





Effects of mixed feeding strategies with different dietary energy: protein ratios on juvenile Nile tilapia (*Oreochromis niloticus*)

Efeitos de estratégias de alimentação mista com diferentes relações de energia:proteína para juvenis de tilápia-do-Nilo (*Oreochromis niloticus*)

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Abstract

The evaluation of feeding strategies is necessary to ensure the sustainability of aquaculture. This study assessed the effect of two diets with different E:P ratios (9.6 and 10.3 kcal of digestible energy per gram of crude protein) on Nile tilapia juveniles. The growth, feed and nutrient use, economic parameters, whole-body composition, and liver steatosis of fish were evaluated. There was no significant effect of treatments on the growth, feed intake, feed conversion ratio, uniformity, and survival of the fish. The lower feed cost ($P>0.05$) per biomass or 1000 units produced was registered in Nile tilapia juveniles fed with 10.3 kcal DE/g CP diet for seven days. The contribution of ether extract in fish weight gain was reduced ($P=0.055$) by the increased use of the 10.3 kcal DE/g CP diet in the feeding strategies. The same trend was observed in fish whole-body lipid levels. Body indexes were similar ($P>0.05$) among fish from the different treatments. Mixing diets with different E:P ratios in a weekly feeding protocol does not impair productive performance of Nile tilapia juveniles. However, considering the cost of feeding, the recommendation is to supply a diet with 33% CP and 3.4 kcal/DE for seven days per week.

Keywords: feeding management; productive performance; fish nutrition; aquaculture

Resumo

A avaliação das estratégias de alimentação é necessária para garantir a sustentabilidade da aquicultura. Este estudo avaliou o efeito de duas dietas com diferentes proporções de E:P (9,6 e 10,3 kcal de energia digestível por grama de proteína bruta) para juvenis de tilápia do Nilo. Foram avaliados o crescimento, uso da dieta e nutrientes, parâmetros econômicos, composição corporal e esteatose hepática de peixes. Não houve efeito significativo dos tratamentos sobre o crescimento, consumo de ração, conversão alimentar, uniformidade e sobrevivência dos peixes. O menor custo de ração ($P>0,05$) por biomassa ou 1000 unidades produzidas foi registrado nos juvenis de tilápia do Nilo alimentados com a dieta 10,3 kcal DE/g PB por sete dias. A contribuição do extrato etéreo no ganho de peso dos peixes foi reduzida ($P=0,055$) pela maior utilização da dieta 10,3 kcal DE/g PB nas estratégias de alimentação utilizadas. A mesma tendência foi observada nos níveis de lipídios corporais em peixes. Os índices corporais foram semelhantes ($P>0,05$) entre os peixes dos diferentes tratamentos. A mistura de dietas com diferentes relações E:P em um protocolo de alimentação semanal não prejudica o desempenho produtivo de juvenis de tilápia do Nilo. No entanto, considerando os custos de alimentação, a recomendação é fornecer uma dieta com 33% PB e 3,4 kcal/DE por sete dias por semana.

Palavras-chave: manejo alimentar; desempenho produtivo; nutrição de peixes; aquicultura

1. Introduction

Industrialized diets are the main source of nutrients for fish, comprising up to 40-60% of the total cost of intensive tilapia production⁽¹⁾. Thus, adequate feed management is essential to ensure economic and environmental sustainability of aquaculture^(2,3). Usually, fish farmers feed the early stages of Nile tilapia daily to meet higher growth rates due to the higher nutritional requirements in this phase⁽⁴⁾. However, warm-water fish show lower feed intake and feed efficiency when water temperatures are below the range of optimal growth.

Therefore, in regions where seasonality influences water temperature, specific feeding strategies may be necessary to improve the efficiency of Nile tilapia production systems.

In this context, feeding strategies based on periods of feed restriction and refeeding to stimulate compensatory growth responses have been previously evaluated for different species of aquatic organisms^(5,6,7,8,9). However, compensatory growth responses in fish are not necessarily achieved after a period of complete and severe feeding restriction. In fact, the nutritional profile of

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the diets can regulate the compensatory response in fish, as previously observed in juveniles of olive flounder (*Paralichthys olivaceus*)⁽¹⁰⁾, channel catfish (*Ictalurus punctatus*)⁽¹¹⁾, common carp (*Cyprinus carpio*)⁽¹²⁾ and yellow catfish (*Pelteobagrus fulvidraco*)⁽¹³⁾ due to changes in dietary energy:protein ratio (E:P). The E:P is a relevant factor for the manufacture of sustainable aquafeeds, since high E:P ratios can reduce fish feed intake, limiting the amount of nutrients available to meet their nutritional requirements. On the other hand, lower E:P ratios increase the use of catabolized protein for energy purposes, and increase nitrogen excretion, which negatively affects the water quality⁽¹⁴⁾.

