

## Dietary Energy: Protein Ratios and Their Impact on Juvenile Nile Tilapia Growth in Mixed Feeding Approaches

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### Abstract

To guarantee the sustainability of aquaculture, feeding practices must be evaluated. In this research, two diets were given to young Nile tilapia with varying Energy: Protein (E: P) ratios (10.3 and 9.6 kcal of digestible energy per gram of crude protein). Economic factors, whole-body composition, hepatic steatosis, feed, growth, and nutrient consumption were assessed in the fish. The fish's uniformity, feed consumption, feed conversion ratio, growth, and survival were unaffected by the treatments. Nile tilapia juveniles fed a diet containing 10.3 kcal DE/g CP during a seven-day period saw lower feed costs ( $P>0.05$  per biomass or 1,000 units generated). The feeding protocols increased the amount of the 10.3 kcal DE/g CP meal consumed, which reduced ( $P=0.055$ ) the contribution of the ether extract to fish weight gain. The whole-body lipid levels of fish showed a similar pattern. Fish from various treatments had comparable body indices ( $P>0.05$ ). Nile tilapia juveniles perform productively when given a weekly feeding program that combines meals with various E: P ratios.

**Keywords:** Fish nutrition, feeding management, Aquaculture, Productive performance

### Introduction

The sustainability of aquaculture from an economic and environmental perspective depends on effective feed management. Nile tilapia is routinely fed daily by fish farmers to encourage higher growth rates because of their greater nutritional needs in the early stages (1). For a variety of fish species, a mixed feeding schedule (MFS) has been used to improve growth performance, boost feed utilization, and decrease waste from a residual feed. From the fry stage to adulthood, Nile tilapia has weight-dependent protein needs that range from high to low. As a result, the switch from HP to LP feed types typically takes place during the rising stage. For fry, fingerlings, broodstock, and young Nile tilapia, MFS cycles have been enhanced (2). One of the fish species that are most frequently cultivated is the Nile tilapia. Farming practices have seen a significant improvement during the past 40 years, which has boosted production growth. By utilizing the resources that are available as effectively as possible, increasing tilapia output seeks to boost productivity. As it does for other animal species, the use of prepared meals has risen, promoting the growth of Nile tilapia aquaculture (3). Fish oxidize the fatty acids, amino acids, and monosaccharides created by the digestion and absorption of lipids, dietary proteins, and carbohydrates to get the energy they require to survive and thrive. In intensive and semi-intensive aquaculture systems, the proportionate contributions of proteins, lipids, and carbohydrates to providing fish with the energy they

need for maintenance and growth are governed by feed composition. Thus, by modifying the diet's components, fish nutritionists can improve how well-farmed species utilize macronutrients (4).

The tilapia fish is one of the most extensively raised fish in the world. This species was once found only in the Middle East and Africa before it began to spread over the globe in the 1900s. Production of tilapia is believed to be profitable because the fish are thought to be disease-resistant and require little maintenance. It grows and multiplies in a variety of conditions and takes about six months to reach commercial sizes of 600-900 g (5). In recent years, Egypt's aquaculture industry has grown to be one of the most significant. It has also attracted major investment and raised national income in addition to providing protein that cannot be acquired from animal sources. In Egypt, aquaculture increases more than 80% of the produced fish. The majority of these species are freshwater fish kept in tanks, like tilapia, catfish, and carp (6). Tilapia is a low-cost fish that is widely raised throughout the world and is continually growing in popularity. No matter what the consequences of Covid-19 were, this was the situation. The output of tilapia is growing at a little over 7% a year and will continue to expand in 2021. Large volumes of feed are produced by the numerous tilapia farms (7). More than 100 million of the 180 million tons of fish and aquatic products consumed globally in 2018 came from aquaculture. Aqua cultural production is probably going to keep expanding because of the demands of the expanding human population and the generally consistent catch from capture fisheries (8). The crucial and scarce components of aqua feed diets are proteins, particularly fishmeal, which is becoming increasingly hard to come by due to the expanding demand from the aquaculture industry (9). Living standards are expected to rise as the world's population increases. This will increase the need for aquatic animal protein. High-quality protein can be added in a variety of inventive ways to aquaculture systems, particularly those that raise tilapia (10).

