Article published online: 2024-05-31

Original Article

Impact of Dehydrated Anchovy Powder on the Growth and Nutritional Well-Being of the Human Undernourished Population: Assessing Bioavailability and Nutritional Effectiveness through In Vivo Experimental Models

Akshay Pradeep[1] Gopika Remadevi[1] Amruth Padmaprakashan[1] Rosemol Jacob Mannuthy[1] Preethy Treesa Paul[1] Joshy Chalil George[2] Suseela Mathew[1*]

[1] Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India
[2] Fish Processing Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India

Address for correspondence Suseela Mathew, PhD, Biochemistry and Nutrition Division, ICAR-Central Institute of Fisheries Technology, Cochin, Kerala 682029, India (e-mail: suseela1962@gmail.com).

J Health Allied Sci[NU]

Abstract

The research focuses on assessing the efficacy of dried anchovy powder as a dietary supplement, examining its bioavailability and impact on growth and nutritional status. The study, centered on Wistar male rats, acknowledges anchovies for their rich essential nutrients and potential health benefits. Employing a meticulously controlled experimental design, the research exposes experimental animals to various dietary interventions by integrating dried anchovy powder. This study investigates the availability of vital nutrients like proteins, omega-3 fatty acids, vitamins, and minerals in the dried anchovy powder to Wistar male rats. The effects of dried anchovy powder on growth parameters, encompassing body weight, length, organ development, and the nutritional status of the rats, are explored. Examining hematological and biochemical markers provide insights into the overall health of the experimental subjects. Additionally, the research delves into potential mechanisms underlying the observed effects, including nutrient absorption and metabolism. The outcomes of this study offer valuable insights into the potential of dried anchovy powder as a nutritional supplement and its role in enhancing the nutritional status of the malnourished people of India. These findings may have a direct impact on dietary interventions aimed at improving human nutrition and health.

Keywords
- dried anchovy powder
- malnutrition
- growth parameters
- animal feeding
- wistar rat

Introduction

Malnutrition presents a multifaceted challenge in India, marked by insufficient access to a well-rounded and nourishing diet. The nutritional concerns in India, within the context of fisheries management, are intricately linked to the sustainability of fish as a valuable source of nutrients.[1] The depletion of fish stocks due to overexploitation has the potential to compound malnutrition by restricting access to essential nutrients. Therefore, implementing effective fisheries management strategies that prioritize sustainability is paramount to preserving ecological equilibrium and supporting the livelihoods of fishing communities.[2] Effectively addressing malnutrition entails advocating for consuming diverse fish

DOI https://doi.org/10.1055/s-0044-1786690.
ISSN 2582-4287.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

species, considering socioeconomic implications, and actively involving local populations in sustainable fishing practices.[3]

Recent research reflects a growing interest in exploring natural sources for nutritional supplements, driven by the increasing recognition of their potential health benefits.[4] Anchovies are renowned for being a rich source of protein, with higher levels than many other animal protein sources. Additionally, fish contains almost essential amino acids, polyunsaturated fatty acids, vitamins and minerals, making it a valuable dietary choice. Specifically, as Kari et al[5] emphasize, dried anchovy powder has garnered attention due to its abundant essential nutrient content. As small pelagic oily fish found in trophic and temperate waters, anchovies are scientifically significant owing to their nutritional value and associated health advantages. These encompass pivotal roles in cardiovascular health, inflammatory disease prevention, infant neuronal development, fat glycemic control, and other health aspects.[6] Anchovies, classified in the Clupeiformes order of the family Engraulidae, are distributed in the Indian and Pacific Oceans, primarily in shallow coastal waters.[7] They vary in size, generally 8 to 32 cm in length, with distinct coloration.

According to the 2017 Food and Agriculture Organization report, anchovies and sardines constitute around 52% of global landings for small pelagic fish. In Malaysia, anchovy landings were reported at 28,894 tons in 2019, with Kedah contributing significantly. The role of anchovies remains prominent within Indian pelagic fisheries resources. In the year 2003, the respective contributions of anchovies to the total marine fish landings were 6%. Indian waters are home to 28 recorded species of anchovies, with significant contributions attributed to the *Stolephorus, Engraulis, Thryssa, Setipinna*, and *Coilia* genera. The anchovy fishery in India focuses on the coastal states of Andhra Pradesh, Tamil Nadu, and Kerala, collectively making significant contributions to the national anchovy catch.[8]

Recognized as an excellent protein source, anchovies offer a complete amino acid profile crucial for bodily functions. Their highly bioavailable protein ensures efficient absorption and contributes omega-3 fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), known for cardiovascular and cognitive health benefits. With essential vitamins and minerals, anchovies enhance their nutritional value, making them a versatile and health-promoting addition to diets.[9,10] Despite their ecological significance and nutritional richness, anchovies may be labeled as "trash fish" due to factors like small size or perceived economic value. Since anchovies are consumed whole, they are considered as a source of calcium and phosphorus. However, recognizing their role in marine ecosystems and promoting sustainable harvesting challenges this perception.[11] Efforts to transform anchovies into a valuable and eco-friendly protein source involve identifying suitable species, ensuring sustainable harvesting, and developing efficient processing techniques.[12] Anchovies have significant potential in Indian fisheries, particularly in the Arabian Sea and the Bay of Bengal, addressing nutritional deficiencies with their protein and nutrient richness. Sustainable harvesting practices and efforts to promote their consumption could enhance their economic and healthy contributions to the Indian fisheries sector.[13]

This study aims to evaluate dried anchovy powder's nutritional effectiveness and bioavailability as a dietary supplement. The drying process is essential for anchovy preservation, extending its shelf life, concentrating nutrients, and promoting economic sustainability in fishing communities. Recognizing the pivotal role of anchovies in fisheries management is crucial for fostering a diverse fisheries industry, addressing protein deficiencies, and creating economic opportunities. Utilizing Wistar male rats, the research investigates the impact of dried anchovy powder on growth parameters, health markers, and underlying mechanisms such as nutrient absorption and metabolism. The findings carry implications for the development of functional food products or dietary interventions that can contribute to human well-being in the context of nutrition and health.

Materials and Methods

Production of Anchovy-Dried Fish Powder Employing Oven Drying

The manufacturing process of dried anchovy powder began with the acquisition of fresh anchovies from the local market, the species being identified as *Stolephorus commersonnii* based on the Food and Agriculture Organization species identification sheet. These anchovies were meticulously cleaned to eliminate sand particles and dirt before being arranged on a metallic tray. Subsequently, the anchovies underwent a 3-day drying period in an electric drier set at 50°C. Once dried, they were finely ground into a powder using a pulverizer equipped with a 250 µm mesh sieve (Model 160B; Jacobson Machinery Works, Minneapolis, Minnesota, United States). The resulting fish powder was then carefully packed in polypropylene bags and stored at −18°C until used. The detailed process flow chart for the production of dried anchovy powder is illustrated in ►**Fig. 1**.

