Effects of protein level on zootechnical performances of male Nile tilapia, *Oreochromis niloticus* (Deroua fish farm, Morocco)

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Abstract. This study aimed to assess the impact of three feeds with varying protein levels (26%, 28%, and 31%) on the zootechnical performance of Nile tilapia (*Oreochromis niloticus*) reared in earthen ponds at Deroua fish farm, with the aim of reducing the protein load, which impacts both the feed cost and, implicitly, the overall cost of Nile tilapia farming. A total of 900 male fish were distributed across nine earthen ponds measuring 300 m^2 each. The stocking density was 100 fish per pond, nearly identical biomass in all nine ponds (11 kg). For each group of three ponds, the fish were fed daily with one of the three experimental feeds for a duration of 8 weeks, with a feeding frequency of five times per day. A daily monitoring of physical parameters and a bi-monthly analysis of the chemical parameters of pond water were conducted. Additionally, a bi-monthly sampling of approximately 30 fish per pond were taken to monitor their growth. The findings revealed that the fish fed a diet containing 31% protein exhibited the highest performance in terms of mean weight gain (258.02±17.15 g), daily weight gain (4.53±0.30 g day^{-1}), and specific growth rate (2.11±0.08% day^{-1}). These parameters demonstrated an improvement with increasing protein levels. Moreover, the diet with 31% protein yielded the best performing feed conversion ratio (1.22±0.08), which exhibited a decrease with increasing protein levels. The highest protein efficiency rate value (2.78±0.47) was observed in fish fed a diet containing 26% protein. however the differences in all these parameters between the three diets were not statistically significant ($p > 0.05$). None of the three feeds had a significant effect on the survival rate of the reared fish. Throughout the rearing period, the physicochemical parameters of the pond water were maintained within the recommended optimal ranges for rearing *O. niloticus*. Comparative economic analysis of the three diets reveals that the 26% protein diet offers the best economic efficiency in terms of production cost per weight gained, while also showing the highest return on investment. However, higher protein diet (31%) generate higher net profits, underlining the importance of a balanced feeding strategy that optimizes both production costs and revenues in the context of sustainable aquaculture.
Key Words: economic efficiency, earthen ponds, feed cost, *Oreochromis niloticus*, protein levels.

Introduction. The global population growth, overexploitation of fishery resources, and changing dietary habits have led to the emergence of aquaculture as a promising solution to enhance food security worldwide. Aquaculture involves the production of fish, crustaceans, molluscs, and algae for human consumption, providing a mean of 20.5 kg per capita per year globally (FAO 2020). Significant efforts have been made to develop aquaculture, resulting in a world production of 82.1 million tons of aquatic animals in 2018, with fish accounting for 54.3 million tons (FAO 2020).

Nile tilapia (*Oreochromis niloticus*) is one of the most widely cultured fish species globally, with commercial importance in various regions such as China, South-East Asia, Africa, the United States, and Latin America (Lim & Webster 2006). This fish species is well-suited for aquaculture due to its hardiness, fast growth, resistance to stress and disease, and low feed requirements (El-Sayed 1999).

According to FAO (2012), global tilapia production increased from less than 500,000 tons in the early 1990s to 3.5 million tons in 2011. The latest FAO statistics report a production of 4.525.400 tons of Nile tilapia, ranking second after carp with 10,492,500 tons (FAO 2020).

Effective husbandry practices are crucial for successful Nile tilapia farming, with a focus on compliance and optimizing various factors, including the availability of high-quality and sufficient feed (Abdel-Tawwab 2016) and proper feed management (Hanssen et al 2012). Feeding frequency also plays a role in fish growth (Byamungu et al 2001), body composition (Villarroel et al 2011; Daudpota et al 2016), and overall health (El-Araby et al 2020). Understanding the optimal nutritional requirements of fish is vital to prevent stress and disease outbreaks (Li et al 2014; Shah et al 2017) and to minimize production costs.

In tilapia farming, feed constitutes a significant portion of production costs (Bamba et al 2008; Elegbe et al 2015), accounting for over 50% of the operational expenses for Nile tilapia (El-Sayed 2006). However, feed quality, particularly in terms of protein content, can vary significantly and impact fish growth (Amoussou et al 2016). Extensive research has focused on fish nutrition, specifically studying dietary protein and energy requirements. The efficiency of these nutritional elements is crucial for formulating efficient, cost-effective, and environmentally sustainable feeds for fish farming (Duarte et al 2007; Chai et al 2013).

Protein represents the costliest component of intensive aquaculture feeds, accounting for approximately 50% of the total feed costs and exerting a significant influence on the growth of Nile tilapia (El-Sayed 2006). Studies indicate that protein requirements for Nile tilapia vary depending on age, size, dietary energy content, protein source (animal or plant), water quality, and growth conditions (FAO 2020). For instance, larval stages of *O. niloticus* require around 35-45% dietary protein for optimal growth performance. Juveniles have protein requirements ranging from 30 to 40%, while adults require 20 to 30% dietary protein for optimal growth (FAO 2020).

Therefore, the protein content in the diet should be precisely adjusted to meet the fish's growth requirements. Excessive protein in fish diets can be wasteful and unnecessarily increase feed costs (Ahmad et al 2004). Thus, reducing feed costs is a key factor for the successful development of aquaculture.

In Morocco, Nile tilapia was introduced in 2004 as part of efforts to diversify high-value fish species, and its production has steadily increased in the past decade, rising from 100 tons in 2011 to 300 tons in 2018 (FAO 2020). However, the availability of efficient and reasonably priced feeds in the national market remains a significant challenge for the development of fish farming in the country.

The importance of developing efficient and cost-effective feeds for the grow-out of Nile tilapia fish has motivated the present study, which aims to contribute to determining the optimal protein level in terms of zootechnical performance. To address this issue, three artificial floating feeds with varying protein levels (31%, 28%, and 26%) were tested to evaluate their impact on the growth of Nile tilapia fish in earthen ponds at the Deroua fish-farming station.

Material and Method

Study site. This study was conducted at Deroua fish farm, which is situated in a semi-arid region with a mild winter, approximately 25 km west of the town of Beni Mellal in central Morocco. The farm is operated by the National Centre for Hydrobiology and Fish Farming, National Water and Forestry Agency.

Fish and experimental design. For the purposes of this experiment, 900 male Nile tilapia fish were chosen based on their enhanced and more uniform growth rates relative to female counterparts, and to prevent uncontrolled breeding during the experiment.

The selected fish had an mean weight of 110.57±0.32 g and an mean size of 18.47±0.03 cm. These fish were sourced from the breeding stock at the Deroua fish farm, ensuring reliable origin and consistent genetic characteristics for the entire experiment.

The experimental phase was conducted during the period from 03/09/2020 to 02/11/2020. The study utilized a total of nine earthen ponds, each with an area of 300 m^2. Prior to the start of the experiment, these ponds were allowed to dry for approximately three weeks before being filled with water. Throughout the entire experiment, the water level in all nine ponds was consistently maintained at a depth equal to or greater than one metre.

Each pond was stocked with 100 male Nile tilapia fish, resulting in a stocking density of one fish per three m^3. Additionally, each of the three ponds was assigned a specific diet for feeding purposes.