The study (11) looked at how Nile tilapia's growth performance, intestinal histology, blood health, and economic viability were affected by refeeding, cyclical fasting, and protein-rich diets. The type and frequency of the diet significantly affect the price and income of fish farming. More work is being done to improve wheat bran's nutritional value and to address the issue of the byproduct's high fiber content in the study (12). Wheat bran (WB) was produced by baker's yeast (*Saccharomyces cerevisiae*) in order to examine the effects of WB on the crude fiber (CF), crude protein (CP), and amino acids profiles as well as the feed utilization, growth, blood indices, and intestinal and liver histology in Nile tilapia (*Oreochromis niloticus*). The study's (13) main goal is to add chamomile flower meal to the diet of Nile tilapia fish in order to improve their floc quality and amino acid content. also dinner marjoram. The long-term objective of sustainable development is to increase economic prosperity and living standards over time without jeopardizing the capacity of subsequent generations to meet their own needs. The study (14), Nile tilapia, *Oreochromis niloticus*, growth performance, intestinal morphology, blood indicators, endogenous enzyme activity, digestibility, and immunological response were assessed. Further, FM was replaced

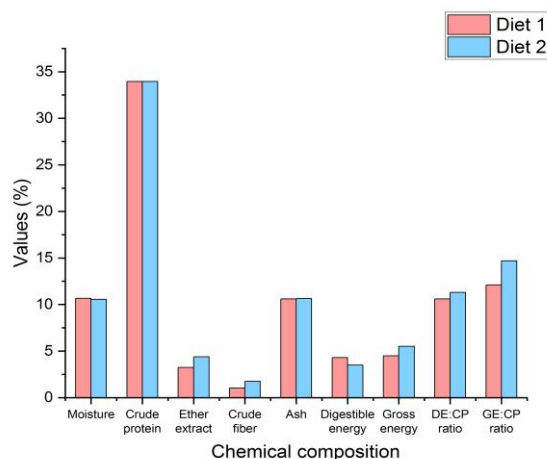
with sources of plant protein. The source of animal protein is grass, which is naturally composed of lignocellulosic organic material. There is little reason to think that the supply would ever increase again as it has really dropped and declined significantly. According to "The fish site," tilapia production will rise by 3.3% to 6 million tonnes in 2020 (15). The study (16) investigated what happened when red wigglers (*Eisenia fetida*) worm meal (RWWM) was given to fingerling Nile tilapia (*Oreochromis niloticus*) instead of fishmeal. The fish were bred in 1000 L fiberglass tanks in a recirculating aquaculture system for 90 days, receiving two hand feeds every day. The study's (17) objective was to determine the effects of different vitamin K (VK) dosages on the developmental capacities, hematopoietic traits, and immune responses of juvenile all-male Tilapia Nile. Five isonitrogenous (30% cp) isocaloric meals were given VK3 (menadione) at five different concentrations. In the study (18), the effectiveness of additional nano-selenium (Se-Nps) treatment on Nile tilapia fingerling growth was investigated. Nanoselenium was mixed with basal feed at the necessary concentrations and administered to Nile tilapia fingerlings for 30 days before growth performance measures were evaluated. The nano selenium was produced using high-energy ball milling (HEBM) and a 10-hour dry milling process at a 10:1 ball-to-powder ratio (BPR). The study (19) set out to determine how different lengths of an early carbohydrate (CHO) feeding stimulus might affect Nile tilapia's later growth performance and CHO metabolism. At the end of the stimulation period, fry on the short- and long-HC/LP diets had less growth performance than controls, as was to be expected. This was linked to altered protein and glucose metabolism gene expression, with later compensatory growth. Red drum, tilapia, and hybrid striped bass (HSB) were utilized in the study to assess the effects of swimming vs. not needing to swim in a continuous, sluggish current of around one body length per second (1bl/s) (20). In this work, we investigate the impact of diverse feeding strategies on the growth of juvenile Nile tilapia and the link between dietary energy and protein consumption.

## Materials and Methods

All of the study's protocols (Protocol #15/2020) have previously received approval from the Palotina Sector of the Federal University of Paraná's Ethics Committee on Animal Use.

## Medication and preparation for experiments

Figure (1) displays the ingredients of experimental diets. We looked at two authentic, isonitrogenous fish meals that were eaten by omnivores and had different digestible energy (DE) contents. Table (1) provides a description of the diets' overall chemical makeup.



