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Attempting to Optimize Nile Tilapia Growth Using Indigenous Crop-Based Feeds in Cooled Geothermal Groundwater Breeding Systems for Sustainable Aquaculture

Intento de optimizar el crecimiento de la tilapia del Nilo utilizando alimentos a base de cultivos autóctonos en sistemas de cría en aguas subterráneas geotérmicas refrigeradas para una acuicultura sostenible

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ABSTRACT

Background: In light of the growing global population, estimated to reach 9.7 billion by 2050, the aquaculture sector faces an increasingly urgent challenge to meet the escalating demand for high-quality protein sources. Nile Tilapia (*Oreochromis niloticus* L., 1758) is a pivotal candidate in the realm of aquaculture due to its adaptability, rapid growth, and excellent nutritional attributes. To achieve sustainable aquaculture expansion, it is imperative to develop efficient, eco-friendly, and cost-effective aquafeeds. **Methodology:** This study was carried out at the National Institute of Marine Science and Technology's experimental center in Béchima, Tunisia. The experiment involved the selection of male *O. niloticus* fry. Three distinct isoprotein and isoenergetic feeds were meticulously formulated, substituting conventional feed ingredients with alternative sources such as rapeseed meal, durum wheat, and field beans, while assessing the economic implications of these novel formulations. These feeds were designed to meet the essential dietary requirements for optimal fish growth. The study rigorously assessed various zootechnical parameters to gauge the performance of the fish under different feed formulations. **Results and Conclusion:** The results indicated that tilapia fed with the rapeseed-based feed exhibited remarkable growth performance, characterized by higher growth rates, elevated survival rates, and enhanced conversion rates. Within 80 days of rearing, these fish achieved an impressive average weight of 108.71 ± 5.55 g. In contrast, fish fed with the faba bean-based feed displayed suboptimal performance across multiple parameters. These findings highlight the pivotal role of feed formulation in bolstering the sustainability and productivity of aquaculture. This research significantly contributes to the ongoing endeavor to optimize aquafeeds, fostering food security and economic viability in aquaculture.

Keywords: *Oreochromis niloticus,* Feed formulation, geothermal water, faba bean, rapeseed.

RESUMEN

Antecedentes: A la luz de la creciente población mundial, que se estima alcanzará los 9,700 millones en 2050, el sector de la acuicultura enfrenta un desafío cada vez más urgente para satisfacer la creciente demanda de fuentes de proteínas de alta calidad. En este sentido, la tilapia del Nilo (*Oreochromis niloticus* L., 1758) es un excelente candidato en el ámbito de la acuicultura debido a su adaptabilidad, rápido crecimiento y excelentes atributos nutricionales. Para lograr una expansión sostenible de la acuicultura, es imperativo desarrollar alimentos acuícolas eficientes, ecológicos y rentables. **Metodología**: Este estudio se llevó a cabo en el centro experimental del Instituto Nacional de Ciencia y Tecnología Marinas en Béchima, Túnez. El experimento implicó la selección de alevines macho de *O. niloticus*. Se formularon meticulosamente tres alimentos isoproteicos e isoenergéticos distintos, sustituyendo los ingredientes de los alimentos convencionales por fuentes alternativas como harina de colza, trigo duro y habas, mientras se evaluaban las implicaciones económicas de estas nuevas formulaciones. Estos alimentos fueron diseñados para satisfacer los requisitos dietéticos esenciales para un crecimiento óptimo de los peces. El estudio evaluó rigurosamente varios parámetros zootécnicos para medir el rendimiento de los peces bajo diferentes formulaciones de alimento. **Resultados y conclusión**: Los resultados indicaron que la tilapia alimentada con alimento a base de colza exhibió un rendimiento de crecimiento notable, caracterizado por tasas de crecimiento más altas, tasas de supervivencia elevadas y tasas de conversión mejoradas. A los 80 días de cría, estos peces alcanzaron un elevado peso promedio de 108,71 ± 5,55 g. Por el contrario, los peces alimentados con pienso a base de habas mostraron un rendimiento subóptimo en múltiples parámetros. Estos hallazgos resaltan el papel fundamental de la formulación de piensos para reforzar la sostenibilidad y la productividad de la acuicultura. Esta investigación contribuye significativamente al esfuerzo continuo por optimizar los alimentos acuícolas, fomentando la seguridad alimentaria y la viabilidad económica en la acuicultura.

Palabras clave: *Oreochromis niloticus*, formulación de piensos, agua geotérmica, habas, colza.

INTRODUCTION

The global fisheries and aquaculture sector stand at the forefront of efforts to address the ever-increasing global demand for high-quality protein sources (FAO, 2020; Hua *et al.*, 2019). With a projected world population exceeding 9 billion by 2050, the necessity for optimizing food production is urgent, particularly in aquaculture (FAO, 2020). Within the realm of aquaculture, Nile Tilapia (*Oreochromis niloticus*) holds a special place due to its adaptability, rapid growth, and nutritional value (Hernández-Vergara *et al.*, 2018). The sustainable expansion of this sector is intricately linked to the development of innovative approaches, with particular emphasis on the formulation of aquafeeds (Magbanua and Ragaza, 2023, 2022; Syed *et al.*, 2022) Aquaculture has made significant contributions to global seafood production, with the Food and Agriculture Organization (FAO) reporting a consistent increase in global aquaculture production over recent years (FAO, 2023, 2022a). This upward trajectory is projected to continue, with aquaculture expected to supply a majority of the fish consumed by humans by 2030. As the industry expands, the optimization of aquafeeds becomes imperative (Tacon and Metian, 2015).

One of the central challenges in aquaculture lies in the formulation of efficient, eco-friendly, and cost-effective aquafeeds. Aquafeeds represent a substantial proportion of the operating costs in aquaculture, making the development of nutritionally balanced and sustainable feeds a top priority (Naylor *et al.*, 2021). Achieving economic and environmental sustainability in aquaculture necessitates the utilization of innovative dietary formulations, which align with the principles of the circular economy and environmental responsibility (Hua *et al.*, 2019; Paredes *et al.*, 2020).

Recent scientific studies have deepened our understanding of the dietary requirements of fish species, including Nile Tilapia. In particular, the pivotal role of essential nutrients such as proteins, lipids, carbohydrates, vitamins, and minerals in fish growth and overall health has been underscored by recent research (Hardy, 2010). Furthermore, advancements in feed technology have facilitated precise control over feed composition and nutrient delivery, enabling more effective dietary formulations tailored to the specific needs of fish species and the prevailing environmental conditions (Glencross *et al.*, 2023) substantial progress has been made in improving feeds and feeding technologies for most aquaculture species. Notable improvements in feed conversion efficiency (through a better understanding of requirements and improved feed management.

