DETERMINING THE DAILY DIGESTIBLE PROTEIN INTAKE FOR NILE TILAPIA AT DIFFERENT GROWTH STAGES*

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ABSTRACT

This study aimed to estimate the utilization efficiency and optimal intake of digestible protein to maximize weight gain and feed conversion in Nile tilapia juveniles at different development stages. Four trials, each lasting 45 days, were performed using sexually inverted Nile tilapia juveniles with an initial average weight of 2.01 g (phase A), 14.26 g (phase B), 59.96 g (phase C), and 149.11 g (phase D). The experimental design was completely randomized with five treatments and four replicates. The levels of digestible protein ranged from 175 to 425 g kg^{-1} (phase A), 163 to 390 g kg^{-1} (phase B), 150 to 360 g kg^{-1} (phase C), and 138 to 330 g kg^{-1} (phase D). The maximum responses in terms of weight gain were obtained with protein intakes (digestible protein) of 88, 328, 713, and 855 mg $fish^{-1}$ day^{-1}, respectively, for phases A, B, C, and D. The maximum feed conversion ratio was obtained with protein intakes of 78, 272, 697, and 793 mg $fish^{-1}$ day^{-1}, respectively, for phases A, B, C, and D. The protein utilization efficiency was 52, 51, 51, and 50% for phases A, B, C, and D, respectively.

Keywords: Feed conversion; *Oreochromis niloticus;* protein deposition; weight gain

DETERMINAÇÃO DA INGESTÃO DIÁRIA DE PROTEÍNA DIGESTÍVEL PARA TILÁPIA-DO-NILO EM DIFERENTES FASES DE CRESCIMENTO

RESUMO

Objetivou-se estimar a eficiência de utilização e a ingestão ótima de proteína digestível para maximizar o ganho de peso e a conversão alimentar de juvenis de tilápia do Nilo em diferentes fases de crescimento. Cada um dos quatro ensaios durou 45 dias, utilizando-se juvenis revertidos sexualmente com peso médio inicial de 2,01g (fase A), 14,26 g (fase B), 59,96 g (fase C) e 149,11 g (fase D). O delineamento adotado foi o inteiramente ao acaso, com cinco tratamentos e quatro repetições. Os níveis de proteína digestível variaram de 175 a 425 g kg^{-1} (fase A), de 163 a 390 g kg^{-1} (fase B), de 150 a 360 g kg^{-1} (fase C) e de 138 a 330 g kg^{-1} (fase D). As máximas respostas para ganho de peso foram estimadas com ingestões de proteína de 88, 328, 713 e 855 mg $peixe^{-1}$ dia^{-1} nas fases A, B, C e D, respectivamente. Para maximizar a conversão alimentar estimaram-se ingestões de proteína de 78, 272, 697 e 793 mg $peixe^{-1}$ dia^{-1} para as fases A, B, C e D, respectivamente. As eficiências de utilização da proteína encontradas para as fases A, B, C e D, foram 52, 51, 51 e 50%, respectivamente.

Palavras-chave: conversão alimentar; *Oreochromis niloticus*; deposição de proteína; ganho de peso

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INTRODUCTION

The rapid growth of aquaculture in recent decades has intensified production systems, which have begun using high stocking densities and balanced diets with high nutritional quality (EL-SAYED and KAWANNA, 2008). The correct balance of these diets, especially in relation to the quantity of protein, can increase the nutrient use efficiency (PONTES *et al.*, 2010), reduce the cost of feed supply (HISANO *et al.*, 2015), and help reduce nitrogen excretion into the environment (BOTARO *et al.*, 2007; ABDEL-TAWWAB and AHMAD, 2009; FERNANDES JR *et al.*, 2016; BOSISIO *et al.*, 2017). Therefore, the search for diets with high nutritional value that are environmentally friendly and ensure high profitability depends upon increased knowledge on the species produced, especially in relation to feed management and meeting their nutritional requirements.

Several studies have aimed to determine the requirement of Nile tilapia for protein (BOTARO *et al.*, 2007; MEURER *et al.*, 2007; TRUNG *et al.*, 2011; OLIVEIRA *et al.*, 2014); however, the results obtained in those studies have been conflicting, with values ranging from 240 to 400 g kg^{-1}, depending on the stage of development. In addition, there have been few studies on the daily recommended requirement of protein in milligrams per fish (LIEBERT *et al.*, 2006; EL-DAHHAR, 2007).