Determination of Proximate Composition

The samples were analyzed for the proximate composition. The moisture, protein, fat, and ash content were analyzed using the validated method.[14]

Moisture

The moisture content was determined by drying a 10 g sample at 105°C in a thermostatically controlled hot air oven. The samples were taken in a preweighed petri dish and kept in an oven; the weight reduction was checked by repeatedly weighing and then cooling the sample in desiccators till the weight became constant. Moisture content was expressed in percentage.

$$\text{Moisture content (\%)} = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

$W1$= Weight of dry petri plate
$W2$= Weight of the sample in the petri plate
$W3$= Weight of the sample in the petri plate after drying

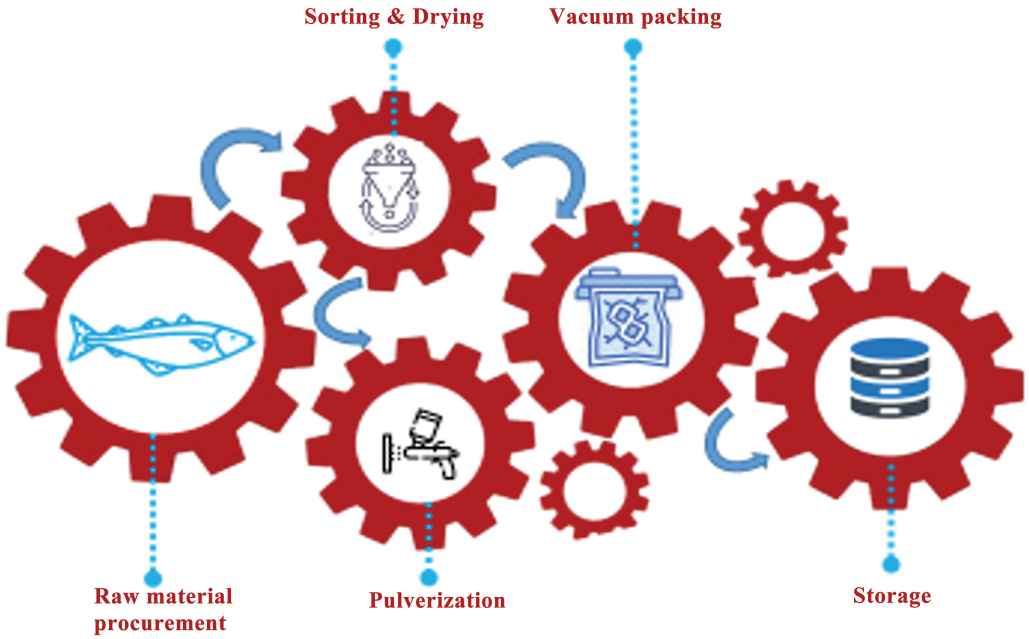

Fig. 1 Flowchart outlining the steps involved in producing dried anchovy powder.

Crude Protein

Crude protein content was determined by the Kjeldahl nitrogen method, which involves digestion, distillation, and titration. About 100 mg of the sample was taken into a Kjeldahl digestion flask of 100 mL capacity. A few glass beads, a pinch of digestion mixture (CuSO4, K_2SO_4), and 10 mL of sulfuric acid were added. This was then subjected to digestion over a burner till the solution turned colorless. The digested and cooled solution was made up to 100 mL by adding distilled water. Ten milliliters of the made-up solution was then added to the reaction chamber of the micro-Kjeldahl distillation apparatus and rinsed down with distilled water. Two drops of phenolphthalein indicator and 40% NaOH were added till the indicator changed to pink. Distillation was performed for 4 minutes, and liberated ammonia was collected in 2% boric acid (10 mL) containing a drop of Tashiro's indicator. The amount of ammonia was determined by titrating with N/100 sulfuric acid until the solution turned pink.

$$\text{Protein content (\%)} = \frac{x \times 0.14 \times V \times 6.25}{V1 \times W \times 1000} \times 100$$

x = Titer value of the sample
V = Total volume of digest
$V1$ = Volume of digest taken for distillation
W = Weight of sample taken

Crude Fat

Crude fat content was determined by the Soxhlet method. One gram of sample was weighed into a thimble and placed in a Soxhlet apparatus. The sample was extracted using petroleum ether for 3 hours at 100°C. After extraction, the solvent was evaporated at 80 to 100°C, and the fat content was weighed after cooling it in a desiccator.

$$\text{Fat content (\%)} = \frac{(W3 - W2)}{W1} \times 100$$

$W1$ = Weight of the sample
$W2$ = Weight of the Soxhlet beaker
$W3$ = Weight of fat and beaker

Ash

To determine the ash content, silica crucibles were cleaned and kept in a muffle furnace at 600°C for an hour. This was then cooled in a desiccator, and the weight of the empty crucible ($W1$) was noted down. Sample (2 g) was taken in a crucible and was subjected to heating until the material got charred. The charred material was then transferred to a muffle furnace, previously set at a temperature of 650°C and heated to 6 to 8 hours until the material became white or grayish-white ash. The crucibles were cooled in a desiccator, and weight was noted ($W3$).

$$\text{Ash content(\%)} = \frac{(W3 - W1)}{W2} \times 100$$

$W1$ = Weight of crucible
$W2$ = Weight of sample
$W3$ = Weight of ash in the crucible

Determination of Fatty Acid Profile

The analysis of fatty acids involves three steps: lipid extraction, preparation of fatty acid derivatives, and gas chromatographic (GC) analysis. The crude lipids in the gutted fish samples were extracted using a chloroform-methanol mixture.[15] Saponification of fats liberates fatty acids from triglycerides. The fatty acids are derivatized into their corresponding fatty acid methyl esters by reflexing with BF_3 methanol reagent,[16] and the fatty acid profile is analyzed using the gas liquid chromatography—Flame Ionization Detector (FID) method.

The GC is set at the required temperature with the optimum flow rate of nitrogen gas (N_2), the carrier gas in the chromatograph. Program of GC Injector 260°C; FID—275°C; capillary column, PE Elite 225 (30 m, 0.25 mm 1.d, 0.25 um) Carrier gas-Nitrogen at 0.6m/min; Air 30ml/min and Hydrogen 30 mL/min for FID temperature program-110°C. After an initial hold of 4 minutes, the temperature is programmed to rise from 2.7°-C/min to 240°C and maintained at that temperature for 5 minutes; the split flow is 12 mL. Samples are identified by retention time by comparing with respective standards using the software. The area of each component is obtained from the computer-generated data, and concentration is calculated using the software by an external standard method. The fatty acid composition samples are expressed as g/100 g of total fatty acid content.

In Vivo Studies of Anchovy Dried Fish Powder in Male Wistar Rats

Preparation of the Feed

The feed was prepared for the experimental control animals by incorporating the anchovy dried fish powder (1.5%) into the standard diet. Positive control feed was prepared by incorporating casein (1.5%) into the standard diet, and the negative control was given a regular diet without any alterations.[17] The standard feed was powdered to maintain consistency, and the required ingredients were added, pelletized, and dried to attain a consistent shape and size (►**Fig. 2**). Feed and water are provided ad libitum, ensuring that animals have constant access to both.

Animal Feeding Trials

The experimental rats were handled following the guidelines for the regulation of scientific experiments on animals set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India. These procedures were approved by the Institutional Animal Ethics Committee of ICAR-Central Institute of Fisheries Technology, Cochin, with approval no: CIFT/B&N/IAEC/2020–1(6).

The initial trials assessed the efficacy of various feeds in male Wistar rats, considering parameters such as feed intake, average weight gain, feed conversion ratio (FCR), and specific growth rate (SGR). Thirty-three Wistar albino male rats weighing between 190 and 210 g were chosen for the feeding trials. The rats were initially given a basal diet for 1 week to facilitate adaptation. Following this period, the rats were divided into three groups, each containing 11 rats. The first group/ negative control continued with the basal diet (control feed), the second group/positive control group received casein-incorporated feed, and the third group/experimental group received anchovy dried fish powder incorporated feed. Throughout the study, all rats had unrestricted access to both food and water. They were paired and housed in individual cages, adhering to a 12-hour dark/light cycle. Weekly recordings were made of the rats' body weights and measurements of their daily food and water intake.[18]

After the experiment, rats were euthanized in a CO_2 chamber maintained at a flow rate of 3 mL/min for 5 to 10 minutes, and tissues were collected after rinsing with an ice-cold saline solution. A portion of the liver tissue was preserved in a 10% buffered formalin solution for subsequent histopathology analysis. Blood samples were obtained from animal models without anticoagulants through cardiac puncture. The serum, separated through centrifugation, was then employed for hematological parameters.