Experimental diets. The objective of this experiment was to examine and assess the impact of three different diets, based on floating artificial feeds with three protein levels, on the zootechnical performance of Nile tilapia. Feeds with the same particle size of 4.5 mm were allocated as follows:

- F1: a feed containing 31% protein (commercially available in the national market) was allocated to ponds n°: 1, 4, and 7;
- F2: a feed containing 28% protein was allocated to ponds n°: 2, 5, and 8;
- F3: a feed containing 26% protein was allocated to ponds n°: 3, 6, and 9.

The composition of the three diets delivered by the supplier is shown in Table 1.

Table 1

Composition of artificial feeds used

Percentage of composition	*Feed for tilapia*		
	F1	*F2*	*F3*
Crude protein (%)	30-31	28-29	26-27
Crude fat acids (%)	7-8.50	8-9	6.50-7
Fiber (%)	2-3.50	1-2	1-2
Ash (%)	7-11	10-11	13-15
Total phosphorus (%)	0.80-1.60	0.80-1.60	0.80-1.60
Vit A (UI kg^{-1})	8000	8000	8000
Vit D3 (UI kg^{-1})	1400	1400	1400
Vit E (mg kg^{-1})	160	160	160
Vit C (mg kg^{-1})	90	90	90
Price in Moroccan dirham per kg (MAD kg^{-1})	11	9.70	8.80

The daily amount of feed distributed (Q) in each pond was calculated based on the total biomass of fish present in the respective pond. Initially, a feeding rate of 4% of the total biomass was employed, gradually reducing to 1.5% towards the end of the experiment. The calculation of Q was determined using the formula: Q = (total biomass * 4)/100.

The daily feed distribution was divided into five equal portions and administered at fixed times (8:00 am, 10:30 am, 1:00 pm, 3:00 pm, and 6:00 pm). The feed was provided at the same location within each pond. The amount of feed given to each pond was periodically adjusted after each fish sampling event and calculation of the new total biomass.

Water quality parameters. Throughout the entire experimental period, in-situ measurements of temperature and dissolved oxygen (DO) were conducted three times a day (at 7:00 am, 1:00 pm, and 6:00 pm) using a YSI Pro ODO oximeter. Additionally, pH measurements were taken once a day at 1:00 pm using a HANNA HI 98191 pH meter.

Chemical analysis of nitrates (NO_3^-), nitrites (NO_2^-), ammonium (NH_4^+), and orthophosphates (PO_4^{3-}) in the rearing environment was carried out in the laboratory of Deroua fish farm. A JASCO V-630 spectrophotometer was utilized, with measurements taken at wavelengths of 415 nm, 537 nm, 630 nm, and 700 nm, respectively. The initial analyses were performed one day after filling the ponds, followed by subsequent analyses at 15 day intervals, along with fish sampling events.

Growth performance. Random sampling of a minimum of 30 fish per pond was conducted every two weeks using a seine net and a small mesh net. At the end of the experiment, and after draining the nine ponds, measurements of weight and total length were obtained for all the fish. The following zootechnical indices and parameters were measured and calculated.

- mean weight: the weight of each individual fish was measured using a SARTORIUS electric scale. The mean weight (MW) is an important parameter for the calculation of the new feed ration and other parameters. It was calculated using the formula:

$$MW (g) = \sum Wi / Nt$$

where: Wi = weight of individual fish in grams ; Nt = number total of individuals.

- mean total length: the measurement of fish size was conducted simultaneously with weight measurement, using a graduated ruler. The mean total length (MTL) was calculated as:

$$MTL (cm) = \sum T.Li / Nt$$

where: T.Li = total length of individual fish in centimeters.

- mean weight gain (MWG): this parameter allows the evaluation of weight growth during a specific period. It is calculated as the difference between the mean final weight (MWf) and the mean initial weight (MWi):

$$MWG (g) = MWf - MWi$$

- daily growth rate (DGR): this parameter indicates the mean growth rate per day during the rearing period. It is determined using the formula:

$$DGR (g\ day^{-1}) = MWG * \Delta T^{-1}$$

where: ΔT = rearing time (feeding time) of fish in days.

- specific growth rate (SGR): is a coefficient used to evaluate the daily weight gain of fish as a percentage of their live weight. It is calculated as:

$$SGR (\%\ day^{-1}) = [(Ln\ MWf - Ln\ MWi) * \Delta T^{-1}] * 100$$

where: Ln Awf = natural logarithm of the mean weight final ; Ln Awi = natural logarithm of the mean weight initial.

- feed conversion rate (FCR): measures the efficiency of feed conversion to live weight. It is calculated as:

$$FCR = Af * (Bmf - Bmi)^{-1}$$

where: Af = amount of feed distributed between two weighings in grams; Bmf = final biomass in grams; Bmi = initial biomass in grams.

- protein efficiency rate (PER): PER indicates the fish's ability to utilize dietary protein in relation to the amount of feed consumed. It is calculated as:

$$PER = (Bmf - Bmi) * Ap^{-1}$$

where: Ap = amount of protein distributed between the two weights in grams.

Survival rate. Survival rate was calculated based on the final number of fish and the initial number of fish at the beginning of the rearing period, using the following formula:

$$Survival (\%) = (final\ number\ of\ fish / initial\ number\ of\ fish) * 100$$

Economic analysis. In addition to evaluating the zootechnical parameters resulting from the rearing of Nile tilapia males fed with three tested dietary regimes, this study undertakes an economic analysis to compare the economic profitability based on the production cost per kilogram of weight gained, the net profit margin and return on investment associated with each experimental feed. The economic analysis conducted solely considers the cost of feeding to produce one kilogram of weight gain, and sets the selling price of one kilogram of fish at 30 MAD. The unit prices of the three feeds are determined by the feed supplier as follows: 11; 9.70; and 8.80 Moroccan Dirhams (MAD) respectively for 1 kilogram of feeds containing 31%, 28%, and 26% protein (Table 1).

Statistical analysis. The growth parameter results were expressed as mean±standard deviation. A one-way analysis of variance (ANOVA) was employed for the three tested diets, with $p < 0.05$ considered as statistically significant.

Results

Water quality parameters. Regarding the physical and chemical parameters of water quality, the mean values recorded during the fish farming period are presented in Tables 2 and 3.

Table 2

Means±SD of temperature, dissolved oxygen (DO), and pH measurements in breeding ponds for the three protein levels

Parameters	*Protein levels in diets*		
	31%	28%	26%
Temperature (°C)	23.40±1.90^a	23.49±2.22^a	23.40±1.71^a
Dissolved oxygen (mg L^{-1})	11.46±2.23^a	11.95±2.18^a	9.40±1.24^a
pH	8.08±0.18^a	8.13±0.26^a	7.89±0.16^a

Note: Different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA results. Values that share a common letter are not significantly different.

Table 3

The chemical parameters of water (means±SD) in the breeding tanks for the three protein levels

Parameters (mg L^{-1})	*Protein levels in diets*		
	31%	28%	26%
Nitrites (NO_2^-)	0.03±0.03^a	0.02±0.03^a	0.02±0.02^a
Nitrates (NO_3^-)	1.37±2.01^a	0.99±1.06^a	1.72±2.08^a
Ammonium (NH_4^+)	0.01±0.14^a	0.01±0.11^a	0.03±0.12^a
Orthophosphates (PO_4^{3-})	0.12±0.02^a	0.10±0.02^a	0.12±0.04^a

Note: Different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA results. Values that share a common letter are not significantly different.

In our study, maximum temperatures were generally recorded at 6 p.m. with the highest mean value of 27.06±0.73°C recorded during the first fortnight in ponds assigned to the 28% protein regime, while minimum temperatures were observed at 7 a.m. with the lowest mean value of 20.09±0.25°C recorded during the last fortnight in ponds assigned to the 26% regime.

