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# EVALUATING HEMPSEED MEAL BY-PRODUCT AS A FUNCTIONAL ADDITIVE IN *Cyprinus carpio* var. *specularis* DIETS

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***Abstract***

*Hempseed meal, a nutrient-rich by-product resulted from oil extraction from Cannabis sativa L., represents a promising candidate for high value functional feed additive due to its favorable amino acid profile, increased levels of essential fatty acids, and availability as an agro-industrial residue. This study investigates the nutritional value and functional potential of hempseed meal as a partial supplementary alternative to traditional protein ingredients in the diet of common carp (Cyprinus carpio), one of the most economically important freshwater species in global aquaculture. The analysis focuses on the biochemical composition, its digestibility, and its influence on growth performance, feed utilization, and metabolic efficiency. Preliminary evidence suggests that the inclusion of moderate levels of hempseed meal in carp diets can enhance feed efficiency without compromising growth or health, while supporting a more sustainable production system through the valorization of agricultural by-products. The findings highlight the relevance of hempseed meal as a functional ingredient that aligns with circular economy principles and contributes to reducing the environmental impact of aquaculture.*

***Key words****: common carp, functional ingredients, hempseed meal.*

## INTRODUCTION

The rapid growth of aquaculture necessitates a transition toward ecologically responsible, cost-efficient feed alternatives to mitigate the high costs and environmental burdens associated with traditional fishmeal and soybean meal (Borrelli et al., 2013; Henriksson et al., 2018).

A critical challenge in contemporary fish nutrition is the replacement of conventional protein sources, such as fishmeal, with sustainable and cost-effective alternatives (Sainsbury et al., 2000; Van Huis et al., 2013). Current research increasingly focuses on the synergistic effects of diet composition and environmental variables on the life traits and heritability of growth-related parameters (Mansilla et al., 2017; Mohamed & House, 2024). Understanding the digestive physiology of this species, particularly its reliance on alkaline enzymatic hydrolysis, is fundamental for formulating feeds that maximize nutrient retention and minimize nitrogenous waste discharge into aquatic ecosystems. In this context, industrial hemp (*Cannabis sativa* L.) derivatives, specifically hemp seed oil and meal, have emerged as promising candidates.

Integration of advanced processing technologies, such as cold-pressing and pelletizing equipment tailored for small-to-medium scale operations, is essential for localizing production and reducing the carbon footprint of the feed supply chain (Cassano et al., 2013; Catchpole et al., 2011; Nenciu et al., 2022).

Unlike traditional plant-based ingredients, hemp products offer a unique profile of bioactive compounds, essential fatty acids, and highly digestible proteins that may enhance the immune response and metabolic health of teleost fish.

For the Mirror Carp variety specifically, dietary formulation is highly important due to its rapid growth potential and unique physiology. Unlike other carp varieties, the *specularis* variety often exhibits faster mass accumulation and a higher tendency for lipid deposition. Consequently, their diets must be carefully balanced with lipid levels between 8% and 12% to ensure high-quality muscle development rather than excessive abdominal fat. For Mirror Carp, the digestive process is highly temperature-dependent; their metabolic rate and enzyme activity, such as amylase and cellulase, peak in warmer waters (20-25°C), making them exceptionally efficient at processing the plant-based carbohydrates found in hemp and other oilseed meals. To support their robust frame and rapid weight gain, Mirror Carp require elevated levels of bioavailable phosphorus (0.6-0.7%). Without this mineral, the fish are prone to structural issues that can decrease their commercial value and welfare (Andre et al., 2009; House et al., 2010).

