFIMAX = FICF \times W^{FIEX} \quad \textbf{(6.13)}$$

where W is the body weight (g) and FICF and FIEX are constants. FEDRTini was estimated using the equation for PHYFIMAX, i.e. at the beginning of each feeding the calculated value of the right-hand side in Equation 6.13 was considered as the initial feeding rate in g d^{-1} (see Figure 6.3).

The main difference between FGS3 and FGS4 is that FGS4 uses daily variation in ambient DO and regulates feed intake continuously based on the oxygen supply/demand ratio. FGS3 used the average ambient DO for each day, calculated the maximum feeding rates prior to the beginning of feeding and assumed that oxygen was the only factor determining feed intake. In FGS4, an empirical equation is introduced to limit feed intake when ambient DO is not limiting.

2.5. Model implementation and error analysis

First, the equations for feeding metabolism in FGS4 were calibrated to obtain best agreement between predicted and observed final fish weights using datasets with known quantities of feed intake and no DO limitation as input for the model. Then, the equations for oxygen demand and supply and feed intake regulation were calibrated to obtain best agreement between predicted and observed feed intake and final fish weight. Data on maximum feed intake at different DO concentrations were used. Since the DOs in the datasets of Tran-Duy et al. (2008a; 2008c) were measured at intervals of 1-2 hours, the time step for integration in FGS4 was reduced to 0.03125 day (45 minutes) and O2MAX was calculated at each time step. In the simulation, if no DO measurement was available for at a certain time step, DO was calculated by linear interpolation between the previous and next known DO.

To evaluate model performance with respect to the effects of DO and body size on feed intake and growth, simulations were run for Nile tilapia of two weight classes (initial weights: 20 and 100 g) reared at 28^{o}C and at 10 constant DOs (1 to 10 mg L^{-1}) for 200 days. Although the DO corresponding to 100% saturation at 28^{o}C is 7.8 mg L^{-1} (Colt, 1984), the simulations were also run with DOs above this saturation value to assess the incipient DO at which feed intake and growth start to level off. In the simulations, food was made available in excess. The diet contained 95% dry matter, 32% crude protein, 50% carbohydrate and 6% crude fat. To compare feed intake of fish of different sizes, feed intake was also expressed in g $kg^{-0.77}$ d^{-1}, the weight exponent being the value of GSEX in the allometric relationship between the maximum oxygen supply and body weight (Equation 6.6) after model calibration (see 3.2).

To assess the effect of diel DO variation (DOV) on feed intake and growth, simulations were run for Nile tilapia of 100 g (initial weight) reared at 28^{o}C under three DOV regimes (Figure 6.4). The first regime mimics the DOV in a pond where DO is predominantly influenced by photosynthesis, with lowest and highest DOs just after sunrise and in the early afternoon (at 6:00 and 14:00 in the scenario) (Boyd, 1982; 1990). The second regime mimics the DOV in a tank where DO is largely influenced by oxygen consumption of the fish. After the beginning of each feeding (at 8:00 and 16:00 in the scenario), the elevated oxygen consumption rate causes a drop in DO which then gradually increases until the next feeding (Tran-Duy et al., 2008a). The third regime represents a constant DO. The average daily DOs in all the three regimes are identical (4.5 mg L^{-1}). In the simulations, food was made available in excess.

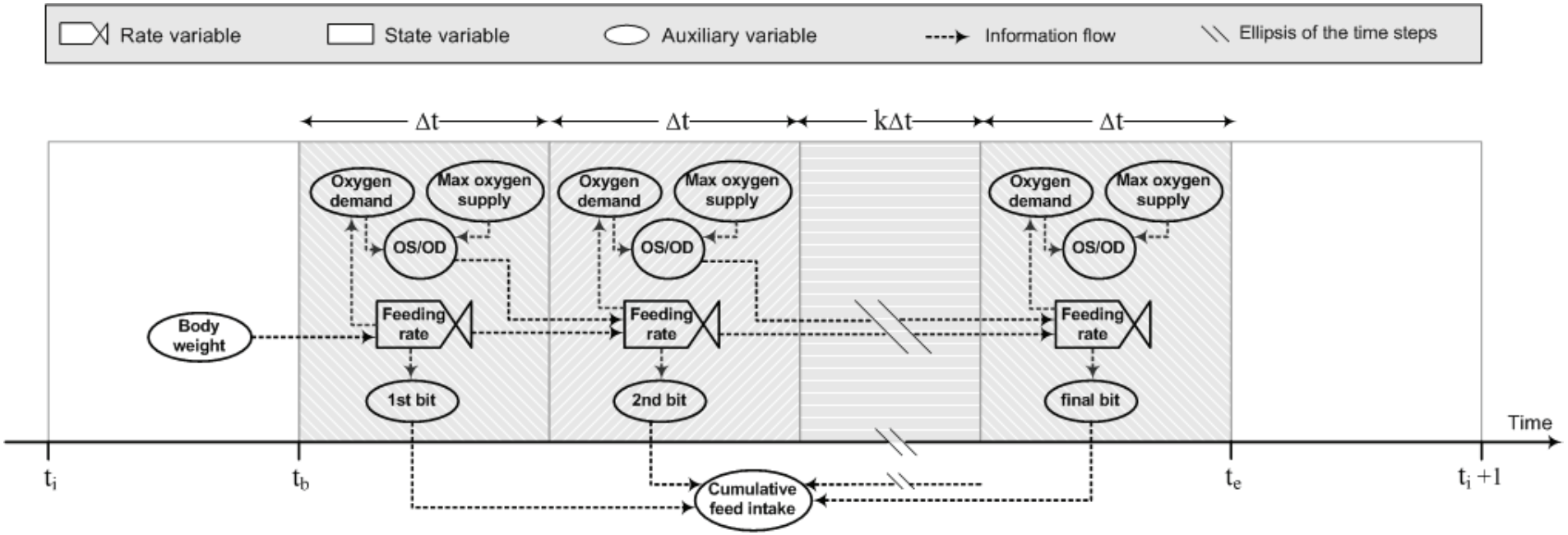

Figure 6.2. Concepts of daily feed intake regulation. During feeding period, feed intake is adjusted at each time step based on the feeding rate and the ratio of oxygen supply (OS) to oxygen demand (OD) at the previous time step. t_i = time at the beginning of day i (in day); t_b = time when feeding starts (in day); t_e = time when feeding stops (in day); Δt = length of one time step (in day).

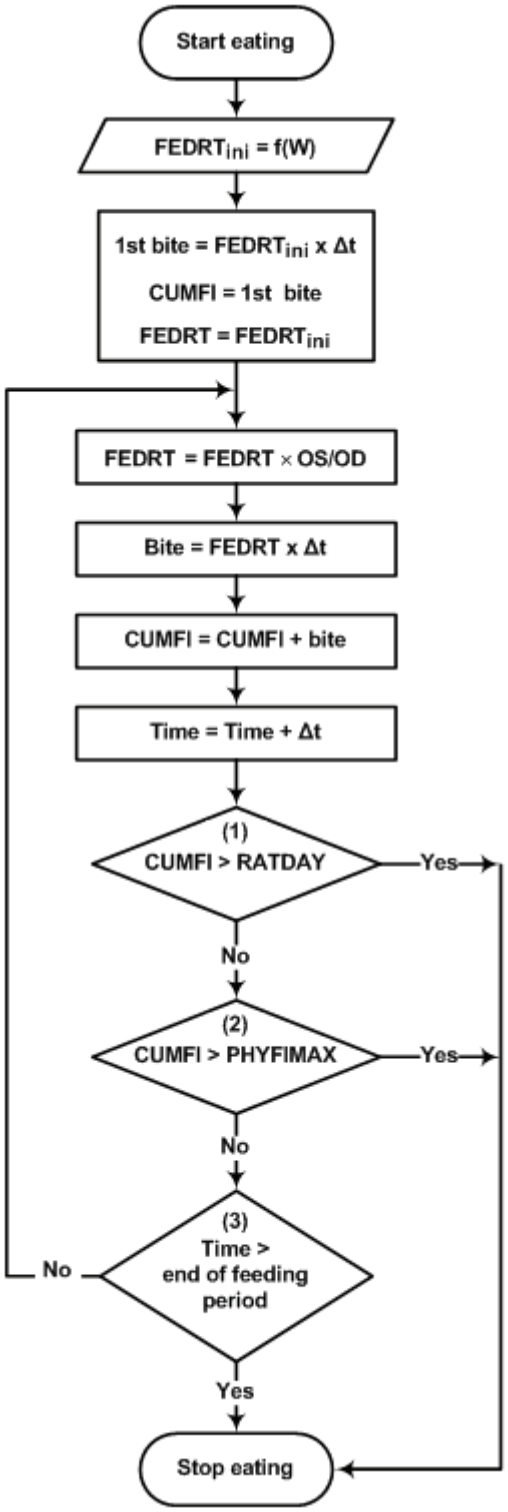

Figure 6.3. Algorithm for determination of daily feed intake. $FEDRT_{ini}$ = initial feeding rate as a function of body weight (g d^{-1}); bite = a bite of food taken during each time step (g); Δt = length of a time step (d); FEDRT = feeding rate at each time step (g d^{-1}); OS/OD = ratio of oxygen supply to oxygen demand; CUMFI = cumulative feed intake (g); RATDAY = daily ration (g); PHYFIMAX = maximum daily feed intake determined by a factor other than ambient DO concentration (g).

For the simulations of the growth experiments, agreement between the simulated and observed variables was assessed based on the relative error (RE) and the average relative error (ARE), which were calculated as follows:

$$RE_i = 100 \times \frac{SW_i - EW_i}{\frac{1}{2}(SW_i + EW_i)} \quad \textbf{(6.14)}$$

$$ARE = \frac{1}{n}\sum_{i=1}^{n} RE_i \quad \textbf{(6.15)}$$

where RE_i is the relative error for case i, SW_i and EW_i are the simulated and observed values for case i, respectively, and n is the number of cases.

The model was written in Borland Delphi 7 (Borland Software Corporation, Texas, USA).

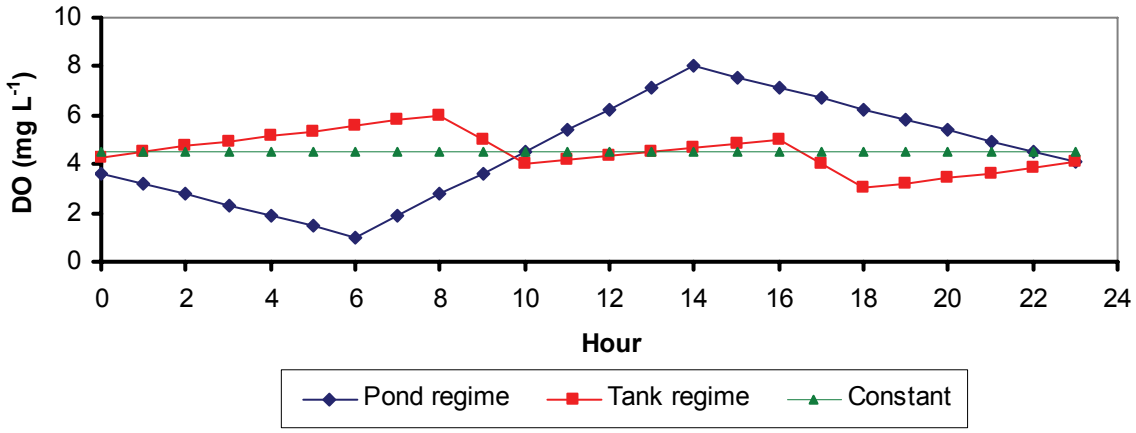

Figure 6.4. Three regimes of diel variation in dissolve oxygen concentrations (DO) used in simulations to investigate effect of diel DO variation on feed intake and growth.

2.6. Data sources and parameterization

To relate feeding activity to feeding level based on the differences between daily feeding metabolism as calculated by FGS2 and the daily costs of absorption and transport, data from Osman (1999), Magouz (1990) and Wee and Tuan (1988) with known quantities of feed and no oxygen limitation were used (see dataset description in van Dam and Penning De Vries, 1995).

To calibrate FGS4 with the improved equations for feeding metabolism, data from Tran-Duy et al. (2008a) and Tran-Duy et al. (2008c) for Nile tilapia were used. Data from Verdegem et al. (2000) were used to validate the model. In all datasets, each case represented

a growth trial with a group of fish in an aquarium. Fish were fed at different feeding levels (Table 6.1).

To calibrate FGS4 with the new module for feed intake regulation based on ambient DO, data from Tran-Duy et al. (2008a) and Tran-Duy et al. (2008c) were used. Data from Kolding et al. (2008) were used to validate the model (Table 6.1). In this new module, under the conditions that ambient DO does not limit feed intake, maximal feed intake (PHYFIMAX) was empirically determined as a function of body weight (Equation 6.13). To estimate the the parameters in this equation (FICF and FIEX), data from Tran-Duy et al. (2008b) and Tran-Duy et al. (2008c) were used. FICF and FIEX were estimated by a linear regression of the logarithmic transformations of the body weights and the corresponding maximum feed intake (N = 10) using PROC REG in SAS 9.1 (SAS Institute Inc., 2004).

An overview of all parameter settings for the different datasets is provided in Tables 6.2 and 6.3. GSEX (Equation 6.6) was set at 0.75 (Fernandes and Rantin, 1986). O2CF1 and O2CF2 (Equation 6.7) were set at 0.251×10^{-3} and 24.642×10^{-3}, respectively (van Dam and Pauly, 1995). Measured digestibility of protein (88-92%), lipid (93-97%) and carbohydrate (69-75%) were used for the simulation with the datasets TRNL11-14 and TRNL21-24 (Tran-Duy et al., 2008c). Since digestibility was not determined by Tran-Duy et al. (2008a) and Kolding et al. (2008), protein, lipid and carbohydrate digestibilities in the datasets TRVN1-4 and KONO1-3 were set at 90%, 95% and 70%, respectively. Digestibilities for the dataset VERNL were reported to be 90%, 90% and 60% for protein, lipid and carbohydrate, respectively (Verdegem et al., 2000). Because Nile tilapia display a predominantly diurnal rhythm of feeding activity, fish were allowed to eat between 6:00 and 18:00 in the simulations (Toguyeni et al., 1997).

Table 6.1. Summary of the data used for calibration and validation

Source[1]	Name	No. of cases	Initial weight (g)	Final weight (g)	Mean Temp (^oC)	Mean DO (mg L^{-1})	No. of DO measurements per day	Growth period (day)
Calibration datasets								
1	TRVN1	3	20.3-22.7	50.4-54.4	32.7	2.7-2.8	6	25
1	TRVN2	3	21.0-21.5	58.7-71.3	32.7	5.4-5.6	6	25
1	TRVN3	3	142.2-153.8	211.6-230.9	32.7	3.2	6	25
1	TRVN4	3	142.1-147.5	241.7-264.9	32.7	5.7-5.9	6	25
2	TRNL11	3	193.7-197.7	195.8-199.6	28.0	1.5-1.6	24	16
2	TRNL12	3	197.6-198.2	229.7-239.6	28.0	2.5-2.6	24	16
2	TRNL13	3	194.4-198.3	247.8-265.0	28.0	3.9-4.2	24	16
2	TRNL14	3	195.9-198.6	268.9-279.2	28.0	6.1	24	16
2	TRNL21	3	56.4-60.2	79.9-81.5	28.0	1.3-1.4	24	20
2	TRNL22	3	57.3-58.0	85.1-86.6	28.0	1.6-1.8	24	20
2	TRNL23	3	57.5-59.8	99.0-102.0	28.0	2.6-2.7	24	20
2	TRNL24	3	58.0-60.5	100.3-100.8	28.0	3.6-3.8	24	20
Validation datasets								
3	VERNL	34	11.8-122.6	19.7-204.0	27.0-28.0	-	-	30-35
4	KONO1	18	1.9-34.0	3.2-43.5	27.0-28.3	0.9-1.7	1-7	13-15
4	KONO2	18	1.8-51.7	3.4-66.9	26.9-28.4	2.1-3.5	1-7	13-15
4	KONO3	18	1.8-65.4	3.7-79.3	27.0-28.3	4.9-7.7	1-7	13-15

[1] Sources: 1: Tran-Duy et al., 2008a; 2: Tran-Duy et al., 2008c; 3: Verdegem et al., 2000; 4: Kolding et al., 2008.