Assessment of Growth Parameters in Experimental Animals

Feed Conversion Ratio

In animal studies, the FCR is a parameter used to measure the efficiency of converting feed into body weight gain. It indicated how much feed was required to produce an inevitable weight gain in the animals. The FCR was calculated using the following formula:

$$\text{FCR} = \frac{\text{Total feed intake (g)}}{\text{Total weight gain (g)}}$$

A lower FCR value indicates better feed efficiency, meaning less feed was required for a specific weight gain. Conversely, a higher FCR value suggested poorer feed efficiency, indicating that more feed was needed to gain the same weight.[19]

Specific Growth Rate

The SGR is a parameter used to measure animal growth rate over a specific period. It indicates how quickly the animals

Fig. 2 (**A**) Control feed, (**B**) casein incorporated feed, and (**C**) anchovy dried fish powder incorporated feed.

are gaining weight. The SGR is calculated using the following formula:

$$\text{SGR} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})(\text{g})}{(\text{final time} - \text{initial time})} \times 100$$

The SGR indicated the relative growth rate of the animals over the study period. A higher SGR value indicated a faster growth rate, while a lower SGR value suggested a slower growth rate. SGR can be useful for comparing the growth performance of different treatments or groups in an animal study.[20]

Blood Profiling

Blood serum parameters like lipid profile, liver enzymes, and renal function tests were analyzed by using an automated analyzer FUJI DRI-CHEM- NX 500.[21]

Histopathological Evaluation of Liver Tissue

The liver tissue was fixed in a 10% buffered formalin fixative overnight. Hematoxylin and eosin (H and E) staining was performed on sections of the liver samples that were 5 µm thick and placed in paraffin wax. The stained slides of liver tissues were observed using a light microscope fitted with a digital camera.[22]

Statistics

A two-way analysis of variance was performed, the significance of the hypothesis was tested at a 5% significance level ($p < 0.05$), and means were compared using Tukey's test. Statistical analysis was performed using SAS 9.3.

Results and Discussions

Dehydrated Anchovy Powder

Traditionally, anchovies were converted into a dried form due to their vulnerability to deterioration after death. The conventional process involves cleaning the fish, stacking them one upon another, and exposing them to sunlight for drying.[23] Value addition processes such as filleting, drying, and flavoring enhance the market appeal of anchovies by addressing challenges like their small size, seasonal availability, and perishability.[24] By creating diverse products with extended shelf life and improved flavor profiles, value addition broadens consumer options and increases the overall marketability and demand for anchovy-based products. Various drying techniques, such as sun drying and open solar drying, have been employed globally to preserve fish.[25] Solar drying, considered superior to traditional sun-drying methods, produces higher-quality dried fish regarding nutritional value and hygiene, reducing the risk of insect infestation.[26] Additionally, the drying process decreases moisture content, providing a stable protein source for individuals with limited access to fresh fish.[27] Various techniques include washing, drying, and boiling fish in saltwater. The drying process involves either sun-drying or artificial drying, with artificial methods utilizing mechanical or electrical equipment such as radiation drying for efficient moisture removal.[28] Despite technological advancements, some Southeast Asian countries, such as Thailand, Indonesia, and Malaysia, still prefer traditional open-air drying methods to preserve anchovies.[23,29]

In our study, oven drying at an optimal temperature was opted. Oven drying offers benefits such as a controlled environment, reduced drying time, year-round availability, hygienic conditions, consistent quality, and the preservation of nutritional value. The controlled temperature and humidity settings of the oven contribute to a faster, more reliable drying process compared with traditional methods, particularly advantageous when consistent sunlight is unavailable throughout the year. The controlled environment minimizes contamination risks, ensuring a more hygienic drying process and a compatible, high-quality end product. Moreover, oven drying aids in preserving the nutritional content of anchovies by controlling the drying conditions.[30] The dried anchovy fish was finely ground into a powder using a pulverizer. The resulting fish powder (►**Fig. 3**) was then carefully packaged in polypropylene bags and stored at -18°C until used.

Anchovies are commonly dried and integrated into various cuisines. In Malaysia, for instance, anchovies are promptly washed and boiled in a 10% brine solution upon landing, followed by sun-drying.[23] In Indonesia, dried anchovies are prepared by boiling them in a salt solution (3–4%) followed by air drying.[31] The salting process serves as a pre-treatment, improving the product's ability to dry quickly and reducing its water activity, thereby extending shelf life and altering the sensory characteristics of the food.[32]

However, drying may compromise nutritional quality, leading to vitamin A degradation, lipid oxidation, and protein breakdown.[33] Furthermore, the open-air drying method exposes the product to contaminants, posing hygiene concerns for consumers.[25] Distributing dried anchovy powder offers numerous advantages, including extended shelf life, storage and transportation convenience, culinary application versatility, retained nutritional value, cost-effectiveness,

Fig. 3 Packed dried anchovy fish powder.

Table 1 Proximate composition of dried anchovy fish

Proximate composition of dried anchovy	Percentage (%)
Moisture	10.0 ± 1.02
Protein	75.52 ± 0.12
Ash	9.27 ± 0.23
Fat	14.43 ± 0.03

reduced fishy odor, improved ingredient homogeneity, and extended usability. These attributes collectively position dried anchovy powder as a valuable and widely applicable ingredient in the global food market.

Determination of Proximate Composition

The nutritional analysis of the sample yielded insightful information about its composition. The proximate composition of dried fish is depicted in ►**Table 1**.

Preserving anchovies involves drying to reduce water content and preventing microbial growth and spoilage.[34] The choice of fish drying method varies based on species and desired end-product; standard methods include sun drying and solar drying. According to studies,[27] a decreased moisture content and increased crude protein, crude fat, and ash were observed in sun-dried anchovies. As shown in the ►**Table 1**, dried anchovies' moisture content at 10% was consistent with the typical range of 10 to 20% for sun-dried fish.[35] Discrepancies may arise from variations in drying duration and temperature. Heat application lowers fish tissue's water activity, reducing the moisture content of the dry anchovies.[36] A study has reported higher protein values in solar-dried fish compared with traditional sun drying.[37]

Determination of Fatty Acid Profiling

A detailed examination of fatty acids in dried anchovy powder sheds light on their nutritional composition, as depicted in ►**Table 2**.

Palmitic acid emerges as the predominant component, presenting the highest concentration at 705.17 ± 2.71 mg/100 g. Following closely is cis-4, 7, 10, 13, 16, 19- DHA, a vital omega-3 fatty acid crucial for brain and cardiovascular health, measuring 552.04 ± 2.11 mg/100 g. Stearic acid, another saturated fatty acid with a neutral impact on cholesterol levels, is also identified at 286.24 ± 1.42mg/100 g. Based on the findings of [38] Sankar et al, anchovies exhibited elevated levels of fatty acids, with palmitic acid constituting 8.79% of the total composition. Additionally, Kaya and Turan[39] indicated that the combined presence of DHA and EPA in anchovies accounted for 25.39% of their composition.