By-products from the agricultural and food sectors offer a viable pathway toward a circular bioeconomy by repurposing residues into high-value aquafeeds (Kroes et al., 2021; Majewski & Jurgoński, 2021). Among these, hemp (*Cannabis sativa* L.) has emerged as a particularly versatile candidate (Campbell et al., 2001; Nenciu et al., 2023; Palmegiano et al., 2006). The defatted meal resulting from cold-press oil extraction, containing 30-35% protein alongside essential fatty acids and bioactive compounds, remains an underexploited nutritional resource (Naylor et al., 2021; Olusegun et al., 2017). Given its favorable amino acid profile and potential palatability enhancers, hempseed meal is well-suited for omnivorous species like carp (Gibb et al., 2015; Oprescu et al., 2023; Oyugi et al., 2011). Empirical evidence suggests that partially substituting conventional proteins with hempseed derivatives does not adversely affect growth or feed conversion in salmonids and cyprinids (Callaway, 2004; Gengmao et al., 2015; Karimi et al., 2020). Furthermore, the inclusion of omega-3 and omega-6 fatty acids from hemp may bolster lipid metabolism and immune function (Elzinga et al., 2015; Ross et al., 2000). Beyond nutrition, the integration of this industrial waste stream aligns with sustainable development goals by reducing reliance on marine stocks and leveraging the agronomic benefits of hemp, such as carbon sequestration and minimal pesticide requirements (Kocour et al., 2010; Makkar et al., 2014). Despite this potential, there is a scarcity of field-scale data regarding the application of cold-pressed hempseed meal in pond-based carp culture (Ahmed et al., 2019; Russo & Reggiani, 2015). This study addresses this gap through a proof-of-concept investigation involving *Cyprinus carpio* var. *specularis*. By evaluating young specimens (100 g each) over a 90-day period, this research bridges the gap between laboratory potential and applied feasibility. The objective is to validate hempseed meal as a functional, circular ingredient that enhances feed sustainability without compromising fish performance or environmental integrity.

## MATERIALS AND METHODS

The research was performed at the National Institute of Research-Development for Machines and Installations Designed for Agriculture and Food Industry (INMA Bucharest), utilizing controlled pond systems during a trial period from May to July 2025. A completely randomized block design was applied with four diets containing increasing levels of hempseed meal, each trial being replicated in three separate ponds to improve statistical robustness and reduce environmental effects. Over a 90-day nutritional study, environmental parameters and water quality metrics were strictly regulated to ensure they remained within the ideal thresholds for carp aquaculture.

Hemp seeds were processed by mechanical cold pressing at temperatures below 50°C to protect heat-sensitive constituents, including omega-3 and omega-6 fatty acids, tocopherols, and other natural antioxidants. The method produces an unrefined oil with preserved sensory qualities, nutritional value, and good oxidative stability, suitable for food and cosmetic uses. In contrast to thermal extraction methods, cold pressing preserves the chemical stability of the meal by avoiding heat-induced breakdown and impurities, offering an eco-friendlier production route.

The hemp seeds used belonged to the Jubileu Secuieni cultivar, (SCDA Secuieni, Romania), with a typical production of 1200-1600 kg/ha. Seeds were pre-dried before processing to improve extraction efficiency calculations. Cold pressing produced an average oil yield of about 29%, corresponding to roughly 84% of the total seed oil, while the remaining fraction formed hempseed cake containing approximately 10-12% residual oil.

The hemp meal has a promising fish fodder value due to its balanced composition: 30-35% crude protein, 8-12% lipids, 20-25% fiber, 5-7% ash, and 25-30% nitrogen-free extract. Its protein provides a favorable amino acid spectrum, particularly arginine, methionine, and glutamic acid, supporting fish growth and metabolism. The residual lipid fraction is rich in polyunsaturated fatty acids - mainly linoleic (50-60%) and α-linolenic acid (15-20%) - which contribute to membrane function, immunity, and overall physiological performance. Natural antioxidants, including tocopherols and phenolics, further enhance oxidative stability and may promote fish health when included in feed formulations.

To produce the oil for this research, a specialized mechanical pressing unit - designed and developed by INMA Bucharest - was employed to ensure controlled extraction parameters.



Figure 1. Industrial cold-pressing installation for extraction of hemp oil from seeds (original source)

The mechanical extraction of lipids via cold pressing represents a highly efficient, non-thermal processing technique that simultaneously yields two distinct fractions: cold-pressed hemp oil and residual hemp meal. Both derivatives exhibit exceptional nutritional profiles characterized by high biological value for both human and animal consumption. The lipid fraction is particularly distinguished by its favorable ratio of polyunsaturated fatty acids, while the resulting meal serves as a concentrated source of high-quality vegetable protein and dietary fiber. By maintaining low processing temperatures, this method ensures the preservation of thermosensitive bioactive compounds, including tocopherols and phenolic antioxidants, thereby enhancing the functional properties and oxidative stability of the final products within a circular bioeconomy framework.



Figure 2. Primary products obtained through cold-pressing process: hemp oil and hemp meal (original source)

The experiment lasted three months and evaluated the influence of hempseed meal inclusion in diets on somatic growth of mirror carp (*Cyprinus carpio* var. *specularis*). Four feeds containing graded hempseed meal levels (0-20%) were formulated to examine effects on nutrient composition and performance. Each treatment was tested in triplicate, with fish of similar initial size distributed evenly among tanks (100 individuals per tank) to maintain uniformity. Fish were fed twice daily, with rations adjusted to biomass, while water temperature, dissolved oxygen, and pH were kept stable throughout the trial.