**Figure (1):** The make-up of the experimental diets

**Table (1):** The composition of the experimental diets

| Chemical structure           | D1    | D2    |
|------------------------------|-------|-------|
| Moisture (%)                 | 10.67 | 10.52 |
| Ether extract (%)            | 3.24  | 4.39  |
| Crude protein (%)            | 33.96 | 34.11 |
| Crude fiber (%)              | 1.04  | 1.75  |
| Ash (%)                      | 10.61 | 10.64 |
| DE:CP ratio (kcal.g-1)       | 10.5  | 11.2  |
| Ingestible power (kcal.g-1)* | 4.3   | 3.5   |
| Total energy (kcal.g-1)      | 4.5   | 5.2   |
| GE:CP ratio (kcal.g-1)       | 12.10 | 14.7  |

The therapies combined various experimental diets with various eating schedules:

1. 7D1 Treatment: For 7 days, the fish were given D1.
2. 7D2 Treatment: D2 was fed to the fish for 7 days.
3. 4D2-3D1 Treatment: Fish were cycled with food D1 for 3 days, then with food D2 for 4 days.
4. 5D2-2D1 Treatment: Fish were given D1 for two days, followed by D2 for five days, in a cycle.

Six duplicates of each treatment were used in the fully randomized design of the experiment.

### Conditions in laboratories and fish

Young *Oreochromis niloticus* (Nile tilapia) was bought from an industrial fish farm in Palotina, Brazil, Paraná, and spent 45 days adjusting to the lab environment. 1000L circular

tanks with biological filters and additional aeration were used to house the fish. Daily, water in the tanks was replenished in part (around one-third of the amount). A multiparameter probe (Akso®, model Ak88) was used to monitor dissolved oxygen throughout this time, and daily tests for ammonia, nitrite, and pH were conducted using commercial kits (LabconTest®). Fish were fed D2 until they appeared full three times a day at 12:00 pm, 8:00 am, and 4:00 pm. Six round tanks are part of an outdoor water recirculation system, each with a 1000-liter capacity, which was used to conduct the experiment. The tanks were equipped with biological filters, increased aeration, and a biological filter and filled with dechlorinated tap water.

### Experimental Procedures

600 young Nile tilapia were first given a benzocaine alcoholic solution (50 mg.L<sup>-1</sup>) to put them to sleep after a 24-hour fast. 6% of biomass was the daily maximum feeding rate, divided into two meals at 11:00 am and 5:00 pm. Changes in water temperature during the study led to adjustments in the frequency and rate of feeding for Nile tilapia using the NRC feeding regimen.

- The fish were given 1% of their biomass once daily when the water temperature was at least 15 °C lower ;
- Fish were given one meal per day at 60% of their maximum rate of consumption in water that ranged in temperature from 16 and 19 °C ;
- The experimental diets included two meals each day at the maximum feeding rate while the water temperature was between 25 and 29 °C;
- Although the water was between 30 and 32 °C, the food intake was similar to that previously reported for temperatures between 20 and 24 °C;

Dissolved oxygen levels and water temperature were measured twice daily between the hours of 6:00 pm and 8:00 am using a multi-parameter probe. Weekly measurements of total ammonium, total nitrite, and total alkalinity were made in accordance with the American Public Health Association.

### Processing, evaluation, and sampling of the parameters

Twenty fish from the initial population were accidentally overdosed on the anesthetic. For further chemical analysis, they were then homogenized, crushed, and frozen. During the final day of the experiment, the fish were not fed.

$$\text{Weightgain}(h) = \text{Finalweight} - \text{Initialweight}; \quad (1)$$

$$\text{Feedintake}(g.fish^{-1}); \quad (2)$$

*Thermalgrowthcoefficient*

$$(\%) = \left[ \frac{\text{initial weight}^{1/3} - \text{final weight}^{1/3}}{\text{sum of water temperatures (mean values}^\circ\text{C)}} \right] \quad (3)$$

*feed conversion ratio*

$$(h. h) = \left[ \frac{\text{Total feed intake}}{\text{initial number of fish}} \times 100 \right] \quad (4)$$

*survival rate*

$$(\%) = \left[ \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100 \right] \quad (5)$$