Notably, the fatty acid composition of dried fish anchovy (►**Fig. 4**) revealed a higher concentration, suggesting that the drying process concentrates these beneficial fatty acids. Recognizing the significance of the fatty acid profile is crucial for nutrition, given their roles in cardiovascular and cognitive health. The composition underscores the potential of dried anchovy powder as a nutritious dietary source, offering

Table 2 Fatty acid profiling of dried fish powder

Fatty acids	Molecular formula	(mg/100 g)
Saturated fatty acids		
C6:0 (Caproic)	$C_6H_{12}O_2$	3.41 ± 0.31
C12:0 (Lauric)	$C_{12}H_{24}O_2$	10.76 ± 0.74
C13:0 (Tridecanoic)	$C_{13}H_{26}O$	5.64 ± 0.20
C14:0 (Myristic)	$C_{14}H_{28}O_2$	118.45 ± 1.21
C16:0 (Palmitic)	$C_{16}H_{32}O_2$	705.17 ± 2.71
C17:0 (Heptadecanoic)	$C_{17}H_{34}O_2$	70.09 ± 1.10
C17:1 (cis-10-Heptadecanoic)	$C_{17}H_{32}O_2$	22.34 ± 0.38
C18 (Stearic)	$C_{18}H_{36}O_2$	286.24 ± 1.42
C20:0 (Arachidic)	$C_{20}H_{32}O_2$	6.77 ± 0.29
C22:0 (Behenic)	$C_{22}H_{44}O_2$	6.08 ± 0.54
C24 (Lignoceric)	$C_{24}H_{48}O_2$	12.45 ± 0.39
Monounsaturated fatty acids		
Cis:11 (Eicosenoic)	$C_{20}H_{38}O_2$	23.04 ± 0.14
C14:1 (Myristoleic)	$C_{14}H_{26}O_2$	2.71 ± 0.54
C16:1 (Palmitoleic)	$C_{16}H_{30}O_2$	101.09 ± 1.31
C18:1n9c (Oleic)	$C_{18}H_{34}O_2$	15.17 ± 0.11
C24:1 n9 (Nervonic)	$C_{24}H_{46}O_2$	7.69 ± 0.61
Polyunsaturated fatty acids		
C18:2n6c (Linoleic)	$C_{18}H_{32}O_2$	139.57 ± 1.20
C18:3n6 (γ-Linolenic)	$C_{18}H_{30}O_2$	32.87 ± 0.36
C20:5n3 (cis-5.8.11.14. 17-eicosapentaenoic)	$C_{20}H_{30}O_2$	147.97 ± 1.33
C22:6n3 (cis-4.7.10.13.16. 19-docosahexaenoic acid)	$C_{22}H_{32}O_2$	552.04 ± 2.11
Other fatty acids		
C15:0 (Pentadecanoic)	$C_{15}H_{30}O_2$	45.84 ± 0.97
cis:11,14 Eicosadienoic acid methyl ester	$C_{21}H_{38}O_2$	16.93 ± 0.61
cis-11,14,17-Eicosatrienoic	$C_{21}H_{36}O_2$	19.70 ± 0.26
C23:0 (Tricosanoic)	$C_{23}H_{48}O_2$	49.52 ± 0.88

essential nutrients with potential health benefits for consumers seeking to boost their omega-3 intake. The fatty acid composition analysis highlights the prevalence of palmitic acid, cis-4, 7, 10, 13, 16, 19-DHA, and stearic acid in dried anchovy fish, which positions it as a valuable nutritional source for growth and nutrition in human diets.

Estimation of Growth Parameters in Experimental Animals

Weight Gain

The fish powder-fed group had the highest average weekly weight gain in 3 out of the 4 weeks. This suggested a relatively rapid increase in weight compared with the other

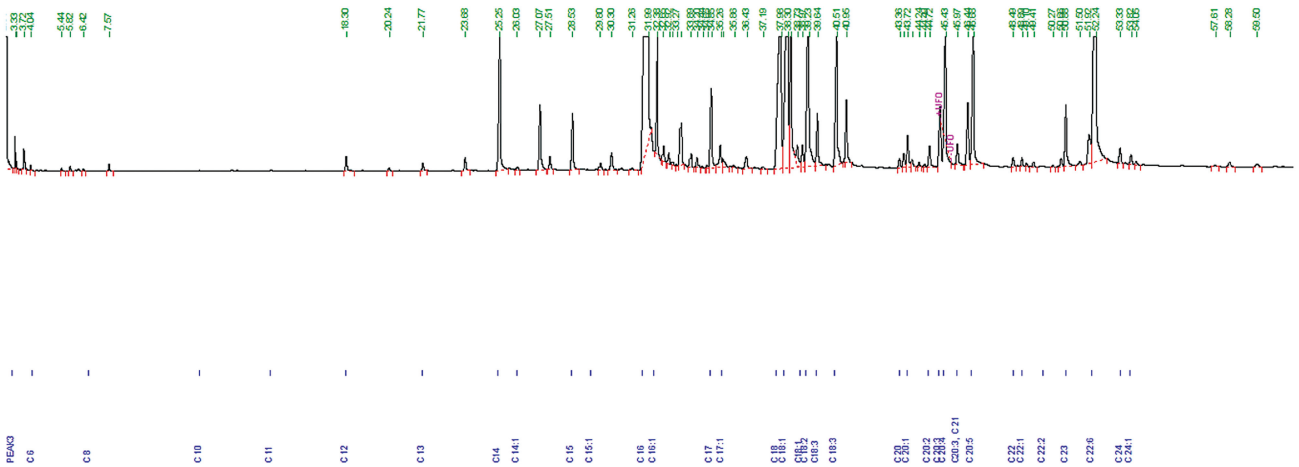

Fig. 4 Fatty acid chromatogram of dried fish powder.

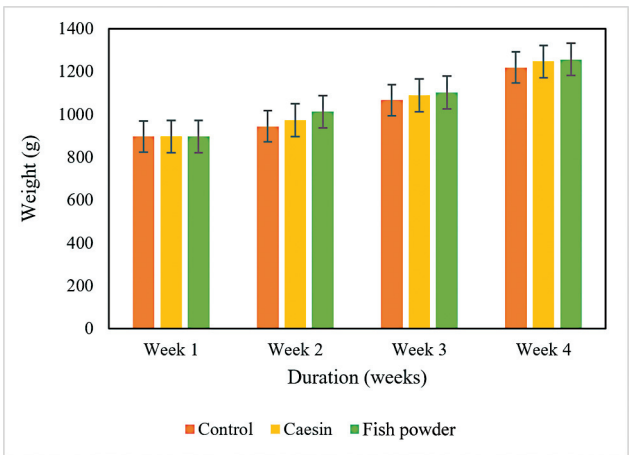

Fig. 5 Bar graph showing weight gain in experimental animals.

two groups. The casein-fed group had the highest average weight gain in the third week, indicating a significant growth during that period. However, their average weight gain in the first and fourth weeks was lower compared with the fish powder-fed group. The control group had the lowest average weight gain throughout the study period, indicating a slower weight increase rate than the other two groups (►**Fig. 5**).

In summary, the fish powder-fed group exhibited the highest overall weight gain and a relatively rapid rate of increase throughout the study period. The casein-fed group showed consistent weight gain with a moderate growth rate, whereas the control group had the lowest weight gain and a slower pace of increase. The highest weight gain among the experimental subjects can be attributed to several factors, including the nutrient composition of the test feed, its enhanced digestibility, higher energy density, and potential palatability, leading to increased feed intake.[40] The optimal FCR in the test feed group suggests that the feed conversion into body mass was more efficient.[41] Including unique ingredients or additives in the test feed, well-controlled experimental conditions and genetic factors may have further contributed to the observed weight gain.[42] The extended duration of the study likely allowed for the cumulative effects of the nutritional advantages in the test feed to become more pronounced. Overall, these results underscore the significance of feed composition and dietary factors in influencing the growth and weight gain of the experimental subjects, highlighting the potential benefits of the test feed formulation in promoting optimal animal performance.