Body weight was recorded monthly from the start (month 0) to the end (month 3). For each diet and replicate, batch averages were calculated, generating longitudinal data at four time points across four treatments (R1-R4) with three replicates each. Individual ponds served as experimental units (n = 3 per treatment), and growth parameters were expressed as pond-level means derived from all fish within a unit.

Juvenile fish with an initial body mass of 100 g each specimen, were stocked in ponds with a volume of 3 m³. Prior to the experiment, fish underwent a two-week acclimation period

during which they received a control diet without hempseed meal. Throughout the experimental phase, essential aquatic parameters - including pH, temperature, dissolved oxygen, and ammonia levels - were strictly regulated and held within the ideal thresholds for carp aquaculture.

The tanks operated within a recirculating aquaculture system equipped with a filtration unit (Hailea G12000) manufactured by Guangdong Hailea Group Co., Ltd. in China, an automated feeder (Eheim Twin Duo) produced by EHEIM GmbH & Co. KG. in Germany, and a monitoring system (Atlas Scientific Wi-Fi Hydroponics Kit) developed by Atlas Scientific Environmental Robotics in United States. These components enabled stable environmental control throughout the experimental period.



Figure 3. Experimental installation for juvenile fish development (original source)

**Composition and Preparation of Experimental Rations**

Four experimental diets (R1-R4) have been formulated to investigate the effects of hempseed meal on the development of juvenile cyprinids, with inclusion levels of 0% (control), 5%, 10%, and 20%. Hempseed meal partially replaced conventional plant protein sources while maintaining isonitrogenous and isoenergetic compositions. Crude protein content was maintained at 30%, a level considered optimal for juvenile carp with elevated metabolic requirements, by gradually decreasing the inclusion of pea flour and soybean meal as the proportion of hempseed meal increased. This approach preserved amino acid balance and metabolizable energy consistency. The hemp by-product, derived from defatted seeds, contributed 25-50% protein along with balanced amino acids and polyunsaturated fatty acids.

Constant proportions of wheat bran (15%), corn flour (10%), and alfalfa meal (15%) were retained to ensure similar pellet structure, density, and acceptability across diets. Minor differences in lipid (2.23-3.52%), fiber (9-10.3%), and ash were considered beneficial for energy supply, digestion, and gut function. Dry ingredients were thoroughly blended using a high-shear mixer, cold-extruded through a 3-mm mesh, air-dried to 8-10% moisture, sieved for uniformity, and stored under refrigeration until use. Fish were fed at 09:00 and 17:00 h at 3% of their average body weight, with rations adjusted weekly based on biomass estimates. Feeding activity and consumption were monitored to detect any influence of hemp-derived components on palatability.

Table 1. Ingredient composition (%) and estimated proximate nutritional composition of the experimental diets containing different levels of hempseed meal for mirror carp

| Ingredient (%) | Formulation 1 Hempseed Meal 0% | Formulation 2 Hempseed Meal 5% | Formulation 3 Hempseed Meal 10% | Formulation 4 Hempseed Meal20% |
|---|---|---|---|---|
| Ingredient composition - Composition for 1 kg of feed (g/kg) | | | | |
| *Cannabis sativa* seed-meal | 0 | 50 | 100 | 200 |
| Pea flour | 100 | 75 | 50 | 0 |
| Defatted Soybean meal | 500 | 475 | 450 | 400 |
| Wheat bran | 150 | 150 | 150 | 150 |
| Maize meal | 100 | 100 | 100 | 100 |
| Dehydrated Lucerne meal | 150 | 150 | 150 | 150 |
| Estimated proximate composition - for 1 kg of feed (g/kg) | | | | |
| Proteins | 300 | 300 | 300 | 300 |
| Crude lipid | 22.3 | 25.6 | 28.8 | 35.2 |
| Fiber content | 90 | 93.3 | 96.5 | 103 |
| Mineral content | 53 | 53.6 | 54.2 | 55.5 |
| Soluble fraction (NFE) | 534.7 | 527.5 | 520.5 | 506.3 |

Although diets were formulated to be isonitrogenous and isoenergetic, minor differences in lipid (± 1.3%), fiber (± 0.8%), and ash (± 0.6%) occurred due to partial replacement of soybean and pea meals with hempseed meal, which naturally contains higher fiber and residual oil. These small compositional shifts may have affected

digestibility and energy utilization; however, overall growth performance indicated efficient use of the mixed plant protein sources without negative effects on feed conversion. The proportional reduction of soybean and pea ingredients alongside hemp inclusion was intended to reflect practical feed production scenarios, where multiple plant proteins are typically combined to achieve nutritional balance. Total feed for each pond daily was 0.4 kg in the first month, 0.6 kg in the second month and 0.9 kg in the third month.

