

Original Article

Cannabinoid Detection in Hemp Seed Oil, Hemp Seed, and Blood of Rohu (*Labeo rohita*) Following 50-Day Dietary Supplementation

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Received: 25 June 2023

Revised: 08 August 2023

Accepted: 16 August 2023

Published: 27 September 2023

HOW TO CITEAfridi, A. J., Zuberi, A., Yousafzai, A. M., Mehmood, H., & Sabur, A. (2023). Level of Cannabinoids in Hemp seed oil, Hemp seed and Blood serum of Rohu (*Labeo rohita*) after feeding Hemp seed oil, and Hemp seed Supplemented in Diet for fifty days. *Journal of Health and Rehabilitation Research*, 3(2). <https://doi.org/10.61919/jhrr.v3i2.2040>**ARTICLE INFORMATION**

Author Contributions	Concept: AJA; Design: AZ; Data Collection: AMY, HM; Analysis: AS; Drafting: AJA, AZ, AMY, HM, AS.
Ethical Approval	Ethical Approval: Ethical approval was obtained from the Bioethical Committee of the Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, under approval number BEC-FBS-QAU2017-67.
Informed Consent	Not applicable, as the study involved experimental fish and no human participants.
Conflict of Interest	The authors declare no conflict of interest.
Funding	No external funding.
Data Availability	Available from the corresponding author on reasonable request.
Acknowledgments	N/A.
AI Declaration	No generative AI was used for conceptualization, analysis, or content generation. AI use was limited to language refinement, and the authors remain fully responsible for the accuracy, originality, validity, and integrity of the manuscript's content.

ABSTRACT

Background: Hemp (*Cannabis sativa* L.) seed and seed oil are increasingly considered as potential feed ingredients because of their nutritional composition. However, the presence of cannabinoids in hemp-derived ingredients raises questions regarding their possible detection in animals following dietary exposure. This study evaluated cannabinoids in hemp seed oil and whole hemp seed and investigated their detection in the blood of Rohu (*Labeo rohita*) following dietary supplementation for 50 days. **Methods:** Cannabinoids were analyzed using gas chromatography-mass spectrometry (GC-MS). Seven experimental diets were used, comprising a basal control diet, three diets supplemented with hemp seed oil at 3%, 6%, and 9%, and three diets supplemented with hemp seed at 5%, 10%, and 15%. Rohu fingerlings received the respective diets for 50 days. At the end of the feeding period, blood samples were collected and analyzed for the targeted cannabinoid compounds. **Results:** Three target compounds were detected in hemp seed oil: delta-9-tetrahydrocannabinol (Δ^9 -THC; $9.77 \pm 0.66\%$), cannabinol (CBN; $9.30 \pm 0.43\%$), and resorcinol ($22.96 \pm 0.87\%$). In whole hemp seed, cannabidiol (CBD) was the only target cannabinoid detected, at 0.06%. None of the targeted cannabinoids were detected in blood samples from the control group or from Rohu fed diets supplemented with hemp seed oil or hemp seed. **Conclusion:** Under the conditions of this 50-day feeding experiment, the targeted cannabinoids were not detected in the blood of Rohu receiving diets containing up to 9% hemp seed oil or 15% hemp seed. These findings describe cannabinoid detection in the tested hemp-derived feed ingredients and their absence from Rohu blood within the analytical conditions used in this study. **Keywords:** *Cannabis sativa*; cannabinoids; hemp seed; hemp seed oil; *Labeo rohita*; gas chromatography-mass spectrometry; dietary supplementation.

INTRODUCTION

Hemp (*Cannabis sativa* L.) is one of the oldest cultivated plant species and has historically been used for food, fiber, oil, medicinal preparations, and a variety of industrial purposes. The plant belongs to the family Cannabaceae and occurs in diverse cultivated forms that differ considerably in their morphological characteristics, chemical composition, and intended use. Through prolonged cultivation and selection, *Cannabis sativa* has been utilized both as an agricultural crop and as a source of biologically active compounds, resulting in considerable diversity among

fiber-type, seed-producing, and drug-type cultivars (1). Hemp seed and its derivatives have attracted particular interest because they provide nutritionally valuable lipids, proteins, minerals, vitamins, and other bioactive constituents, while the plant as a whole has also been investigated for pharmaceutical, cosmetic, food, and non-food applications (2-4).

Hemp seed is recognized as a nutritionally dense plant material. It contains substantial quantities of protein and oil together with dietary fiber, minerals, and vitamins. Approximately 25% of the seed mass may consist of protein, whereas the oil fraction can exceed 30%, although the exact composition varies among cultivars, geographical locations, growing conditions, and processing methods (2). The major hemp seed proteins include edestin and albumin, which provide a broad spectrum of essential and non-essential amino acids. Hemp protein is particularly notable for its relatively high arginine content and has attracted interest as an alternative plant-derived protein source for human and animal nutrition (2). Functional studies of hemp protein isolates have also demonstrated physicochemical and nutritional properties that support their potential application in food and feed formulations (5).