Table 6.2. Glossary of parameters

Name	Description	Unit
b1, b2	Coefficients (slopes) in the equation representing the diphasic allometric relationship between feeding activity and feeding level	% d $kg^{0.8}$ g^{-1}
C	Abscissa of the intersection of the two segments of the curve representing the diphasic allometric relationship between feeding activity and feeding level	g $kg^{-0.8}$ d^{-1}
DIGCA	Carbohydrate digestibility	%
DIGLI	Lipid digestibility	%
DIGPR	Protein digestibility	%
F	Feeding level at which feeding activity equals 50% of PMAX	g $kg^{-0.8}$ d^{-1}
FEDCF1	Coefficient 1 (slope) in the equation representing the linear relationship between feeding activity (as % routine metabolism) and feeding level	% d $kg^{0.8}$ g^{-1}
FEDCF2	Coefficient 2 (intercept) in the equation representing the linear relationship between feeding activity (as % of routine metabolism) and feeding level	%
FEEDCA	Feed carbohydrate	% in dry matter
FEEDDM	Feed dry matter	% of fresh weight
FEEDLI	Feed lipids	% in dry matter
FEEDPR	Feed protein	% in dry matter
FICF	Coefficient in the relationship between physiological maximum feed intake and body weight	g g^{-FIEX}
FIEX	Exponent in the relationship between physiological maximum feed intake and body weight	-
GARF	Gill area reduction factor	-
GSCF	Coefficient of gill size-body weight relationship	m^2 g^{-1}
GSEX	Exponent of gill size-body weight relationship	-
K	Krogh's permeability coefficient	g d^{-1} m^{-1} $(mm\ Hg)^{-1}$
O2CF1	Coefficient 1 (slope) in the equation representing the linear relationship between oxygen limitation empirical factor and ambient oxygen pressure	$(mm\ Hg)^{-1}$ $g^{0.25}$ d^{-1}
O2CF2	Coefficient 2 (intercept) in the equation representing the linear relationship between oxygen limitation empirical factor and ambient oxygen pressure	$g^{0.25}$ d^{-1}
PMAX	Maximum feeding activity (as % of routine metabolism) in the equation representing the Holling type II relationship between feeding activity and feeding level	%
r	Smoothness parameter in the equation representing the diphasic allometric relationship between feeding activity and feeding level	g $kg^{-0.8}$ d^{-1}
WBD	Water blood distance	m

Table 6.3. Parameter settings for different datasets

Parameter	Dataset[1]				
	TRVN1-4	TRNL11-14	TRNL21-24	VERNL	KONO1-3
Feed composition					
FEEDDM	95	95	95	91	94
FEEDPR	34	36	36	44-54	56
FEEDCA	52	38	38	27-39	17
FEEDLI	5	15	15	15-16	13
Digestibility					
DIGPR	90	88-92	92	90	90
DIGCA	70	73-75	69-70	60	70
DIGLI	95	95-97	94-95	90	95
Feeding activity					
FEDCF1	1.57	1.57	1.57	1.57	1.57
FEDCF2	-0.36	-0.36	-0.36	-0.36	-0.36
PMAX	55.0	55.0	55.0	55.0	55.0
F	17.0	17.0	17.0	17.0	17.0
b1	16.4	16.4	16.4	16.4	16.4
b2	0.1	0.1	0.1	0.1	0.1
r	2.0	2.0	2.0	2.0	2.0
C	17.0	17.0	17.0	17.0	17.0
Gills					
GSEX	0.75	0.75	0.75	0.75	0.75
GSCF	5.76×10^{-4}	5.76×10^{-4}	5.76×10^{-4}	5.76×10^{-4}	5.76×10^{-4}
GARF	0.65	0.65	0.65	0.65	0.65
K	5×10^{-6}	5×10^{-6}	5×10^{-6}	5×10^{-6}	5×10^{-6}
WBD	3.6×10^{-6}	3.6×10^{-6}	3.6×10^{-6}	3.6×10^{-6}	3.6×10^{-6}
Oxygen limitation					
O2CF1	0.251×10^{-3}	0.251×10^{-3}	0.251×10^{-3}	0.251×10^{-3}	0.251×10^{-3}
O2CF2	24.642×10^{-3}	24.642×10^{-3}	24.642×10^{-3}	24.642×10^{-3}	24.642×10^{-3}
Physiological maximum feed intake					
FICF	0.11	0.11	0.11	0.11	0.11
FIEX	0.73	0.73	0.73	0.73	0.73

[1] See Table 6.1 for sources and characteristics of the datasets.

3. Results

3.1. Feeding metabolism

A plot of feeding activity (FDACPER; in % routine metabolism) against feeding level (FEDLEV; g $kg^{-0.8}$ d^{-1}) is shown in Figure 6.5 (see 2.2). FEDCF1 and FEDCF2 were estimated to be 1.57 and -0.36, respectively (R^2 = 0.986).

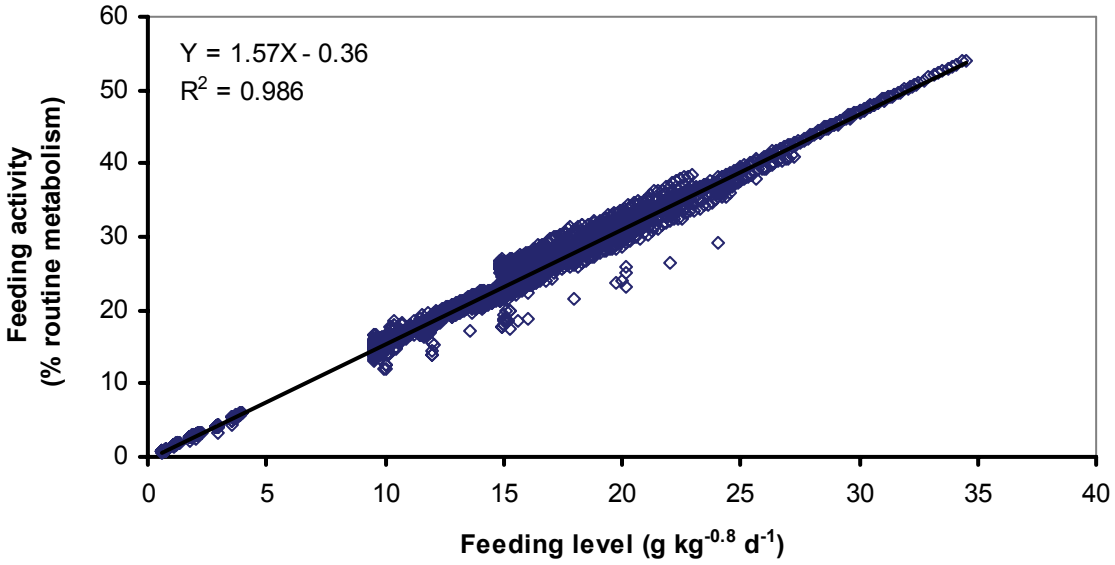

Figure 6.5. Relationship between feeding activity (in % of routine metabolism) and feeding level (g $kg^{-0.8}$ d^{-1}) in Nile tilapia (*Oreochromis niloticus*). Feeding activity refers to food capture and handling and motor activity of the gastrointestinal tract.

Using this linear relationship (Equation 6.1) in FGS4, best agreement between observed and simulated final fish weight after calibration was achieved with FEDCF1 = 1.64 and FEDCF2 = -0.10. The REs ranged from -24.17% to 7.48% with ARE = -0.60%. Validation of the linear model resulted in REs ranging from -20.57 to 27.88% with ARE = -0.29%. The agreement between the simulated (Y) and observed (X) values for fish weight was Y = 1.00 X + 1.40 (R^2 = 0.952).

A plot of RE against feeding level (Figure 6.6) shows that REs decreased with increasing feeding levels, with positive and negative values at feeding levels lower and higher than 17 g $kg^{-0.8}$ d^{-1}, respectively. This implies that the model overestimated fish weight at low feeding levels (< 17 g $kg^{-0.8}$ d^{-1}) while underestimating fish weights at high (> 17 g $kg^{-0.8}$ d^{-1}) feeding levels. Better weight prediction would be achieved if the model could increase the feeding metabolism of the fish fed low feeding levels while reducing that of fish fed high feeding levels. This was attempted with the curvilinear models (Equation 6.2 and 6.3).

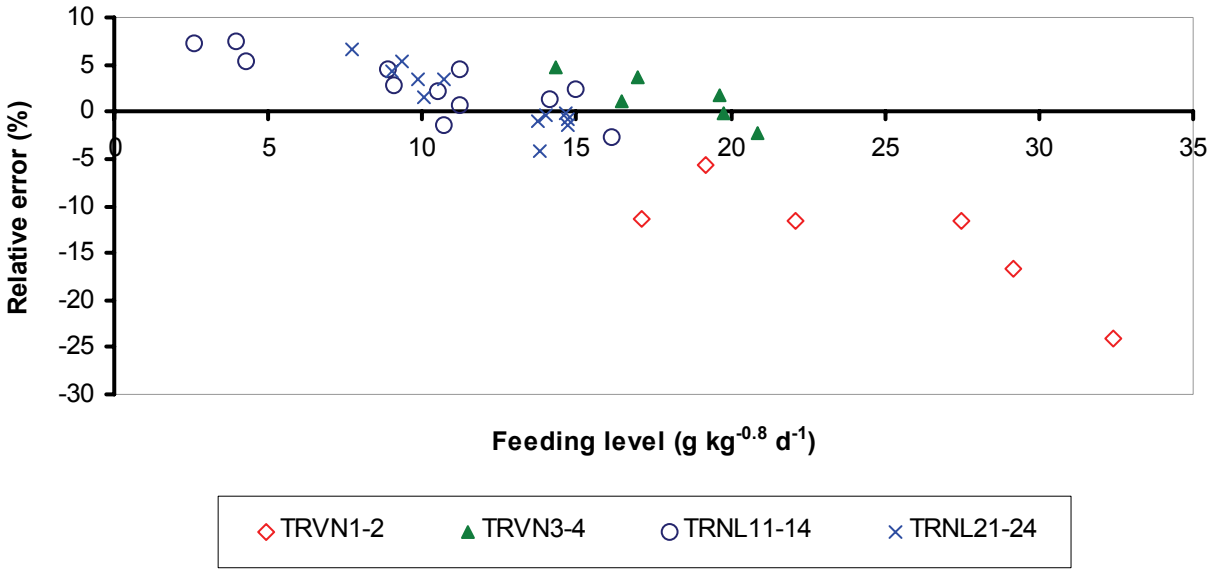

Figure 6.6. Variation in relative errors of simulated fish weight with varying feeding levels when the linear relationship between feeding activity (in % routine metabolism) and feeding level (g $kg^{-0.8}$ d^{-1}) was used for the simulation. Points of the same symbol indicate cases in the same dataset (see Table 6.1 for characteristics).

The greatest feeding level and feeding activity calculated by FGS2 with the data from Osman (1999), Magouz (1990) and Wee and Tuan (1988) were 35 g $kg^{-0.8}$ d^{-1} and 54.6%, respectively (see Figure 6.5). Based on these values, PMAX and F in Equation 6.2 (Holling Type II) was set at 54.6% and 17.5 g $kg^{-0.8}$ d^{-1} (half of 35 g $kg^{-0.8}$ d^{-1}), respectively. After calibration, best agreement between observed and simulated final fish weight was achieved with PMAX = 55.0% and F = 17.0 g $kg^{-0.8}$ d^{-1}. The REs ranged from -15.52% to 7.15% with ARE = -0.04%. Validation results of this model showed REs ranging from -19.66% to 26.77% with ARE = -0.84%. The agreement between the simulated (Y) and observed (X) fish weights was $Y = 0.99 X + 1.71$ ($R^2 = 0.952$).

For the diphasic allometric model (Equation 6.3), b1 was set at 1.64, which is the slope in the linear relationship between FDACPER and FEDLEV after calibration; b2 was set at 0.1, assuming that the increase in FDACPER with increasing FEDLEV above the intersection point (C) is minimum; C was set at 17.0 based on the observation that the linear model overestimated fish weight at feeding levels lower, while underestimating fish weight at feeding levels higher than 17.0 g $kg^{-0.8}$ d^{-1} (Figure 6.6); r was arbitrarily set at 2. After calibration, best agreement between observed and simulated final fish weight was achieved with b1 = 1.9, b2 = 0.1, C = 16.0 and r = 2.0. The REs ranged from -13.69% to 7.30% with ARE = 0.19%. Validation results of this model showed REs ranging from -20.62% to 26.93%

with ARE = -0.86%. The agreement between the simulated (Y) and observed (X) fish weights was Y = 0.99 X + 1.29 (R^2 = 0.952).

The Holling type II and diphasic allometric relationships between FDACPER and FEDLEV are shown in Figure 6.7. Table 6.4 shows the relative errors of the simulated final fish weights resulting from the different approaches for feeding metabolism. The Holling type II and diphasic allometric relationships resulted in a narrower range of REs than the linear relationship. Moreover, application of these curvilinear relationships decreased and increased the REs at low and high feeding levels, respectively, compared to the linear relationship (plot not shown). Because the differences in the range of REs and ARE between the Holling type II and diphasic allometric relationships are relatively small, the diphasic allometric relationship was arbitrarily used in FGS4 for simulation of effect of DO on maximum feed intake. The calibration and validation results of the diphasic allometric model are shown in Figure 6.8 and 6.9, respectively.

Table 6.4. Relative errors of simulated final fish weights resulted from different equations for feeding metabolism in the model

	Calibration[1]			**Validation**[2]					
	RE_{min} (%)	RE_{max} (%)	ARE (%)	RE_{min} (%)	RE_{max} (%)	ARE (%)	Slope[7]	Inter-cept[7]	R^2 [7]
Empirical equations[3]	-12.88	7.81	1.86	-20.09	28.15	-0.06	1.00	1.26	0.951
Linear equation[4]	-24.17	7.48	-0.60	-20.57	27.88	-0.29	1.00	1.71	0.952
Holling type II equation[5]	-15.52	7.15	0.04	-19.66	26.77	-0.84	0.99	1.71	0.952
Diphasic allometric equation[6]	-13.69	7.30	0.19	-20.33	27.10	-0.70	0.99	1.29	0.952

[1] Data from Tran-Duy et al. (2008a) and Tran-Duy et al. (2008c).

[2] Data from Verdegem et al. (2000).

[3] Feeding metabolism was set as 4.5% of the gross energy intake.

[4] Feeding activity (in percent of routine metabolism) and feeding level are linearly related. Feeding activity comprises food capture and handling and motor activity of the gastrointestinal tract. See text for details.

[5] The relationship between feeding activity and feeding level follows the Holling type II equation. See text for details.

[6] The relationship between feeding activity and feeding level follows the diphasic allometric equation. See text for details.

[7] Coefficients in the linear regression on predicted (dependent variable) and observed (independent variable) fish weights.