The fisheries and aquaculture sector in Tunisia play a vital role in the country's socio-economic landscape. Contributing approximately 8% to the value of agricultural production and 1.1% to the gross national product, this sector generates employment for around 53,000 individuals, fostering economic development and livelihoods (Ministry of Agriculture, Water Resources and Fisheries, 2016). However, per capita seafood consumption displays stark regional disparities, with inland regions significantly trailing coastal areas (FAO, 2022b).

Aquaculture in Tunisia has witnessed steady growth, with an annual average growth rate of 15.21% in 2019, when exchange rate fluctuations introduced a period of stability. As of 2019, current aquaculture production reached approximately 22 893 tones, contributing about 20% of Tunisia's total fish production (FAO, 2022b).

A recent study published by Mili *et al.* (2023) primarily focusing on the tilapia (*Oreochromis niloticus*) and its adaptation to geothermal waters in Tunisia, an area that has witnessed an agricultural revolution due to the utilization of geothermal water resources, stated that one noteworthy development within Tunisia's aquaculture landscape is the exploitation of dam reservoirs for fish farming, particularly in the country's interior regions. This extensive fish farming approach empowers local farmers to produce affordable fish, which can be sold or consumed locally. Various species are commonly fished in these regions, including carp, pikeperch, mullet, eel, catfish, barbel, and tilapia.

Aquaculture in geothermal water resources in Tunisia offers an emerging and promising avenue for sustainable fish farming. Geothermal waters have brought about significant agricultural advancements in the southern regions of Tunisia, but aquaculture in geothermal waters presents unique challenges, primarily related to water cooling and availability. Currently, only two active aquaculture projects in Tunisia are dedicated to the production of Nile Tilapia (*O. niloticus*) using geothermal waters (Mili *et al.*, 2023).

Nile Tilapia (*O. niloticus*) is a fascinating species with a rich history. Its natural distribution spans the African continent, covering the Nile basin and extending to central and western regions, including the Chad and Niger basins, as well as south to lakes Ethiopia and Turkana (Lévêque *et al.*, 2008). However, *O. niloticus* has transcended its native range, with numerous introductions and transfers to various African countries, such as Rwanda, Madagascar, Côte d'Ivoire, Cameroon, Tunisia, South Africa, Tanzania, and Libya, for the purpose of diversifying fish populations in natural lakes and dams, as well as for aquaculture development (Bonham, 2023).

Interestingly, *O. niloticus* has even reached at least 100 countries and has become among the main important aquaculture species globally (Shuai *et al.*, 2023). Amongst, it has been introduced in thermal water discharges of thermoelectric and electronuclear power plants, including the cooling water of the Thiang nuclear power plant. The adaptability of *O. niloticus* to diverse environments, including geothermal waters, underscores its significance in the context of aquaculture expansion and innovation (Mili *et al.*, 2023).

To ensure the economic viability of aquaculture, one of the key aspects to consider is the cost of feed. In typical aquaculture operations, feed expenses can account for a substantial portion of production costs, ranging from 30% to 60% in semi-intensive and intensive systems (O'Shea *et al.*, 2019). This cost factor necessitates innovative approaches to feed formulation that can reduce expenses while maintaining or even improving the growth performance of aquaculture species.

This study, conducted at state-of-the-art aquaculture research facilities, sets out to investigate the potential of innovative dietary formulations in optimizing the growth and health of Nile Tilapia (*Oreochromis niloticus*). It takes into account the most recent findings in the field, with an in-depth examination of the impact of these formulations on essential zootechnical parameters. Through rigorous experimentation and comprehensive data analysis, this research aims to identify the most effective dietary components and their influence on growth rates, conversion efficiency, and survival rates.

In an era when environmental sustainability and food security are paramount, the imperative to optimize aquaculture practices has never been more pronounced. Focused on optimizing aquaculture practices in Tunisia, this study seeks to evaluate the effects of replacing conventional feed ingredients, notably soybean, with locally sourced alternatives on the growth performance of Nile Tilapia in geothermal groundwater systems. By emphasizing cost-effective and nutritionally balanced feed formulations, our research aims to address the challenges posed by scarce soybean availability and high import costs. Furthermore, the utilization of geothermal groundwater is justified as a means to enhance water resource sustainability and mitigate water scarcity issues in aquaculture.

The findings of this research have the potential to revolutionize the aquaculture industry, offering valuable insights for aquaculturists, researchers, and the aquafeed industry. This research represents a pivotal step in the quest to harness the power of dietary formulations to enhance the growth, efficiency, and sustainability of Nile Tilapia aquaculture, aligning with the global drive towards eco-friendly and responsible food production practices.

MATERIAL AND METHODS

Study Location and setup. The research was conducted at the Tilapia breeding center in Bechima, ElHamma, Gabès, Tunisia, a collaborative project between the National Institute of Marine Science and Technology (INSTM) and the Technical Center for Aquaculture. This center spans approximately 10,000 square meters and features a gravity-based water supply system. The research center is strategically located near an artesian well that yields geothermal water.

Geothermal water, initially at temperatures between 40°C and 70°C, was cooled using a vertical cooling tower system. This system involved pumping the hot water to the top of a vertical structure equipped with obstacles. As the water descended, it lost thermal energy through evaporation and heat exchange, reducing the temperature to a more suitable range for tilapia growth (25°C to 28°C). The cooled water ensured optimal dissolved oxygen levels and minimized stress on the fish, thereby promoting better growth and health. In our study, the geothermal water used had the following initial properties: pH of 7.5-8.0, dissolved oxygen levels between 4-5 $mg.L^{-1}$, salinity of 1.5-2.0 PSU, and total ammonia levels below 0.1 $mg.L^{-1}$, dry residue of 2.98 $g.L^{-1}$, and key ions (32.5 $mg.L^{-1}$ of K^+, 708 $mg.L^{-1}$ of Na^+, 710 $mg.L^{-1}$ of Cl^-, 892.8 $mg.L^{-1}$ of SO_4^-, 18.9 $mg.L^{-1}$ of CO_3^-, and 141 $mg.L^{-1}$ of HCO_3^-). These parameters were monitored weekly to ensure consistency. Minor variations were observed, but the conditions remained within the tolerance range for Nile Tilapia as established by previous studies (Mili *et al.*, 2023). Monitoring these parameters was crucial as they directly affect the metabolic and homeostasis processes in Nile Tilapia, influencing growth performance and survival rates. The water quality parameters were maintained consistent with the values reported by Singha *et al.* (2021), emphasizing the importance of keeping optimal water quality in aquaculture systems.