Although the best E:P for Nile tilapia has already been established in other studies^(14, 15), little is known about how diets with different E:P can influence the compensatory growth response of fish species⁽¹⁶⁾. Therefore, this study aimed to evaluate the effects of varying E:P ratios associated with different feeding strategies on the growth, feed and nutrient use, economic parameters, whole-body composition, and liver steatosis of juvenile Nile tilapia.

2. Material and methods

All procedures performed in this study were previously approved by the Ethics Committee on Animal Use of the Federal University of Paraná, Palotina Sector (Protocol #15/2020).

2.1 Treatments and experimental design

Two isonitrogenous practical feeds (33% crude protein – CP) for omnivorous fish with different digestible energy (DE) levels were used in trial. Diet 1 (D1) contained 3.2 kcal DE.g⁻¹, while Diet 2 (D2) contained 3.4 kcal DE.g⁻¹. The complete chemical composition of the diets is described in Table 1.

Table 1. Chemical composition (wet basis) of the experimental diets

Chemical composition	Diet 1	Diet 2
Moisture (%)	9.67	9.55
Crude protein (%)	32.95	33.10
Ether extract (%)	3.23	3.38
Crude fiber (%)	1.03	0.74
Ash (%)	10.60	9.65
Digestible energy (kcal.g ⁻¹)*	3.2	3.4
Gross energy (kcal.g ⁻¹)	4.3	4.5
DE:CP ratio (kcal.g ⁻¹)	9.6	10.3
GE:CP ratio (kcal.g ⁻¹)	12.9	13.6

* Values informed by the manufacturer

The treatments combined different experimental diets with varying times of feeding:

- Treatment 7D1: fish were fed D1 for seven days.
- Treatment 7D2: fish were fed D2 for seven days.
- Treatment 5D2-2D1: fish were cyclically fed D2 for five days, and D1 for two consecutive days.
- Treatment 4D2-3D1: fish were cyclically fed D2 for four days, and D1 for three consecutive days.

The experiment was conducted as a completely randomized design with six replicates per treatment.

2.2 Fish and laboratorial conditions

Nile tilapia (*Oreochromis niloticus*) juveniles were purchased from a commercial fish farm (Piscicultura Luciana Peretti, Palotina, Paraná, Brazil) and adapted to laboratorial conditions for 45 days. Fish were kept in circular tanks (1000L) equipped with biological filters and supplementary aeration. The water in the tanks was partially replaced (1/3 of the volume) on a daily basis. During this period, the water quality parameters were monitored daily: ammonia (0.039 mg.L⁻¹), nitrite (0.5 mg.L⁻¹), and pH (7.5) using commercial kits (LabconTest®); and dissolved oxygen (5.35 mg.L⁻¹) with a multiparameter probe (Akso®, model Ak88). Fish were fed daily with D2 at 8:00 am, 12:00 pm, and 4:00 pm until apparent satiation.

An outdoor water recirculation system with six circular tanks (1000L each) supplied with dechlorinated tap water and equipped with a biological filter and supplementary aeration was used for the experiment.

2.3 Experimental procedures

To begin the experiment, six hundred Nile tilapia juveniles was fasted for 24 h and anesthetized with alcoholic solution of benzocaine (50 mg.L⁻¹). The fish were then weighed (4.51±0.09 g) and randomly assigned to four cages of 60L per 1000L tank (25 fish per cage), constituting one experimental unit of each treatment per tank. A maximum feeding rate of 6% of biomass per day divided into two meals (11:00 am and 5:00 pm) was established according to Huang et al.⁽⁴⁾. Due to the variations in water temperature throughout the trial, the feeding rate and frequency were adjusted according to the feeding protocol described in the NRC⁽¹⁴⁾ for Nile tilapia as follows:

- When the water temperature was under or equal to 15 °C, the fish were fed at 1% of their biomass at one meal per day;
- When the water temperature ranged from 16 to 19 °C, the fish were fed at 60% (3.6% of their biomass) of the maximum feeding rate at one meal per day;
- When the water temperature oscillated from 20 to 24 °C, the fish were fed at 80% (4.8% of their biomass) of the maximum feeding rate at two meals per day;
- When the water temperature ranged from 25 to

29 °C, the experimental diets were supplied at the maximum feeding rate (6.0% of their biomass) at two meals per day;

- When the water temperature ranged from 30 to 32 °C the feeding rate used was similar to the one previously described for 20 to 24 °C.

The water temperature and dissolved oxygen ($7.40 \pm 0.68 \text{ mg.L}^{-1}$) were monitored daily at 8:00 am ($22.97 \pm 2.89 \text{ °C}$) and 6:00 pm ($26.55 \pm 3.41 \text{ °C}$) using a multi-parameter probe (Akso®, model AK88). The total ammonium ($4.0 \pm 0.033 \text{ } \mu\text{mol.L}^{-1}$) (method n° 4500B), nitrite ($0.005 \pm 0.032 \text{ } \mu\text{mol.L}^{-1}$) (method n° 4110B), and total alkalinity (95 mg.L^{-1}) (method n° 2320B) were monitored weekly according to the American Public Health Association⁽¹⁷⁾.