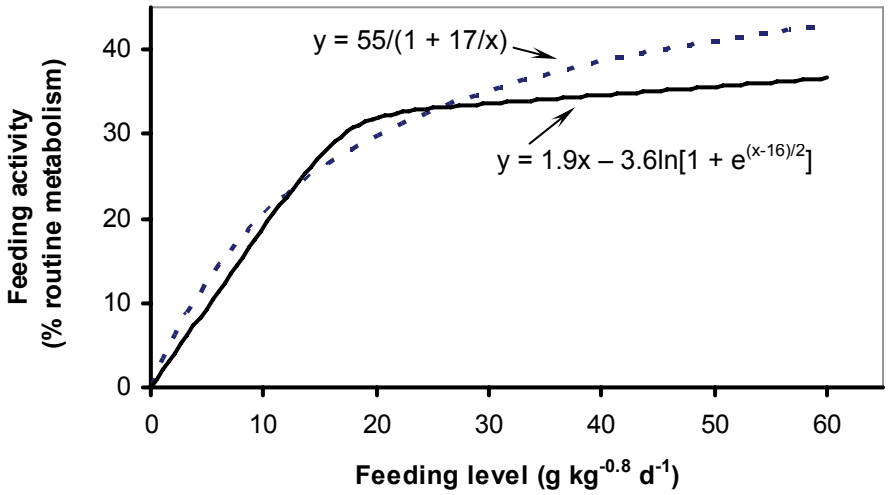

Figure 6.7. Holling type II (broken line) and diphasic allometric (solid line) relationships between feeding activity (in % of routine metabolism) and feeding level. Feeding activity refers to the costs for food capture and handling and motor activity of the gastrointestinal tract.

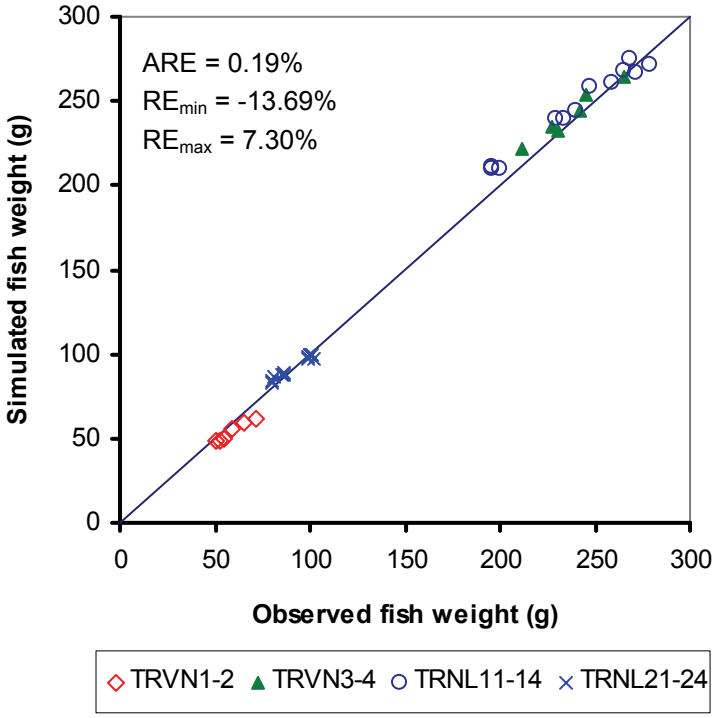

Figure 6.8. Calibration results of a model using the diphasic allometric relationship between feeding activity (in % routine metabolism) and feeding level (g $kg^{-0.8}$ d^{-1}). The bisector represents best agreement between simulated and observed values. Points of the same symbol indicate cases in the same dataset (see Table 6.1 for characteristics).

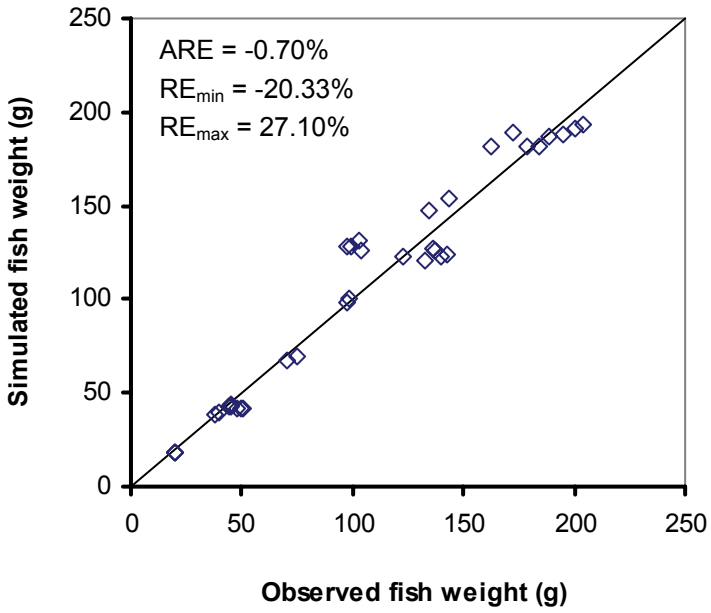

Figure 6.9. Validation results of a model for feeding metabolism using the diphasic allometric relationship between feeding activity (in % routine metabolism) and feeding level (g $kg^{-0.8}$ d^{-1}). The bisector represents best agreement between simulated and observed values. Dataset VERNL was used for the simulation (see Table 6.1 for characteristics).

3.2. Effect of DO on feed intake and growth

Estimation of the parameters for PHYFIMAX (Equation 6.13) resulted in FICF = 0.11 and FIEX = 0.73 (R^2 = 0.967) (Figure 6.10). After calibration, best agreement between observed and simulated values for total feed intake during the whole experimental period and final fish weight (Figure 6.11) was obtained with FICF = 0.13, FIEX = 0.76, O2CF1 = 0.285×10^{-3}, O2CF2 = 28.012×10^{-3} and GSEX = 0.77. The REs of the simulated total feed intake ranged from -63.90% to 64.67% with ARE = -7.25%. The Pearson's correlation coefficient between simulated and observed total feed intake (N = 36) was 0.941. The REs of the simulated fish weight ranged from -47.75% to 18.87% with ARE = -5.05%. A plot of simulated total feed intake against mean DO over the whole growth trial period of each case (Figure 6.12) shows that simulated total feed intake of fish of the same initial weight class increased with increasing DOs. A plot of RE against DO (Figure 6.13) shows no systematic change in RE with changing DO. The Pearson's correlation coefficient between RE and DO is -0.28, indicating that the model is similarly precise in all ranges of DO. However, it should be noted that the highest RE occurred with the dataset TRNL1 and lowest RE with the datasets TRVN1 and TRVN2.

Validation results are shown in Figure 6.14, with ARE = 6.93% and REs ranging from -36.17% to 40.45% for final fish weights. The model overestimated growth of fish with final weights greater than 40 g.

Model performance with respect to the effects of DO and body size on feed intake and growth is shown in Figure 6.15. With increasing DO, feed intake and growth increased to a ceiling where they are limited by PHYFIMAX. The incipient DO was about 9 mg L^{-1}. Although at a given DO absolute feed intake was higher in big fish (Figure 6.15a), feed intake was smaller in big fish than in small fish when expressed as g $kg^{-0.77}$ d^{-1} (Figure 6.15c).

Effect of diel DO variation on feed intake and growth is shown in Figure 6.16. Every day, fish under pond and constant DO regimes consumed the largest and smallest amounts of food, and attained highest and lowest body weights, respectively. Feed intake and fish weight under each regime and the differences in feed intake and fish weight among the regimes increased over time. Average feed intake was 3.57, 3.13 and 3.00 g $fish^{-1}$ d^{-1}, and specific growth rates were 2.03, 1.80 and 1.75% under pond, tank and constant regimes, respectively.

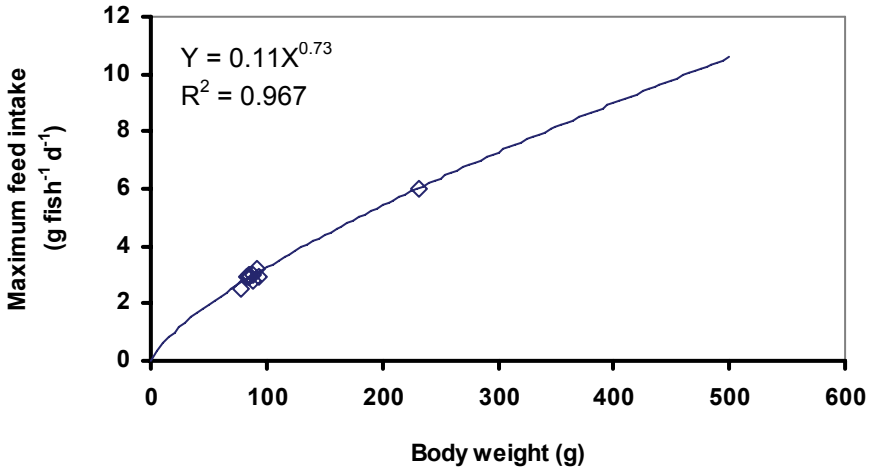

Figure 6.10. Relationship between maximum feed intake under the condition that ambient DO does not limit feed intake (PHYFIMAX; g $fish^{-1}$ d^{-1}) and body weight (W; g). The curve represents the following equation: PHYFIMAX = $0.11 \times W^{0.73}$ ($R^2 = 0.967$). The diamonds are the experimental data points used for curve-fitting.

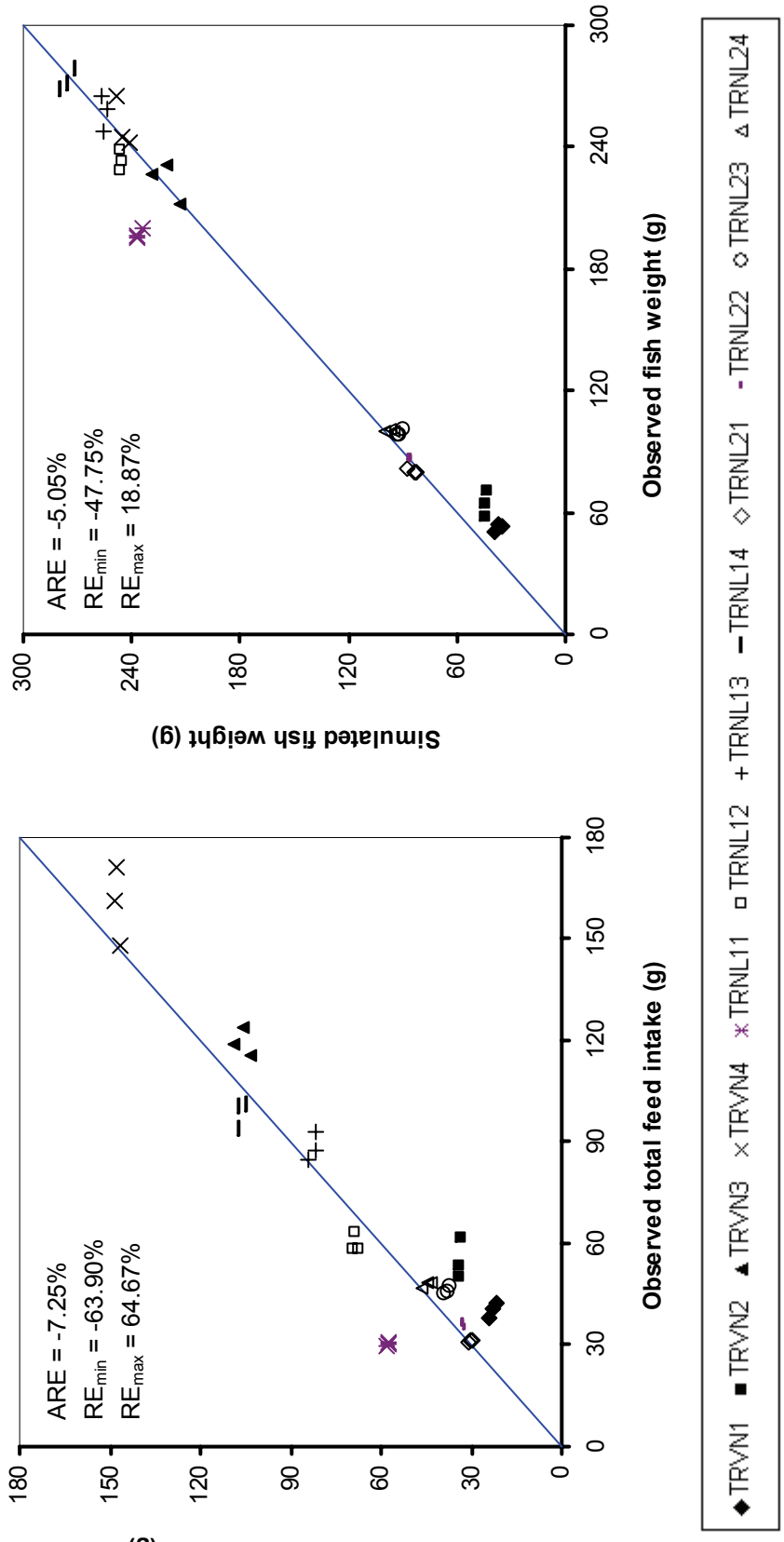

Figure 6.11. Calibration results of the model for the effects of dissolved oxygen on feed intake. The bisectors represent perfect agreement between simulated and observed values. The symbols represent different datasets pertaining to different fish sizes and dissolved oxygen levels. Points of the same symbol indicate cases in the same dataset (see Table 6.1 for characteristics).

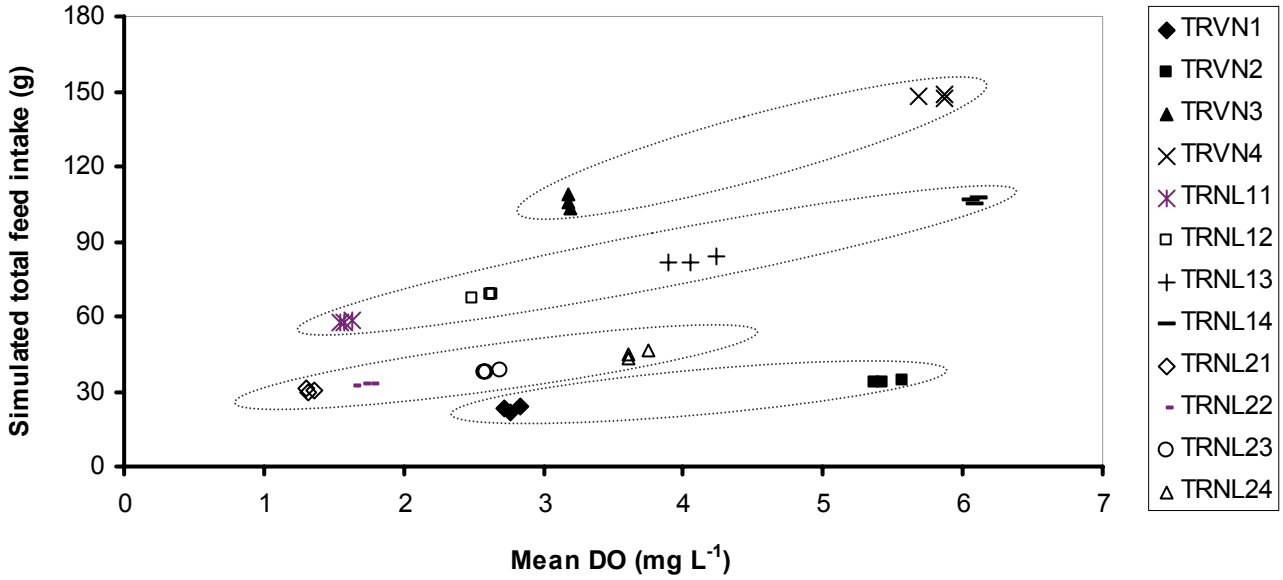

Figure 6.12. The relation between simulated total feed intake and mean dissolved oxygen concentration over the whole growth trial for the calibration runs of FGS4. Points inside the same oval represent cases with similar initial fish weights. Points of the same symbols indicate cases in the same dataset (see Table 6.1 for characteristics).

