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ARTICLE

Influence of Processed Mustard Seed Meals on Growth and Health Parameters in Indian Major Carps

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ABSTRACT

The current investigation focuses on intertwined relationships of ecology and aquaculture for the benefit of farmers involved in fish farming practices. The study evaluated glucosinolate reduction in black, brown, and white mustard meals as fish feed ingredients for Indian Major Carps. Fish were fed with 10% mustard meal-supplemented diets in three forms: Raw (R), Anti-nutritional Rich (AR), and Anti-nutritional Lowered (AL), alongside a control group using floating feed. The three-month indoor experiment (September-November 2023) was conducted in FRP tanks with triplicate treatments. Blood analysis revealed compromised health in AR-fed carps, with reduced hemoglobin levels in rohu, catla

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and mrigal and elevated total leukocyte counts indicating inflammation in all the three carps studied here. Liver function was impaired in AR-fed fish, shown by increased alanine transaminase levels, highest in rohu followed by mrigal and catla. Histopathological examination of AR-fed carps liver tissue revealed necrotic spots, deformed hepatocytes, and significant vacuolation. In contrast, AL-fed fish demonstrated improved health parameters through Complete Blood Count analysis, liver function tests, and histo-pathological observations, suggesting successful reduction of anti-nutritional factors in the processed mustard meals. In near future, replacement of unprocessed seed meal with processed seed meal will lead to economic gains in fish farming.

Keywords: Biochemical Parameters; Glucosinolates; Growth Parameters; Histo-Pathological Analysis; Indian Major Carps; Liver Function Test

1. Introduction

Mustard is globally used for oil extracted from its seeds and the oil cake left after and is used in preparing feed of farmed animals. However, it is interesting to note that protein content of mustard seed meal is higher (32–38%) thus, it becomes appropriate to be included in diet. But, it is the involvement of anti-nutritional factors that limits seed meals excessive use, only to be used as a supplement in animal feed[1]. The freshwater fishes lose their 65% of protein content in form of nitrogen (N) as they are ammonotelic in nature, excreting ammonia (NH_3). So, a protein rich diet becomes crucial for the survival and growth rate of freshwater fishes[2]. Mustard serves this role in form of seed meals but at the same time it is also limited by presence of anti-nutritional factors (phytic acid, glucosinolates and sinapine)[3]. By the soxhlet led oil extraction, the mustard seed meals loose most of their phytic acid and sinapine as the oil is extracted out, but the action of enzyme myrosinase inflicted upon by glucosinolates takes place as soon as the seeds are crushed to fill in a thimble for oil extraction and leads to formation of glucosinolate breakdown products (GBPs) which remain present in mustard seed meals and thereby, serves a major role in limiting the mustard's utilization as an ingredient only[4].

These total glucosinolates and GBPs can be measured by spectrophotometric methods, titrimetric methods and by advanced instrumental analysis like High Performance Liquid Chromnatography (HPLC)[5]. The processing techniques available can lower down the concentration of GBPs, especially isothiocyanates in these mustard meals and such meals can be preserved to be used as a feed supplement[6].

The body growth parameters of Indian major carps are key indicators of their aquaculture potential. Among Indian Major Carps the growth of Catla relatively exceeds over the Rohu and least growth potential is related to Mrigal over the due course of time. Thereby it becomes crucial to link the observations of growth related to feed optimizations. These management practices including aquatic environment optimization leads to better farming outputs and thereby enhances economic gain benefits to fish farmers[7, 8]. The aquatic environment management is governed by water quality parameters *viz.,* temperature, dissolved oxygen (DO), pH, total dissolved solids (TDS) and total alkalinity which are in all required at key points and their measurable variation necessarily affects the fish growth[9].

Among the biochemistry behind blood are the parameters that govern the fish health and thereby growth. These crucial parameters maintain the flow of essential to survival metabolites derived from feed supplied. The complete blood count (CBC) parameters oversees the inflammation, infection and regulates the metabolism by modulating essential oxygen supplying protein to tissues known as hemoglobin (Hb). The crucial cells associated with the protein Hb are red blood cells (RBC) and white blood cell (WBC) indicating the platelet count (PC) and total leukocyte count (TLC). The CBC parameters variation above and below the defined threshold levels are usually a sign of inflammation, tissue degeneration and susceptibility to further infection[10]. The largest organ of animal system, the liver is an indicator of overall health of that species. The diagnosis of liver cells, its metabolism and histo-pathology studies reveals the entanglement of various parameters of CBC that are the indicators of organism's growth and health. Moreover, enzymes found in liver [alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALPase)] are crucial to dietary absorption, assimilation and metabolic regulation of

the organism[11].

A homogenous biological structure of hepatocytes arranged in distinct cords is reflected in liver tissue sections of animal kingdom. The nuclei are located centrally in hepatocytes of fishes in polyhedral shape. The liver architecture is well-organized, with hepatic cords radiating from the central veins. Catla's liver histology shows hepatocytes organized in rows between sinusoids, with a similar structure to Rohu but slightly larger cell size, likely due to faster metabolism. The liver of Mrigal displays hepatocytes with distinct borders and round nuclei, similar to other carps but slightly more compact in arrangement[12, 13]. The damage to liver is reflected in its histo-pathology screening whereby the signs like necrotic spots, leukocyte infiltration, increased vacoulation and deformation of hepatocytes is visible under microscopic examination[14].