**Growth Performance Calculations**

For each experimental unit (replicate tank and batch), growth parameters were calculated and subsequently averaged by treatment based on diet type. When individual-level data were required, body weights recorded at each sampling interval were used.

Absolute weight gain (WG), representing the overall increase in body weight throughout the experimental period, was determined using the following formula:

$$WG = Wf - Wi \quad (1)$$

where Wf is the final body weight (g) and Wi is the initial body weight (g).

Average daily gain (ADG), defined as the mean daily increase in body weight, was determined using:

$$ADG = (Wf - Wi) / t \quad (2)$$

where Wf is the terminal mass of the fish specimens (g), and Wi is the initial body weight (g), and t is the duration of the period (days).

Specific growth rate (SGR), which expresses growth as a percentage of body weight per day while accounting for exponential growth patterns, was calculated as:

$$SGR = [(ln\, Wf - ln\, Wi) / t] \times 100 \quad (3)$$

where Wf and Wi represent final and initial body weights (g), respectively, t is time (days), and ln denotes the natural logarithm.

Feed conversion ratio (FCR), indicating feed efficiency as the quantity of feed required to produce a unit of weight gain, was calculated as the ratio between total feed consumed (TFC and the weight gain (Wg):

$$FCR = T_{fc}/W_g \quad (4)$$

**Ethical statement**

All experimental procedures were conducted in accordance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the for Ethical Research of National Institute of Research-Development for Machines and Installations Designed for Agriculture and Food Industry-INMA Bucharest, (Protocol Code 02/ May 2021). The stocking density (100 individuals per 3 $m^3$) was selected based on aquaculture practices and did not induce stress conditions, as confirmed by stable water quality parameters and high survival rates (≥95%) across all treatments.

**Statistical Analysis**

All data were expressed as expressed as the mean values of the recorded parameters, for each experimental group. Differences between the experimental months were analyzed using one-way Analysis of Variance (ANOVA). When significant differences were detected, Tukey's Honestly Significant Difference (HSD) post-hoc test was applied for multiple comparisons. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using Microsoft Excel 2024 Software.

## RESULTS AND DISCUSSIONS

The Mirror Carp's natural behavior as a benthic (bottom) feeder necessitates the use of high-stability, sinking pellets. Their diet often functions as a supplement to the natural protein-rich biota found in pond sediments. By integrating hempseed meal into these sinking formulations, farmers can provide a balanced amino acid profile - specifically supplementing Lysine and Methionine - to mirror the nutrition found in their natural environment.

Figure 4 illustrates the evolution of the average body weight of mirror carp specimens during the three-month experimental period for the four dietary treatments. All groups showed a progressive increase in body weight, indicating normal growth conditions and good feed acceptance. Statistical analysis revealed that fish fed diets R2 and R3 exhibited significantly higher final weights compared to the control group (R1) ($P < 0.05$), while no significant differences were observed between R2 and R3.

The R4 group showed lower values compared to R2 and R3, although differences were not always statistically significant. These results suggest that moderate inclusion levels (5-10%) optimize growth performance.

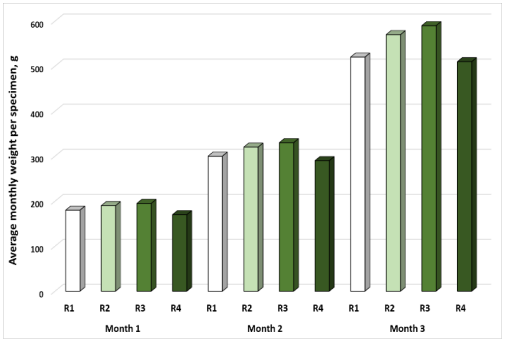


Figure 4. Average monthly weight per specimen for the four feeding recipes for mirror carp

Figure 5 presents the average daily growth rate of mirror carp under the four feeding regimes. Daily growth rate followed a similar trend, with significantly higher values recorded in R2 and R3 compared to R1 ($P < 0.05$). The highest values were observed in R3 across all months. The R4 diet resulted in lower DGR values, supporting the hypothesis that excessive inclusion levels may limit nutrient utilization efficiency. Diets containing moderate levels of hempseed meal tended to produce higher daily growth rates, which may be associated with the balanced amino acid profile and the presence of beneficial polyunsaturated fatty acids in the hemp by-product.