2.4 Sampling, processing, and parameters evaluated

From the initial population, 20 fish were euthanized by anesthetic overdose (benzocaine at 500 mg.L^{-1}). They were then ground, homogenized and frozen until chemical analysis. For the last 24 h of the experiment (on day 67), the fish were not fed. Subsequently, all animals were sedated, weighed, and counted as previously described. A sample of six euthanized fish per cage was pooled to analyze the whole-body composition, as previously described.

The following parameters were calculated:

• Weight gain (g) = Final weight - Initial weight;

• Feed intake (g.fish^{-1});

• Thermal growth coefficient

$$(\%) = \left[\frac{\text{Initial weight}^{1/3} - \text{Final weight}^{1/3}}{\text{Sum of water temperatures (mean values } ^\circ\text{C)}} \right];$$

• Feed conversion ratio

$$(\text{g:g}) = \left[\frac{\text{Total feed intake}}{\text{Weight gain}} \right];$$

• Survival rate

$$(\%) = \left[\frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100 \right];$$

• Uniformity

$$(\%) = \left[\frac{N}{N_t} \times 100 \right];$$

• Gross energy participation in weight gain

$$(\%) = \left[\frac{(\text{Wf} \times \text{BGEf}) - (\text{Wi} \times \text{BGEi})}{(\text{Wf} - \text{Wi})} \times 100 \right];$$

• Ether extract participation in weight gain

$$(\%) = \left[\frac{(\text{Wf} \times \text{BEEf}) - (\text{Wi} \times \text{BEEi})}{(\text{Wf} - \text{Wi})} \times 100 \right];$$

• Crude protein participation in weight gain

$$(\%) = \left[\frac{(\text{Wf} \times \text{BCPf}) - (\text{Wi} \times \text{BCPi})}{(\text{Wf} - \text{Wi})} \times 100 \right];$$

• Energy productive value

$$(\%) = \left[\frac{(\text{Wf} \times \text{BGEf}) - (\text{Wi} \times \text{BGEi})}{\text{GEi}} \times 100 \right];$$

• Lipid productive value

$$(\%) = \left[\frac{(\text{Wf} \times \text{BEEf}) - (\text{Wi} \times \text{BEEi})}{\text{EEi}} \times 100 \right];$$

• Protein productive value

$$(\%) = \left[\frac{(\text{Wf} \times \text{BCPf}) - (\text{Wi} \times \text{BCPi})}{\text{CPI}} \times 100 \right]$$

Where N: total number of animals; Nt: total number of animals with weight $\pm 20\%$ within the mean live weight in each experimental unit (cage) as described by Furuya et al.⁽¹⁸⁾; BGEi: initial body gross energy; BGEf: final body gross energy; GEi: total gross energy intake; BEEi: initial body ether extract; BEEf: final body ether extract; EEi: total ether extract intake; BCPi: initial body crude protein; BCPf: final body crude protein; CPI: total crude protein intake; Wi: average initial weight; Wf: average final weight^(19, 20).

The profitability of the feeding strategies was calculated using the amount of money spent with feed per kilogram of fish or one thousand fish produced. The price of D1 and D2 was USD 0.72 and USD 0.65 per kilogram, respectively. The conversion from Brazilian Real (BRL) to US Dollars used the exchange rate on May 18, 2021: 1.00 USD = 5.255 BRL.

Another three fish per cage, also previously euthanized, were weighed and necropsied to calculate the following indexes:

• Hepatosomatic index

$$(\%) = \left[\frac{\text{Liver weight}}{\text{Body weight}} \times 100 \right]$$

• Intraperitoneal fat index

$$(\%) = \left[\frac{\text{Visceral fat weight}}{\text{Body weight}} \times 100 \right]$$

To determine liver steatosis, tissue fragments were fixed in Davidson's solution for 24 hours, dehydrated in graded ethanol solutions, and embedded in paraffin. Then, paraffin-embedded tissues were cut transversely ($7 \text{ } \mu\text{m}$ thickness) using an automatic microtome (SLEE medical GmbH®, CUT 6062, Mainz, PP, Germany), and then stained with Hematoxylin and Eosin (H&E). The slides were photographed (40x objective lens) using a trinocular microscope with a camera (Carl Zeiss® — Primo Star). The images were processed and analyzed using the IMAGEJ software. A 36-point test system (6 x 6 points) was superimposed on each photomicrograph to determine the volume density of hepatic steatosis⁽²¹⁾ using the following formula:

$$\text{Steatosis} = \frac{P_p}{P_t} \times 100$$

Where: Pp = test points that hit liver fat vesicles and Pt = total test points (36 points).