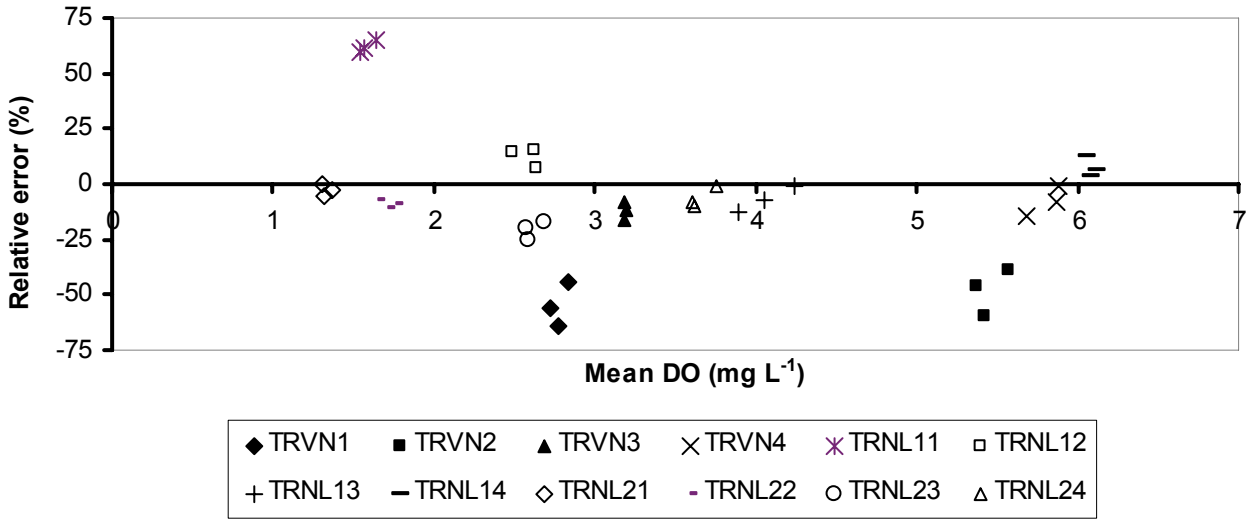

Figure 6.13. Variation in relative errors of simulated total feed intake with varying mean dissolved oxygen concentrations over the whole growth trial for the calibration runs of FGS4. Points of the same symbols indicate cases in the same dataset (see Table 6.1 for characteristics).

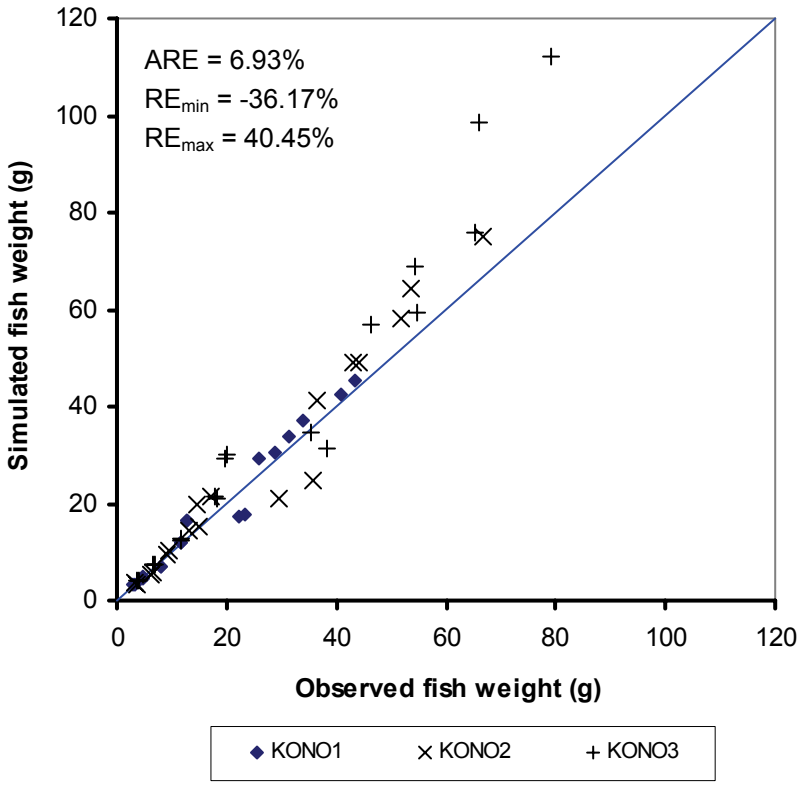

Figure 6.14. Validation results of the FGS4 model for the effects of dissolved oxygen on feed intake. The bisectors represent perfect agreement between simulated and observed values. The symbols represent different datasets pertaining to different dissolved oxygen levels. Points of the same symbol indicate cases in the same dataset (see Table 6.1 for characteristics).

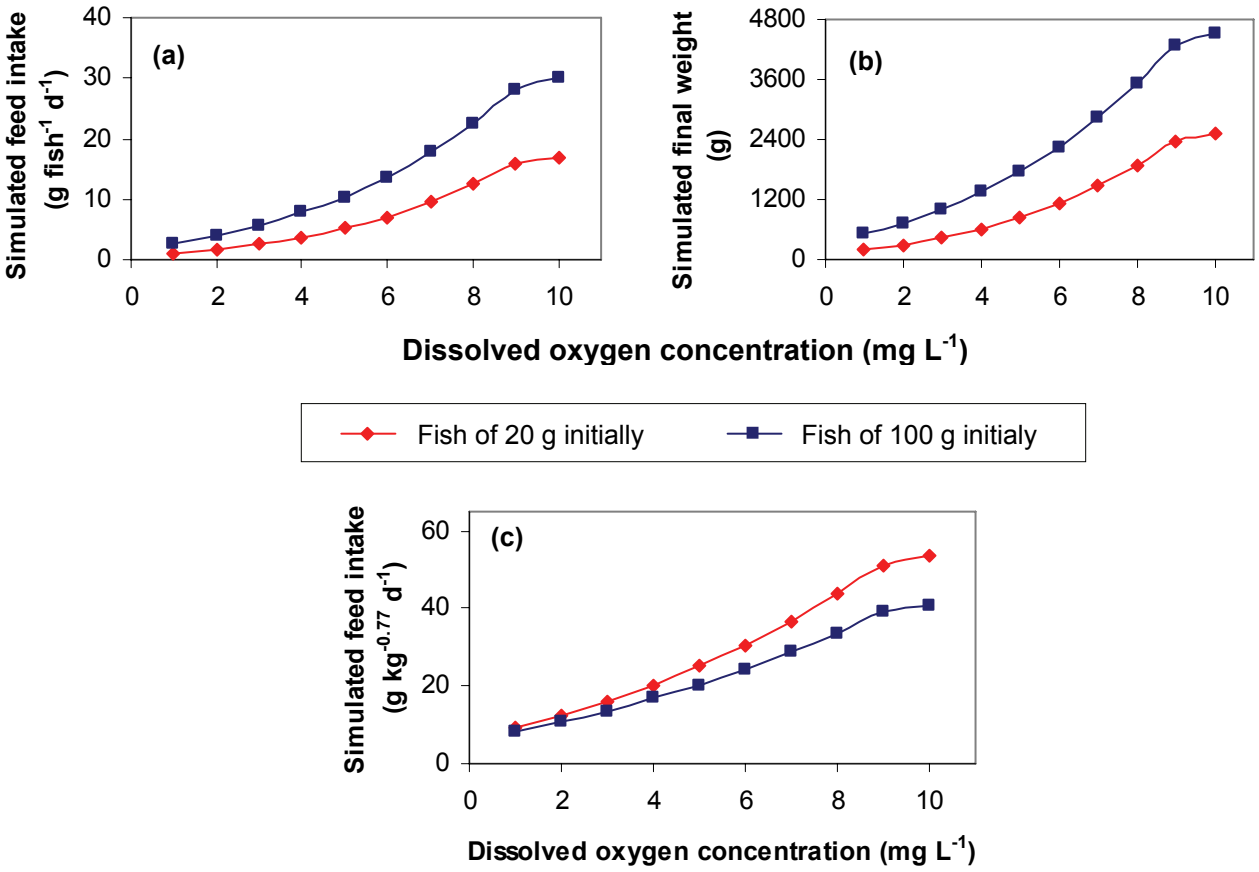

Figure 6.15. Model performance regarding effects of dissolved oxygen concentration and body size on feed intake and growth of two sizes of Nile tilapia (*Oreochromis niloticus*) fed *ad libitum* for 200 days at 28°C with a diet containing 32% protein (see text for details).

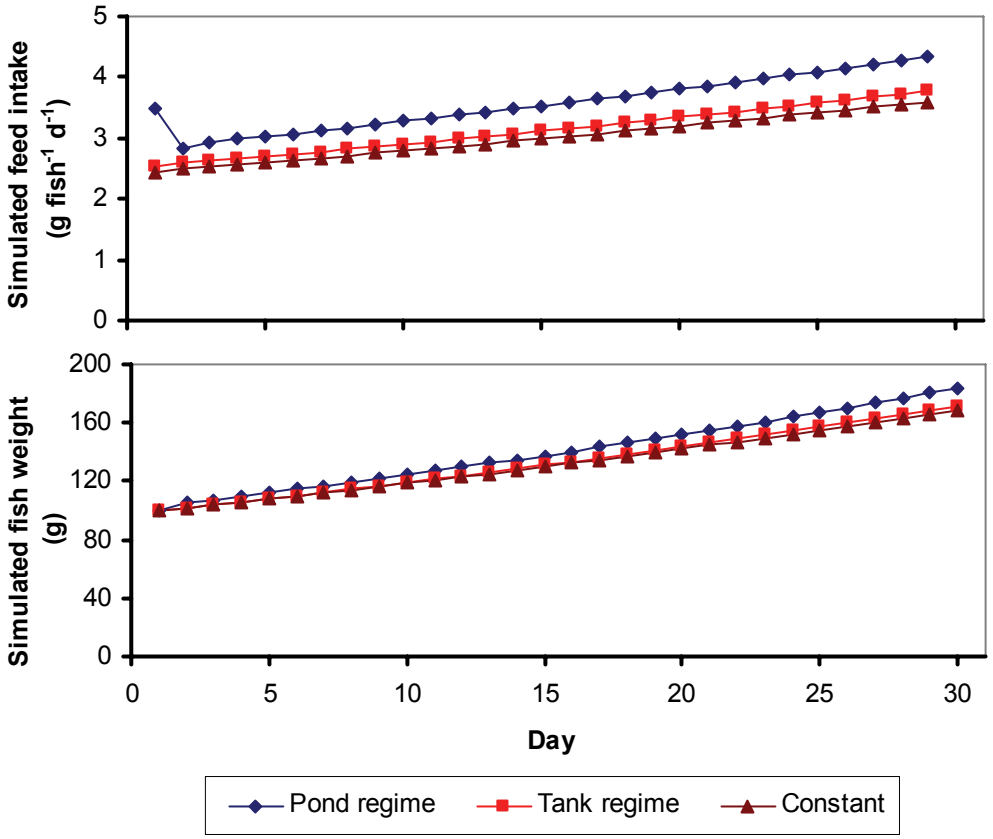

Figure 6.16. Simulated daily feed intake and growth of Nile tilapia (*Oreochromis niloticus*) reared at 28°C under three regimes of diel variation in dissolved oxygen concentration (DO) for 30 days. See Figure 6.4 for diel DO variation regimes.

4. Discussion

4.1. Feeding metabolism

The linear equation between FDACPER and FEDLEV decreased the predictive quality of FGS4 compared to FGS2, showing a larger range of REs. Application of the curvilinear relationships (Holling type II and diphasic allometric equations) improved the simulation quality compared to the linear relationship. Both estimated curvilinear relationships (Figure 6.7) indicate that the increase in feeding metabolism declines with increasing feeding levels. This reflects the physiological and behavioral responses of fish to the availability of food. As food availability increases, fish increase the activity of food capture and handling and of mechanical digestion, but meanwhile reduce their search activity for food (Holling, 1959b). However, the curvilinear relationships estimated for feed intake and feeding metabolism could be an artifact as FGS4 was calibrated using datasets obtained from experiments in which fish were fed to apparent satiation at different DO levels. Thus, low feed intake was caused by low

DO. Consequently, the curvilinear relationship between FDACPER and FEDLEV might partially reflect a change in energy expenditure associated with gill ventilation with changing DO. Under low DO condition, fish increase breathing frequency (Gerald and Cech, 1970; Lomholt and Johansen, 1979; Rantin et al., 1992; Fernandes and Rantin, 1994). As DO increases, the cost of gill ventilation decreases to a certain level at which it becomes independent of further increase in DO. Therefore, the estimated relationships between feeding activity and feeding level (Figure 6.7) might be explained by inclusion of the cost of gill ventilation into feeding activity.

Furthermore, it was not clear whether the relationships between FDACPER and FEDLEV were influenced by changes in energy requirements for maintenance with changing DO. Tran-Duy et al. (2008c) demonstrated that energy requirements for maintenance decreased with decreasing DO concentrations, which was suggested to be partly related to a reduced swimming activity at low DO level. Further improvement of the model might be made by incorporation of the impact of DO on the cost of gill ventilation and energy requirements for maintenance. More data on fish growth under different feeding levels and DOs without a correlation between feed intake and DO are needed to further evaluate the Holling type II and diphasic allometric equations for feeding activity.

4.2. Effect of DO on feed intake and growth

The calibration results showed that the model predicted total feed intake during the experimental periods and final fish weights satisfactorily. The effect of DO on feed intake is visualized in Figure 6.12, which shows that predicted feed intake of fish of the same size at lower DO is lower than at higher DO.

In the validation, the model overestimated fish weights in 42 out of 54 cases. Figure 6.14 shows that the REs increase with increasing final fish weights above 40 g. This overestimation in heavier fish (> 40g) might be due to differences in growth and feed intake between males and females. The datasets used for calibrating FGS4 were all derived from studies with only male Nile tilapia, whereas the validation datasets comprised mixed populations of Nile tilapia. Generally, male Nile tilapia have a higher growth potential than females (El-Sayed, 2006). This was also found in the study that generated the validation datasets (see also Haug, 1998). This growth potential difference might involve differences in the energy expenditure for gonad development as females have a higher gonadosomatic index (Haug, 1998). Moreover, the overestimation might be due to differences in social interactions between fish whereby feed intake is affected. With increasing age (weight), tilapias exhibit increased territorial/hierarchal behavior coinciding with more aggressive interactions. Richard (2007) showed that feed intake in heavy Nile tilapia (>375) was strongly reduced with increasing aggressive behavior.

Another explanation for the bias in weight prediction of FGS4 with the data from Kolding (2008) is that during the two-week growth periods there were only a few days (< 4 days in most of the datasets) on which DOs were measured more than 2 times per day. Interpolated DO at each time step of 0.75 hour was based on only one or two measured values on most days. Usually, DO in the fish tank drops considerably right after feeding and then gradually increases until the next feeding (Tran-Duy et al., 2008a). If DOs were measured at the beginning of two feedings, the model would overestimate the DOs for the time between these measurements using the linear interpolation. As a consequence, the model may have overestimated feed intake and thus growth of the fish.

While the approach for feed intake regulation in FGS4 is promising for prediction of feed intake of fish fed *ad libitum*, it requires information about the diurnal variation in DO. A better method for interpolation of DO should be investigated based on the trend of DO variation in the culture system. Because the rate of oxygen consumption influences DO in tanks, DO variation would depend on feeding levels, feed composition and feeding frequency. In ponds, diel variation in DO depends on photosynthesis and respiration. The simulation results in Figure 6.16 showed that feed intake and growth of fish are influenced by the regimes of diel DO variation. This influence on final fish weight will be more pronounced when the growth period is longer. However, the simulated effect of diel DO variation on feed intake and growth could not be validated with experimental data because no information on this issue could be found in the literature. This should be a topic for future research. Moreover, research is needed to provide a trajectory of DO dynamics under different circumstances, which could be used as a guide for the DO interpolation algorithm. An alternative is to link an existing model for prediction of DO in culture systems (e.g. Romaire and Boyd, 1979; Piedrahita, 1989) to FGS4.