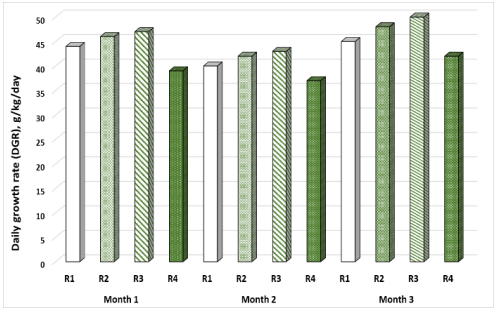


Figure 5. Daily average growth rate depending on the diet for mirror carp

Figure 6 shows the average final body length of mirror carp at the end of the experimental period. Differences among treatments were relatively small, indicating that all diets supported normal somatic development. Nevertheless, fish fed diets supplemented with hempseed meal displayed slightly higher mean lengths, suggesting a positive influence of this ingredient on overall growth performance.

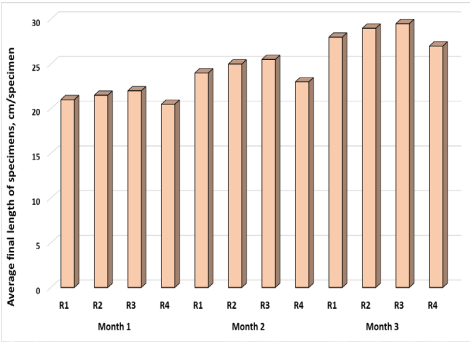


Figure 6. Average final length for mirror carp specimens

Figure 7 presents the survival rate of mirror carp in each experimental treatment. Survival remained high across all dietary groups, indicating that the inclusion of hempseed meal in the feed did not negatively affect fish health or environmental adaptability. The results confirm that hempseed meal can be safely incorporated into carp diets without compromising fish viability.

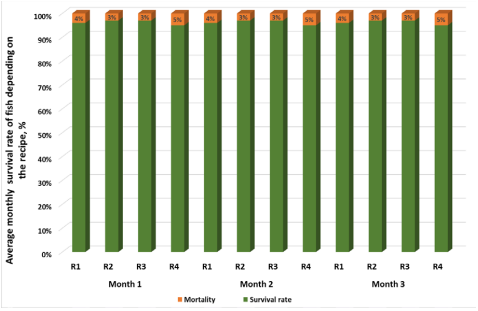


Figure 7. Average survival rate depending on diet in each pool

Figure 8 illustrates the feed conversion ratio (FCR) recorded for each dietary treatment. Feed conversion ratio (FCR) values were significantly improved in R2 and R3 compared to the control group ($P < 0.05$), indicating more efficient feed utilization. In contrast, R4 showed higher FCR values, suggesting reduced efficiency. This trend may be associated with

increased dietary fiber content, which can negatively affect digestibility.

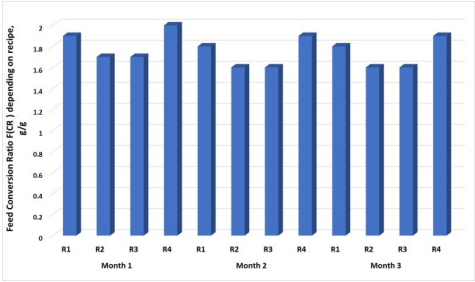


Figure 8. Feed conversion ratio depending on died for mirror carp

The growth performance recorded in the experimental groups indicates that moderate inclusion levels did not negatively affect fish development and, in some cases, even supported slightly improved growth indicators. This effect may be associated with the nutritional characteristics of hempseed meal, which contains a balanced amino acid profile, digestible proteins and beneficial fatty acids. In addition, the presence of bioactive compounds and residual lipids may contribute to improved feed palatability and nutrient utilization. Similar findings have been reported in previous studies investigating plant-based protein sources as substitutes for conventional feed ingredients in carp diets. However, further studies evaluating long-term growth performance, digestibility and carcass composition would be beneficial in order to determine the optimal inclusion level for carp nutrition.