The chemical composition of the diets and fish were analyzed according to the Association of Official

Analytical Chemists⁽²²⁾; moisture was determined using a forced-air oven at 105 °C until a constant weight was achieved; ash by combustion in a muffle furnace at 550 °C (No. 942.05); ether extract by Soxhlet method (No. 920.39); crude protein by Kjeldahl method (N×6.25) (No. 936.15); crude fiber by non-enzymatic gravimetric method (No. 993.21); and gross energy by calorimetric bomb.

2.5 Statistical analysis

To verify the assumptions of variance analysis (ANOVA), all data were subjected to exploratory analysis to assess normality (Shapiro–Wilk test) and homogeneity of variances (Bartlett's test). There was an outlier experimental unit in treatment 7D2 that differed from the others in terms of mortality rate (over 60%). Therefore, it was excluded from the statistical analyses. A one-way

analysis of variance (ANOVA) was performed, and significant means ($P < 0.05$) were compared using Tukey's test. All analyses were carried out using Statistical Analysis System (SAS) software, version 9.1.

3. Results

The average survival rate of the fish was 83.7%, which was similar ($P > 0.05$) among treatments. There was no significant effect of feeding strategies on fish growth. However, it is important to highlight that fish from the 7D1 treatment had a 12% lower growth performance ($P > 0.05$). The same trend was observed in the cost per kilogram or one thousand units of fish produced. The productive performance parameters of Nile tilapia juveniles are summarized in Table 2.

Table 2. Productive performance of Nile tilapia juveniles submitted to mixed feeding strategies varying the dietary E:P ratio after a 67-day trial.

Parameters	Feeding strategies				P value
	7D1	5D2-2D1	4D2-3D1	7D2	
Average initial weight (g.fish ⁻¹)	4.5±0.1	4.5±0.1	4.5±0.1	4.5±0.1	0.7849
Average final weight (g.fish ⁻¹)	28.5±1.2	27.7±1.2	28.1±1.9	25.9±2.1	0.0691
Average weight gain (g.fish ⁻¹)	24.1±1.1	23.1±1.2	23.6±1.9	21.4±2.1	0.0663
Feed intake (g.fish ⁻¹)	35.1±4.4	33.0±3.1	33.3 ±1.3	30.5±1.5	0.4100
Thermal growth coefficient (%)	0.086±0.002	0.083±0.002	0.084±0.004	0.079±0.005	0.0612
Feed conversion ratio (g:g)	1.3±0.07	1.3±0.03	1.3±0.06	1.4±0.09	0.3098
Survival rate (%)	80±11.3	86.1±6.0	82±6.6	86.8±7.8	0.4516
Uniformity (%)	34.7±8.0	42.7±12.6	35.5±10.9	39.5±7.1	0.5092
Feed cost (USD.kg fish ⁻¹)	0.89±0.12	0.84±0.06	0.83±0.06	0.78±0.05	0.0780
Feed cost (USD.1000 juveniles ⁻¹)	46.02±13.95	39.01±5.43	40.48±5.96	35.46±3.02	0.241

7D1: fish were fed D1 for seven days; 5D2-2D1: fish were cyclically fed D2 for five days and D1 for two consecutive days; 4D2-3D1: fish were cyclically fed D2 for four days, and D1 for three consecutive days; 7D2: fish were fed D2 for seven days. D1 = 3.2 kcal DE.g⁻¹ and 9.6 kcal DE: g CP ratio; D2 = 3.4 kcal DE.g⁻¹ and 10.3 kcal DE: g CP ratio

Table 3. Dietary nutrients use of Nile tilapia juveniles submitted to mixed feeding strategies with variation in dietary E:P ratio after a 67-day trial.

Parameters	Feeding strategies				P value
	7D1	5D2-2D1	4D2-3D1	7D2	
Gross energy participation in weight gain (%)	174±4.59	171.7±8.27	170±6.13	168.1±6.59	0.5214
Ether extract participation in weight gain (%)	9.7±0.25 ^a	9.3±0.5 ^{ab}	9.0±0.6 ^{ab}	8.8±0.5 ^b	0.0550
Crude protein participation in weight gain (%)	13.5±0.21	13.9±0.6	13.9±0.3	13.8±0.2	0.2726
Energy productive value (%)	26.9±3.76	27.1±2.7	27.3±2.8	27.2±1.9	0.9964
Lipid productive value (%)	16.6±2.22	16.6±1.7	16.4±2.0	16.8±1.5	0.9904
Protein productive value (%)	26.4±3.5	28.8±2.6	28.2±2.5	28.4±2.0	0.3980

Different superscripts within rows indicate significant differences by Tukey's test ($P < 0.05$). 7D1: fish were fed D1 for seven days; 5D2-2D1: fish were cyclically fed D2 for five days and D1 for two consecutive days; 4D2-3D1: fish were cyclically fed D2 for four days and D1 for three consecutive days; 7D2: fish were fed D2 for seven days. D1 = 3.2 kcal DE.g⁻¹ and 9.6 kcal DE: g CP ratio; D2 = 3.4 kcal DE.g⁻¹ and 10.3 kcal DE: g CP ratio

The participation of ether extract in fish weight gain was reduced ($P=0.055$) by the increased participation of D2 in feeding strategies. There were no differences ($P>0.05$) in the other dietary nutrient use indices by fish (Table 3).