The lipid fraction of hemp seed is of particular nutritional importance because hemp seed oil contains a high proportion of polyunsaturated fatty acids. More than 80% of the fatty acids in hemp seed oil may occur as polyunsaturated fatty acids, with linoleic acid and α -linolenic acid representing major components (2,6). Oleic acid and γ -linolenic acid are also present, while tocopherols and other constituents of the unsaponifiable fraction contribute to the nutritional and oxidative characteristics of the oil (3,6). The presence of both omega-6 and omega-3 fatty acids has encouraged investigation of hemp seed oil as an ingredient in animal feeds, including aquaculture diets. Previous work in fish has indicated that dietary incorporation of hemp-derived ingredients may influence tissue fatty-acid composition and the balance of nutritionally important fatty acids (7).

In addition to its nutritional constituents, *Cannabis sativa* produces a chemically diverse group of terpenophenolic compounds collectively known as phytocannabinoids. These compounds are predominantly associated with glandular trichomes occurring on the aerial reproductive parts of the plant, particularly the flowering structures of female plants (8,9). A large number of naturally occurring cannabinoids and cannabinoid-related compounds have been described, although their abundance varies considerably with genotype, plant part, developmental stage, environmental conditions, post-harvest handling, and analytical methodology (8-10). Delta-9-tetrahydrocannabinol (Δ 9-THC) is the principal cannabinoid associated with the characteristic psychoactive effects of cannabis, whereas cannabidiol (CBD) and cannabinol (CBN) are among the other extensively studied cannabinoids (8,11).

The relative concentrations of Δ 9-THC, CBD, CBN, and other cannabinoids contribute to the chemical distinction between cannabis chemotypes intended for drug use and those developed primarily for fiber, seed, or industrial applications. Chemical phenotype has historically been characterized using the relative proportions of major cannabinoids, including relationships between THC, CBN, and CBD concentrations (12-15). Modern industrial hemp production has therefore emphasized the selection and cultivation of low-THC varieties. Considerable variation may nevertheless occur among plants and derived products because cannabinoid concentration is influenced by genetic characteristics, sex, maturity, environmental conditions, harvested plant material, storage, and the interval between harvesting and chemical analysis (13-15).

Hemp seed itself is generally considered to contain little or no biosynthesized cannabinoid because cannabinoids are principally formed in glandular structures located on other parts of the plant. Nevertheless, measurable traces of cannabinoids have been reported in commercial seeds and hemp seed-derived products (16). Such residues may arise from contact of the seed surface with cannabinoid-rich flowers, leaves, or resinous plant material during harvesting and processing. Consequently, the cannabinoid concentration of hemp seed and hemp seed oil can depend not only on plant chemotype but also on seed cleanliness and processing conditions. Cannabinoids present on the external surface of seeds may subsequently enter the oil during extraction or pressing, resulting in detectable cannabinoid residues even when the seed itself is not considered an important site of cannabinoid biosynthesis (16,17).

The occurrence of cannabinoids in hemp-derived food and feed ingredients is particularly relevant because Δ 9-THC and several related compounds are biologically active. Industrial hemp cultivars with low THC concentrations have been developed to facilitate agricultural and commercial utilization while minimizing psychoactive exposure (17-19). Studies of commercially available hemp products have nevertheless demonstrated variability in residual Δ 9-THC

concentrations, emphasizing the importance of analytical characterization of hemp-derived products before their use for nutritional or feeding purposes (17). This consideration becomes especially important when hemp seed, hemp seed oil, seed cake, or related products are incorporated repeatedly into animal diets, because the degree to which plant-derived cannabinoids may be absorbed, distributed, metabolized, accumulated, or eliminated can influence the safety assessment of animal feed and animal-derived food products.

The nutritional composition of hemp has resulted in increasing interest in its utilization as an animal-feed ingredient. Whole hemp plant material, hemp seed, hemp seed cake, and hemp seed oil have been investigated in several livestock and poultry species. Cold-pressed hemp seed cake has been evaluated as a protein-rich feed ingredient for growing cattle, demonstrating its potential nutritional value in ruminant diets (20). Hemp seed and hemp seed oil have also been incorporated into poultry diets, where their effects on performance, egg characteristics, and blood biochemical parameters have been examined (21). In dairy cattle, increasing dietary inclusion of hemp seed cake has been studied in relation to feed utilization, milk production, and milk composition (22). These findings collectively demonstrate the potential of hemp-derived materials as feed resources, although nutritional benefits must be considered together with the chemical characteristics and possible biological disposition of cannabinoid residues.

Aquaculture represents another area in which alternative plant-derived feed ingredients are increasingly being investigated. Fish feeds require appropriate concentrations of protein, lipid, essential fatty acids, vitamins, and minerals to support growth, physiological function, and the nutritional quality of the final product. Hemp seed and hemp seed oil provide several of these components and may therefore represent potentially useful ingredients in formulated fish diets. Previous research involving Indian major carps has indicated that hemp-derived dietary supplementation can modify the fatty-acid profile of fish and may contribute nutritionally important polyunsaturated fatty acids (7). However, the incorporation of a novel plant-derived feed ingredient requires assessment not only of its nutritional contribution but also of potentially bioactive compounds that may enter the animal following prolonged dietary exposure.