Therefore, the objectives of this study were to determine the daily intake of digestible protein (DP) required to optimize the performance of Nile tilapia in different phases of development, and to determine the protein utilization efficiency.

MATERIAL AND METHODS

All the procedures used in the present study were approved by the Ethics Committee on Animal Use (CEUA), the Faculty of Agrarian and Veterinary Sciences, UNESP (Protocol No. 009999/14).

The study was divided into four developmental phases (A, B, C, and D), of 45-days duration each. Two-thousand genetically improved farmed Nile tilapia - GIFT (*Oreochromis niloticus*) juveniles were used in phase A (2.01 ± 0.29 g), 1200 in phase B (14.26 ± 0.26 g), 1000 in phase C (59.96 ± 9.60 g), and 1000 in phase D (149.11 ± 11.71 g).

The experimental design was completely randomized. The growth phases comprised five treatments, with increasing levels of DP (varying from 175 to 425 g kg^{-1} in phase A; 163 to 390 g kg^{-1} in phase B; 150 to 360 g kg^{-1} in phase C; and 138 to 330 g kg^{-1} in phase D), with four replicates.

The experimental diets were prepared using the "dilution technique", proposed by FISHER and MORRIS (1970). Two diets were formulated for each phase, one with a high protein content and the other, protein-free. The diet with the high protein content was formulated by maintaining the recommended levels of energy, minerals, vitamins, amino acids, and at least 1.3-times the requirement for DP, according to FURUYA (2010). The protein-free diet was formulated to contain the same levels of energy, minerals, and vitamins as the high- protein diet (Tables 1 and 2). The intermediate levels of digestible protein used in each phase were obtained by appropriately blending the two basal diets (high protein content diet and protein-free diet).

After dilution, the following results were obtained: D1 - diets meeting 50% of the digestible protein requirement; D2 – meeting 70%; D3 – meeting 100%; D4 - meeting 110%, and D5 – meeting 120% (LIEBERT, 2015). The proportions used are shown in Table 3. After diets were prepared for each test, both diets were mixed at the proper ratios and pelleted. The pellets were broken and classified according to each growth phase; phase A – from 1 to 2 mm; phase B – from 2 to 3 mm; phase C – from 3 to 4 mm, and phase D from 4 to 5 mm.

A closed recirculation system was used, comprising 20 rectangular tanks (2000 L) under controlled temperature. Water quality parameters, such as temperature (28.08 ± 1.53ºC), concentration of dissolved oxygen (6.00 ± 0.84 mg L^{-1}) and pH (6.54 ± 0.51), were monitored daily, whereas total alkalinity (46.66 ± 4, 72 mg L^{-1}), ammonia concentration (54.39 ± 36.18 µg L^{-1}), nitrate concentration (613.01 ± 24.41 µg L^{-1}), and nitrite concentration (21.38 ± 11.96 µg L^{-1}) were monitored weekly by a colorimetric method, and read using a spectrophotometer, in accordance with the method described by GOLTERMAN *et al*. (1978). The observed values were within the appropriate levels for fish farming (SIPAÚBA-TAVARES and SANTEIRO, 2013).

Table 1. Formulation (g kg^{-1}) of the high protein and protein-free diets used for Nile tilapia juveniles in each growth phase.

Ingredients	High protein diets				Protein-free diets			
	Phase A	Phase B	Phase C	Phase D	Phase A	Phase B	Phase C	Phase D
Soybean bran 48%	466.0	-	421.0	410.9	-	-	-	-
Soybean bran 45%	-	521.0	-	-	-	-	-	-
Corn gluten meal 60%	296.0	246.3	200.0	186.0	-	-	-	-
Wheat bran	100.0	88.3	-	59.4	-	-	-	-
Corn grain	54.3	53.8	15.62	151.6	-	-	-	-
The viscera meal	30.0	-	41.7	-	-	-	-	-
Rice bran	-	-	150.0	150.0	-	-	-	-
Fish viscera 55%	-	9.0	-	-	-	-	-	-
Rice husk	9.8	40.1	-	-	-	-	-	-
Starch	-	-	-	-	810.1	835.2	846.8	863.6
Soyabean oil	-	-	-	-	135.8	116.5	113.0	96.2
Dicalcium phosphate	26.8	24.9	12.2	17.4	40.5	35.1	27.6	27.6
Limestone	3.7	2.0	3.0	4.3	3.1	2.7	2.1	2.1
Minerals and vitamin premix[1]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
BHT[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
L-Lysine HCL	-	-	1.0	2.9	-	-	-	-
DL-Methionine	0.1	1.1	1.0	2.2	-	-	-	-
L-Threonine	1.8	2.3	2.5	3.7	-	-	-	-
L-Tryptophan	1.0	0.7	0.9	1.1	-	-	-	-