*Uniformity*

$$(\%) = \left[ \frac{M}{M_s} \times 100 \right] \quad (6)$$

*Gross energy involvement in weight gain*

$$(\%) = \left[ \frac{(U_e \times AHF_e) - (U_j \times AHF_j)}{(U_e - U_j)} \times 100 \right] \quad (7)$$

*Ether extract participation in weight gain*

$$(\%) = \left[ \frac{(U_e \times AFF_e) - (U_j \times AFF_j)}{(U_e - U_j)} \times 100 \right] \quad (8)$$

*Rudeproteinparticipationinweightgain*

$$(\%) = \left[ \frac{(U_e \times ADF_e) - (U_j \times ADF_j)}{(U_e - U_j)} \times 100 \right] \quad (9)$$

*Efficacy of energy*

$$(\%) = \left[ \frac{(U_e \times AHF_e) - (U_j \times AHF_j)}{HF_j} \times 100 \right] \quad (10)$$

*Lipid productive value*

$$(\%) = \left[ \frac{(U_e \times AFF_e) - (U_j \times AFF_j)}{FF_j} \times 100 \right] \quad (11)$$

*Protein productive value*

$$(\%) = \left[ \frac{(U_e \times ADO_e) - (U_j \times ADO_j)}{DO_j} \times 100 \right] \quad (12)$$

Then, as described before, all the creatures were put to sleep, weighed, and added up. Six dead fish per cage were used as a sample to determine the composition of the entire body. Ms is the total number of animals in each experimental unit (cage) whose weight is 20% or less of the mean live weight, where M is the total number of animals overall, and so on.; AHFe, AHFj, and HFj stand for the initial and final body gross energies, respectively. The average starting weight is Ue, while the average finishing weight is Uj. The Brazilian Real (BRL) to USD exchange rate utilized for the conversion was 1 USD = 5.255 BRL on May 18, 2021. In order to determine the following indexes, three additional fish per cage that had previously

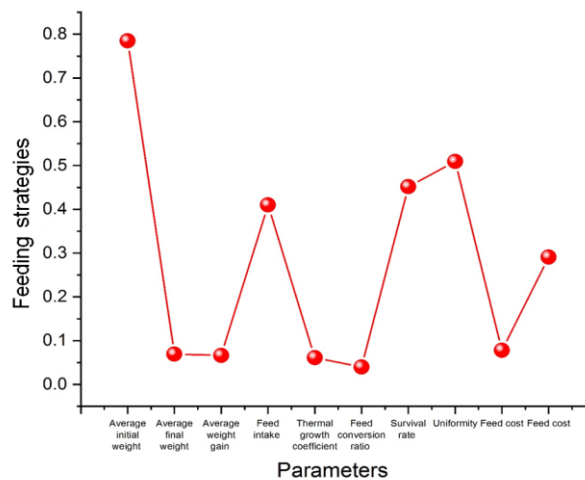
been put to death were weighed and necropsied. An automatic microtome was then used to produce transverse slices of paraffin-fixed tissues that were 7 m thick and Eosin and hematoxylin (E&H) were then used to stain these slices. A camera and a trinocular microscope were used to record the slides.

### Statistic evaluation

All data were subjected to exploratory analysis to determine the homogeneity and normality of variances in order to support the fundamental assumptions of variance analysis. In treatment 7D2, one peculiar experimental unit exhibited a higher death rate than the others. As a result, it was not included in the statistical evaluation. Tukey's test was used to compare significant means ( $P < 0.05$ ) after a one-way analysis of variance. Version 9.1 of the Statistical Analysis System (SAS) program was used for all analyses.

### Results and discussion

Fish survived on average 83.7% of the time, and differences in treatment were not statistically significant ( $P > 0.05$ ). Fish growth was not significantly impacted by feeding practices. But it's crucial to note that fish from the 7D1 treatment performed 12% worse in terms of growth ( $P > 0.05$ ). The cost per kilogram or 1,000 generated units of fish showed the same trend. Table (2) provides a summary of the Nile tilapia juveniles' productive performance metrics. Figure (2) examines the productive conduct of young Nile tilapia.