The current investigation revealed the impact of lowered anti-nutritional factor (glucosinolate) on health and growth parameters of Indian major carps where the AL feed supplement raised protein bioavailability to the metabolic system of carps. This influenced the carp growth as well as improved biochemical parameters (CBC and LFT) along with improved condition of the liver tissue, thus sustained better functioning. Since, the growth of carps also improved under the influence of AL supplemented diet, the fish farming upon the inclusion of AL diet shall provide benefits to those engaged in fish farming. Since, the mustard types studied here are consumed globally[3] (largely for their oil), the seed meal left after may be implemented (after lowering of the glucosinolates) in fish feed so as to influence the sector of fish farming as is evidenced by their inclusion in seed meal given as feed supplement to fishes here in this study. The food functional studies along with phytochemical screening have proved the essential phytochemical components in mustard to be considered in diet but, largely limited due to involvement of glucosinolates, the subsequent lowering of which may prove beneficial in feed and food[3].

2. Materials and Methods

The present experiment was conducted from September 2023 to November 2023 in circular FRP tanks kept indoors under polycarbonate house at Instructional Fish Farm, College of Fisheries, G.B. Pant University of Agriculture and Technology, Pantnagar, District Udham Singh Nagar, Uttarakhand, India. Geographical location of Pantnagar is 28°58′ N latitude and 79°25′ E, longitude with an altitude of 243.8 meter above mean sea level, in Tarai belt of the Shivalik range of Himalayas.

The set of seed meals with processing and lowered allylisothiocyanate (AITC) were named as Anti-nutritional Lowered (AL), the ones without processing and lowering of AITC were named as Anti-nutritional Rich (AR) and raw (R) seeds (non-defatted and un-processed) were named as R. The floating feed was used as Control (C). The fish treatments were named accordingly to the type of feed given. Rohu designated as 'r'; catla 'c' and mrigal 'm' were further named in tables according to fish feed type given, like r AR, r AL and r R. Likewise, c AR, c AL, c R; m AR, m AL and m R. The Control tank was designated as Control rcm.

2.1. Experimental Carps

In the present investigation, Indian Major Carps Rohu (*Labeo rohita*), Catla (*Labeo catla*) and Mrigal (*Cirrhinus mrigala*) were used as the experimental animals (because of their economic importance to farmers). The fish fingerlings were used at the start of experimentation and their health and growth was observed for a period of 90 days.

2.1.1. Feeding Experimentation with Mustard Seed

Mustard seed meal from three mustard *viz.,* black, brown and white were chosen for fish feeding 10% in addition to commercial floating feed that was obtained from market. The seeds were procured from Norman E. Borlaug Crop Research Centre, Gobind Ballabh Pant University of Agriculture and Technology (GBPUA&T), Pantnagar, which were inevitably provided by (Indian Council of Agricultural Research-Directorate of Rapeseed Mustard Research (ICAR-DRMR), Bharatpur, Rajasthan. Black, Brown and White Mustard were given in equal proportion (1:1:1) to carps in each feed treatment every day.

Total glucosinolates in seed meals were checked by spectrophotometric methods in Black, Brown and White mustard[5]. HPLC analysis was done to estimate isothiocyanates present in un-processed seed meals of Black, Brown and White mustard[15] so as to measure GBPs. The levels of phytic acid[16] and sinapine content[17] were assessed in ad-

dition to proximate analysis[18] of individual and combined seed meals. Only combined seed meals were fed to fishes in each fish feed treatment type.

The treatments were named according to the lowering and non-lowering of glucosinolate breakdown products (GBPs). The seeds were finely grounded and defatted using soxhlet assembly[19]. The determination and removal of AITC, a major GBP was done in seed meals[6, 20]. The modification done includes reduced incubation time from 8 hours to 7 hours at 80 °C and with added incubation time at 4 °C for further use after moderate air drying for 2 hours.

2.2. Water Quality Parameters

The multi-meter (Pro Dss YSI) was used to assess water temperature (°C), TDS (mgL^{-1}), pH, DO (mgL^{-1} & %). The total alkalinity (mgL^{-1}) was calculated using titration by using 0.02 N H_2SO_4. The phenophthalein and methyl orange were used as indicators. These parameters were assessed after every 15 days[21].

2.3. Physical Parameters

2.3.1. Body Growth Parameters

The body growth parameters were noticed with respect to initial weight gain (g), final weight gain (g), weight gain (g), length gain (cm), Specific Growth Rate (SGR) (% day^{-1}), FCR and survival (%)[22]. All these parameter were also assessed after every 15 days.

2.4. Biochemical Parameters

2.4.1. Complete Blood Count Analysis

The complete blood analysis was outsourced from Central Pathology Laboratory, Udham Singh Nagar using Hematology analyzer (PE6800). The parameters like Hb (g/dL), Total RBC (million/cumm), Total Leukocyte Count (TLC) (million/cumm), Differential Leukocyte Count (DLC): Neutrophils (N) (million/cumm), DLC: (Lymphocytes) L (million/cumm), DLC:E (Eosinophils) (million/cumm), DLC: M (million/cumm), DLC: Basophils (B) (million/cumm), Packed Cell Volume (PCV) (%), Mean Cell Volume (MCV) (fL), Mean Cell Hemoglobin (MCH) (pg), Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dL) and Platelet Count (PC) (lacs/mm3) were assessed thrice in rohu, catla and mrigal fed with all treatments at the end of every month. For every treatment type, 5 replicates of fishes were used for CBC analysis.