[1] *Mineral and vitamin premix (warranty levels* kg^{-1} *of product): moisture = 2%; iron = 25,714 mg; copper = 1,960 mg; manganese = 13,345 mg; iodo = 939 mg; selenium = 30 mg; sulfur = 1.16%; choline = 71.64%; vitamin A = 600,000 IU; vitamin D3 = 600,000 IU; vitamin E = 12,000 mg; vitamin K3 = 631 mg; vitamin B1 = 1,176 mg; vitamin B2 = 1,536 mg; vitamin B6 = 1,274 mg; vitamin B12 = 4,000 mg; folic acid = 192 mg; vitamin B3 = 3,920 mg; vitamin C = 40,250 mg; biotin = 20 mg and niacin = 19,800 mg.* [2]*Antioxidant (BHT) = butylhydroxy toluene.*

The fish were fed to apparent satiety four times each day. Performance was evaluated by assessing weight gain (WG), feed conversion (FCR), specific growth rate (SGR), and DP intake (DPI). Protein deposition (PD) was determined through a slaughter comparative study of a sample (10% of each experimental unit) at the beginning and end of each test. The animals were euthanized after 24 h of fasting by dipping in an anesthetic solution containing benzocaine. Fish were then stored in a freezer at -20°C, ground, and dried in an oven at 55°C. The samples were crushed in a ball mill, and sent to the laboratory for protein and dry matter analyses (AOAC, 2016). PD was calculated as follows:

$$PD(\%) = 100 \times \frac{(Final\ body\ weight\ \times Final\ body\ protein - Initial\ body\ weight\ \times Initial\ body\ protein)}{(Total\ feed\ intake\ \times dietary\ protein)}$$

Table 2. Composition (g kg-1) of the high protein and protein-free diets used for Nile tilapia juveniles at each growth phase.

Energy and other nutrients[1]	High protein diets				Protein-free diets			
	Phase A	Phase B	Phase C	Phase D	Phase A	Phase B	Phase C	Phase D
Digestible protein	420	390	360	330	-	-	-	-
Digestible energy	3200	3100	3100	3000	3200	3100	3100	3000
Crude fiber	45	58	46	50	-	-	-	-
Fat	26	22	44	40	135.8	116.5	113,0	96.2
Calcium	11	10	8	8	11	10	8	8
Available phosphorus	8	7	5	5	8	7	5	5
Dry matter	898	897	892	891	916.6	914.0	912.8	911.0
Mineral matter	45	47	52	48	11.5	11.6	11.8	11.9
Arginine	25.9	25.2	23.4	20.8	-	-	-	-
Histidine	12.1	11.5	10.3	9.3	-	-	-	-
Isoleucine	19.0	18.3	16.2	14.8	-	-	-	-
Leucine	53.7	48.7	43.0	39.3	-	-	-	-
Lysine	17.6	17.8	16.6	15.8	-	-	-	-
Methionine	9.7	9.6	9.1	9.1	-	-	-	-
Phenylalanine	29.0	26.7	23.9	21.5	-	-	-	-
Threonine	18.3	18.1	17.0	16.7	-	-	-	-
Tryptophan	4.7	4.6	4.0	3.9	-	-	-	-
Valine	21.4	20.2	18.6	16.7	-	-	-	-

[1]*Composition calculated as described by FURUYA (2010).*

Table 3. Proportions of the high protein and protein-free diets and calculated concentration of digestible protein used for each growth phase.