Technically, there is still room for improvement of the model. In the current model, a fish takes a bite of food which may lead to a situation in which oxygen demand (OD) is higher than oxygen supply (OS). Based on the OS/OD ratio, the fish adjusts the size of the next bite to compensate for the shortage or redundancy of oxygen. In reality, fish might regulate feed intake more finely, ingesting the exact amount of feed that balances oxygen demand with maximum oxygen supply. A model can simulate this regulation by repeatedly adjusting the size of a bite of food at each time step until oxygen demand equals oxygen supply. Only then, the size of the food bite is registered as food intake for the current time step and the model repeats the procedure for the next time step. This would increase the number of calculations and simulation time. A smart algorithm for the adjustment of the size of the food bite should be developed to accelerate the simulation speed.

The evaluation of model performance showed that the model simulated reasonably the effect of DO on feed intake and growth: Feed intake and growth increased with increasing DO

up to a point where PHYFIMAX started to limit feed intake and thus growth (Figure 6.15a; b). The simulation results also showed that the smaller fish had a higher feed intake capacity due to higher oxygen uptake capacity than the bigger fish (Figure 6.15c). This is in line with the findings of Tran-Duy et al. (2008c; Figure 6.17). However, model behaviour is not perfect. In the experiments, the incipient DO of the smaller fish would be lower than for the bigger fish. However, the simulated incipient DO of the smaller fish and big fish were the same (compare Figures 6.15 and 6.17). Furthermore, the simulated incipient DO, which is around 9 mg L^{-1} or 115% air saturation at 28°C, seems to be too high. Prevailing DO in a well aerated fish tank is normally in the range of 5.0-7.5 mg L^{-1}. Within this range, maximum feed intake of fish can be independent of DO. Tran-Duy et al. (2008b) demonstrated that maximum feed intake of Nile tilapia fed diets different in carbohydrate and energy levels at an average DO of about 6.1 mg L^{-1} was limited by either stomach volume or blood glucose level. Thus, factors other than DO are important in determining the incipient DO.

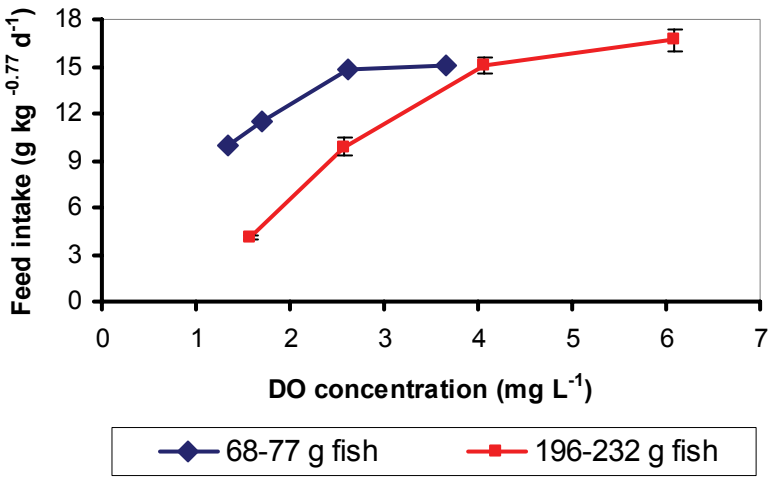

Figure 6.17. Maximum feed intake (g $kg^{-0.77}$ d^{-1}) of Nile tilapia (*Oreochromis niloticus*) of two weight classes (68-77 g and 196-232 g; geometric mean weights) in relation to dissolved oxygen concentrations. Data points represent means of three replicate values; each replicate value was calculated based on an individual fish as a mean of one tank. Vertical bars, which may be obstructed by the symbols, represent two times the standard errors. Data from Tran-Duy et al. (2008c).

In the model, PHYFIMAX represents the maximum feed intake determined by a factor other than DO and has an influence on the incipient DO. Because the parameter estimates for the PHYFIMAX empirical equation were based on only two weight classes (around 90 and 230 g), PHYFIMAX calculations for fish outside that range may be inaccurate. The simulated final weights of the fish in the evaluation of model performance at high DOs (above 5 mg L^{-1}; Figure 6.15b) were far higher than the fish weights used for parameter estimation of PHYFIMAX. At high DOs, when DO is not limiting feed intake, PHYFIMAX might have been overestimated, resulting in high incipient DO values.

Experimental and model results provide strong support for the theory that limiting DO concentrations regulate feed intake in fish. When DO is not limiting, feed intake is affected by a number of other factors, including stomach volume, blood metabolites, body composition and social interaction (see Chapter 1). PHYFIMAX may be related to some of these factors, but more research is needed to elucidate the underlying mechanisms.

Acknowledgments

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Chapter 7

General discussion

1. Introduction

Aquaculture is currently seen as a potential savior to help the overburdened fisheries sector meet the global increasing demand for food fish. However, there are also risks to aquaculture development. Fish production can lead to the discharge of wastes and have negative impacts on the environment. It is therefore important to carefully monitor and plan the development of aquaculture. Since the production of fish biomass is ultimately based on the growth of individual fish, a model that can simulate fish growth on the basis of available fish species and local conditions like water quality, availability and quality of fish feed is needed. Feed consumption is undoubtedly a required input for any model which claims to give insight into fish growth processes. Understanding how fish control feed intake also helps aquaculturists design feeds and feeding strategies for optimizing production while minimizing feed waste. An extensive review of mechanisms controlling feeding and satiation (Chapter 1) showed that feed intake regulation in fish is highly complex, multifactorial and still not well understood.

The general aim of this thesis was to investigate some potential mechanisms for feed intake regulation and to predict maximum feed intake in the light of these hypothesized mechanisms using a theoretical modeling approach. In Chapter 1, different mechanisms influencing feed intake were identified. For the purpose of this thesis, two of these were selected: blood glucose levels and the oxygen uptake capacity of the fish. Glucose and oxygen are representatives of the physiological and environmental factors, respectively which co-determine voluntary feed intake. In this thesis, hypotheses related to glucose and oxygen on feed intake regulation and the possibility to predict maximum feed intake in the light of these hypotheses were investigated. The overall hypotheses were: (1) high blood glucose level has a negative effect on feed intake in fish; and (2) dissolved oxygen concentration is a major determinant of feed intake in fish. The study was developed as a combined set of experiments, which served as a basis for calibration or validation of models in which these mechanisms of glucostatic and oxygen limitation control of feed intake were included. For practical reasons, the glucostatic control was tested (Chapter 2) and modeled (Chapter 3) first. Work on the oxygen limitation mechanism followed later (Chapters 4, 5 and 6). Because of changing views on the applicability of the model, the basic structure of the model applied in Chapter 6 (focusing on the oxygen limitation control mechanism) differed from the model used in Chapter 3 (involving the glucostatic control mechanism).

In this final chapter, the methodology and results of the preceding chapters will be discussed in view of the original objectives. The discussion is grouped into the following issues: modeling approaches (section 2); the role of glucose and oxygen in feed intake regulation (section 3); implications for aquaculture management (section 4); reliability of data on maximum feed intake (section 5) ; and implications for further research (section 6).

2. Modeling approaches

A model for fish growth should ideally be able to predict feed intake under *ad libitum* or voluntary feeding. As feed intake is a function of body weight, a model should predict well fish growth before trying to simulate feed intake. In this thesis, two approaches were used for modeling growth, hereafter referred to as the enzyme-kinetic (Chapter 3) and the oxygen-balance (Chapter 6) approach. Although differing in the degree of aggregation of the biochemical reactions in the intermediary metabolism, these two approaches share some basic principles. In the following text, I first will discuss the basic principles shared by these two approaches, followed by a discussion of the strengths and weaknesses of both approaches, and a final discussion on the complexity in relation to their applicability.

2.1. Principles of nutrient partitioning

Both modeling approaches aimed at explaining the growth process, i.e. growth in relation to feed amount, feed composition and water temperature. In this way, the applicability of the model is not restricted to a certain set of rearing conditions. For this purpose, both approaches relied on principles of biochemical and physiological control of nutrient partitioning. These principles, as generalized by Black and de Lange (1995), comprise the following features:

(1) *Body composition*: Initial body composition is input of the model and is the basis for prediction of final body composition. During simulation, body constituents can be used as regulators of the biochemical reactions, e.g. body fat oxidation (Machiels and Henken, 1986; Machiels and van Dam, 1987) or breakdown of body protein and fat (Pettigrew et al., 1992; Gerrits et al., 1997; Halas et al., 2004; Chapter 3 in this thesis).

(2) *Nutrient intake*: The nutrient intake is input of the model, which is determined by feed amount and composition. In most models, only macronutrients (protein, lipid and carbohydrate) are considered. Effects of vitamins and minerals on growth are too complex and literature provides usually only qualitative information. Therefore, in most models, the intake of vitamins, minerals and essential fatty acids is usually assumed to be sufficient for normal body functioning.

(3) *Availability of nutrients for metabolism*: Availability of nutrients is determined by digestion and absorption efficiencies. The term “digestibility” is commonly used to refer to the absorption into the blood of a nutrient as it passes through the digestive tract of an animal, which is expressed as a percentage of the ingested nutrient. In most animal growth models, the digestibility of a nutrient is assumed to be constant.

(4) *Metabolism and stoichiometric calculation*: An absorbed nutrient can be used either for catabolism or for anabolism. Depending on the objectives of the study, the intermediate reactions for these two processes are more or less aggregated for use in the model. The

aggregation results in an overall pathway with summative stoichiometry for a nutrient. Generally, two overall pathways for a nutrient are considered: synthesis to form the corresponding body constituent and oxidation to generate energy. For instance, in the model developed in Chapter 3 all metabolites were oxidized to acetyl CoA, which was then oxidized to the final products (CO_2 and H_2O). However, in the model developed in Chapter 6 all metabolites were oxidized to the final products, adopting a higher aggregation level compared to the model in Chapter 3. Because the basic biochemical pathways of metabolism have remained essentially unchanged during evolution from the prokaryote cell, the overall pathways are assumed to be common for all species.

(5) *Energy requirement*: Energy requirement determines the rates of utilization of nutrients for oxidation. There are several approaches for partitioning of the total energy requirement. In some models for growth of warm-blooded animals (e.g. Gerrits et al., 1997; Halas et al., 2004), total energy requirement is the sum of costs for maintenance, absorption, biosynthesis and formation and excretion of urea, and additional costs for growth, which represent costs for protein turnover, ion pumping, synthesis of endogenous protein and substrate cycles. In the models based on Machiels and Henken (1986), total energy requirement is the sum of routine metabolism, feeding metabolism and the costs for biosynthesis.

(6) *Growth calculation*: In general, the nutrients available for growth are calculated as the difference between the nutrients available for metabolism and the nutrients being oxidized to meet the total energy requirement. The stoichiometric coefficients calculated in the overall pathways are used to determine the amount of nutrients required for oxidation and amount of body constituents being synthesized from the corresponding nutrients.

2.2. Enzyme-kinetic approach

The enzyme-kinetic approach was used to develop the model in Chapter 3. In this approach, absorbed amino acids, fatty acids and glucose go to corresponding pools from which they can be oxidized, deposited or converted to other possible nutrients. Determination of the rates of these fluxes was based on the theory of enzyme action and kinetics, in which concentration of the metabolites are the driving forces for metabolism. According to Gill (1989), Michaelis-Menten type equations (Equation 3.2-3.5) can best be used to express effects of nutrients input on metabolism. First, all the parameters in the equations have the same dimension of the substrate concentration and therefore are biologically relevant. Second, the priorities of the conversions utilizing the same substrate can be established by setting the value of the affinity constant (K_i), i.e. a conversion which has a higher priority must have a lower value of K_i in the rate equation. Third, effects of hormones on metabolism can be mediated through changes in K_i and maximum rates of utilization of the substrates (Ru_{max}), i.e. for the anabolic

processes, decreasing K_i represents rising levels of anabolic hormones and increasing Ru_{max} represents rising levels of catabolic hormones (see Chapter 3).

Although this approach seems to properly reflect the mechanisms of nutrient partitioning, parameter estimation in the Michaelis-Menten-type equations provides highly uncertain results due to a gap in our knowledge about the quantitative relations in the intermediary metabolism in fish. There is no published information about the maximum rate of utilization of a substrate in a conversion. Some processes may be mainly regulated by hormones rather than substrate concentration (Young, 1980), but this can hardly be simulated due to a lack of quantitative information about the effect of hormones on the reaction rates. Moreover, it is still unclear under which conditions the maximum rates of utilization of amino acids occur. The priority of conversions utilizing the same substrate may change according to the nutritional status, but the affinity constants in the equations representing these conversions, as the names suggest, are fixed during the simulation. Estimation of the affinity and inhibition constants in the Michaelis-Menten type equations in most of cases is arbitrary and characterized by "trial and errors".

Temperature is a factor affecting the rate of a (bio)chemical reaction. At high temperature, it is easier for a reaction to occur. For enzyme-catalyzed reactions, high temperature may also exert a negative effect. The enzyme protein can be denatured at a certain temperature and lose activity (Bender, 2002). Due to a lack of information about the relationship between temperature and the conversion rates used in the model, effects of temperature were ignored.

While the estimation of parameters remains a problem, the enzyme-kinetic approach offers a flexible structure to simulate effects of the metabolites on feed intake. Theoretically, 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. Although the concentrations of the metabolites were considered at whole body level in this thesis, these concentrations can still be used to investigate the relationship between the accumulation of the metabolites in the whole body and feeding rate. When sufficient data is available, the structure of the model can be expanded to include blood as a compartment. However, the trade-off of this expansion is that the model becomes more complex and less general for use with a wide range of fish species.

2.3. Oxygen-balance approach

The oxygen-balance approach was used to develop the model in Chapter 6. In this approach, absorbed amino acids, fatty acids and glucose are directly converted into body protein or fat. Energy requirement is supplied by oxidation of body protein and fat. In this way, growth simulation to a great extent is a matter of determination of how much body protein and fat are

used for energy. Key parameters in the oxygen-balance-based model are those in the equation determining the proportion of total energy requirement supplied by fat oxidation. This makes the oxygen-balance-based model much less complicated and easier to handle than the enzyme-kinetic-based model.

Although the oxygen-balance approach offers a relatively simple structure to simulate fish growth, it does not allow simulation of the effects of metabolites on feed intake. While the model can simulate the effect of oxygen on feed intake, the lack of metabolite pools may prevent obtaining insight into the mechanisms by which fish sense a shortage of oxygen supply. It was hypothesized that fish stop eating when oxygen supply does not satisfy oxygen demand. However, the question is what signals the shortage of oxygen. The common result of a shortage of oxygen supply in tissue is the production of lactic acids (Heisler, 1993). On the other hand, oxygen shortage may lead to accumulation of amino acids in the blood (Pauly, 1981). If oxygen limitation is linked to high blood metabolite levels in signally satiety, the oxygen-balance approach is an alternative of the enzyme-kinetic approach for simulating feed intake regulation. Lactic and amino acids are both considered potentially toxic of which high concentrations may induce a loss of appetite. Unfortunately there have been no systematic studies on this issue.

2.4. On the complexity of a model for feed intake and growth of fish

In general, simplified models have fewer input requirements and parameters for calibration. This makes them less expensive to use and thus allows more thorough analysis with a given budget. Moreover, they are easier to transfer and combine with other models, and to interpret. With a small number of state variables and parameters, the behaviour of a model is easier to understand and improve (Meisel and Collins, 1973). Due to these advantages, simplification of models has been advocated and several simplification methods have been developed.