2.4.2. Liver Function Test Analysis

The Liver function test with respect to three enzymes *viz.*, ALT, AST and ALPase was done in all treatment and control fed rohu, catla and mrigal thrice at the end of every month by using Liver Function Test (LFT) kit (Sigma-Aldrich). The work was carried out at Metabolite Research Lab, Department of Biochemistry, CBSH-GBPUA&T. Pantnagar. For every treatment type, 5 replicates of fishes were used for CBC analysis.

2.4.3. Liver Histopathology Studies

The liver histopathology studies were conducted at Indian Council of Agricultural Research-Directorate of Cold Water Fisheries (ICAR-DCFR), Bhimtal, Uttarakhand in the month of January, 2024. The liver histopathology studies were carried at the end of 90 day experimentation. The liver tissue sections of 5 fish per treatment were used and from every replication a total of three slides were mounted so as to gain deeper insights into the histology studies.

Slaughter Method of Fishes

Fishes were left unharmed during blood and serum extraction by small 22 gauge needles and fin labeled at the end of every month. For liver tissue collections, the labeled fishes were collected in a tub size of 40 liters and 10 ml of clove oil is added to anesthetize them. After subjecting to incubation for 40 minutes in tub, the destruction of fish brain was done by physically hitting the individual fish head wrapped in cloth by surgical hammer and no movement left is ensured. Thereafter the fishes were dissected with surgical scissor and liver tissue sections were preserved in bouin solution for histological studies[23].

Fish Sample Procurement

For every feed treatment type *viz.*, AL, AR, R and C, three fishes were chosen from each type *viz.,* rohu, catla and mrigal. The samples were preserved in ice box with ice packs and carried to ICAR-DCFR, Bhimtal for further analysis. The liver tissue sections were cut from each replica and preserved in bouin's solution until further analysis. The fishes were named according to their treatments (**Table 1**).

Embedding and Section Cutting

The cut liver tissue sections were embedded using em-

Table 1. Fish, treatment types and feed composition.

Fish/Treatment Type	Anti-Nutritional Lowered (AL)	Anti-Nutritional Rich (AR)	Raw (R)
Rohu (r)	rAL	rAR	rR
Catla (c)	cAL	cAR	cR
Mrigal (m)	mAL	mAR	mR

bedding machine (Merck: Tissue Embedding Centre). The embedded sections were finely cut with microtome machine (Thermo Scientific HM 340E) to a trim size of 20 and size 4 micrometer. The cut embedded sections were placed on histology slides and made wet with water at a temperature of 45 °C to a brief period of 15 seconds each. The slides were further processed for staining.

Staining & Microscopic Examination

The experimental work pertaining to histo-pathological analyses was done after the completion of 90 days. The slides were mounted with hematoxylin and eosin stains with involvement of hexane and ethanol diluted to a range of 70–95% with an exposure to a time range of 5–15 minutes. Histology staining procedures were followed for three carps having three replicates and five slide sections per replicate, to an overall mounting of 180 slides. Liver tissue sections were dissected and preserved in bouins solution (composed of 5% acetic acid, 9% formaldehyde and 1.6% picric acid) until further analysis. Tissue sections were further embedded into wax and steps of de-paraffinization and dehydration were followed under staining procedures The hematoxylin and eosin dyes were used successively with xylene and ethanol as solvents at concentrations ranging from 10–95%. Following further steps the organs were processed[24]. With Thermo Scientific MICROM HM 340 E manual microtome, 4 μm liver tissue sections were cut and further processed for staining with hematoxylin and eosin[25]. The slides were permanently mounted and analyzed using the Differential Interference Contrast (DIC), Nikon, Model Eclipse Ci-L microscope.

The mustard seed meal feeding experimentation was done so as to assess the impact of dietary glucosinolates and their breakdown products present in mustard seed meals distinctly on Rohu, Catla and Mrigal. After the lowering of GBPs through biochemical processing, the titrimetric methods reflected the GBPs presence with same concentration in three different seed meals, making them AL feed type. While the GBPs were higher in seed meal with AR feed type due to non-involvement of biochemical processing. The feed treatment of R type contained only crushed seeds (which were not defatted). The defatted seed meals were obtained by soxhlet assembly. Further by biochemical processing, the AL and AR feed types of mixture of Black, Brown and White Mustard were given in equal proportion (1:1:1) to carps as 10% of floating feed diet. Meals were tested for proximate analysis (protein content) and total glucosinolate content before and after the alteration.

3. Results

The Rohu, Catla and Mrigal fed with AL seed meal were observed to have better body growth parameters than the fishes fed with AR and R seed meal. The control group seemed to be comparable to R seed meal while the body growth parameter values of AR seed meal fed carps were altogether lower than all the treatment groups. Moreover, CBC and LFT parameters of AL treatment group were better than AR, R and control group. The AL treatment group outperformed the control group among all the parameters when compared and thus proved better for fish growth and health.