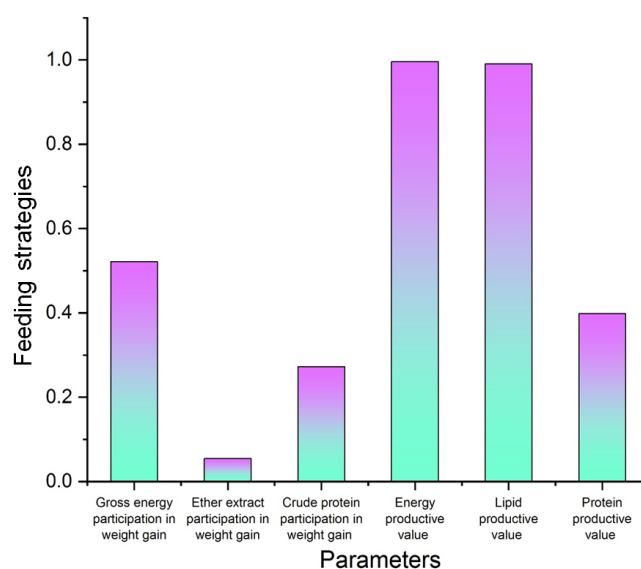


**Figure (2):** The productive conduct of young Nile tilapia

**Table (2):** Nile tilapia juveniles' productive behaviour

| Parameters                                     | Feeding techniques |         |         |       |         |
|--|--------------------|---------|---------|-------|---------|
|  | 7D1                | 5D2-2D1 | 4D2-3D1 | 7D2   | P value |
| Feed prices (USD.kg $fish^{-1}$ )              | 1.01               | 0.9     | 0.89    | 0.83  | 0.0780  |
| Feed cost (USD.1000 $juveniles^{-1}$ )         | 59.97              | 44.44   | 46.44   | 38.48 | 0.291   |
| Feed intake ( $g \cdot fish^{-1}$ )            | 39.5               | 36.1    | 34.6    | 32    | 0.4100  |
| Temperature growth factor (%)                  | 0.088              | 0.085   | 0.088   | 0.084 | 0.0612  |
| Survival rate (%)                              | 91.3               | 92.1    | 88.6    | 94.6  | 0.4516  |
| Uniformity (%)                                 | 42.7               | 55.3    | 46.4    | 46.6  | 0.5092  |
| Average weight gain ( $g \cdot fish^{-1}$ )    | 25.2               | 24.3    | 25.5    | 23.5  | 0.0663  |
| Ratio of feed conversion (g:g)                 | 1.37               | 1.33    | 1.36    | 1.49  | 0.0398  |
| Average initial weight ( $g \cdot fish^{-1}$ ) | 4.6                | 4.6     | 4.6     | 4.6   | 0.7849  |
| Average final weight ( $g \cdot fish^{-1}$ )   | 29.7               | 28.9    | 30.8    | 28    | 0.0691  |

The enhanced involvement of D2 in feeding strategies lowered ( $P=0.055$ ) the role that ether extract plays in fish weight increase. Other dietary nutrient utilization indices for fish were similar  $P>0.05$ ; Table (3). Figure (3) depicts the Nutritional intake of juvenile Nile tilapia.

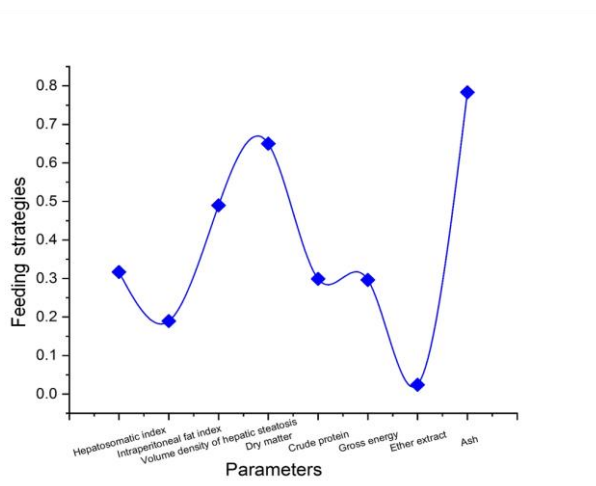
**Figure (3):** Nutritional intake of juvenile Nile tilapia