Phytocannabinoids are generally lipophilic compounds, and their biological disposition depends on the compound involved, route and duration of exposure, dose, metabolism, and characteristics of the exposed organism (23). Following absorption, cannabinoids may undergo extensive metabolism and distribution among biological compartments. Therefore, the detection of cannabinoids in an animal feed ingredient does not necessarily establish that the same compounds will be measurable in circulating blood or edible tissues after dietary exposure. Conversely, failure to characterize potential transfer from feed ingredients leaves uncertainty regarding their biological availability and possible accumulation. Regulatory assessments of hemp as an animal-feed material have consequently considered both the composition of hemp-derived feed ingredients and the potential exposure of animals to Δ^9 -THC and related substances (24).

Despite increasing interest in the nutritional utilization of hemp, information regarding dietary exposure to cannabinoids in fish remains limited. Most cannabinoid research has focused on the chemical characterization of cannabis materials, human pharmacology, medicinal applications, or terrestrial animal exposure rather than the transfer of cannabinoids from hemp seed and hemp seed oil into fish following sustained dietary supplementation (8,23,25). This represents an important knowledge gap because aquaculture species differ physiologically from terrestrial animals, and the presence of cannabinoids in a feed ingredient cannot be assumed to result in measurable circulating concentrations without direct experimental evaluation.

Rohu (*Labeo rohita*) is an economically important freshwater carp widely cultured in South Asia and is an appropriate model for evaluating novel feed ingredients intended for regional aquaculture. Previous investigation of hemp supplementation in this species has demonstrated potential effects on nutritionally important fatty-acid profiles (7). However, limited evidence is available regarding whether cannabinoids detected in hemp seed or hemp seed oil remain detectable in the circulating blood of Rohu after repeated dietary exposure. Determining this relationship is important for understanding the analytical fate of cannabinoid constituents associated with hemp-based aquaculture feeds and for informing subsequent investigations of their nutritional and safety implications.

The present study was therefore designed to determine the cannabinoid profile of hemp seed oil and whole hemp seed using gas chromatography-mass spectrometry (GC-MS) and to investigate whether the targeted cannabinoids could be detected in the blood of Rohu following dietary exposure. Fish were fed diets containing graded

concentrations of hemp seed oil or hemp seed for 50 days, together with a basal control diet without hemp supplementation. It was hypothesized that cannabinoids detected in the hemp-derived dietary ingredients might become detectable in the blood following repeated dietary exposure. Accordingly, the study evaluated hemp seed oil, whole hemp seed, and blood obtained after the feeding trial to characterize the targeted cannabinoid compounds and examine their detection following 50 days of hemp-supplemented feeding.

MATERIALS AND METHODS

Study Design and Experimental Overview

The study was conducted as a controlled 50-day feeding experiment to evaluate cannabinoids in hemp seed oil, whole hemp seed, and the circulating blood of Rohu (*Labeo rohita*) following dietary supplementation. Seven dietary treatments were evaluated: a basal control diet without hemp supplementation; three diets supplemented with hemp seed oil at 3%, 6%, and 9%; and three diets supplemented with whole hemp seed at 5%, 10%, and 15%. All experimental diets were formulated to contain approximately 35% crude protein. Following the feeding period, blood was collected from fish in the control and hemp-supplemented groups, and plasma was separated for cannabinoid analysis. Hemp seed oil, whole hemp seed, and fish plasma were analyzed using gas chromatography-mass spectrometry (GC-MS).

Collection of Hemp Seed and Extraction of Hemp Seed Oil

Hemp (*Cannabis sativa* L.) seed was collected from the Tirah Valley, District Khyber, Khyber Pakhtunkhwa, Pakistan. Oil extraction was carried out at the Medicinal Botanic Centre, Pakistan Council of Scientific and Industrial Research (PCSIR), Peshawar. Hemp seed was placed in the extraction chamber of a Soxhlet apparatus and extracted with n-hexane (Fisher Scientific, UK) for 6–8 hours. Following extraction, the solvent-containing oil fraction was concentrated using a rotary vacuum evaporator (PLC/FTC/Chem, Eyela, Japan). The recovered oil was transferred into clean vials and left overnight to allow residual n-hexane to evaporate before its use in diet preparation and subsequent chemical analysis.

Preparation of Experimental Diets

Seven experimental diets containing approximately 35% crude protein were prepared. One diet served as the basal control and contained no hemp-derived ingredient. Three experimental diets contained hemp seed oil at inclusion levels of 3%, 6%, and 9%, designated H03, H06, and H09, respectively. Three additional diets contained whole hemp seed at inclusion levels of 5%, 10%, and 15%, designated HS5, HS10, and HS15, respectively.

All feed ingredients, including hemp seed, were dried and finely ground before diet preparation. The dry ingredients were thoroughly mixed, after which the designated quantity of oil was incorporated according to the treatment formulation. Water was gradually added to produce a homogeneous dough. The dough was passed through a meat grinder to produce pellets. The prepared pellets were dried at room temperature, transferred to sealed Ziploc bags, and stored at low temperature until use.

Experimental Fish

Rohu (*Labeo rohita*) fingerlings were produced through induced breeding at the Fisheries and Aquaculture Research Station, Quaid-i-Azam University, Islamabad, Pakistan. Following absorption of the yolk sac, swim-up fry were transferred to earthen nursery ponds and subsequently moved to rearing ponds until they reached the fingerling stage.