Feed Utilization

The fish powder-fed group showed a feed utilization of 29.19 ± 0.18 units, whereas the control group had a relatively 27.99 ± 0.65 units over the 28 days. The casein group expressed 28.79 ± 0.77 units over the 28 days (►**Table 3**). Overall, there are minor differences in the average daily feed intake among the three groups. The fish powder-fed group had the highest intake, followed by the casein and control groups. This suggests that the experimental subjects in the fish powder group consistently consumed a moderate to the high amount of feed over the duration of the study. The relatively narrow range of feed intake indicates a degree of consistency in the feeding behavior of the group, and the slightly higher average daily intake suggests a tendency for the subjects to consume a relatively consistent amount of

Table 3 Assessment of growth parameters (different letters on the superscript indicate significance $p < 0.05$)

Groups	FCR	SGR	Feed utilization
Control	10.75 ± 1.26^{A}	1.27 ± 0.22^{A}	27.99 ± 0.65^{A}
Casein fed	10.14 ± 1.20^{A}	1.38 ± 0.27^{A}	28.79 ± 0.77^{B}
Fish powder fed	8.31 ± 1.26^{B}	1.52 ± 0.18^{A}	29.19 ± 0.18^{C}

Abbreviations: FCR, feed conversion ratio; SGR, specific growth rate.

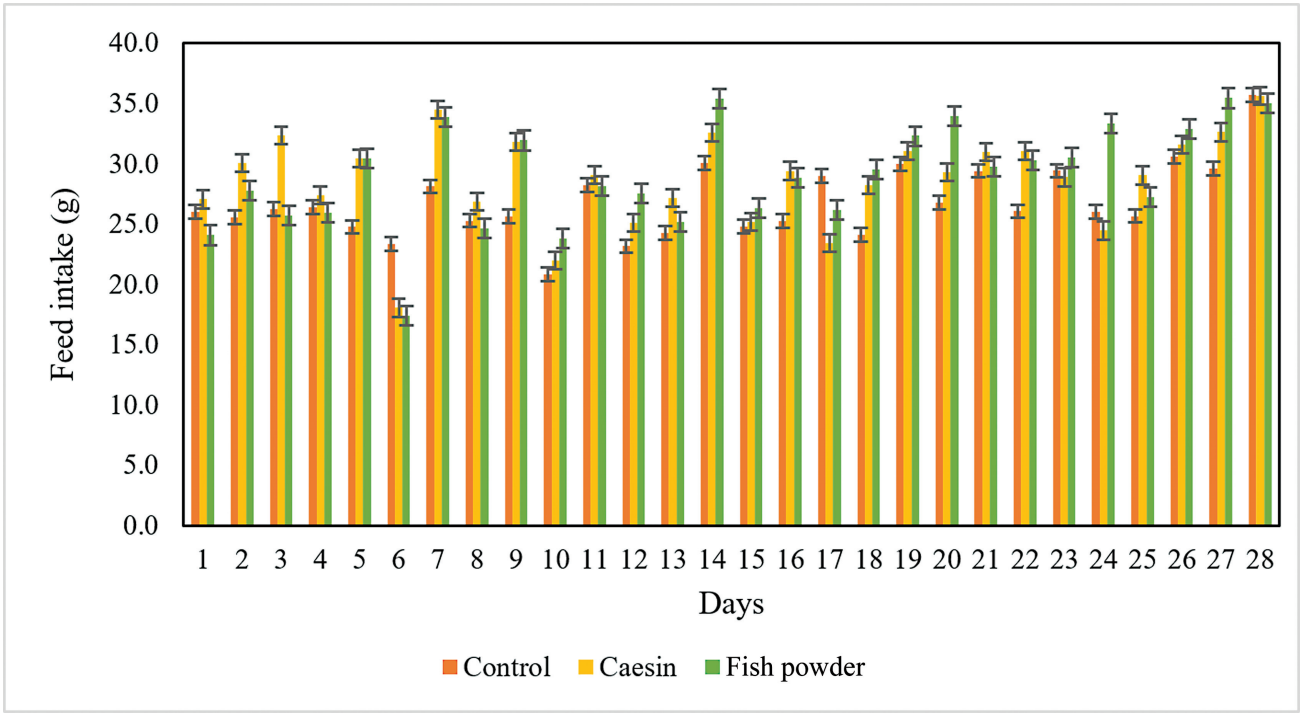

Fig. 6 Bar graph showing feed utilization profile of experimental animals.

fish powder daily.[43] Understanding the group's feeding patterns and average intake provides valuable insights into the experimental subjects' dietary preferences and consumption habits over the specified 28-day period (►**Fig. 6**).

Feed Conversion Ratio

The fish powder-fed group exhibited a lower FCR (8.31 ± 1.26 units), while the control group (10.75 ± 1.26 units) and casein group (10.14 ± 1.20 units) exhibited a slightly higher FCR. This signifies enhanced feed conversion efficiency for the fish powder-fed groups, reflecting superior feed conversion efficiency among the three groups. These findings suggest that the fish powder-fed group achieved the most effective feed conversion, followed by the casein group, while the control group displayed the least efficient conversion. This implies that incorporating casein or fish powder into the diet could enhance feed efficiency in weight gain compared with the control group in 28 days. This result underscores the effectiveness of the fish powder diet in promoting optimal growth and nutrient utilization among the experimental subjects. Factors contributing to this efficiency include the favorable nutrient composition of the fish powder, improved digestibility, and the diet's palatability, encouraging consistent and sufficient feed intake.[44] In summary, the results highlight the positive impact of the fish powder formulation on achieving economical feed conversion and supporting the overall growth of the experimental subjects.

Specific Growth Rate

The detailed examination of SGRs among the three groups reveals notable differences. The fish powder-fed group exhibited a substantially higher growth rate (1.52 ± 0.18 units) than the control and casein-fed groups. This result strongly suggests that organisms in the fish powder-fed group experienced superior growth compared with their counterparts in the control and casein-fed groups. The elevated SGR in the fish powder-fed group indicates a faster rate of development and increased body mass over the given period. The presence of essential nutrients or bioactive compounds in the fish powder might have played a crucial role in promoting enhanced growth and development among the organisms in this group.[45]

Overall findings underscore the positive impact of the fish powder feed on SGRs, highlighting its potential as a valuable dietary component for fostering accelerated growth in the studied organisms.

Blood Serum Profiling

The three groups of animals were of a control diet, a casein diet, and a fish powder diet. The biomarkers analyzed included total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), SGPT/ALT (alanine aminotransferase), SGOT/AST (aspartate aminotransferase), serum creatinine, and blood urea nitrogen (BUN).

Serum Lipid Profile

The comprehensive examination of lipid profiles (►**Fig. 7**) in the three groups of rats revealed notable distinctions in cholesterol levels. The control group demonstrated the lowest total cholesterol levels at 73 ± 2.21 mg/dL, followed by the fish powder group at 74 ± 2.25 mg/dL and the casein group at 81 ± 2.49 mg/dL. A significant difference was observed between the control and casein groups, indicating that the casein diet led to a moderate increase in total cholesterol compared with the control diet. Likewise, the control group exhibited the lowest levels of triglyceride at