Repro-modeling is a technique involving mapping a set of input to a set of output using appropriate algorithms, the simplest of which is linear regression analysis. Searching for a relationship between input and output using statistical procedures has also been realized in fish growth modeling. Von Bertalanffy, logistic and Gompertz growth equations are among the well-known models which relate growth rate to body size (von Bertalanffy, 1957; Ricker, 1979). The parameters in these models are found by fitting the curves to growth data. The values of these parameters therefore represent only the conditions of the experiments from which the data were derived. Outside these conditions, these empirical models are not applicable. However, because of their simplicity, these models have been widely used especially for prediction of growth in fish population.

Reducing the number of state variables is another method of simplification. For this, variable aggregation is a commonly-used technique in which several state variables are

combined into one while the integrity of the model output is maintained. In the oxygen-balance approach, the absence of pools of amino acids and fatty acids can be interpreted as their aggregation with the body protein and body fat state variables, respectively. This results in a reduction of some eight parameters to be estimated. In the enzyme-kinetic approach, variable aggregation is usually done by combining pools of a metabolite from different compartments (e.g. organs) to one pool. In Chapter 3, the whole body was selected as the only one compartment for the pools of metabolites. Although concentration of the metabolites in the blood rather than that in the whole body is involved in feed intake control, partitioning the whole body into blood and cells compartments would double the parameters of the original model, generating some 60 or even more parameters, of which most of the values are completely unknown and hard to determine experimentally.

It will be hard to obtain reliable data for parameterization and calibration of enzyme-kinetic-based models, especially those with more than one compartment. Measurement of fluxes of metabolites between and within the compartments is not an easy task. To estimate the parameters in each Michaelis-Menten equation, at least several data points at different substrate concentrations must be determined. Establishing different levels of concentrations of a metabolite and measuring the corresponding reaction rates would require very sophisticated techniques, and may be impossible for some metabolites. For example, acetyl CoA has an extremely high turnover rate and its metabolism is completely intracellular (Stryer, 1988), making it very difficult to measure its utilization rates. In general, it is easy to add more state variables to a model to make it more realistic, but much harder to get data for calibration and validation of the model.

Jørgensen (1995), in an overview of modeling in limnology, wrote:

> "… *Given a certain amount of data, the addition of new state variables or parameters beyond a certain model complexity does not add to our ability to model the ecosystem, but only adds to unaccountable uncertainty*…"

Obviously, fish growth and feed intake modeling is not an exception to this judgment. If we define knowledge gained from a model as a product of the information generated by the model and its accuracy, and model complexity as the number of state variables (or rate variables or parameters), the relationship between knowledge gain and complexity for two levels of data quality and quantity is visualized in Figure 7.1. Knowledge gain increases with increasing complexity up to a certain level. Increasing complexity beyond this level will not increase the knowledge gain. When the complexity is high, knowledge gain goes down due to high uncertainty caused by a high number of unknown parameters. The optimum range of complexity is that resulting in highest knowledge gain. When the data are more comprehensive and have better quality, the knowledge gain at a given complexity will be higher and the optimum range of complexity larger with higher lower bound value.

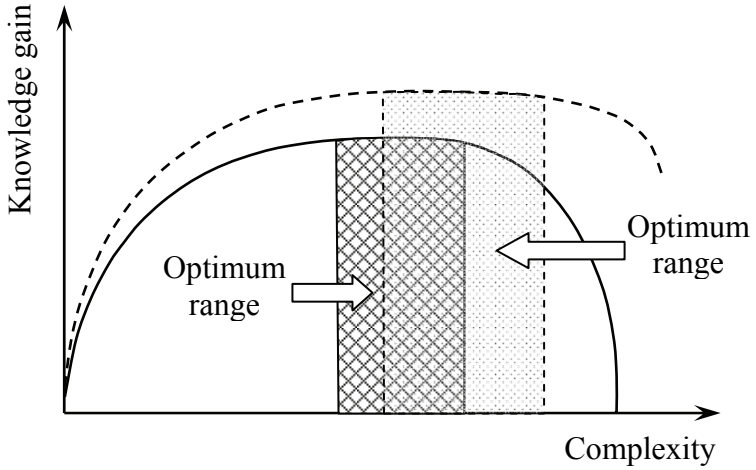

Figure 7.1. Relationship between knowledge gain and complexity of a model. The broken line corresponds to more comprehensive data with better quality than those corresponds to the solid line. Adapted from Jørgensen (1993).

A similar trend is observed in the relationship between the applicability of a model and its complexity. If a model is very simple, its potential use will be limited. The model may simulate well some particular features of a system, but as soon as conditions change, the model can no longer be used. As more processes are included in the model, it covers a wider range of factors influencing the behaviour of the system and thus becoming more applicable. However, when the model consists of too many processes, it becomes very specific for the system modeled and cannot be applied to other, similar systems. The optimum range of complexity is that giving widest applicability to a model.

It is therefore important to select a complexity level of a model for feed intake and growth of fish. This certainly depends to a large extent on the objectives of the research. A highly complex, realistic model (like the enzyme-kinetic-based model) can help the researchers synthesize and identify the gaps in the current knowledge of intermediary metabolism in fish. However, in the current context of aquaculture development, in which many new fish species are being introduced for culture, models with moderate complexity, like the oxygen-balance-based ones, are probably a more practical choice.

3. Feed intake in relation to blood glucose and dissolved oxygen concentration

3.1. Blood glucose

Oral administration of glucose resulted in a persistent hyperglycemia in a number of fish species, including rainbow trout, common carp (*Cyprinus carpio*), red sea bream *(Pagellus bogaraveo*), yellowtail (*Seriola quinqueradiata*) and channel cattish (*Ictalurus punctatus*) (Wilson, 1994). Based on these findings, some researchers (e.g. Peter, 1979; Carter et al., 2001) suggest that fish are unable to regulate blood glucose, arguing against the important

role of glucose in feed intake regulation in fish. Nevertheless, no systematic studies have been done to justify this, i.e. to relate blood glucose level to feed intake. A few observations showed contradictory ideas about the role of blood glucose on feed consumption. For instance, skipjack tuna (*Katsuwonus pelamis*) with elevated blood glucose levels consumed more food than fish with lower blood glucose levels resulting from food deprivation (Magnuson, 1969). In contrast, red piranha (*Rooseveltiella nattereri*) ingested greater meals in response to lower preceding levels of blood glucose (Bellamy, 1968). In the present study, an experiment was designed to test the hypothesis that the use of digestible carbohydrate as a major source of energy would reduce feed intake due to the effect of blood glucose (Chapter 2). By comparing the digestible energy intake of fish fed high-starch diets with that of fish fed low-starch diets, the study suggested that high blood glucose suppresses feed intake in Nile tilapia.

This study is likely to be the first which supports the role of blood glucose in feed intake regulation in fish. However, some aspects still need to be further elucidated. First, the overall hypothesis is that *high blood glucose level has a negative effect on feed intake fish*. To test this hypothesis directly and experimentally, blood glucose concentrations of fish must be measured. However, this was not done in the experiment of Chapter 2. Based on our observations, Nile tilapia easily get stressed when disturbed. The stress related to taking blood samples from the experimental fish would definitely interfere with their feed intake, thus defeating the purpose of the experiment. Moreover, stress response is characterized by an elevation in plasma cortisol and catecholamine levels. The latter cause an increase in plasma glucose (Wendelaar Bonga, 1997), confounding the effect of diets with the stress response. Due to these direct and indirect effects of stress on feed intake, it is difficult to measure experimentally a relationship between blood glucose level and feed intake. Experiments with individual fish may help to avoid disturbing the remaining fish when sampling. However, in this approach feed intake is subject to individual variation, which is still not clear in Nile tilapia and in many other fish species. If a glucose threshold exists above which fish stop eating, this threshold so far has not been found.

The arguments in Chapter 2 about the effect of blood glucose on feed intake were based on the hypothesis that fish eat to meet their energy requirements. For Nile tilapia, we found only one study that supports this hypothesis (Kubaryk, 1980). Generally, animals eat to satisfy their nutrients and energy requirements. It is therefore possible that feed ingestion is driven by protein content. The arguments in Chapter 2 would be better justified if more evidence from Nile tilapia which eat to achieve a constant level of digestible energy were available.

Using the enzyme-kinetic modeling approach, we tested the potential role of glucose concentration on feed intake (Chapter 3). In this model, glucose concentration was considered at the whole body level, i.e. with no distinction between blood and cells. This was done to

reduce the uncertainty in parameter estimation due to a lack of data (see 2.2 and 2.4). In the model, fish cease to eat when glucose concentration surpasses a threshold until glucose concentration drops below that point. The simulation results for rainbow trout found a glucose threshold of 0.25 mmol g^{-1}. This threshold could not be validated with experimental data because no information on this could be found in the literature, as mentioned above. Because we do not know how glucose is distributed among blood and cells, this whole-body concentration cannot be converted to blood concentration to ascertain whether this threshold is realistic, i.e. within the possible range of blood glucose concentrations found experimentally. The enzyme-kinetic-based model may be useful in identifying priorities for research in this area.

3.2. Dissolved oxygen (DO)

Both experimental and model results in this thesis strongly support the hypothesis that limiting DO concentrations regulate feed intake in fish. Whether DO is a major determinant of feed intake in fish depends to a large extent on fish size. The results from Chapter 5 showed that in smaller fish the incipient DO at which feed intake starts to level off was lower than in bigger fish. Specifically, incipient DO for Nile tilapia of 60-100 g was around 2.6 mg L^{-1} while that of 200-270 g fish was probably not below 5 mg L^{-1}. In a well aerated system, prevailing DO can be often higher than 5 mg L^{-1}. This means that DO is important for feed intake only when fish become bigger than 200 g. The model results in Chapter 6 showed that feed intake in fish depended on the balance between oxygen supply, determined by DO and the gill surface area (and therefore body weight), and oxygen demand, determined by the amount and composition of the feed and the activity level of the fish. This implies that whether or not DO becomes limiting also depends on feed composition.

When DO is not limiting, the incipient DO is determined by a factor other than DO which limits feed intake. Elucidating the relationship between this "other" factor and feed intake would allow a good evaluation of the importance of DO in feed intake regulation. In the model, the equation for maximum feed intake determined by a factor other than DO was empirically formulated as a function of body weight, which resulted in unsatisfactory simulation results. Thus, to further investigate the overall hypothesis that oxygen is a determinant of feed intake in fish, research into underlying mechanisms of feed intake regulation in fish in relation to other factors like stomach volume, blood metabolites, body composition and social interaction is needed.

4. Reliability of data on maximum feed intake

Regardless of the techniques used, measuring maximum feed intake of a group of fish is often influenced by the social interactions among the individuals. Social hierarchies and aggressive behaviour are the main problem to obtaining reliable data on maximum feed intake

measurements in relation to physical and environmental factors. Dominant fish can monopolize a disproportionate share of the available feed in comparison with the subordinates (Koebele, 1985; Metcalfe, 1986; Jobling and Baardvik, 1994). A persistence of social hierarchy alters feeding behaviour of the subordinate fish, causing reduced feeding rate, positioning on the sites with less food and suppressed reaction towards food (McCarthy et al., 1992; Huntingford et al., 1993; Hart and Salvanes, 2000; Harwood et al., 2003). Moreover, social hierarchies can induce a stress response and loss of appetite. As a consequence, the measured feed intake may be underestimated in the experiments designed to investigate only the effects of physiological or environmental factors.

The experiments in this thesis were conducted with male Nile tilapia, which exhibit strong aggressive behaviour, especially during breeding (Balarin and Haller, 1982). The social rank becomes more pronounced in big fish. During the experiments it was observed that males of about 150 g started to be very territorial, with a dominant male in the group trying to establish himself on top of the hierarchy. A stocking density of more than 20 fish per 100 L considerably reduced the aggressive behaviour. Because the fish were fed to apparent satiation, the dominant fish became less territorial after it reached satiety, giving the subordinate fish an opportunity to obtain food. However, it was not clear to what extent the effects of social hierarchy contributed to the cessation of feeding of the subordinates in the low density tanks.

In an experiment conducted to investigate effects of carbohydrate and energy levels on maximum feed intake (Richard, 2007), Nile tilapia of about 380 g were stocked at a density of 10 fish per 120 L-tank and fed to apparent satiation with different diets. The resulting feed intake was not significantly different among the treatments due to a high variation in feed intake within each treatment related to large differences in group behavior among the replicates. When the aggression levels of the fish, which were scored during the experimental period, were included into the analysis as co-variables, they partly explained the variation in feed intake. This indicates that group feed intake in big tilapia at low density can be strongly affected by the aggressive behavior.

Feeding frequency may influence maximum feed intake through its effect on dominance hierarchy and gastric evacuation rates. Jobling (1983), using the results of an experiment with Arctic charr (*Salvelinus alpinus),* suggested that effects of social interactions can be reduced with increased feeding frequency, leading to a more homogenous size distribution within a population. In an experiment where rainbow trout were fed one to six meals per day, maximum feed intake occurred with two feedings per day (Grayton and Beamish, 1977). This was attributed to the low metabolic and gastric evacuation rates of trout. According to Riche et al. (2004), the return of appetite following a satiation meal is about 4 h in Nile tilapia held at 28^{o}C. This suggests that maximum feed intake in Nile tilapia may occur with satiation

feeding at 4-h intervals. In the experiments in this thesis, Nile tilapia were fed twice per day; the time between two meals were 7-9 hours. During the adaptation period of at least 2 weeks, fish were adapted to this feeding frequency. It is questionable whether or not after a period of adaptation to a low frequency of feeding, fish will increase their feeding rates during the first meal in compensation to the long feeding intervals.

5. Implications for aquaculture management

The studies in this thesis contribute to the understanding of the effects of DO on feed intake and growth of fish. A growth model with explanatory characteristics is an efficient mean to predict fish growth in different scenarios. It would be very time consuming and expensive to obtain the same knowledge from field experiments as that gained from the model, knowing that the combinations of different temperatures, oxygen levels, dietary nutrient levels and feeding levels are enormous. Being based on the biochemical reaction equations of the intermediary metabolism, the model can relate feed intake and feed composition to oxygen consumption and waste production and thus give an implication for the impact of fish culture on environment. In closed production systems like tanks and ponds, knowing oxygen demands in relation to fish size and feed amount and composition can help the farmers determine optimal aeration or oxygen regimes. In open production systems like pens and cages, quantifying the impact of reduced DO as a result of global warming on fish growth can help the stakeholders take appropriate actions to counteract the situation (WWF, 2005).

Besides the significance of studies on oxygen as a growth limiting factor, prediction of maximum feed intake and growth based on feed composition and environmental factors can help farmers design feeds and feeding strategies for optimizing production while minimizing waste. Normally, feed accounts for over 50% of the operating costs of growing fish. Wastage of food in feeding not only reduces economic benefit but also adversely affects the water quality. So far the commercial fish culture has relied heavily on scattered empirical knowledge or experience in determining feeding regimes. This approach is prone to bias in the amount of feed supplied when diets and rearing conditions change. An explanatory model for feed intake and growth can solve this problem.

6. Implications for further research

6.1. From the modeling perspective

Two approaches were used in this thesis to develop the models: an enzyme-kinetic and an oxygen-balance approach. For both types of models, more data on maximum feed intake and growth of different fish species in relation to body size, feed composition, temperature and oxygen are needed for model calibration and validation.

To improve the enzyme-kinetic-based model, a number of experiments are needed. At the whole body level, effects of dietary protein, lipid and carbohydrate and their interactions on maximum feed intake should be investigated. At the organ level, the relationships between plasma metabolite levels and maximum feed intake should be quantified. Measurements of maximum rates of utilization of the metabolites in the intermediary metabolism are also important to reduce the uncertainty of parameter estimates.