The core units within the research center are the Breeding Unit and the Food Manufacturing Unit. The Breeding Unit, covering an area of 300 square meters, accommodates three shelter houses, each designated for various aquaculture activities, including breeding, larval rearing, and nursery functions. This unit also comprises two earthen basins with a unit volume of 100 cubic meters, dedicated to pre-fattening activities. The breeding process takes place in 12 raceway tanks, each with a capacity of 7 cubic meters, while larval rearing is conducted in square and cylindrical-conical tanks of varying sizes, ranging from 0.5 to 2 cubic meters.

The Food Manufacturing Unit is a vital component of the research, tasked with producing three distinct feed types tailored to specific phases of tilapia farming. These feeds are categorized based on their protein content, and the unit is equipped with essential machinery, including a hammer mill, a meat grinder, an electronic scale, and two food storage freezers.

Experimental Approach

Subjects Selection

Male fry of tilapia (*Oreochromis niloticus*) were carefully chosen as the subjects for this research. The selection criteria were based on an average weight of 16.89 ± 0.9 grams. These fry were accommodated in rectangular tanks, each having a unit volume of 400 liters, with a stocking density of 30 individuals per tray.

Physico-Chemical Parameters

Throughout the course of the experiment, each tank was subjected to a continuous water flow of approximately 10 $L.min^{-1}$, ensuring consistent water exchange. Several physico-chemical parameters were meticulously monitored. The dissolved oxygen level was maintained above 3 $mg.L^{-1}$, while the water temperature was regulated within the range of 25 to 30°C, achieved by adjusting the inlet water flow to the breeding tanks. Both dissolved oxygen and temperature were measured using a multiparameter device of type Multi 3620 IDS (WTW). The photoperiod was naturally maintained, with uniform 12h-13h/day lighting conditions observed in all tanks. The rearing tanks were siphoned daily before the first feeding to remove fecal matter. The tanks were completely emptied and thoroughly cleaned every two weeks.

Formulation of Experimental Diets

The nutritional requirements of growing tilapia fry were met through the formulation of three isoprotein and isoenergetic feeds. These feeds were designed to deliver approximately 31% crude protein, 8% crude fat, and 14 $Kj.g^{-1}$ of crude energy. The selection of ingredients adhered to a series of critical criteria, encompassing chemical composition, availability, purchase price, and sustainability. The chosen ingredients included fishmeal (FP), soybean meal (TS), corn (M), rapeseed meal (TC), field beans (F), and durum wheat (B), all sourced from local suppliers.

The feed formulations in this study were specifically designed not only for cost-effectiveness but also to ensure nutritional adequacy under the unique conditions presented by geothermal water, such as potential variations in dissolved oxygen and mineral content. To reduce the cost of exotic Ingredients, mainly soybean which is not produced locally and not regularly available in local markets, native fodder crops like faba beans, rapeseed and durum wheat were chosen for their nutritional profiles and potential to support fish health under these conditions.

In our study, the commonly used formulation, Feed 1, was adopted as the benchmark diet. This formulation is widely used by tilapia breeders in the region. Based on Feed 1, we developed two isoprotein formulations (Feeds 2 and 3), where we partially substituted some ingredients, primarily soybean meal, with locally available crops. This strategy was designed to mitigate the high costs and limited availability of imported ingredients, thereby promoting more sustainable and eco-friendly aquaculture practices.

The feed formulation process involved finely grinding all ingredients into a powder, which was then combined with a vitamin-mineral supplement (CMV) and soybean oil. Water was gradually added to achieve the desired consistency. This mixture was subsequently processed through a kitchen meat grinder, extruded through 3 mm holes, and sun-dried for a period of 24 hours. The dry feed was stored in polyethylene bags at a temperature of -20°C.

NIR Spectrometry estimation of the diets nutritional composition

The determination of feed composition was performed by Techna Tunisia Lab utilizing the advanced Allix³ software. Prior to the formulation of feeds, a comprehensive analysis of the nutritional attributes of individual ingredients and the combined composition of the three formulated feeds was conducted. This analysis leveraged Near-Infrared Spectroscopy (NIRS), calibrated with an extensive dataset of feedstuff raw materials, including the specific ingredients under consideration. NIRS facilitated the accurate prediction of essential parameters, encompassing Dry Matter, Crude Proteins, Crude Fats, Crude Fibers, Ash, Calcium, Phosphorus, Total Energy, and Essential Amino Acids. The reliability and precision of these predictions were rigorously validated using robust Partial Least Square (PLS) regression models, ensuring the utmost accuracy for each assessed parameter.

Experimental design and zootechnical Parameters

The experimental design followed a completely randomized layout, comprising a total of 9 fish batches labeled T1 to T9. Each batch was housed in a tank and fed one of the tested diets (Control, Feed1, Feed2). Importantly, each diet was presented in triplicates, with three tanks randomly assigned to each diet.

Feeding occurred four times daily, initially at a rate of 5% of their biomass until reaching 30 g in weight (after 4 weeks), followed by a reduced rate of 4% for the remaining duration of the 81-day experiment. The specific daily feed quantities per tank throughout the experimental period are outlined in Table S1.

Regrettably, a significant mortality event resulting from thermal shock transpired after the first month, impacting tanks T3, T6, and T7 (Table S1). These tanks were situated adjacent to each other, and the thermal shock was an isolated incident affecting only these specific tanks on one side of the setup. The thermal shock was related to issues in water aeration and temperature management in those particular tanks and had no relation to the type of feed formulation being tested. Consequently, biometric assessments from day 52 onward were conducted with two replicates per treatment instead of the initial three.

To evaluate the performance of fish growth, several key zootechnical parameters were calculated, serving as crucial indicators for the research outcomes.