**Table (3):** Nile tilapia juvenile’s dietary nutrient consumption

| Parameters  | Feeding techniques |         |         |        |         |
|---|--------------------|---------|---------|--------|---------|
|   | 7D1                | 5D2-2D1 | 4D2-3D1 | 7D2    | P value |
| Efficacy of energy (%)                              | 30.66              | 29.8    | 30.1    | 29.1   | 0.9564  |
| Participation of crude protein in weight growth (%) | 13.71              | 14.5    | 14.2    | 29.1   | 0.2726  |
| Protein productive value (%)                        | 29.9               | 31.4    | 30.7    | 30.4   | 0.3980  |
| Ether extract participation in weight gain (%)      | 9.95               | 9.8     | 9.6     | 9.3    | 0.0550  |
| Lipid productive value (%)                          | 18.82              | 18.3    | 15.4    | 18.3   | 0.9904  |
| Gross energy participation in weight gain (%)       | 178.59             | 25.97   | 176.13  | 174.69 | 0.524   |

The value of the whole-body ether extract dropped ( $P < 0.05$ ) in fish fed only Diet 1. When the feeding approach was changed, there was no discernible difference in the other body indices and nutrients of juvenile Nile tilapia (Table 4). Figure (4) represents the physical characteristics of young Nile tilapia, including body mass index.



**Figure (4):** Physical characteristics of young Nile tilapia, including body mass index

**Table (4):** Juvenile Nile tilapia physical composition, including body index

| Parameters                    | Feeding techniques |         |         |        |         |
|-------------------------------|--------------------|---------|---------|--------|---------|
|                               | 7D1                | 5D2-2D1 | 4D2-3D1 | 7D2    | P value |
| Ether extract (%)             | 8.63               | 8.56    | 8.3     | 8.01   | 0.0239  |
| Intraperitoneal fat index (%) | 1.9                | 1.83    | 2.53    | 0.59   | 0.4898  |
| Gross energy (kcal/kg)        | 1644.1             | 16441.7 | 1618.7  | 1583.7 | 0.2963  |
| Crude protein (%)             | 13.48              | 14.09   | 13.83   | 13.77  | 0.2988  |
| Dry matter (%)                | 27.40              | 27.97   | 27.51   | 27.16  | 0.4400  |
| Ash (%)                       | 4.05               | 3.91    | 4.03    | 4.00   | 0.7831  |
| Hepatosomatic index (%)       | 4.4                | 3.7     | 4.6     | 4.3    | 0.30666 |

Brazilian Nile tilapia meals range from 35% CP (juveniles) to 40% CP. These values are significantly (29.9%) higher than the lowest values for dietary CP content needed for the same developmental period. As a result, there was no discernible difference between treatments in terms of feed consumption or energy retention ( $P>0.05$ ).

When fish are fed until they appear full, their protein consumption is really restricted, and their energy intake is constant, making it challenging to determine the ideal E:P ratio. The increase in energy in diets often corresponds positively with the fish body's lipid content. Fish fed D1 had more body lipids than fish fed D2 did, nevertheless. Juvenile Nile tilapia grows more quickly in water that is between 26 and 30 °C. The daily average temperature gradually dropped throughout the trial, and there was significant variation in the minimum and highest water temperatures. According to research done on the Nile tilapia GIFT strains, the water temperature can affect batch consistency. The low homogeneity noted in the investigation may therefore be explained by the temperature differential. Hepatic steatosis is a condition caused by an increase in lipid deposition in the liver brought on by dietary carbs or high fat intake. The therapies had little to no effect on the massive intracytoplasmic vacuoles and misplaced nuclei that were present in the Nile tilapia livers. Similar to this study, it was observed that Nile tilapia had a high hepatosomatic index. These authors hypothesized that the excessive intake of dietary digestible carbs was the cause of this outcome. Whether the feeding plans in the current study contained mixed meals or not, nutrient-productive levels were unaffected.

## Conclusion

In this study, maintaining a proper diet's energy-to-protein ratio is crucial for fostering juvenile Nile tilapia's growth and development while using diverse feeding strategies. Finding the ideal ratio of protein to energy would improve growth rates, maximize feed utilization, and ultimately lead to the success of tilapia aquaculture operations. Different dietary E:P ratios can be fed to juvenile Nile tilapia without having any negative effects on their ability to reproduce. However, it's important to take production expenses into account while selecting a feeding strategy. A proper diet's energy-to-protein ratio is crucial for achieving the highest growth rates in Nile tilapia, according to research in this area. Because protein is essential for the growth of muscles and the entire body, a diet with more protein tends to promote better growth. However, high protein levels without sufficient energy might lower feed intake and lead to ineffective nutrient utilization, limiting growth potential.

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