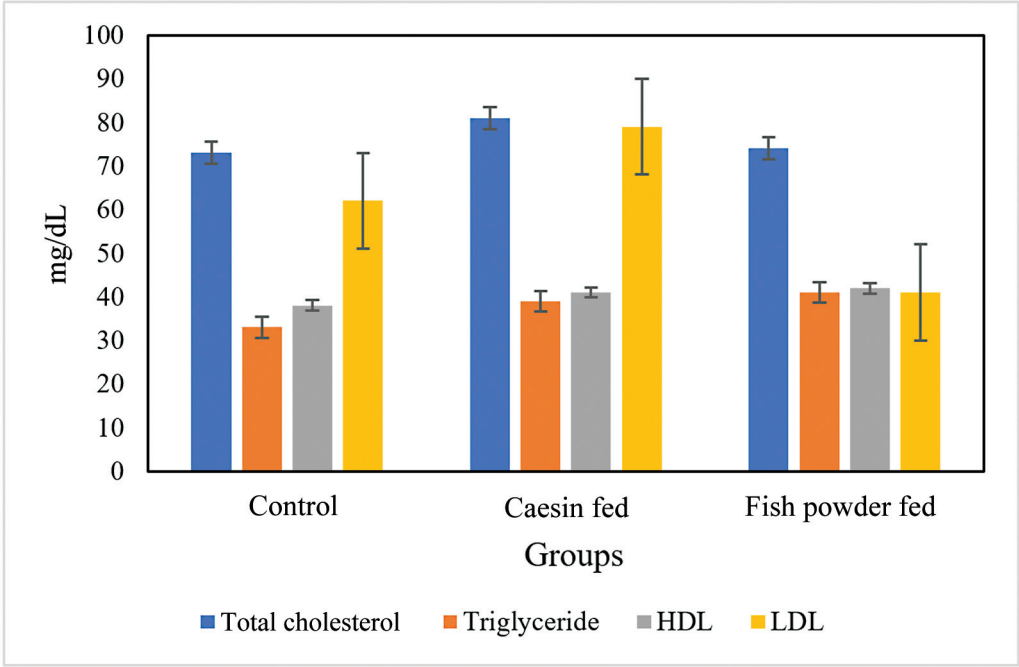

Fig. 7 Bar graph showing lipid profile of experimental animals.

33 ± 1.0 mg/dL. The casein and fish powder groups displayed slightly higher levels at 39 ± 1.19 mg/dL and 41 ± 1.33 mg/dL, respectively. The HDL levels followed a similar trend, with the fish powder group showing the highest levels at 42 ± 1.28 mg/dL, followed by the casein group at 41 ± 1.27 mg/dL and the control group at 38 ± 1.19 mg/dL. Meanwhile, LDL levels in the fish powder group were significantly lower (41 ± 1.29 mg/dL) compared with both the control group (62 ± 1.89 mg/dL) and the casein group (79 ± 2.39 mg/dL). This substantial reduction in LDL levels in the fish powder group suggested a potentially positive impact on cardiovascular health.[46] In summary, the results underscore the varied effects of the three diets on lipid profiles, highlighting potential cardiovascular benefits associated with the fish powder diet, which demonstrated a significant reduction in the major cardiovascular risk factor, LDL cholesterol, and higher levels of "good" cholesterol, HDL.

Liver Function Markers

The analysis of liver function markers (►**Fig. 8**) revealed that the control group had slightly elevated SGPT/ALT levels at 34 ± 1.05 U/L, compared with the casein-fed group at 29 ± 0.96 U/L and the fish powder group at 27 ± 0.85 U/L. Despite the numerical differences, these variations were not statistically significant. This implies that the dietary interventions, namely the casein and fish powder diets, did not

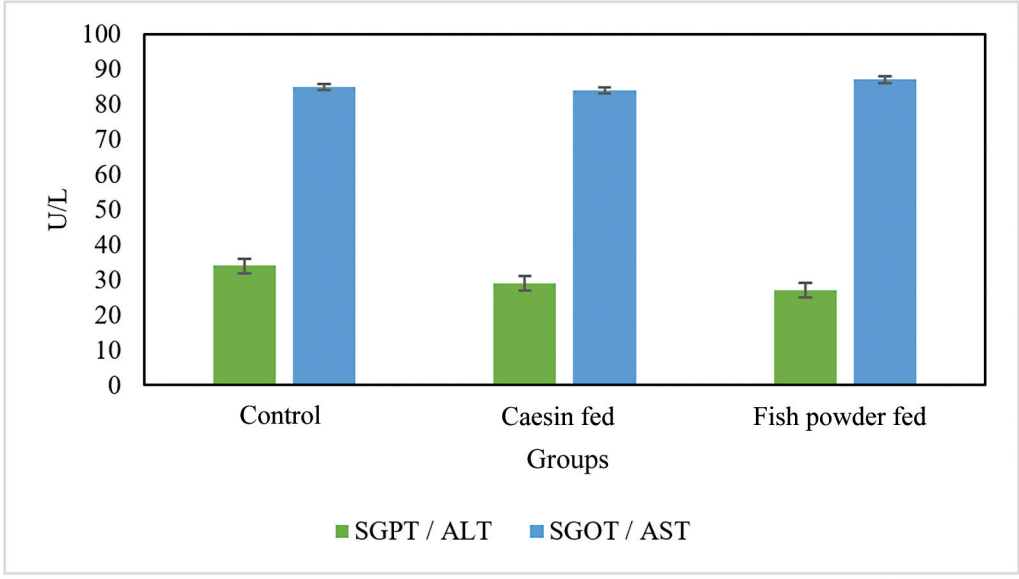

Fig. 8 Bar graph showing liver enzymes of experimental animals. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

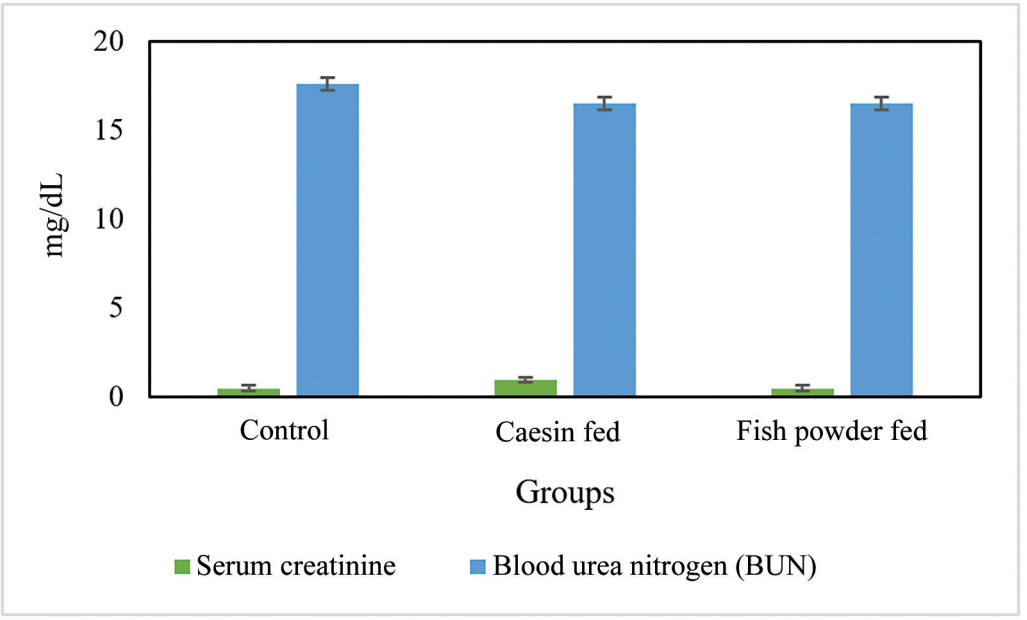

Fig. 9 Bar graph showing kidney function markers of experimental animals.

substantially influence SGPT/ALT levels, and the differences observed were likely within the normal range of variation. On the other hand, SGOT/AST levels were relatively consistent across all three groups, with no statistically significant differences observed. The casein-fed group had slightly lower SGOT/AST levels at 84 U/L compared with the control group at 85 ± 2.59 U/L and the fish powder group at 87 ± 2.69 U/L. This uniformity suggests that the dietary interventions had minimal impact on liver function, as measured by SGOT/AST levels. In conclusion, the study indicates that the tested diets did not significantly alter liver function, as reflected by SGPT/ALT and SGOT/AST levels, suggesting that these dietary interventions may have a limited effect on hepatic health in the animal models studied.