Uniform and apparently healthy fingerlings were selected for the feeding experiment. At the beginning of the experiment, mean body weight was 40.60 ± 7.95 g and mean total body length was 15.32 ± 1.28 cm. Before initiation of the feeding trial, fish were acclimatized to laboratory conditions for one week in well-aerated circular fiberglass tanks with a capacity of 8,000 L.

Experimental Conditions and Dietary Treatments

Following acclimatization, uniform-sized Rohu fingerlings were transferred to glass aquaria measuring 120 × 60 × 60 cm. Fish were maintained at a target stocking density of approximately 2 g/L, with 15 fingerlings stocked per

aquarium. Fish were assigned to the seven dietary treatments consisting of the control diet, hemp seed oil diets H03, H06, and H09, and hemp seed diets HS5, HS10, and HS15. The experimental feeding period lasted 50 days.

Fish were fed twice daily at approximately 09:00 and 16:00 hours. The experimental aquaria were continuously aerated using air stones connected to aerators, and water heaters were used to maintain the required temperature. Water temperature was maintained at approximately 26.5°C. Water-quality parameters, including temperature, dissolved oxygen, hardness expressed as CaCO₃, and pH, were monitored regularly before and during the feeding experiment. At the completion of the 50-day feeding period, fish were deprived of feed for 24 hours before blood collection.

Anesthesia and Blood Collection

At the end of the feeding experiment, fish were captured individually from the aquaria using a dip net and anesthetized with clove oil at a concentration of 0.03 mL/L. Blood was collected from the caudal vasculature using a 3-mL syringe containing heparin as an anticoagulant. Where direct venous collection was not possible, the caudal peduncle vasculature was accessed using a sterile sharp instrument, and the resulting blood sample was collected immediately. Because an anticoagulant was used during blood collection, the separated blood fraction was considered plasma. The collected blood was centrifuged at 3,000 rpm for 15 minutes to separate the plasma from cellular components. Plasma samples were transferred to appropriately labeled containers and stored at -20°C until cannabinoid analysis.

Cannabinoid Analysis

Cannabinoid analysis of hemp seed oil, whole hemp seed, and Rohu plasma was performed at the Medicinal Botanic Centre, PCSIR, Peshawar. Samples were analyzed using a gas chromatograph coupled with a mass spectrometer (GC-MS-QP 2010 Plus, Shimadzu, Japan). The extraction and sample-preparation procedure for cannabinoid analysis was based on the GC-MS method described by Qureshi et al., with modifications appropriate to the matrices examined in the present study (26). The targeted compounds included tetrahydrocannabivarin (THCV), cannabivarin (CBV), cannabidiol (CBD), cannabichromene (CBC), cannabipinol (CBP), cannabigerol (CBG), hexahydrocannabinol (HHCBN), delta-9-tetrahydrocannabinol (Δ^9 -THC), cannabinol (CBN), and resorcinol.

Sample Preparation for GC-MS Analysis

Whole hemp seed was finely ground before extraction. Ground hemp seed and fish plasma samples were initially treated with acetonitrile and filtered through Whatman filter paper. Hemp seed oil was analyzed by weighing approximately 0.2 g of the oil sample. The hemp seed oil, prepared hemp seed extract, and plasma samples were processed separately using the same subsequent extraction procedure. Approximately 15 mL of n-hexane (Fisher Scientific, UK) was added to each sample-containing tube, and the samples were vortexed for 5 minutes using a Grant-Bio PV-1 vortex mixer (England). Approximately 30 mL of acetonitrile saturated with n-hexane was then added, and the mixture was transferred to a separating funnel. After phase separation, the lower acetonitrile layer containing the extracted compounds was collected. The recovered fraction was transferred to a larger separating funnel containing approximately 600 mL of a 2% sodium chloride solution prepared in double-distilled water. Approximately 100 mL of n-hexane was subsequently added, and the mixture was shaken thoroughly and allowed to stand until phase separation was complete.

Residual water in the organic fraction was removed using anhydrous sodium sulfate. The solution was then filtered through Whatman No. 42 filter paper, 125 mm in diameter. The resulting extract was concentrated using a rotary vacuum evaporator (RV10DS99-IKA-BH10, Germany). The dried residue was reconstituted in 1 mL of analytical-grade cyclohexane (Scharlau, Spain). The reconstituted solution was filtered, and a 1- μ L aliquot was injected into the GC-MS system for analysis.

GC-MS Instrumentation and Operating Conditions

Chromatographic analysis was performed using a gas chromatograph coupled to a mass spectrometer and equipped with an AOC-20S autosampler and AOC-20i autoinjector. Separation was achieved using a capillary column measuring 30 m in length, with an internal diameter of 0.25 mm and a film thickness of 0.25 μ m.

The GC-MS interface temperature was maintained at 280°C, and the electron-ionization source temperature was maintained at 280°C. The solvent cut time was 2 minutes. A sample injection volume of 1 µL was used for chromatographic analysis. Helium was used as the carrier gas.