3.1. Variation in Biochemical Parameters of Seed & Seed Meals

Three different mustard (brown, black and white) seed meals (AL and AR) and raw seed (R) were fed to Indian major carps (Rohu, Catla and Mrigal) with variation in GBPs concentration. The objective of the experiment was to analyze the impact of glucosinolates (present in varying concentration in different mustard types) on health parameters of Indian major carps, as they are large scale farmed fishes providing high economic benefits. Spectrophotometric analysis of total glucosinolates (μmol/g) was made in fresh seeds, in fresh seed meals through HPLC (% area) and titrimetric methods (%) were adopted to assess GBPs in biochemically processed and unprocessed seed meals (**Table 2**). Total glucosinolates (were found to be highest in brown mustard seed

(88.11) followed by black (71.30) and least in white (57.04). Using titration procedures, the processed seed meals were shown to have almost similar concentration of GBPs (0.31 for brown, 0.32 for black and 0.29 for white mustard seed meals) while, unprocessed seed meals were having GBPs as 0.91 for brown, 0,74 for black and 0.58 for white mustard seed meal. The phytic acid levels were almost similar ranging from (1.13 to 1.19 (mg/100 g) and sinapine content varied from (2.04 to 3.05 %). **Table 2** shows the concentration of glucosinolates in seed and seed meals. The HPLC analysis in un-processed seed meals with respect to isothiocyanates (major GBPs) shows close similarity with GBPs found in unprocessed seed meals quantified by titrimetric analysis. The amount of phytic acid and sinapine were also calculated. The phytic acid was found to be statistically non-significant (NS).

Table 2. Glucosinolate, Phytic Acid and Sinapine Content Analysis in Mustard Seeds and Seed Meals.

Type of Plant Part	**Processed Seed Meals**	**Un-Processed Seed Meals**	**Un-Processed Seed Meals**	**Phytic Acid (mg/100 g) (Seed)**	**Sinapine Content (%) (Seed Meal)**
Instrumentation	**Titrimetric Method**	**Titrimetric Method**	**HPLC**	**Spectrophotometer**	
Kind of Glucosinolates	**Glucosinolate Breakdown Products (%)**	**Glucosinolate Breakdown Products (%)**	**Isothiocyanates (% Area)**		
Black Mustard	0.32 ± 0.002	0.74 ± 0.006	69.83	1.19 ± 0.12	3.05 ± 0.03
Brown Mustard	0.31 ± 0.007	0.91 ± 0.005	82.07	1.17 ± 0.32	2.08 ± 0.11
White Mustard	0.29 ± 0.009	0.58 ± 0.008	63.74	1.13 ± 0.21	2.04 ± 0.07
C.D.	0.32	0.44	-	NS	0.42
C.V.	3.56	3.12	-	3.56	2.19

*The results were explicated as mean ± standard deviation. The statistical analysis of experimental analysis used ANOVA at $p < 0.05$.

The comparative proximate analysis of floating feed and uniformly combined three mustard seed meals were done, before and after alteration and found to have higher crude protein, fiber, fat and moisture content than floating fish feed (**Table 3**).

The HPLC peak analysis of three mustard seed meals with respect to isothiocyanates shows highest concentration of isothiocycantes (as % area) in brown (82.07) (**Figure 1**) followed by black (69.83) (**Figure 2**) and least in white mustard (63.74) (**Figure 3**).

Table 3. Comparative proximate analysis of floating feed and combined seed meals.

Proximate Analysis	**Floating Feed**	**Combined Mustard Seed Meals After Alteration in Processing**	**Combined Mustard Seed Meals Before Alteration in Processing**	**C.D.**	**C.V.**
Crude Protein (%)	30.00	33.85 ± 0.05	33.23 ± 0.13	NS	2.54
Crude Fat (%)	5.0	4.96 ± 0.12	4.82 ± 0.15	0.43	2.61
Crude Fiber (%)	5.5	8.87 ± 0.06	8.61 ± 0.11	0.38	2.52
Moisture (%)	12.0	14.76 ± 0.07	15.79 ± 0.04	0.58	3.78

*The results were explicated as mean ± standard deviation. The statistical analysis of experimental analysis used ANOVA at $p < 0.05$.

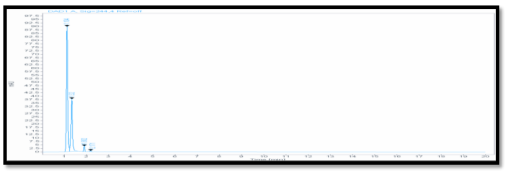

Figure 1. HPLC peak of brown mustard un-processed seed seals.

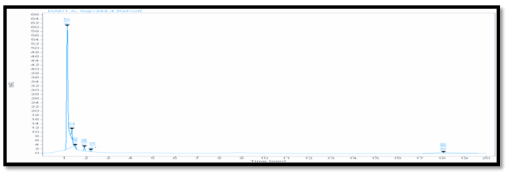

Figure 2. HPLC peaks of black mustard un-processed seed meals.

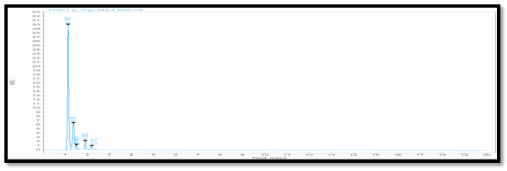

Figure 3. HPLC peaks of white mustard unprocessed seed meals.

3.2. Assessment of Water Quality Parameters

Among water quality parameters the uniform variation was observed in all the treatment tanks with regards to water temperature (27.6–30.5 °C), total dissolved solids (121.3–190.5 (mgL^{-1}), pH (7.3–8.6), dissolved oxygen (7.2–9.3 (mgL^{-1}) and total alkalinity (149.3–180.4 (mgL^{-1}) (**Table 4**).

3.3. Assessment of Growth Parameters of Fishes

The growth parameters of Indian major carps reared for 90 days were assessed monthly. The weight gain was found to be highest in Catla (83.0, 94.7, 84.4 and 92.3 g for AR, AL, R and control, respectively. Likewise, length gain (11.2, 13.5, 11.4 and 13.7cm) and SGR (1.24, 1.29, 1.23 and 1.32) were noted highest for Catla with respect to different feed treatments during experimental period. FCR and percent survival were noted highest for Mrigal in respect to every feed treatment type including control during wholesome period. Rohu was found to have middle scale values of weight gain and length gain (**Table 5**).