Kidney Function Markers

The comparison of serum creatinine levels revealed similar values (►**Fig. 9**) for the control and fish powder groups (0.47 ± 0.02 mg/dL) and a slight elevation in the casein group (0.94 ± 0.04 mg/dL). The significant difference between the control and casein groups suggested that casein may have adverse effects on kidney function, as indicated by elevated creatinine levels. On the other hand, BUN levels were comparable across all three groups, with no significant differences. This suggests that the dietary interventions, including the casein and fish powder diets, did not significantly impact kidney function, as measured by BUN levels. In summary, the study indicates that the fish powder diet had neutral effects on serum creatinine and BUN levels, suggesting a limited impact on kidney function. On the contrary, the casein diet significantly increased serum creatinine levels, indicating potential adverse effects on kidney function. These findings underscore the importance of considering specific dietary components in assessing their impact on renal health.

Histopathological Evaluation of Liver Tissue

The histopathological examination of liver tissue in animals exposed to diverse diets yielded significant insights (►**Fig. 10**). No thickening of the epithelial layer was observed in any dietary group, indicating the absence of notable abnormalities in the outermost liver cell layer and affirming the structural integrity of liver cells across all diet groups. Additionally, the

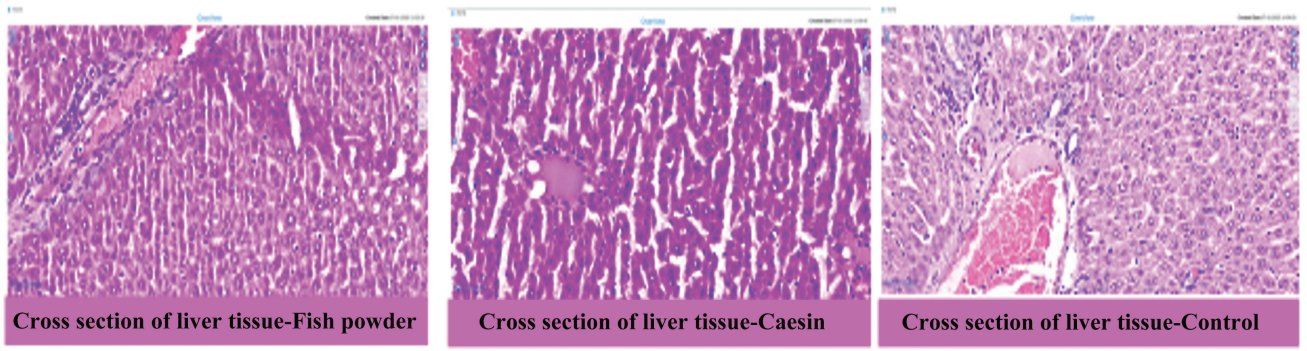

Fig. 10 Liver tissue cross-section: (**A**) Experimental control, (**B**) positive control, and (**C**) negative control.

lack of submucosal inflammatory cell infiltration in the liver tissue of all groups is noteworthy, suggesting an absence of indications of inflammation, a crucial aspect as liver inflammation is often associated with various pathological conditions. A distinctive finding was the identification of collagen deposition, specifically in the liver tissue of the fish powder-fed group. Collagen, a fibrous protein linked to tissue repair and scar formation, may indicate a reparative response or potential fibrosis in the liver tissue of the fish powder-fed group, necessitating further investigation to understand the implications of this specific observation. The absence of lymphoid follicles in all groups is reassuring, signaling no significant immune-related changes in the liver tissue.[47]

The histopathological examination also revealed positive outcomes regarding cell health, with no necrotic or apoptotic cells detected across all dietary groups, crucial for maintaining overall liver health.[48] The absence of fatty infiltrations in all groups is a favorable outcome, indicating no excessive fat accumulation in the liver tissue. This is crucial as excessive fat can lead to fatty liver disease, and the absence of such infiltrations indicates good liver health in experimental animals.[49] In summary, the histopathological examination suggests that different diets did not induce significant structural or inflammatory changes in liver tissue. While collagen deposition in the fish powder-fed group warrants further investigation, other observations, such as the absence of inflammation, necrosis, and fatty infiltrations, collectively indicate the overall well-being of the liver tissue in the studied animals. The most notable finding, particularly in the fish powder-fed group, is the detection of collagen deposition, signifying a potential reparative response in the liver tissue. It is essential to note that this finding, alongside other positive indicators, points toward an overall healthy state of the liver tissue in the fish powder-fed group.

Conclusion

The research focused on evaluating the effectiveness of dried anchovy powder as a dietary supplement, its impact on the bioavailability of essential nutrients, and its influence on the growth and nutritional status of male Wistar rats. Acknowledging anchovies' rich nutritional content and potential health benefits, the study employs a meticulously controlled experimental design to expose rats to various dietary interventions, including incorporating dried anchovy powder. Biochemical analyses were conducted to investigate the availability of critical nutrients such as proteins, omega-3 fatty acids, vitamins, and minerals. The research explores the effects of dried anchovy powder on growth parameters, including body weight, length, and organ development, while assessing the rats' nutritional status. Hematological and biochemical markers are examined to gain insights into the overall health of the experimental subjects. Furthermore, the study investigates potential mechanisms underlying the observed effects, focusing on nutrient absorption and metabolism. The outcomes of this research provide valuable insights into the potential of dried anchovy powder as a nutritional supplement, shedding light on its role in enhancing experimental animals' growth and nutritional status. These findings have implications for developing functional food products or dietary interventions to improve human nutrition and health.

Conflict of Interest

None declared.

Acknowledgments

The authors would like to express their sincere gratitude to the WorldFish project for providing funds to carry out the research work. The authors acknowledge the Director, ICAR-Central Institute of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India for providing the facilities to carry out this work and also for granting permission to publish the data acquired from the study.

References

1 Narayan J, John D, Ramadas N. Malnutrition in India: status and government initiatives. J Public Health Policy 2019;40(01):126–141
2 Hernández-Delgado EA. The emerging threats of climate change on tropical coastal ecosystem services, public health, local economies and livelihood sustainability of small islands: Cumulative impacts and synergies. Mar Pollut Bull 2015;101(01):5–28
3 Vianna GMS, Zeller D, Pauly D. Fisheries and policy implications for human nutrition. Curr Environ Health Rep 2020;7(03):161–169
4 Ghosh S, Sarkar T, Pati S, Kari ZA, Edinur HA, Chakraborty R. Novel bioactive compounds from marine sources as a tool for functional food development. Front Mar Sci 2022;9:832957
5 Kari N, Ahmad F, Ayub M. Proximate composition, amino acid composition and food product application of anchovy: a review. Food Res 2022;6(04):16–29
6 Ganias K. Biology and Ecology of Sardines and Anchovies. CRC Press Greece2014
7 Hopman LJ, Gilbert CR. Engraulidae: Anchovies. Freshwater Fishes of North America. Baltimore: John Hopkins University Press; 2014:332–353
8 Mohanty B, Mahanty A, Ganguly S, et al. Amino Acid compositions of 27 food fishes and their importance in clinical nutrition. J Amino Acids 2014;2014:269797
9 Di Bella G, Litrenta F, Pino S, et al. Variations in fatty acid composition of Mediterranean anchovies (Engraulis encrasicolus) after different cooking methods. Eur Food Res Technol 2022;248 (09):2285–2290
10 Sofoulaki K, Kalantzi I, Machias A, Pergantis SA, Tsapakis M. Metals in sardine and anchovy from Greek coastal areas: public health risk and nutritional benefits assessment. Food Chem Toxicol 2019;123:113–124
11 Tacon AG, Hasan MR, Subasinghe RP. FAO. Use of Fishery Resources as Feed Inputs to Aquaculture Development: Trends and Policy Implications. Rome: Food and Agriculture Organization of the United Nations; 2006
12 Venugopal V, Sasidharan A, Rustad T. Green chemistry to valorize seafood side streams: an ecofriendly roadmap toward sustainability. J Agric Food Chem 2023;71(46):17494–17509
13 Srinivasa Gopal T, Edwin L. Development of fishing industry in India. J Aquat Biol Fish 2013;1(01):38–53
14 Ravishankar CN. Recent Advances in Harvest and Post-Harvest Technologies in Fisheries. Rosemol Jacob M, Paul PT, Mathew S, Anandan R. Profiling of macro and micronutrients in seafood. , India: Cochin; 2021:270–282
15 Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226(01):497–509