For the oxygen-balance-based models, some other aspects need to be considered. The proportion of total energy requirement supplied by fat oxidation (AALIRAT) is the key variable to determine fish growth. In the model developed by Machiels and Henken (1986) for growth of African catfish (*Clarias gariepinus*), AALIRAT was formulated as a function of the ratio of body fat to body protein. Dietary protein level and the ratio of dietary protein to energy contents (P/E ratio) are also important factors influencing the use of fuel for energy generation in fish (Ogino and Saito, 1970; Huisman, 1976; Mazid et al., 1979; Huisman and Valentijn, 1981; Jauncey, 1982; Siddiqui et al., 1988; de la Higuera et al., 1989; Magouz, 1990; Sargent et al., 2002). In the model developed by van Dam and Penning De Vries (1995) for Nile tilapia and rainbow trout, AALIRAT was formulated as a function of protein feeding level and P/E ratio. It gave satisfactory results, but the equation in the model of Machiels and Henken (1986) did not work for Nile tilapia and rainbow trout. This suggests that there should be one general equation for AALIRAT which is applicable for a wide range of fish species. For this, extensive studies on the effects of feed and body composition on feed intake and energy metabolism in different fish species should be carried out.

In the oxygen limitation module, maximum feed intake is regulated based on the balance between maximum oxygen supply and oxygen demand. Maximum oxygen supply was calculated based on Fick's law of diffusion, in which water-blood distance is the only diffusion barrier. To quantify the oxygen diffusion more precisely, effects of other possible diffusion barriers, including water and mucus on the gill surface, blood plasma and erythrocyte membrane (Hughes, 1966; Hughes, 1972) should be taken into consideration. Apart from that, the relationships between water-blood distance, effective gill size area and Krogh's diffusion constant and activity level of the fish species need to be determined.

6.2. From the experimental perspective

In this thesis, effects of dietary carbohydrate and water dissolved oxygen concentration on maximum feed intake were studied. As maximum feed intake can be influenced by aggressive behaviour, two following approaches should be considered. First, the establishment of social hierarchies must be reduced as much as possible so that the maximum feed intake is affected only by the factors studied. Second, the effect of social interactions on maximum feed intake must be quantified and incorporated into the model.

Reduction of the social hierarchies can be achieved in several ways. Increasing satiation feeding frequencies can reduce competition for food. For Nile tilapia, increasing stocking density or limiting space can reduce the aggressive behaviour (Balarin and Haller, 1982). However, this may be a problem in an experiment where two or more weight classes are required or for the water-purification system in experiments carried out in closed recirculation systems (as is often the case in experimental facilities). For example, stocking 30-40 fish of 100 g in a 100-L tank seems to be sufficient to prevent male from establishing a territory. This results in a stocking density of 30-40 kg m^{-3}. If fish of 20 g are also used in the experiment, 150-200 fish should be stocked per tank to achieve the same stocking density as the big fish. As fish grow, they require more oxygen supply and excrete more wastes. This can surpass the carrying capacity of the system in the end.

Experiments with individual fish completely remove the effect of the social interactions. This requires a high number of replicates to avoid the risk of losing data due to mortality. In practice, fish are reared in a group and thus a model should predict the average group feed intake. When the individual feed intake is used for model calibration, it is important to quantify the individual variation in feed intake and growth. If the variation is high, it would be ideal to know the probability distribution of the measured variables so that the model can be expanded with stochastic components to predict feed intake and growth of a population. In a study with African catfish (*Clarias gariepinus*), Martins et al. (2005) found that fish housed individually exhibited a pronounced individual variation in feed intake (34.3%) and growth (52.8%) and that differences in these observed variables were consistent over time. She suggested that the differences in individual feed intake, feed efficiency, feeding behaviour and stress response are genetically linked; and these differences, instead of the dominance hierarchy, are the cause of the pronounced individual variation in growth of African catfish. So far, these aspects have not been considered in model development which aims at simulating feed intake regulation and growth of an individual fish. For Nile tilapia, it is still unclear to what extent genetic-based factors and social interactions contribute to differences in feed intake and growth. This would be an interesting study in the future for the model improvement.

If in practice social hierarchy of a certain fish species is a common factor influencing feed intake, this effect should be quantified. For this, studies on the relative social status and feeding ranks are needed. Feed intake of each individual in the group can be determined using X-radiography technique (Talbot and Higgins, 1983; Jobling et al., 1995). Because the establishment of dominance hierarchies depends on many factors, including fish size, feeding frequency, feed distribution and stocking density, studying the effects of social interactions on food intake requires labor and time as many experiments are needed. On the other hand, dominance hierarchies induce stress response, characterized by an elevation in plasma cortisol and catecholamine levels. It is suggested that the increased secretion of catecholamines acts as

a satiety signal by its influence on glucose metabolism (Russek, 1981). Thus, there is a link between social and physiological factors in feed intake regulation, suggesting that effects of these factors on maximum feed intake should be studied in combination.

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Summary

Worldwide, aquaculture of fish and other aquatic organisms has grown very rapidly, increasing from 3.9% of total production by weight in 1970 to 32.4% in 2004. As fishery resources are showing a declining trend, aquaculture is seen as a potential savior to help the overburdened fisheries sector meet the global increasing demand for food fish. However, there are also risks to aquaculture development. Fish production can lead to the discharge of wastes and have negative impacts on the environment. It is therefore important to carefully monitor and plan the development of aquaculture. Since the production of fish biomass is ultimately based on the growth of individual fish, a model that can simulate fish growth on the basis of available fish species and local conditions (like water quality and quantity and quality of fish feed) could be extremely useful.

During the past decades, an explanatory simulation model for fish growth (Fish Growth Simulator, FGS) was developed at Wageningen University in The Netherlands. Being based on very basic processes underlying growth, FGS was considered general and applicable to a wide variety of fish species. However, no regulatory mechanisms controlling feed intake were included. Feed consumption is a required input for any model which claims to give insight into fish growth processes. Understanding how fish control their feed intake also helps farmers design feeds and feeding strategies to optimize production and at the same time to minimize feed waste. However, little effort has been devoted to the prediction of maximum feed intake under various conditions. This is probably due to a lack of knowledge on feed intake regulation in fish, which is therefore the topic of this thesis.

The mechanisms controlling feeding and satiation in fish are highly complex, multifactorial and still not well understood (Chapter 1). The general aim of this thesis was to investigate some hypotheses about feed intake regulation in fish and to predict maximum feed intake in the light of these hypotheses using a theoretical modeling approach. Among a wide range of factors involved in feed intake regulation, glucose and oxygen are two factors of particular interest. In mammals the role of glucose on feed intake is obvious and explained by the glucostatic theory, which states that the satiety center in the brain is stimulated by an increase of glucose in the blood and causes a reduction in feed intake. In fish this role of glucose is far less clear. In contrast, the availability of oxygen might play a major role in feed intake regulation in fish, while in mammals this is not the case. The overall hypotheses were: (1) high blood glucose level has a negative effect on feed intake in fish; and (2) dissolved oxygen concentration of the water is a major determinant of feed intake in fish.

To investigate the effects of blood glucose on feed intake in Nile tilapia (*Oreochromis niloticus*) an experiment was done with feeds containing different combinations of carbohydrate and energy levels. It was hypothesized that the use of digestible carbohydrate as

a major source of energy would reduce feed intake due to the effect of elevated blood glucose, and that the stomach volume may play a role in regulating feed intake in fish fed low-energy (i.e., high-volume) diets. The results (Chapter 2) showed that fish fed a diet high in carbohydrate but low in energy failed to achieve their digestible energy requirement. In this case, the stomach volume was found not to be the cause of this failure. Because blood glucose and dietary carbohydrate are positively correlated, the results suggested that a high level of blood glucose suppresses feed intake in Nile tilapia.

A dynamic explanatory model for fish growth which incorporated metabolite pools as potential regulators of feed intake was then developed based on FGS (Chapter 3). In line with the glucostatic theory, the model was used to test the potential role of blood glucose on feed intake. In the model, fish cease to eat when glucose concentration surpasses a threshold, until glucose concentration drops below that point. The model was parameterized and calibrated for rainbow trout (*Oncorhynchus mykiss*) using published data from growth trials and predicted fresh weight of the fish with an average relative error (ARE) of −2.91% (range -32.47% to +26.89%). A glucose threshold lower than 0.25 mmol (g body weight)$^{-1}$ reduced feed intake and growth when feeding level was high. A glucose threshold higher than 0.25 mmol (g body weight)$^{-1}$ did not change the simulation results. This threshold could not be validated with experimental data because no information on this could be found in the literature. The parameterization and simulation results showed the gaps in our quantitative knowledge about the intermediary metabolism in fish.

The effects of dissolved oxygen (DO) concentration of the water and body weight on maximum feed intake and growth of Nile tilapia were investigated in Chapters 4 and 5. These studies also aimed at obtaining data for calibration of a simulation model for the effect of DO on feed intake (Chapter 6). In a first experiment, two weight classes of fish (21 g and 147 g) and two DO levels (about 3.0 mg L^{-1} and 5.6 mg L^{-1}) were employed (Chapter 4). The study demonstrated that (1) feed intake and growth of the fish at high DO level were significantly higher than at low DO level; and (2) relative feed intake and growth of smaller fish were significantly higher than of bigger fish. These findings provide strong support for the theory that limitation of oxygen supply through the gill surface area results in lower feed intake and growth of fish at low DO concentration. The allometric relationship between gill surface area and body weight results in lower relative feed intake, and therefore lower relative growth in bigger fish than in smaller fish.

Since there were only two DO levels, the experiment in Chapter 4 could not indicate whether there is one incipient DO (the oxygen concentration above which maximum feed intake of fish remains unchanged) for all sizes of fish. Moreover, as energy metabolism was not investigated, it was unclear how Nile tilapia partitioned its energy budget under different DOs. Chapter 5 therefore aimed at determining, for Nile tilapia of different body weights, the

incipient DO and the effect of DO on energy metabolism. The study demonstrated that no single incipient DO for Nile tilapia exists. The incipient DO of Nile tilapia < 100 g individual weight was about 2.6 mg L^{-1} while for bigger fish (> 200 g individual weight) it was probably above 5 mg L^{-1}. The results showed that Nile tilapia reduce energy for maintenance of body weight as DO declined.

The short-term effect of DO on *ad libitum* feed intake was then simulated based on the balance between oxygen demand (determined by the sum of routine metabolism, feeding metabolism and the energy costs for biosynthesis) and oxygen supply (derived from an allometric relationship with body weight based on gill surface area and Fick's law of diffusion). The model (Chapter 6) was calibrated and validated using the experimental data from Chapter 4 and 5 on the effect of oxygen on feed intake and growth of Nile tilapia and some additional data from earlier published experiments. The calibration results showed an average relative error (ARE) of simulated total feed intake of -7.25% with a range of relative errors (RE) of -63.90 to +64.67%. The REs of the simulated fish weight ranged from -47.75% to +18.87% with ARE = -5.05%. Validation results showed an ARE of the simulated final fish weight of 6.93% with a range of RE of -36.17 to +40.45%. The model results, together with the experimental results in Chapters 4 and 5, provide strong support for the theory that limiting DO concentrations regulate feed intake in fish. However, feed intake when DO was not limiting was not simulated satisfactorily. This was attributed to the empirical equation for maximum feed intake determined by a factor other than DO. More research into these factors (such as stomach volume, blood metabolites, body composition and social interaction) is needed to further elucidate the underlying mechanisms of feed intake regulation.

In the concluding chapter (Chapter 7), the methodology and results of the preceding chapters are discussed in view of the original objectives and implications for aquaculture management and further research are reviewed. Main conclusions are: (1) Feed intake in fish is suppressed by a high level of dietary starch possibly due to the effect of blood glucose, but the relationship between blood glucose level and feed intake remains to be explored; (2) Incorporation of pools of metabolites into a growth model provides a possibility to simulate effect of blood glucose and other metabolites on feed intake. The trade-off of this approach is that the model becomes highly complex and less general for use with a wide range of fish species; (3) Limiting DOs regulate feed intake in fish. Feed intake and growth of fish increase with increasing DO up to a point where they are independent of further increases in DO. This incipient DO depends strongly on fish size and probably on feed composition. The relationship between the incipient DO and stomach volume, blood metabolite level and social interactions needs to be elucidated; (4) Short-term effect of DO on feed intake can be simulated based on the balance between oxygen demand and oxygen supply. The oxygen balance-based model developed in this study may be used to determine optimal feeding, aeration or oxygenation regimes.

Samenvatting

Over de hele wereld heeft de aquacultuur een sterke groei laten zien met een toename van het aandeel in de totale productie van vis en andere aquatische producten (gemeten in gewicht) van 3.9% in 1970 tot 32.4% in 2004. In een tijd waarin veel visserijen dalende bestanden en opbrengsten laten zien wordt de aquacultuur gezien als een verlosser die de overbelaste visserijsector kan helpen aan de stijgende vraag naar vis te voldoen. Er zijn echter ook risico's verbonden aan deze ontwikkeling. Aquacultuur brengt afvalproductie met zich mee en kan negatieve effecten op het milieu hebben. Om die reden is het van belang de ontwikkeling van de aquacultuur zorgvuldig te plannen en begeleiden. Aangezien de productie van vis zijn oorsprong vindt in de groei van individuele vissen kan een model dat de visgroei voorspelt op basis van de vissoort en teeltomstandigheden (waterkwaliteit, hoeveelheid en samenstelling van het voer) daarbij goede diensten bewijzen.

Gedurende de afgelopen twintig jaar werd bij Wageningen Universiteit een verklarend simulatiemodel voor visgroei ontwikkeld (de Fish Growth Simulator, FGS). Aangezien FGS is gebaseerd op de fundamentele processen die aan visgroei ten grondslag liggen wordt het model gezien als algemeen toepasbaar op een groot aantal vissoorten. FGS bevatte echter geen mechanisme voor regulering van de voedselopname. Voedselopname is een onmisbare input voor elk model dat pretendeert de processen te verklaren die aan de groei van vissen ten grondslag liggen. Een goed begrip van de regulering van de voedselopname bij vissen kan vistelers helpen bij het samenstellen van voeders en voederstrategieën die de productie optimaliseren en de afvalproductie zoveel mogelijk beperken. Tot nu toe is echter weinig moeite gedaan om de maximale voederopname van vissen onder verschillende omstandigheden te voorspellen. Dit komt waarschijnlijk door een gebrek aan kennis over de regulering van de voederopname bij vissen. Dat is dan ook het thema van deze dissertatie.

De mechanismen die de voedselopname en verzadiging bij vissen reguleren zijn zeer complex, worden beïnvloed door meerdere factoren en worden nog niet volledig begrepen (Hoofdstuk 1). De algemene doelstelling van deze dissertatie was om een aantal hypothesen over de voedselopnameregulering bij vissen te onderzoeken en om de maximale voedselopname te voorspellen met een theoretisch model gebaseerd op de hypothesen. Onder de vele factoren die betrokken zijn bij de regulering van de voedselopname zijn vooral glucose en zuurstof interessant. In zoogdieren is de invloed van glucose op de voedselopname evident en verklaard door de glucostatische theorie, die stelt dat het verzadigingscentrum in de hersenen wordt gestimuleerd door een toename van de glucosespiegel van het bloed en daardoor een afname van de voedselopname veroorzaakt. Bij vissen is deze rol van glucose veel minder duidelijk. Daarentegen speelt zuurstof mogelijk een hoofdrol in de voedselopname bij vissen terwijl dat bij zoogdieren niet het geval is. De hypothesen bij dit

onderzoek waren: (1) een hoge concentratie glucose in het bloed heeft een negatief effect op de voedselopname bij vissen; en (2) de zuurstofconcentratie van het water bepaalt in belangrijke mate de voedselopname van vissen.