According to Table 2, the results show that average water temperatures over the entire experimental period were very close for all three regimes, with the 31% and 26% protein groups remaining similar, with the 28% group showing a slightly higher average temperature. These differences in mean temperatures and their variabilities are very small, and the standard deviations show that the water temperatures in each group varied within a similar range. These variations in mean temperature between the three regimes were not statistically significant ($p > 0.05$).

With regard to DO, the rearing media for the 31% and 28% protein diets show a similar, relatively high mean DO saturation with almost identical variability, while the media for the 26% diet show a lower mean DO concentration, but with reduced variability compared with the other two diets. However, these variations in DO between regimes were not statistically significant ($p > 0.05$).

DO concentrations in the nine ponds provide crucial information on ecosystem dynamics. Observations made at different times of the day revealed temporal variations, demonstrating a close correlation between the time of measurement and DO levels, as well as spatial variations between ponds.

The data show a general downward trend in DO concentration during the night, with lower values in the morning (7 a.m.) and higher in the evening (6 p.m.). This variation is mainly influenced by the photosynthetic activity of plants, as well as by respiration and decomposition processes.

pH is an essential operational indicator for assessing fish water quality. During our study, mean pH values tended to be slightly higher (more alkaline) in the media for the 31% and 28% protein diets compared to the 26% diet, indicating small variations between groups. All three diets show comparable profiles, indicating a trend towards increasing pH over time. Variations in pH measurements over the experimental period in the ponds of each diet showed no statistically significant differences ($p > 0.05$).

Nitrogen compounds play a fundamental role in aquatic environments, acting as key elements in the nitrogen cycle. For all three dietary protocols tested (31%, 28% and 26%), initial nitrite concentrations were trace levels. Subsequent analysis revealed an absence of nitrites in the first sample for all diets, followed by a gradual appearance and rise from the second sample onwards. A peak in concentration was observed in the third sample for the 31% protein diet, with a value of 0.056 mg L^{-1}, before experiencing a slight regression in the fourth sample to 0.045 mg L^{-1}. In contrast, for the 28% and 26% diets, nitrite progression was more linear, reaching a maximum in the last sample, with a particularly tempered rise in the 26% diet.

When we consider the averages of nitrite concentrations adjusted for data variability, the 31% protein diet shows an average concentration of 0.026 mg L^{-1}, with a moderate dispersion of values. The 28% diet recorded a slightly lower average concentration, albeit with greater variability. The 26% diet, on the other hand, has the lowest average concentration, at 0.018 mg L^{-1}. Taking into account the mean nitrite concentrations adjusted for data variability, the 31% protein diet showed a mean concentration of 0.03 mg L^{-1}, with a moderate dispersion of values. The 28% diet had a slightly lower average concentration than the 31% diet, while maintaining comparable variability. On the other hand, the 26% diet showed an average concentration equivalent to that of the 28% diet, at 0.02 mg L^{-1}, but with the lowest dispersion of the results observed. Although all regimes show an upward trend in nitrite concentrations over the duration of the experiment, the values remain low overall.

For nitrates, initial concentrations were identical for all groups, with a starting value of 0.08 mg L^{-1}. Subsequent readings showed no significant increase in nitrate levels at first sampling for all regimes. Subsequently, a divergent increase in concentrations was observed.

For the group fed 31% protein, a gradual rise in concentrations peaked at 2.03 mg L^{-1} at the third sampling, followed by a marked acceleration to a peak of 4.63 mg L^{-1} at the last sampling. This peak contrasts with the more temperate curve of the 28% protein diet, which saw a more modest rise in nitrates, peaking at 2.54 mg L^{-1}. The 26% diet showed a similar pattern initially, but with a noticeable rise from the second sample onwards, leading to the highest concentration of 5.09 mg L^{-1} at the last sampling.

The mean nitrate concentration and standard deviation for each diet also show deviations. The 31% regime has a mean concentration of 1.37 mg L^{-1} with a fairly high variability of 2.01, which may indicate significant fluctuations in nitrate levels. The 28% diet has the lowest mean concentration of 0.99 mg L^{-1} and the lowest variability at 1.06. As for the 26% regime, it reveals the highest mean concentration of 1.72 mg L^{-1} and the greatest variability at 2.08, reflecting a wider dispersion of measured values.

In sum, although all three regimes showed an overall increase in nitrate concentrations over the course of the experiment, there were notable variations in the evolution and stability of these concentrations, with the 28% regime standing out for its more consistent nitrate management.

Initial ammonium concentrations were of the order of 0.035 mg L^{-1} for all regimes. Subsequent measurements revealed a marked decline in ammonium levels from the second sample onwards for all diets. As the experiment progressed, the 31% and 28% protein treatments showed a tendency to stabilize, with ammonium concentrations remaining low or undetectable after the first sample. This regulation manifested itself in similar average concentrations of 0.01 mg L^{-1}.

In contrast, the 26% protein diet showed a significant increase from the first sample, with a concentration that climbed to 0.089 mg L^{-1}, before falling back to undetectable levels. The higher mean concentration of 0.03 mg L^{-1} and the more consistent standard deviation of 0.04 for this group reflect greater variability, indicating more significant fluctuations in ammonium levels over the course of the study.

In summary, although the initial concentration is the same for all diets, the 26% diet stands out for its higher average ammonium concentrations and greater variability, while the 31% and 28% diets show lower, more constant levels of ammonium in the rearing water.

At the start of the experiment, orthophosphate concentrations were undetectable for all groups, ensuring a fair basis for comparison. The data collected reveal that no increase in orthophosphates was recorded in the first samples of each diet, maintaining the continuity of the initial conditions.

With the 31% protein diet, a modest rise is noted in the second sample, followed by a significant peak in the third sample, where the concentration reached 0.31 mg L^{-1}, before decreasing slightly in the fourth sample to 0.23 mg L^{-1}. This translates into an average orthophosphate concentration of 0.12 mg L^{-1}, with some variation within measurements. The group fed 28% protein also showed a slight increase in the second sample, but experienced more linear growth, culminating in a similar concentration of 0.23 mg L^{-1} in the final sample. This diet is distinguished by a lower average concentration of 0.10 mg L^{-1} and less variation. For the 26% protein diet, a more marked rise was observed from the second sample, with a concentration of 0.13 mg L^{-1}, followed by gradual increases to reach 0.25 mg L^{-1} by the fourth sample. With an average concentration equivalent to that of the 31% diet and an identical variation, this diet shows a constant increase and stability in orthophosphate values throughout the experiment.

In summary, all three diets started with zero orthophosphate concentrations and all showed an increasing trend over time. The 26% diet showed continuous growth, while the 31% diet showed slight fluctuation. The 28% regime showed the lowest mean and variability, indicating a more stable progression of orthophosphate levels in the water.

The mean concentrations of these four parameters showed no statistically significant difference ($p > 0.05$) between the three diets.

Zootechnical performances. The overall results of the growth performance of male Nile tilapia fed on three diets with different protein contents (31%, 28% and 26%) based on the data measured and calculated at the end of the experiment are summarised in Table 4 below.