Table 4. Values of water quality parameters in different treatments.

Parameters	r AR	r AL	r R	c AR	c AL	c R	m AR	m AL	m R	Control rcm	C.D.	C.V.
Water Temp. (°C)	27.61 ± 0.13	30.59 ± 0.04	30.08 ± 0.06	30.12 ± 0.03	30.21 ± 0.08	29.81 ± 0.06	30.12 ± 0.16	29.71 ± 0.14	29.72 ± 0.19	29.76 ± 0.08	1.04	4.11
TDS (mgL^{-1})	121.30 ± 0.14	138.07 ± 0.06	131.45 ± 0.05	129.36 ± 0.07	144.88 ± 0.17	132.50 ± 0.07	190.56 ± 0.11	123.20 ± 0.18	130.34 ± 0.14	137.63 ± 0.04	2.56	3.16
DO (mgL^{-1})	8.53 ± 0.07	8.65 ± 0.23	8.51 ± 0.17	8.77 ± 0.12	7.21 ± 0.11	7.45 ± 0.08	7.38 ± 0.13	7.80 ± 0.15	8.55 ± 0.17	9.34 ± 0.23	0.46	3.19
DO (%)	81.66 ± 0.08	105.72 ± 0.02	86.4 ± 0.06	82.31 ± 0.18	70.48 ± 0.18	85.11 ± −0.19	79.93 ± 0.02	117.59 ± 0.09	99.73 ± 0.18	122.60 ± 0.17	5.53	3.78
Total Alk. (mgL^{-1})	180.45 ± 0.22	156.91 ± 0.05	172.40 ± 0.04	160.53 ± 0.14	156.82 ± 0.08	163.29 ± 0.16	185.32 ± 0.07	172.81 ± 0.04	166.95 ± 0.07	149.31 ± 0.16	6.49	2.84
pH	7.30 ± 0.015	8.37 ± 0.11	8.62 ± 0.14	8.17 ± 0.07	8.64 ± 0.04	7.94 ± 0.19	8.11 ± −0.02	8.30 ± 0.07	8.68 ± 0.08	8.14 ± 0.09	1.32	2.78

*The results were explicated as mean ± standard deviation. The statistical analysis of experimental analysis used ANOVA at $p < 0.05$.

Table 5. Growth parameters of experimental fishes.

Parameters	AR			AL			R			Control				
	r	c	m	r	C	m	r	c	m	R	C	M	C.D.	C.V.
Initial mean weight (g)	3.6 ± 0.02	6.8 ± 0.03	2.2 ± 0.04	3.7 ± 0.03	6.9 ± 0.04	2.6 ± 0.05	3.6 ± 0.04	7.1 ± 0.05	2.7 ± 0.04	3.5 ± 0.13	6.4 ± 0.11	2.5 ± 0.09	1.56	3.18
Final mean weight (g)	37.8 ± 0.03	89.8 ± 0.06	19.4 ± 0.02	42.5 ± 0.02	101.6 ± 0.03	39.8 ± 0.07	39.5 ± 0.08	91.5 ± 0.06	21.3 ± 0.02	41.3 ± 0.17	98.7 ± 0.10	35.4 ± 0.08	6.43	3.29
Weight gain (g)	34.2 ± 0.01	83.0 ± 0.03	17.2 ± 0.04	38.8 ± 0.12	94.7 ± 0.05	37.2 ± 0.05	35.9 ± 0.19	84.4 ± 0.02	18.6 ± 0.05	37.8 ± 0.13	92.3 ± 0.12	32.9 ± 0.06	5.67	2.82
Length gain (cm)	6.8 ± 0.05	11.2 ± 0.04	6.1 ± 0.06	7.3 ± 0.05	13.5 ± 0.06	7.2 ± 0.03	6.4 ± 0.13	11.4 ± 0.04	6.3 ± 0.07	7.1 ± 0.14	13.7 ± 0.15	6.9 ± 0.02	2.55	3.17
SGR (% day^{-1})	1.13 ± 0.04	1.24 ± 0.05	1.05 ± 0.04	1.17 ± 0.08	1.29 ± 0.07	1.31 ± 0.07	1.15 ± 0.06	1.23 ± 0.03	0.99 ± 0.03	1.19 ± 0.05	1.32 ± 0.11	1.27 ± 0.03	0.38	2.58
FCR	2.19 ± 0.03	0.90 ± 0.06	4.36 ± 0.02	1.76 ± 0.03	0.79 ± 0.02	2.01 ± 0.07	2.08 ± 0.07	0.88 ± 0.04	4.03 ± 0.07	1.98 ± 0.06	0.81 ± 0.18	2.27 ± 0.06	0.75	3.42
Survival 1%	89.3 ± 0.01	84.4 ± 0.03	86.4 ± 0.05	97.2 ± 0.05	98.1 ± 0.05	97.5 ± 0.03	85.6 ± 0.03	84.1 ± 0.05	85.6 ± 0.06	83.3 ± 0.03	85.2 ± 0.03	88.6 ± 0.05	2.71	4.88

*The results were explicated as mean ± standard deviation. The statistical analysis of experimental analysis used ANOVA at $p < 0.05$.

