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Daily rhythms of locomotor activity, feeding behavior and dietary selection in Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Fish do not feed at any time of the day and on whatever food item they encounter in the wild, but they show daily rhythms of feeding activity and dietary selection. The aim of this research was to investigate the daily rhythms of behavior in Nile tilapia self-fed with plant-based diets supplemented with different levels of exogenous phytase (an enzyme that hydrolyzes non-digestible phytate and improves the nutritional value of the diet). To this end, ten males were individually kept in 50-L tanks, each equipped with two self-feeders and one infrared photo-cell connected to a computer. The selection factors investigated were the level of phytase supplementation (0 IU kg^{-1} vs 1500 IU kg^{-1}; 1500 IU kg^{-1} vs 4000 IU kg^{-1}) or sodium phytate (1% phytate vs 1% phytate + 1500 IU kg^{-1} phytase). The results revealed that 66.7% of total daily activity occurred during the day, while feeding was strictly nocturnal, with 93.0% of the daily food demands occurring at night. Tilapia preferred the diet with 1500 IU kg^{-1} phytase rather than the control or 4000 IU kg^{-1} diets. When exogenous sodium phytate was added to the diet, tilapia preferred the phytase diet. In conclusion tilapia self-feed at night (although locomotor activity was mostly diurnal) and chose plant-diets containing phytase, which should be taken into account when designing feeding strategies and practical diets for tilapia aquaculture.

1. Introduction

As most animals, fish feed rhythmically, consuming meals at specific times during the day or night. The fish circadian rhythm is, however, more flexible than that of higher vertebrates, and fish show inter-specific and even intra-specific differences in phasing (Madrid et al., 2001). Such high flexibility is exemplified by the dual phasing behavior of European sea bass, which shows both diurnal and nocturnal feeding rhythms (Sánchez-Vázquez et al., 1995). To further complicate things, different behavioral patterns (e.g. feeding and locomotor activities) may or may not match. For instance, goldfish display diurnal feeding but nocturnal activity (Sánchez-Vázquez et al., 1996). In the case of tilapia, this species has been described as strictly diurnal (Toguyeni et al., 1997), though a recent paper reported both males and females displayed looser daily activity patterns (Vera et al., 2009). The feeding rhythms of tilapia, however, have not yet been described and it has been assumed that they prefer to feed during the day.

Dietary selection has been extensively investigated in mammals, which reduce their intake of an imbalanced diet to avoid negative nutritional effects (Badman and Flier, 2005). Food selection is based on the premise that animals, including fish, possess "dietary wisdom" and thus select a diet that optimally restores a metabolic imbalance resulting from a nutritional challenge (Simpson and Raubenheimer, 2001). According to that hypothesis, the source of nutrients can be detected by gastrointestinal receptors during digestion, as they are released inside the stomach and then pass into the intestine. Those receptors would trigger signals (neural activity and hormones), that would inform brain centres about the nutritional properties of food and so modify feeding behavior (Vivas et al., 2003; Almaida-Pagan et al., 2006; Rubio et al., 2009). Apart from the "feed intake" approach widely used in animal nutrition, other methods can greatly improve the understanding of physiological mechanisms, such as the use of self-feeders in diet selection. Fish can learn to self-feed and select among diets, according to their nutritional requirements (Sánchez-Vázquez et al., 1998; Sánchez-Vázquez et al., 1999; Aranda et al., 2000; Vivas et al., 2006). Studies have examined food selection of diets with different ingredients, such as zinc in trout (Cuenca et al., 1993), methionine in European sea bass (Hidalgo et al., 1988), and feeding stimulants (Adron and Mackie, 1978) in trout. All these studies confirm that fish can discriminate among levels of the test substance in diets, and suggest that the self-selection feeding method is useful to determine nutrient requirements and to help develop quality feeds for fish.

The aim of the present work was to investigate the daily rhythms of locomotor and feeding activity, and to analyze whether Nile tilapia

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(*Oreochromis niloticus*) can detect and select among plant-based diets supplemented with different levels of exogenous phytase and phytate. Phytase is an enzyme proved capable that hydrolyze non-digestible phytate, can be added to plant-based diets to improve protein digestibility (Sugiura et al., 2001), bone mineralization (Apines et al., 2003), reduce phosphorus excretion (Sajjadi and Carter, 2004) and total phosphorus effluent (Ai et al., 2007) (reduce water pollution), improved calcium and phosphorus availability in Nile tilapia (Furuya et al., 2001).

2. Material and methods

2.1. Animals and housing conditions

Feeding trials were carried out in the fish laboratory at the University of Murcia (UMU), Murcia, Spain. Nile tilapia (*O. niloticus*) was provided by the Polytechnic University of Madrid, Madrid, Spain, from a mono-sex male population (male offspring, GMT®). Ten males, with an average initial body mass of 65 ± 3.3 g (mean ± S.E.M.), were housed individually in 50-L tanks (connected to a recirculation unit). The number of fish used in this work was in accordance with previous studies of diet selection and feeding patterns obtained using self-feeders (Sánchez-Vázquez et al., 1998; Sunuma et al., 2007; Vera et al., 2009). Each tank had two battery-powered self-feeders (EHEIM 3581, Germany) that delivered approximately 3 pellets of feed (about 0.12 g) each time a fish activated a string sensor located 0.5 cm below the water surface. A photo-switch (Omron, model E3S-AD62, Japan) connected to a computer was attached to the front of each aquarium, approximately 5.0 cm from the bottom. Tanks were kept indoors at a chronobiology lab with restricted access (to avoid disturbing fish behavior) and constant water temperature (30 ± 1 °C). The photoperiod was 12L:12D (lights on at 7:00 h). Water parameters were measured daily and maintained at appropriate levels (Kubitza, 2000).