Table 4

Growth parameters (mean±SD) of male *O. niloticus* at the end of the experimental period, subjected to three protein levels

Parameters	*31%*	*28%*	*26%*
Mean initial weight (g)	110.32±0.82	110.93±2.30	110.45±1.14
Mean final weight (g)	368.35±16.83^a	337.17±15.47^a	324.87±31.99^a
Mean initial total length (cm)	18.44±0.07	18.49±0.15	18.49±0.04
Mean final total length (cm)	25.27±0.32^a	24.80±0.40^a	24.69±0.90^a
Quantity of feed distributed (kg)	90.10±0.72	91.08±1.40	83.08±1.83
Mean weight gain (g)	258.02±17.15^a	226.24±13.20^a	214.42±31.47^a
DGR (g day^{-1})	4.53±0.30^a	3.97±0.23^a	3.76±0.55^a
SGR (% day^{-1})	2.11±0.09^a	1.95±0.04^a	1.89±0.16^a
FCR	1.22±0.09^a	1.39±0.05^a	1.39±0.14^a
PER	2.65±0.19^a	2.57±0.10^a	2.79±0.29^a
Survival rate (%)	97^a	97.66^a	96^a

Note: Different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA results. Values that share a common letter are not significantly different.

The evolution of these zootechnical parameters along our experiment, obtained at each sampling, are the following.

Mean weight. The Figure 1 shows the evolution of the mean weight of Nile tilapia males as a function of the protein levels during the experiment.

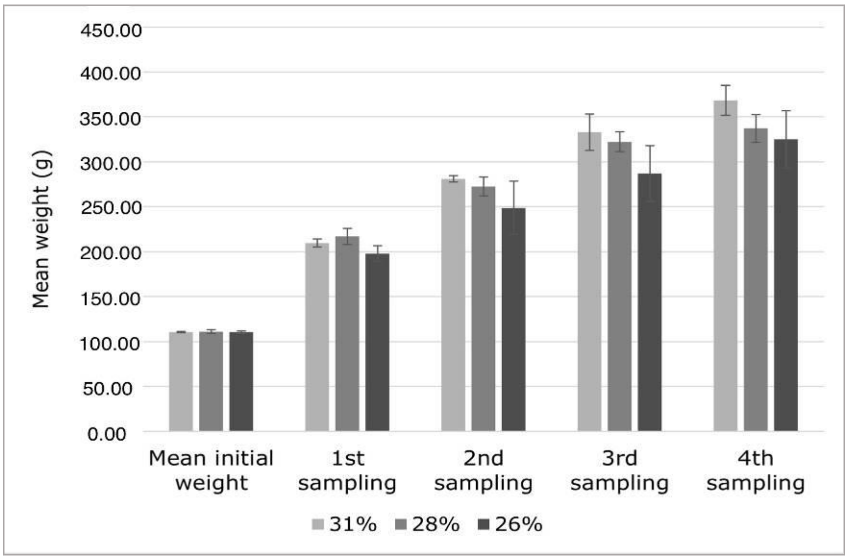

Figure 1. Time-dependent variation in mean weight±SD of male Nile tilapia fed different protein levels (26%, 28%, and 31%).

According to Figure 1, the evolution of the mean weight of fish on different diets reveals positive trends. Initially homogeneous, the groups fed the 31%, 28% and 26% protein diets showed distinct growth patterns over the course of the experiment. Fish fed the 31% diet showed steady growth, reaching a significantly higher final weight (368.35 ±16.83) compared to the other two diets. However, although the 28% and 26% diets had slightly lower final weights, their weight development remained significant. An analysis of the standard deviation values shows a general upward trend for subsequent sampling of all three diets, particularly for the 26% protein diet. This suggests increased variability in fish weights over time.

Although the 31% diet retained its significant weight advantage, variations in mean fish weight between the three diets did not show statistically significant differences ($p > 0.05$). On the other hand, the variations recorded between the values of each sampling reveal a statistically significant difference ($p < 0.05$), underlining the impact of the duration of the experiment on fish growth, independently of diet.

Mean total length. The evolution of the mean total length of the fish as a function of the protein levels during the experiment is pre-sented in Figure 2.

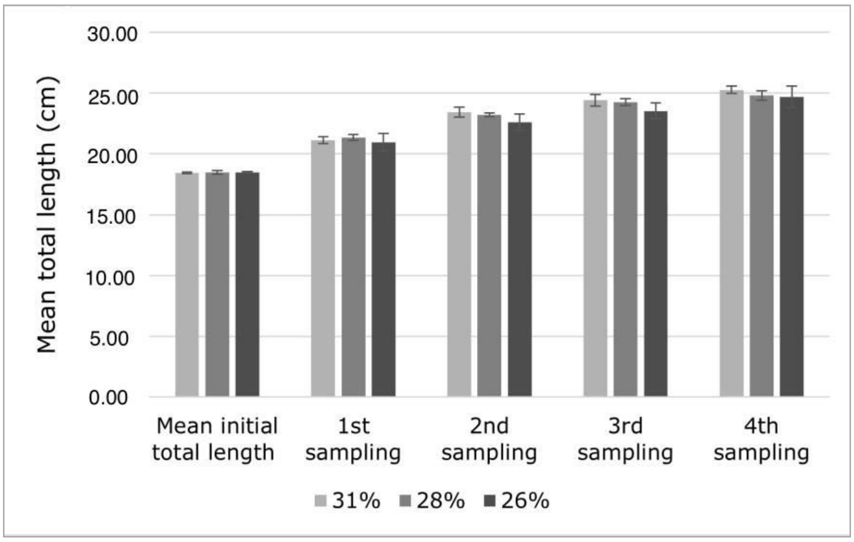

Figure 2. Time-dependent variation in mean total length±SD of male Nile tilapia fed different protein levels (26%, 28%, and 31%).

Observation the evolution of relating mean total fish length data, presented in Figure 2 for the three diets (31%, 28%, and 26%), reveals significant trends both with the increase in protein levels and during the experiment. In contrast to the mean weight, the standard deviation values remain low for all three diets and during all four samplings.

Initially, the groups appear homogeneous, with very similar initial mean total length values between fish fed the three diets, all around 18.50 cm. However, as the experiment progresses, distinct variations become apparent.

The results of successive sampling show a steady increase in mean total length for each diet. The 31% protein diet stands out as showing the most marked growth, with mean total length reaching 25.27 cm by the fourth measurement. The 28% and 26% protein diets also showed increases, albeit slightly less pronounced, with respective mean total lengths of 24.80 cm and 24.69 cm at the last sampling. However, the results of the analysis of variance showed that the three diets had no significant effect ($p > 0.05$) on the total length of Nile tilapia.

Mean weight gain (MWG). Figure 3 shows the evolution of the mean weight gain every two weeks of the fish during the experiment as a function of the protein levels.

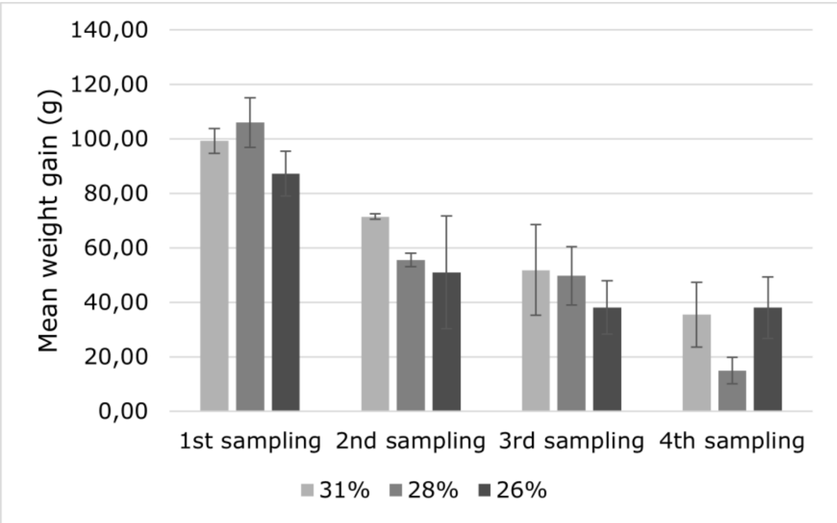

Figure 3. Time-dependent variation in weight gains (mean±SD) of male Nile tilapia as a function of protein levels (31%, 28%, 26%).