Diets	Phase A			Phase B			Phase C			Phase D		
	HPD[1]	PFD[2]	DP[3]	HPD	PFD	DP	HPD	PFD	DP	HPD	PFD	DP
	%	%	g kg-1	%	%	g kg-1	%	%	g kg-1	%	%	g kg-1
D1	41.7	58.3	175	41.8	58.2	163	41.7	58.3	150	41.8	58.2	138
D2	58.3	41.7	245	58.5	41.5	228	58.3	41.7	210	58.5	41.5	193
D3	83.3	16.7	350	83.3	16.7	325	83.3	16.7	300	83.3	16.7	275
D4	91.7	8.3	385	91.8	8.2	358	91.7	8.3	330	91.8	8.2	303
D5	100	0	420	100	0	390	100	0	360	100	0	330

[1]*HPD - high protein diets;* [2]*PFD - protein-free diets;* [3]*DP - levels of digestible protein (g kg-1).*

Performance data were analyzed by the SAS program (2008), using analysis of variance (ANOVA). When statistical significance was observed, Duncan test ($P < 0.05$) was applied to compare means. The broken line model was adjusted according to the procedures described by ROBBINS *et al*. (2006). The DPI was obtained by meeting the ascending line with a plateau. To verify the adjustment of the obtained equations, the coefficient of determination (R^2 = SQ model / SQ total) was taken into account.

The results for the PD of fish were submitted to linear regression analysis, considering the DPI corrected for maintenance (cDPI) as an independent variable in the model broken line

(ROBBINS *et al.*, 2006). The cDPI was calculated as follows:

$$cDPI = DPI\ (b \times PC^{0.67});$$

where: b = 437.5 mg, parameter used to estimate the requirement of DP for maintenance obtained from LIEBERT *et al.* (2006). PC = body weight.

The efficiency of protein utilization (*k*) was obtained by the relationship between the parameters L and R generated by the "broken line" model when adjusted to the PD and the cDPI, and *k* was calculated by the following equation:

$$k = (L\ /\ R) \times 100.$$

The results obtained for WG (mg $fish^{-1}$ day^{-1}) and FCR per day were submitted to linear regression analysis, considering the DPI (mg $fish^{-1}$ day^{-1}) as the independent variable, by the broken line mathematical model (ROBBINS *et al.*, 2006):

$$Y = L + U \times (R - X),$$

where: Y and X are the model variables and L, U, and R are model parameters; L (maximum model response), U (slope of the line), and R (intake for maximum response). The estimates of this function are valid if $X < R$; when $X \geq R$, then $Y = L$.

RESULTS

The level of DP in the diet significantly affected the growth, utilization, and protein intake of tilapia in all phases of development (Table 4). The recommended level of digestible dietary protein for Nile tilapia, considering weight gain, were 350, 325, 300, and 275 g kg^{-1} for phases A, B, C, and D, respectively.

In phase A, the level of DP increased to 350 g kg^{-1}, increasing the WG and reducing the FCR, with no difference observed between the fish fed 385 and 425 g kg^{-1} DP. The SGR responded positively to increased levels of DP up to 385 g kg^{-1}, with the intake and PD being proportional to the increased protein level (Table 4). In phase B, the increase in the level of DP up to 325 g kg^{-1} increased weight gain, feed intake, SGR, and reduced the average FCR. The intake of protein and SGR were proportional to the increase in protein (Table 4).

In phases C and D, when the levels of DP were increased to 300 and 275 g kg^{-1}, respectively, the WG, SGR, and PD also increased, and the average FCR was reduced, since the intake of protein was proportional to the increase in protein level. In phase D, the feed intake was inversely proportional to the increased level of protein (Table 4). Differences in DP intake were observed in all phases for all weight groups, with smaller WG and higher FCR reported for fish fed with diets containing lower levels of DP. The SGR values also declined when fish were fed with diets containing lower levels of DP in each growth phase. The results of the variables in question also reduced linearly with the increase in fish size. In the present study, the SGR values ranged from 0.8 to 3.9% per day for the fishes that received the D1 and D2 diets in all stages of growth.

The results for PD (mg $fish^{-1}$ day^{-1}), adjusted in line with the cDPI (mg $fish^{-1}$ day^{-1}) by the broken line mathematical model to the four phases of development (A, B, C, and D), are presented in Figure 1. The maximum estimated response (L) by mathematical model broken line for PD was 50, 223, 370, and 390 mg $fish^{-1}$ day^{-1}, with cDPI (R) of 96, 434, 722, and 779 mg $fish^{-1}$ day^{-1} for phases A, B, C, and D, respectively. The efficiencies of protein utilization (k) were 0.52 (k = 50/96), 0.51 (k = 223/434), 0.51 (k = 370/722), and 0.50 (k = 390/779) for growth phases A, B, C, and D, respectively.