Survival rate (SC): Survival rate (equation 1) is calculated as the percentage of the final number of individuals compared to the initial stocking. This parameter provides insights into the overall health and adaptability of the tilapia fry.

$$SC = \frac{Final\ Number\ of\ Fish}{Initial\ Number\ of\ Fish} \times 100 \qquad \text{equation (1)}$$

Conversion rate (TC): The conversion rate (equation 2) indicates the efficiency of converting the provided feed into biomass and is calculated as the quantity of feed distributed divided by the difference between the final and initial biomass.

$$TC = \frac{Quantity\ of\ Feed\ Distributed}{Final\ Biomass\text{-}Initial\ Biomass} \qquad \text{equation (2)}$$

Daily growth rate (DBR): The daily growth rate measures the change in average weight per day, providing a precise indication of growth rates.

$$DBR = \frac{Final\ Mean\ Weight\text{-}Initial\ Mean\ Weight}{Ageing\ Time\ (Days)} \qquad \text{equation (3)}$$

Specific growth rate (SSR): Expressed as a percentage, the specific growth rate is used to assess growth relative to the duration of rearing (equation 4). It offers insights into the growth efficiency of the tilapia fry.

$$SSR = \frac{100}{Duration\ of\ Rearing} \times log_{10}\left(\frac{Final\ Biomass}{Initial\ Biomass}\right) \qquad \text{equation (4)}$$

Table S1. Quantity of feed (g) distributed daily during the experiment

Feeds	Tanks/ day	D_0 - D_{14}	D_{15} - D_{28}	D_{29} - D_{52}	D_{53} - D_{66}	D_{67} - D_{81}
	T4	377.34	413.91	607.72	856.24	1245.44
Feed 1	T9	377.34	413.91	602.4	820.8	1244.32
	T3	377.34	413.91	-	-	-
	T1	377.03	404.11	521.4	752.64	1046.08
Feed 2	T5	377.03	404.11	524.6	722.07	1008
	T7	377.03	404.11	-	-	-
	T2	377.55	493.02	610.08	442.176	616
Control	T8	377.55	493.02	678	948.08	1272.32
	T6	377.55	493.02	-	-	-

Statistical Analysis. The data collected throughout the research were subjected to statistical analysis, which was performed using the GraphPad Prism 5.0 software. The analytical approach encompassed variance assessments of weight monitoring, quantities of food distributed per bin, and the number of fish under investigation. The verification of the normal distribution of the dataset was computed *via* Shapiro-Wilk test ($P>0.05$). To examine the effect of different food types over different dates, a two-way ANOVA procedure was employed, followed by the Bonferroni test for comparing means, with a significance level set at $P<0.05$. The analysis also involved the comparison of means *via* the Bonferroni test following a one-way ANOVA, exploring variations attributed to the impact of different food types on conversion rates, survival rates, and calculated growth rates. A significance level of $P<0.05$ was employed as the threshold for identifying statistically significant results.

RESULTS

Nutritional Composition Patterns of Fish Feed Ingredients

The nutritional composition table sheds light on the diverse ingredients used in fish feed formulations, emphasizing soybean meal, rapeseed meal, faba bean, and wheat. Soybean meal stands out with a substantial 42.86% protein content, positioning it as a crucial protein source. Rapeseed meal follows closely, boasting a noteworthy 36.16% protein content. Faba bean contributes significantly with a commendable 29.12% protein content, while durum wheat provides a moderate protein supply at 13.05%.

In terms of lipid content, soybean meal is lean with 1.38%, whereas rapeseed meal contributes more at 2%. Faba bean and durum wheat both supply moderate lipids, registering at 2.56% and 2.51%, respectively.

Analyzing mineral composition, soybean meal has a moderate ash content of 6.80%. Rapeseed meal demonstrates a richer mineral composition with 7.41% ash. Faba bean leads with the highest ash content at 13.47%, emphasizing its significant mineral contribution. Durum wheat aligns closely with faba bean at 13.47%.

Examining the amino acid profile, soybean meal provides a balanced composition with essential amino acids like lysine (2.62%), valine (1.34%), and leucine (3.83%). Rapeseed meal contributes slightly lower amounts. Faba bean showcases a notable amino acid profile, including lysine (2.12%), valine (1.71%), and leucine (2.56%). Durum wheat, with a comparatively lower amino acid profile, necessitates strategic blending with other ingredients for optimal nutrition.

These insights underscore the importance of comprehending the distinct nutritional attributes of each ingredient, enabling strategic feed formulation to enhance fish growth and overall health.

Given the nutritional insights derived from the previous composition analysis (Table 1), we aimed to formulate isoprotein and isoenergetic fish feeds. The formulations, outlined in the Table 2, maintain consistency in protein and energy levels while strategically adjusting the proportions of key ingredients. The variations in soybean meal, rapeseed meal, faba bean, and wheat content reflect an intentional effort to optimize nutritional patterns and achieve a balanced and tailored feed composition for enhanced fish growth and health.

This feed formulation aims to capitalize on the distinct nutritional attributes of each ingredient, aligning with the observed patterns in the nutritional composition analysis. The goal is to create feeds that not only meet the dietary requirements of the fish but also leverage the unique contributions of each component for optimal growth and overall health.

Table 1. Approximate composition (expressed as % dry matter) and essential amino acid profile of ingredients used in test foods

(% DM)	Fish meal	Soybean Meal	Maize	Rapeseed meal	Faba bean	Durum wheat
Dry matter	91.92	90.85	90.04	90.25	90.78	90.86
Crude Proteins	47.63	42.86	7.58	36.16	29.12	13.05
Crude Fats	3.58	1.38	1.72	2	2.56	2.51
Crude Cellulose	1.86	6.60	7.13	12.98	11.31	12.32
Ash	28.59	6.80	1.16	7.41	6.88	13.47
Calcium	6.62	0.31	0.03	0.72	0.39	0.61
Phosphorus	4.29	0.68	0.26	1.07	0.53	1.23
Total Energy ($Kj.g^{-1}$)	14.08	16.08	14.83	14.18	14.69	14.92
Lysine	4.63	2.62	0.14	2.15	2.12	0.28
Valine	4.18	1.34	0.26	1.75	1.71	0.60
Leucine	4.44	3.83	0.73	2.38	2.56	0.91
Histidine	1.23	1.01	0.15	0.92	0.64	0.33
Arginine	3.65	2.48	0.32	2.45	2.01	0.47
Threonine	3.18	1.69	0.17	1.61	1.37	0.32
Isoleucine	2.32	1.38	0.29	1.47	1.77	0.53
Methionine + cysteine	2.49	0.59	0.20	0.86	0.31	0.52
Phenylalanine	2.29	1.85	0.47	1.61	1.49	0.64

The Table 2 illustrates the composition of these three feed formulations (% dry weight), namely the Control, Feed 1, and Feed 2. In terms of macronutrients, Feed 1 stands out with a slightly higher dry matter content (89.44%) compared to the Control (88.66%) and Feed 2 (88.72%). Feed 1 also exhibits the highest crude protein content (32.45%), followed by the Control (32.19%) and Feed 2 (30.68%). Concerning crude fats, Feed 1 surpasses the other formulations with 8.69%, while the Control and Feed 2 have 7.94% and 7.71%, respectively.