The average weight gains obtained during the rearing period showed distinct trends. The overall data shows a progressive decrease in this parameter over the four sampling periods for all the protein diets studied. The group fed a 28% protein diet started the study with the highest weight gain, which can be interpreted as an initial positive response to this protein level. However, this growth rate was not maintained, and this diet ended up with the lowest weight gain at the final sampling.

In comparison, the 31% diet showed a less spectacular start but maintained a more consistent growth rate, avoiding the precipitous drop seen in the 28% diet. The 26% group, despite its more modest start, showed resilience in its growth, approaching the weight gains observed in the 31% diet towards the end of the experiment.

Overall, fish fed the 31% protein diet showed the most favourable mean total weight gain (258.02±17.15 g) compared to the other diets. The increase in mean weight gain with higher protein levels was not statistically significant ($p > 0.05$). Fish fed the 28% protein diet showed only a 12.29 g increase in mean weight, while those fed the 31% protein diet showed a substantial 43.47 g increase over fish fed the 26% protein diet.

The convergence of average weight gains observed between the diets, in particular between 26% and 31%, suggests that fish growth tends to balance out independently of the protein content of their diet. This observation could suggest that the adaptability of Nile tilapia allows them to optimise their growth over time despite variations in protein intake.

This overall analysis underlines the importance of considering feed efficiency over the entire duration of the experiment rather than focusing on short-term responses, and highlights the homogenisation of long-term growth between the different diets.

Daily growth rate (DGR). The evolution of the mean DGRs recorded by the fish during this experiment and as a function of the protein levels are presented in Figure 4 below.

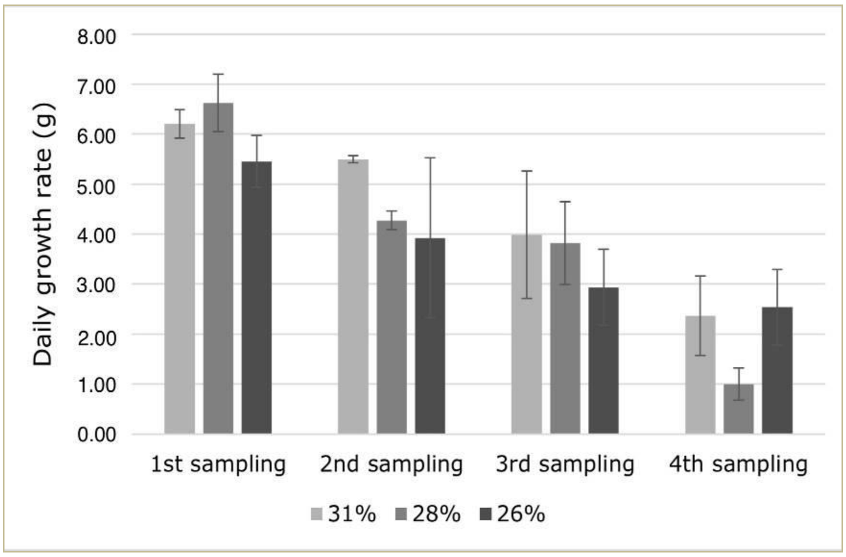

Figure 4. Variation in daily growth rate (mean±SD) of male Nile tilapia as a function of protein levels (31%, 28%, 26%) and time.

The mean DGRs observed during the experiment showed significant values. At the start of the experiment, fish on the 28% protein diet showed the highest daily growth rate (6.62 g d^{-1}), slightly outperforming those on the 31% and 26% diets.

However, as the experiment progressed, there was a downward trend in the DGR for all diets. The 31% diet showed a more gradual decrease, maintaining a higher growth rate (2.36 g d^{-1}) at the fourth sampling compared to the more drastically reduced rate of the 28% diet (1.00 g d^{-1}). The latter regime, although starting with the best rate, ultimately showed the most significant decline.

The 26% diet starts with the lowest rate, but its decline is less pronounced, finally stabilising at a higher growth rate than the 31% diet at the last sampling (2.54 g d^{-1}) (Figure 4). These values reflect a convergence of growth rates at the end of the experiment, with a slightly greater variability for the 26% regime. This suggests that despite lower initial performance, growth can be sustained more stably on a lower protein diet.

The final mean DGRs of the experiment showed that the highest rate (4.53 g d^{-1}) was recorded by fish fed the 31% protein diet, while those fed the 26% protein diet had the lowest rate (3.76 g d^{-1}) (Table 4). The differences in DGRs between the three protein level diets were not statistically significant ($p > 0.05$).

In summary, the progression of DGR over the period of the experiment shows that initial high levels do not necessarily guarantee better long-term performance. The data suggest that the 31% and 26% diets may offer a better balance between initial growth.

Specific growth rate (SGR). Figure 5 shows the evolution of the mean SGR in fish as a function of protein levels during the experiment.

Analysis of the evolution of SGRs shows a general downward trend over the four sampling periods for all diets, with no statistically significant difference between them ($p > 0.05$). The final mean SGR show a slightly better performance for the 31% protein diet with a rate of 2.11±0.09% day^{-1}, closely followed by the 28% diet with a rate of 1.95±0.04 and finally the 26% diet with a rate of 1.89±0.16 (Table 4).

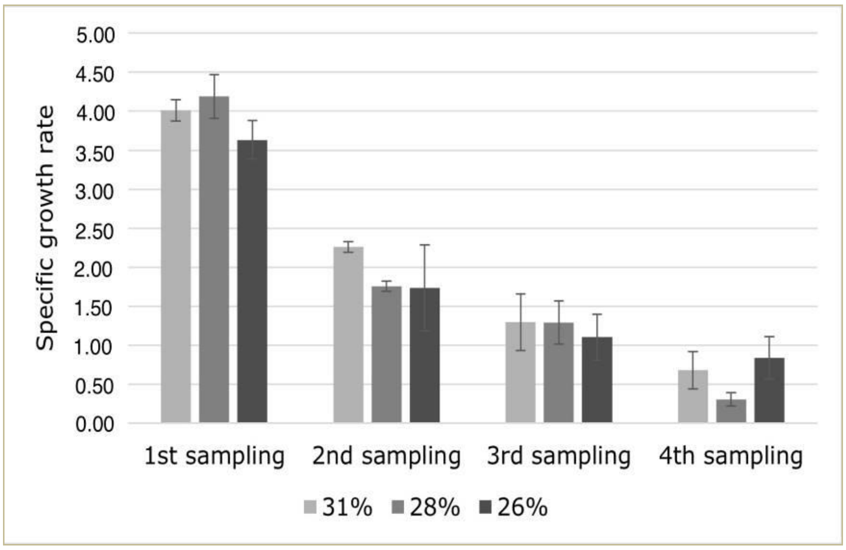

Figure 5. Time-dependent variation of specific growth rate (mean±SD) of male Nile tilapia as a function of protein levels (31%, 28%, and 26%).