Fish fed only with Diet 1 had a lower ($P<0.05$) whole-body ether extract value. There was no significant difference in other body indices and nutrients of Nile tilapia juveniles when the feeding strategy was varied (Table 4).

Table 4. Body index and whole-body composition (wet basis) of Nile tilapia juveniles submitted to mixed feeding strategies with variation in dietary E:P ratio after a 67-day trial.

Parameters	Feeding strategies				P value
	7D1	5D2-2D1	4D2-3D1	7D2	
Hepatosomatic index (%)	3.6±0.8	3.5±0.2	4.0±0.6	3.90±0.4	0.3066
Intraperitoneal fat index (%)	1.6±0.3	1.53±0.3	1.93±0.6	1.42±0.3	0.1892
Volume density of hepatic steatosis (%)	0.47±0.15	0.50±0.17	0.49±0.05	0.48±0.11	0.4898
Dry matter (%)	27.0±0.40	27.1±0.87	26.8±0.71	26.7±0.46	0.6400
Crude protein (%)	13.3±0.18	13.6±0.49	13.6±0.23	13.6±0.17	0.2988
Gross energy (kcal/kg)	1602±42.1	1576±68.7	1564±54.7	1537±46.7	0.2963
Ether extract (%)	8.4±0.23 ^a	8.1±0.46 ^{ab}	7.8±0.50 ^{ab}	7.6±0.41 ^b	0.0239
Ash (%)	3.9±0.15	3.8±0.11	3.9±0.13	3.9±0.10	0.7831

Different superscripts within rows indicate significant differences by Tukey's test ($P<0.05$). 7D1: fish were fed D1 for seven days; 5D2-2D1: fish were cyclically fed D2 for five days and D1 for two consecutive days; 4D2-3D1: fish were cyclically fed D2 for four days and D1 for three consecutive days; 7D2: fish were fed D2 for seven days. D1 = 3.2 kcal DE.g⁻¹ and 9.6 kcal DE: g CP ratio; D2 = 3.4 kcal DE.g⁻¹ and 10.3 kcal DE: g CP ratio

4. Discussion

The Nile tilapia require between 3.0 kcal.g⁻¹ (15, 23) and 3.4 kcal.g⁻¹ (14) of DE in their early growth phase. Increasing dietary digestible energy — especially from lipids and carbohydrates — is essential to reduce protein levels in aquafeeds to meet the body's protein synthesis requirements. Therefore, the estimated crude protein requirement for juvenile Nile tilapia in the post-sexual reversion phase is around 268-297 g.kg⁻¹ (14, 15, 24), resulting in a DE:CP ratio between 11 and 13 kcal DE: g CP. Nile tilapia feeds in Brazil have between 40% CP (fingerlings) and 35% CP (juveniles) (25). These values are much higher than the minimum dietary CP content (29.7%) required values (15) for the same development stage. Brazilian law does not require fish feed industries to inform the protein content in its digestible form. This is a plausible hypothesis to explain the predominant availability of commercial diets for tilapia with values of proteins knowledgeable excessive, despite it being the most expensive macronutrient in aquafeeds. Experimental diets showed CP levels (33%) slightly higher than the minimum nutritional requirement, although DE was within the recommended range. Therefore, both diets meet the nutritional requirements for Nile tilapia, especially in terms of DE:CP ratio, when supplied alone or in mixing strategies, considering similar ($P>0.05$) productive performance among groups. However, it is important to highlight the non-significant trend towards

lower growth on fish fed only D2. It is possible that differences in feed formulation resulted in an E:P imbalance when CP is expressed on a digestible basis.

Feeding restriction is largely used by fish farmers to optimize feed conversion ratio. However, this strategy decreases the effect of dietary energy levels on voluntary feed intake, in contrast to when fish are fed to apparent satiation. Thus, feed consumption and energy retention between the different treatments were similar ($P>0.05$). The amount of digestible carbohydrate, mostly consisting of starch, can restrict voluntary feed intake (26, 27). Therefore, the decrease in dietary energy intake in fish fed to apparent satiation is due to differences in non-protein and protein energy sources in diets. In fact, when fish is fed to apparent satiety, it is difficult to estimate the ideal E:P ratio because energy intake remains constant and limits protein intake (28).

The body lipid content in fish usually has a positive correlation with the energy increment in diets. However, fish fed D1 (3.2 kcal.kg⁻¹) had a higher body lipid content than those fed D2 (3.4 kcal.kg⁻¹). Low whole-body lipid content in fish fed D2 was probably a result of their smaller growth ($P>0.05$) despite similar feed intake among treatments.