3.4. Assessment of Biochemical Parameters of Fishes

3.4.1. Complete Blood Count (CBC) Analysis of Fishes

Among CBC parameters, significant observations were noted with respect to feed treatments in all fishes for CBC. Among The AR consistently exhibited lower hemoglobin, RBC, PCV, and MCHC values, indicating persistent anemia. Total leukocyte counts remained within the reference range but showed a slight elevation in lymphocyte counts, hinting at immunogenic response involvement. Platelet counts were consistently lower than in other groups. This arising was later confirmed due to variation found in liver tissue sections. AL values fluctuated but generally remained within normal limits. Hemoglobin and RBC counts were relatively stable, with some improvement over time. Slight variability in TLC and DLC values was observed, but nothing extreme was found (**Supplementary Table S1**).

3.4.2. Liver Function Test (LFT) & Liver-Histo Pathology Analysis

The LFT test shown to have elevated enzymatic activity for ALT, AST and ALP in AR type feed treatment while, the lower scale monthly values of AL and Control were found to be similar to each other for all three carps (**Table 6**). AR

Table 6. LFT parameters of Indian Major Carps for the months of September, October and November, respectively.

Treatment Type	SGOT/AST (U/l)			ALPase (U/l)			SGPT/ALT (U/l)		
	N	O	S	N	O	S	N	O	S
Control	112.33 ± 0.24 (r)	108.15 ± 0.32 (m)	121.34 ± 0.17 (c)	39.76 ± 0.24 (r)	41.26 ± 0.91 (m)	39.63 ± 0.18 (c)	63.72 ± 0.71 (r)	61.98 ± 047 (m)	62.34 ± 0.63 (c)
r AR	195.43 ± 0.09	189.76 ± 0.56	187.99 ± 0.28	79.70 ± 0.31	76.31 ± 0.35	71.74 ± 0.27	99.81 ± 0.07	94.19 ± 0.13	91.23 ± 0.58
r AL	119.87 ± 0.14	105.87 ± 0.42	104.88 ± 0.34	45.21 ± 0.52	42.25 ± 0.47	41.17 ± 0.48	69.12 ± 0.85	68.76 ± 0.18	68.97 ± 0.42
r R	151.37 ± 0.25	145.64 ± 0.18	133.65 ± 0.39	54.86 ± 0.73	51.98 ± 0.59	48.90 ± 0.67	89.87 ± 0.88	81.98 ± 0.29	89.01 ± 0.71
c AL	117.70 ± 0.36	114.87 ± 0.27	101. 44 ± 0.33	48.14 ± 0.47	46.13 ± 0.14	41.11 ± 0.22	67.13 ± 0.18	66.76 ± 0.15	67.88 ± 0.13
c AR	182.87 ±	174.12 ± 0.28	173.09 ± 0.41	89.87 ± 0.30	87.65 ± 0.08	82.18 ± 0.20	95.76 ± 0.10	94.66 ± 0.82	92.34 ± 0.18
c R	151.26 ±	145.65 ± 0.35	148.77 ± 0.54	54.55 ± 0.31	53.27 ± 0.06	52.83 ± 0.23	85.61 ± 0.27	86.12 ± 0.33	87.84 ± 0.17
m AR	197.16 ±	188.12 ± 0.33	186.47 ± 0.36	83.12 ± 0.27	80.66 ± 0.22	78.14 ± 0.58	99.67 ± 0.23	97.13 ± 0.16	96.54 ± 0.25
m AL	123.45 ±	116.97 ± 0.38	113.12 ± 0.38	46.71 ± 0.18	42.92 ± 0.26	41.59 ± 0.61	60.98 ± 0.35	61.11 ± 0.25	63.01 ± 0.78
m R	135. 14 ±	126.89 ± 0.41	118.01 ± 0.28	65.87 ± 0.11	53.79 ± 0.10	56.12 ± 0.24	89.02 ± 0.48	84.81 ± 0.22	87.33 ± 0.18
C.D.	5.41	6.52	7.73	6.37	5.44	6.58	7.14	4.35	5.49
C.V.	2.58	2.64	2.93	3.11	2.85	3.08	2.74	2.67	2.33

*The results were explicated as mean ± standard deviation. The statistical analysis of experimental analysis used ANOVA at $p < 0.05$.

and R type feed treatments of all three carp were found to be elevated than AL and Control fed diet, reflected by observable signs of necrotic spots, inflammation and persistent vacuolation.

Since, body growth parameters, CBC and LFT reflected a pattern of variation among all three carps with AR, AL and Control feed type, the liver-histopathology becomes an inevitable tool to verify the above biochemical results. For this, the fishes were analyzed for the anomalies in their liver tissue. For every replica, the tissue sections were mounted on the slides with the help of embedding machine, microtome and following the staining procedures. The carps fed with AR type treatment were found to have vacuolation, hepatocyte deformations along with necrosis (**Supplementary Figure S1**).

Table 7 describes These observations were not recorded with AL type feed treatment in all experimental fishes**.** A system of semi-quantitative scoring was used so as to assess the intensity of lesions due to AR feed (**Table 7**). The intensity of lesion observed in three carps in relation to feed supplement are shown. The intensity increases progressively in ascending order from 1 to 5, reflected highest in AR fed diet and lowest in AL feed while R showed middle scale values. Moreover, LFT, CBC and growth were also observed to be better in all the three carps when fed with AL feed supplement.

Table 7. The semi-quantitaive scoring to assess the intensity of lesions.