The 28% protein diet initially showed the highest SGR at 4.19% day^{-1}. However, this rate fell dramatically in the second half of the year. However, this rate fell considerably in the fourth sample, ending up at 0.30. This profile indicates a marked reduction in specific growth over time.

Fish on the 31% diet showed a steadier decrease in SGR, retaining a higher final mean, which may indicate a long-term benefit of higher protein content. In contrast, the 26% diet started with the lowest rate, but showed a less pronounced decline across sampling, suggesting some resilience or adaptation to lower protein levels.

Feed conversion ratio (FCR). Figure 6 shows the variation of the mean FCR in fish as a function of protein levels during our study.

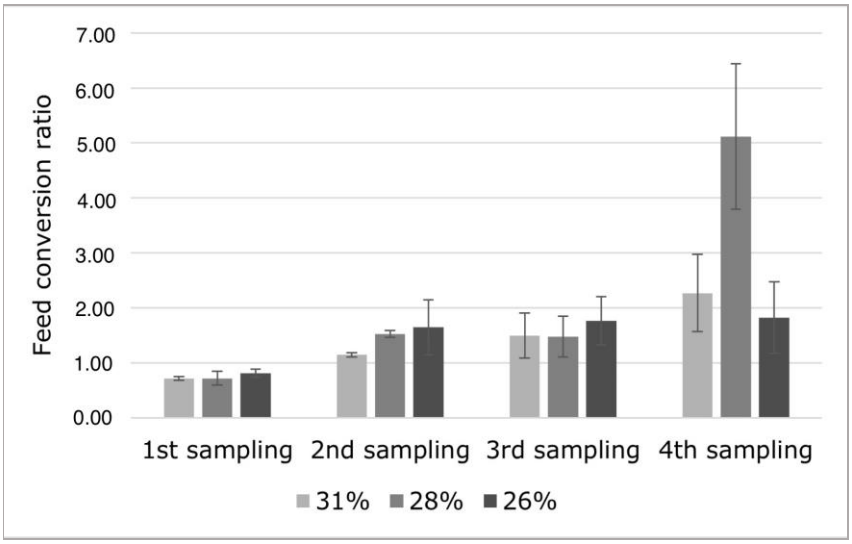

Figure 6. Time-dependent variation of the feed conversion ratio (mean±SD) of male Nile tilapia as a function of protein levels (31%, 28%, and 26%).

According to this graph, at first sampling, the 31% and 28% protein groups show similar and relatively low FCRs (0.71 and 0.72, respectively), indicating a high initial feed efficiency for these diets. The 26% protein group started with a slightly higher FCR (0.81), suggesting lower initial feed efficiency.

As the experiment progresses, there is a gradual increase in FCR for all diets, which is typical as the fish grow and their feed efficiency may decrease. However, the 28% diet shows a disproportionate increase in FCR by the fourth sampling (5.12), which could indicate a drastic reduction in feed efficiency at this stage. It is important to note that this value is much higher than the others and could be due to factors specific to this sample or group of fish.

The 31% diet showed a more gradual and steady increase in FCR, finishing at 2.27 by the fourth sampling. This diet maintained a final mean FCR of 1.22±0.09, reflecting a relatively stable feed efficiency throughout the study.

The 26% diet also shows a gradual increase, but retains a final RCF of 1.82, which is comparable to the 31% diet at the last sampling. The final mean FCR for this diet was 1.39±0.14, identical to that of the 28% diet (1.39±0.05), suggesting that over the duration of the study, fish on these two diets achieved similar feed efficiency.

Statistical analysis indicated that differences in FCR between fish fed the three different protein diets (31%, 28% and 26%) were not statistically significant ($p > 0.05$).

Despite fluctuations during sampling, the close final means indicate that the differences between the diets in terms of feed efficiency faded towards the end of the experiment. While the 31% diet showed the best overall performance with the lowest average FCR, the results suggest that in the long term, a diet with a lower protein content may result in comparable feed efficiency.

Protein efficiency rate (PER). Figure 7 shows the variation of the mean PER in Nile tilapia as a function of protein levels during the experiment.

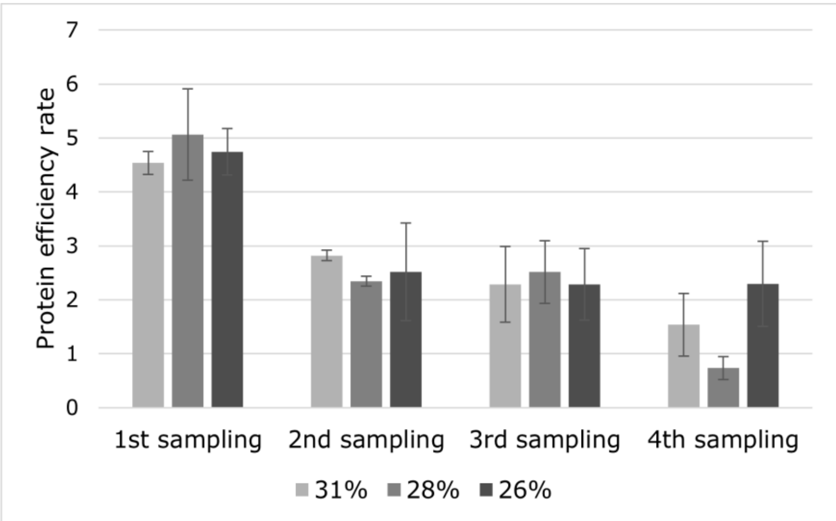

Figure 7. Time-dependent variation of the protein efficiency rate (mean±SD) of male Nile tilapia as a function of protein levels (31%, 28%, and 26%).

Longitudinal analysis of PER reveals a progressive decline for the three protein diets tested (31%, 28% and 26%) over the four sampling periods. This trend is consistent with the physiological development of the fish.

The 31% diet starts with a PER of 4.54, and although this figure decreases over the course of the samplings, ending up at 1.54, the final average PER is 2.65. This indicates a steady reduction in PER over the course of the four samplings. This indicates a steady reduction in protein efficiency, with modest variability around the mean.

Fish receiving the 28% diet start with the highest PER of 5.06, but undergo a noticeable drop, ending at 0.73 at the last sampling. The final average PER for this diet was 2.57, with little variability in performance for this group.

The 26% regime starts with a PER of 4.74 and shows a more linear decline to 2.29, distinguished by the highest final PER of 2.79. This may reflect a more constant protein utilization over the rearing period, although the variability indicated by the standard deviation is slightly higher compared to the other diets.

It is pertinent to note that the absence of statistically significant differences ($p > 0.05$) between diets, despite the distinct evolution of PER, suggests that all three diets offer comparable protein efficiency for tilapia growth. The standard deviation associated with each diet provides a perspective on the consistency of protein efficiency, with the 26% diet demonstrating the greatest variability. In an aquaculture context, this information can guide decisions regarding the formulation of diets to optimize growth and protein efficiency, while considering the relative costs and benefits of different protein levels.

***Survival rate**.* The survival rate of fish fed with each diet at the end of the experiment is presented in Table 5.

Table 5

Survival rate (mean±SD in%) of male Nile tilapia fed with three diets at different protein levels (31%, 28%, and 26%) at the end of the experiment

Protein level	*31%*	*28%*	*26%*
Survival rate (%)	97±1.73[a]	97.67±2.52[a]	96±1.73[a]

Note: Different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA results. Values that share a common letter are not significantly different.