The maximum response predicted by the mathematical model broken line for WG was estimated at 265, 1085, 1894, and 1966 mg $fish^{-1}$ day^{-1} with a DPI of 88, 328, 713, and 855 mg $fish^{-1}$ day^{-1} for growth phases A, B, C, and D, respectively. Through the respective equations, the maximum estimated response for FCR was calculated at 0.999, 1.048, 1.485, and 1.542 mg $fish^{-1}$ day^{-1}, with an PD intake of 78, 272, 697, and 793 mg $fish^{-1}$ day^{-1} for growth phases A, B, C, and D, respectively (Table 5).

With increasing levels of DP in the diet, a linear increase in the PD was observed at different stages of Nile tilapia growth. Thus, by adjusting the model broken line, the appropriate DPI to maximize PD was estimated at 96, 434, 722, and 779 mg fish^{-1} day^{-1} in phases A, B, C, and D, respectively. The protein utilization efficiency observed in this study i.e., 52, 51, 51, and 50% for groups A, B, C, and D, respectively, were obtained with the responses within the range where protein was not a limiting factor.

Table 4. Performance of Nile tilapia juveniles fed different levels of digestible protein (DP) during developmental stages A, B, C, and D. IW = initial weight; WG = weight gain; FC = feed consumption; FCR = feeding conversion ratio; SGR = specific growth rate; IDP = ingestion of digestible protein, PD = protein deposition.