2.2. Experimental diets

Before developing the experimental diets, a basal diet (diet D_0) with no sodium phytate or phytase was formulated to contain 35% crude protein and 9.4 MJ kg^{-1} digestible energy (Table 1). Subsequently, four more diets were developed containing only phytase at 1500 IU kg^{-1} (diet D_1); only phytase at 4000 IU kg^{-1} (diet D_2); only 1% sodium phytate (diet P_0) and, lastly, a diet containing sodium phytate (1%) and phytase (1500 IU kg^{-1}; diet P_1).

Regarding phytase concentration, 1 IU of phytase represented the amount of phytase that releases inorganic phosphorus from a 1.5 mM solution of sodium phytate at a rate of 1 μmol min^{-1} at pH 5.5 and 37 °C. The phytase was purchased as 5000 IU kg^{-1} (Natuphos®5000 EC 3.1.3.8, BASF-Germany) and sodium phytate ($C_6H_{18}0_{24}P_{6X}NA_YH_{2-}O$), was from Sigma-Aldrich™, USA. The phytase contained no additives that could affect its palatability for fish. To avoid enzyme denaturing during feed processing (Hughes and Soares, 1998), the phytase was diluted in 50 ml of distilled water and added to 1 kg of the basal diet with a spray and dried at room temperature. Phytate was added to 1 kg of the basal diet. The same treatments were applied to all other diets using distilled water only. The diets were made into 3 mm pellets using an electric pelleting machine, which consists basically of a mill that presses the wet diets (100 parts of ingredients with 30 parts of distilled water) through small holes in a die. The diets were stored at 4 °C. The digestible energy of the experimental diet was calculated according to Alanärä et al. (2001). Dietary moisture was determined by drying the samples for 24 h at 110 °C, crude protein was estimated by micro-Kjeldahl (Nx6.25), crude fat by diethyl ether extraction, ash by heating at 450 °C for 24 h and nitrogen free extract (NFE) as the remainder.

Table 1
Ingredients and chemical composition of the basal diet (%).

Ingredients	D_0	D_1	D_2	P_0	P_1
Fish meal	5.9	5.9	5.9	5.9	5.9
Soybean meal-51 (hamlet)	52.0	52.0	52.0	52.0	52.0
Wheat bran	19.3	19.3	19.3	19.3	19.3
Wheat	8.2	8.2	8.2	8.2	8.2
Soybean oil	11.8	11.8	11.8	11.8	11.8
Celite	2.0	2.0	2.0	2.0	2.0
Vit. and mineral premix[a]	0.8	0.8	0.8	0.8	0.8
Phytase (U kg^{-1})	–	1500	4000	–	1500
Sodium phytate	–	–	–	1.0	1.0
Calculated chemical composition					
Digestible energy (MJ kg^{-1})	9.4				
Dry matter	91.3				
Ash	7.8				
Crude protein	35.0				
Ether extract	15.0				

[a] Composition per kilogram of product: vitamin A — 1.000.000 IU; vitamin D_3 — 150.000 IU; vitamin E — 3.000 mg; vitamin K — 1.000 mg; vitamin B_1 — 1.600 mg; vitamin B_2 — 3.200 mg; vitamin B_6 — 3.200 mg; vitamin B_{12} — 2.400 mg; vitamin C — 15.000 IU; folic acid — 600 mg; panthotenic acid — 9.600 mg; biotin — 28.0 mg; niacin — 12.000 mg; $CuSO_4 \cdot 5H_2O$ — 1.0 g; $FeCO_3$ — 8.0 g; MnO_2 — 3.0 g; IK — 0.4 g.

2.3. Experimental design

The self-feeders were connected to a computer that continuously recorded the number of string activations (Sánchez-Vázquez et al., 1996). The photo-switch continuously emitted an infrared light beam, and every interruption caused by a fish was registered on a computer. During 30 days, fish were acclimated with a commercial diet (38.0% protein; 9.0% fat; 6.8% moisture; 7.0% ash) which were provided by self-feeders. After the adaptation period, fish were fasted for five days to minimise possible preferences for a particular, string sensor, be for providing the experimental diets.

Every day the feed remaining in the feeder was weighed and the feeder recipient refilled (phase I). After feed demand had stabilized, on day 6 feeds were crossed over between feeders (phase II). On day 18, the feeds were crossed over again (phase III). This procedure was used to ensure $D_0 \times D_1$ selection was based on the feed and not the feeder. The same procedure was used to test $D_1 \times D_2$ and $P_0 \times P_1$ selection, although only phases I and II were used. Fish underwent 48 h of fasting between each experimental phase to boost the active search for their preferred diet. The fish were weighed at the beginning and at the end of the experiment. Real feed intake was monitored by counting the number of uneaten food pellets every day and subtracting their weight from the total feed demanded. Activity rhythms were recorded for 55 days, after which an average daily waveform was calculated per fish.

2.4. Data analysis

Data were analyzed using chronobiology software (Temps, v.1, 179 by Dr. Díez Noguera, Barcelona), Microsoft Excel and SPSS. The Temps software provides actograms, which were double-plotted for convenient visualization. Data from the data-loggers was transferred to the computer and exported to Microsoft Excel for analysis and plotting. Feed consumption rates were expressed as g/100 g BW day^{-1} and the relative phytase selection was expressed as the percentage of diet. Results are expressed as the average of ten tanks, with the corresponding standard errors (S.E.M). Significant differences between average diet selection and the main effect between treatments were assessed using Student's *t*-test. The statistical significance was considered at ($p<0.05$).