When examining fiber content, Feed 1 presents the highest level (4.03%), indicating a greater inclusion of fibrous materials. In terms of ash content, all formulations are relatively close, with Feed 2 having the lowest ash content (6.38%).

Table 2 Approximate composition (expressed as % dry matter) and essential amino acid profile of the foods tested

Components (% dry weight)	Control	Feed 1	Feed 2
Soybean Meal	45	34.4	35
Fish Meal	14	14	14
Maize	36	17.6	25
Durum Wheat	0	15	11
Rapeseed Meal	0	15	0
Faba Bean	0	0	10
Soybean Oil	4	3	4
Mineral Vitamin Supplement (MVS)	1	1	1
Dry Matter	88.66	89.44	88.72
Crude Proteins	32.19	32.45	30.68
Crude Fats	7.94	8.69	7.71
Crude Fibers	2.75	4.03	3.23
Ash	6.53	6.9	6.38
Calcium	1.14	1.23	1.13
Phosphorus	0.76	0.83	0.76
Total Energy (Kj.g^{-1})	13.92	13.92	13.61
Essential Amino Acids (g.Kg^{-1})			
Lysine	19.5	19	13.8
Valine	15.3	15.4	14.4
Leucine	24.5	23.7	22.8
Histidine	8.7	8.7	8.3
Arginine	22.6	22	21.8
Threonine	12.5	12.7	11.7
Isoleucine	14.2	14	13.3
Methionine + Cysteine	10.2	11	9.5
Phenylalanine	15.4	15.1	14.4

Calcium and phosphorus levels are similar across formulations, with Feed 1 showing a slight increase in calcium (1.23%) compared to the Control (1.14%) and Feed 2 (1.13%). Phosphorus content remains consistent, ranging from 0.76% to 0.83%.

The total energy content (Kj.g^{-1}) is comparable among the formulations, with Feed 1 and the Control both at 13.92 Kj.g^{-1}, and Feed 2 slightly lower at 13.61 Kj.g^{-1}.

Essential amino acid profiles reveal variations among formulations. Feed 1 contains higher amounts of lysine (19 g.Kg^{-1}), valine (15.4 g.Kg^{-1}), leucine (23.7 g.Kg^{-1}), and arginine (22 g.Kg^{-1}) compared to the Control and Feed 2. Feed 2, on the other hand, shows higher methionine + cysteine (9.5 g.Kg^{-1}) and phenylalanine (14.4 g.Kg^{-1}) levels compared to the Control and Feed 1. In light of the nuanced nutritional profiles observed in the ingredients, we endeavored to create isoprotein and isoenergetic feed formulations. The proportions of key components in these formulations are detailed in the Table 2. The variations in soybean meal, rapeseed meal, faba bean, and wheat content reflect an intentional effort to optimize nutritional patterns and achieve a balanced and tailored feed composition for enhanced fish growth and health. Subsequently, these formulations underwent an 80-day trial, where their impact on biometric and zootechnical traits in Nile tilapia was meticulously assessed.

Effect of dietary formulation substitutions on the growth performance of Nile Tilapia

Evolution of the Average Weight

The evolution of the average weight of fish during the experimental period is a critical indicator of the success of the dietary formulations (Fig. 1). The initial average weight of 16.89±0.9 g significantly increased, reaching 36.5±7.09 g within the first month of rearing. The subsequent growth patterns showed notable distinctions between the dietary groups. After 80 days of rearing, fish fed with feed 1 (rapeseed) reached an average weight of 98.3±2.63 g, while those fed with feed 2 (faba bean) reached 108.71±5.55 g. In contrast, fish fed the control feed achieved an average weight of 110.47±7.9 g. These observations underline the impact of different dietary formulations on the growth of Nile tilapia.

The substantial weight gain in the first month demonstrates the active feeding behavior and adaptability of the fish to the provided rations. However, statistical analysis reveals significant differences in the average weights among the various dietary groups, indicating that diet plays a pivotal role in the growth of tilapia fry. The Bonferroni test further illustrates the significance of these differences, with comparable average weights among all feeds for the initial 51 days of rearing. Thereafter, a clear divergence emerges, with fish fed the control and feed 1 surpassing those fed with feed 2. These findings highlight the influence of diet on the growth trajectory of Nile tilapia.

Zootechnical Parameters

The survival rate of fish is a critical parameter in aquaculture, as it directly impacts the success and profitability of fish farming. The results of this study indicate notable variations in survival rates among the different dietary groups (Fig. 2-A). Fish fed with feed 1 (rapeseed) and the

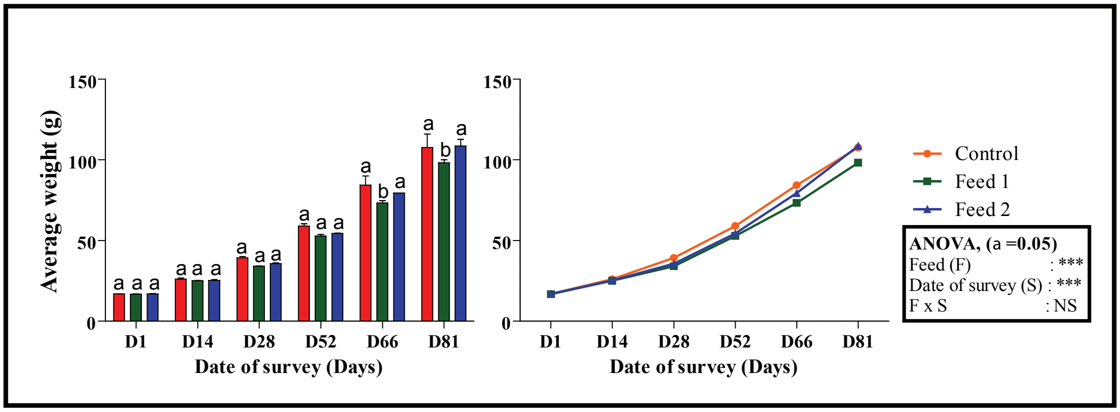

Figure 1. Evolution of average weights within Tilapias fed by the three different feeds during the experiment period. Values represent averages with different letters (a, b) indicating significant differences according to Bonferronni test P<0.05.

control feed exhibited high survival rates of 93.33%, whereas those fed with feed 2 (faba bean) had a lower survival rate of 83.33%. Statistical analysis confirms the significant difference in survival rates between the dietary groups, underscoring the impact of diet on the survival of tilapia fry. The reduced survival rate in the feed 2 group suggests the importance of dietary composition in ensuring the health and viability of the fish. Feed 2, which included faba beans, resulted in the highest weight gain but also showed increased mortality rates. This can be attributed to the presence of anti-nutritional factors such as tannins and vicine in faba beans, which can impair nutrient absorption and overall fish health. These anti-nutritional compounds may have contributed to the observed mortality, highlighting the need for processing methods to mitigate their effects in future formulations.