Diets (gDP kg^{-1})	IW (g)	WG (g)	FC (g)	FCR	SGR (% day^{-1})	IDP (g)	PD (g)
Phase A							
D1 - 175	2.01 ± 0.12	7.70 ± 0.26^{c}	11.87 ± 0.14	1.54 ± 0.04^{c}	3.50 ± 0.06^{d}	2.08 ± 0.03^{e}	1.30 ± 0.04^{d}
D2 - 245	2.05 ± 0.10	9.50 ± 0.21^{b}	11.77 ± 0.22	1.24 ± 0.04^{b}	3.88 ± 0.04^{c}	2.88 ± 0.06^{d}	1.65 ± 0.04^{c}
D3 - 350	2.09 ± 0.11	11.52 ± 0.24^{a}	11.61 ± 0.35	1.01 ± 0.01^{a}	4.24 ± 0.04^{b}	4.06 ± 0.12^{c}	2.15 ± 0.10^{b}
D4 - 385	2.04 ± 0.12	12.35 ± 0.79^{a}	12.00 ± 0.50	0.97 ± 0.03^{a}	4.37 ± 0.12^{a}	4.61 ± 0.19^{b}	2.37 ± 0.05^{a}
D5 - 420	2.05 ± 0.13	11.49 ± 0.35^{a}	11.69 ± 0.18	1.02 ± 0.04^{a}	4.23 ± 0.06^{b}	4.91±0.08^{a}	2.24 ± .17^{ab}
Protein	*p-value*	<0.0001	0.4465	<0.0001	<0.0001	<0.0001	<0.0001
	CV	4.05	2.60	2.74	1.77	2.05	4.87
Phase B							
D1 - 163	14.26 ± 0.31	29.17 ± 0.84^{c}	50.32 ± 1.22^{b}	1.73 ± 0.06^{d}	2.47 ± 0.04^{c}	8.18 ± 0.20^{d}	5.00 ± 0.23^{d}
D2 - 228	14.20 ± 0.27	36.87 ± 1.43^{b}	47.19 ± 0.96^{c}	1.28 ± 0.08^{c}	2.84 ± 0.06^{b}	10.73 ± 0.22^{c}	6.60 ± 0.39^{c}
D3 - 325	14.29 ± 0.43	47.99 ± 1.45^{a}	53.44 ± 1.07^{a}	1.11 ± 0.05^{b}	3.27 ± 0.05^{a}	17.37 ± 0.35^{b}	8.79 ± 0.28^{b}
D4 - 358	14.25 ± 0.39	47.97 ± 1.56^{a}	49.30 ± 0.73^{b}	1.03 ± 0.03^{a}	3.27 ± 0.06^{a}	17.63 ± 0.26^{b}	9.39 ± 0.67^{b}
D5 - 390	14.28 ± 0.28	50.47 ± 2.30^{a}	50.41 ± 0.76^{b}	1.00 ± 0.05^{a}	3.36 ± 0.08^{a}	19.66 ± 0.30^{a}	10.09 ± 0.32^{a}
Protein	*p-value*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	CV	3.74	1.93	4.49	1.96	1.84	5.12
Phase C							
D1 - 150	59.89 ± 1.32	57.20 ± 4.28^{c}	122.36 ± 4.43	2.15 ± 0.20^{c}	1.51 ± 0.23^{c}	18.35 ± 0.66^{d}	10.00 ± 0.36^{c}
D2 - 210	59.19 ± 1.48	76.91 ± 7.67^{b}	131.21 ± 11.24	1.71 ± 0.05^{b}	1.85 ± 0.16^{b}	27.55 ± 2.36^{c}	14.30 ± 1.21^{b}
D3 - 300	59.50 ± 1.10	87.76 ± 5.34^{a}	131.27 ± 3.83	1.50 ± 0.06^{a}	2.02 ± 0.17^{a}	39.38 ± 1.15^{b}	16.51 ± 1.12^{a}
D4 - 330	59.73 ± 1.35	82.94 ± 1.93^{ab}	121.92 ± 6.71	1.47 ± 0.09^{a}	1.95 ± 0.19^{ab}	40.23 ± 2.21^{b}	16.71 ± 0.38^{a}
D5 - 360	59.42 ± 1.28	84.94 ± 2.93^{a}	127.06 ± 13.01	1.50 ± 0.19^{a}	1.98 ± 0.19^{a}	45.74 ± 4.68^{a}	16.79 ± 2.03^{a}
Protein	*p-value*	<0.0001	0.1442	<0.0001	<0.0001	<0.0001	<0.0001
	CV	6.23	4.96	6.17	4.01	7.45	8.26
Phase D							
D1 - 138	149.11 ± 2.98	63.55 ± 7.67^{b}	149.59 ± 7.22^{a}	2.37 ± 0.23^{c}	0.79 ± 0.13^{b}	20.57 ± 0.99^{e}	11.70 ± 2.67^{b}
D2 - 193	149.54 ± 2.23	69.64 ± 6.90^{b}	142.20 ± 5.93^{ab}	2.06 ± 0.26^{b}	0.86 ± 0.11^{b}	27.37 ± 1.14^{d}	13.70 ± 2.49^{b}
D3 - 275	149.23 ± 2.78	86.92 ± 2.42^{a}	133.45 ± 7.33^{b}	1.54 ± 0.11^{a}	1.02 ± 0.08^{a}	36.70 ± 2.01^{c}	18.35 ± 0.89^{a}
D4 - 303	149.18 ± 2.26	87.27 ± 5.97^{a}	136.24 ± 4.18^{b}	1.57 ± 0.12^{a}	1.03 ± 0.07^{a}	41.21 ± 1.26^{b}	17.94 ± 1.32^{a}
D5 - 330	149.32 ± 2.56	89.66 ± 4.92^{a}	133.82 ± 4.36^{b}	1.49 ± 0.04^{a}	1.05 ± 0.10^{a}	44.16 ± 1.44^{a}	17.01 ± 1.40^{a}
Protein	*p-value*	<0.0001	0.0106	<0.0001	<0.0001	<0.0001	<0.0001
	CV	6.66	4.43	8.79	5.24	4.31	10.39

CV – coefficient of variation (%). Values followed by the same letter in the columns do not differ by Duncan test at 5% probability for different levels of DP within the same phase of Nile tilapia development.