Nile tilapia juveniles show better growth at a water temperature of 26 to 30 °C (29). Throughout the experiment, there was high variability in the minimum

and maximum water temperatures, and a gradual decline in the daily average temperature. Therefore, feeding rate adjustments were constantly necessary, as well as the reduction from two to one meal when the water temperature was lower than 20 °C. Water temperature can influence batch uniformity, as observed in Nile tilapia GIFT strain by Santos et al. (30). Thus, the temperature gradient may explain the low uniformity (under 40%) observed in this study.

Ingestion of high levels of lipids or dietetic carbohydrates can increase lipid deposition in the liver, resulting in a condition called hepatic steatosis. This is a common disorder in fish lipid metabolism, especially in cultured fish, which can result in poor feed efficiency and growth (31, 32). However, diagnosing steatosis as a pathology can be erroneous, as fat storage varies between species and fat accumulation indicates a good dietary status rather than a nutritional disorder (33). Nile tilapia livers presented large intracytoplasmic vacuoles and displaced nucleus (average steatosis degree around 48%), without significant effect of the treatments. To determine if the steatosis degree is indicative of either a healthy dietary status or nutritional disease, it would be necessary to test commercial Nile tilapia feeds with wider variances in E:P ratios.

Hepatosomatic and intraperitoneal fat indexes are indirect measures of the fish's body energy stock. Sgnaulin et al. (24) observed a high hepatosomatic index in Nile tilapia similar to this study. These authors considered the high amount of dietary digestible carbohydrates responsible for this result. The physiological response to high dietary carbohydrate concentration was previously studied in Nile tilapia fed increasing levels of starch (34) or after intraperitoneal glucose injection (35, 36).

In the present study, the feeding protocols (with mixed diets or not) did not influence nutrient-productive values. In fact, only ether extract participation in weight gain decreased in opposition to energy levels of diets, corroborating with the trend registered in weight gain and whole-body ether extract content. A similar finding was reported by Haidar et al. (28).

5. Conclusion

It is possible to feed Nile tilapia juveniles with different dietary E:P ratios without affecting the productive performance. However, the choice of feeding protocol should also consider production costs. Thus, it is recommended to feed Nile tilapia juveniles with a commercial diet with 10.3 kcal DE per gram of crude protein for seven days per week.

Declaration of conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

Conceptualization: I. Litaiff and A. J. A. Bicudo. *Data curation:* I. Litaiff and A. J. A. Bicudo. *Formal Analysis:* A. J. A. Bicudo. *Funding acquisition:* R. Y. Sado and A. J. A. Bicudo. *Investigation:* I. Litaiff, S. Moesch and R. Y. Sado. *Methodology:* A. J. A. Bicudo and R. Y. Sado. *Project Management, Supervision and Validation:* A. J. A. Bicudo and R. Y. Sado. *Visualization and Writing (original draft):* I. Litaiff and S. Moesch. *Writing (review & editing):* I. Litaiff, S. Moesch, R. Y. Sado and A. J. A. Bicudo.

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References

1. Lovell R. Diet and fish husbandry – Salmonids. In: Halver JE, Hardy RE. Fish Nutrition. 3rd ed. California: Academic Press-Elsevier Science; 2002. p. 720-732. Available from: <https://www.agrifs.ir/sites/default/files/Fish%20Nutrition.pdf>
2. Santos MM, Calumby JA, Filho PAC, Soares EC, Gentelini AL. Nível de arraçoamento e frequência alimentar no desempenho de alevinos de tilápia-do-Nilo. [Feeding level and feeding frequency on Nile tilapia fingerling performance]. Boletim do Instituto da Pesca, 2015; 41(2): 387-395. Available from: <https://doi.org/10.4025/actascianimsci.v31i4.6300>. Portuguese.
3. Leonardo FA, Baccarin AE, Filho JDS, Scorvo CMDF. Custo de produção tilápia-do-Nilo (*Oreochromis niloticus*) e pacu (*Piaractus mesopotamicus*) no vale do Ribeira, Estado de São Paulo. [Production cost of Nile tilapia (*Oreochromis niloticus*) and pacu (*Piaractus mesopotamicus*) in vale do ribeira, state of São Paulo]. Informações Econômicas, 2018; 48(1): 21-33. Portuguese.
4. Huang Q, Huang K, Ma Y, Qin X, Wen Y, Sun L, Tang L. Feeding frequency and rate effects on growth and physiology of juvenile genetically improved farmed Nile tilapia. North American Journal of Aquaculture, 2015; 77(4): 503-512. Available from: <https://doi.org/10.1080/15222055.2015.1066472>
5. Abdel-Tawwab M, Khattab YAE, Ahmad MH, Shalaby AME. Compensatory growth, feed utilization, whole-body composition, and hematological changes in starved Juvenile Nile tilapia, *Oreochromis niloticus* (L.). Journal of Applied Aquaculture. 2008; 18 (3): 17–36. Available from: https://doi.org/10.1300/J028v18n03_02
6. Ali M, Nicieza A, Wootton RJ. Compensatory growth in fishes: a response to growth depression. Fish and Fisheries. 2003; 4(2): 147-190. Available from: <https://doi.org/10.1046/j.1467-2979.2003.00120.x>
7. Myszkowski L. Compensatory growth, condition and food utilization in barbel *Barbus barbus* juveniles reared at different feeding periodicities with a dry diet. Journal of Fish Biology, 2013; 82(1): 347–353. Available from: <https://doi.org/10.1111/j.1095-8649.2012.03482.x>

