Aquaculture 489 (2018) 28–35

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effect of constant digestible protein intake and varying digestible energy levels on energy and protein utilization in Nile tilapia

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ARTICLE INFO

Keywords:
Digestible energy
Digestible protein
Protein efficiency
Protein/Energy ratio
Energy utilization
Feed evaluation

ABSTRACT

In literature, the variability in the estimated optimal digestible protein to digestible energy ratio (DP/DE) is high. The present study aimed to estimate the optimal DP/DE ratio in Nile tilapia (*Oreochromis niloticus*) using different criteria (performance, energy and nitrogen balances parameters). Duplicate aquaria were randomly assigned to one of 16 diets. These diets covered a wide range of dietary DP/DE ratio (from 16.7 to 27 g MJ^{-1}). DP levels ranged between 36 and 50% and DE levels between 17.5 and 22 MJ kg^{-1}. Fish were fed restrictively based on a similar digestible protein amount at all 16 diets. Initial fish weight was 6.7 g. Broken line analysis showed that no optimal DP/DE ratio was present for Nile tilapia within the DP/DE ratio range studied. Regression analysis showed that growth declined as DP/DE ratio increased and seemed to level off at high DP/DE ratio (25 g MJ^{-1}). FCR ranged between 0.8 and 1.1 and increased linearly with increasing DP/DE ratio. Decreasing the DP/DE ratio resulted in a linear increase in protein efficiency to a highest value of 53%. However, protein efficiency did not show a plateau or a maximum value. Moreover, decreasing the DP/DE ratio resulted in a very high fat content of the fish (over 16%). In conclusion, an optimal DP/DE ratio in Nile tilapia being fed restrictively seems to be absent or to be below 16 g MJ^{-1}. A maximum protein deposition level is not present in 5–40 g Nile tilapia.

1. Introduction

The variability in dietary ingredient composition of fish feed is expected to increase, due to the limited availability of fishmeal and fish oil, the growth of the aquaculture sector and the increasing demand for the same ingredients for the production of biofuel and terrestrial animal feeds (Tacon et al., 2011). This increased variability will also coincide with a larger variability in ingredient and nutrient digestibility. The ratio between digestible protein to digestible energy (DP/DE) is important for formulating optimal fish diets (NRC, 2011). At very high dietary protein levels (high DP/DE ratios), part of the protein is catabolized for meeting the energy demands. In several fish species it is shown that increasing the dietary non-protein energy level in protein rich diets minimizes the amount of protein used as energy source, which increases the protein efficiency (i.e., the protein sparing effect) (Kaushik and de Oliva Teles, 1985; Kim and Kaushik, 1992). This protein sparing effect by reducing the DP/DE ratio also increases growth and reduces nitrogen excretion (Kaushik, 1998). However, at very low DP/DE ratios reducing the DP/DE ratio is expected to reduce growth and to have no impact anymore on protein efficiency. This effect is most likely dependent on the feeding method (ad lib vs restricted). Ali and Jauncey (2005) suggested that at too high dietary energy levels, growth is reduced due to a hampered feed intake. An alternative explanation for the reduced growth at low DP/DE ratios is that the maximal (genetic) potential for protein gain is reached. It has been demonstrated in pigs, that each animal has a maximal capacity (limit) for protein gain (Costa-Orvay et al., 2011). In fish, no maximal daily limit for protein deposition has been reported, expect for the study of Dumas et al. (2007) on rainbow trout. They reported that the increase in protein deposition potential with body weight diminished at higher body weights. This suggests that at high body weights rainbow trout has a maximum potential for protein retention.