Another crucial zootechnical parameter in aquaculture is the feed conversion rate (FCR), which indicates the efficiency of converting feed into fish biomass. In this study, FCR varied among the dietary groups. The control feed exhibited an FCR of 1.48±0.14, the rapeseed feed had an FCR of 1.38±0.07, and the field bean feed showed the highest FCR at 1.57±0.02 (Fig. 2-B). Statistical analysis confirms the significant effect of diet on FCR, emphasizing the role of dietary composition in the utilization of feed. The highest FCR in the field bean feed group suggests potential inefficiencies in converting feed into fish biomass compared to the other diets.

Daily growth rates (DGR) provide insights into the rate of weight gain in fish and are essential for assessing the performance of dietary formulations. Fish fed with the rapeseed feed exhibited a daily growth rate of 1.15±0.07 g.day^{-1}, slightly higher than the control feed (1.14±0.14 g.day^{-1}), while the field bean feed group showed a lower daily growth rate of 1.02±0.03 g.day^{-1} (Fig. 2-C). Statistical analysis highlights the significant difference in daily growth rates between the dietary groups, indicating the profound impact of diet on the growth performance of tilapia fry.

Specific growth rates (SGR) offer a measure of the relative growth of fish and provide further insights into the impact of diet on growth. The specific growth rates were comparable among the three dietary treatments. Grown fish fed with the rapeseed and control feeds recorded SGR of 2.45±0.03 day, while those fed with the field bean feed achieved an SGR of 2.39±0.01 day (Fig. 2-D). These results indicate that specific growth rates were consistent across the dietary groups, suggesting that this particular parameter was less influenced by dietary composition.

The economic implications of the dietary formulations were also examined, revealing differences in the cost of ingredients between the diets. The control feed's ingredients were found to be 5.95% more expensive than the rapeseed-based feed and 5.03% higher than the field bean feed. This cost analysis underscores the potential economic advantages of certain dietary formulations in aquaculture practices.

Correlation Analysis: Relationships Between Feed Composition and Nile Tilapia Performance Parameters. The correlation matrix Table 3 presents a detailed correlation matrix illustrating the relationships between various biometric and zootechnical parameters (weight, survival rate "SR", feed conversion rate "FCR", daily growth rate "DGR", and specific growth rate "SGR") and the composition of fish feed. The correlation coefficients indicate the strength and direction of these relationships, providing valuable insights into how different feed components influence fish growth and health.

Positive correlations were observed between weight and several feed components, including dry matter (0.4389), crude proteins (0.5571), crude fats (0.9567), and total energy (Kj.g^{-1}) (0.8462). These findings suggest that higher levels of these feed components are associated with increased weight gain in Nile Tilapia. For instance, the strong correlation between crude fats and weight (0.9567) indicates that higher fat content in the diet significantly enhances weight gain. Similarly, the positive correlation with crude proteins (0.5571) highlights the essential role of protein in fish growth.

Survival rate (SR) exhibited strong positive correlations with several feed components, particularly crude proteins (0.9907) and total energy (0.8462). This suggests that diets rich in proteins and energy not only support growth but also improve fish survival, possibly by boosting their overall health and immune response.

Daily growth rate (DGR) showed extremely high positive correlations with crude proteins (0.9991), total energy (0.9956), and specific growth rate (SGR) (0.9989). These near-perfect correlations underscore

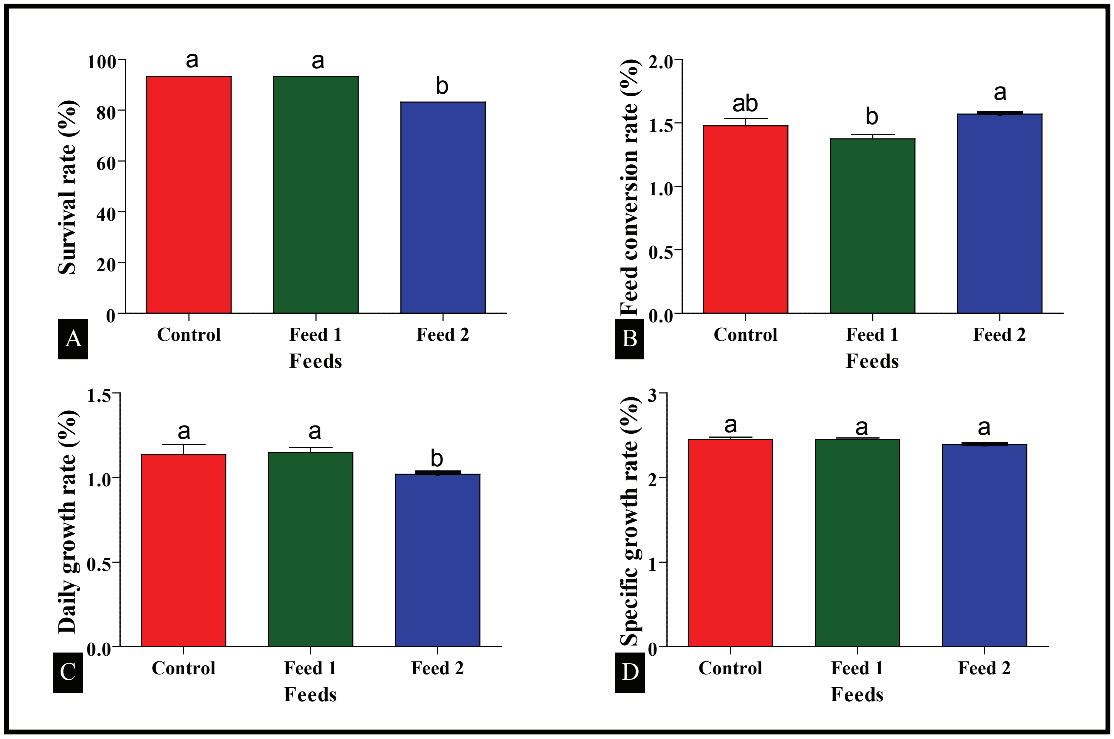

Figure 2. Effects of feed formulations on Tilapia's zootechnical attributes; Values represent averages with different letters (a, b) indicating significant differences according to Bonferronni test P<0.05.

the critical role of protein and energy in daily growth increments and overall growth efficiency. This implies that optimizing these nutrients in the diet can lead to maximal growth rates.