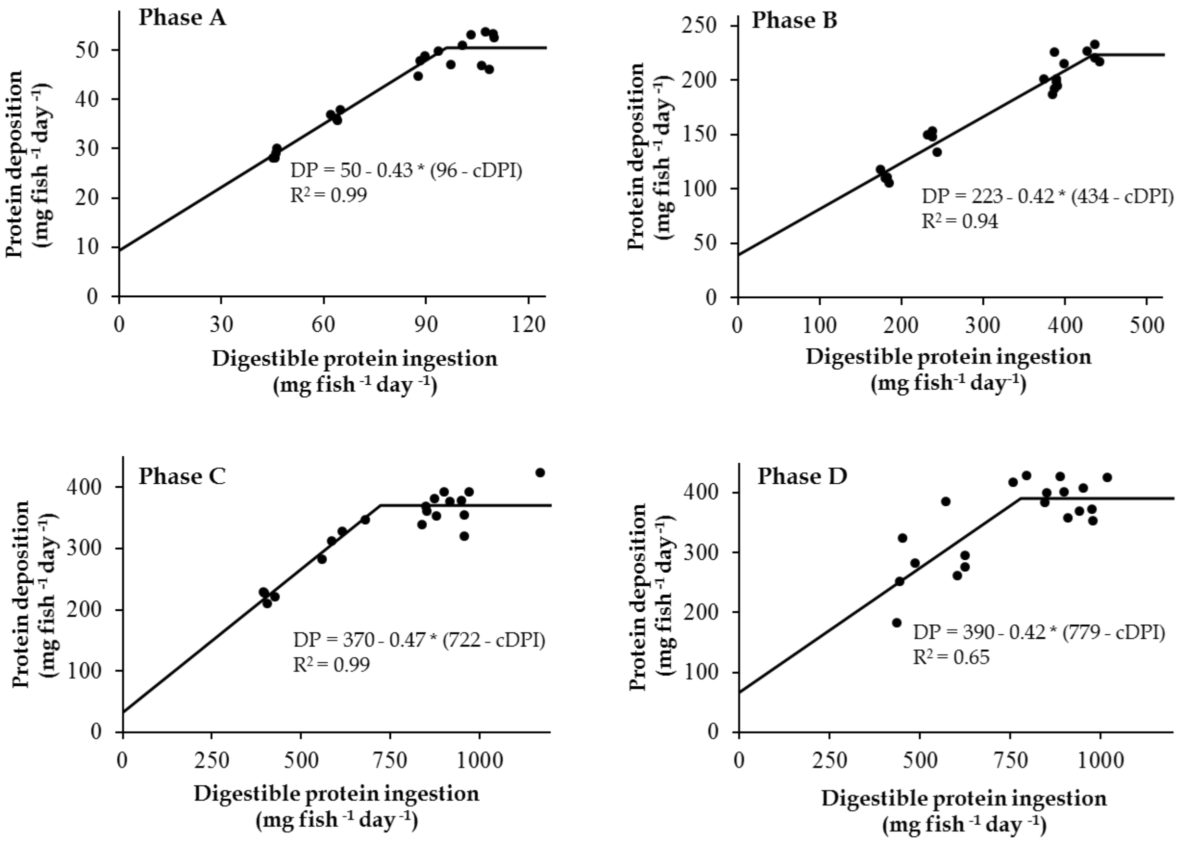

Figure 1. Graphical representation of the broken line mathematical function for Nile tilapia body protein deposition in growth phases A, B, C, and D receiving increasing levels of digestible protein (DP). (•) Fish response to diets (D1 and D5 in growth stages A, B, C, and D).

Table 5. Equations with the broken line model for the productive performance variables (weight gain and feed conversion ratio) in Nile tilapia fed diets containing different levels of digestible protein.

Variable		**Optimum level of digestible protein** (mg fish^{-1} day^{-1})	**R²**
Phase A			
Weight gain (mg fish^{-1} day^{-1})	Y = 265 – 2.22 (88 – X)	88	0.97
Feed conversion ratio	Y = 990 + 17 (78 – X)	78	0.98
Phase B			
Weight gain (mg fish^{-1} day^{-1})	Y = 1085 – 3 (328 – X)	328	0.99
Feed conversion ratio	Y = 1040 + 7 (272 – X)	272	0.93
Phase C			
Weight gain (mg fish^{-1} day^{-1})	Y = 1894 – 2.04 (713 – X)	713	0.98
Feed conversion ratio	Y = 1480 + 2 (697 – X)	697	0.85
Phase D			
Weight gain (mg fish^{-1} day^{-1})	Y = 1966 – 1.47 (855 – X)	855	0.98
Feed conversion ratio	Y = 1540 + 3 (793 – X)	793	0.80

DISCUSSION

The recommended digestible dietary protein required for Nile tilapia, considering weight gain, are close to those reported by ABDEL-TAWWAB *et al*. (2010), who determined the optimum value of 450 g kg^{-1} crude protein for tilapia, with 0.5 g and 350 g kg^{-1} for tilapia fish with 17 to 43 g of live body weight. EL-SAYED and TESHIMA (1991) found that dietary protein requirements decreased with increasing fish weight and age, and ranged from 400 to 450 g kg^{-1} for fish weighing less than a 1 g, and declined to 300 g kg^{-1} CP for fish weighing 46–260 g.