The data showed high and very similar survival rates for all three diets, with male Nile tilapia fed a 28% protein diet having a slightly higher mean survival rate than tilapia fed a 31% protein diet. The 26% protein diet had the lowest mean survival rate.

The lower standard deviation for the 31% and 26% protein diets indicates consistency in survival rates between replicates in these groups, indicating a uniform response of the fish to these diets. In contrast, the slightly higher standard deviation observed for the 28% protein diet reflects more variability than the other diets, suggesting that fish responses to this diet may be more variable.

Based on ANOVA analysis, no statistically significant differences were detected ($p > 0.05$) in survival rates between the different protein diets tested. This implies that the variations measured could be the result of random factors, such as accidental mortalities during sampling manipulations or the effects of predation, rather than the direct effect of the protein composition of the diets. In other words, in the context of this study, it is likely that the protein content of diets does not have a determining impact on the survival of Nile tilapia.

***Economic analysis**.* Analyzing the data provided for the three diets, we observe interesting dynamics in terms of production cost per kilogram of fish gained, net profit, and return on investment. The results reveal a statistically significant difference ($p < 0.05$) in the cost of production per kilogram of fish gained between the diets, with the third diet showing the lowest cost (12.09 MAD kg^{-1}), indicating superior efficiency compared to the other two diets, where costs are slightly higher (13.37 MAD kg^{-1} and 13.48 MAD kg^{-1} respectively) (Table 6).

However, when examining net profit and return on investment, the differences between the schemes are not statistically significant ($p > 0.05$), suggesting that these parameters are influenced by other factors beyond production cost alone. The 31% protein diet showed the highest net profit (411.07 MAD), while the second and third diets generated comparable net profits (360.88 MAD and 361.03 MAD respectively). As for return on investment (ROI), although the third scheme has the highest rate (148.14%), this measure does not differ significantly between schemes from a statistical point of view, implying that investments in all schemes can be considered equally financially viable. The ROIs of 124.43% and 122.54% for the 31% and 28% plans respectively, although slightly lower, still reflect substantial profitability.

Table 6

Economic performance indicators (mean±SD) for feeding male Nile tilapia with the three diets studied

	31%	*28%*	*26%*
Initial biomass (kg)	11.03±0.08	11.09±0.23	11.05±0.11
Final biomass (kg)	35.75±2.23	32.94±1.97	31.20±3.37
Biomass gained (kg)	24.71±2.25	21.85±1.77	20.16±3.33
Quantity of feed consumed (kg)	30.03±0.72	30.36±1.40	27.69±1.83
Cost of feed consumed (MAD)	330.36±7.97	294.51±13.60	243.71±16.12
Production cost per kg of fish gained (MAD kg^{-1})	$13.37±0.95^a$	$13.48±0.51^a$	$12.09±1.23^b$
Profit (MAD)	741.43±67.41	655.39±53.16	604.74±99.98
Net profit (MAD)	$411.07±60.62^a$	$360.88±39.83^a$	$361.03±85.11^a$
Return on investment (%)	$124.43±15.81^a$	$122.54±8.29^a$	$148.14±25.50^a$

Note: Different superscript letters in the same row indicate statistically significant differences ($p < 0.05$) based on ANOVA results. Values that share a common letter are not significantly different.

Discussion

Water quality parameters. During this study, the physical parameters of the rearing water, including temperature, DO and pH, were carefully managed and their recorded values were on the one hand very similar between the three diets studied, and on the other hand maintained within ranges favorable to Nile tilapia rearing.

Water temperature is a crucial physical parameter that significantly influences the chemical and biochemical reactions in aquatic organisms, including fish, ultimately direct impacting their growth processes. The mean water temperature during the rearing period fluctuated between 20.09°C recorded in the morning and 27.06°C observed in the afternoon, which aligns with the preferred range for the species. The daily temperature variations in the nine ponds were influenced by the combined effects of ambient temperature, particularly the change of season (early autumn), and the rate of water filling from the borehole (21°C).

Previous studies by Lazard (2009) and Ndour et al (2011) have confirmed that Nile tilapia is a thermophilic species, and they also indicated nearby optimal temperature ranges for its growth, ranging between 26 and 32°C. The influence of temperature on the growth of other fish species, such as largemouth bass (*Micropterus salmoides*) and grass carp (*Ctenopharyngodon idella*), was also demonstrated on the same fish farm by Ouizgane et al (2017) and Farid et al (2019).

DO concentrations measured in the nine ponds show high and close average levels, exceeding a mean value of 9 mg L^{-1}, with variations ranging from 2.2 to 26.22 mg L^{-1}. Despite these fluctuations, which are influenced by several factors, including water temperature, atmospheric pressure, water transparency and agitation, as well as the presence of aquatic plants, algae and bacteria, low values are observed in the morning and higher values towards the end of the day, implying a diurnal period with DO concentrations highly favorable to growth. And even the lowest DO values recorded during the experiment were considered favorable for Nile tilapia rearing. Studies by Kestemont et al (1989) and Mélard (1999) have indicated that DO levels above 3 mg L^{-1} are considered optimal for the growth of Nile tilapia. In addition, the species has demonstrated tolerance to DO concentrations as low as around 0.1 mg L^{-1}, as reported by Mélard & Philippart (1981).

The mean pH values recorded in all ponds ranged from 7.39 to 8.74, with an exception in pond no. 2, where the pH reached a maximum of 9.21. These pH levels are within the range between 7 and 9 considered suitable for the growth of Nile tilapia, as indicated by Pouomogne et al (1998). Kestemont et al (1989) also confirmed that Nile tilapia can thrive in water with a pH between 5 and 11, showcasing the species' adaptability to varying pH conditions.

The chemical parameters during our experiment were found to be within the recommended ranges for the culture of Nile tilapia. The results of the chemical analyses, including NO_3^-, NO_2^-, NH_4^+, and PO_4^{3-}, were all within acceptable levels in the ponds. These parameters play a critical role in the fish's environment, as the concentration of nitrogenous waste (excreted by gills, urine, and faeces) depends on factors such as temperature, fish size, ammonia concentration, and feed quality. It is essential to maintain these values below the critical threshold for Nile tilapia. Adhering to these guidelines contributes to creating a suitable environment for optimal growth and minimizing potential adverse effects on the fish's health.

Studies by Beveridge & McAndrew (2000) recommend specific mean chemical analysis values during rearing, with suggested limits of 15 mg L^{-1} for nitrate, 2 mg L^{-1} for nitrite, 0.95 mg L^{-1} for ammonium ions, and 0.3 mg L^{-1} for orthophosphate, these limits being still far from the values recorded during our study.

Zootechnical performances. Regardless of the protein levels of the three feeds, we noticed a high and continuous growth for all fish. This could be explained by the fact that the three diets perfectly meet the different metabolic needs of Nile tilapia. The results obtained from the body growth (weight and size) indicate that fish fed with the 31% protein diet show higher zootechnical performance than fish fed with the other diets (28 and 26% protein).