Conversely, feed conversion rate (FCR) demonstrated strong negative correlations with most feed components, including dry matter (-0.8404), crude proteins (-0.9183), crude fats (-0.9618), and total energy (-0.8559). The strong negative correlation between FCR and weight (-0.9878) indicates that as feed conversion efficiency improves (lower FCR), weight gain increases. This is a crucial finding, suggesting that diets rich in proteins and fats not only promote growth but also improve feed efficiency.

Specific feed components, such as dry matter, crude proteins, and crude fats, showed strong positive correlations with multiple performance parameters, underscoring their significant influence on fish growth and health. For example, the positive correlation between specific growth rate (SGR) and both crude proteins (0.4805) and total energy (0.8462) highlights the importance of these nutrients in achieving high growth rates.

Overall, the correlation matrix reveals the complex relationships between feed composition and the performance of Nile Tilapia, emphasizing the importance of specific nutrients in achieving optimal growth and health outcomes. By understanding these relationships, we can better optimize feed formulations to enhance growth performance and sustainability in aquaculture practices, particularly in geothermal groundwater systems.

DISCUSSION

Addressing the imperative for sustainable aquaculture, this study focuses on optimizing feed formulations for Nile Tilapia in geothermal aquaculture settings, emphasizing economic viability and enhanced food production within Tunisia's aquaculture sector. The exploration of locally sourced feed ingredients, such as rapeseed meal, durum wheat, and field beans, seeks to reduce costs while potentially improving growth performance. By inspecting the substitution of conventional feed components with these alternatives, we evaluate the economic implications of novel formulations. The study's outcomes offer crucial insights into the utilization of locally available protein sources derived from plant-based ingredients to bolster fish growth in sustainable aquaculture systems. In examining both economic and environmental dimensions, this research aligns with global initiatives promoting eco-friendly and sustainable food production practices, fostering advancements in Tunisia's aquaculture sector.

The variations in raw material proportions within the formulated feeds underscore the necessity of precision in feed formulation. The positive correlation between crude protein content in the feeds and key growth parameters is consistent with existing literature (Ng and Romano, 2013; Singha *et al.*, 2021). Rapeseed meal, a prominent ingredient in our formulations, has been acknowledged for its high protein content and balanced amino acid profile (Kaiser *et al.*, 2022). The notable growth advantage observed in fish fed rapeseed-based feed aligns with studies emphasizing the significance of protein quality in promoting growth (Han *et al.*, 2022; Kaiser *et al.*, 2022; Zhang *et al.*, 2023).

Table 3. Correlations Between Feed Composition and Nile Tilapia Performance Parameters

	Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	Dry Matter	**1**																					
2	Crude Proteins	0.557	1																				
3	Crude Fats	0.957	0.775	**1**																			
4	Crude Fiber	0.952	0.276	0.822	**1**																		
5	Ash	0.938	0.810	**0.998**	0.787	**1**																	
6	Calcium	0.987	0.682	0.991	0.891	0.981	**1**																
7	Phosphorus	**0.998**	0.613	0.974	0.929	0.960	0.996	**1**															
8	Total Energy	0.439	0.991	0.682	0.143	0.723	0.577	0.500	**1**														
9	Lysine	0.366	0.977	0.621	0.064	0.666	0.510	0.430	0.997	**1**													
10	Valine	0.519	**0.999**	0.745	0.232	0.782	0.648	0.577	0.996	0.986	**1**												
11	Leucine	-0.035	0.810	0.257	-0.339	0.313	0.125	0.034	0.882	0.917	0.836	**1**											
12	Histidine	0.439	0.991	0.682	0.143	0.723	0.577	0.500	**1.000**	0.997	0.996	0.882	**1**										
13	Arginine	-0.343	0.589	-0.055	-0.614	0.003	-0.189	-0.277	0.693	0.748	0.625	0.951	0.693	**1**									
14	Threonine	0.601	**0.999**	0.808	0.327	0.840	0.721	0.655	0.982	0.964	0.995	0.778	0.982	0.545	**1**								
15	Isoleucine	0.239	0.939	0.511	-0.070	0.560	0.391	0.305	0.977	0.991	0.954	0.962	0.977	0.830	0.920	**1**							
16	Methionine + Cysteine	0.850	0.911	0.967	0.648	0.980	0.923	0.885	0.846	0.801	0.891	0.496	0.846	0.203	0.932	0.714	**1**						
17	Phenylalanine	0.157	0.908	0.438	-0.153	0.489	0.313	0.225	0.956	0.976	0.926	0.981	0.956	0.874	0.884	0.997	0.653	**1**					
18	Weight	-0.988	-0.680	-0.990	-0.893	-0.981	**-1.000**	-0.996	-0.573	-0.507	-0.645	-0.121	-0.573	0.193	-0.718	-0.387	-0.922	-0.309	**1**				
19	SR	0.439	0.991	0.682	0.143	0.723	0.577	0.500	**1.000**	0.997	0.996	0.882	**1.000**	0.693	0.982	0.977	0.846	0.956	-0.573	**1**			
20	FCR	-0.840	-0.918	-0.962	-0.634	-0.976	-0.916	-0.876	-0.856	-0.812	-0.899	-0.512	-0.856	-0.221	-0.938	-0.727	**-1.000**	-0.667	0.915	-0.856	**1**		
21	DGR	0.521	**0.999**	0.747	0.234	0.784	0.650	0.579	0.996	0.985	**1.000**	0.835	0.996	0.623	0.995	0.953	0.892	0.925	-0.647	0.996	-0.900	**1**	
22	SGR	0.480	0.996	0.715	0.189	0.754	0.614	0.540	**0.999**	0.992	**0.999**	0.860	**0.999**	0.659	0.990	0.966	0.870	0.942	-0.611	**0.999**	-0.879	**0.999**	**1**

Coefficients in bold denote significant correlations according to Pearson test (P<0.05)

In-depth investigations into the interplay of raw material proportions reveal that optimal growth is not solely contingent on protein content but also on the synergistic effects of other nutrients. The variations in lipid content, amino acid profiles, and mineral composition contribute to the observed differences in growth trajectories among the formulated feeds. This aligns with recent studies highlighting the multifaceted nature of feed formulation and the need for a holistic approach to achieve optimal growth (Miles and Chapman, 2006; Rahimnejad *et al.*, 2021).

Beyond proximate analyses, the nature of raw materials plays a pivotal role in shaping the nutritional landscape of formulated feeds. The inclusion of durum wheat introduces a carbohydrate source with implications for energy availability and utilization. Carbohydrates, often an underrated component in aquafeeds, have been shown to influence growth performance and feed utilization efficiency. Our findings underscore the importance of considering the energetic contributions of raw materials, shedding light on the nuanced interactions that influence overall growth dynamics (Rutegwa *et al.*, 2019).

