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The influence of ration size on energetics and nitrogen retention in tilapia (*Oreochromis niloticus*)

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ABSTRACT

Proper nutrient management is essential for the environmental sustainability of aquaculture. While increasing daily rations generally may lead to improved growth rates, this does not necessarily mean that nutrients are utilized more efficiently. To investigate how ration size affects partitioning of dietary nutrient intake, the effects of meal size on growth and metabolism were examined in triplicate groups of adult Nile tilapia (*Oreochromis niloticus*) receiving daily rations corresponding to 1, 2, 3, or 4% of their biomass. While biomass gain and specific growth rates were positively correlated with ration size, feed conversion and protein retention were most efficient at ration sizes of 3%. Although the magnitude of the SDA response following feeding also increased with ration size, this was not proportionate to meal size. Therefore the metabolic cost of meal processing (SDA coefficient) was found to be lowest in the 3% ration group. The lowest rates of nitrogen excretion as well as the lowest SDA coefficients were also observed for fish receiving meal sizes corresponding to 3% of their body mass. In contrast, fish fed ration sizes of 1% displayed a reduction in apparent digestibility of protein, nitrogen free extract and dry matter, in addition to excreting a disproportionate amount of ingested nitrogen as ammonia and urea.

1. Introduction

Nutrient intake in fish is used to fuel a variety of biological processes, such as hormone synthesis, cellular homeostasis and activity. A proportion of the nutrients retained are used for the synthesis of new tissue, or simply growth. Growth in fishes is important for fisheries management and aquaculture production, and has been described by a suite of bioenergetic models of varying complexity (Kitchell et al., 1977; Rasmussen and From, 1991). In wild fish populations where feed availability may be patchy, nutritional values fluctuate, and prey items vary in composition models require a higher level of complexity. In aquaculture production, simpler calculations may be more appropriate (Kaushik and Médale, 1994; NRC, 2011). In its simplest definition, growth is the difference between dietary energy intake and the sum of energy lost (Jobling, 1994), where lost energy encompasses faecal, urinary, and branchial excretions, in addition to energy spent on meal processing, activity and standard metabolism (NRC, 2011).

Protein is undoubtedly the most expensive ingredient in commercial fish feeds, and fish feed is the biggest expense in the majority of aquaculture production. From a monetary and environmental perspective, it is of great interest that fish retain as much ingested protein as possible for protein deposition, rather than it being excreted to the environment in solid or dissolved form. The performance of fish under particular conditions can be assessed by growth trials, in which growth rates and feed conversion ratios are determined (Larsen et al., 2012). Digestibility studies, in which the residual quantities of dietary nutrients in the faeces are determined, are typically used to determine how efficiently fish digest a given diet, *e.g.* by using a Guelph type digestibility system (Bureau and Cho, 1999) or some modification hereof (Dalsgaard and Pedersen, 2011), which allow for the collection of faecal matter. While digestibility studies are often conducted in the testing of novel raw materials, they can also be applied to test the effects of biological and physical variables, such as phenotypical traits (Olsen and Ringo, 1999; Olsen et al., 2005) and water quality parameters (Skov et al., 2013) on faecal nutrient losses.

Digestibility studies provide information on how much of a given nutrient is digested by, and theoretically available to the fish. A further assessment of the metabolic fate of dietary amino acids from protein can be achieved by measurements of nitrogen excretion as ammonia and urea, to assess how efficiently they are used in the deposition of new protein. Nitrogen excretion during digestion represents the sum of amino acids that are deaminated for *de novo* lipogenesis or glycogenesis, as well as those oxidised for fuel. For example, if fish are fed excessive amounts of protein, compared to the amount of other dietary

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energy sources in the feed, an increasing fraction of protein will be deposited as fat (Ekmann et al., 2013), accompanied by an increase in nitrogen excretion. In addition, nitrogen excretion has been shown to vary in response to meal size (Muir and Niimi, 1972; Jobling and Davies, 1980; Soofiani and Hawkins, 1982; Fu et al., 2006), temperature (Frisk et al., 2013), or dissolved gas levels (Jordan and Steffensen, 2007; Skov et al., 2013).

Oxygen consumption (MO_2) measurements in fish serve as a proxy for overall energy expenditure (Frisk et al., 2013). Specifically, MO_2 during digestion is referred to as the specific dynamic action (SDA), and, in fishes, represents the increase in MO_2 above standard metabolic rate (SMR). The SDA response represents the summated costs of ingesting a meal, including feed handling, digestion, absorption and assimilation (Secor, 2009). Variables of the SDA response that are typically assessed are total SDA, peak MO_2, and the energetic equivalent of the SDA response in comparison with the dietary energy intake (SDA coefficient).

It is well documented for a multitude of species, that increasing ration size increases daily growth rates (Brett et al., 1969; Ahmed, 2007; Desai and Singh, 2009; Mizanur et al., 2014), although there is an upper limit to growth, beyond which excess feeding provides no further gain (Storebakken and Austreng, 1987). Ration size studies also commonly examine the effects on proximate composition, frequently demonstrating that higher ration sizes lead to increased lipid content of fish and reduced protein retention efficiency (PER) (Desai and Singh, 2009; Mizanur et al., 2014). Reduced PER and/or increased fat deposition may imply, that the fate of ingested protein N is either faecal or branchial excretion, yet no efforts have been made to identify which is the case in ration size studies, despite the pivotal role of nitrogen metabolism in the proper environmental and financial management of aquaculture (Wood, 2001).