Om de effecten van verhoogde glucosespiegels in het bloed in de Nijltilapia (*Oreochromis niloticus*) te onderzoeken werd een experiment gedaan met voeders die verschillende combinaties van koolhydraat- en energieniveaus bevatten. De hypothese was dat het gebruik van verteerbare koolhydaten als belangrijkste energiebron de voedselopname zou verminderen via het effect van een verhoogde glucosespiegel in het bloed, en dat het maagvolume een rol zou kunnen spelen bij de regulering van de voedselopname in vissen die een dieet kregen met een laag gehalte aan energie (en dus een hoog volume). Uit de proef bleek (Hoofdstuk 2) dat vissen met een koolhydraat-rijk maar energie-arm dieet er niet in slaagden aan hun behoefte aan verteerbare energie te voloen. Het maagvolume bleek hiervan niet de oorzaak te zijn. Aangezien glucose in het bloed en koolhydraatgehalte van het diet positief gecoreleerd zijn kan uit de resultaten worden opgemaakt dat een hoog glucosegehalte in het bloed de voedselopname van de Nijltilapia onderdrukte.

Vervolgens werd een dynamisch verklarend model voor visgroei ontwikkeld op basis van FGS waarin de niveau's van metabolieten als regulatoren van de voedselopname waren opgenomen (Hoofdstuk 3). Uitgaande van de glucostatische theorie werd het model gebruikt om de mogelijke rol van glucose bij de voedselopnameregulering te onderzoeken. In het model stoppen de vissen met eten op het moment dat de glucoseconcentratie een drempelwaarde overschrijdt, totdat de glucoseconcentratie weer tot onder de drempel is gedaald. De groei van de vis werd berekend op basis van de relatie tussen lichaamsgewicht and eiwitbiomassa. Het model werd geparameterizeerd en gecalibreerd voor de regenboogforel (*Oncorhynchus mykiss*) met gegevens uit eerder gepubliceerde groeiproeven en voorspelde het gewicht met een gemiddelde relatieve fout (average relative error, ARE) van -2.91% (bereik van -32.47% tot +26.89%). Een glucosedrempel lager dan 0.25 mmol per gram lichaamsgewicht verlaagde de voedselopname en groei als het voederniveau hoog was. Een glucosedrempel hoger dan 0.25 mmol per gram veranderde de simulatieresultaten niet. De resultaten van de parameterizering en simulaties toonden aan dat er nog lacunes zijn in de kwantitatieve kennis van de intermediaire stofwisseling van vissen.

De effecten van het zuurstofgehalte van het water en het lichaamsgewicht op de maximale voedselopname en groei van Nijltilapia werden onderzocht in Hoofdstukken 4 en 5. Deze studies waren ook gericht op het verzamelen van gegevens voor de calibratie van een simulatiemodel voor de effecten van zuurstof op de voedselopname (Hoofdstuk 6). In een eerste experiment werden twee gewichtsklassen (21 g en 147 g) en twee zuurstofgehalten (ongeveer 3.0 mg L-1 en 5.6 mg L^{-1}) gebruikt (Hoofdstuk 4). Het onderzoek liet zien dat (1) voedselopname en groei van de vissen bij een hoog zuurstofgehalte significant hoger waren

dan bij een laag zuurstofgehalte; en dat (2) de relatieve voedselopname en groei (uitgedrukt ten opzichte van het lichaamsgewicht) in kleine vissen significant hoger waren dan in grote vissen. Deze resultaten vormen een sterke bevestiging van de theorie die de beperkte zuurstoftoevoer door het kieuwoppervlakte aanmerkt als de oorzaak van verminderde voedselopname en groei bij vissen onder lage zuurstofconcentratie. De allometrische relatie tussen kieuwoppervlakte en lichaamsgewicht leidt tot een lagere relatieve voedselopname, en daarmee een lagere relatieve groei in grotere vissen dan in kleine vissen.

Aangezien slechts twee zuurstofconcentraties werden gebruikt kon uit het experiment in Hoofdstuk 4 niet worden afgeleid of er één kritische zuurstofconcentratie is (de concentratie waarboven de maximale voedselopname van de vis niet verandert) voor alle visgrootten. Evenmin werd duidelijk hoe de energiebalans van de Nijltilapia eruitziet bij verschillende zuurstofconcentraties, aangezien het energiemetabolisme geen deel uitmaakte van de studie. Daarom was het doel van Hoofdstuk 5 om voor Nijltilapia's van verschillende gewichtsklassen de kritische zuurstofconcentratie en het effect van zuurstofconcentratie op het energiemetabolisme te bepalen. Uit de resultaten bleek dat er niet één enkele kritische zuurstofconcentratie bestaat voor de Nijltilapia. De kritische zuurstofconcentratie voor Nijltilapia kleiner dan 100 g individueel lichaamsgewicht was ongeveer 2.6 mg L^{-1} terwijl voor grotere vissen (boven 200 g individueel gewicht) de kritische concentratie boven de 5 mg L^{-1} lag. Tevens bleek dat de energie voor onderhoud van het lichaamsgewicht van de Nijltilapia afnam bij afnemende zuurstofconcentratie.

Vervolgens werd het korte termijn-effect van de zuurstofconcentratie op de *ad libitum* voedselopname gesimuleerd op basis van het verschil tussen de zuurstofvraag van de vis (bepaald door de som van routinemetabolisme, voedermetabolisme en de energie benodigd voor biosynthese) en het zuurstofaanbod (afgeleid van de allometrische relatie met het lichaamsgewicht op basis van het kieuwoppervlak en de diffusiewet van Fick). Het model (zie Hoofdstuk 6) werd gecalibreerd en gevalideerd met de experimentele gegevens over het effect van zuurstof op voedselopname en groei van de Nijltilpia uit de studies in Hoofdstukken 4 en 5 en aanvullende gegevens van eerder gepubliceerde experimenten. De resultaten van de calibratie lieten een gemiddelde relatieve fout (ARE) in gesimuleerde voedselopname zien van -7.25% met relatieve fouten (relative error, RE) tussen -63.90 en +64.67%. De RE van het gesimuleerde visgewicht variëerde van -47.75% tot +18.87% met een ARE van -5.05%. De validatie gaf gesimuleerde eindgewichten van de vissen met een ARE van 6.93% en RE variërend van -36.17% tot +40.45%. De modelresultaten, in combinatie met de resultaten uit Hoofdstukken 4 en 5, ondersteunen de theorie dat lage zuurstofconcentraties van het water de voedselopname van vissen beperken. Daarentegen was de simulatie van de maximale voedselopname bij niet-limiterende zuurstofconcentraties onbevredigend. Dit werd toegeschreven aan de empirische vergelijking in het model voor maximale voedselopname bepaald door andere factoren dan de zuurstofconcentratie. Meer onderzoek naar die andere

factoren (zoals maagvolume, concentraties van metabolieten in het bloed, lichaamssamenstelling en sociale interacties) is nodig om de mechanismen die de voedselopname reguleren verder op te helderen.

In het afsluitende hoofdstuk (Hoofdstuk 7) worden de methoden en resultaten uit de voorgaande hoofdstukken besproken in het licht van de oorspronkelijke doelstellingen van het onderzoek en wordt hun betekenis voor de praktijk van de aquacultuur en voor verder onderzoek belicht. De belangrijkste conclusies zijn: (1) De voedselopname van vissen wordt onderdrukt door een hoog gehalte koolhydraten in het diëet, mogelijk via hoge concentraties van glucose in het bloed, maar de relatie tussen glucoseconcentratie in het bloed en voedselopname dient verder onderzocht te worden; (2) Opname van de concentratie van metabolieten in een groeimodel biedt mogelijkheden om het effect van glucose en andere metabolieten op de voedselopname te simuleren. De keerzijde van deze benadering is dat het model zeer complex wordt en daardoor minder algemeen toepasbaar op een groot aantal verschillende vissoorten; (3) Lage zuurstofconcentraties in het water limiteren de voedselopname van vissen. De voedselopname en groei van vissen nemen toe met toenemende zuurstofconcentratie tot een concentratie waarboven ze onafhankelijk worden van verdere zuurstoftoename. Deze kritische zuurstofconcentratie is sterk afhankelijk van het lichaamsgewicht. De relatie van de kritische zuurstofconcentratie met maagvolume, metabolietenniveaus in het bloed en sociale interacties vereist nader onderzoek; (4) Het korte termijn-effect van de zuurstofconcentratie op de voedselopname kan worden gesimuleerd op basis van het verschil tussen zuurstofvraag en zuurstofaanbod. Het zuurstofbalansmodel ontwikkeld in dit onderzoek kan gebruikt worden voor het bepalen van optimale voeder-, aëratie- en oxygenatieregimes.

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Training and Supervision Program

Training and Supervision Program		**Graduate School WIAS**	
Name PhD student	Tran Duy An		
Project title	Modeling the effects of dietary carbohydrate and ambient oxygen concentration on feed intake and growth in fish		
Group	Aquaculture and Fisheries		
Daily supervisors	Dr. Johan Schrama and Dr. Anne van Dam		
Supervisor	Prof. Dr. Johan Verreth		
Project term	from September 2003 until October 2008		
EDUCATION AND TRAINING (minimum 30, maximum 60 credits)			
The Basic Package (minimum 3 credits)		**year**	**ECTS ***
• WIAS Introduction Course (mandatory)		2004	1.5
• Course on philosophy of science and ethics (mandatory)		2004	1.5
Subtotal Basic Package			**3.0**
Scientific Exposure (conferences, seminars and presentations, minimum 8 credits)			
International conferences *(minimum 3 credits)*			
• MODSIM05, Melbourne, Australia, 12-15 December		2005	1.2
• Asian-Pacific Aquaculture 2007, Hanoi, Vietnam, 5-8 August		2007	1.2
• Aquaculture Europe 07, Istanbul, Turkey, 25-28 Oct		2007	1.2
Seminars and workshops			
• WIAS/VLAG seminar plus "Seafood and Fatty acids", Wageningen, NL		2003	0.3
• INREF-POND annual scientific meeting		2003, 2005	0.6
• WIAS seminar: INREF-POND, ZAFIRA & POND-LIVE, Wageningen, NL		2003	0.3
• WIAS seminar Plus " Dietary protein", Wageningen, NL		2004	0.3
• DIARP Netherlands-Israel workshop, Wageningen, NL		2004	0.6
• PhD-retreat Workshop “Unity in diversity”, Nijmegen, NL		2004	0.6
• WIAS Science Day 2004, 2005, 2007, Wageningen, NL		2004-2007	0.9
• WIAS seminar plus "Vitality of fish", Wageningen, NL		2005	0.3
• HarmoniQuA workshop, Melbourne, Australia		2005	0.3
Presentations *(minimum 4 original presentations of which at least 1 oral)*			
• Poster presentation, WIAS Science Day		2004	1.0
• Oral presentation, DIARP Netherlands-Israel workshop, Wageningen		2004	1.0
• Oral presentations, INREF-POND meeting 2004, Wageningen, NL		2003, 2005	2.0
• Oral presentation, MODSIM05, Melbourne, Australia		2005	1.0
• Oral presentation, WIAS Science Day, Wageningen, NL		2007	1.0
• Oral presentation, Scientific meeting, UNESCO-IHE Institute, Delft, NL		2007	1.0
• Oral presentation, Asian-Pacific Aquaculture 2007, Hanoi, Vietnam		2007	1.0
• Oral presentation, Aquaculture Europe 07, Istanbul, Turkey		2007	1.0
Subtotal International Exposure			**16.8**

In-Depth Studies (minimum 6 credits, of which minimum 4 at PhD level)		
Disciplinary and interdisciplinary courses		
• Biology underpinning animal sciences, Wageningen, NL	2003	1.5
• The Art of Modelling, Wageningen, NL	2004	3.0
• Design of animal experiments, Wageningen, NL	2007	1.0
Advanced statistics courses		
• Advanced statistics, Wageningen, NL	2005	1.5
MSc level courses		
• Nutritional physiology (HAP-30304), Wageningen, NL	2003	6.0
Subtotal In-Depth Studies		**13.0**
Professional Skills Support Courses (minimum 3 credits)		
• Techniques for Writing and Presenting a Scientific Paper, Wageningen, NL	2004	1.2
• Laboratory Animal Science, Utrecht, NL (mandatory)	2004	4.5
Subtotal Professional Skills Support Courses		**5.7**
Didactic Skills Training (optional)		
Supervising practicals and excursions *(real time)*		
• MSc course "Environmental modelling", UNESCO-IHE Institute, Delft, NL	2005, 2007	4.2
Supervising theses		
• MSc thesis, Henrik Weitkamp, Wageningen University/Humboldt University	2007	2.0
Subtotal Didactic Skills Training		**6.2**
Education and Training Total		**44.7**

* One ECTS credit equals a study load of approximately 28 hours

List of Publications

Peer-reviewed articles

Tran-Duy, A., Schrama, J.W., van Dam, A.A., Verreth, J.A.J., 2008. Effects of oxygen concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia, *Oreochromis niloticus*. Aquaculture 275, 152-162.

Tran-Duy, A., Smith, B., van Dam, A.A. and Schrama, J.W., 2008. Effects of dietary starch and energy levels on maximum feed intake, growth and metabolism of Nile tilapia, *Oreochromis niloticus*. Aquaculture 277, 213-219.

Tran-Duy, A., van Dam, A.A. and Schrama, J.W., 2008. Feed intake, growth and metabolism of Nile tilapia (*Oreochromis niloticus*) in relation to dissolved oxygen concentration. Aquaculture, *submitted*.

Tran-Duy, A., van Dam, A. A. and Schrama, J.W, 2008. Simulation of feed intake regulation and growth in *Oreochromis niloticus* with special reference to ambient oxygen concentration. Ecological modeling, *submitted*.

Tran-Duy, A. and van Dam, A. A., 2008. Fish growth simulator: a software tool for studying and predicting fish growth. Computer and Electronics in Agriculture, *in preparation*.

Conference proceedings

Tran Duy, A., van Dam, A.A, Verreth, J.A.J. and Schrama, J.W., 2005. Modeling fish growth using the concentration of metabolites to regulate feed intake and metabolism. In: Zerger, A. and Argent, R.M. (Eds). MODSIM 2005 International Congress on Modelling and Simulation. Modelling and Simulation Society of Australia and New Zealand, December 2005, pp. 170-176. ISBN: 0-9758400-2-9. http://www.mssanz.org.au/modsim05/papers/tran_duy.pdf .

Tran Duy, A., Schrama, J.W., van Dam, A.A. and Verreth, J.A.J., 2007. Effects of oxygen concentration and body weight on maximum feed intake and growth of Nile tilapia *Oreochromis niloticus*. In: Asian-Pacific Aquaculture 2007, "Prospering from Dynamic Growth", Hanoi, Vietnam, 5 - 8 August, 2007 (pp. 232).

Tran Duy, A., Weitkamp, H., Schrama, J.W. and Verreth, J.A.J., 2007. Effects of dissolved oxygen on feed intake, growth and metabolism of Nile tilapia (*Oreochromis niloticus*). In: Proceedings of the International conference and industry forum. Aquaculture Europe 2007, "Competing Claims", Istanbul, Turkey, 24-27 October 2007 (pp. 562-563).

About the author

Tran Duy An was born on 15th of April, 1972 in Hue, Vietnam. After finishing high school in 1990 he enrolled in a 5-year course in Aquaculture at the Nha Trang University of Fisheries in Vietnam. He obtained the degree of Aquaculture Engineer in 1995 and was then recruited by the Hue University of Agriculture and Forestry (HUAF) as a lecturer in Aquaculture. During his tenure at HUAF, he was also taking part in research projects on rural development and natural resources management and pursued a second university education in informatics at the Hue University of Sciences. After obtaining a Bachelor degree of Informatics in 2000 he enrolled in the MSc programme in Aquaculture at Wageningen University in The Netherlands. He obtained the degree of Master of Science with distinction in 2002 and returned to work at HUAF. In 2003, he was awarded a scholarship by The Netherlands Foundation For the Advancement of Tropical Research (WOTRO) to carry out his PhD study at the Aquaculture and Fisheries Group at Wageningen University. As part of his PhD project, he also worked as a visiting researcher and teaching assistant at the UNESCO-IHE Institute for Water Education in Delft, The Netherlands for about six months.

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