GC-MS Solution software supplied with the analytical system was used for instrument control, chromatographic acquisition, and evaluation of the resulting analytical data.

Outcome Assessment

The primary analytical outcome was the detection and measured concentration of the targeted cannabinoid-related compounds in hemp seed oil and whole hemp seed. The corresponding plasma samples obtained from Rohu after the feeding trial were examined for the presence or absence of the same targeted compounds. The fish groups evaluated consisted of the basal control group, three hemp seed oil groups receiving H03, H06, or H09 diets, and three hemp seed groups receiving HS5, HS10, or HS15 diets.

Statistical Analysis

Quantitative cannabinoid measurements were expressed as percentages and summarized as mean $\pm$ standard error (SE). Five samples were used for the reported cannabinoid measurements ($n = 5$). Compounds that were not detected under the analytical conditions were reported as ND. Because all targeted compounds in the Rohu plasma samples were reported as not detected, these findings were presented descriptively according to dietary treatment rather than as between-group quantitative comparisons.

Ethical Approval

All procedures involving experimental fish were conducted in accordance with animal-welfare standards applicable to the use of animals in scientific research. Ethical approval was obtained from the Bioethical Committee of the Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad. The approved protocol number was BEC-FBS-QAU2017-67.

RESULTS

Target Compounds Detected in Hemp Seed Oil

Gas chromatography-mass spectrometry analysis of hemp seed oil was performed for ten targeted compounds. Three compounds were detected in the analyzed hemp seed oil samples: delta-9-tetrahydrocannabinol (Δ^9 -THC), cannabinol (CBN), and resorcinol. The remaining seven targeted compounds, tetrahydrocannabivarin (THCV), cannabivarin (CBV), cannabidiol (CBD), cannabichromene (CBC), cannabipinol (CBP), cannabigerol (CBG), and hexahydrocannabinol (HHCBN), were not detected.

Resorcinol showed the highest measured concentration among the detected compounds, with a mean concentration of $22.96 \pm 0.87\%$. Delta-9-tetrahydrocannabinol was detected at $9.77 \pm 0.66\%$, while cannabinol was detected at $9.30 \pm 0.43\%$. The complete analytical profile of the targeted compounds in hemp seed oil is presented in Table 1.

Table 1. Concentrations of Targeted Compounds Detected in Hemp Seed Oil by GC-MS

Target compound	Abbreviation	Hemp seed oil (%)
Tetrahydrocannabivarin	THCV	ND
Cannabivarin	CBV	ND
Cannabidiol	CBD	ND
Cannabichromene	CBC	ND
Cannabipinol	CBP	ND
Cannabigerol	CBG	ND
Hexahydrocannabinol	HHCBN	ND
Delta-9-tetrahydrocannabinol	Δ^9 -THC	9.77 ± 0.66
Cannabinol	CBN	9.30 ± 0.43
Resorcinol	—	22.96 ± 0.87

Data are presented as mean $\pm$ standard error (SE), $n = 5$. ND, not detected; GC-MS, gas chromatography-mass spectrometry.

Target Compounds Detected in Whole Hemp Seed

Whole hemp seed samples were analyzed for the same ten targeted compounds. Cannabidiol was the only targeted cannabinoid detected in whole hemp seed, with a mean concentration of $0.06 \pm 0.01\%$. Tetrahydrocannabivarin, cannabivarin, cannabichromene, cannabipinol, cannabigerol, hexahydrocannabinol, Δ^9 -THC, cannabinol, and resorcinol were not detected. The concentration of CBD detected in whole hemp seed was substantially lower than the concentrations of the compounds detected in hemp seed oil. The complete analytical profile of whole hemp seed is presented in Table 2.

Table 2. Concentrations of Targeted Compounds Detected in Whole Hemp Seed by GC-MS

Target compound	Abbreviation	Whole hemp seed (%)
Tetrahydrocannabivarin	THCV	ND
Cannabivarin	CBV	ND
Cannabidiol	CBD	0.06 ± 0.01
Cannabichromene	CBC	ND
Cannabipinol	CBP	ND
Cannabigerol	CBG	ND
Hexahydrocannabinol	HHCBN	ND
Delta-9-tetrahydrocannabinol	Δ^9 -THC	ND
Cannabinol	CBN	ND
Resorcinol	—	ND

Data are presented as mean $\pm$ standard error (SE), $n = 5$. ND, not detected; GC-MS, gas chromatography-mass spectrometry.

Detection of Target Compounds in Rohu Plasma Following Dietary Supplementation

Plasma samples collected from Rohu after the 50-day feeding experiment were analyzed for the same targeted compounds evaluated in hemp seed oil and whole hemp seed. None of the ten targeted compounds was detected in plasma obtained from fish maintained on the basal control diet. Similarly, none of the targeted compounds was detected in plasma from Rohu fed diets supplemented with hemp seed oil at 3%, 6%, or 9%. Plasma obtained from Rohu fed whole hemp seed at dietary inclusion levels of 5%, 10%, and 15% also showed no detectable THCV, CBV, CBD, CBC, CBP, CBG, HHCBN, Δ^9 -THC, CBN, or resorcinol. Thus, despite the detection of Δ^9 -THC, CBN, and resorcinol in the hemp seed oil and CBD in the whole hemp seed used in the experiment, none of these targeted compounds was detected in Rohu plasma following 50 days of dietary exposure at the tested inclusion levels. Results for all experimental groups are presented in Table 3.