Feed Treatment/ Intensity of Lesion	Rohu (r)	Catla (c)	Mrigal (m)
Anti-nutritional Lowered (AL)	1	1	1
Anti-nutritional Rich (AR)	4	5	4
Raw (R)	2	3	3

A comparison has also been drawn between histopathology of liver tissue sections from all three carps with respect to control and raw feed treatment. Careful analysis revealed higher signs of vacuolation and hepatocyte deformation with respect to raw feed treatment in comparison to control for all three carps (**Supplementary Figure S2**).

Moreover, the AL and Control feed groups with respect to liver histo-pathology were noticed for little to no signs of vacuolation with negligible observations for necrosis as well as for hepatocyte deformations. Thereby the carps fed with AL treatment were observed to have better body growth, CBC and LFT parameters compared to fishes fed with AR and R kind of feed treatments. Although, the biochemical parameter results of control type were comparable to AL type but body growth parameters of experimental fishes with AL feed found better than control group, suggesting the enhanced utilization of AL feed by all three carps, resulting in better health of fishes at medium to large scale fish farming systems. These all parameters were found to be co-related with results of liver histo-pathological assays.

3.5. Statistical Analysis

Statistical analysis has been done through ANOVA. For CBC (3 way), LFT (2 way) and for growth parameters (1 way) ANOVA has been assessed with $p \leq 0.05$. The reared carp population showed $p \geq 0.05$.

4. Discussion

Mustard seed meal is an important feed supplement due to abundance of dietary proteins but is also limited to certain extent by presence of anti-nutritional factors [1, 4]. In order to increase its amount as a feed supplement it is necessary to remove or, lower anti-nutritional compounds from mustard seed meals. These seed meals can be obtained from different mustard seed types based on their genotype, coat color and chemical composition [26]. The seed meals obtained from brown, black and white mustard have varying phytochemical composition with respect to glucosinolates, protein content and other metabolites [3]. The usage of mustard seed as seed meal can be enhanced by lowering the concentration of AITC (a major glucosinolate breakdown product) through already existing biochemical protocols and the changes in GBPs concentration can be measured titrimetrically [6, 20]. The RP-HPLC methodology can measure the amount of GBPs with isothiocyanates as standard [15]. This type of seed meal prepared from utilizing existing processes but with necessary alteration is utilized here as fish feed supplement. For comparative assessment, three different types of mustard types were chosen for this study differing widely in the concentration of glucosinolates but varying very minutely for phytic acid content (another major anti-nutritional factor) [27], so as to ascertain studying the effect of glucosinolates solely on biochemical and growth parameters of three different carps with overseen variation in seed meals and seeds. Liver cells or, hepatic cells showing signs like vacoulation (due to glycogen depletion in liver), cell degeneration or, cell death (necrosis), nuclei deformation or, fused nuclei are indicators of disease inflicted or, pathological condition of liver [28, 29].

In a previous study [30] species-specific variations in growth rates among Indian major carps under different feeding regimes, findings that align with the observations in this study. Mrigal exhibited the highest FCR and percent survival across all treatments, suggesting its greater resilience and efficiency in feed utilization compared to other species.

The superiority observed in performance for Mrigal indicates that it withholds the potential of better growth when fed with diverse feeding supplements. The growth performance of Rohu turns out to be intermediate in terms of length/weight gain. The feed type thus definitely has the effect on growth performance of carps. The authors of same research group have also highlighted the crucial role of varying feed supplements in optimizing the growth, the same concept validated by the current investigation. The AL fed Catla showed highest weight gains (101.6 g) in contrast to control (only floating feed fed Catla) which showed lowest weight gains comparatively. The reports indicated a direct positive correlation between feed quality and RBC production, the same revealed in the current investigation where AL fed three carps reflected higher RBC count in contrast to lowest reflected in AR fed carps [31]. Similarly, the PC was highest in AL fed carps, while TLC count, an indicator of inflammation was reflected highest in AR fed carps. An earlier study [32] reported TLC as an indicative of immune function, showing in this investigation as Rohu, Catla and Mrigal fed with AL feed having low TLC values will be immune to diseases. However, the immunity profiling studies are not accompanied in the current investigation posing a limitation. The reports [33] linked the CBC parameters like Packed Cell Volume (PCV) to hematological parameters the same pattern of which was noticed in the current investigation. The AL fed carps were observed to be with better PCV values and no lesions in liver tissue sections were observed in AL fed three carps. In contrast, the PCV values were lower in AR fed carps and deformations in terms of necrosis were seen in the current investigation. This altogether reinforces the trend of better health in carps fed with AL feed in comparison to AR, R and control diet. The study [34] reflected the role of nutrients and anti-nutrients in affecting the carp health as is observed in the current investigation by means of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) which were noticed altogether highest in AL fed carps. These CBC parameters are an indicative of enhanced oxygen-carrying capacity and hemoglobin content. Conversely, these lower values of CBC parameters of carps fed with AR in current investigation shows their deteriorating health in response to certain type of fish feed enriched with anti-nutritional factors (glucosinolates). The histo-pathology of liver tissue sections of AR fed carps in the current investigation reflected signs of necrosis and cell deformations as were found [34], where they observed hepatotoxic effects in response to glucosinolates as are observed in the current study.