The reduced DP intake of fish fed diets with low levels of DP hindered optimal growth of animals, because the high demand of amino acids necessary for protein biosynthesis is indispensable for the deposition of tissues and subsequent weight gain (NRC, 2011; SILVA, 2014). The highest values of FCR for juvenile tilapia were also observed in animals fed diets with low levels of DP. These results are close to those found by FURUYA *et al*. (2000) and MEURER *et al*. (2007) (1.4 and 2.4, respectively). This can be explained by the deficiency of essential amino acids and excess digestible energy in relation to PD favoring the imbalance in the diet and consequently reducing weight gain (SILVA, 2014).

The results of this study more accurately describe the protein requirements during different phases of growth, since possible variations within the limits of 138 to 425 g kg^{-1} DP were tested. Variations in the requirements for crude protein associated with fish weights can be attributed to differences in the requirement of proteins in different weight classes (ABDEL-TAWWAB and AHMAD, 2009). EL-SAYED and TESHIMA (1991) concluded that the dietary protein requirement decreases with an increase in of the weight and age of fish, both for fry and juveniles of Nile tilapia.

It was also possible to confirm that reduced DP in the diets directly affected the PD of fish, and this was independent of the size of the fish that ingested the D1 and D2 diets with the lowest values of PD. Similar data were observed by MEURER *et al.* (2007), who observed that Nile tilapia weighing 10 to 30 g, fed with DP levels less than 200 g kg^{-1}, had a lower PD (16.07%) compared with those fed with higher levels of DP (300 g kg^{-1}), that presented a PD of 17.81%. Protein intake levels interfere with the maintenance of whole body total protein and with protein synthesis and degradation (MOREIRA and POZZA, 2014). Linear relationships also exist between the ingestion of protein, growth, synthesis, capacity of protein synthesis and RNA activity (MENTE *et al*., 2011). Increased protein intake, which occurs with diets containing high levels of protein, led to an increase in the concentration of RNA (capacity of protein synthesis) and/or activity of RNA and alteration in the equilibrium between the synthesis and degradation of protein. Thus, diets containing high protein levels led to the production of fishes with high body protein. (MOREIRA and POZZA, 2014).

Similar results regarding protein utilization efficiency were observed by FURUYA *et al*. (2005) and GONÇALVES *et al*. (2009), who worked with Nile tilapia from a Thai lineage, and reported values of 519.7 and 506.8 g kg^{-1}, with average weight ranging from 5 to 125 g, and from 30 to 194 g, respectively. The results of the present study showed that protein utilization efficiency was very close between the different stages, showing that protein utilization in Nile tilapia is similar in all phases of growth, indicating that this species uses protein in the same way throughout its development. Thus, a single value for protein efficiency (51%) is suitable for use in the growth of Nile tilapia, as recommended by TRUNG *et al*. (2011).

Although several studies have been performed to determine the requirement of crude protein by tilapias (FURUYA *et al*., 2000; BOTARO *et al*., 2007), the proper levels required in the diet are not sufficient when consumption is not considered. Thus, protein intake expressed as an absolute value of the body mass of the fish, or weight gain, and then as a percentage of feed will provide a clearer understanding of the efficiency of the species studied (GLENCROSS *et al*., 2011; LUPATSCH, 2012).

CONCLUSION

The responses obtained from the broken line mathematical model determine recommendations to optimize the performance of Nile tilapia in different stages of growth. To maximize the animals WG and feed conversion, the recommended daily intake of protein is 88 and 78 mg $fish^{-1}$ day^{-1} (for fish weighting between 2.01 and 14.36 g), 328 and 272 mg $fish^{-1}$ day^{-1} (14.26 to 64.73 g), 713 and 697 mg $fish^{-1}$ day^{-1} (59.96 to 147.72 g), and 855 and 793 mg $fish^{-1}$ day^{-1} (149.11 to 238.77 g), respectively. Growing Nile tilapia (2 to 250 g) have a protein utilization efficiency of 51%.

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