Faba bean, rich in essential amino acids and minerals, contributes to the nutritional diversity of the formulated feeds (Martineau-Côté *et al.*, 2022). This aligns with the emerging perspective that the inclusion of multiple protein sources with complementary amino acid profiles can optimize growth performance (Ng and Romano, 2013). The observed differences in growth patterns among the feeds emphasize the need for a comprehensive understanding of the nutritional attributes of each raw material, transcending traditional proximate analyses.

Cereals such as wheat make up a significant proportion of the dietary carbohydrates in animal feeds, so optimizing the use of these carbohydrates can improve their effectiveness in aquaculture (Singha *et al.*, 2021). The inclusion of rapeseed and faba beans aims to provide high quality protein and other essential nutrients that are critical for fish growth and health. This is consistent with previous research highlighting the importance of protein quality in aquafeeds (Gule and Geremew, 2022). These ingredients are not only cost effective but also locally sourced, reducing the carbon footprint associated with feed transport and supporting local economies. Sustainable feed formulations help reduce reliance on conventional protein sources such as fishmeal, which have a higher environmental impact and are subject to overfishing concerns (Hussain *et al.*, 2024). Despite advances in fish nutrition and the development of species-specific diets that promote optimal growth and healthy fish production, there are still gaps in understanding the precise nutrient requirements and efficient feeding strategies for tilapia, especially in regions such as Africa. In addition, the effects of additives such as enzymes, hormones and pre/probiotics in tilapia diets show mixed results, with some studies reporting significant improvements in growth and nutrient digestibility, while others show minimal effects (Consuegra *et al.*, 2023). These discrepancies therefore highlight the importance of continued research to optimize feed formulations and feeding strategies.

The sustainability of aquaculture practices hinges not only on economic viability but also on the environmental footprint of feed formulations. The use of locally sourced ingredients, as exemplified in our study, aligns with the broader agenda of sustainable aquaculture (FAO, 2020). Rapeseed meal, in particular, has been recognized for its potential as a sustainable protein source with a lower environmental impact compared to traditional fishmeal (Kaiser *et al.*, 2022). This resonates with global initiatives advocating for the responsible use of feed resources in aquaculture (FAO, 2022a).

The adoption of alternative ingredients in tilapia feeds offers a pathway to reduce reliance on conventional protein sources, contributing to the overall resilience and sustainability of the aquaculture sector. The economic advantages, coupled with positive growth outcomes, position these formulations as viable contenders in the quest for environmentally responsible feed practices (FAO, 2022b; Ministry of Agriculture, Water Resources and Fisheries, 2016).

While faba bean-based feed resulted in the highest weight gain, the associated high mortality rates make it less desirable. In contrast, the rapeseed-based feed provided a balance of good growth performance, high survival rates, and overall fish health. Therefore, we conclude that rapeseed is a more reliable ingredient for sustainable aquafeeds.

The superior feed conversion efficiency observed in the rapeseed-based diet aligns with contemporary discussions on the economic viability of aquafeeds. The cost-effectiveness of alternative ingredients, particularly rapeseed meal, positions them as attractive options for aquaculture operations seeking to optimize production costs without compromising growth performance (Tacon and Metian, 2015). The economic impact of the diets was calculated by comparing the cost of ingredients and the overall feed conversion ratio (FCR). Costs were analyzed based on local market prices, and the FCR was used to determine the efficiency of each diet in converting feed into biomass. This analysis demonstrated that the rapeseed-based diet offered the most cost-effective solution, combining lower ingredient costs with efficient feed conversion, thereby supporting its recommendation for sustainable aquaculture practices. The economic implications of such formulations resonate with the broader industry goal of achieving sustainable intensification in aquaculture (Mili *et al.*, 2023).

While our study demonstrates the potential of alternative feeds, it is essential to acknowledge the challenges associated with their adoption. Variability in raw material quality, availability, and potential anti-nutritional factors necessitates a meticulous approach to sourcing and processing (Ng and Romano, 2013; Tacon and Metian, 2015). Future studies should consider comparing the experimental diets with traditional fish meal-based diets to further validate the findings. Furthermore, the influence of water quality, system dynamics, and fish behavior on feed utilization warrants continuous scrutiny for a comprehensive understanding of growth determinants (Mili *et al.*, 2023). The comparison of the experimental diets with a commercial diet adds significant value to the discussion on environmentally friendly and responsible food production. In the context of optimizing feed formulations for Nile tilapia, our present study shows that the rapeseed-based diet not only promotes comparable growth rates but also exhibits superior feed conversion efficiency, highlighting its potential as a cost-effective and environmentally friendly alternative. This finding is in line with global initiatives to promote sustainable aquaculture practices by reducing reliance on conventional protein sources such as fishmeal, which have a higher environmental impact (Boyd *et al.*, 2020). In addition, local sourcing of ingredients such as rapeseed meal supports economic viability and reduces the carbon footprint associated with feed transport (Sarker, 2023). In another context, the health benefits associated with omega-3 long-chain polyunsaturated fatty acids are well documented, with regular consumption linked to a reduced risk of cardiovascular disease (Tacon *et al.*, 2020). However, the inclusion of terrestrial vegetable oils, a common alternative to fish oil, has led to a significant reduction in omega-3, negatively impacting the nutritional value of farmed fish (Napier and Betancor, 2023).

CONCLUSION

This study delves into aquafeed formulations, spotlighting raw material proportions' nuanced impact on Nile Tilapia's growth. Local ingredients like rapeseed meal, durum wheat, and faba bean show promise for sustainable aquaculture. Optimizing feed formulations becomes key for economic and environmental sustainability amid global seafood demands. Ongoing research and innovation are crucial for refining feed practices and advancing aquaculture resilience.

This integrated exploration spans Nile Tilapia adaptation to geothermal waters and formulating cost-effective aquafeeds, contributing significantly to sustainable aquaculture. Balancing food security and environmental responsibility, this multidimensional approach sets the stage for future aquaculture advancements.

In conclusion, our study unveils the transformative potential of rapeseed-based feed for Nile tilapia in geothermal waters, enhancing sustainability and economic viability. Future research can explore physico-chemical analyses and the integration of supplements for further aquaculture advancements.

CREDIT AUTHOR STATEMENT

Conceptualization ST, HD and KE, HL; Supervision: ST, HD and HL; Experiments and data curation: ST, KE, HL; Statistical analysis: ST; Writing and drafting: ST; Funding: HD and HL.

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