Many studies have addressed the importance of the dietary protein to energy ratio, regarding its effect on growth and protein retention in various fish species (e.g., rainbow trout (*Oncorhynchus mykis*) (Kim and Kaushik, 1992; Lanari et al., 1995); African catfish (*Clarias gariepinus*) (Ali and Jauncey, 2005; Henken et al., 1986); Nile tilapia (*Oreochromis niloticus*) (Al Hafedh, 1999; Ali et al., 2008; El-Sayed and Teshima, 1992; Kaushik et al., 1995; Li et al., 2012; Shiau and Huang, 1990; Winfree and Stickney, 1981); Atlantic salmon (*Salmo salar*) (Einen and

Abbreviations: DP/DE, digestible protein to digestible energy ratio; BW, body weight; RE, retained energy; FCR, feed to gain ratio; PER, protein efficiency ratio

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https://doi.org/10.1016/j.aquaculture.2017.12.035
Received 16 December 2016; Received in revised form 17 December 2017; Accepted 20 December 2017

Roem, 1997; Hillestad and Johnsen, 1994); carp (*Cyprinus carpio*) (Watanabe et al., 1987); and gilthead seabream (*Sparus aurata*) (Lupatsch et al., 2001)). Comparison of the impacts of dietary DP/DE ratios between studies is difficult, because of the large between-study variability in factors like: fish weights; nutrient digestibility (diet quality: fishmeal vs. plant based diets); the selected criteria for estimating the optimal DP/DE ratio; feeding level (ad lib vs. restricted); experimental design (e.g., number of DP/DE levels); and the range of DP/DE ratios studied.

In the majority of aforementioned studies on dietary DP/DE ratios, fish were fed to satiation. Consequently, the impact of DP/DE ratio on growth (energy and nitrogen balances) are the combined effect of the protein sparing effect and the impact of dietary composition on feed intake. Despite the fact that most studies on dietary DP/DE ratio intend to estimate an optimum, often a limited amount of DP/DE levels were used (normally 4 to maximal 9 levels). Moreover, most studies apply a very narrow range of DP/DE ratios and seldom levels below 18 g kJ^{-1}. In few studies, an objective method for estimating the optimal DP/DE ratio was applied, like the broken line analysis (used by e.g., Booth et al., 2007). In Nile tilapia, broken line analysis have not been applied and limited information is available on the impact DP/DE ratios on the protein sparing effect especially at low levels.

In the current study, Nile tilapia (*Oreochromis niloticus*) were fed 16 diets with differing DP/DE ratios. Fish were fed restrictively equal amounts of digestible protein for studying the impact of DP/DE ratio on the protein sparing effect without interference of changes in voluntary feed intake. Under these conditions, we: 1) assessed the effect of DP/DE ratios on energy and nitrogen balances; 2) studied if Nile tilapia have a maximal protein deposition level; and 3) estimated the optimal DP/DE ratio by broken line analysis. We hypothesised that when fish have an equal protein intake, their protein retention and protein efficiency will show a maximum value when dietary DP/DE ratio decreases.

2. Materials and methods

2.1. Diets and feeding

Sixteen experimental diets were produced and randomly assigned to 32 aquaria, which were stocked with Nile tilapia (*Oreochromis niloticus*). Ten major ingredients were used to formulate these 16 diets (Table 1). These ingredients were included at different levels in these diets with the aim to create a large contrast in dietary DP/DE ratio, which was calculated on forehand to range between 14.6 and 26.3 mg kJ^{-1}. Based on the digestibility measurements, the realized range in DP/DE ratio between diets was 16.6 to 27.4 mg kJ^{-1}. The measured DP and DE contents of the experimental diets ranged from 364 to 483 g kg^{-1} and from17.5 and 22.2 MJ kg^{-1} on dry matter basis, respectively. Based on data obtained from older Nile tilapia (NRC, 1993; 2011), it was expect that the 6-g tilapia in the current study would have an optimal DP/DE ratio between 20 and 23 mg kJ^{-1}. The selected range of DP/DE ratios between the diets in this study were aimed to have the expected optimal in the middle of the selected range. The experimental diets were supplemented with synthetic amino acids, a premix and monocalcium phosphate in order to meet and to be above the essential nutrient requirement (amino acids, essential fatty acid, mineral and vitamins) as recommended for Nile tilapia by NRC (2011). The ingredient composition and analysed nutrient content of the 16 experimental diets is given in Supplemental Table 1.