The present study showed that the protein level influences the growth performance of Nile Tilapia. The mean weight gain, DGR and SGR were significantly improved with increasing protein levels from 26 to 31%. The best results were obtained with a dietary protein level of 31%. This is because the utilization of the total dietary protein incorporated, and the good digestibility of the feed, is a function of the increase in dietary protein levels up to 31%. Many authors have obtained similar results on the effect of dietary protein levels on the growth of Nile tilapia. The dietary protein requirement of several tilapia species has been estimated to be between 20 and 56% (El-Sayed & Teshima 1991). Siddiqui et al (1988) and El-Sayed & Teshima 1991) reported that the optimal level of dietary protein for growth in Nile tilapia fingerlings (initial weight 0.83 g) is 40-45% and it is 30% for fish weighing 40-250 g. And according to Wee & Tuan (1988) a diet containing around 27.5-35% protein seemed optimal for rearing Nile tilapia juveniles with a mean weight of 24 g. As well, Al Hafedh (1999) showed that larger tilapia (96-264 g) should be reared on a diet containing 30% protein. Bahnasawy (2009) demonstrated that a diet with 30% protein is considered economical and optimal for rearing Nile tilapia monosex fry with an mean weight of 2.5 g with fertilisation of rearing ponds. However, optimal growth performance of adult Nile tilapia fish (40.5 g $fish^{-1}$) was obtained with 35% and 45% protein diets with a non-significant difference ($p > 0.05$) (Ahmad et al 2004). Closer results from Hooley et al (2014) show that the greatest weight gain for Nile tilapia juveniles with an initial weight of 34.5 g was observed when the fish were fed 36% dietary protein. and for Nile tilapia weighing between 17 and 43 g, growth was affected by protein levels, with optimum performance at 35% according to Abdel-Tawwab et al (2010).

The FCR is a factor that provides information on feed quality. According to Craig et al (2017), the lower the FCR value, the better the feed utilization and conversion. For the three diets tested in this study, the best FCR was 1.22 for fish fed the 31% protein diet, reflecting better utilization and conversion of dietary protein for body growth. These results agree with those of numerous studies, which confirmed that better FCR values were obtained with increasing dietary protein levels up to certain thresholds. Bahnasawy (2009) proved a threshold of 30% protein, Wee & Tuan (1988) found that 42.5% protein had the best FCR, similarly Al Hafedh (1999) confirmed that FCR decreased progressively and linearly with increasing dietary protein level specifying that a protein content between 35 and 45% resulted in a better performing FCR than diets with 25 to 35% protein. In our experiment, the low FCR values recorded for the three diets can be explained, on the one hand, by the low stocking density of the fish during rearing and, on the other hand, by the abundance and/or nutritive quality of the algal biomass in the nine ponds. However, over the course of the experiment, the FCR showed a gradual increase from the first to the last sampling, due to the observed drop in the temperature of the rearing water (early autumn). This drop in temperature directly affects the way the fish feel and behave when feeding and can affect their digestion and absorption of nutrients. Tilapia, which are sensitive to temperature variations, react by slowing down their feeding when the temperature drops. This metabolic slowdown leads to a reduction in their appetite and activity, which can influence their FCR. To ensure optimum rearing performance, it is essential to carefully monitor the temperature of tilapia rearing waters and adapt feeding strategies accordingly during the autumn season.

The PER is used as an indicator of the quality of protein in a fish diet and the balance of amino acids (number and proportion). This parameter is therefore used to assess the use of dietary protein and its conversion into flesh gain; in other words, the profitability of breeding is also directly defined by this parameter. In our experiment, PER was inversely affected by dietary protein levels and reflects that protein utilization decreases with increasing dietary protein levels. The mean PER of fish fed the different diets (26, 28 and 31% protein) varied from 2.567±0.095 to 2.789±0.476. The fish fed the 26% protein diet had the highest mean PER, while fish fed the 28% protein diet had the lowest, although these values reflect the good quality of the diets tested as protein sources. The negative correlation between PER and dietary protein level has been demonstrated in the rearing of several fish species, including Nile tilapia (Jauncey & Ross 1982; Wee & Tuan 1988; Ahmad et al 2004; Bahnasawy 2009). Indeed, excess protein in diets would be used as energy (Kim et al 1991; Bahnasawy 2009). In addition, De Silva et al (1989) reported that PER and FCR decrease with increasing dietary protein levels.

***Survival rates**.* The values recorded for this parameter ranged between 96% and 97.67%. These consistently high rates signify the remarkable adaptability of Nile tilapia fish to the different diets used, as well as to the general conditions of our experiment. Furthermore, it is likely that the mortality recorded and the slight variations in survival rates may be attributed to stress induced during the sampling operations or potential predation by piscivorous birds present at the fish farm. Nonetheless, the overall survival rates remained impressively high.

Comparisons with studies conducted by Bahnasawy (2009) and Elfeki et al (2018) further validate our results, as they reported similar survival rates in their respective experiments. The remarkable resilience of Nile tilapia, as indicated by these survival rates, reaffirms its ability to thrive in various rearing conditions and dietary regimes.

***Economic analysis**.* Economic analysis at the end of experimental rearing of male Nile tilapia on three diets with different protein levels reveals valuable information on optimal cost management and increased profitability in rearing this species. The 26% protein diet stands out for its superior economic efficiency in converting feed into biomass. This observation suggests that a strategy reducing the level of protein in the feed can be economically advantageous without significantly affecting fish growth.

However, it is important to note that the 31% protein diets recorded higher net profits than the other diets, demonstrating that a higher protein intake, although associated with higher costs, can lead to an increase in revenue.

In terms of ROI, all three diets proved beneficial, but the 26% protein diet had the highest ROI. This indicates that, despite a lower gross profit, this diet offers the best cost-benefit ratio. This investment efficiency suggests that it may be preferable in contexts where reducing financial risk is crucial.

In conclusion, diet selection for Nile Tilapia should result from a thorough evaluation of costs, benefits and return on investment, while taking into account biological conditions and market dynamics. Tilapia production should adopt a flexible, well-informed strategy to maximize the profitability of the aquaculture operation. Such an approach will align farming practices with the long-term objectives of the business, ensuring economically viable and sustainable operations.

Conclusions. This research explored the impact of various protein levels (26%, 28%, and 31%) on the growth and survival of Nile tilapia, enriching our understanding of its nutritional requirements in an aquaculture setting. Through experimentation carried out in earthen ponds, supplemented by careful monitoring of water physico-chemical parameters, to study the feeding efficiency and adaptability of this species. Through experimentation carried out in earthen ponds and supplemented by careful monitoring of water physico-chemical parameters, we studied the feeding efficiency and adaptability of this species. Our observations confirm that the 31% protein diet favors the best zootechnical performance (WG, DGR, SGR, FCR), although the 26% protein diet has the highest PER.

Incorporating an economic perspective, our analysis revealed that, although the 26% diet offered the best economic efficiency in terms of cost per weight gained and a higher return on investment, the higher protein diets (28% and 31%) generated greater net benefits, highlighting a complex relationship between dietary composition, fish growth, and the economic viability of tilapia aquaculture. These results suggest that fine-tuning protein levels in diets can not only optimize the growth and survival of Nile tilapia, but also improve the profitability of its production.

In conclusion, our study highlights the crucial importance of protein content in diets for Nile tilapia, highlighting its significant impact on growth performance, survival, and feed conversion efficiency. The remarkable adaptability of this species to the various diets tested reflects its resilience and potential in aquaculture. In addition, the integration of in-depth economic analysis provides essential insights for sustainable and profitable aquaculture, directing practices towards the efficient production of high-value fish proteins to meet local and international market demands.

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Conflict of interest. The authors declare that there is no conflict of interest.

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