Table 3. Detection of Targeted Compounds in Plasma of Rohu (*Labeo rohita*) Following 50 Days of Dietary Hemp Seed Oil or Hemp Seed Supplementation

Target compound	Control	H03	H06	H09	HS5	HS10	HS15
Tetrahydrocannabivarin (THCV)	ND	ND	ND	ND	ND	ND	ND
Cannabivarin (CBV)	ND	ND	ND	ND	ND	ND	ND
Cannabidiol (CBD)	ND	ND	ND	ND	ND	ND	ND
Cannabichromene (CBC)	ND	ND	ND	ND	ND	ND	ND
Cannabipinol (CBP)	ND	ND	ND	ND	ND	ND	ND
Cannabigerol (CBG)	ND	ND	ND	ND	ND	ND	ND
Hexahydrocannabinol (HHCBN)	ND	ND	ND	ND	ND	ND	ND
Delta-9-tetrahydrocannabinol (Δ^9 -THC)	ND	ND	ND	ND	ND	ND	ND
Cannabinol (CBN)	ND	ND	ND	ND	ND	ND	ND
Resorcinol	ND	ND	ND	ND	ND	ND	ND

ND, not detected; H03, 3% hemp seed oil; H06, 6% hemp seed oil; H09, 9% hemp seed oil; HS5, 5% whole hemp seed; HS10, 10% whole hemp seed; HS15, 15% whole hemp seed. Reported analytical sample size: $n = 5$.

GC-MS Chromatographic Profiles

The GC-MS chromatographic profiles obtained from hemp seed oil demonstrated detectable peaks corresponding to Δ^9 -THC, cannabinol, and resorcinol, consistent with the quantitative findings presented in Table 1.

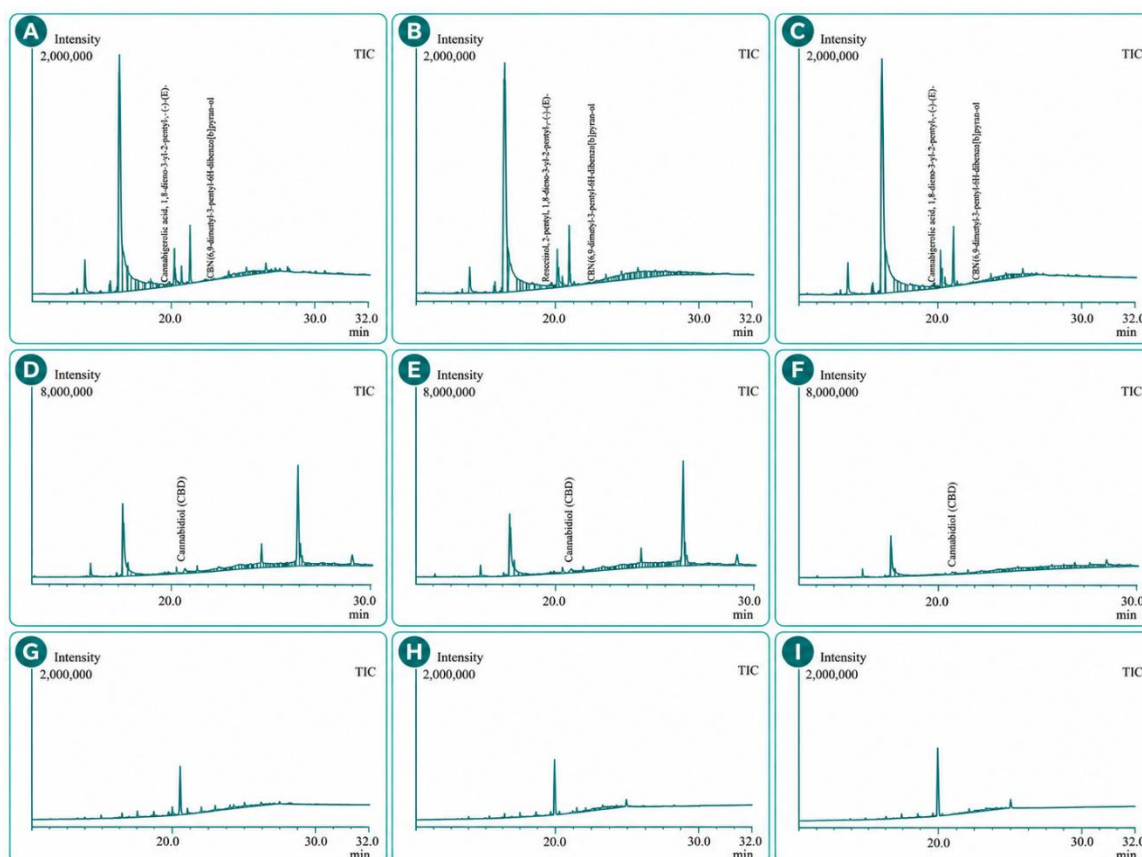


Figure 1 GC-MS total ion chromatograms (TICs) of hemp-derived samples and Rohu plasma. Panels A–C show chromatographic profiles of hemp seed oil, with detectable peaks corresponding to delta-9-tetrahydrocannabinol ($\Delta 9$ -THC), cannabidiol (CBD), and resorcinol. Panels D–F show chromatographic profiles of whole hemp seed, with cannabidiol (CBD) detected as the principal targeted cannabinoid. Panels G–I show chromatographic profiles of Rohu (*Labeo rohita*) plasma following dietary hemp supplementation, in which the targeted cannabinoids were not detected under the analytical conditions used. The chromatograms are presented as total ion current against retention time (min).