8. Takahashi LS, Biller JD, Criscuolo-Urbinati E, Urbinati EC. Feeding strategy with alternate fasting and refeeding: effects on farmed pacu production. *Journal of Animal Physiology and Animal Nutrition*, 2011; 95(2): 259-266. Available from: <https://doi.org/10.1111/j.1439-0396.2010.01050.x>
9. Yarmohammadi M, Shabani A, Pourkazemi M, Soltanloo H, Imanpour MR, Ramezanpour S, Smith-Keune C, Jerry DR. Effects of starvation and re-feeding on compensatory growth performance, plasma metabolites and IGF-I gene expression of Persian sturgeon (*Acipenser persicus*, Borodin 1897). *Iranian Journal of Fisheries Sciences*, 2013; 12(2): 465- 483. Available from: <http://jifro.ir/article-1-1003-en.html>
10. Cho SH, Heo TY. Effect of dietary nutrient composition on compensatory growth of juvenile olive flounder *Paralichthys olivaceus* using different feeding regimes. *Aquaculture nutrition*, 2011; 17(1): 90-97. Available from: <https://doi.org/10.1111/j.1365-2095.2010.00788.x>
11. Gibson T, Gatlin GDM. Dietary protein and energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*). *Aquaculture*, 2001; 194(3-4): 337-348. Available from: [https://doi.org/10.1016/S0044-8486\(00\)00523-8](https://doi.org/10.1016/S0044-8486(00)00523-8)
12. Sardar P, Sinha A, Datta S. Effect of mixed feeding schedules with varying dietary protein levels on the growth performances of common carp (*Cyprinus carpio* Linn.). *Indian Journal of Animal Sciences*, 2011; 81(5): 105–110.
13. Dong GF, Yang YO, Yao F, Wan Q, Yu L, Zhou JC, Li Y. Response Of Yellow catfish (*Pelteobagrus fulvidraco* Richardson) to low-protein diets and subsequent recovery. *Aquaculture Nutrition*. 2013; 19(3): 430-439. Available from: <https://doi.org/10.1111/j.1365-2095.2012.00978>
14. NRC, National Research Council. Nutrients requirements of fish and shrimp. Washington: The National Academies Press; 2011.
15. Furuya WM, Pezzato LE, Barros MM, Boscolo WR, Cyrino JEP, Furuya VRB, Feiden A. Tabelas brasileiras para nutrição de tilápias. Toledo: GFM; 2010. 100p. Portuguese. Available from: https://www.academia.edu/8857270/Tabelas_Brasileiras_para_a_Nutri%C3%A7%C3%A3o_de_Til%C3%A1pias
16. Azaza MS, Khiari N, Dhraief MN, Aloui N, Kraïem MM, Elfeki A. Growth performance, oxidative stress indices and hepatic carbohydrate metabolic enzymes activities of juvenile Nile tilapia, *Oreochromis niloticus* L., in response to dietary starch to protein ratios. *Aquaculture Research*, 2015; 46(1): 14-27. Available from: <https://doi.org/10.1111/are.12153>
17. American Public Health Association - APHA. Standard Methods for the Examination of Water and WasteWater. 22nd Edition, American Public Health Association, American Water Works Association, Water Environment Federation, 2012.
18. Furuya WM, Souza SR, Furuya VRB, Hayashi C, Ribeiro RP. Dietas peletizada e extrusada para machos revertidos de tilápias do Nilo (*Oreochromis niloticus* L.), na fase de terminação. *Ciência Rural*, 1998; 28(3): 482–487. Available from: <https://doi.org/10.1590/S0103-84781998000300022>
19. Sandre LCG, Buzollo H, Nascimento TMT, Neira LM, Jomori RK, Carneiro DJ. Productive performance and digestibility in the initial growth phase of tambaqui (*Colossoma macropomum*) fed diets with different carbohydrate and lipid levels. *Aquaculture Reports*, 2017; 6: 28-34. Available from: <https://doi.org/10.1016/j.aqrep.2017.02.003>
20. Oliveira-Júnior JCD, Aguiar GACC, Carneiro CLS, Ladeira ALF, Campelo DAV, Furuya WM, Santos FAC, Zuanon JAS, Luz RK, Salario AL. Effects of different ratios of crude protein and non-fibrous carbohydrates on growth, metabolism, physiology, nutrient utilization and muscle cellularity of *Lophiosilurus alexandri*, a carnivorous freshwater fish. *Aquaculture*, 2021; 540: 736685. Available from: <https://doi.org/10.1016/j.aquaculture.2021.736685>
21. Mandarim-de-Lacerda CA. Stereological tools in biomedical research. *Biomedical and Medical Sciences*, 2003; 75(4): 469–486. Available from: <https://doi.org/10.1590/S0001-37652003000400006>