Aquaculture production of tilapias reached 3.2 million tons in 2012 (FAO, 2013). With an annual growth of 12–15% during the past 2 decades, the production of this species has grown twice as fast as the average of global aquaculture production. The high resilience of tilapias to suboptimal water quality and the possibility of green water production, has probably facilitated its wide spread farming, from household subsistence farming to intensive production systems (Fitzsimmons, 2006). Despite this large production volume, questions pertaining to the feed requirements and nutrient utilization of tilapia remain unanswered. The aim of the present study was to investigate how meal sizes influence the efficiency of protein retention in Nile tilapia. This was achieved through a conventional growth study, supplemented by the determination of the digestibility coefficients for macronutrients. The magnitude of the SDA response was determined in order to quantify the proportion of dietary energy that was used to fuel the digestive processes, along with a determination of the amount of dissolved nitrogen waste excreted to the aquatic environment. There are numerous examples in the literature on how feed formulations or water quality parameters might influence feed intake (*e.g.* Frisk et al., 2013; Fu et al., 2005; Fu et al., 2006; Jordan and Steffensen, 2007). How meal size *per se* influences the efficiency with which its nutritional properties are utilized, are poorly understood.

2. Materials and methods

2.1. Fish and husbandry

Experiments were performed on a laboratory stock of Nile tilapia, *Oreochromis niloticus*, originally obtained as larvae from a commercial producer (Til-Aqua International, Velden, Netherlands). Fish were housed in a common aquarium facility at DTU Aqua, Technical University of Denmark until their time of use, maintained at 25 °C on a 12 h:12 h light cycle and fed daily with a commercial diet (Efico Alpha 830F, BioMar, France), containing 30% crude protein, 8% lipid, and 7.0% ash, with an energy content of 13.25 MJ kg^{-1}, and a DP:DE of 22.4.

2.2. Experimental procedures

Nutrient digestibility, growth, feed conversion, and post-prandial oxygen consumption and nitrogen excretion were assessed for groups of fish fed single meals corresponding to 1, 2, 3 or 4% of the tank biomass. Experiments were performed in a modified Guelph type system, consisting of 12 cylindro-conical tanks with a volume of 135 L each. Water supply to each tank was supplied from a central pump in a 1.5 m^3 aerated reservoir thermostatted at 25 °C. Approximately 20% of the total water volume in the system was replaced on a daily basis. The water inlet to each tank was controlled by an angle-seat valve, adjusted to deliver 100 L h^{-1} $tank^{-1}$. Continuous aeration was provided directly in each tank.

A total of 120 fish with an average body mass of 77.9 ± 1.7 g (mean tank range 74.7–86.9 g) were evenly distributed in the digestibility tanks. Fish were conditioned to the digestibility tanks for 7 feeding days. Ration sizes were randomly assigned to different tanks, and each tank was fed its experimental ration of 1, 2, 3 or 4% of the tank biomass. Following 7 days of feeding, feed was withheld for 24 h, fish were anaesthetised with a buffered solution of benzocaine (40 mg L^{-1}, Benzoak, ACD Pharmaceuticals, Leknes, Norway), weighed and returned to their respective tanks. All weighing procedures were always performed after fish had been fasted to avoid including any feed in the body mass determinations.

Digestibility studies were performed during the following 9 days. Fish were fed their respective ration sizes at 08:00 h (2 h after lights on), with feeding lasting approximately 20 min. Any uneaten feed was removed and quantified. Faeces were collected in settling columns immersed in an ice-slurry to prevent degradation, and settled faeces were collected daily prior to feeding and frozen at −20 °C until analysed. Faeces from each tank were pooled in 3 day periods as described previously (Dalsgaard and Pedersen, 2011). On day 10, fish were anaesthetised once again, weighed and returned to their tanks.

Feeding resumed the following day, and fish were allowed to recover from handling for 48 h before experiments were performed to determine nitrogen excretion and oxygen consumption following feeding. Experiments were performed on all 12 tanks, using 4 tanks at a time that included all feeding levels. On the day prior to feeding, each of the experimental tanks was scrubbed and ~50% of the water was replaced. Fibre optic oxygen sensors connected to a 4-channel optical oxygen meter (FireStingO2, Pyro Science GmbH, Aachen, Germany) were calibrated and mounted in the centre of each of the 4 tanks. A small mixing pump (Eheim Compact 300) was mounted in each tank to ensure mixing and a continuous flow of water across the sensor. A circular piece of bubble wrap was floated on the water surface to avoid oxygen diffusion, and the water supply to the tank was turned off. The aeration to each tank was switched to run in on:off intervals, consisting of a 45 min on period, followed by a 15 min off period. Oxygen saturation in each tank was logged every minute from 15:00 h, using an interfaced PC running the oxygen logger software supplied with the instrument. Background oxygen consumption measurements were performed in tanks with no fish at the end of the experiments. As this contributed <3% to fish MO_2, no corrections were performed.

In parallel with oxygen consumption measurements, ammonia and urea excretion rates following feeding were determined during meal digestion. For this purpose, 15 mL water samples were taken manually from each tank, starting immediately prior to feeding, and at 1, 2, 3, 6, 9, 12, and 24 h after feeding. Water samples were filtered (0.2 μm, Filtropur Sarstedt, Nümbrecht, Germany) and stored at −18 °C until analysed. TAN levels were measured periodically in all tanks. During the digestibility trial, TAN levels never exceeded 0.6 mg L^{-1}. During the TAN excretion experiments, TAN levels were allowed to reach 2.0 mg L^{-1}. These values are considered to be within safe limits for tilapia.