A study [35] showed signs of vacuolation and deformation of hepatocytes in response to raw seed diets aligned with

the current investigation findings. Conversely, the AL and control groups exhibited negligible signs of vacuolation, hepatocyte deformation, or necrosis. These results corroborate the findings [36] where it was observed that liver health improved and pathological lesions reduced in fish fed with optimized diets. In terms of growth and biochemical parameters, fish fed with the AL treatment demonstrated superior body growth, complete blood count (CBC), and liver function test (LFT) parameters compared to those on AR and raw feed diets. Similar improvements in fish growth and physiological parameters due to optimized yet different feed compositions were reported [37]. Interestingly, while the biochemical parameters of the control and AL groups were comparable, the AL feed group exhibited enhanced body growth, suggesting a higher efficiency of nutrient utilization. These findings align with the observations where it was demonstrated that modified feeds reduce the bioavailability of toxic glucosinolates, thereby promoting better growth and health outcomes in fish [38–40].

In a previous study [15], it was demonstrated that isothiocyanates are major bioactive components in mustard oil and seed extracts, often present in higher concentrations compared to other compounds. These findings align with the current chromatographic results, where the main peak exhibited a significantly higher absorbance (~97.5 AU) indicative of an elevated concentration of isothiocyanates. In another study, Singh, R emphasized the importance of tailored feeding strategies, a concept further validated by the current study [41–43]. The AL treatment led to the highest weight gains in Catla, with final weights reaching 101.6 g, indicating the potential of this feed formulation for maximizing growth. In contrast, the control group exhibited significantly lower growth rates, reinforcing the need for specific feed strategies to enhance the performance of individual carp species. In terms of growth and biochemical parameters, fish fed with the AL treatment demonstrated superior body growth, complete blood count (CBC), and liver function test (LFT) parameters compared to those on AR and raw feed diets. Similar improvements in fish growth and physiological parameters due to optimized yet different feed compositions have been reported . Interestingly, while the biochemical parameters of the control and AL groups were comparable, the AL feed group exhibited enhanced body growth, suggesting a higher efficiency of nutrient utilization. These findings align with the observations [44], where it was demonstrated that modified feeds reduce the bioavailability of toxic glucosinolates, thereby promoting better growth and health outcomes in fish.

Overall, this study reinforces the detrimental effects of glucosinolate compounds and other toxic substances present in certain feed formulations, as previously noted [35, 39]. The reduced impact of these compounds in the AL feed diet not only improved liver histology but also positively influenced growth parameters, supporting its use in medium- to large-scale aquaculture systems. The observed correlations between histopathological and physiological parameters further emphasize the critical role of feed optimization in promoting fish welfare and productivity.

These findings contribute to the growing body of research on the growth responses of Indian major carps and underscore the importance of species-specific feed formulations in aquaculture.

Since, double zero genotypic lines of mustard seed already carry lower abundance of glucosinolates and erucic acid than normal germplasm accessions, so these may be utilized for preparation of seed meal but these are also scientifically harnessed to meet global demand of mustard for humans and their production is limited [40]. Thus, there is a need to develop novel fish seed meal from those mustard seed types that have higher glucosinolate proportion but still may be utilized in a greater proportion as feed for commercially important carps.

5. Conclusions

The present study reflects the involvement of glucosinolates and their breakdown products supplied through mustard seed meals in hampering the fish health. The types of feed included here were biochemically checked for the presence and abundance of glucosinolates in three distinct mustard seed meals (brown, black and white) through spectrophotometric, titrimetric and HPLC methods. The replacement of fish floating feed with mustard seed cakes is possible only when the anti-nutritional factors in mustard seed meals would be present in too low amounts so as not to hamper the fish growth. In this experiment, fishes fed with biochemically processed mustard seed meals (with lowest concentration of glucosinolates, AL) as compared to the seed meals (with

naturally high concentration of glucosinolates, AR) were experimentally proved to have the better health. Moreover, the histo-pathological study done on liver of the different feed type treatments has shown the results of negligible liver anomalies in AL and as well as in C group, while AR fed fish liver sections were identified with increased vacuolation, signs of necrosis, hepatocyte nuclei deformation and with some signs of leukocyte infiltration (an indication of inflammation). Similar observations were noticed in liver tissue sections for carps fed with R type treatment.

Since, the Indian major carps are meant for farming and consumption practice, their health becomes an important parameter to study. Fish farming when done with improved fish feed supplement shall prove beneficial to farming practices and shall also improve ecological practices in aquaculture. In the near future, the emphasis should be laid onto the involvement of anti-nutritional factor lowered different mustard types seed meal for fish farming, aiming at better fish growth and improved health.

Supplementary Materials

The following supporting information can be downloaded at https://journals.bilpubgroup.com/public/RE-9401-Supplementary-File.pdf.

Author Contributions

Writing—review & editing, Writing—original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization S.G. (Shivanshu Garg); Visualization, Validation, Supervision, Project administration H.P.; Writing—review & editing, Visualization, Validation, Supervision, Project administration A.M.; Visualization, Validation, Supervision, Project administration A.K.V.; Visualization, Validation, Supervision, Project administration N.P.; Software, Formal analysis R.S.; Supervision, Project administration S.G. (Saurabh Gangola).

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Institutional Review Board Statement

All procedures were performed in compliance with relevant laws and institutional guidelines and that the appropriate institutional committee has approved them under letter number CBSH/BC/1343 in accordance to University Animal Ethical Committee approved on 15th February, 2023, with synopsis number CBSH/BC/1360. The sflaughter method used for fishes is mentioned in material and methods section with EU 2010 as laid in directive 2010/63/EU.

Informed Consent Statement

Informed consent was obtained from all participants involved in the study.

Data Availability Statement

The data is available in the form of **Supplementary Material**.

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Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

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