DISCUSSION

The present study evaluated the occurrence of selected cannabinoid-related compounds in hemp seed oil and whole hemp seed and subsequently examined their detectability in the plasma of Rohu (*Labeo rohita*) after 50 days of dietary supplementation. The principal findings were that $\Delta 9$ -tetrahydrocannabinol ($\Delta 9$ -THC), cannabidiol (CBD), and resorcinol were detected in hemp seed oil, whereas cannabidiol (CBD) was the only targeted cannabinoid detected in whole hemp seed. Despite dietary exposure to hemp seed oil at inclusion levels of up to 9% and whole hemp seed at inclusion levels of up to 15%, none of the targeted compounds was detected in Rohu plasma under the analytical conditions used in the study.

Hemp seed and hemp seed oil are nutritionally distinct from the cannabinoid-rich flowering and resinous portions of *Cannabis sativa*. Hemp seed is valued primarily for its protein, lipid, essential fatty-acid, mineral, and vitamin composition, whereas phytocannabinoids are produced predominantly in glandular trichomes associated with the aerial reproductive tissues of the plant (2,8,9). Hemp seed oil is particularly rich in polyunsaturated fatty acids, including linoleic and α -linolenic acids, and has therefore attracted interest as a nutritional ingredient for human and animal diets (2,3,6). Previous work involving Indian major carps has also demonstrated that dietary hemp-derived ingredients can influence the fatty-acid profile of fish, supporting their potential use as aquaculture feed ingredients from a nutritional perspective (7).

The detection of $\Delta 9$ -THC and CBN in the hemp seed oil used in the present experiment is consistent with previous reports showing that cannabinoid residues can occur in hemp seed-derived products even though the seed itself is not regarded as a major site of cannabinoid biosynthesis. Cannabinoid contamination of seeds can occur through contact with cannabinoid-containing flowers, leaves, or resinous plant surfaces during harvesting, handling, and processing (16,17). Investigations of commercial hemp seeds and oils have similarly reported detectable

concentrations of $\Delta 9$ -THC and other cannabinoid-related compounds, with substantial variation among products and production methods (17,27).

The cannabinoid content of hemp-derived materials can be influenced by several factors, including plant genotype, chemotype, maturity, environmental conditions, harvested plant part, storage, and processing procedures (13-15). Oil extraction may also transfer surface-associated or contaminating cannabinoid material into the lipid fraction. This provides a plausible explanation for the presence of $\Delta 9$ -THC and CBN in hemp seed oil despite the substantially lower cannabinoid detection in whole seed. Earlier analytical studies have emphasized that the cannabinoid content of hemp seed-derived foods can vary markedly according to seed cleanliness and the extent of contamination from other parts of the cannabis plant (17,27,28).

Whole hemp seed in the present study contained detectable CBD at $0.06 \pm 0.01\%$, whereas the remaining targeted compounds were not detected. This pattern differs from the hemp seed oil, in which CBD was not detected but $\Delta 9$ -THC, CBN, and resorcinol were identified. Such differences between whole seed and extracted oil may reflect differences in the distribution, extraction, and concentration of plant-derived chemical constituents during processing. Previous studies have demonstrated considerable variability in cannabinoid profiles among cannabis accessions and plant-derived products, emphasizing that cannabinoid composition cannot be assumed to be uniform across different hemp matrices (10,14,15).

The most important biological finding of the present study was the absence of detectable targeted cannabinoids in Rohu plasma following 50 days of dietary hemp supplementation. This finding was observed across all three hemp seed oil treatments and all three whole hemp seed treatments. Plasma from control fish similarly showed no detectable targeted compounds. Accordingly, increasing dietary inclusion from 3% to 9% hemp seed oil and from 5% to 15% whole hemp seed did not result in detectable circulating concentrations of the targeted compounds under the conditions of the analytical procedure employed.

The absence of detectable cannabinoids in plasma should not, however, be interpreted as evidence that gastrointestinal absorption, metabolism, tissue distribution, or elimination did not occur. Cannabinoids are generally lipophilic compounds and may undergo extensive absorption, metabolism, redistribution, and sequestration in lipid-rich tissues following exposure (23). Pharmacokinetic behavior also differs according to the individual cannabinoid, route of administration, dose, duration of exposure, and biological species. Consequently, circulating blood concentrations may not directly represent the total amount of a cannabinoid or its metabolites distributed throughout an organism (23).