The analysis of growth performance revealed a highly significant progression across all monitored parameters, as evidenced by the robust one-way ANOVA results. The substantial increase in body mass ($F(2, 18) = 624.5$, $p < 0.001$) suggests a steady anabolic trajectory, with Tukey's HSD post-hoc test confirming that each monthly interval represented a statistically distinct developmental stage. This consistent weight gain was mirrored by a significant optimization of the Feed Conversion Ratio ($p = 0.032$), where the transition from the first to the second month marked a shift toward higher nutrient utilization efficiency. While the Daily Growth Rate exhibited minor mid-term fluctuations, the significant recovery observed in the final phase ($p < 0.01$ vs. Month 2) indicates that the specimens reached a state of metabolic equilibrium rather than growth stagnation. Collectively, the convergence of stabilized FCR values and peak DGR in the third month provides a strong statistical basis for the efficacy of the experimental protocol, demonstrating that the culture conditions successfully supported maximum biomass accumulation without compromising metabolic efficiency.

The experimental diets containing different inclusion levels of hempseed meal supported normal development of the fish throughout the experimental period, indicating that this ingredient can represent a viable alternative protein source in aquaculture feeds. Growth indicators such as final body weight, length and daily growth rate showed comparable values among the experimental groups, suggesting that moderate inclusion levels of hempseed meal are well tolerated by mirror carp. In addition, the feed conversion ratio values indicate that the experimental diets were efficiently utilized by the fish, highlighting the nutritional potential of this plant-based ingredient.

Future studies will aim to optimize these formulations through detailed physiological and metabolic analyses. This will allow for the creation of precision diets tailored to the life stages of the Mirror Carp, ensuring that the shift toward sustainability also results in maximum production efficiency and fish health.

The significant increase in body mass, corroborated by the optimized FCR values in the latter stages of the experiment, suggests an efficient metabolic adaptation of the specimens to the diet. The stabilization of the FCR between Month 2 and Month 3 (as shown by the Tukey post-hoc test) indicates that the peak of feed conversion efficiency was reached (for their specific age and environmental conditions), maintaining a steady plateau despite the increase in total biomass.

The reduced performance observed at the highest inclusion level (R4, 20% hempseed meal) may be explained by the increased crude fiber content (10.3%). High dietary fiber is known to reduce nutrient digestibility by limiting enzyme access and accelerating intestinal transit time. Excessive fiber intake may impair protein and energy utilization

efficiency. This likely explains the plateau or slight decrease observed in growth and feed efficiency parameters at higher inclusion levels. While the dietary fiber content appears to be a primary driver of the observed performance trends, several contextual factors inherent to the experimental design must be considered. First, the study utilized juvenile specimens with an initial mean weight of 100 g; it is well-established that metabolic rates and nutrient requirements shift as fish progress through different life stages. Consequently, the observed sensitivity to fiber-rich hempseed meal may vary in larger, more mature individuals with more developed digestive tract capacities.

Furthermore, the three-month experimental duration, while sufficient to capture significant growth trends, represents only a snapshot of the species full production cycle. Long-term effects of high-fiber inclusion on intestinal morphology or chronic nutrient absorption remain to be fully elucidated over an extended period. Lastly, the pond dimensions and stocking density employed in this study provided a specific controlled environment that may influence energy expenditure and social behavior. The physical constraints of the culture system, combined with the juvenile stage of the specimens, create a unique physiological context that should be factored into the generalization of these growth and feed efficiency parameters to larger-scale or different-aged cohorts.

## CONCLUSIONS

These findings support the use of hempseed meal as a viable alternative feed ingredient in carp aquaculture, offering promising prospects for improving feed sustainability and resource efficiency in fish production systems. By repurposing this agro-industrial by-product, the study provides a "proof-of-concept" for circular bioeconomy practices that reduce waste and reliance on traditional fishmeal. Moderate inclusion levels (5-10%) proved optimal, improving growth performance and feed conversion efficiency without negatively affecting fish health or survival. In contrast, higher inclusion (20%) resulted in reduced performance, likely due to increased dietary fiber limiting nutrient digestibility. However, the outcomes are influenced by factors such as fish developmental stage, experimental duration, and controlled rearing conditions. Therefore, further long-term studies are recommended to evaluate digestibility, carcass quality, and physiological responses across different life stages and production systems.

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