22. Association of Official Analytical Chemists - AOAC. Official Methods of Analysis. 15th edition .Arlington, Virginia, USA: Association of Official Analytical Chemists, Inc.v.1,1990.
23. Durigon EG, Lazzari R, Uczay J, Lopes DLA, Jerônimo G T, Sgnaulin T, Emerenciano M G C. Biofloc technology (BFT): Adjusting the levels digestible protein and digestible energy in diets of Nile tilapia juveniles raised in brackish water. *Aquaculture and Fisheries*, 2019; 5(1): 42-51. Available from: <https://doi.org/10.1016/j.aaf.2019.07.001>
24. Sgnaulin T, Durigon EG, Pinho SM, Jerônimo GT, Lopes DLA, Emerenciano MGC. Nutrition of genetically improved farmed tilapia (GIFT) in biofloc technology system: Optimization of digestible protein and digestible energy levels during nursery phase. *Aquaculture*, 2020; 521: 734998. Available from: <https://doi.org/10.1016/j.aquaculture.2020.734998>
25. Montanhini Neto, R., Ostrensky, A. Evaluation of commercial feeds intended for the Brazilian production of Nile tilapia (*Oreochromis niloticus* L.): nutritional and environmental implications. *Aquaculture Nutrition*, 2015, 21(3): 311-320. Available from: <https://doi.org/10.1111/anu.12154>
26. Saravanan S, Geurden I, Figueiredo-Silva AC, Kaushik SJ, Haidar MN, Verreth JAJ, Schrama JW. Control of voluntary feed intake in fish: A role for dietary oxygen demand in Nile tilapia (*Oreochromis niloticus*) fed diets with different macronutrient profiles. *British Journal of Nutrition*, 2012; 108(8): 1519–1529. Available from: <https://doi.org/10.1017/S0007114511006842>
27. Tran-Duy A, Smit B, Vandam AA, Schram JW. Effects of dietary starch and energy levels on maximum feed intake, growth and metabolism of Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, 2008; 277(3–4): 213–219. Available from: <https://doi.org/10.1016/j.aquaculture.2008.03.004>
28. Haidar MN, Bleeker S, Heinsbroek LTN, Schrama JW. Effect of constant digestible protein intake and varying digestible energy levels on energy and protein utilization in Nile tilapia. *Aquaculture*, 2018; 489: 28–35. Available from: <https://doi.org/10.1016/j.aquaculture.2017.12.035>
29. Azaza MS, Dhraief MN, Kraïem MM. Effects of water temperature on growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia. *Journal of Thermal Biology*. 2008; 33(2): 98-105. Available from: <https://doi.org/10.1016/j.jtherbio.2007.05.007>
30. Santos VB, Mareco EA, Silva MDP. Growth curves of Nile tilapia (*Oreochromis niloticus*) strains cultivated at different temperatures. *Acta Scientiarum - Animal Sciences*, 2013; 35(3): 235–242. Available from: <https://doi.org/10.4025/actascianimsci.v35i3.19443>
31. Dai YJ, Cao XF, Zhang DD, Li XF, Liu WB, Jiang GZ. Chronic inflammation is a key to inducing liver injury in blunt snout bream (*Megalobrama amblycephala*) fed with high-fat diet. *Developmental and Comparative Immunology*. 2019; 97: 28–37. Available from: <http://dx.doi.org/10.1016/j.dci.2019.03.009>
32. Xu F, Xu C, Xiao S, Lu M, Limbu SM, Wang X. Effects of

- α -lipoic acid on growth performance, body composition, antioxidant profile and lipid metabolism of the GIFT tilapia (*Oreochromis Niloticus*) fed high-fat diets. *Aquaculture Nutrition*, 2019; 25(3): 585–596. Available from: <https://doi.org/10.1111/anu.12881>
33. Caballero MJ, Kjorsvik E, Fernandez AJ, Rosenlund G. Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short-orlong-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. *Journal of Fish Diseases*. 2004; 27(9): 531-541. Available from: <https://doi.org/10.1111/j.1365-2761.2004.00572.x>
34. Wang XX, Chen MY, Wang K, Ye, JD. Growth and metabolic responses in Nile tilapia (*Oreochromis niloticus*) subjected to varied starch and protein levels of diets. *Italian Journal of Animal Science*, 2017; 16: 308–316. Available from: <https://doi.org/10.1080/1828051X.2016.1275953>
35. Chen YJ, Wang XY, Pi RR, Feng JY, Luo L, Lin SM, Wang DS. Preproinsulin expression, insulin release, and hepatic glucose metabolism after a glucose load in the omnivorous GIFT tilapia *Oreochromis niloticus*. *Aquaculture*. 2018; 482: 183–19.