16 Metcalfe L, Schmitz A, Pelka J. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal Chem 1966;38(03):514–515
17 Addass P, Midau A, Perez I, Magaji M. The effect of type and levels of animal protein supplements on the growth rate of rats. Agric Biol J N Am 2010;1(05):841–844
18 Zynudheen AA, Anandan R, Ninan G, Ashok Kumar K. Effect of dietary supplementation of fermented fish silage on growth performance in male Wistar rats. Fishery Technology 2016;53:301–306
19 Bautista-Teruel MN, Fermin AC, Koshio SS. Diet development and evaluation for juvenile abalone, Haliotis asinina: animal and plant protein sources. Aquaculture 2003;219(1–4):645–653
20 Toledo A, Frizzo L, Signorini M, Bossier P, Arenal A. Impact of probiotics on growth performance and shrimp survival: a meta-analysis. Aquaculture 2019;500:196–205
21 Kim KS, Jang MJ, Fang S, et al. Anti-obesity effect of taurine through inhibition of adipogenesis in white fat tissue but not in brown fat tissue in a high-fat diet-induced obese mouse model. Amino Acids 2019;51(02):245–254
22 Hoffmann G, Ijzer J, Brinkhof B, et al. Comparison of different methods to obtain and store liver biopsies for molecular and histological research. Comp Hepatol 2009;8(01):3
23 Karim NU, Sufi NFA, Hasan S, Hasan S. Quality analysis of anchovies, Stolephorus commersonnii dried in drying racks. J Sustain Sci Manag 2017;3:111–118
24 Xavier M, Piyadarshini B, Ninan G, et al. Enrobed snack product from Devis's Anchovy (Stolephorus commersonnii) and its quality evaluation during frozen storage. J Aquat Food Prod Technol 2018; 27(07):859–867
25 Wang Y, Zhang M, Mujumdar AS. Trends in processing technologies for dried aquatic products. Dry Technol 2011;29(04):382–394
26 Sablani SS, Rahman MS, Mahgoub O, Al-Marzouki A. Sun and solar drying of fish sardines. . Paper presented at: 13th International Drying Symposium; 27-30 August 2002; China
27 Abraha B, Samuel M, Mohammud A, Habte-Tsion HM, Admassu H, Al-Hajj NQM. A comparative study on quality of dried anchovy (Stelophorus heterolobus) using open sun rack and solar tent drying methods. Turk J Fish Aquat Sci 2017;17(06):1107–1115
28 Moses JA, Norton T, Alagusundaram K, Tiwari BK. Novel drying techniques for the food industry. Food Eng Rev 2014;6:43–55
29 Chotimarkorn C. Quality changes of anchovy (Stolephorus heterolobus) under refrigerated storage of different practical industrial methods in Thailand. J Food Sci Technol 2014;51(02):285–293
30 Chukwu O. Influences of drying methods on nutritional properties of tilapia fish (Oreochromis nilotieus). World J Agric Sci 2009; 5(02):256–258
31 Dewi EN. Chemical analysis during the processing of dried salted anchovy. J Coast Dev 2002;5(02):55–65
32 Chabbouh M, Hajji W, Hadj Ahmed SB, Farhat A, Bellagha S, Sahli A. Combined effects of osmotic dehydration and convective air drying on kaddid meats: Kinetics and quality. Dry Technol 2011; 29(13):1571–1579
33 Czerner M, Tomás MC, Yeannes MI. Ripening of salted anchovy (Engraulis anchoita): development of lipid oxidation, colour and other sensorial characteristics. J Sci Food Agric 2011;91(04): 609–615
34 Modibbo U, Osemeahon S, Shagal M, Halilu M. Effect of moisture content on the drying rate using traditional open sun and shade drying of fish from Njuwa Lake in North Eastern Nigeria. IOSR Journal of Applied Chemistry. 2014;7(01):41–45
35 Jahan SN, Joadder AR, Islam S. Assessment of proximate composition of oven dried Channa punctatus at three different temperatures. Int J Fish Aquat Stud 2019;7(05):311–314
36 Kumar GP, Xavier KM, Nayak BB, Kumar HS, Venkateshwarlu G, Balange AK. Effect of different drying methods on the quality characteristics of Pangasius hypophthalmus. Int J Curr Microbiol Appl Sci 2017;6(10):184–195
37 Ojutiku R, Kolo R, Mohammed M. Comparative study of sun drying and solar tent drying of Hyperopisus bebeoccidentalis. Pak J Nutr 2009;8(07):955–957
38 Sankar TV, Anandan R, Mathew S. Chemical composition and nutritional value of Anchovy (Stolephorus commersonnii) caught from Kerala coast, India. Eur J Exp Biol 2013;3(01):85–89
39 Kaya Y, Turan H. Fatty acids composition of Anchovy (Engraulis encrasicolus L. 1758) oil produced in Sinop-Turkey. Journal of Fisheries Sciences. 2008;2(05):693–697
40 Houlihan Dominic. Food intake in fish. de la Higuera M. Effects of nutritional factors and feed characteristics on feed intake. USA: Wiley; 2001:250–268
41 Khan MA, Abidi SF. Optimum ration level for better growth, conversion efficiencies and body composition of fingerling Heteropneustes fossilis (Bloch). Aquacult Int 2010;18:175–188
42 Ferket P, Van Heugten E, Van Kempen T, Angel R. Nutritional strategies to reduce environmental emissions from nonruminants. J Anim Sci 2002;80(02):E168–E182
43 Ashcroft J, Semmler C, Carnell S, van Jaarsveld CH, Wardle J. Continuity and stability of eating behaviour traits in children. Eur J Clin Nutr 2008;62(08):985–990
44 Alagawany M, Elnesr SS, Farag MR, et al. Omega-3 and omega-6 fatty acids in poultry nutrition: effect on production performance and health. Animals (Basel) 2019;9(08):573
45 Ashraf SA, Adnan M, Patel M, et al. Fish-based bioactives as potent nutraceuticals: exploring the therapeutic perspective of sustainable food from the sea. Mar Drugs 2020;18(05):265
46 Chiesa G, Busnelli M, Manzini S, Parolini C. Nutraceuticals and bioactive components from fish for dyslipidemia and cardiovascular risk reduction. Mar Drugs 2016;14(06):113
47 Whittaker P, Hines FA, Robl MG, Dunkel VC. Histopathological evaluation of liver, pancreas, spleen, and heart from iron-overloaded Sprague-Dawley rats. Toxicol Pathol 1996;24(05):558–563
48 Krishna M. Patterns of necrosis in liver disease. Clin Liver Dis (Hoboken) 2017;10(02):53–56
49 Edozien JC, Switzer BR. Fatty liver in experimental protein-energy malnutrition in the rat. Exp Mol Pathol 1978;29(01):1–11