This distinction is particularly relevant to $\Delta 9$ -THC. Assessments of hemp-derived animal feeds have recognized that THC and biologically active metabolites may be distributed to different tissues following exposure and that lipid-rich tissues can represent important compartments because of the lipophilic properties of these compounds (24). Food-safety assessments have similarly considered the potential transfer of THC and related compounds into foods of animal origin following dietary exposure (29). Therefore, the present plasma findings establish only that the targeted compounds were not detectable in the circulating plasma under the experimental and analytical conditions used; they do not establish the complete absence of cannabinoids or metabolites from other tissues.

The route of exposure may also influence cannabinoid disposition. Oral exposure differs substantially from inhalational exposure because orally ingested cannabinoids undergo gastrointestinal absorption and extensive presystemic metabolism before reaching the systemic circulation (23). The dietary exposure used in the present study therefore represents a fundamentally different biological context from studies involving inhaled cannabis or cannabinoid preparations. This difference should be considered when interpreting the absence of detectable circulating cannabinoids after prolonged consumption of hemp-containing feed.

The findings are also relevant when considered in the broader context of hemp use in animal nutrition. Hemp seed, hemp seed cake, and hemp seed oil have previously been evaluated as feed ingredients in cattle and poultry (20-22). In laying hens, diets containing hemp seed and hemp seed oil have been evaluated in relation to production performance, egg characteristics, and blood biochemistry, demonstrating that these feed materials can be incorporated into formulated diets under controlled conditions (21). In cattle, hemp seed cake has been investigated as a protein-rich feed ingredient and has also been evaluated for its effects on milk production and composition

(20,22). These studies support continued investigation of hemp-derived ingredients in animal nutrition while also emphasizing the need for chemical characterization and safety assessment of the particular material being used.

For aquaculture, this issue is especially important because the nutritional value of the final fish product is influenced by feed composition. Previous work in Rohu and Mrigal has shown that hemp seed and hemp seed oil supplementation can modify muscle fatty-acid composition and improve selected indices of essential fatty-acid balance following dietary exposure (7). The present findings complement that nutritional evidence by providing information on the detection of selected cannabinoid-related compounds in the hemp-derived ingredients and in circulating plasma after dietary administration. Nevertheless, the present experiment was designed primarily to examine cannabinoid detection rather than growth performance, therapeutic efficacy, tissue nutritional quality, or clinical outcomes.

The measured percentages reported for hemp seed oil and whole hemp seed should be interpreted within the specific analytical framework used in this experiment. GC-MS permits sensitive chemical identification, but quantitative interpretation depends on calibration procedures, reference standards, recovery, extraction efficiency, and clearly defined analytical limits. The study reported concentrations as percentage values and classified compounds in plasma as not detected. Consequently, these percentages should not automatically be interpreted as directly equivalent to regulatory mass-concentration measurements reported in units such as mg/kg or µg/g unless equivalence is established by the underlying analytical calibration procedure.

A further consideration is that “not detected” is an analytical rather than an absolute biological designation. An analyte may be present below the detection capability of a particular analytical method while still being present at a very low concentration. Reliable interpretation of non-detection therefore depends on the analytical sensitivity of the assay. The findings of this experiment are most appropriately interpreted as showing that the targeted cannabinoids were below detectable levels in the tested Rohu plasma samples under the GC-MS conditions employed.

The study has several limitations that should be considered. First, circulating plasma was examined, whereas lipid-rich tissues, liver, muscle, and other organs were not analyzed for cannabinoid residues or metabolites. Second, the experiment evaluated a 50-day exposure period and therefore does not establish the consequences of longer-term feeding. Third, only the targeted compounds included in the analytical panel were evaluated, and cannabinoid metabolites were not separately quantified. Fourth, the reported analytical information does not provide compound-specific limits of detection and quantification, preventing precise definition of the maximum possible concentration represented by an ND result. Finally, the reported percentage concentrations require interpretation within the analytical methodology used and should not be extrapolated directly to regulatory exposure thresholds without corresponding validated mass-based measurements.

Despite these limitations, the findings provide experimental information regarding the relationship between cannabinoid-containing hemp-derived feed ingredients and circulating cannabinoid detection in Rohu. The detection of selected compounds in hemp seed oil and CBD in whole hemp seed demonstrates the importance of chemical characterization of hemp-derived feed materials before dietary use. At the same time, the absence of detectable targeted cannabinoids in Rohu plasma after 50 days of feeding provides a basis for further studies examining cannabinoid disposition in edible tissues, metabolites, longer exposure periods, and analytically validated residue concentrations.

CONCLUSION

Hemp seed oil contained detectable Δ^9 -THC, CBN, and resorcinol, while CBD was the only targeted cannabinoid detected in whole hemp seed. Following 50 days of dietary supplementation with up to 9% hemp seed oil or 15% whole hemp seed, none of the targeted compounds was detected in the plasma of Rohu (*Labeo rohita*) under the GC-MS conditions used. These findings indicate an absence of detectable circulating targeted cannabinoids under the experimental conditions but do not establish their complete absence from other tissues or at concentrations below the analytical detection limit. Further studies incorporating validated limits of detection and quantification, cannabinoid metabolites, and edible tissue analysis are warranted to characterize the disposition and food-safety implications of cannabinoids associated with hemp-based aquaculture feeds.

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