Yttrium oxide (Y_2O_3) was added to both diets as an inert marker for digestibility measurements. The experimental feeds were extruded by BioMar TechCenter (Brande, Denmark). The pellet size was 2 mm.

Fish were fed restrictively twice daily at 09:00 and at 15:00. The daily feed intake at the diet with the lowest DP/DE ratio was aimed to be 90% of the expected satiation level of Nile tilapia. At all other diets, fish were fed the same amount of digestible nitrogen as the fish received at the lowest DP/DE diet. This resulted in decreasing levels of digestible energy intake between the diets with increasing DP/DE ratios (Fig. 1). This procedure was aimed at having equal DP intakes at all experimental diets. The calculated feed rations were based on the analysed crude protein content and predicted DP digestibility of all diets.

The daily feeding ration at the lowest DP/DE diet was calculated based on the mean initial fish weight, the feeding level of the treatment (in g kg$^{0.8}$ BW per d) and the expected growth of the fish. The daily growth of the feed ratio calculated was estimated from the expected feed to gain ratio (FCR).

Table 1
The mean, minimum (Min) and maximum (Max) inclusion level of ingredients in the 16 experimental diets (for the individual ingredient composition of each experimental diet see Supplemental Table 1).

Ingredients (%)	Inclusion level[b]			
	N[a]	Mean	Min	Max
Fishmeal	16	12.9	9.5	22.8
Rape cake	4	6.7	2.4	10.0
Full fat soybean	4	4.7	3.4	8.1
Defatted soybean meal	14	26.3	5.8	40.0
Soya protein concentration	15	23.2	3.8	30.0
Corn gluten	5	8.2	2.8	12.0
Sunflower meal	1	1.5	1.5	1.5
Wheat	16	25.6	15.6	33.7
Wheat gluten	7	4.5	2.8	7.0
Rapeseed oil	14	11.6	0.8	22.7
Premix	16	0.45	0.45	0.45
Methionine	16	0.31	0.10	0.40
Lysine	16	0.81	0.49	1.17
Threonine	16	0.18	0.02	0.29
Mono calcium phosphate	16	2.42	1.74	3.17
Yttrium oxide	16	0.10	0.10	0.10

[a] The number of diets in which the ingredient was included.
[b] The presented mean and minimum (Min) inclusion levels were calculated using only the diets in which the ingredients were added.

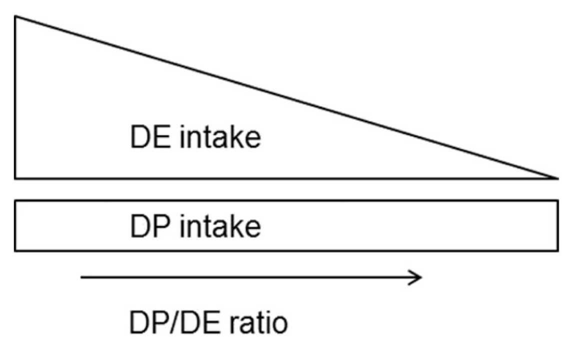

Fig. 1. Schematic illustration of the experimental setup. At the different experimental diets, fish were fed similar amounts level of digestible protein (DP) and varying amount of digestible energy (DE).

2.2. Fish and housing

Mixed sex of Nile tilapia (*Oreochromis niloticus*) were obtained from the brood stock of the aquatic research facility (CARUS) of Wageningen University. The experiment was approved by the Ethical Committee judging Animal Experiments of Wageningen University. The experiment was carried out according to the Dutch law on animal experiments. At the start of the experiment fish were randomly distributed over 32 tanks of 120 l. Per tank, 60 fish were stocked. All tanks were connected to the same recirculation system for maintaining proper water quality. This system comprised of: a common water reservoir; a lamella sedimentation unit for solids removal; a trickling filter for gas exchange and nitrification of NH_4^+. Water flow through each aquarium was kept constant at 7 l min^{-1} (except for the first week, when the